Studies towards understanding the regulation and function of *bällchen* in different stem cell systems of *Drosophila melanogaster*

PhD Thesis

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AFFIDAVIT

Here I declare that my doctoral thesis entitled “Studies towards understanding the regulation and function of *bällchen* in different stem cell systems of *Drosophila melanogaster*” has been written independently with no other sources and aids than quoted.

Toma Yakulov
Göttingen, September 2008

Most experiments in this thesis were performed at the Max Planck Institute for Biophysical Chemistry in the Department of Molecular Developmental Biology, in the Bioanalytical Mass Spectrometry Group (Göttingen, Germany) and at the Institute of Molecular Biology and Tumor Research in AG Brehm (Marburg, Germany).

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Content

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Abstract

Bällchen (BALL) is a conserved Serine/Threonine kinase of *Drosophila melanogaster* suggested to participate in stem cell function. Here, I report the pattern of the *ball* transcripts and the *ball* protein (BALL). The results show that in the central nervous system (CNS) *ball* transcripts are enriched in the stem cells, referred to as neuroblasts (Nbs) and germline stem cells (GSCs), respectively. BALL is expressed not only in the stem cells, but also in their differentiating progeny. I identified the *cis*-acting regulatory region of *ball* gene, which is both necessary and sufficient to drive the expression of a reporter gene in a *ball*-like pattern in the CNS. I used the corresponding DNA region to isolate proteins of nuclear extracts of staged embryos which bind to the corresponding DNA *in vitro.* Using mass spectrometry and rigorous *in silico* selection criteria, a total of 296 factors were assigned to a putative *ball trans*-acting factor proteome. I also addressed the function of BALL in the two stem cell populations by asking whether *ball* activity is required in larval Nbs and adult male GSCs and in which processes BALL is involved. Removal of the BALL activity in male GSCs causes them to leave the stem cell niche and to differentiate. Removal of the BALL activity from Nbs caused the loss of the functional marker protein Miranda and resulted in fewer differentiating cells. The results indicate that *ball* is essential for the maintenance of the stem cell character in both stem cell systems analyzed.

List of figures

List of tables

Abbreviations

ahs	after heat-shock
ALH	After Larval Hatching
AP	Alkaline phosphatase
BALL	Bällchen protein
ball	*bällchen*
bp	base pairs
C	Celsius
CNS	Central Nervous System
DaPKC	*Drosophila* atypical protein kinase C
Dlg	Discs large
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ESI-MS	Electrospray Ionization Mass Spectrometry
FBgn	Flybase gene number
Fig	Figure
FISH	Fluorescent *In Situ* Hybridization
FLP	Flipase
FRT	Flipase recognition target sequence
GAL4	GAL4 transcription factor
GFP	Green Fluorescent Protein
GMC	Ganglion Mother Cell
GO	Gene Ontology
GSC	Germline Stem Cell
h	hours
Hts	Hu-li tai shao
IgG	Immunoglobulin G
IgY	Immunoglobulin Y
l	liter
lacZ	the gene that encodes Beta-galactosidase
LB	Luria Bertani broth
Lgl	Lethal(2) giant larvae (Lgl)
Loco	Locomotion defective
MARCM	Mosaic Analysis with a Repressible Cell Marker
MASCOT	Matrix Science
mg	milligram
µg	microgram
min	minute
µl	microliter
ml	milliliter
mRNA	messenger Ribonucleic Acid
MS/MS	Tandem Mass Spectrometry
NaCl	Sodium chloride
Nano LC	Nano liquid chromatography
Nb	Neuroblast
NH_4HCO_3	Ammonium Hydrogen Carbonate
PBS	Phosphate buffered saline
PCI	Past Clone Induction
PCR	Polymerase Chain Reaction
Pins	Partner of Inscuteable
Pol	Polymerase
ppm	parts per million
Pros	Prospero
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
Tris	Trishydroxymethylaminomethane
UAS	Upstream Activating Sequence
UTR	Untranslated region

1. Introduction

Multicellular organisms consist of different types of differentiated cells, which can be distinguished by morphological and functional criteria. The majority of cells is short lived as compared to the lifespan of the organism and they are continuously replenished from pools of tissue specific stem cells (Kay, 1965; Reya et al., 2001). A fundamental feature of stem cells is their ability to undergo multiple asymmetric divisions, through which they self-renew and produce differentiating cells (Kay, 1965; Reya et al., 2001). The balance between the processes of self-renewal and differentiation is very important, since imbalance in the regulation of these processes can lead to malignancy (Al-Hajj & Clarke, 2004; Singh et al., 2003; Alison & Lovell, 2005). A major scientific challenge is to understand how a stem cell retains its stem cell character through multiple cell divisions and how the differentiation process is initiated in the daughter cells.

In recent years, the neuroblasts (Nbs) and the male and female germline stem cells (GSCs) of *Drosophila melanogaster* (referred to as *Drosophila*) have been established as model stem cell systems and thus, well characterized. (Doe, 2008; Egger et al., 2008; Fuller & Spradling, 2007; Gilboa & Lehmann, 2004). Nbs and GMCs have in common that they undergo self-renewing divisions and generate cells with distinct fates. However, there are fundamental differences in the way Nbs and GSCs maintain the stem cell character. In GSCs, the stem cell behavior is governed predominantly by extrinsic signaling from a group of somatic cells situated nearby, which form the so called “niche” (Gilboa & Lehmann, 2004; Spradling et al., 2001; Li & Xie, 2005). In contrast, Nb self-renewing division is dependent on the asymmetric distribution of fate determinants between the two daughter cells (Knoblich, 2008; Wodarz & Huttner, 2003; Egger et al., 2008). Unraveling the mechanisms that govern stem cell maintenance in those two systems will bring further insights into the fundamental principles of stem cell biology and contribute to the understanding of the mammalian stem cell system.

1.1 *Drosophila* neuroblast system

The central nervous system (CNS) of *Drosophila* is derived from the neuroectoderm, which is an epithelial sheet of cells with apico-basal polarity on the ventral side of the embryo (Campos-Ortega & Hartenstein, 1997; Wheeler, 1893; Wheeler, 1891). From embryonic stage eight onwards the Nbs delaminate from the neuroectoderm (Campos-Ortega & Hartenstein, 1997; Wheeler, 1893; Wheeler, 1891). They divide continuously during embryonic development and, after a period of quiescence, continue proliferation during larval and pupal development to generate approximately 90% of the adult CNS cells (Prokop et al., 1998; Prokop & Technau, 1991; Truman & Bate, 1988). Nbs represent stem cells, which proliferate by dividing in an asymmetric fashion, thereby generating another Nb, a process termed self-renewal, and a smaller daughter cell, called a ganglion mother cell (GMC) (Campos-Ortega & Hartenstein, 1997; Doe, 1992). GMCs divide once to produce two lineage-specific post-mitotic cells, giving rise to either neurons or a glia cells (Campos-Ortega & Hartenstein, 1997; Doe, 1992).

Within the epithelial sheet, neuroectodermal cells divide parallel to the epithelial layer and thus, the cell fate determinants are symmetrically distributed between the daughter cells (Kaltschmidt et al., 2000). Once Nbs delaminate, their mitotic spindles rotate by 90°, which orients the following divisions perpendicular to the epithelial layer and along the apico-basal axes. This rotation and the asymmetric divisions of Nbs lead to an unequal distribution of cell fate determinants to the daughter cells (Broadus & Doe, 1997): the Nbs inherit the apico-basal polarity from the neuroectodermal cells and thus, the basally localized cell fate determinants (Broadus & Doe, 1997) segregate into the GMCs (Kaltschmidt et al., 2000).

In Nbs, a group of proteins organized in two complexes at the apical cortex directs cell-fate determinants to the basal cortex. The first apical complex (Par complex) consists of Bazooka, Par6 and *Drosophila* atypical protein kinase C (DaPKC) (Kuchinke et al., 1998; Schober et al., 1999; Wodarz et al., 1999; Petronczki & Knoblich, 2001). This complex binds through Bazooka to Inscuteable (Kraut & Campos-Ortega, 1996) which in turn binds to the second apical complex. The second complex (Pins/ Gαi complex) includes the proteins Partner of Inscuteable (Pins), Locomotion defective (Loco) and the heterotrimeric G protein subunit Gαi (Kraut & Campos-Ortega, 1996; Parmentier et al., 2000; Yu et al., 2000; Yu et al., 2003; Yu et

al., 2005; Schaefer et al., 2001). These complexes have distinct functions in Nb asymmetric divisions. The role of the Pins/ Gαi complex is to orient the mitotic spindle perpendicular to the neuroectodermal layer, whereas the Par complex serves to localize cell fate determinants to the basal cortex (Wodarz et al., 1999; Schober et al., 1999; Izumi et al., 2004).

The Par complex functions together with two tumor suppressor proteins, Discs large (Dlg) and Lethal(2) giant larvae (Lgl), and is required to localize the cell fate determinants Prospero (Pros), Brat and Numb as well their adaptor proteins Miranda and Partner of Numb at the basal cortex (Ohshiro et al., 2000; Peng et al., 2000; Betschinger et al., 2003; Doe, 2008; Egger et al., 2008). Miranda is an adaptor protein that associates with Pros and Brat. Once this complex is formed and segregated to the GMC, Miranda is rapidly degraded, thereby releasing its cargo (Ikeshima-Kataoka et al., 1997; Shen et al., 1997; Matsuzaki et al., 1998; Lee et al., 2006). Miranda is therefore only present in the Nb and not in the GMC and the descending neural cell lineage. The presence of both Pros and Brat in GMCs is important for fate decisions of these cells (Broadus et al., 1998; Bello et al., 2006; Betschinger et al., 2006). In contrast, Numb (Uemura et al., 1989) continues to be segregated from the GMC and is required to distinguish fates of sibling neurons that derive after GMC division (Spana et al., 1995; Spana & Doe, 1996; Buescher et al., 1998).

The localization process resulting in the asymmetrical distribution of factors in Nbs is based on the DaPKC-dependent phosphorylation of Lgl in the apical cortex region, causing Lgl inactivation. This process prevents the association of Lgl with the Par complex (Betschinger et al., 2003). In contrast, Lgl is not phosphorylated at the basal cortex and thus, it can recruit Miranda in this region of the cell (Betschinger et al., 2003). Aside from these distinct factors and complexes, the cytoskeleton also plays a role in assembly of these apical/basal complexes involving motor proteins Myosin II and Myosin VI in differential protein localization (Barros et al., 2003; Petritsch et al., 2003). Myosin II is restricted to the apical cortex and associates with phosphorylated Lgl to prevent an apical localization of cell fate determinants (Barros et al., 2003). Conversely, Myosin VI is necessary for basal localization of these determinants (Petritsch et al., 2003).

The balance between self-renewal and differentiation depends on correct asymmetric localization of cell fate determinants in Nbs and their segregation into GMCs (Gonzalez, 2007; Yu et al., 2006). It was recently shown that the DaPKC activity is essential for the Nb self-renewal (Lee et al., 2006). During mitosis, DaPKC segregates to the Nb and not to the GMC. Its proper localization depends on *lgl* gene activity (Lee et al., 2006) as observed with *lgl* mutants where DaPKC is partially delocalized to the basal cortex in some of the Nbs. As a result both Nb daughter cells develop into Nbs again and no GMC is generated. In *pins lgl* double mutants DaPKC is uniformly distributed throughout the Nb cortex, and a Nb overproliferation phenotype was found. Furthermore, ectopic expression of DaPKC, which is localized uniformly in the cortical region of Nbs, results also in an excessive number of Nbs (Lee et al., 2006). Thus, Lgl and DaPKC negatively regulate each other's activity. As already mentioned (see above), DaPKC phosphorylates and thereby inactivates Lgl at the apical cortex (Betschinger et al., 2003; Mayer et al., 2005). Conversely, Lgl restricts DaPKC activity to the apical cortex in Nbs (Lee et al., 2006).

Both Brat and Pros play a role in Nb maintenance. Pros is a homeodomain transcription factor (Hirata et al., 1995; Doe et al., 1991; Vaessin et al., 1991; Matsuzaki et al., 1992), which can act as a tumor suppressor in larval brain (Bello et al., 2006; Betschinger et al., 2006; Lee et al., 2006). In the embryonic nervous system, Pros is thought to activate genes required for terminal differentiation and repress Nb specific genes (Choksi et al., 2006). It acts in the process that decides whether stem cells undergo self-renewal or form GMCs. Consistent with this conclusion, GMCs are transformed into Nbs in *pros* mutant embryos (Choksi et al., 2006). Brat, a third factor required to distinguish between Nb and GMC identity, acts as a negative regulator of cell growth and ribosomal RNA synthesis (Frank et al., 2002). *brat* mutant Nbs divide asymmetrically as wild type, but the smaller putative GMCs grow in size and start expressing again Nb cell marker proteins such as Deadpan and Miranda. In addition, cell clones originating from a single larval *brat* mutant Nb produce an excess of cells which express Nb cell markers. This finding not only confirms the results obtained with embryonic Nbs, but also indicates that Brat function is required in Nb cells and thus acts in a cell-autonomous fashion (Bello et al., 2006; Betschinger et al., 2006; Lee et al., 2006). It remains unclear, however, how Brat inhibits the proliferation of GMCs which inherit the protein (see above). One possibility is that Brat inhibits the cell cycle inhibitor dMyc in GMCs. This proposal is

based on studies where Brat activity was abolished in Nbs and elevated levels of dMyc in all Nb daughter cells, which normally do not express this gene (Betschinger et al., 2006). In addition, there might be a functional connection between Pros and Brat, since *brat* expression is downregulated in *pros* mutant clones and the correct localization of Pros depends on Brat (Betschinger et al., 2006; Lee et al., 2006). In addition, expression of *pros* in *brat* mutant clones can rescue the Nb overproliferation phenotype. Thus, Brat function might be mediated directly or indirectly by Pros (Bello et al., 2006).

1.2 Germline stem cells in testis

The stem cell character of Nbs is maintained predominantly by Nb intrinsic cues. In contrast, maintenance of germline stem cells (GSCs) depends on external signals that derive from their microenvironment called “niche”, which refers to cells and the extracellular matrix that surround the stem cells (Gilboa & Lehmann, 2004; Tulina & Matunis, 2001; Kiger et al., 2000; Tran et al., 2000). In testis, the niche is termed “hub” and represents a distinct group of somatic cells, on average nine, at the very tip of the organ (Hardy et al., 1979). Stem cell division generates another stem cell, which remains directly attached to the hub, and a so called gonialblast, which moves away from the hub and differentiates (Hardy et al., 1979). Both stem cells and gonialblasts can be identified by the presence of a spherical organelle termed “spectrosome”, which consists of cytoskeletal proteins and vesicles (Lin et al., 1994; Roper & Brown, 2004; Leon & McKearin, 1999; McKearin & Ohlstein, 1995). The gonialblast undergoes four rounds of mitotic divisions to produce the 16-cell germline cyst (Fuller, 1993). The germline cyst cells remain interconnected by cytoplasmic bridges, the “fusomes” (Lin et al., 1994; Roper & Brown, 2004; Leon & McKearin, 1999). Subsequently, the germline cells enter meiosis and generate a total of 64 interconnected spermatids, which continue differentiation into mature sperms (Fuller, 1993). The hub region also contains somatic stem cells in addition to the GSCs (Lindsley & Tokuyasu, 1980). These somatic stem cells divide in a stem cell fashion to produce new stem cells which remain attached to hub and cyst cells, surrounding the differentiating germline cells (Lindsley & Tokuyasu, 1980; Hardy et al., 1979).

Physical attachment of stem cells to the niche cells seems to be a general requirement for stem cell maintenance in different niche-controlled stem cell systems of *Drosophila* (Yamashita et al., 2003; Song et al., 2002; Song & Xie, 2002) as well

as in vertebrates (Mitsiadis et al., 2007). In the *Drosophila* male GSC stem cell system, both hub cells and GSCs express high levels of DE-cadherin, a component of adherens junctions. They accumulate at the membranes where the two cell types are in contact (Yamashita et al., 2003). In addition, Armadillo, another component of adherens junctions, also co-localizes with DE-cadherin (Yamashita et al., 2003). If GSCs lack DE-cadherin, they leave the hub and differentiate (Yamashita et al., 2003).

In addition to being in physical contact with the niche, GSCs receive niche-dependent signals that are essential to maintain the GSC character in both the male and female germline (Xie & Spradling, 1998; Xie & Spradling, 2000; Song et al., 2004; Silver & Montell, 2001; Kiger et al., 2001; Tulina & Matunis, 2001; Kawase et al., 2004; Schulz et al., 2004; Shivdasani & Ingham, 2003). In males, the self-renewing process of GSCs depends on JAK-STAT signaling (Kiger et al., 2001; Tulina & Matunis, 2001; for review of the pathway see Arbouzova & Zeidler, 2006), whereas in females, GSC maintenance depends mainly on Decapentaplegic and Glass Bottom Boat signaling independent of JAK-STAT signaling (Xie & Spradling, 1998; Xie & Spradling, 2000; Song et al., 2004). The major components of the JAK-STAT signaling pathway are the ligand Upd, its receptor Domeless, the JAK kinase Hopscotch and the transcription factor Stat92E (Arbouzova & Zeidler, 2006). In testis, Upd is expressed in hub cells. Experimentally induced overexpression of *upd,* which causes constitutive activation of the signaling pathway, results in overproliferation of GSCs (Kiger et al., 2001; Tulina & Matunis, 2001). However, if the GSCs carry a mutation of the *hopscotch* gene, and thus lack the kinase activity which acts downstream of the Upd and its receptor, the GSCs get lost because they differentiate (Kiger et al., 2001; Tulina & Matunis, 2001). In addition to JAK-STAT signaling, Decapentaplegic signaling plays also a role in male GSCs maintenance, but its effect is less pronounced as compared to female GSCs (Schulz et al., 2004; Kawase et al., 2004; Shivdasani & Ingham, 2003).

1.3 Molecular and cellular functions of *bällchen*

The *bällchen* (*ball*) gene has recently been implicated to participate in the proper establishment of the male germline (Klinge, 2006). It encodes a conserved nuclear Ser-Thr protein kinase of the VRK1 family of kinases, but the conserved portion of BALL is restricted to the kinase domain (Klinge, 2006). This domain is 42% identical

with the human VRK1 (SwissProt ID: Q99986) and 43% with mouse VRK1 (SwissProt ID: Q80X41) (Klinge, 2006), whereas both the N- and C-terminal regions of BALL are not conserved. In *Drosophila* there is a second gene, CG8878 (Flybase ID: FBgn0027504), which encodes a protein kinase with 36% sequence identity to the BALL kinase domain (Klinge, 2006). A null mutation of the *ball* gene, however, causes 100% pupal lethality, which shows that *ball* carries an essential function, which cannot be compensated for by the normal activity of CG8878 (Klinge, 2006).

ball encompasses approximately 2,200 bp of genomic sequence at cytological position 97D on the right arm of third chromosome (Klinge, 2006). It has two annotated transcripts *ball-RA* (FlyBase ID: FBtr0085095) and *ball-RB (*FlyBase ID: FBtr0085096). The two transcripts differ in their non-coding 5'UTR due to an intron positioned in the 5'UTR sequence of *ball-RA*. The processed *ball-RA* transcript is 2027 bp long including its 133 bp 5'UTR sequence. The total length of the *ball-RB* transcript is 2049 bp, including its 185 bp 5'UTR sequence. The two transcripts have the same open reading frame (ORF) of 1800 bp which translates into a single protein of 599 amino acids, with a calculated molecular weight of 65.9 kDa. A detailed study of the molecular analysis of the gene is described by (Klinge, 2006).

There is an accumulating body of evidence that *ball* has a conserved function in chromatin organization (Ivanovska et al., 2005; Lancaster et al., 2007; Nichols et al., 2006; Gorjánácz et al., 2007) . In *Drosophila*, *ball* has been described to be required for female meiosis (Ivanovska et al., 2005; Lancaster et al., 2007). A hypomorphic mutation in *ball* results in defective oocytes that fail to build a metaphase I spindle and polar bodies (Ivanovska et al., 2005). In addition, a reduction in the level of *ball* protein in oocytes leads to a less compact karyosome and chromosomes tend to form extensive contacts with the nuclear envelope (Lancaster et al., 2007). Interestingly, *vrk-1*, the *C. elegans* homologue of *ball*, seems to have a different function because it is required for nuclear envelope assembly. *vrk-1* mutants fail to form a functional nuclear envelope and are unable to segregate chromosomes properly. VRK-1 localizes both to the nuclear envelope and the chromosomes, the latter in a cell-cycle specific manner (Gorjánácz et al., 2007).

Consistent with the VRK-1 localization to both nuclear envelope and chromosomes, biochemical studies revealed that the function of *ball* in chromatin organization is mediated by a conserved nuclear protein called BAF (Gorjánácz et al., 2007;

Ivanovska et al., 2005; Lancaster et al., 2007). BAF binds to both DNA (Lee & Craigie, 1998; Zheng et al., 2000; Suzuki & Craigie, 2002) and the LEM-domain proteins (Furukawa, 1999; Lee et al., 2001; Shumaker et al., 2001; Holaska et al., 2003; Mansharamani & Wilson, 2005) at the inner nuclear membrane. These features are consistent with the proposed function of BAF in chromatin organization during replication (Shumaker et al., 2001; Shimi et al., 2004). During interphase, BAF is found predominantly at the nuclear periphery (Shimi et al., 2004), where it is thought to participate in anchoring DNA at the inner nuclear membrane. During mitosis, the BAF distribution pattern becomes gradually diffuse and later, in anaphase, BAF is found associated with telomeres (Shimi et al., 2004; Haraguchi et al., 2001). At that stage, BAF was shown to be required for reassembly of the nuclear envelope (Haraguchi et al., 2001). In support of a functional interaction between BAF and BALL, *in vitro* phosphorylation studies have shown that BALL is able to phosphorylate BAF (Lancaster et al., 2007; Nichols et al., 2006; Gorjánácz et al., 2007). Phosphorylation of the extreme N-terminus of the human BAF protein by the human homologues of BALL, VRK1 and VRK2, disturbs its interaction with DNA and reduces its interaction with the LEM domain at the same time (Nichols et al., 2006). In *Drosophila*, overexpression of a BAF mutation which cannot be phosphorylated in oocytes causes an extensive contact of chromosomes with the nuclear envelope as has been observed in *ball* mutants (Lancaster et al., 2007). In *C. elegans,* downregulation of VRK-1 during mitosis results in an abnormal accumulation of BAF on chromatin, suggesting that VRK-1 activity is required for the dissociation of BAF from chromatin (Gorjánácz et al., 2007).

The hypomorphic allele *ball*^{1} (EP0863) of *Drosophila* carries a transposable element inserted in the 5'UTR of the *ball* gene (Klinge, 2006). The *ball*^{1} homozygous mutants are viable, but mutant males are sterile. Examination of the testes of *ball*^{1} homozygous mutants reveals that the organs are reduced in size and lack GSCs. This observation suggests that *ball* is required for spermatogenesis. The mutant testes contained a few cyst cells at early stages of differentiation and a few mature but immobile spermatozoa (Klinge, 2006).

Mobilization of the EP0863 insertion led to the generation of the amorphic *ball*^{2} allele. In this allele, 35 bp of the genomic DNA upstream of the translation start site and 117 bp of the open reading frame of the *ball* gene were deleted, which deleted the coding

sequence for the ATP binding site of the kinase domain, thereby abrogating functional BALL production (Klinge, 2006). In fact, *ball*^{2} homozygous mutants die in early pupal stage. Examination of the pupae revealed that no adult tissues are formed, which is consistent with the fact that *ball*^{2} homozygous mutant larvae lack imaginal discs from which adult appendages are formed (Klinge, 2006). The mutant larvae show also strong defects in CNS development as shown by their reduced brains, in which the optic lobes (Bate & Arias, 1993) were either remnants or absent (Klinge, 2006).

The expression pattern of *ball* is spatially and temporally controlled. Maternally deposited *ball* transcripts are ubiquitously distributed in the early embryo (Klinge, 2006). Later, *ball* mRNA, probably due to zygotic expression of the gene, is enriched in the elongating germ band. From embryonic stage eleven onwards, mitotic proliferation starts also in the CNS, the epidermis and the germline cells (Campos-Ortega & Hartenstein, 1997). At this stage, enrichment of *ball* mRNA correlates with the mitotic pattern in the CNS, where *ball* mRNA shows a dynamic spatiotemporal profile (Klinge, 2006). Towards the end of embryogenesis, *ball* transcripts are also detectable in germline cells which enter mitosis at that stage. The expression of *ball* in the CNS and the germline of both males and females remains persistent throughout development. *ball* transcripts are detectable in the germaria of the females as well as at the tips of the male testes where the proliferation of GSCs takes place. Taken together, the expression pattern of *ball* as well as the phenotype of the *ball* mutants suggest a role of *ball* during the proliferation of yet undetermined cells, including the stem cells (Klinge, 2006).

Here I report a detailed analysis of the expression patterns of both *ball* mRNA and BALL protein in the embryonic and larval CNS. I report also the characterization of the cis-acting control region of the *ball* gene which is required for its expression in the CNS. In addition, I isolated proteins which are able to bind specifically to DNA intervals of the control element. The identified proteins represent candidates which may act as transacting factors that regulate CNS expression of *ball.* In order to examine the function of *ball* in both Nbs and GSCs, I performed genetic studies involving mitotic recombination to generate *ball* mutant cell clones that were characterized with a variety of molecular markers to identify specific cell types. The

results show that BALL acts in a cell-autonomous fashion in both cell types and that its activity is required to maintain their stem cell character.

2. Materials and methods

2.1 Molecular biology

2.1.1 Quantification of nucleic acid concentrations

For the determination of nucleic acid concentrations, a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, USA) was used, and measurements were done according to the protocol of the manufacturer. In addition, nucleic acids were loaded on agarose gels containing 0.5 µg/µl ethidium bromide along with nucleic acids marker (GeneRuler, Fermentas, Burlington, Canada) of known molecular weight.

2.1.2 Polymerase chain reaction (PCR)

PCR to amplify DNA fragments was carried out according to standard protocols (Ausubel et al., 1999). Annealing temperatures and extension times were adjusted to fit the respective primer melting temperature and the length of the expected PCR product. The PCR conditions were varied to optimize the product yield. *Pfu* polymerase (Stratagene, Cedar Creek, USA) was used for the amplification reactions.

2.1.3 Primer design

Primers were designed with the program Lasergene (DNASTAR, Madison, USA). The primers used in this study are shown in Table 3.

2.1.4 Preparation of plasmid DNA

Plasmid DNA was purified using the QIAGEN Mini or Midi Kits (QIAGEN, Hilden, Germany) following the manufacturer's instructions.

2.1.5 Sequencing of DNA

DNA for sequencing was submitted to MWG (Eurofins MWG Operon, Ebersberg, Germany). The submitted DNA and primers were prepared according to the instructions.

Table 1: List of oligo-nucleotides.

Name	Purpose	Tag	Sequence 5'->3'
ball-CDS-5'SpeI	Generating *pBSSK+AgBall2*	SpeI	ccaaactagtaatgccgcgtgtagccaag
ball-CDS-3'NheI	Generating *pBSSK+AgBall2*	NheI	cctagctagcctatccctggtatttccg
ABgBall2-5'XhoI	Generating *pBSSK+ABgBall2 and pBSSK+AgBall2*	XhoI	cctactcgaggaaatttttcggggaaaagacg
gBall2-d-3'	Generating *pBSSK+ABgBall2, pBSSK+BgBall2, pBSSK+AgBall2*	None	ccgagtgcccagagtcacg
BgBall2-XhoI5'		XhoI	cctactcgagaatatatccttgaaatcataatcatc
AgBall2-5'BamHI	Generating *pBSSK+gBall2-AgBall2*	BamHI	ctcaggatccctgtcactcacttgacaacc
AgBall2-3'BamHI	Generating *pBSSK+gBall2-AgBall2*	BamHI	cagtggatccgatagacagctctgcaaaacgg
BallA5'	Generating *pC4 -AgBall2-lacZ-SV40*	none	catgggatccgaaatttttcggggaaaagacgcc
BallA3'	Generating *pC4 -AgBall2-lacZ-SV40*	none	catgagatctcaatgtgcggccacaaaaggaag
ball-A-5'	*pBSSK+AgBall2*	none	gatccgtaatatatccttgaaatcataatcatccttttttat ttttatgtttcaatgactcggcaacagccc
ball-A-3'	*pBSSK+AgBall2*	none	tcgagggctgttgccgagtcattgaaacataaaaata aaaaaggatgattatgatttcaaggatatattacg
attB-PstI-for	PCR of *attB* site	PstI	gatcctgcaggtcgatgtaggtcacgg
attB-XhoI-rev	PCR of *attB* site	XhoI	gatcctcgagtgtcgacatgcccgccgtg
bgal-BsiWI-for	Generating *pC4-ABgBall2-lacZ(-ball3')*	BsiWI	catgcgtacgtcttcccgagcgaaaacg
bgal-XbaI-rev	Generating *pC4-ABgBall2-lacZ(-ball3')*	XbaI	catgtctagattatttttgacaccagacc
pC4gBallLacZseq	Sequencing	none	gattaacccttagcatgtccgtg
Ball-Prom-01-for	DNA-Protein binding Oligo 1	Biotin	bio-gaaatttttttcggggaaaagacgccgtcagcggac
Ball-Prom-02-for	DNA-Protein binding Oligo 2	Biotin	bio-gtcagcggacgccaggggtggaacggaatatatcg atgtg
Ball-Prom-03-for	DNA-Protein binding Oligo 3	Biotin	bio-tatcgatgtgtggccaatcgatatgccctcacccctag at
Ball-Prom-04-for	DNA-Protein binding Oligo 4	Biotin	bio-acccctagatggtagcttccttttgtggccgcacattgc c
Ball-Prom-05-for	DNA-Protein binding Oligo 5	Biotin	bio-gcacattgccaagtggcgccgttttgcagagctgtcta tc
Ball-3UTR-for	DNA-Protein binding Oligo6	Biotin	bio-tttcaaggatatattacgatagacagctctgcaaaacg gc
Ball-Prom-01-rev	DNA-Protein binding Oligo 1	none	gtccgctgacggcgtcttttccccgaaaaaatttc
Ball-Prom-02-rev	DNA-Protein binding Oligo 2	none	cacatcgatatattccgttccacccctggcgtccgctg ac
Ball-Prom-03-rev	DNA-Protein binding Oligo 3	none	atctaggggtgagggcatatcgattggccacacatcg ata
Ball-Prom-04-rev	DNA-Protein binding Oligo 4	none	ggcaatgtgcggccacaaaaggaagctaccatcta ggggt
Ball-Prom-05-rev	DNA-Protein binding Oligo 5	none	gatagacagctctgcaaaacggcgccacttggcaat gtgc
Ball-3UTR-rev	DNA-Protein binding Oligo6	none	gccgttttgcagagctgtctatcgtaatatatccttgaaa

2.1.6 Restriction digest of DNA

Restriction endonucleases (New England Biolabs, Ipswich, USA or Fermentas Burlington, Canada) were used according to the manufacturer's instructions. For cloning, PCR-products or preparative amounts of plasmid DNA (10-20μg) were incubated overnight with 10-folds excess of enzyme. In case a partial digest was desired, a time course of 0 min to 60 min was performed to define the optimal reaction time.

2.1.7 DNA extraction from agarose gels

Ethidium bromide stained DNA fragment was visualized with UV light, excised with a clean scalpel from the agarose gel and purified using the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

2.1.8 DNA ligation

Ligation reactions were carried out according standard protocols with the following modifications. The reaction was carried out most often for 2 h at 12 °C in 10 µl reaction volume using 1 µl T4 DNA ligase (Fermentas, Burlington, Canada) with a total of approximately 100 ng of DNA and a molar ratio of insert DNA to vector DNA of 5:1. When optimization was required, these conditions were varied (Ausubel et al., 1999).

2.1.9 Agarose gel electrophoresis of DNA

Agarose gel electrophoresis of DNA was used to analyse PCR products and restriction digests, as well as for preparative agarose gels according to standard protocols (Ausubel et al., 1999). Depending on the fragment size, 0.8-2.0% agarose gels were prepared in 1x TBE buffer and ethidium bromide (0.5 μg/ml) was added to the gels. DNA bands were visualized with UV light (254 nm).

2.1.10 Transformation of bacterial cells

Escherichia coli DH5α or Top10 cells were obtained from Invitrogen (Karlsruhe, Germany). Chemically competent cells were prepared according to a standard protocol (Inoue et al., 1990). For transformation of ligation reactions (see section 2.1.8), an aliquot of chemically competent cells was added to approximately 100 ng of plasmid DNA followed by 30 min incubation on ice. Cells were then heat shocked in a water bath at 42°C for 45 s and immediately transferred back on ice. After 2 min

on ice, Luria-Bertani (LB) medium was added, and the cells were allowed to recover for 30 min at 37°C. The cells were distributed on LB agar plates containing antibiotics and incubated overnight at 37°C.

Table 2: List of plasmids.

Name	Backbone	Purpose	Reference
pC4-gBall-lacZ	*pCaSpeR 4*	Promoter analysis	Alf Herzig
pC4-gBall2-lacZ(-ball3')	*pCaSpeR 4*	Promoter analysis	This study
pC4-gBall2-lacZ-SV40	*pCaSpeR 4*	Promoter analysis	This study
pC4-gBall2-lacZ	*pCaSpeR 4*	Promoter analysis	This study
pC4-ABgBall2-lacZ	*pCaSpeR 4*	Promoter analysis	This study
pC4-ABgBall2-lacZ(-ball3')	*pCaSpeR 4*	Promoter analysis	This study
pC4-ABgBall2-lacZ-SV40	*pCaSpeR 4*	Promoter analysis	This study
pC4-AgBall2-lacZ	*pCaSpeR 4*	Promoter analysis	This study
pC4-BgBall2-lacZ	*pCaSpeR 4*	Promoter analysis	This study
pC4-attB-gBall2-lacZ	*pCaSpeR 4*	Promoter analysis	This study
pC4-attB-ABgBall2-lacZ	*pCaSpeR 4*	Promoter analysis	This study
pC4-attB-BgBall2-lacZ	*pCaSpeR 4*	Promoter analysis	This study
pC4-attB-ABgBall2-lacZ-SV40	*pCaSpeR 4*	Promoter analysis	This study
pC4-AgBall2-lacZ(-ball3')	*pCaSpeR 4*	Promoter analysis	This study
pC4 -AgBall2-lacZ-SV40	*pCaSpeR 4*	Promoter analysis	This study
pC4-attB-AgBall2-lacZ-SV40	*pCaSpeR 4*	Promoter analysis	This study
pUAS-attB	*pUAST*	Promoter analysis	Alf Herzig
pBSSK+gBall2-lacZ	*pBSSK*	Promoter analysis	This study
pBSSK+ABgBall2-lacZ	*pBSSK*	Promotor analysis	This study
pBSSK+gBall2	*pBSSK*	Rescue	Alf Herzig
pBSKS-betagal	*pBSKS*	Promotor analysis	Alf Herzig
pCaSpeR4	*pCaSpeR4*	Promotor analysis	Alf Herzig
pBSSK+gBall2-ball	*pBSSK*	Rescue	This study
pC4-gBall2-ball	*pCaSpeR*	Rescue	This study
pBSSK+BgBall2	*pBSKS*	Promotor analysis	This study
pBSSK+BgBall2-lacZ	*pBSKS*	Promotor analysis	This study
pBSSK+gBall2-AgBall2	*pBSKS*	Promotor analysis	This study
pBSSK+AgBall2	*pBSKS*	Promotor analysis	This study
pBSSK+AgBall2-lacZ	*pBSKS*	Promotor analysis	This study

2.1.11 Generation of DNA vectors

The primers used to generate DNA vectors are shown in Table 3. All constructs used in this study are shown in Table 4.

pBSSK+gBall2-ball: A *ball* coding sequence was amplified from a LD27410 cDNA clone with primers ball-CDS-5'SpeI and ball-CDS-3'NheI. The PCR product was

digested with SpeI and NheI and inserted into the SpeI and NheI sites of *pBSSK+gBall2.*

pC4-gBall2-ball: A 2618 bp fragment was excised with XhoI/ XbaI from *pBSSK+gBall2-ball* and inserted into the XhoI/ XbaI sites of *pCaSpeR4*.

pBSSK+ABgBall2: This construct was generated by PCR amplification of a 3629 bp fragment from the *pBSSK+gBall2* vector with primers gBall-d-3' and ABgBall2-5'XhoI, subsequent digest of the PCR product with XhoI and re-ligation of the restriction product.

pBSSK+ABgBall2-lacZ: A *lacZ* fragment with SalI and XbaI sites on both ends was inserted in the SalI and NheI sites of *pBSSK+ABgBall2.*

pC4-ABgBall2-lacZ: A 4110 bp fragment was cut out from *pBSSK+ABgBall2-lacZ* with XhoI and XbaI and inserted into the XhoI/ XbaI sites of *pCaSpeR4*.

pBSSK+BgBall2: This construct was generated by PCR amplification of the *pBSSK+gBall2* vector with primers BgBall2-XhoI5' and gBall2-d-3', digestion of the product with XhoI and subsequent re-ligation.

pBSSK+BgBall2-lacZ: A *lacZ* fragment with SalI and XbaI sites on both ends was inserted in the SalI and NheI sites of *pBSSK+BgBall2.*

pC4-BgBall2-lacZ: A 3955 bp fragment was cut out from *pBSSK+BgBall2-lacZ* with XhoI and XbaI and inserted into the XhoI/ XbaI sites of *pCaSpeR4*.

pBSSK+gBall2-AgBall2: This construct was generated by PCR amplification of the *pBSSK+gBall2* vector with primers AgBall2-5'BamHI and AgBall2-3'BamHI, digestion of the product with BamHI and subsequent re-ligation.

pBSSK+AgBall2: A 3515 bp fragment was amplified by PCR from *pBSSK+gBall2-AgBall2* with the primer pair ABgBall2-5'XhoI and gBall2-d-3', digested with XhoI and re-ligated.

pBSSK+AgBall2-lacZ: A *lacZ* fragment with SalI and XbaI sites on both ends was inserted into the SalI and NheI sites of *pBSSK+AgBall2.*

pBSSK+gBall2-lacZ: A *lacZ* fragment with SalI and XbaI sites on both ends was inserted into the SalI and NheI sites of *pBSSK+gBall2.*

pC4+AgBall2-lacZ: A 3996 bp fragment was cut out from *pBSSK+AgBall2-lacZ* with XhoI and XbaI and inserted into the XhoI/ XbaI sites of *pCaSpeR4*.

pC4-attB-BgBall2-lacZ: An *attB* fragment was generated by PCR from *pUAS-attB* with primers attB-PstI-for and attB-XhoI-rev. The PCR product was digested with PstI and XhoI and inserted into the PstI/ XhoI sites of the linearized *pC4-BgBall2-lacZ.*

pC4-attB-gBall2-lacZ: An *attB* fragment was generated by PCR from *pUAS-attB* with primers attB-PstI-for and attB-XhoI-rev. The PCR product was digested with PstI and XhoI and inserted into the PstI/ XhoI sites of the linearized *pC4-gBall2-lacZ.*

pC4-ABgBall2-lacZ(-ball3'): A *lacZ* fragment was generated by PCR from *pC4-hs43-lacZ* with primers bgal-BsiWI-for and bgal-XbaI-rev and subsequently digested with BsiWI and XbaI. *pC4-ABgBall2-lacZ* was digested with BsiWI and XbaI and the 11133 bp fragment was ligated with the *lacZ* PCR product.

pC4-ABgBall2-lacZ-SV40: An *SV40* fragment with terminal XbaI and BamHI restriction sites (Alf Herzig) was inserted into the XbaI/ BamHI sites of *pC4-ABgBall2-lacZ(-ball3')*.

pC4-AgBall2-lacZ(-ball3'): A *lacZ* fragment was generated by PCR from *pC4-hs43-lacZ* with primers bgal-BsiWI-for and bgal-XbaI-rev and subsequently digested with BsiWI and XbaI. *pC4-AgBall2-lacZ* was digested with BsiWI and XbaI and the 11019 bp fragment was ligated with the *lacZ* PCR product.

pC4 -AgBall2-lacZ-SV40: An SV40 fragment with terminal XbaI and BamHI restriction sites (Alf Herzig) was inserted into the XbaI/ BamHI sites of *pC4-AgBall2-lacZ(-ball3')*.

pC4-attB-AgBall2-lacZ-SV40: An *attB* fragment was generated by PCR from *pUAS-attB* with primers attB-PstI-for and attB-XhoI-rev. The PCR product was digested with PstI and XhoI and inserted into the PstI/ XhoI sites of the linearized *pC4-AgBall2-lacZ-SV40.*

pC4-gBall2-lacZ(-ball3'): A *lacZ* fragment was generated by PCR from *pC4-hs43-lacZ* with primers bgal-BsiWI-for and bgal-XbaI-rev and subsequently digested with BsiWI and XbaI. *pC4-gBall2-lacZ* was digested with BsiWI and XbaI and the 10978 bp fragment was ligated with the *lacZ* PCR product.

pC4-gBall2-lacZ-SV40: An SV40 fragment with terminal XbaI and BamHI restriction sites (Alf Herzig) was inserted into the XbaI/ BamHI sites of *pC4-gBall2-lacZ(-ball3')*.

pC4-attB-gBall2-lacZ-SV40: An *attB* fragment was generated by PCR from *pUAS-attB* with primers attB-PstI-for and attB-XhoI-rev. The PCR product was digested with PstI and XhoI and inserted into the PstI/ XhoI sites of the linearized *pC4-gBall2-lacZ-SV40.*

pC4-attB-ABgBall2-lacZ-SV40: An *attB* fragment was generated by PCR from *pUAS-attB* with primers attB-PstI-for and attB-XhoI-rev. The PCR product was digested with PstI and XhoI and inserted into the PstI/ XhoI sites of the linearized *pC4-ABgBall2-lacZ-SV40.*

pC4-attB-ABgBall2-lacZ: An *attB* fragment was generated by PCR from *pUAS-attB* with primers attB-PstI-for and attB-XhoI-rev. The PCR product was digested with PstI and XhoI and inserted into the PstI/ XhoI sites of the linearized *pC4-ABgBall2-lacZ.*

2.2 Biochemistry

2.2.1 Quantification of protein concentration

For determining the concentration of proteins, the Bio-Rad Protein Assay (Bio-Rad Laboratories, Munich, Germany) was used following the manufacturer's instructions. Briefly, the dye reagent was diluted 1:5 in water to a final volume of 1 ml. 980 µl of the diluted dye reagent were mixed with 20 µl of protein and the absorbance was measured at 598 nm. A standard curve, generated with a dilution series of bovine serum albumine was used to deduce protein concentration.

2.2.2 Polyacrylamide gel electrophoresis (SDS-PAGE)

Denaturing polyacrylamide gel electrophoresis based on the method developed by Laemmli 1970 was used according to standard protocols (Ausubel et al., 1999). For separation of the proteins on gels, the Bio-Rad MiniProtean 2 system (Bio-Rad, Munich, Germany) was used.

2.2.3 Preparation of nuclear extracts from staged embryos

Nuclear extracts from 0-12 hours old embryos were prepared in the laboratory of A. Brehm, Institute of Molecular Biology and Tumor Research, Marburg according to a standard protocol (Sandaltzopoulos et al., 1995) with modifications. Briefly, embryos were stored for up to 72 hours at 4°C. The embryos were collected in three sieved embryo collection apparatus by using distilled tab water and a 1 inch paint brush. The embryos were transferred into a beaker and soaked in 100 ml water with 30 ml commercial bleach for 3 min, followed by rinsing with 1 l embryo wash buffer and then extensively with distilled tab water. The embryos were transferred into a pre-chilled and weighted beaker and their weight was determined. From here on, all steps were carried out at 4°C in the cold room. The embryos were resuspended in 2 ml/g embryos in buffer 1, poured into Yamato LH-21 homogenizer at 1000 rpm and disrupted with six subsequent passes. The homogenate was filtered through a single layer of miracloth (Calbiochem, San Diego, USA). Buffer 1 was added to the filtrate to a final volume of 5ml/g embryos. The nuclei were pelleted in a pre-cooled GSA rotor at 8000 rpm for 15 min. The supernatant was removed and the lipids were wiped from the walls of the tube. The pellet was resuspended in 1 ml/g embryos of buffer AB. The volume was measured and the liquid was distributed in ultracentrifuge tubes. To each tube was added 1/10 of the measured volume $(NH_4)_2SO_4$ pH 7.9. The centrifuge tubes were rotated for 20 min head-over-tail. The lysate was centrifuged at 35000 rpm for 2 h. The supernatant was transferred in a beaker placed on ice by pipetting from underneath the white lipid layer. For each ml of supernatant 0,3 g of finely ground $(NH_4)_2SO_4$ was slowly added for 5 min on ice, followed by spinning down the supernatant at 15000 rpm for 20 min. The supernatant was poured off and the pellet was resuspended in 0.2 ml/g pellet of Buffer C. The protein concentration was measured to be 18.5 mg/ml. The nuclear extract was dialysed against 2 l of buffer C for 4 h, followed by spinning down for 5 min at 9000 rpm.

2.2.4 DNA-protein binding assay

The complementary single stranded DNA oligo-nucleotides were annealed by applying a temperature gradient to the reactions from 95°C to 25°C with steps of 5°C for 5 min. The double stranded oligo-nucleotides were biotinylated at their 5’ end. 200 pmol of each double stranded (Table 1) oligo were incubated with 1 ml of nuclear extract (Section 2.2.3) for three hours head-over-tail at 4°C. In the meantime, 40 µl

streptavidin coated magnetic bead slurry (Dynabeads M280, Invitrogen, Karlsruhe, Germany) was prepared according to the manufacturer's instructions. The reaction was incubated for one hour at 4°C. The beads were collected using a magnetic tubes holder (Invitrogen, Karlsruhe, Germany), rinsed three times with Buffer C and washed 3 times for 20 min in Buffer C. The beads were collected and the supernatant was removed. The bound proteins were eluted by heating the beads at 99°C for 5 min and separated on 4-12% Bis-Tris Acrylamide gels (Invitrogen, Karlsruhe, Germany).

2.2.5 Mass spectrometric identification and analysis of proteins

Mass spectrometric identification of proteins was performed according to a published protocol (Takamori et al., 2006). Briefly, total protein is separated on 4-12% Bis-Tris Gel (Invitrogen, Karlsruhe, Germany). Each lane is cut into 24 equally sized gel pieces and *in-gel* digested. Extracted peptides from each piece were separated by Nano LC and directly analyzed by ESI-MS followed by MS/MS sequencing on LTQ OrbitrapXL (Thermo Fisher Scientific, Waltham, USA). Raw data were searched through MASCOT daemon on an in-house MASCOT search engine (Perkins et al., 1999). Peak lists of the 24 gel pieces are combined for a single search. Standard search parameters for this type of data were 5 ppm mass accuracy against a National Center for Biotechnology Information non-redundant database, two missed cleavages, variable modifications allowed, oxidation of methionine and carbamidomethylation of cystein residues. Cut off criteria were ions score greater than 25, one bold red peptide. The analysis and the figures were made with the programs Filemaker (Unterschleißheim, Germany) and R (open source graphical software http://www.r-project.org)

2.3 Immunostaining and FISH of embryos and organs

2.3.1 Fixation of embryos

Embryos for immunostaining or *in situ* hybridization were collected on apple juice agar plates supplemented with yeast. After dechorionation (3 min, 50% commercial bleach), embryos were fixed for 20 min on a rotating wheel in a mix of 750 µl heptane and 750 µl 7,4% formaldehyde in PBT. The lower aqueous phase was removed, 500 µl methanol were added and the embryos were devitelinized via osmotic shock (shaking in heptane/ methanol). All the liquid was removed from the embryos, which

settled on the bottom of the tube. The embryos were rinsed three times in methanol and stored in methanol at -20 °C (Hauptmann, 2001).

2.3.2 RNA *in situ* hybridization of embryos

The temporal and spatial expression pattern of mRNA transcripts during the embryonic development of *Drosophila melanogaster* were studied by *whole-mount in situ* hybridization using a modified protocol (Hauptmann, 2001). All the following steps were carried out on rotating wheel at room temperature unless otherwise stated. Approximately 50 µl of embryos were stepwise hydrated in PBT (see List of buffers). The hydrated embryos were incubated in 500 µl PBT and 500 µl Hybe (see List of buffers) for 15 min. After rinsing 3x with 500 µl Hybe, the embryos were pre-hybridized for 1 h in 500 µl Hybe at 57 °C. Hybridization was carried out over night with 1 to 2 µl of RNA probe in 30 µl Hybe at 57 °C. On the next day the embryos were washed 2x 15 min with 250 µl Hybe at 57 °C. The following washing steps were carried out at room temperature: 1x 20 min in a mix of 250 µl Hybe and 250 µl PBT, and 3x 20 min in 500 µl PBT. For signal detection the embryos were incubated for 2 h at room temperature with pre-absorbed anti-DIG alkaline phosphatase-coupled antibody (1:2000 diluted in PBT). Then, the embryos were rinsed 3x in 500 µl PBT, washed 3x 20 min in 500 µl PBT, transferred in AP-buffer and washed 3x 10 min in 500 µl AP-buffer. The signal was developed by incubating the embryos with 10 µl NBT/ BCIP substrate (Roche, Basel, Switzerland) in 1 ml of AP-buffer at room temperature. The reaction was stopped by washing the embryos 3x 5 min in 1 ml PBT. The embryos were dehydrated stepwise in ethanol and mounted in Canada balsam (Sigma Aldrich Chemie, Munich, Germany).

2.3.3 Immunostaining of embryos

Immunostaining was performed as described (Mitchison & Sedat, 1983) with modifications. 50 µl fixed embryos stored in methanol were stepwise rehydrated in PBTx and incubated for 30 min in 10% goat serum (Sigma Aldrich Chemie, Munich, Germany) in PBTx. Then, the embryos were incubated over night at 4°C head-over-tail with pre-absorbed primary antibodies in 10% serum in PBTx. All the following steps were carried out on a rotating wheel at room temperature in 10% serum in PBTx. Unbound antibodies were washed 3x 20 min. The embryos were incubated for 2 h with Alexa fluorescently labeled secondary antibodies (Invitrogen, Karlsruhe,

Germany). Subsequently the embryos were washed 3x 20 min and mounted in ProLong Gold (Invitrogen, Karlsruhe, Germany) mounting medium.

2.3.4 Immunostaining of larval brains or adult testes

A modified protocol was used (Wu & Luo, 2006). All dissections were done in Schneiders cell culture medium (Gibco, Eggenstein, Germany) at room temperature (RT) for no longer than 15 min before fixation. Organs were fixed for 10 min in 2% paraformaldehyde in PBTx. After rinsing in PBTx, the organs were transferred in PBT with 1% TritonX-100 for 30 min at room temperature. The organs were rinsed again in PBTx and incubated in 10% goat serum for 30 min (Sigma Aldrich Chemie, Munich, Germany) in PBTx. The organs were incubated with the primary antibody over night at 4°C on a rotating wheel. Primary antibodies were affinity purified rabbit α-BALL (1:400, generated against residues 1-352 of BALL, a kind gift from A. Herzig), rabbit α-VASA (1:2500, generated against full length *Vasa*, a kind gift from A. Herzig), rabbit α-Cleaved-Caspase3 (1:150, Cell Signaling Technologies, Beverly, USA), mouse α-HTS 1B1 (1:10, DSHB, Iowa, USA). The organs were washed 3 times for 20 min followed by incubation for 2 h with secondary antibodies. Secondary antibodies against mouse and rabbit IgGs were coupled to Alexa 488, Alexa 568, Alexa 633 (1:400, Invitrogen, Karlsruhe, Germany). Secondary antibodies against chicken IgY were coupled to Cy5 (1:400, Abcam, Cambridge, USA). Subsequently the embryos were washed 3 times for 20 min. For staining DNA, tissue was treated with RNaseA at 2 mg/ml in PBTx for 30 min, followed by staining with 10 µM Draq5 (Biostatus Ltd., Shepshed, UK). For DNA staining of testes, the concentration of Draq5 was increased 10 fold. For identification of GFP-marked clones direct GFP fluorescence was assayed. The organs rinsed and washed for 10 min in PBTx followed by mounting in Prolong Gold antifade medium (Invitrogen, Karlsruhe, Germany).

2.3.5 Immunostaining combined with RNA *in situ* hybridization of embryos

The RNA *in situ* hybridization was carried out as described in Section 2.3.2 based on a published protocol (Hauptmann, 2001), with the following modifications. As a primary antibody sheep α-DIG antibody from (Roche, Basel Switzerland) was used. After washing out the unbound primary antibody, embryos were incubated for 2 h with biotinylated α-sheep antibody from donkey (Jackson ImmunoResearch,

Willemsdorp, Belgium). Embryos were rinsed 3x in 500 µl PBT, washed 3x 20 min in 500 µl PBT. In the meantime, 10 µl of solution A and 10 µl of solution B from the ABC KITStandard (Vector Labs, Burlingame, USA) were mixed with 980 µl PBT and incubated for 30 min at room temperature. The embryos were incubated for 30 min in this solution for 30 min. Then, the embryos were rinsed 3x in 500 µl PBT, washed 3x 20 min in 500 µl PBT. As much as possible from the liquid was removed. 100 µl of the Tyramide Signal Amplification Kit System (Perkin Elmer, Waltham, USA) and 2 µl Cy3 tyramide reagent (Perkin Elmer, Waltham, USA) were added to the embryos. The reaction was incubated for 10 min in the dark and at all next steps the embryos were kept in darkened vials. The embryos were rinsed 3x in 500 µl PBT, washed 3x 20 min in 500 µl PBT. Then, the immunostaining reaction was performed as described in Section 2.3.3 using a modified protocol (Mitchison & Sedat, 1983).

2.3.6 Immunostaining combined with RNA *in situ* hybridization of larval brains

In this method a standard protocol was modified (Knirr, Azpiazu & Frasch 1999). For *in situ* hybridization the method described in Section 2.3.5 was used with the following modifications: the freshly dissected organs were fixed in 2% paraformaldehyde for 10 min, transferred to a vial with PBTx. The larval brains were incubated in 500 µl PBTx with 2 µl of ProteinaseK (10 mg/ml, Qiagen, Hilden, Germany). The brains were rinsed afterwards with PBTx and the immunostaining carried out as described in Section 2.3.4.

2.3.7 Confocal Laser Scanning Microscopy

Confocal microscopic images were obtained on a Leica AOBS SP2 confocal microscope (Leica Microsystems, Heidelberg, Germany). For quantification of clone frequencies, testes or larval brains were captured as a z-series of confocal images. These images were analyzed for marked cells with Leica TCS software package. Single images were processed using Adobe Photoshop CS3 (Adobe, San Jose, USA).

Table 3: List of antibodies.

Antibody	Dilution	Reference
α-DIG AP-coupled Fab fragment from sheep	1:2000	Roche, Basel, Germany
α-Vasa, rabbit	1:2500	A kind gift from A. Herzig
α-HTS, mouse	1:25	Developmental Studies Hybridoma Bank; (Ding et al., 1993)
α-Prospero, mouse	1:25	Developmental Studies Hybridoma Bank; (Campbell et al., 1994)
α-BALL, rabbit	1:450	A kind gift from A. Herzig
α-Elav, mouse	1:25	Developmental Studies Hybridoma Bank; (O'Neill et al., 1994)
α-Miranda, rabbit	1:1000	A kind gift from J. Knoblich
α-Beta-galactosidase, chicken	1:1000	Abcam, Cambridge, UK
α-Chicken-Cy2	1:400	Jackson Laboratory, Bar Harbor, USA
α-rabbit, Alexa-Fluor-488	1:400	Invitrogen, Karlsruhe, Germany
α-mouse, Alexa-Fluor-488	1:400	Invitrogen, Karlsruhe, Germany
α-rabbit, Alexa-Fluor-568	1:400	Invitrogen, Karlsruhe, Germany
α-mouse, Alexa-Fluor-568	1:400	Invitrogen, Karlsruhe, Germany
α-rabbit, Alexa-Fluor-633	1:400	Invitrogen, Karlsruhe, Germany
α-mouse, Alexa-Fluor-633	1:400	Invitrogen, Karlsruhe, Germany
anti-Cleaved-Caspase3, rabbit	1:150	Cell Signaling Technologies, Beverly, USA

Table 4: List of buffers.

Name	Composition
PBT	137 mM NaCl; 2,7 mM KCl; 10 mM Na_2HPO_4; 2 mM KH_2PO_4; 0,1 % Tween 20
Hybe	50 % formamide; 5x SSC; 0,1 % Tween 20; 400 µg/ml sonicated DNA from fish sperm; 200 µg/ml torula RNA; 100 µg/ml heparin; pH 5,0
AP-buffer	100 mM Tris pH 9,5; 100 mM NaCl; 50 mM $MgCl_2$; 0,1 % Tween20
PBTx	137 mM NaCl; 2,7 mM KCl; 10 mM Na_2HPO_4; 2 mM KH_2PO_4; 0,1 % TritonX 100
Buffer 1 (nuclear extracts from embryos)	15 mM Hepes pH 7.6, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM EGTA, 0.1 mM EDTA, 350 mM Sucrose, 1 mM DTT, 0.2 mM PMSF, 1 mM NaMBS
Buffer AB (nuclear extracts from embryos)	15 mM Hepes pH 7.6, 110 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.2 mM PMSF, 1 mM NaMBS
Buffer C	20 % Glycerol, 25mM Hepes pH 7.6, 100 mM KCl, 12.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.2 mM PMSF, 1 mM NaMBS

Table 5: Chromosomes and insertions.

Genotype	Reference
w^{1118}	(Lindsley & Zimm, 1992)
y^{1} M{*vas-int.Dm*}ZH-2A w*; M{*3xP3-RFP.attP*}ZH-86Fb	(Bischof et al., 2007)
wg^{Sp1}	(Buratovich et al., 1997)
y^{1} *w*^{1118} P{*70FLP*}3F	(Golic & Golic, 1996)
P{*neoFRT*}82B P{*tubP-Gal80*}LL3	(Lee & Luo, 2001)
TM3, Sb1, P{*35UZ*}2	(Duronio et al., 1995)
T(2;3)TSTL, CyO: TM6B,Tb^{1}	(Tio & Moses, 1997)
P{*UAS-lacZ.NZ*}20b	Hiromi and West, personal communication to A. Herzig
P{*neoFRT*}82B	(Xu & Rubin, 1993)
P{*tubP-Gal4*}	(Bello et al., 2003)
P{*Gal4-nos.NGT*}40	(Li & Gergen, 1999)
ball^{2}	(Klinge, 2006)
P{*pC4-attB-gBall2-lacZ*}	This study
P{*pC4-gBall2-ball*}	This study
P{*pC4-attB-AgBall2-lacZ-SV40*}	This study
P{*pC4-attB-ABgBall2-lacZ*}	This study
P{*pC4-attB-ABgBall2-lacZ-SV40*}	This study
P{*UASp.ballE*}2.1	A.Herzig, personal communication
P{*UASt.ballE*}	A.Herzig, personal communication
P{*GAL4-pros.MG*}	(Pearson & Doe, 2003)
P{*GAL4-worniu*}	J. Knoblich, personal communication.

2.4 *Drosophila* genetics

2.4.1 Fly strains and fly culture

Flies were maintained and propagated on a complex cornflour-soyflour-molasse medium supplemented with dry yeast. Fly stocks were kept at 18°C. Fly strains for analysis were maintained at 25°C with 20–30% humidity and 12 h/ 12 h light/ dark cycle. If not noted differently, flies were handled according to standard protocols. w*hite* flies were obtained from the Bloomington stock center (Bloomington, Indiana, USA). The chromosomes, mutants and insertions used in this study are listed in Table 5.

2.4.2 Generation of transgenic flies

Transgenic insertions were generated by microinjection of *Drosophila* embryos using standard techniques (Rubin & Spradling, 1982). *pC4-gBall2-ball* fly strain was generated by injection of embryos of genotype w^{1118}.The transgenic fly strains *pC4-attB-gBall2-lacZ, pC4-attB-AgBall2-lacZ-SV40, pC4-attB-ABgBall2-lacZ, pC4-attB-ABgBall2-lacZ-SV40* were generated by injecting the corresponding DNA into embryos of the genotype y^1 M{*vas-int.Dm*}ZH-2A w*; M{*3xP3-RFP.attP*}ZH-86Fb (Bischof et al., 2007).

2.4.3 Mating schemes

For clonal analysis in adult male testes, the following crossing scheme was used:

Males of the genotype

(1) w^*/ *Y;;* P{*neoFRT*}82B P{*Ubi-GFP*}83/ P{*neoFRT*}82B P{*Ubi-GFP*}83

were mated to virgin females of the genotype

(2) y^1 w^* P{ry^+, *hs-FLP*}1/ y^1 w^* P{ry^+, *hs-FLP*}1; D^3/ *TM3, Sb1*, P{*35UZ*}2

and in the next generation males of the genotype

(3) y^1 w^* P{ry^+, *hs-FLP*}1/ *Y*;; P{*neoFRT*}82B P{*Ubi-GFP*}83/ *TM3, Sb1*, P{*35UZ*}2

were collected and mated with virgin females of the following genotype:

(4) y^1 w^* P{ry^+, *hs-FLP*}1/ y^1 w^* P{ry^+, *hs-FLP*}1; D^3/ *TM3, Sb1*, P{*35UZ*}2

From this cross virgin females of the genotype

(5) y^1 w^* P{ry^+, *hs-FLP*}1/ y^1 w^* P{ry^+, *hs-FLP*}1; P{*neoFRT*}82B P{*Ubi-GFP*}83/ *TM3, Sb1*, P{*35UZ*}2

were collected and mated with males of control genotype (4) or test males carrying the $ball^2$ allele:

(6) w^*/ Y; P{*neoFRT*}82B/ P{*neoFRT*}82B

(7) w^*/ Y; P{*neoFRT*}82B *e* $ball^2$/ *TM3, Sb1*, P{*35UZ*}2

From mating animals of genotype *(3)* with *(4),* males of the genotype

(8) y^1 w^* P{ry^+, *hs-FLP*}1/ Y; P{*neoFRT*}82B P{*Ubi-GFP*}83/ P{*neoFRT*}82B were collected.

From mating animals of genotype *(3)* with *(5)* males with the following genotype were collected:

(9) y^1 w^* P{ry^+, *hs-FLP*}1/ Y; P{*neoFRT*}82B P{*Ubi-GFP*}83/ P{*neoFRT*}82B *e* $ball^2$

For clonal analysis in larval brains, the following crossing scheme was used:

In the first step two stocks were generated by mating males of the genotype

(1) *w*/Y; wg^{Sp1}/ T(2;3)TSTL, CyO: TM6B,Tb^{1}*; +/ *T(2;3)TSTL, CyO: TM6B,Tb^{1}*

with virgin females of the following genotypes:

(2) *w*/w**; P{*UASp.ballE*}2.1/ P{*UASp.ballE*}2.1; P{*neoFRT*}82B *e ball^{2}*/ *TM3, Sb1*, P{*35UZ*}2

(3) *w^{1118}*/ *w^{1118}*; P{*UAS-lacZ.NZ*}20b/ P{*UAS-lacZ.NZ*}20b; P{*neoFRT*}82B *e ball^{2}*/ *TM3, Sb1*, P{*35UZ*}2

From mating flies of genotype *(1)* with flies with genotype *(2)*, males and females of the following genotype were collected and a stock was established by mating those flies:

(4) *w*/ w**; P{*UASp.ballE*}2.1/ *T(2;3)TSTL, CyO: TM6B,Tb^{1}*; P{*neoFRT*}82B *e ball^{2}*/ *T(2;3)TSTL, CyO: TM6B,Tb^{1}*

From mating flies of genotype *(1)* with flies with genotype *(3)*, males and females of the following genotype were collected and a stock was established by mating those flies:

(5) *w*/ w**; P{*UAS-lacZ.NZ*}20b / *T(2;3)TSTL, CyO: TM6B,Tb^{1}*; P{*neoFRT*}82B *e ball^{2}*/ *T(2;3)TSTL, CyO: TM6B,Tb^{1}*

In parallel, males of the genotype

(6) *y^{1} w** / Y; P{*tubP-Gal4*}/ CyO, P{*ftz/ lacB*}E3; P{*neoFRT*}82B P{*tubP-Gal80*}LL3/ P{*neoFRT*}82B P{*tubP-Gal80*}LL3

were mated to virgin females of the genotype

(7) *y^{1} w^{1118}* P{*70FLP*}3F/ *y^{1} w^{1118}* P{*70FLP*}3F; +/ *T(2;3)TSTL, CyO: TM6B,Tb^{1};* P{*Gal4-nos.NGT*}40/ *T(2;3)TSTL, CyO: TM6B,Tb^{1}*

and in F1 males of the following genotype were collected and used in the next crosses

(8) *y^{1} w^{1118}* P{*70FLP*}3F/ *Y*; P{*tubP-Gal4*}/ *T(2;3)TSTL, CyO: TM6B,Tb^{1}*; P{*neoFRT*}82B P{*tubP-Gal80*}LL3 / *T(2;3)TSTL, CyO: TM6B,Tb^{1}*

In the next step, virgins of genotypes (4) and (5) as well as virgins of the genotype

(9) *w^{1118}*/ *w^{1118}*; P{*UAS-lacZ.NZ*}20b/ P{*UAS-lacZ.NZ*}20b; P{*neoFRT*}82B/ P{*neoFRT*}82B

were mated to males of genotype (8). From the mating of males of (8) with females of *(4)* larvae of the following genotype were collected

(10) *y^{1} w^{1118}* P{*70FLP*}3F/ *w**; P{*tubP-Gal4*}/ P{*UASp.ballE*}2.1; P{*neoFRT*}82B P{*tubP-Gal80*}LL3/ P{*neoFRT*}82B *e ball^{2}*.

From the mating of males of *(8)* with females of *(5)* larvae of the following genotype were collected:

(11) y^1 w^{1118} P{*70FLP*}3F/ *w**; P{*tubP-Gal4*}/ P{*UAS-lacZ.NZ*}20b; P{*neoFRT*}82B P{*tubP-Gal80*}LL3/ P{*neoFRT*}82B *e* $ball^2$

From the mating of males of *(8)* with females of *(9)* larvae of the following genotype were collected:

(12) y^1 w^{1118} P{*70FLP*}3F/ w^{1118}; P{*tubP-Gal4*}/ P{*UAS-lacZ.NZ*}20b; P{*neoFRT*}82B P{*tubP-Gal80*}LL3/ P{*neoFRT*}82B

For overexpression of *ball* in the embryonic central nervous system embryos from the following crosses were collected:

+/Y; P{*GAL4-pros.MG*} males were crossed to X/X; P{*UASp.ballE*}2.1 females

+/Y; P{*GAL4-pros.MG*} males were crossed to X/X; P{*UASt.ballE*} females

+/Y; P{*GAL4-worniu*} males were crossed to X/X; P{*UASp.ballE*}2.1 females

+/Y; P{*GAL4-worniu*} males were crossed to X/X; P{*UASt.ballE*} females

2.4.4 Induction of clones in adult testes

Males, 0-7 days old, were kept at 25°C and fed on yeast for at least one day before heat shock. Three subsequent heat shocks for 50 min at 38°C were applied in 12 hours intervals. For the heat shocks, the flies were placed in empty vials with moisturized foam stoppers. Between the heat shocks, flies were kept at 25°C with dry yeast in the vial. After the heat shocks, flies were mated to w^{1118} for the period of the analysis and kept in vials supplemented with dry yeast. Clones were induced in the following genotypes:

(1) y^1 *w* P{ry^+, *hs-FLP*}1/*Y*; P{*neoFRT*}82B P{*Ubi-GFP*}83/P{*neoFRT*}82B

(2) y^1 *w* P{ry^+, *hs-FLP*}1/*Y*; P{*neoFRT*}82B P{*Ubi-GFP*}83/P{*neoFRT*}82B *e* $ball^2$

Heat shock of genotype (1) generated control cell clones and heat shock of genotype (2) produced $ball^{2/2}$ mutant cell clones. Testes were dissected 48, 72 and 96 hours after the first heat shock.

2.4.5 Induction of clones in larval brains

For MARCM studies in larval brains (see section 2.5), flies were mated in fly cages closed with apple juice agar plates supplemented with yeast paste. For collections,

the plates were changed every 4 h. The old plates were kept at 25°C for 24 h. They were rinsed with water to remove larvae that already hatched. After another 2 h, the larvae that hatched in the meantime were collected and placed in vials with yeast paste. The vials were kept at 25°C and 20-30% humidity for 24 h. Then a heat shock was applied by placing the vials at 38°C in a water bath for 40 min. The vials were kept at 25°C for the next 72 h. At approximately 96 h after larval hatching larval brains were dissected. Clones were induced in animals of the following genotypes:

(1) y^1 w^{1118} P{*70FLP*}3F/ *X*; P{*tubP-Gal4*}/ P{*UASp.ballE*}2.1; P{*neoFRT*}82B P{*tubP-Gal80*}LL3/ P{*neoFRT*}82B *e* $ball^2$

(2) y^1 w^{1118} P{*70FLP*}3F/ *X*; P{*tubP-Gal4*}/ P{*UAS-lacZ.NZ*}20b; P{*neoFRT*}82B P{*tubP-Gal80*}LL3/ P{*neoFRT*}82B P{*tubP-Gal80*}LL3

(3) y^1 w^{1118} P{*70FLP*}3F/ *X*; P{*tubP-Gal4*}/ P{*UAS-lacZ.NZ*}20b; P{*neoFRT*}82B P{*tubP-Gal80*}LL3/ P{*neoFRT*}82B *e* $ball^2$

Heat shock of larvae of genotype (2) generated control clones, heat shock of larvae of genotype (3) produced $ball^2$ mutant clones and heat shock of larvae of genotype (1) produced rescue clones.

3. Results

3.1 *ball* expression in the embryonic and larval central nervous systems

ball has a dynamic temporal and spatial expression pattern during embryogenesis and larval development of *Drosophila melanogaster* (Klinge, 2006). The early embryo contains ubiquitously supplied maternal *ball* transcripts (Fig. 1A). These are excluded only from the pole cells, which bud out at the posterior of the embryo (Demerec, 2000) (Fig. 1A). During germ band extension *ball* mRNA is ubiquitous (Fig. 1B). Later on *ball* expression is gradually restricted to the central nervous system (CNS), where it is enriched in the neuroblasts (Nbs; Fig. 1C-F), which are morphologically identifiable both by size and position underneath the neuroectoderm (Doe, 1992). Towards the end of embryogenesis, *ball* mRNA in the CNS is degraded when most Nbs enter a phase of mitotic quiescence (Fig 1H). In late embryonic stages, *ball* transcripts can also be detected in the germline cells, which are included in the embryonic gonads and are derived from the primordial germ cells (Fig. 1G,H). *ball* transcripts in the CNS and the gonads can also be detected during larval development (Klinge, 2006). In third instar larvae, *ball* mRNA is expressed in optic lobes (for description of this organ see (Bate & Arias, 1993) as well as in the gonads of both males and females (Klinge, 2006).

I addressed the question whether the distribution of *ball* protein (BALL) parallels the expression of *ball* mRNA. To study BALL expression in the embryonic CNS, co-immunostainings of embryos in different embryonic stages were performed with α-BALL and α-Prospero (Pros) antibodies (Section 2.3.3). The transcription factor Pros specifically labels the nuclei of the GMC fraction of the CNS (Doe et al., 1991; Spana & Doe, 1995; Vaessin et al., 1991; Matsuzaki et al., 1992), and thus can serve as a GMC-specific marker protein.

Nbs were distinguished morphologically by their size and position underneath the neuroectoderm (Campos-Ortega & Hartenstein, 1997). As Nb divisions are asymmetric and thus directional, the GMCs form a layer underlying the Nbs. Confocal images of immunostained embryos were taken with a laser scanning confocal microscope and 3D reconstructions of embryonic CNS were analyzed (Section 2.3.7).

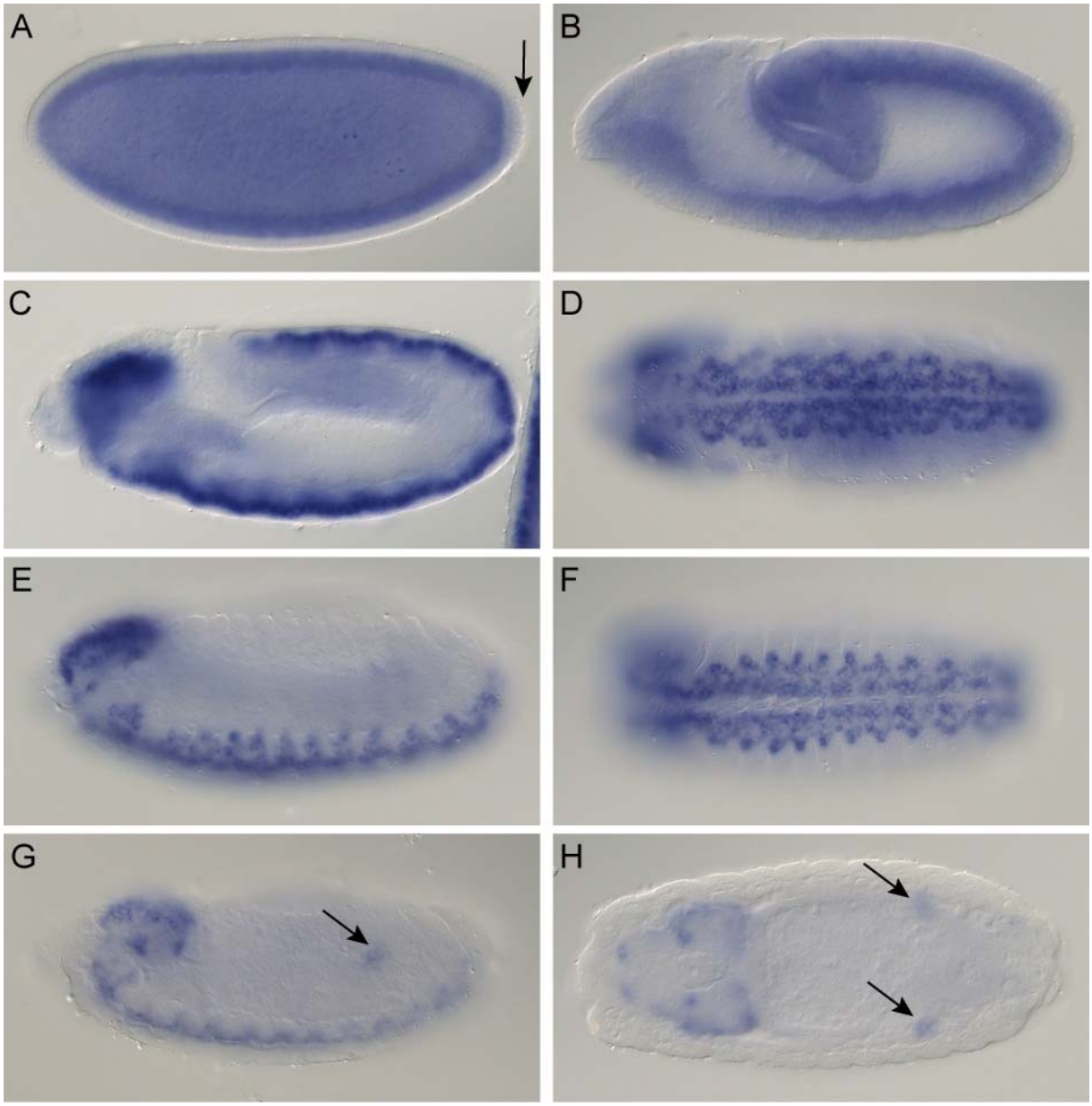


Figure 1: *ball* mRNA expression during embryogenesis.
(A) In embryonic stage 5 *ball* RNA is ubiquitous but excluded from the pole cells (arrow). **(B)** At stage 9 *ball* transcripts are enriched in the germ band (lateral view). **(C-D)** At stage 11 *ball* is expressed in Nb. **(E-F)** At stage 13 *ball* is expressed in Nbs. **(G)** In embryonic stage 15 *ball* expression in the CNS is downregulated. *ball* transcripts can now be detected in the embryonic gonads (lateral view). **(H)** In embryonic stage 16 *ball* is expressed only in a sub-set of cells in the CNS and in the gonads (dorsal view). Orientation of embryos: (A-E) anterior to the left, dorsal side up; (F-G) ventral view; (H) dorsal view.

The results summarized in Fig. 2E-S show that BALL is enriched in Nbs in the embryonic stages 10-13 but it is also present in GMCs. To further detail the distribution of *ball* mRNA in embryonic CNS, fluorescent *in situ* hybridization (FISH) with a *ball* antisense probe was carried out (Section 2.3.5). Immunostaining for Pros

detection was performed in parallel to label the GMCs. *ball* mRNA was enriched in the Nb layer and was very weakly detectable in the GMC layer at embryonic stage 11 (Fig. 2A-D), which correlates with the whole mount *in situ* data (Klinge, 2006). Similar to the expression of *ball* mRNA in embryonic CNS, BALL is enriched in Nbs but not restricted to Nbs of the larval brain. In addition, BALL is present at low levels in post-mitotic neurons (Alf Herzig, personal communication). To further characterize the distribution of *ball* mRNA in the larval brain, FISH with *ball* antisense RNA probe was combined with Elav immunostaining of brains from third instar larvae (Fig. 3A). Elav, a RNA-binding protein required for neurogenesis, localizes to the nuclei of neurons of the central and peripheral nervous systems of both embryos and larvae, and can be used as a neuronal marker (Robinow & White, 1991; Betschinger et al., 2006; Lisbin et al., 2001; Soller & White, 2003; Soller & White, 2005). The strongest signal from *ball* mRNA could be detected in Nbs of the ventral ganglion of the larvae (Fig. 3A); signals from optic lobe Nbs were weaker. Similar to the embryonic CNS, the levels of *ball* transcripts were significantly higher in Nbs than in neurons (Fig. 3B-D). The results indicate that *ball* mRNA is expressed in Nbs of both embryonic and larval CNS, whereas BALL protein is enriched in Nbs and found as well in GMCs and neurons.

The specific expression pattern of *ball* in Nbs prompted me to ask how *ball* expression is established, whether BALL activity has a specific function in Nbs and whether it is required for CNS development.

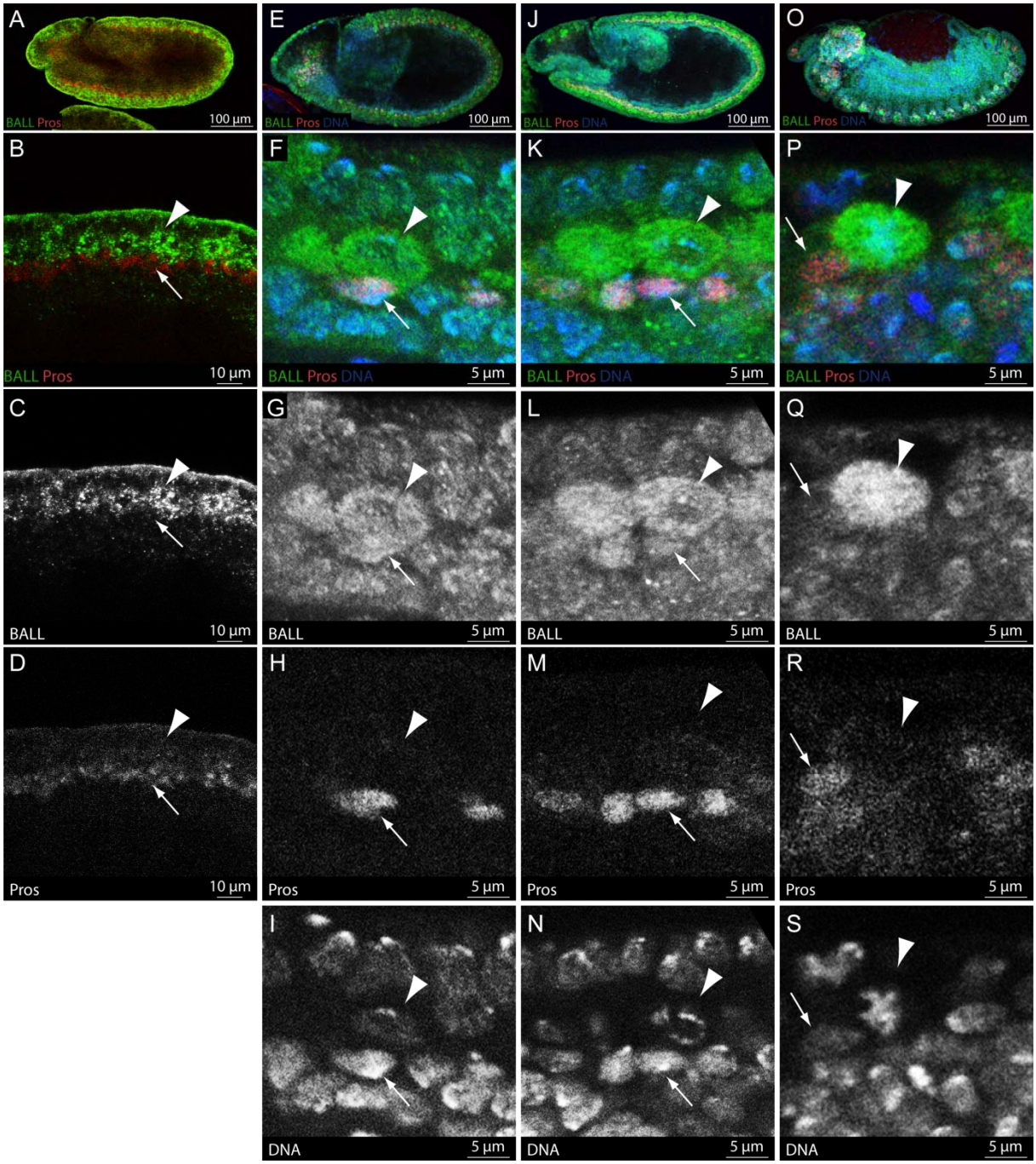


Figure 2: *ball* expression in embryonic neuroblasts.
In all panels staining of w^- embryos are shown. GMCs are labeled with Pros (red) and DNA is stained with Draq5 (blue). (A) *ball* RNA *in situ* of stage 11 embryos co-immunostained for Pros. (B-D) Magnification of Nbs and GMCs of embryo in panel (A). In (C-D) individual channels of panel (B) are shown. *ball* transcripts are detectable at high levels at stage 11 in the Nb layer (arrowhead). The GMC layer reveals lower levels of *ball* RNA. (E) Co-immunostaining for BALL and Pros in a stage 10 embryo. (F-I) Magnification of single cells from panel (E). At stage 10 the BALL protein levels in Nbs (arrowhead) and GMCs (arrow) are comparable. (J) Co-immunostaining for BALL and Pros in a stage 11 embryo. (K-N) Magnification of single cells from panel (J). BALL protein starts accumulating in Nbs (arrowhead) as compared to GMCs (arrow). (O) Co-immunostaining for BALL and Pros in stage 13 embryo. (P-S) Magnification of single cells from panel (O). BALL protein levels are strongly elevated in Nb (arrowhead) in comparison to GMCs (arrow). Orientation of the embryo (A, E, J, O) is anterior to the left, dorsal side up.

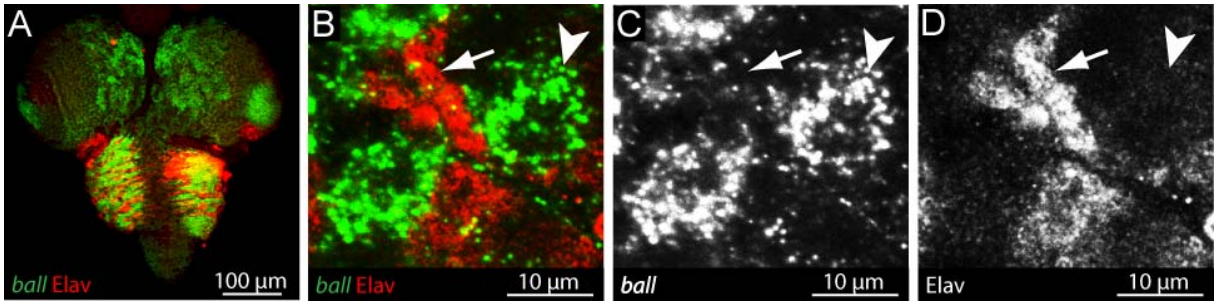


Figure 3: ***ball* mRNA expression in larval brains.**
(A) Fluorescent *in situ* hybridization of *ball* (green) and Elav immunostaining (red) of third instar larval brains. The strongest FISH signal could be detected in the region of the ventral ganglion. **(B-D)** At 10 fold higher magnification it is evident that *ball* mRNA (green) is enriched in Nbs (arrowhead) as compared to neurons (arrow). Panels (C-D) show single channels from panel (B).

3.2 *Cis*-regulatory elements of the *ball* gene

In order to identify the DNA sequences of the *ball* gene that control the temporal and spatial aspects of *ball* expression, I first identified a genomic sequence of the gene sufficient to rescue the homozygous lethal $ball^2$ allele (Klinge, 2006) by integration of the transgene DNA into the genome of the mutants. To generate the rescue transgene *pC4-gBall2-ball*, the *ball* coding sequence was cloned in a *pCaSpeR4* vector containing 568 bp of genomic sequence upstream of the *ball* translation start site and 284 bp downstream of the *ball* stop codon. The upstream genomic DNA contained the *ball* 5'UTR, 123 bp of intergenic sequence and the 5'UTR of the neighboring gene, *his2av* (Fig. 4A). The construct was injected in w^{1118} embryos and four independent transgenic fly lines were obtained. Flies from two of these lines were crossed to flies carrying the $ball^2$ allele, and the potential rescue of the $ball^2$ mutant individuals, which are normally lethal, was assayed. From the homozygous mutant flies with one copy of the transgene only the males survived. However, they showed a neurologic phenotype: their motor activity was impaired, for example, they were not able to climb the walls of the vials they were kept in. A more detailed phenotypic analysis was not performed. In contrast to one copy, two copies of the transgene could rescue the $ball^2$ pupal lethal phenotype of both males and females and led to the development of flies without a visible phenotype. Thus, 568 bp upstream of the *ball* translation start site and 284 bp downstream of the *ball* stop codon must contain *cis*-regulatory elements sufficient to ensure the correct temporal and spatial expression pattern of *ball*.

In order to identify *cis*-regulatory elements, which drive distinct spatial and temporal aspects of *ball* expression, I cloned various genomic subregions of the *ball* rescue transgene into P-element vectors. They carried as a reporter the bacterial *lacZ* gene. For integration of the constructs in the fly genome, the φC31 phage integration system was used (for a detailed description of the DNA integration see Bischof et al., 2007). In this system a φC31 phage integrase catalyses the recombination between a phage attachment site *attP* previously introduced in the fly genome by transposable elements ("landing site") and a bacterial attachment site *attB* present in the vector DNA (Thorpe et al., 2000). All constructs used for this study carried *attB* attachment sites upstream of the *ball* 5' genomic sequences. They were targeted for integration to the same landing site in the cytological position 86F on the 3rd chromosome, since

no positional effects had been reported for this landing site (Bischof et al., 2007). Due to this experimental arrangement, the expression of the different reporter transgenes from the same genomic locus could be directly compared. After successful integration of the transgene DNA, the genomic sequences could be tested for their ability to act as *cis*-regulatory elements for the reporter gene expression observed after whole-mount *in situ* hybridization of embryos with a specific *lacZ* anti-sense RNA probe.

If the temporal and spatial expression pattern of the reporter gene corresponded to that of *ball,* one could conclude that the *ball* genomic sequence included in the transgene would correspond to the respective *cis*-regulatory elements that also drive *ball* expression.

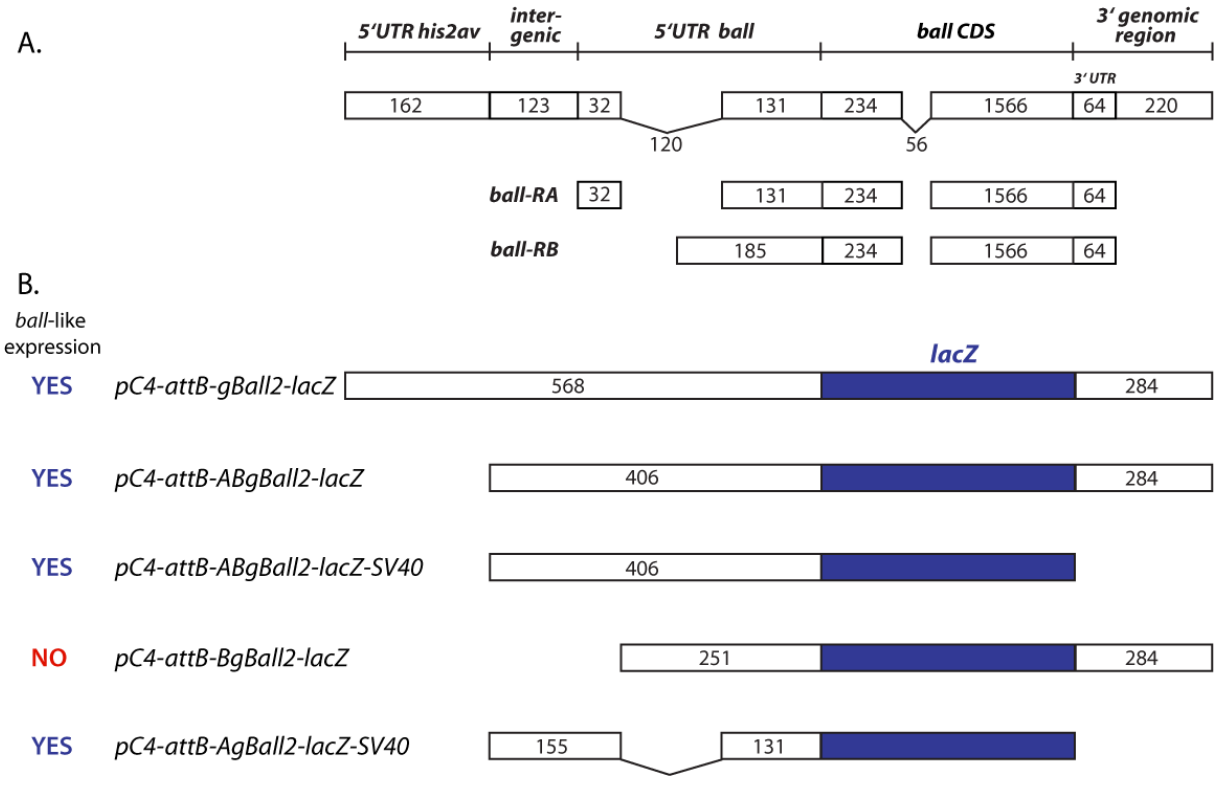


Figure 4: *Cis*-elements that regulate *ball* expression.
(A) The minimal genomic sequence, which is sufficient to rescue *ball*^{2}. The numbers represent base pairs. The drawing is not up to scale. **(B)** Schematic representation of the analysis of the *cis*-regulatory elements of the *ball* gene. All constructs with the exception of *pC4-attB-BgBall2-lacZ* give *ball*-like expression pattern of the *lacZ* reporter gene (blue). The shortest genomic sequence sufficient to reproduce *ball*-like expression pattern of the *lacZ* reporter is 286 bp in *pC4-attB-AgBall2-lacZ-SV40*. For the corresponding DNA sequence see (Klinge, 2006).

The constructs used for this study and the results are summarized in Fig. 4. First, the reporter transgene *pC4-attB-gBall2-lacZ* was generated by replacing the *ball* gene in the *pC4-gBall2-ball* rescue transgenic construct with *lacZ* and by inserting an *attB* attachment site upstream of the *ball* genomic sequence. Transgenic flies were generated and the reporter gene expression was analyzed. Consistent with the functional data, e.g. the above reported rescue of $ball^2$ mutant phenotype, the *lacZ* reporter in *pC4-attB-gBall2-lacZ* embryos showed a *ball*-like expression pattern (Fig. 5A-E). However, only this transgene had significant enrichment of *lacZ* transcripts in the embryonic gonads (Fig. 5E). The *pC4-attB-ABgBall2-lacZ* transgene, which lacked 162 bp of the genomic sequence comprising the 5'UTR of *his2av* (Fig. 4B), was only sufficient to drive the *ball*-like expression pattern of the *lacZ* reporter in the CNS (Fig. 6A-D).

The 5'UTR of *ball* contains two transcription initiation sites and an intron (Klinge, 2006). The 5' *ball* genomic sequence was further shortened by deleting 123 bp of intergenic sequences and 32 bp of the first untranslated exon of *ball* (*pC4-attB-BgBall2-lacZ*; Fig. 4B). This transgene contains only the more proximal transcription initiation start site and the intronic sequence in the 5' direction. This was the only transgene that did not result in *ball*-like expression of the *lacZ* reporter. Thus, the herein deleted 155 bp DNA sequence must contain sequences necessary for *ball*-like expression of *lacZ*. In the next step of the analysis I also asked whether *ball* 3'UTR sequences were necessary for *ball*-like expression of the *lacZ* reporter. Thus, I replaced the 284 bp of genomic sequence downstream of the *lacZ* reporter with the SV40 3'UTR sequence (*pC4-attB-ABgBall2-lacZ-SV40;* Fig. 4B). With this transgene, embryonic *ball*-like expression of the reporter gene was still detectable in embryonic Nbs (Fig. 6E-H), indicating that the *ball* 3'UTR was not necessary for *ball*-like expression of the reporter gene. Finally, I asked whether regulatory elements could be located within the first intron of *ball*, which was tested with *pC4-attB-AgBall2-lacZ-SV40*, lacking 120 bp of intronic sequence (Fig. 4B). The reporter gene was expressed in the CNS in *ball*-like fashion (Fig. 5F-I).

Taken together, 286 bp comprised of 123 bp of intergenic sequence and 163 bp of *ball* 5' region sequence were sufficient to drive *ball*-like expression of the *lacZ* reporter in the CNS. Conversely, the 123 bp intergenic DNA and 32 bp of the first *ball* exon were found to be necessary for *ball*-like expression of *lacZ* in the CNS.

Sequences found to be necessary and/or sufficient for *ball*-like expression of the reporter gene were subsequently used in further studies to identify *trans*-acting factors which may serve as regulators of *ball* expression in the CNS.

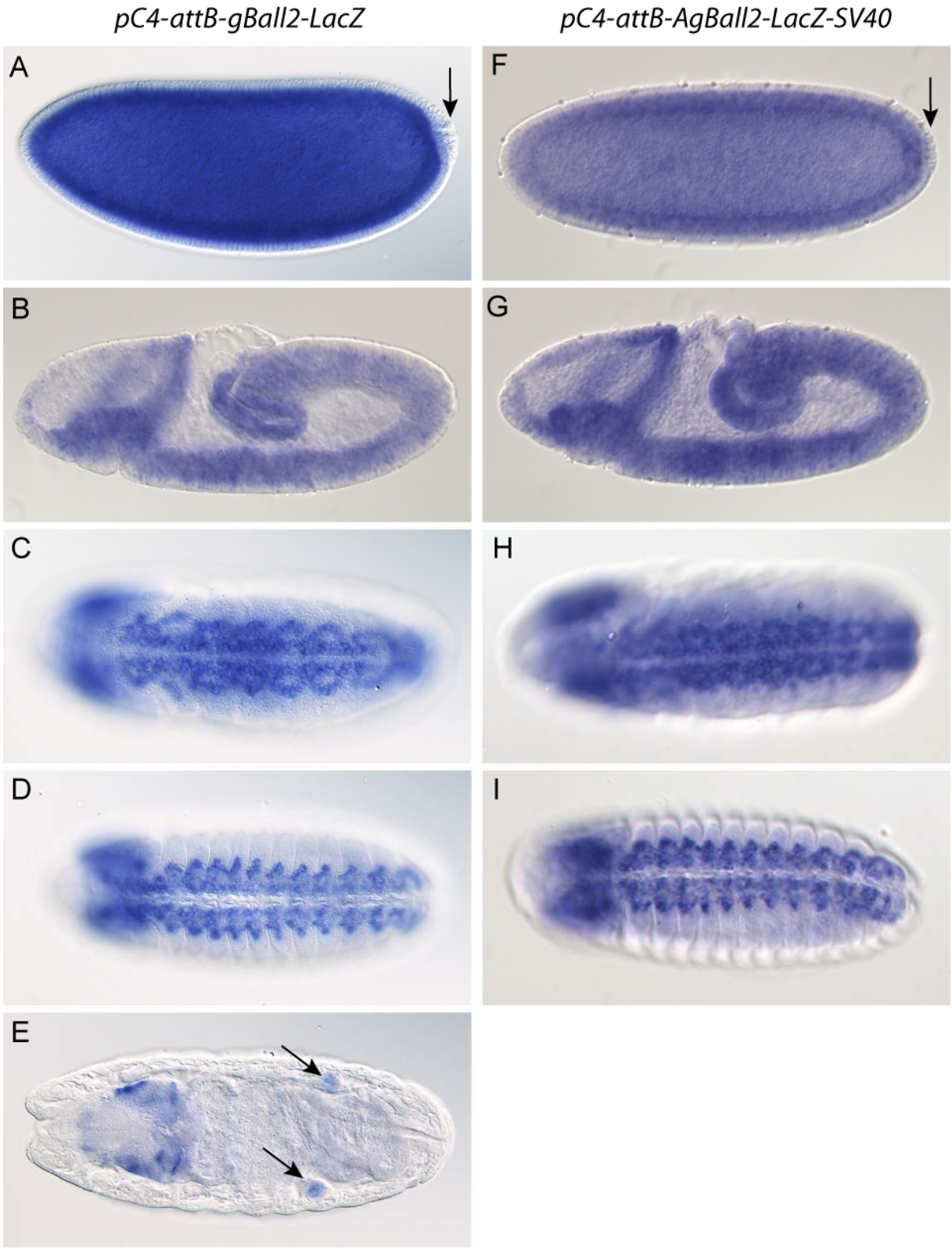


Figure 5: Expression pattern of the *lacZ* reporter gene in *pC4-attB-gBall2-lacZ* and *pC4-attB-AgBall2-lacZ-SV40* transgenic embryos.

(A-D) Expression pattern of *pC4-attB-gBall2-lacZ.* This construct contained the largest genomic fragment. Only in this construct there was clear expression in embryonic gonads (E, arrow). **(F-I)** Expression pattern of *pC4-attB-AgBall2-lacZ-SV40.* This construct contained the shortest genomic fragment. With both constructs maternal *lacZ* transcripts are ubiquitous but excluded from the pole cells (A,F, arrows). Later, *lacZ* expression is restricted to the neuroectoderm (B,G). *lacZ* is expressed in mitotic Nbs and fades away from the CNS in late developmental stages (C-E, H-I). Orientation of embryos is anterior to the left and dorsal up (A,B,F,G) or viewed from the ventral side.

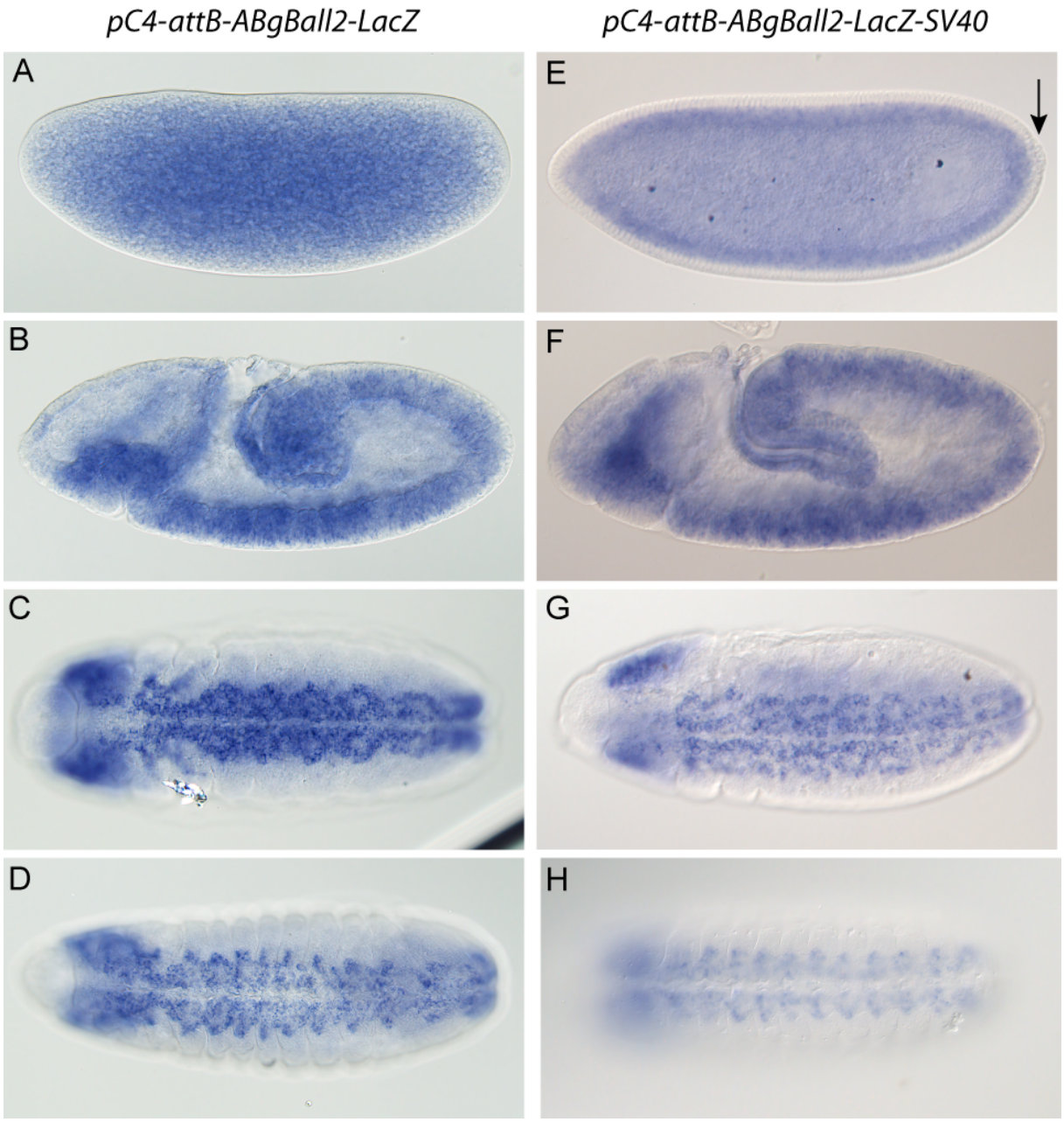


Figure 6: Expression pattern of the *lacZ* reporter gene in *pC4-attB-ABgBall2-lacZ* and *pC4-attB-ABgBall2-lacZ-SV40* transgenic embryos.
(A-D) Expression pattern of *pC4-attB-ABgBall2-lacZ.* **(E-H)** Expression pattern of *pC4-attB-ABgBall2-lacZ-SV40.* With both constructs maternal *lacZ* transcripts are ubiquitous. Later, *lacZ* expression is restricted to the neuroectoderm (B,F). *lacZ* is expressed in mitotic Nbs (C-D, H-I). Orientation of embryos is anterior to the left and dorsal up (A,B,E,F) or viewed from the ventral side (C-H).

3.3 Identification of nuclear proteins binding to the *cis*-regulatory sequences of *ball in vitro*

The genomic sequence sufficient to drive *ball*-like expression of the *lacZ* reporter in the embryonic CNS comprised 286 bp of DNA sequence upstream of the *ball* translation start site. 155 bp of this sequence was also shown to be necessary for *lacZ* expression. I used this genomic DNA sequence, which is necessary for the proper spatiotemporal expression pattern of *ball* as revealed by the reporter gene analysis, to isolate and identify *trans*-acting factors that bind to it.

To identify such putative *trans*-acting regulators of *ball* transcription, I used an approach that utilizes ESI-mass-spectrometry to identify nuclear DNA-binding proteins that are able to specifically interact with double-stranded DNA oligo-nucleotides, which were immobilized onto paramagnetic beads (for description of the method see Nordhoff et al., 1999). In this experiment I used five overlapping 35-40 bp long double-stranded DNA oligo-nucleotides that cover the 155 bp of the *ball* regulatory region (designated test oligo-nucleotides) (Table 1). To ensure that no binding sites were interrupted, the test oligo-nucleotides were designed to overlap by 10bp. In addition, I used a DNA oligo of the 3'UTR of *ball* (designated control oligo-nucleotide), which was shown to be unnecessary for *ball*-like expression of the *lacZ* reporter. The test and the control oligo-nucleotides shared no significant sequence similarity. For immobilization of the oligo-nucleotides on paramagnetic beads, the strong and specific interaction of biotin with streptavidin was utilized, i.e. DNA oligo-nucleotides were biotinylated at their 5' ends.

For binding assays, the biotinylated oligo-nucleotides were incubated with highly concentrated nuclear protein extract (≈30 mg/ ml, Fig. 7), prepared from 0-12 hour old embryos (obtained from A. Brehm, Marburg, Section 2.2.3). After incubation, paramagnetic beads covered with a monolayer of streptavidin were added to the reaction (Section 2.2.4). After incubation, followed by extensive washing, the attached proteins were eluted and separated by 1D SDS-PAGE (Section 2.2.2).

Staining of the proteins revealed a complex pattern on the gels. The protein bands from each individual gel were extracted and subsequently subjected to mass-spectrometric analysis (in collaboration with H. Urlaub, Göttingen, Section 2.2.5). Three independent experiments with each oligo were performed leading to the identification of a total of 1726 unique *Drosophila* proteins from all three repetitions

and all oligo-nucleotides used in the experiments (Appendix). The procedure of the protein attachment to DNA fragments is schematically summarized in Fig. 7 and outlined in Methods 2.2.4.

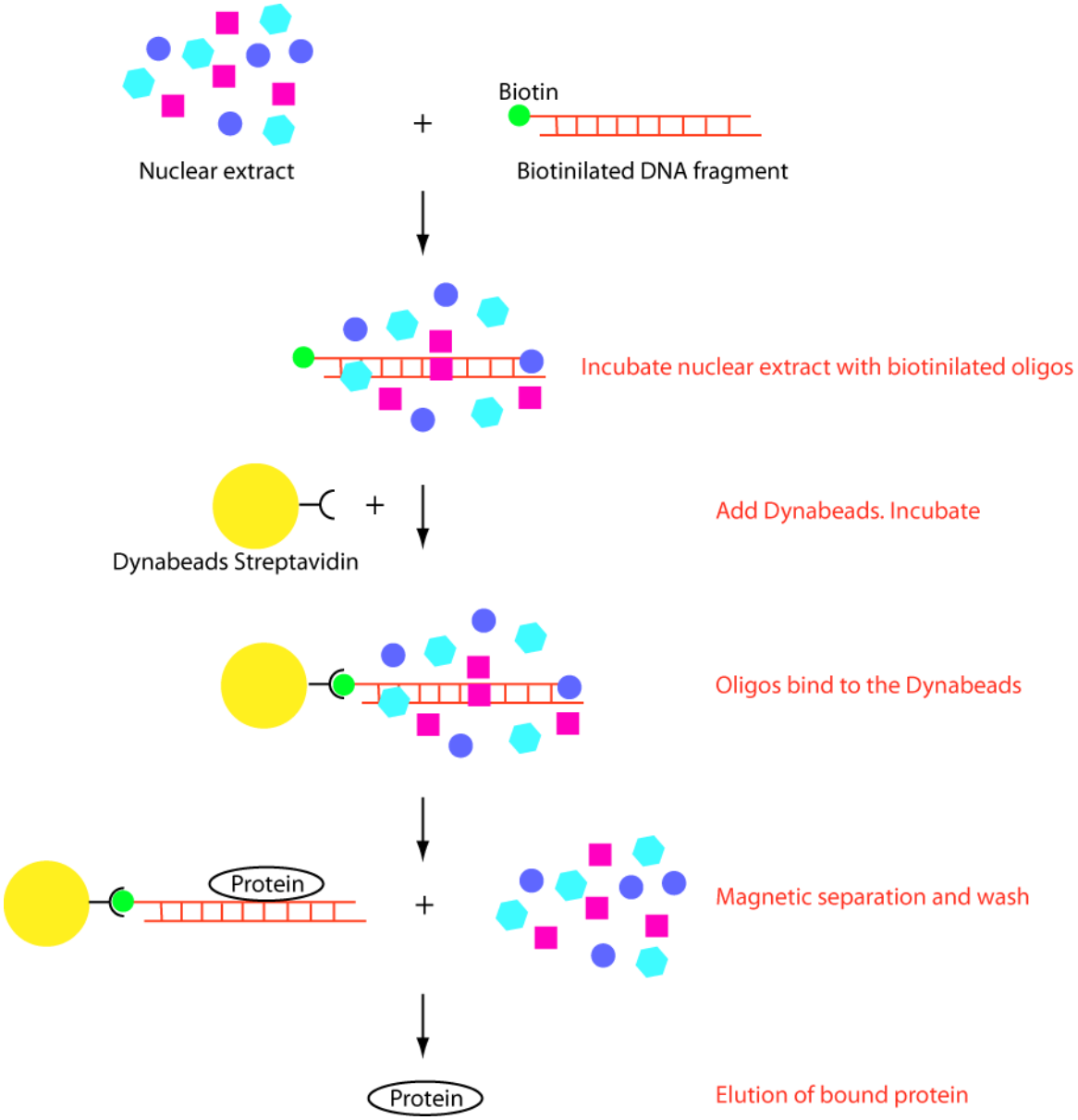


Figure 7: Schematic representation of the experimental procedure for isolation of DNA binding proteins from nuclear extracts.
Embryonic nuclear extract was incubated with biotinylated double-stranded DNA oligo-nucleotides. Subsequently, magnetic particles covered with covalently bound streptavidin were added to the reaction. Using magnetic separation the beads were washed from the unbound proteins. The specifically bound proteins were eluted from the beads.

To distinguish specific from unspecific binding proteins, the isolated proteome was filtered afterwards *in silico* by using the following criteria. Proteins found in two or three of the three experiments with a given oligo were considered “true” identifications. Proteins identified only once with a given oligo were considered “false”

identifications. This pragmatic assignment allowed me to exclude two large groups of proteins: 687 proteins were excluded because they appeared in the "false" identification fraction only; the second group consists of 489 proteins found in the "true" identification fraction for all oligo-nucleotides and were, therefore, excluded as non-specific binders. Finally, 254 proteins with positive identification in the control oligo from the *ball* 3'UTR were subtracted. The remaining 296 proteins bind specifically to one or more oligo-nucleotides from the *ball* 5' regulatory sequence shown to be both necessary and sufficient for driving gene expression in a *ball*-like pattern in the CNS.

To test whether the applied filtering of the data resulted in an enrichment of nuclear proteins with an expected function in transcription, I asked what gene ontology (GO) terms (Harris et al., 2004) are enriched in the filtered proteome in comparison to those found in the initially identified proteome. For that, the software tool for visualizing and analyzing GO annotation data in sets of genes (VLAD) was employed (Cruz et al., 2005). GO terms are grouped in three large classes: Cellular Component, Molecular Function and Biological Process (Harris et al., 2004). Comparable fractions, i.e. 69% of the proteins in the filtered set and 72% of the proteins in the initially identified proteome, were associated with GO terms of the Cellular Component class. A comparison of the filtered protein set with the initially found proteome revealed a significant enrichment for nuclear proteins in the filtered set (p=3.5E-04), (Fig. 8). Next, I examined the relative enrichment for GO terms from the Molecular Function Class. 80% of the proteins in the filtered set and 78% of the proteins in the initially found proteome were associated with this class. In the filtered set, significantly enriched was the group of chromatin insulator sequence binders (p=5.2E-03) and transcription regulators (p=1.4E-03) (Fig. 9). Thus, the filtering significantly increased the relative number of nuclear proteins with functions in transcription and reduced the total number of proteins from 1726 to 296 candidate proteins to be examined in further detail. The individual results of the enrichment analysis are summarized in Figs. 8 and Fig. 9.

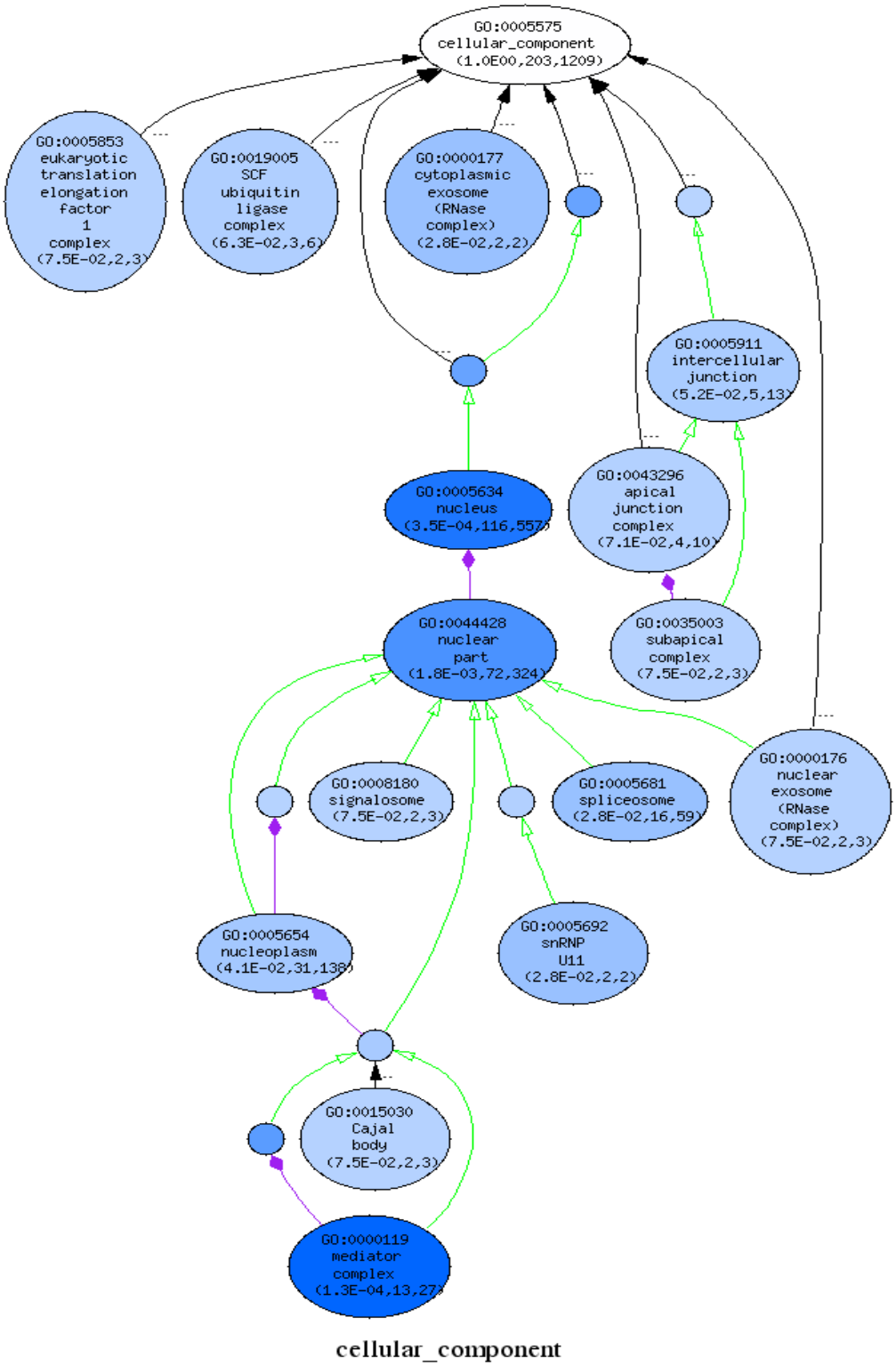


Figure 8: Relative enrichment of GO terms from the cellular component class in the filtered protein set *versus* the initially found sub-proteome.
After filtering based on experimental criteria for specificity to one or more oligo-nucleotides from the *ball* 5' region, the filtered set was significantly enriched for nuclear proteins (p=3.5E-04) in comparison with the set of all found proteins. The online tool VLAD was used for the analysis. Together with the GO term, in brackets are shown the p-value, the number of proteins associated with this GO term in the filtered set and in the reference set, respectively.

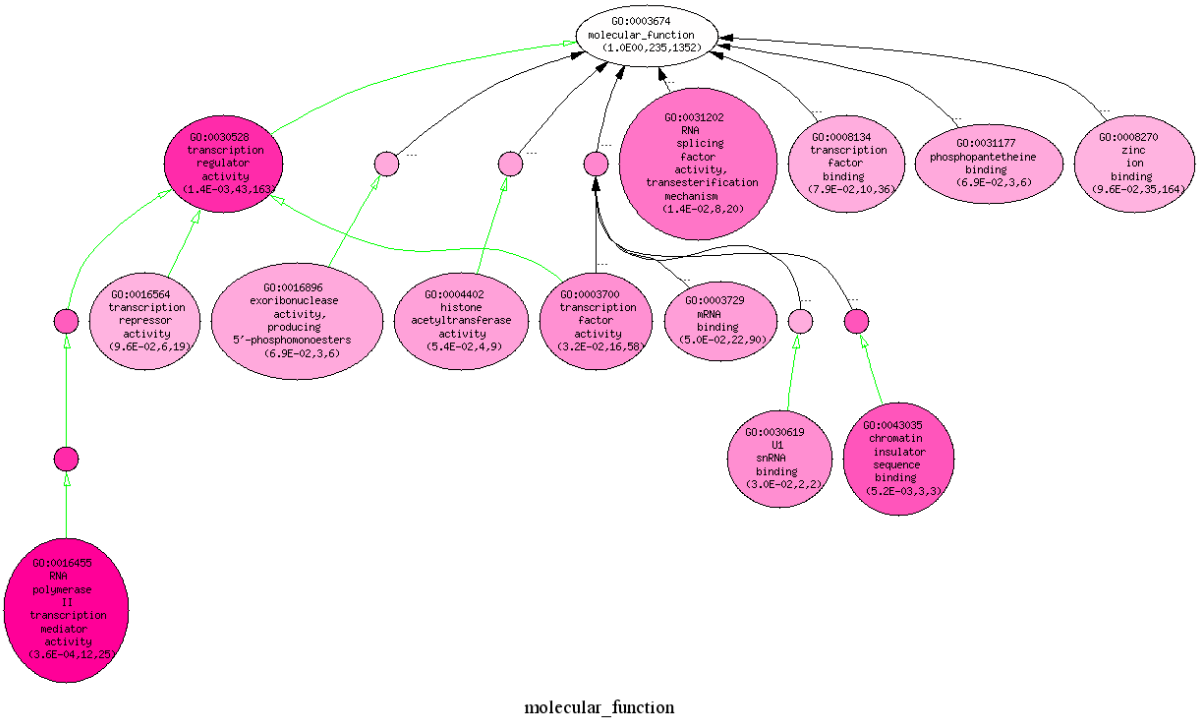


Figure 9: Relative enrichment of GO terms from the molecular function class in the filtered protein set *versus* the initially found sub-proteome.
After filtering based on experimental criteria for specificity to one or more oligo-nucleotides from the *ball* 5' region, the filtered set was significantly enriched for transcriptional regulators (p=1.4E-03) in comparison with the set of all found proteins. The online tool VLAD was used for the analysis. Together with the GO term, in brackets are shown the p-value, the number of proteins associated with this GO term in the filtered set and in the reference set, respectively.

Next, experimental criteria were used to further group the 296 proteins of the candidate proteome. I considered a protein as a specific binder when it bound only with one oligo. 28 specific factors were identified with oligo-nucleotide 1 (Fig. 10), 37 proteins with oligo 2 (Fig. 11), 15 proteins with oligo-nucleotide 3 (Fig. 12), 9 proteins with oligo-nucleotide 4 (Fig. 13) and 38 proteins with oligo-nucleotide 5 (Fig. 14). The remaining 169 proteins bound to more than one of the test oligo-nucleotides but not to the control oligo. Of these, 24 proteins were found more than once with all five test oligo-nucleotides (Fig. 15), 28 with four oligo-nucleotides (Fig. 16), 49 with three oligo-nucleotides (Fig. 2.18) and 68 with two oligo-nucleotides (Fig. 2.17). In each of the groups potential regulators of *ball* expression could be found. They include transcription factors such as Shuttle craft (Stroumbakis et al., 1996) and Dp (Dynlacht et al., 1994), chromatin insulator sequence binders such as BEAF-32 (Zhao et al., 1995) and CTCF (Yusufzai et al., 2004; Gerasimova et al., 2007). In addition, I observed DNA binders with unknown molecular functions such as CG17446 (FlyBase

ID: FBgn0030121) and CG9437 (FBgn0034599) and proteins with unknown functions such as CG5597 (FlyBase ID: FBgn0034920) and CG32295 (FlyBase ID: FBgn0052295). The role of those proteins in *ball* expression remains to be elucidated by functional tests.

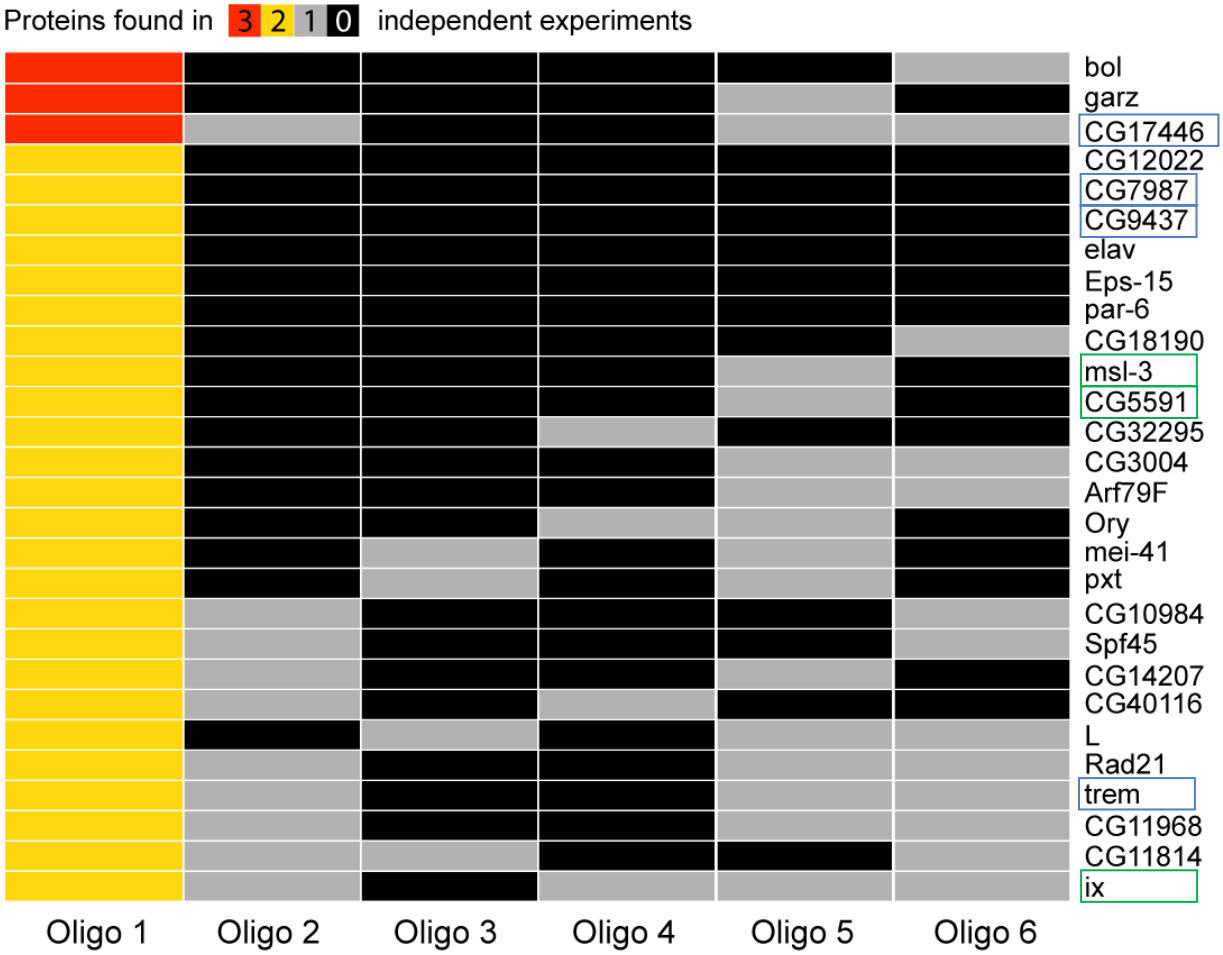


Figure 10: Proteins binding specifically to oligo-nucleotide 1.
28 proteins were found in more than one independent experiments with oligo-nucleotide 1 and in less than two independent experiments with the other oligo-nucleotides. On the figure “Oligo” stands for oligo-nucleotide. Oligo-nucleotide 6 is the control oligo-nucleotide. Annotated specific regulators of transcription are marked in green. Annotated nucleic binders with unknown molecular functions are labeled in blue.

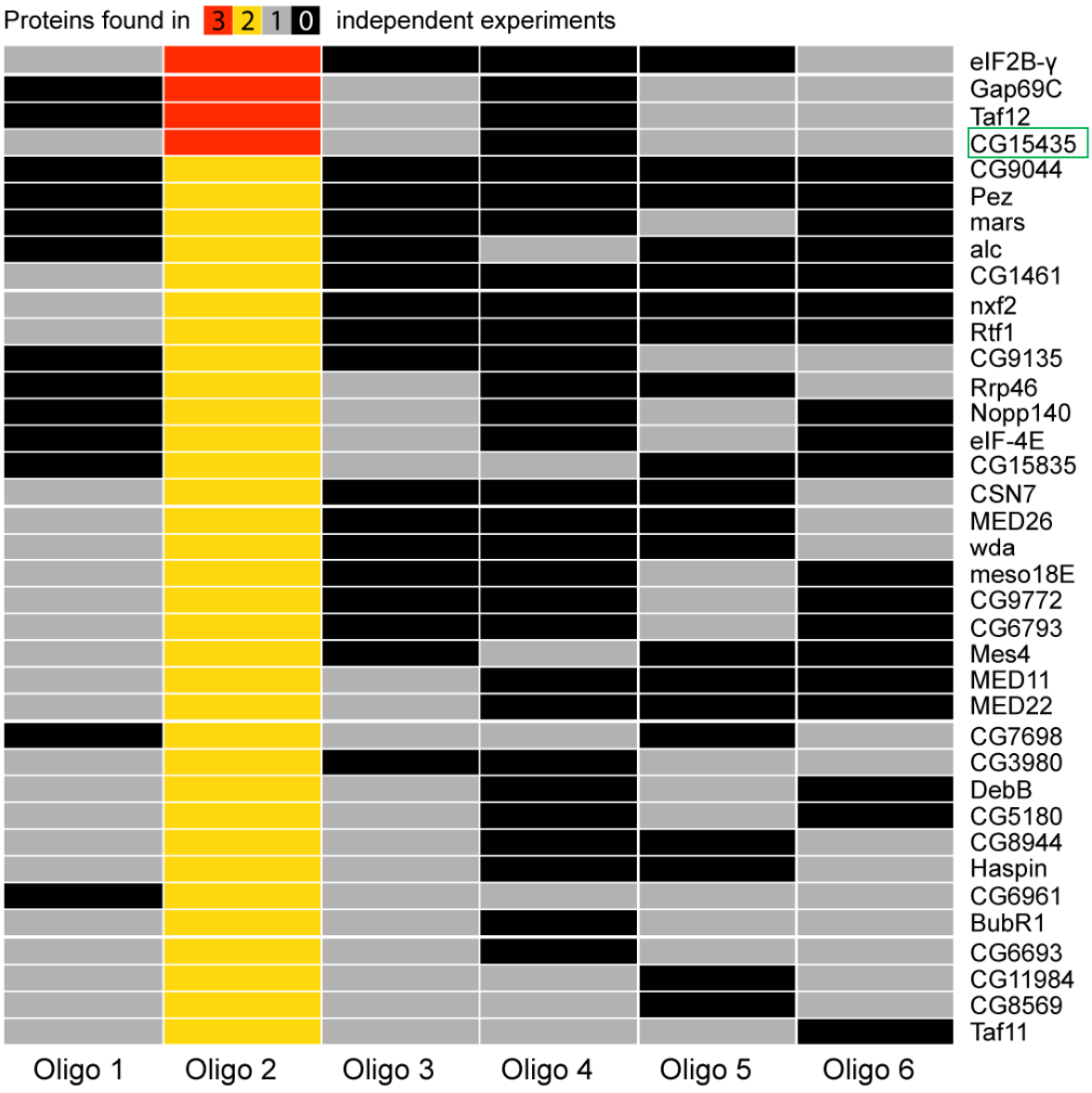


Figure 11: Proteins binding specifically to oligo-nucleotide 2.
37 proteins were found in more than one independent experiments with oligo-nucleotide 2 and in less than two independent experiments with the other oligo-nucleotides. On the figure “Oligo” stands for oligo-nucleotide. Oligo 6 is the control oligo-nucleotide. Annotated specific regulators of transcription are marked in green. Annotated nucleic acid binders with unknown molecular functions are labeled in blue.

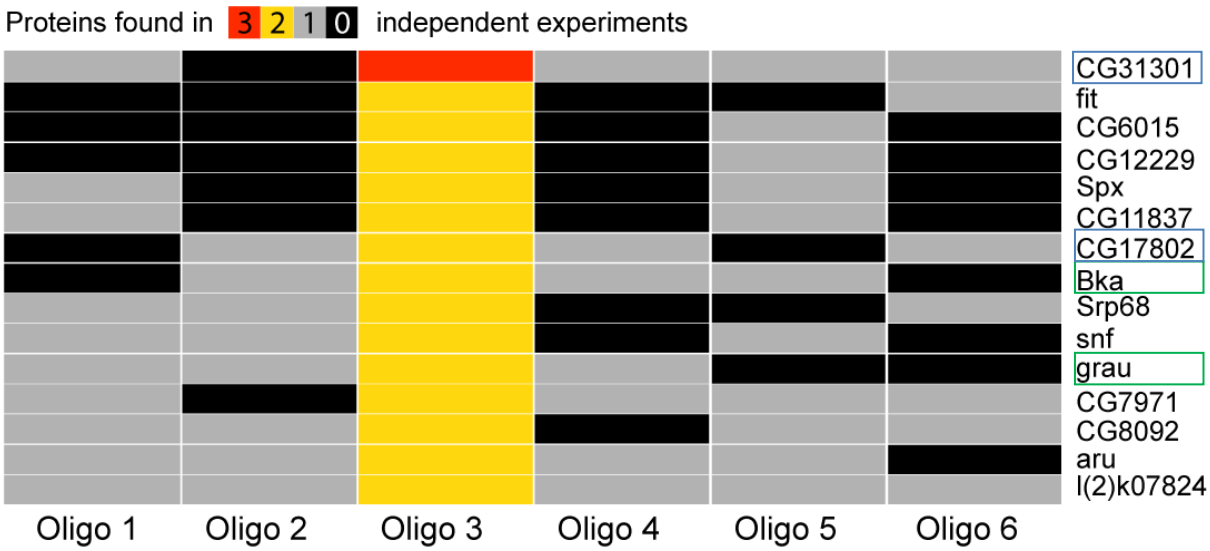


Figure 12: Proteins binding specifically to oligo-nucleotide 3.
15 proteins were found in more than one independent experiments with oligo-nucleotide 3 and in less than two independent experiments with the other oligo-nucleotides. Oligo 6 is the control oligo-nucleotide. Annotated specific regulators of transcription are marked in green. Annotated nucleic acid binders with unknown molecular functions are labeled in blue.

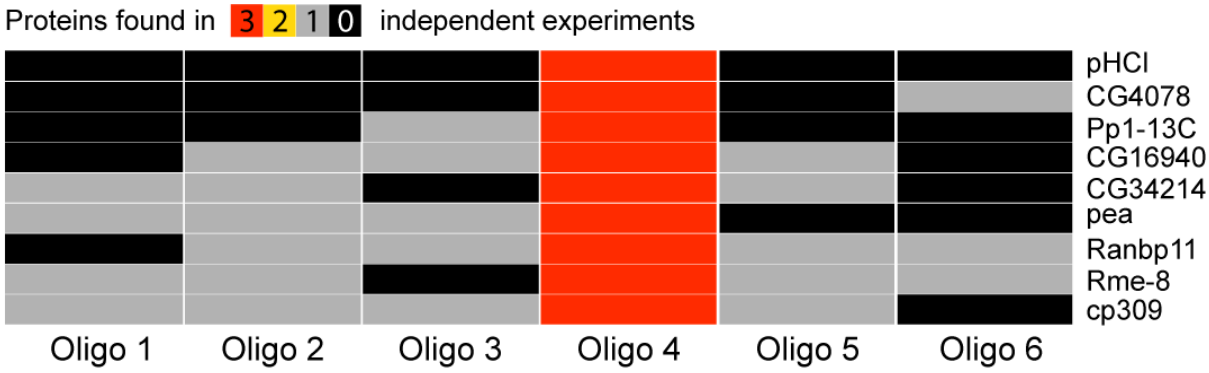


Figure 13: Proteins binding specifically to oligo-nucleotide 4.
9 proteins found in more than one independent experiments with oligo-nucleotide 4 and in less than two independent experiments with the other oligo-nucleotides. On the figure “Oligo” stands for oligo-nucleotide. Oligo 6 is the control oligo-nucleotide.

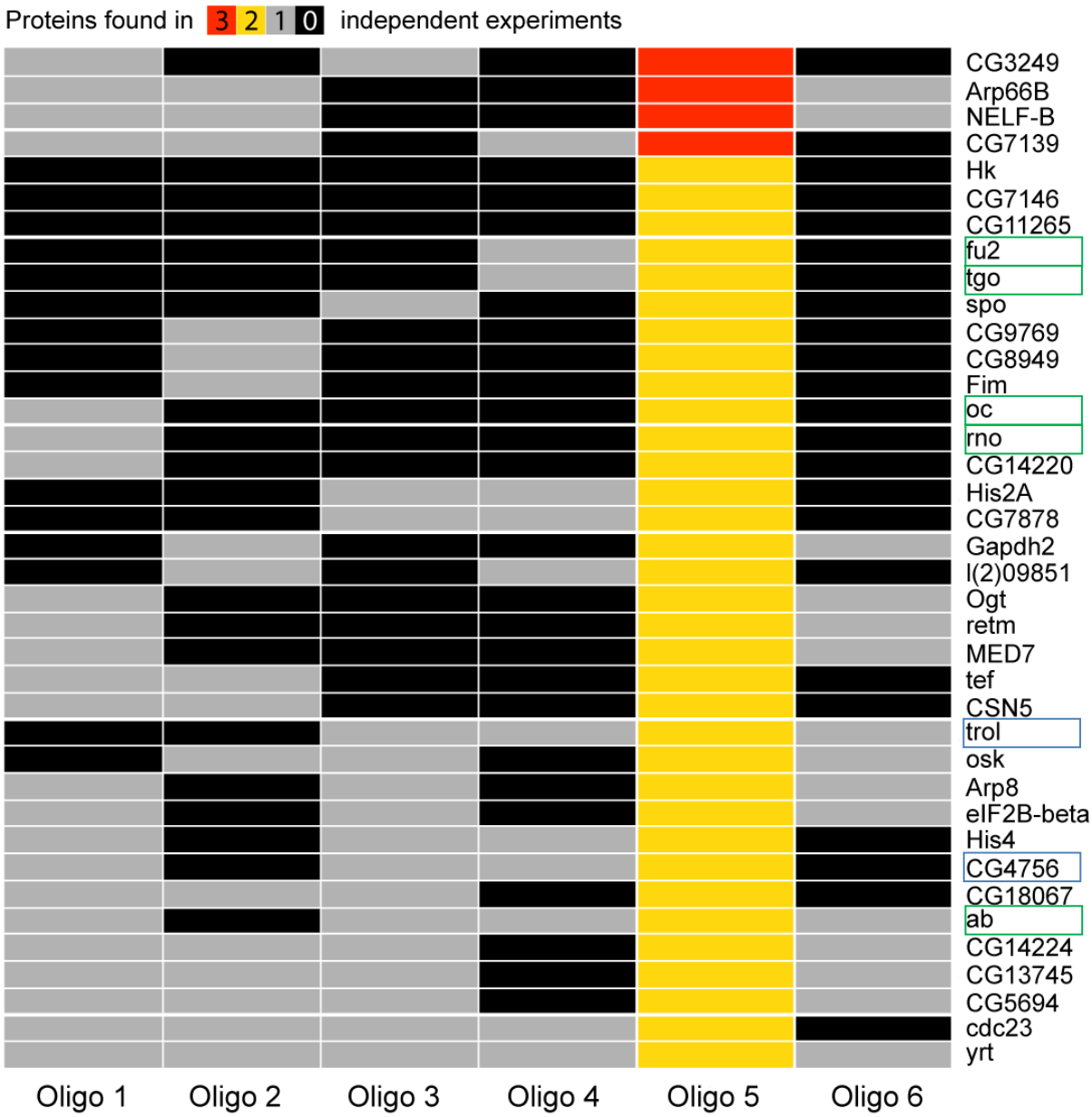


Figure 14: Proteins binding specifically to oligo-nucleotide 5.
38 proteins found in more than one independent experiments with oligo-nucleotide 5 and in less than two independent experiments with the other oligo-nucleotides. On the figure “Oligo” stands for oligo-nucleotide. Oligo 6 is the control oligo-nucleotide. Annotated specific regulators of transcription are marked in green. Annotated nucleic acid binders with unknown molecular functions are labeled in blue.

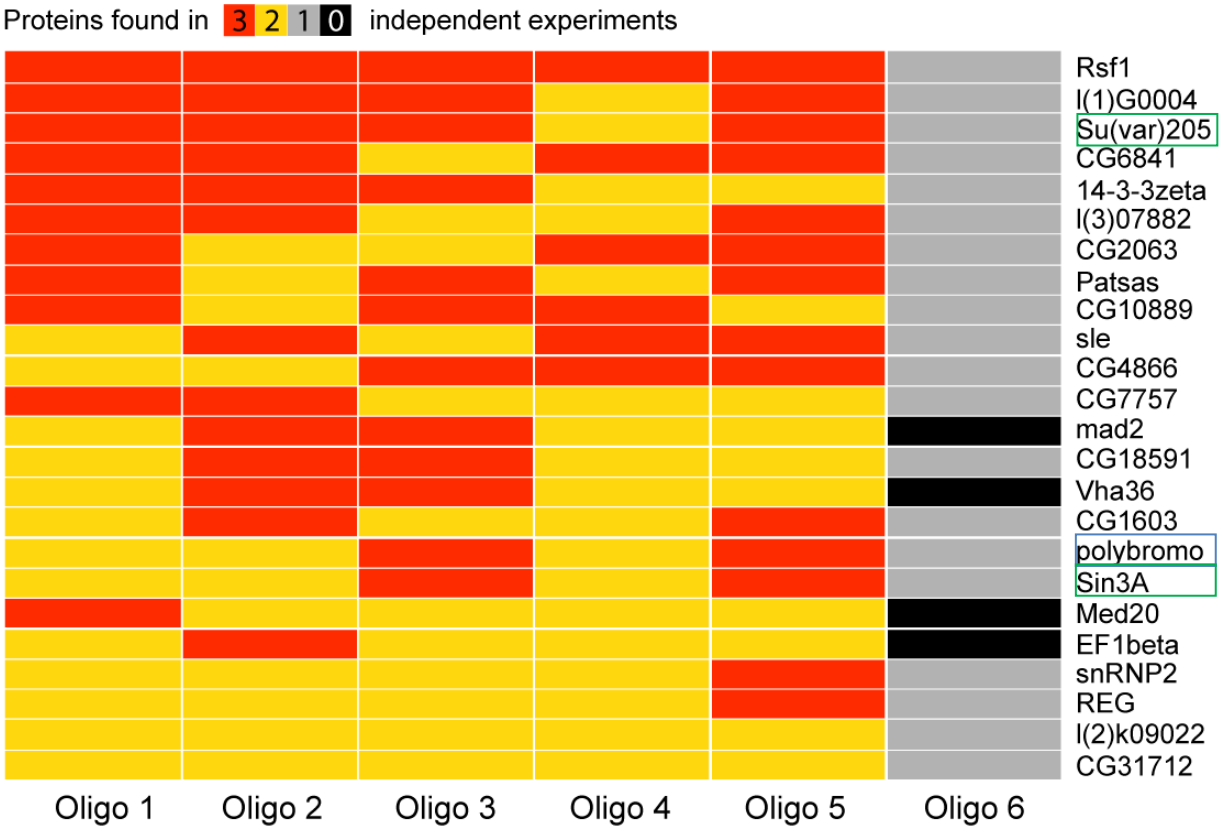


Figure 15: Proteins binding specifically to five test-oligo-nucleotides.
24 proteins were found in more than one independent experiments with all oligo-nucleotides of the *ball* 5' control region and in less than two independent experiments with the control oligo-nucleotide 6. On the figure "Oligo" stands for oligo-nucleotide. Annotated specific regulators of transcription are marked in green. Annotated nucleic acid binders with unknown molecular functions are labeled in blue.

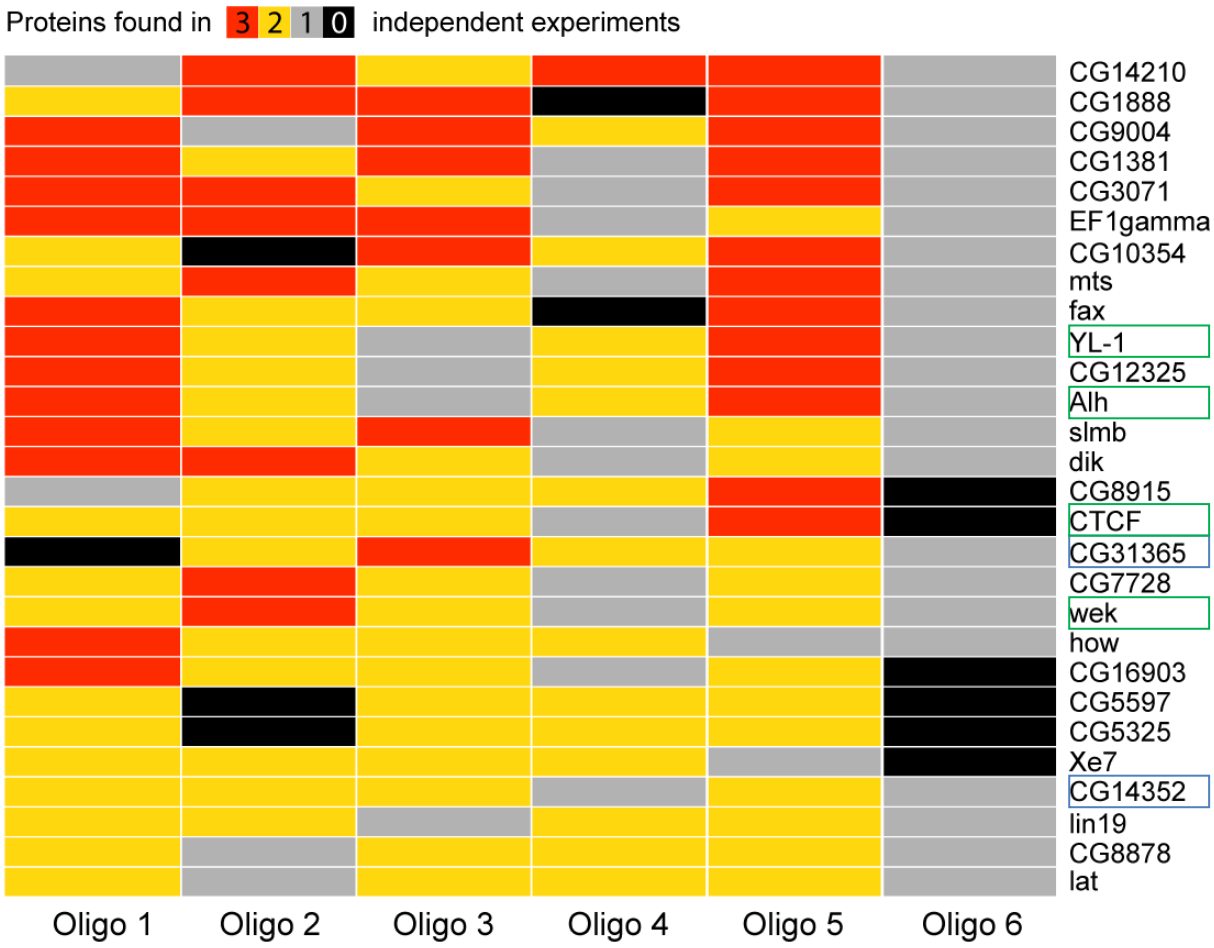
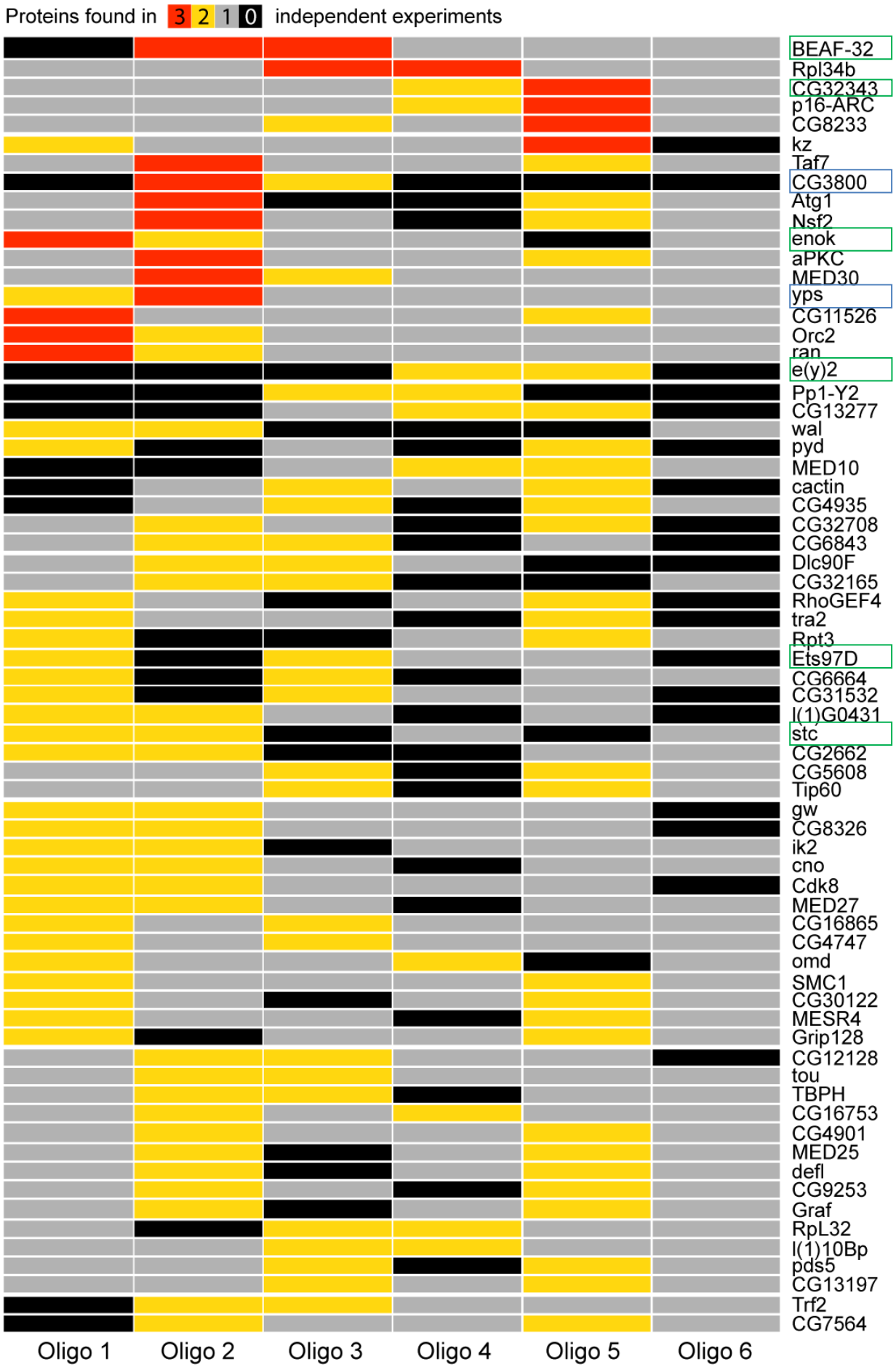


Figure 16: Proteins binding specifically to four test-oligo-nucleotides.
28 proteins were found in more than one independent experiments with four oligo-nucleotides of the *ball* 5' control region and in less than two independent experiments with the other oligo-nucleotides. On the figure "Oligo" stands for oligo-nucleotide. Oligo 6 is the control oligo-nucleotide. Annotated specific regulators of transcription are marked in green. Annotated nucleic acid binders with unknown molecular functions are labeled in blue.

Figure 17: Proteins binding specifically to two test-oligo-nucleotides.
Figure shown on next page. 68 proteins were found in more than one independent experiments with two oligo-nucleotides of the *ball* 5' control region and in less than two independent experiments with the other oligo-nucleotides. On the figure "Oligo" stands for oligo-nucleotide. Oligo 6 is the control oligo-nucleotide. Annotated specific regulators of transcription are marked in green. Annotated nucleic acid binders with unknown molecular functions are labeled in blue


Proteins found in 3 2 1 0 independent experiments
BEAF-32
Rpl34b
CG32343
p16-ARC
CG8233
kz
Taf7
CG3800
Atg1
Nsf2
enok
aPKC
MED30
yps
CG11526
Orc2
ran
e(y)2
Pp1-Y2
CG13277
wal
pyd
MED10
cactin
CG4935
CG32708
CG6843
Dlc90F
CG32165
RhoGEF4
tra2
Rpt3
Ets97D
CG6664
CG31532
l(1)G0431
stc
CG2662
CG5608
Tip60
gw
CG8326
ik2
cno
Cdk8
MED27
CG16865
CG4747
omd
SMC1
CG30122
MESR4
Grip128
CG12128
tou
TBPH
CG16753
CG4901
MED25
defl
CG9253
Graf
RpL32
l(1)10Bp
pds5
CG13197
Trf2
CG7564
Oligo 1
Oligo 2
Oligo 3
Oligo 4
Oligo 5
Oligo 6

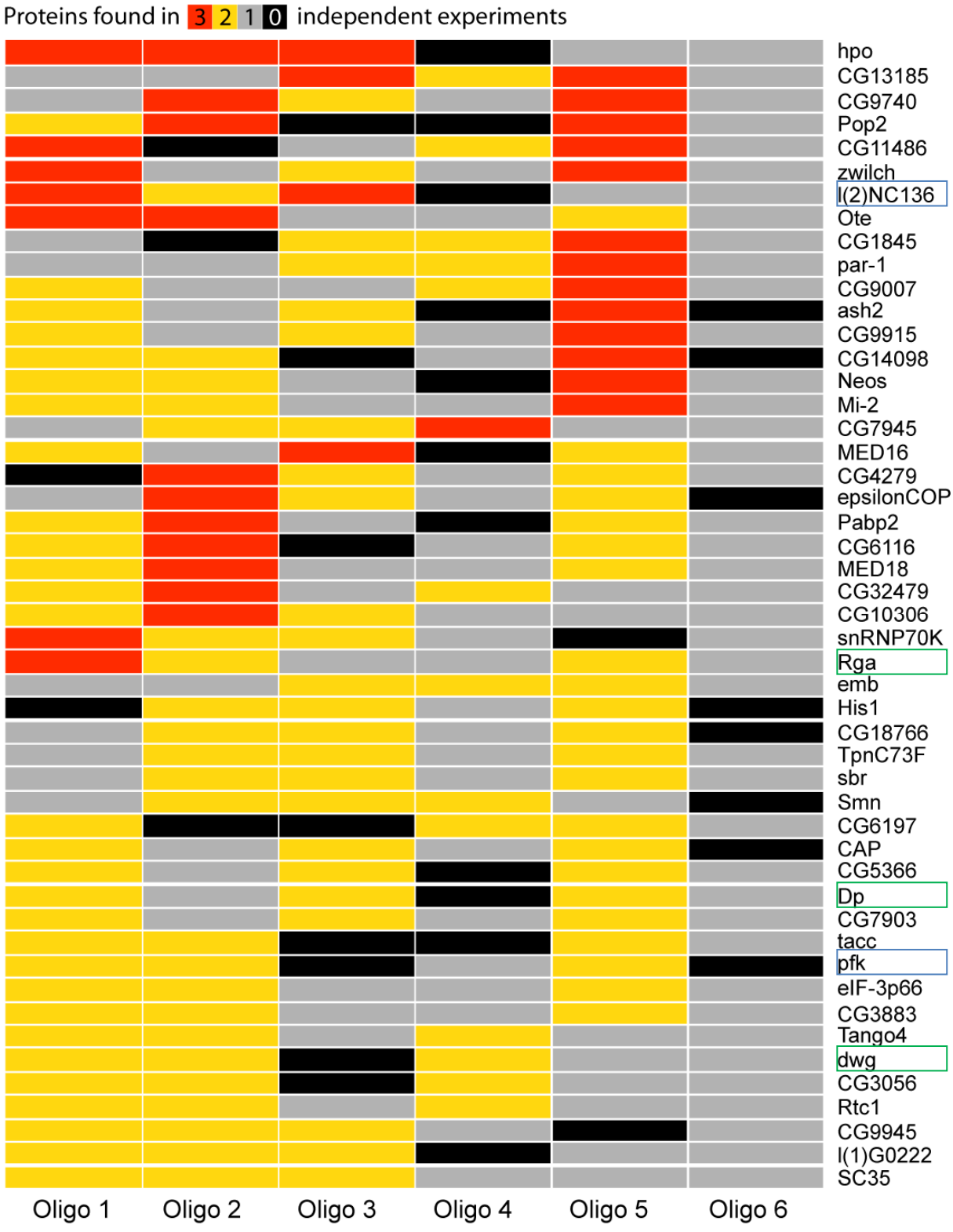


Figure 18: Proteins binding specifically to three test-oligo-nucleotides.
49 proteins were found in more than one independent experiments with three oligo-nucleotides of the *ball* 5' control region and in less than two independent experiments with the other oligo-nucleotides. On the figure "Oligo" stands for oligo-nucleotide. Oligo 6 is the control oligo-nucleotide. Annotated specific regulators of transcription are marked in green. Annotated nucleic acid binders with unknown molecular functions are labeled in blue.

3.4 Impact of UAS-GAL4 driven *ball* expression on embryonic Nbs and GMCs

The specific expression of *ball* in embryonic and larval CNS, and particularly the enrichment of *ball* mRNA in Nbs, suggest a function of *ball* in CNS development. Consistently, defects in CNS development of *ball* mutant larvae were already observed (Klinge, 2006). However, *ball* mutant embryos complete embryogenesis without obvious defects in neural development (Klinge, 2006). A plausible explanation for this observation is that the maternal *ball* transcripts are able to fulfill requirement for embryonic neural development may rest on the presence of the maternal *ball* transcripts in the embryo. Thus, early zygotic function of *ball* is difficult to study through a "loss-of-function" approach, since germline clone-derived eggs, which lack maternal *ball* expression, do not develop (A. Herzig, personal communication). This observation confirms that the maternal component of *ball* carries an essential function which may include early neural development. Therefore, I used a "gain-of-function" assay to examine the role of *ball* in embryonic CNS development after overexpression of *ball* in various cells of the developing nervous system.

To study the possible effect of *ball* in embryonic neural proliferation by a gain-of-function approach, the UAS-GAL4system was used (Brand & Perrimon, 1993). In this system, the expression of the GAL4 transcriptional activator is under the control of a tissue-specific enhancer. GAL4 binds UAS activation sequences and drives the expression of a target transgene, which is cloned downstream of the UAS sites (see schematic representation in Fig. 19). When the transgenes are carried by different parental lines, the UAS target line can be crossed to different GAL4 driver lines and *vice versa*. The same driver line can be crossed to different UAS target lines. Thus, effects of misexpression of genes in specific tissues or sets of cells can be analyzed and conclusions about the functions of these genes can be drawn from possible effects observed.

For this study, I used two different driver lines that express GAL4 under the control of *worniu* and *prospero* enhancers, respectively. The neuronal gene *prospero* is expressed in most if not all embryonic neuronal lineages (Vaessin et al., 1991; Matsuzaki et al., 1992). In the CNS, the *prospero* enhancer drives expression in Nbs and GMCs (Spana & Doe, 1995; Hirata et al., 1995; Knoblich et al., 1995). The

transcription factor encoded by *worniu* is expressed in embryonic Nbs and is required for CNS development, in particular by serving redundant functions in the control of asymmetric Nbs divisions (Ashraf et al., 1999; Ashraf et al., 2004).

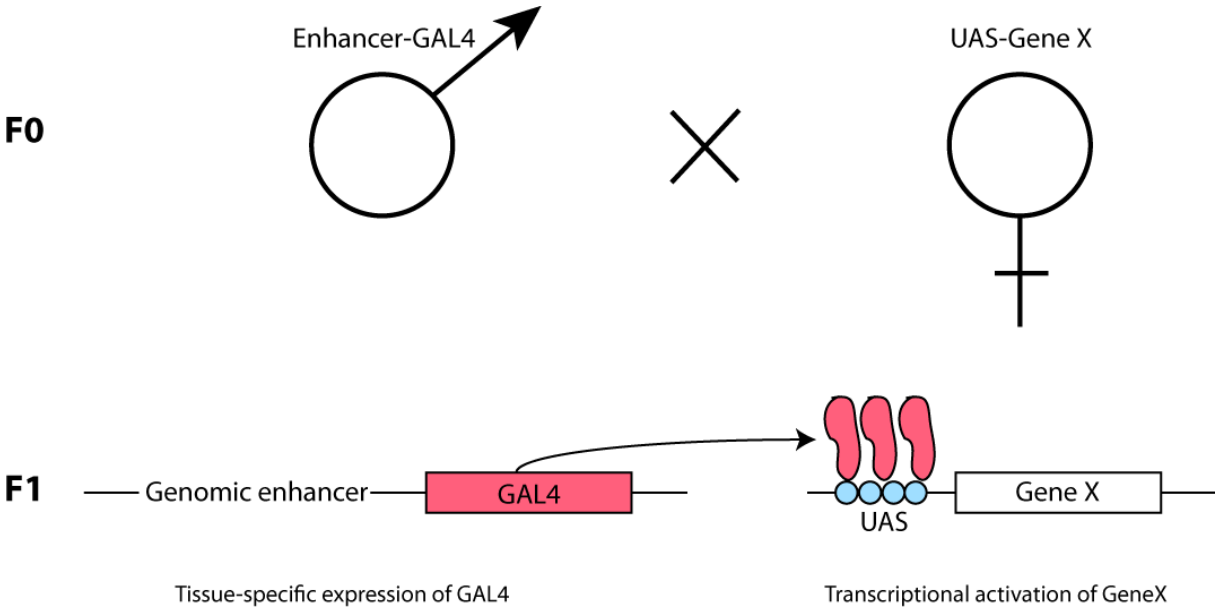


Figure 19: Directed gene expression in *Drosophila* using the UAS-GAL4 system.
Flies carrying the target (UAS-Gene X) are crossed to flies expressing GAL4 (Enhancer-GAL4) under the control of tissue specific enhancer. In the progeny of this cross, Gene X will be expressed in cells where GAL4 is expressed. In this way, the functions of Gene X can be studied by directed misexpression (adapted from Brand & Perrimon, 1993).

Flies carrying a *UAS-ball* transgene on the second chromosome were crossed to flies carrying either a *worniu-GAL4* or a *prospero-GAL4* transgene on the third chromosome. As a control, flies with a *UAS-lacZ* transgene on the second chromosome were crossed to flies of the same driver lines. Embryos from these crosses were collected and the CNS was examined for morphological defects at different developmental stages. The expression of *ball* or *lacZ* was detected by immunostaining with α-BALL or α-Beta-galactosidase antibodies, respectively (Section 2.3.3). Nbs were morphologically identified (Campos-Ortega & Hartenstein, 1997; Wheeler, 1893; Wheeler, 1891). In addition, molecular markers were used to mark individual cell types of the CNS, i.e. α-Pros and α-Elav antibodies were used to mark GMCs and neurons, respectively. Serial sections of confocal images through single embryos were taken with a laser scanning confocal microscope and 3D reconstructions of the CNS were analyzed (Section 2.3.7).

The results confirmed that the levels of BALL were indeed increased in the embryos overexpressing BALL (Fig. 20). I noted, however, that *worniu*, which was previously

described to be expressed in a Nb specific manner (Ashraf et al., 1999; Ashraf et al., 2004), caused *ball* expression also in GMCs and neurons (Fig. 20). This observation could be explained with the distribution of BALL during the Nb divisions to both daughter cells and a subsequently slow degradation of the protein in the resulting GMCs and neurons. Despite these uncertainties, the important result was that overexpression of *ball* in CNS in response to *worniu-GAL4* or *prospero-Gal4* did not cause a scorable phenotype in the embryonic CNS (data not shown). This result is consistent with the finding that BALL is present in GMCs in wild-type and that BALL expression has no determining function in other cells or cell types than in those cells where the gene is normally expressed. Thus, the overexpression study did not bring further insights into a possible function of *ball* in embryonic nervous system development. However, if *ball* has any function in the CNS, it would be permissive rather than instructive, since the *ball* overexpression also did not lead to an excess of Nbs or to the elimination of GMCs.

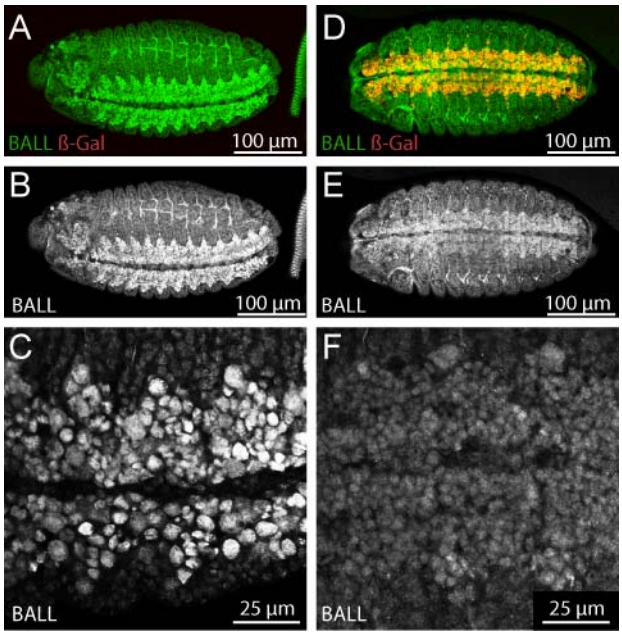


Figure 20: Overexpression of BALL in embryonic CNS with *worniu-Gal4*.
Orientation of the embryos (A-F): anterior left, ventrolateral. The expression of *UAS-ball* and *UAS-lacZ* was driven in embryonic CNS by *worniu-GAL4*. Embryos were pooled together and immunostained with α-BALL and α-ß-Gal antibodies. Images were obtained with the same settings of the microscope. **(A-C)** BALL was overexpressed in the embryonic CNS of a stage 13 embryo. (A) Ventral view. BALL overexpressed in CNS of embryo negative for ß-Gal. (B) Individual channel showing only BALL expression in the CNS. (C) Magnification of a region from panel (B). **(D-F)** l*acZ* overexpressed in a stage 13 control embryo, ventral view. (E) Individual channel showing only BALL expression in the CNS. (F) Magnification of a region from panel (E).

3.5 Mosaic analysis with a repressible cell marker (MARCM) of the *ball* gene

While *ball* zygotic mutants exhibit no defects in embryonic neurogenesis, their larval brains are strongly affected (Klinge, 2006). At third instar larval stage, the brains of homozygous *ball*^{2} larvae are much smaller than those of control larvae (Klinge, 2006). Most significantly reduced in size are the optic lobes (Klinge, 2006). These data indicate that *ball* functions in the development of the *Drosophila* CNS and that its function can be assessed in larvae rather than in embryos. Therefore, I investigated the cell-autonomous role of *ball* in neurogenesis by generating cell clones in the context of either heterozygous *ball* mutant cells, which develop normally, or wild-type cells.

For this study, I used MARCM clonal analysis to generate and label individual *ball* mutant neural cell lineages in a heterozygous background (Lee & Luo, 2001; Lee & Luo, 1999; Fig. 21A). This was possible since heterozygous larvae develop without scorable defects during brain development. The MARCM system is a variation of the classical FLP/ FRT system (Xu & Rubin, 1993), where mutant clones were marked by the absence of marker gene expression. In the MARCM system, mutant cells are generated with 50% probability after mitosis. Due to a recombination event, these cells lose the expression of GAL80, a repressor of the Gal4 transcription factor, and therefore express a UAS-GAL4-induced marker gene, which labels the mutant cell clones. In contrast to the classical FLP-FRT system (Xu & Rubin, 1993), where both mutant clones and twin spots are marked, the MARCM system allows one to specifically label the mutant clones. Thus, clones that result from a recombination event in single Nbs can be unambiguously identified.

A successful recombination event in a Nb can produce two types of cell clones: either a mutant Nb or a mutant GMC which are continuously labeled by marker gene expression. If after the asymmetric division a Nb gets marked, then cell clones of positively marked Nbs, GMCs and neurons are generated (Fig. 21B). In contrast, if the GMC gets marked, then clones with only two positively labeled differentiated cells will be obtained since GMC divide only once and both cells differentiate subsequently (Fig. 21C). In the following experiments cell clones that are derived from marked Nbs were analyzed, since only those clones are informative about a function of *ball* in

Nbs. The principle of the experimental design used for the subsequently described results is summarized in Fig. 21A.

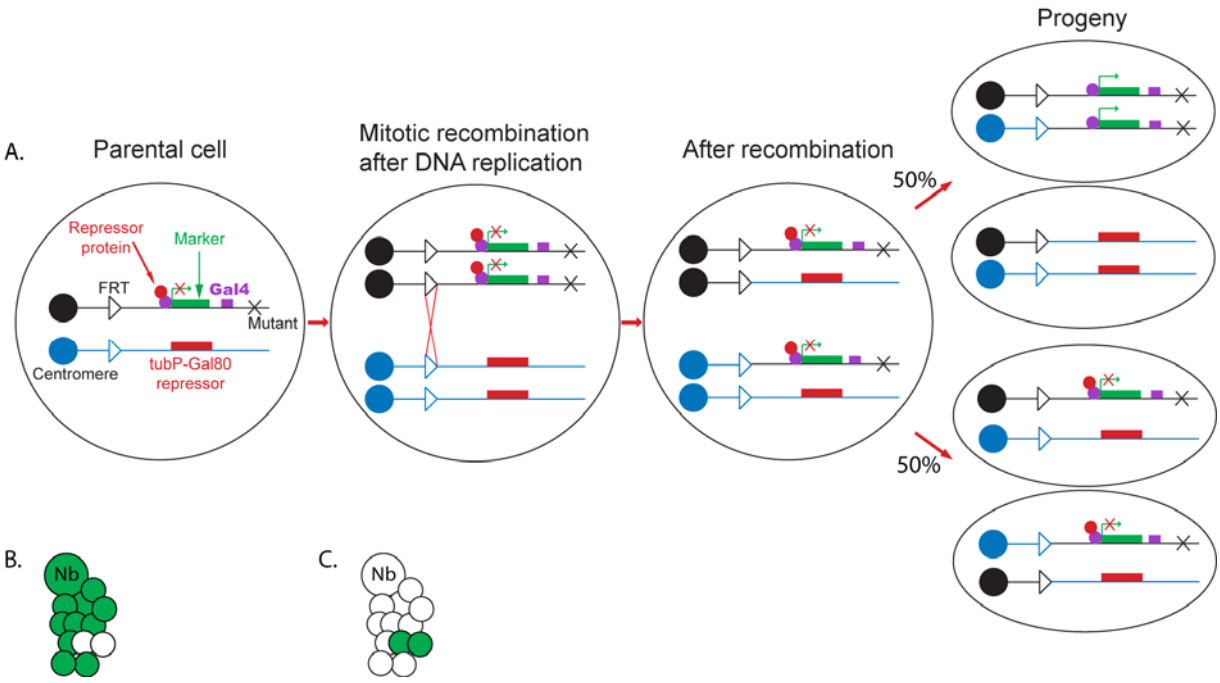


Figure 21: Schematic representation of the mosaic analysis with a repressible cell marker (MARCM).
(A) In a heterozygous mother cell Gal80 is ubiquitously expressed under the control of a tubulin promoter and suppresses GAL4-dependent expression of a UAS-marker gene. GAL80 is inserted on the chromosome arm carrying the wild-type gene of interest, whereas GAL4 and UAS-marker gene are on the chromosome carrying the mutant allele of the same gene. After induction of a site-specific mitotic recombination the chromosome arms distal to the recombination site become homozygous. During independent segregation of chromosomes there is 50% probability that one of the daughter cells will become homozygous for the repressor, whereas the other one becomes homozygous for the mutant gene (x). This cell no longer contains tubP-GAL80 and the marker gene is now turned on by GAL4 (adapted from Lee & Luo, 2001). **(B)** and **(C)** Recombination in Nb can generate two types of cell clones. (B) If the daughter Nb receives two copies of the marked mutant chromosome, a large positively marked cell clone is generated. (C).If the GMC is homozygous mutant, then a cell clone of only two positively marked neurons is formed.

In *Drosophila* most Nbs proliferate during two neurogenic periods, one in embryos and one in larvae. Between these proliferation periods, Nbs remain quiescent (Campos-Ortega & Hartenstein, 1997; Maurange & Gould, 2005). I have studied cell clones that are derived from larval Nbs in the thoracic region of the ventral ganglion (Fig. 22A). Following the quiescent period, post-embryonic Nbs in this region resume asymmetric divisions at around 36 h after larval hatching (ALH) and continuously divide until pupariation (Maurange & Gould, 2005). In my experimental setup, somatic mitotic recombination events were induced by heat-shock controlled *flp* expression at approximately 24 h ALH and the brains of these larvae were analyzed at approximately 96 h ALH in late third instar larval stage. The heat-shock conditions

were varied to optimize the number of clones per brain, so that single *ball*$^{2/2}$ mutant cell clones, which were marked by ß-Gal expression, could be studied. As controls, non-mutant cell clones marked with ß-Gal were induced (referred to as “control clones”). In addition, *ball*$^{2/2}$ mutant cell clones were induced that expressed EGFP instead of ß-Gal. The expression of BALL-EGFP fusion protein was used to identify the mutant cell clone and to examine whether defects in *ball*$^{2/2}$ mutant cell clones can be rescued by *ball* expression (“rescue clones”).

First, I asked whether there are size differences between mutant and control cell clones that were of similar age. For that I quantified the average number of cells per clone in *ball*$^{2/2}$ and control clones. Control MARCM cell clones examined in late third instar larvae typically contain a single large Nb, which is associated with a discrete number of smaller cells (Fig. 22). These cells represent the entire progeny of this Nb, which was generated during larval development (Campos-Ortega & Hartenstein, 1997; Maurange & Gould, 2005). The average number of neurons in control cell clones was 27.1±4.4 per clone (n=63) (Fig. 22D), whereas the *ball*$^{2/2}$ cell clones contained 16.7±4.3 cells (n=37; $p<0.05$) (Fig. 22E). Interestingly, the rescue cell clones (Fig. 22C), in which the BALL-EGFP fusion was expressed in the *ball*$^{2/2}$ mutant cells, contained 40.2±6.9 cells (n=12) (Fig. 22E). This number is significantly higher than the number of cells in both control and *ball*$^{2/2}$ mutant cell clones ($p<0.05$).

The smaller size of the *ball*$^{2/2}$ clones could be explained by a loss of the *ball*$^{2/2}$ mutant Nbs and/ or GMCs or by a lower proliferation rate of these cell types. Therefore, I next asked whether Nbs or GMCs are lost in the *ball*$^{2/2}$ mutant clones. In most mutant clones Nbs could be identified by their size and position on the dorsal side of the ventral ganglion (Fig. 22B). In order to distinguish between GMCs and neurons, I used the pan-neuronal marker Elav, which is expressed in neurons only (Ceron et al., 2001) (Fig. 23A-D). GMCs could be identified in both *ball*$^{2/2}$ mutant and control clones by their size, a lack of Elav expression and their position next to a larger Nb (Fig. 23A-D, 23E-F). The results of the analysis revealed that both Nbs and GMCs were present in *ball*$^{2/2}$ mutant clones (Fig. 23).

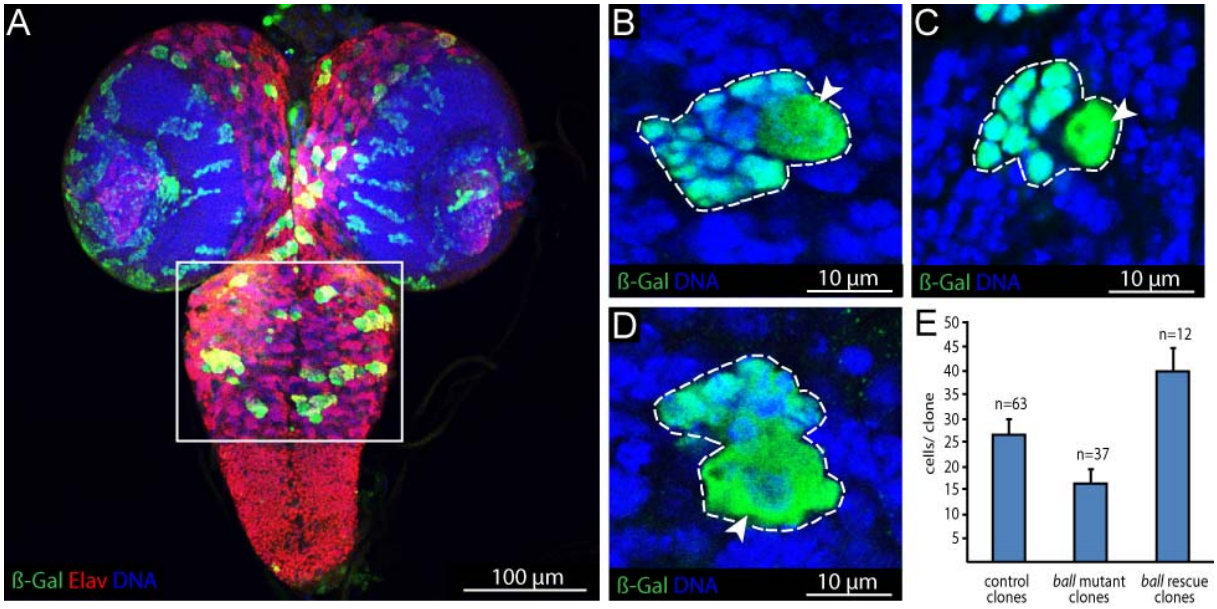


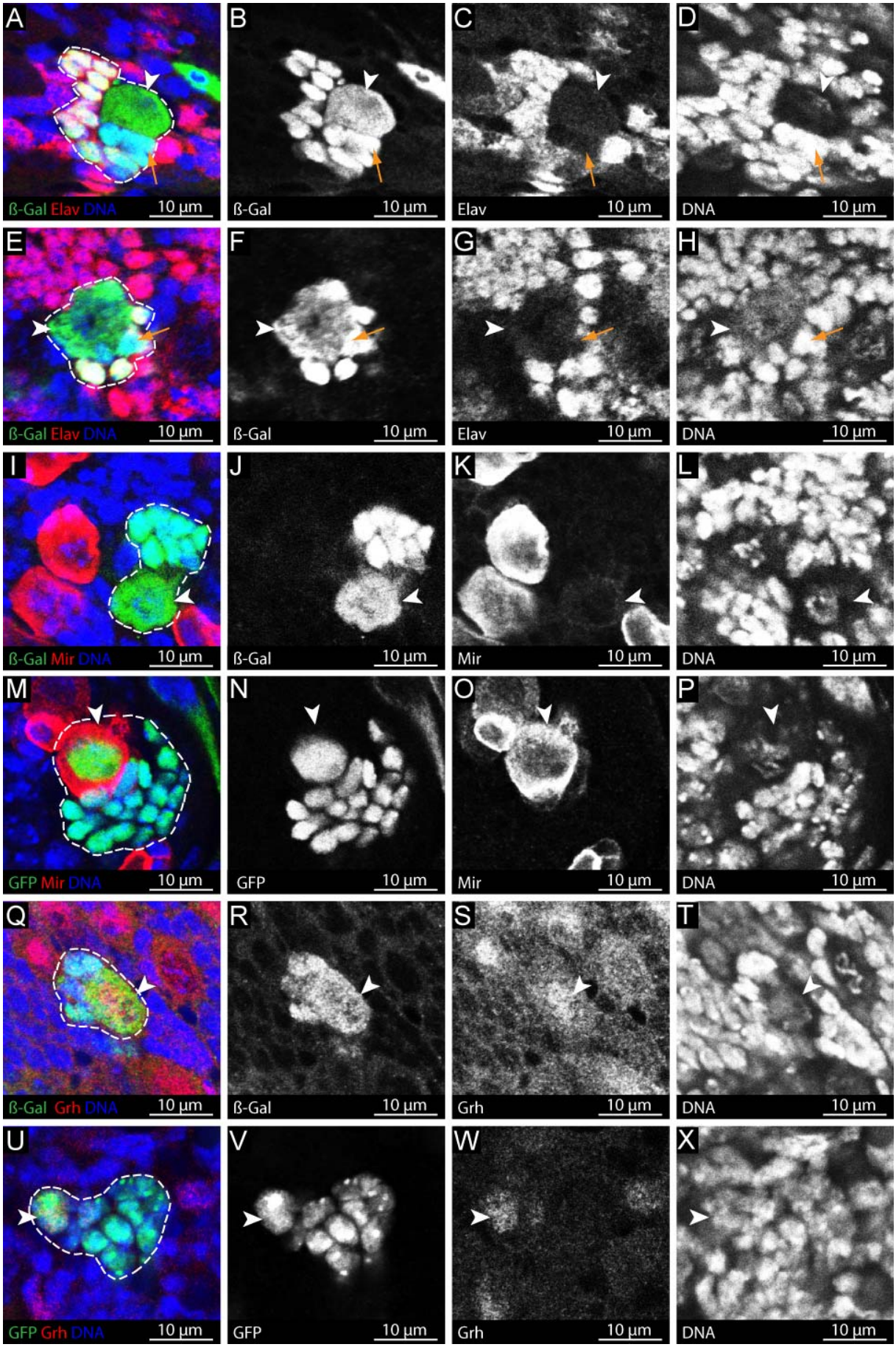
Figure 22: MARCM analysis of *ball* in larval brains.
Wild-type MARCM clones labeled with ß-Gal in third instar larval brain counterstained with the DNA dye Draq5 and the neuronal marker Elav (**A**). In this study clones in the ventral ganglion region (white rectangle) were analyzed. When induced at low frequency in 24 h old larvae, wild-type clones contain progeny of a single Nb occupying a small area of third instar larval brain (single clone shown in **B**). Similar conditions generate *ball* mutant clones of significantly smaller (**D, E**; p<0,01) and *ball-EGFP* rescue clones of significantly larger size (**C, E**; p<0,01). Neuroblasts could most often be identified in individual clones based on their larger size and position in the clone (**B, C, D**, arrowheads). **(E)** Average number of neural progeny per cell clone in three different genotypes 96 h PCI. n represents the number of clones per genotype analyzed. control=27.1±4.4, $ball^{2/2}$=16.7±4.3; BALL-EGFP=40.2±6.9.

The fact that both Nbs and GMCs were present in $ball^{2/2}$ mutant cell clones rules out apoptosis as a major cause of the reduced cell number in $ball^{2/2}$ mutant cell clones. However, minor contribution of cell death to the observed phenotype cannot be excluded, since it was shown in various other cell types, that ball is not essential for cell proliferation (A. Herzig, personal communication). This conclusion is consistent with the finding that $ball^{2/2}$ Nbs could produce the cell types constituting the neuronal lineage, although in reduced numbers. An attractive explanation for this finding would be that loss of BALL activity leads to Nb dysfunction, which would essentially result in a reduced number of neurons. In order to test this proposal, I studied the expression of both Miranda and Grainyhead which are essential for Nb function (Ceron et al., 2001; Almeida & Bray, 2005) in $ball^{2/2}$ mutant cell clones. The results are summarized in Fig. 23.

The results of the immunostaining experiments revealed that the majority of the Nb cells, in $ball^{2/2}$ mutant cell clones, lack Miranda (78 out of 84; Fig. 23I-L). In contrast, all examined Nbs in the rescue clones expressed Miranda (56 out of 56; Fig. 23M-P).

Grainyhead expressing Nbs could be detected in both *ball* mutant cell clones and in all *ball-EGFP* rescue cell clones. However, a detailed and unambiguous quantification of the Grainyhead expressing cells in *ball*$^{2/2}$ clones was not possible due to a low signal to noise ratio that was obtained with the available antibodies. Nevertheless, the fact that Grainyhead was still found in Nbs argues that *ball*$^{2/2}$ mutant Nbs did not lose their Nb identity completely. However, at least one functional Nb marker, Miranda, was lost. The data, therefore, indicate that *ball* is required for the maintenance of neural stem cell character, as defined by functional marker gene expression, in *Drosophila*. The results leave open, whether at least a fraction of these cells could still have Nb function, as evidenced by the weak Grainyhead staining observed. Thus, the reduction of neuronal offspring from those Nbs, as reflected in the cell number in *ball*$^{2/2}$ mutant clones, could relate to this observation.

Figure 23: Expression of Nb markers in *ball* MARCM clones.
Figure shown on next page. Mir stands for Miranda. **(A-H)** *ball* mutant or control clones positively labeled with ß-Gal (green) and immunostained with Elav (red) and the DNA dye Draq5 (blue). Panels (B-D) and (F-H) show the individual channels for (A) and (E) respectively. Elav labels neurons but not ganglion-mother cells or Nbs. GMCs (arrow) are smaller and located next to a bigger Nb (arrowhead). GMCs could be found both in ball mutant (E-H) and in control (A-D) clones. **(I-P)** *ball* mutant or *ball* rescue clones positively labeled with ß-Gal (green) or GFP (green) respectively and immunostained with the Nb marker Miranda (red) and the DNA dye Draq5 (blue). Panels (J-L) and (N-P) show the individual channels for (I) and (M) respectively. The Nbs in *ball* mutant clones lose Miranda (I-L, arrowhead) as compared to *ball* rescue clones (M-P, arrowhead). **(Q-X)** *ball* mutant or *ball* rescue clones positively labeled with ß-Gal (green) or GFP (green) respectively and immunostained with the Nb marker Grainyhead (red) and the DNA dye Draq5 (blue). Panels (R-T) and (V-X) show the individual channels for (Q) and (U) respectively. Grainyhead could be detected in both *ball* mutant (Q-T, arrowhead) and in *ball* rescue (U-X, arrowhead) clones.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DNA
10 µm

3.6 Mosaic analysis of the *ball* gene in *Drosophila* germline stem cells

Asymmetry of Nb division, i.e. maintenance of Nb as a stem cell and differentiation of GMCs, is primarily driven by the asymmetric distribution of cell fate determinants and not by the stem cell niche environment. In this respect Nbs do not resemble a prototype model for most vertebrate stem cells, in which stem cell maintenance is governed by signaling events in response to a stem cell niche (Li & Xie, 2005). In contrast, former studies on the germline stem cells (GSCs) of *Drosophila* indicated that this stem cell system, and its interaction with niche cells, closely resembles the vertebrate model (Mitsiadis et al., 2007; Li & Xie, 2005; Gilboa & Lehmann, 2004). Therefore, I also focused on *ball* function in germline stem cells. I addressed two questions: does *ball* have a function in niche-controlled stem cells and does *ball* have a similar function in GSCs as observed in Nbs, i.e. cell fate maintenance.

GSC maintenance in both females and males is tightly regulated by a combination of internal and external molecular factors, which are to some extent different (Gilboa & Lehmann, 2004; Fuller & Spradling, 2007). In the male germline, the GSC self-renewal is dependent on the JAK-STAT signaling (Kiger et al., 2001; Tulina & Matunis, 2001), in addition to Decapentaplegic and Glass Bottom Boat signaling, which is required for GSC maintenance in both males and females (Xie & Spradling, 1998; Kawase et al., 2004; Shivdasani & Ingham, 2003). In this study I asked whether *ball* plays a role in male GSCs and thus, whether BALL is required not only for neural but also for germline stem cell maintenance which would imply a more general function of BALL in stem cell biology.

In the *Drosophila* testis, around nine GSCs surround a cluster of about twelve non-mitotic somatic cells at the very tip of the organ (Kiger et al., 2001; Tulina & Matunis, 2001). The latter form the stem cell niche, called “hub”. Each GSC divides continuously and asymmetrically to generate another GSC (“stem cell renewal”) and a gonialblast. The daughter GSC remains attached to the hub cells, whereas the differentiating gonialblast is released from the stem cell niche (Kiger et al., 2001; Tulina & Matunis, 2001). In the process of differentiation, the gonialblasts first undergo four incomplete mitotic divisions, leading to the formation of 16 spermatogonia, which remain interconnected by cytoplasmic bridges. Subsequently, spermatogonia enter meiosis to generate spermatocytes. In time, differentiating cells gradually move away from the testis tip (Kiger et al., 2001; Tulina & Matunis, 2001).

To study the role of *ball* in the male germline cells, I analyzed the effects of loss of BALL activity by mosaic analysis of *ball*^{2} heterozygous cells using the classical FLP-FRT system (Xu & Rubin, 1993); the experimental design is outlined in Fig. 24). With this system, a successful recombination event in a heterozygous mother cell generates with 50% probability mutant cells, which can be distinguished by the absence of the GFP marker. At the same time, the recombination event produces a wild-type twin-clone, which is homozygous for the GFP marker and, thereby, labeled by an increased GFP signal. As controls, non-mutant clones were induced, which were also labeled by absence of GFP expression ("control clones"). For a schematic representation of the experimental system see Fig. 24.

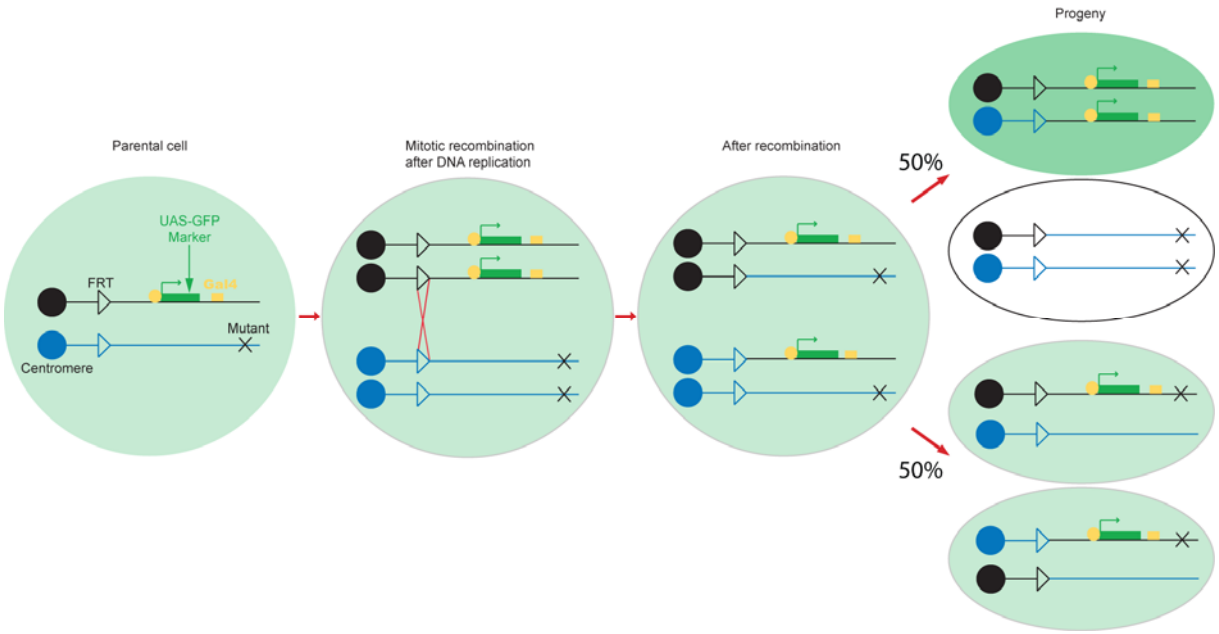


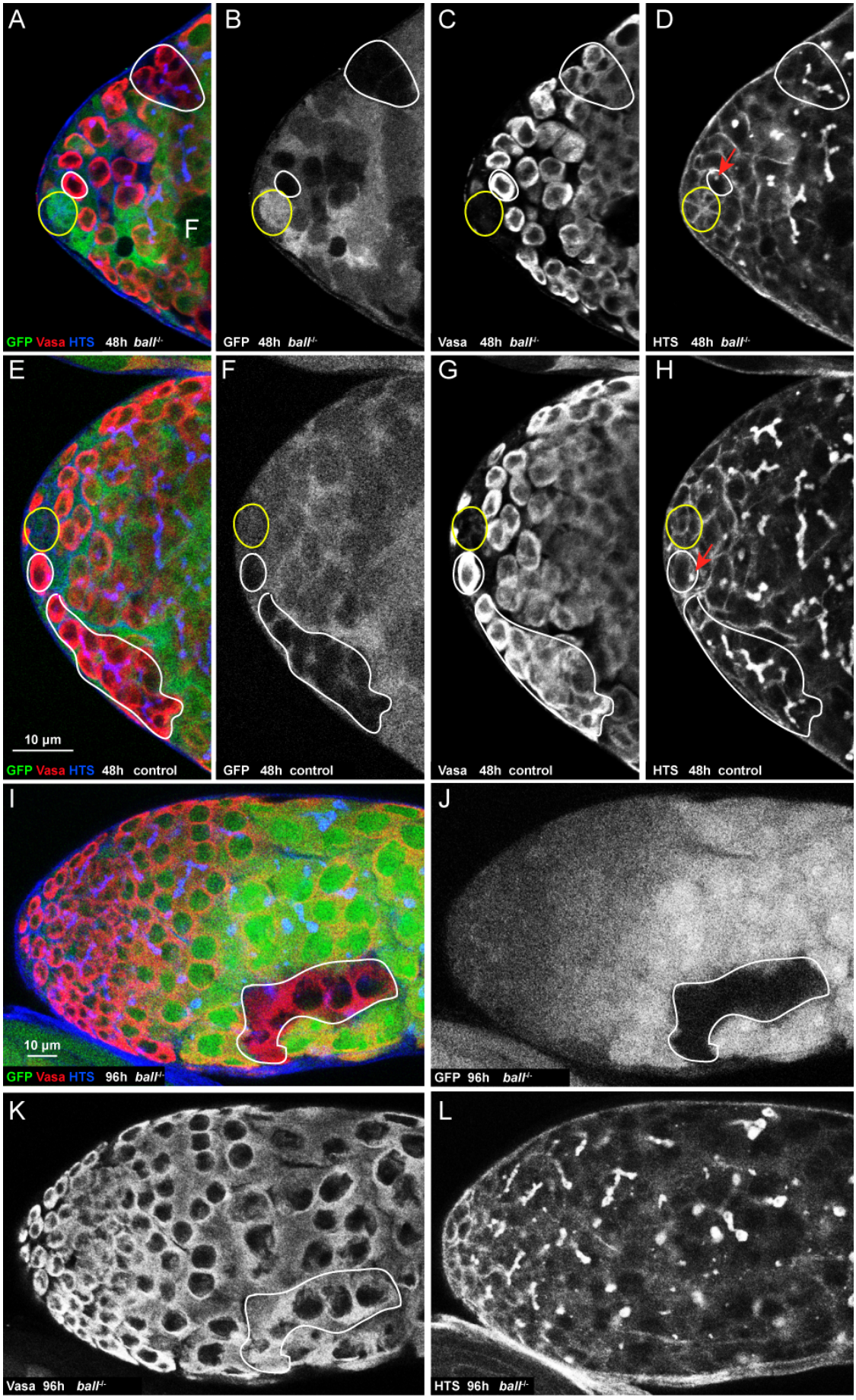
Figure 24: Schematic representation of FLP-FRT mosaic analysis.
Heat-shock induction of the *flp* gene leads to recombination between the FRT sites and exchange of the chromosome arms distal from the recombination site. After independent segregation of the chromosomes there is 50% probability that one of the daughter cells will be homozygous for the mutant gene and will lack the marker and the other cell will carry two copies of the marker (adapted from Xu & Rubin, 1993).

In the experimental setup, young adult males were heat-shocked to induce somatic recombination. GSCs could be identified by their position directly contacting the hub and by the expression of the germline marker *vasa* (Fig. 25A-H). In addition, each GSC possesses a spot-like cytoplasmic structure called spectrosome. The differentiating germ cells remain connected by cytoplasmic bridges called fusomes. The cytoskeletal protein Hu-li tai shao (Hts; Lin et al., 1994) labels spectrosomes,

fusomes and cell membranes, which allows cell types to be distinguished from one another, in addition to the morphological criteria.

The results of the experiment are presented on Fig. 25 and Fig. 26. The number of *ball*$^{2/2}$ mutant and control GSCs in testes was quantified after 48 hours, 72 hours and 96 hours past clone induction (PCI). In wild-type, the average number of control GSCs is about two cells per testis and the number is maintained throughout testis development (Fig. 26I). The average number of *ball*$^{2/2}$ mutant GSCs per testis was 2.04±0.07 at 48 hours PCI, and thus, no difference was observed between wild-type and mutant testes. However, in the next two days the average number of *ball*$^{2/2}$ mutant GSCs per testis significantly decreased. At 72 hours PCI the average number of *ball*$^{2/2}$ mutant GSCs per testis was 0.97±0.10 and only 0.33±0.05 per testis at 96 hours PCI (Fig. 25I). Thus, in contrast to wild-type, the average number of GSCs decreases almost one order of magnitude in the mutants (Fig. 26I).

The observation that *ball*$^{2/2}$ mutant GSCs are lost could be explained in two ways. On the one hand it is possible that the *ball*$^{2/2}$ GSCs undergo apoptosis. On the other hand, they might lose stem cell character and thus, start differentiation and move away from the hub. To tell apart these possibilities, I induced *ball*$^{2/2}$ mutant or control clones and stained testes for the apoptosis marker “cleaved-Caspase-3” (Xu et al., 2006). I observed apoptotic differentiating cell clusters in both *ball*$^{2/2}$ mutant and control clones (Fig. 26J). However, apoptotic GSCs were observed neither in *ball*$^{2/2}$ mutant nor in control GSCs (n≥50 testes at each time point for *ball*$^{2/2}$ mutant clones and n≥30 testes at each time point for control clones). Thus, *ball* is not required for the survival of GSCs. In addition, I frequently observed differentiating mutant spermatocyte clones, which indicates that *ball* does not participate in germline proliferation process in general (Fig. 25I-L). *ball* is, therefore, most probably required for the self-renewal of GSCs in the stem cell niche and prevents them from initiating the differentiation process that leads to spermatogenesis. The results support the conclusion that BALL activity has similar functions in GSCs and in Nbs: It is not essential for the survival of the stem cells but needed to maintain their undifferentiated state.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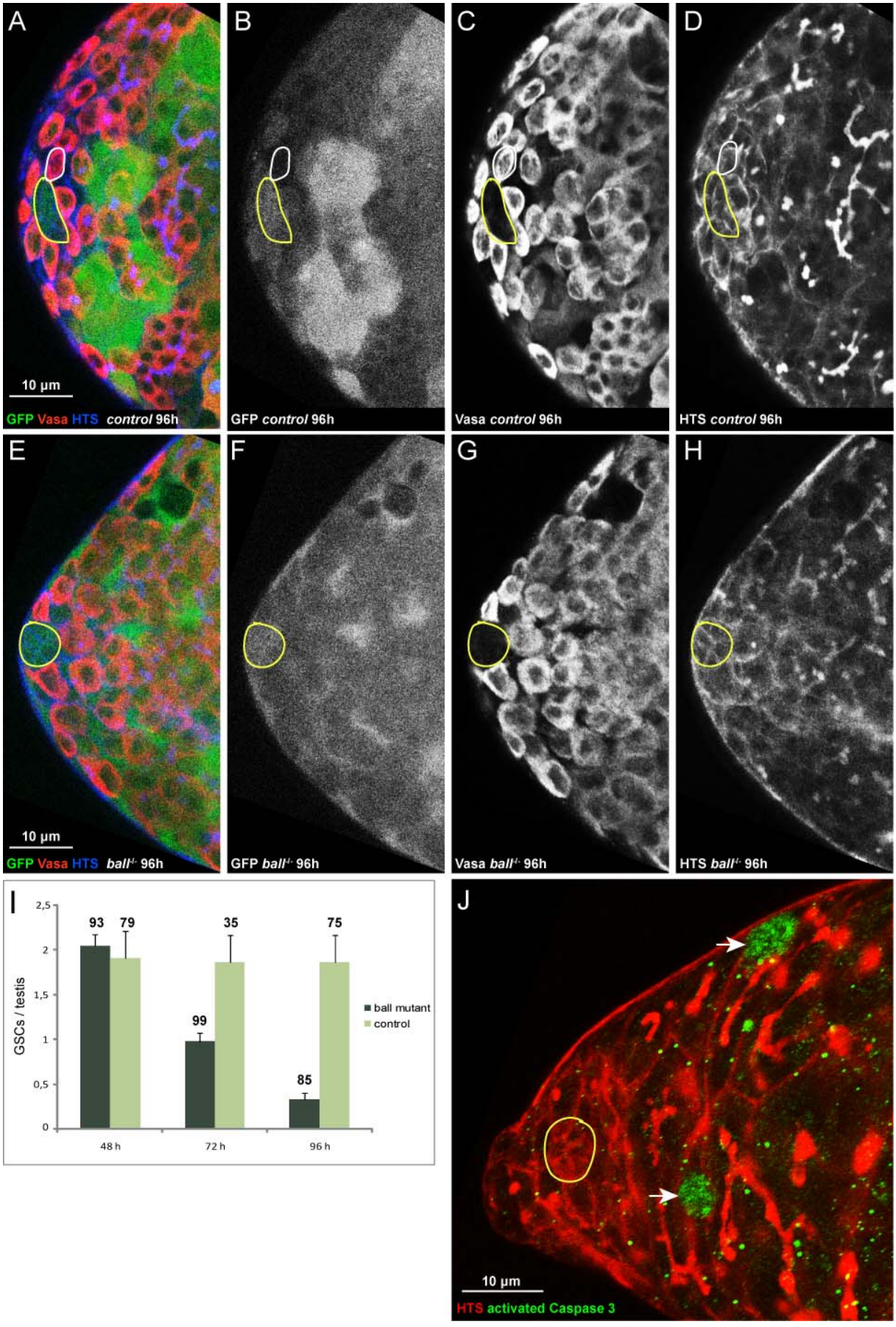
GFP Vasa HTS 96h ball-/-
GFP 96h ball-/-
K
L
Vasa 96h ball-/-
HTS 96h ball-/-

Figure 25: Clonal analysis of *ball* function in *Drosophila* germline stem cells: figure 1.
Figure shown on the previous page. In all panels testis shown tip pointing to the left. Lack of GFP auto-fluorescence (green) marks either *ball*$^{2/2}$ mutant or control cell clones. HTS (blue) and Vasa (red) were detected with α-HTS and α-Vasa antibodies and visualized by immunofluorescence. **(A)** Overlay image of testis tip with *ball*$^{2/2}$ mutant GSCs 48 h PCI. In yellow is encircled the testis tip. In white next to the testis tip is encircled a *ball*$^{2/2}$ mutant GSC. In the upper right corner in white is encircled differentiating spermatocyte clone. **(B)** Individual channel from panel (A) shows GFP auto-fluorescence. **(C)** Individual channel from panel (A) shows Vasa immunofluorescence. **(D)** Individual channel from panel (A) shows HTS immunofluorescence. A red arrow points to a spectrosome. **(E)** Overlay image of testis tip with control GSCs 48 h PCI. In yellow is encircled the testis tip. In white next to the testis tip is encircled a *control* GSC. In the middle down in white is labeled a differentiating spermatocyte control cell clone. **(F)** Individual channel from panel (E) shows GFP auto-fluorescence. **(G)** Individual channel from panel (E) shows Vasa immunofluorescence. **(H)** Individual channel from panel (E) shows HTS immunofluorescence. A red arrow points to a spectrosome. **(I)** Overlay image of testis tip with differentiating *ball*$^{2/2}$ mutant spermatocyte clone 96 h ACI. **(J)** Individual channel from panel (I) shows GFP auto-fluorescence. **(K)** Individual channel from panel (I) shows Vasa immunofluorescence. **(L)** Individual channel from panel (I) shows HTS immunofluorescence.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GFP Vasa HTS ball-/- 96h
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Vasa ball-/- 96h
HTS ball-/- 96h
I
GSCs / testis
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2
1,5
1
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0
93
79
35
75
99
85
48 h
72 h
96 h
ball mutant
control
J
10 µm
HTS activated Caspase 3

Figure 26: Clonal analysis of *ball* function in *Drosophila* germline stem cells: figure 2.
Figure shown on the previous page. In all panels testis shown tip pointing to the left. (A-H) Lack of GFP auto-fluorescence (green) marks either *ball*$^{2/2}$ mutant or control cell clones. HTS (blue) and Vasa (red) were detected with α-HTS and α-Vasa antibodies and visualized by immunofluorescence. **(A)** Overlay image of testis tip with control GSCs 48 h PCI. In yellow is encircled the testis tip. In white next to the testis tip is encircled a *control* GSC. **(B)** Individual channel from panel (A) shows GFP auto-fluorescence. **(C)** Individual channel from panel (A) showing Vasa immunofluorescence. **(D)** Individual channel from panel (A) shows HTS immunofluorescence. **(E)** Overlay image of testis tip where no *ball*$^{2/2}$ mutant GSCs are detectable 96 h PCI. In yellow is encircled the testis tip. **(F)** Individual channel from panel (E) showing GFP auto-fluorescence. **(G)** Individual channel from panel (E) shows Vasa immunofluorescence. **(H)** Individual channel from panel (E) showing HTS immunofluorescence. **(I)** Average number of *ball*$^{2/2}$ mutant or control GSCs 48 h, 72 h and 96 h PCI. The average number of *ball*$^{2/2}$ mutant GSCs for each time point is as follows: *ball*$^{2/2}_{48h}$=2.04±0.13; *ball*$^{2/2}_{72h}$= 0.97±0.10; *ball*$^{2/2}_{96h}$= 0.32±0.08. The average number of control GSCs for each time point is as follows: control$_{48h}$=1.90±0.31; control$_{72h}$=1.86±0.30; control$_{96h}$= 1.86±0.30. The calculated errors represent the average differences from the mean. **(J)** Immunostaining for HTS (red) and activated caspase 3, which labels apoptotic cells. The arrows point to apoptotic cells further away from the hub. Apoptosis was not detectable at 48 h, 72 h or 96 h after clonal induction in *ball*$^{2/2}$ mutant GSCs (n≥50 per time point, where n is the number of testes analyzed).

4. Discussion

The work presented here addresses the expression pattern of the *ball* gene, both in terms of mRNA and protein distributions, and a proteomic approach towards identifying potential *trans*-acting regulators that act in conjunction with the *cis*-acting control region that was delimited to a 286 bp DNA fragment which is sufficient to mediate a *ball*-like CNS expression pattern. Within this fragment 155 bp were also shown to be necessary for this aspect of *ball* expression. In addition, the results provide evidence that BALL is not only expressed in both Nbs and GSCs, but also required, in a cell-autonomous fashion, to maintain their stem cell character. In fact, *ball* mutant Nbs lose the functional marker protein Miranda and produce a smaller number of Nb progenies as compared to the wild-type Nbs. *ball* mutant GSCs leave their position in the stem cell niche and differentiate. Although these observations suggest that the responses of the two different stem cell types are different, these cellular phenotypes might reflect a common biological phenomenon: the exit of the stem cells from active mitosis.

4.1 *ball* expression in embryonic Nbs is controlled by a short *cis*-regulatory genomic sequence

During embryogenesis *ball* transcripts are enriched in actively proliferating Nbs, which represent the stem cell population of the developing CNS of *Drosophila* (Klinge, 2006). I have characterized the distribution of BALL in the embryonic CNS (Section 3.1) and observed that in contrast to the *ball* mRNA, BALL is enriched but not restricted to embryonic Nbs (Section 2.1, Fig. 2). In addition, BALL is also present, although at lower levels, in Nb-derived GMCs and neurons. This suggested that there are in fact differences in the distribution of *ball* mRNA and its corresponding protein. A more detailed analysis of the *ball* mRNA distribution, involving molecular cell markers to unambiguously identify Nb derived cells, showed that *ball* mRNA is indeed enriched in Nbs and virtually absent from differentiating cells (Section 2.1, Fig. 2). The differences in the distribution between *ball* mRNA and its corresponding protein could be explained under the assumption that BALL is exclusively produced in Nbs. Upon cell division, BALL is distributed to both the renewed Nb and the GMC daughter cells and from the latter to the deriving neurons. Alternatively, there could be low and thus undetected levels of *ball* transcription in

these cells. The strong expression of the gene in Nbs and the marked differences in the protein levels between Nbs, GMCs and neurons suggest a functional role for *ball* in Nbs.

The question whether *ball* has a function in embryonic CNS was addressed by a gain-of-function study (Section 2.4). However, overexpression of *ball* in Nbs and GMCs did not result in an apparent phenotype in the CNS (Section 2.4). This observation is consistent with the fact that *ball*$^{2/2}$ homozygous mutant embryos have also no scorable defects in embryonic CNS development (Klinge, 2006). While this observation can be explained through maternally derived *ball* mRNA both in the egg and early embryo, the results of the gain-of-function studies clearly establish that the presence of BALL in cells different from Nbs has no visible effect on neural development. Such a result is, for example, consistent with the argument that a possible substrate of the kinase BALL (Lancaster et al., 2007) might not be present in all cells and that the specific action of BALL in the neural system of the fly might be restricted by the availability of its functional substrate in Nbs only. Alternatively or in addition, BALL may require some cell-specific modifications or cofactors only provided in the stem cells. The results also suggest that, at least in the embryonic CNS, *ball* does not have an instructive function, i.e. overexpression of BALL did neither increase the number of Nbs nor the number of neurons. However, reaching a definite answer to the question whether *ball* could act as an instructive factor in embryonic CNS development, and whether BALL functional targets or modifiers and cofactor are only present in Nbs, would require the employment of a strictly GMCs specific driver, which is currently not available.

The observation, that *ball* is specifically expressed in Nbs during embryonic and larval development raised the question of how the persistent expression of *ball* in Nbs throughout development is brought about. One possibility is that a sequence-specific transcription factor regulates the expression of *ball* in Nbs. However, no single transcription factor is known to be expressed exclusively in Nbs during the period when they proliferate. On the other hand, there are known transcription factors which are expressed in a characteristic temporal sequence (Hunchback → Krüppel → Pdm → Castor) in Nbs (Brody & Odenwald, 2000; Grosskortenhaus et al., 2006; Isshiki et al., 2001; Kambadur et al., 1998) and their activity is required to specify GMC and

neuronal identities (Brody & Odenwald, 2000; Grosskortenhaus et al., 2006; Isshiki et al., 2001;Kambadur et al., 1998). However, the persistent expression of *ball* in Nbs suggests that *ball* expression is turned on early and remains active as long as these cells maintain Nb function. Thus, it is possible that *ball* expression requires a complex array of *cis*-acting regulatory sequences similar to many other developmentally regulated genes such as, for example, *hairy* and *even skipped* (Hooper et al., 1989*;* Fujioka et al., 1999; Small et al., 1992*).* Surprisingly, however, the results show that the *ball* regulatory region is contained within 123bp of intergenic and 217bp of 5'UTR sequence (Section 2.2, for summary see Fig. 4B). *In silico* analysis of this region for transcription factor binding sites revealed in fact putative Huchback (Hb) binding sites, suggesting that Hb could regulate *ball* expression. This hypothesis was tested, but the result revealed that *ball* expression in embryonic Nbs was not affected in Hb mutants (data not shown). Thus, Hb activity by itself is not decisive for *ball* expression in the Nbs.

Identification of the short 286 bp *cis*-regulatory sequence of *ball* argues against a complex *cis*-acting regulatory requirement for the spatiotemporal aspects of ball expression. It is possible that *ball* expression in Nbs is controlled either by a yet unknown transcription factor, a combination of several transcription factors or in a different way that involves cell-specific chromatin remodeling (for reviews on the role of chromatin remodeling in gene transcription see (Williams & Tyler, 2007; Vaissiere et al., 2008). Noteworthy, *ball* embryonic expression closely resembles the pattern of mitotic proliferation (Foe, 1989; Lee & Orr-Weaver, 2003) with the important exception that *ball* is downregulated in GMCs (Section 2.1) Thus, one possibility is that *ball* expression is coupled to the process of cell proliferation, similar to the expression of the transcription factor E2F of the basic cell cycle machinery (Duronio & O'Farrell, 1995). To restrict the expression to Nbs, however, *ball* expression in GMCs would need to be actively downregulated. The deletion study performed with the regulatory region has not been informative about a possible negative regulation in GMCs, since a corresponding upregulation of *ball* expression in GMCs has not been observed upon shortening the *cis*-regulatory region (Fig. 5 and Fig. 6). Therefore, the possibility that a yet unknown transcription factor regulates *ball* expression in Nbs cannot be excluded. Alternatively, the downregulation could be caused by

suppressor(s) which act in the same region of the gene as the transcriptional activators. An assignment of overlapping transcriptional activator and repressor sites within cis-acting control regions of genes have been described (Hoch et al., 1992).

4.2 Snapshot of the *ball*-specific transcription machinery

Sequence-specific transcription factors that are potential regulators of *ball* expression in Nbs were identified by a proteomics approach using nuclear extracts of staged embryos. The consistent binding of proteins to a specific oligo of the *cis*-regulatory region of the *ball* gene was used as a criterion to filter out a large number of unspecific binders.

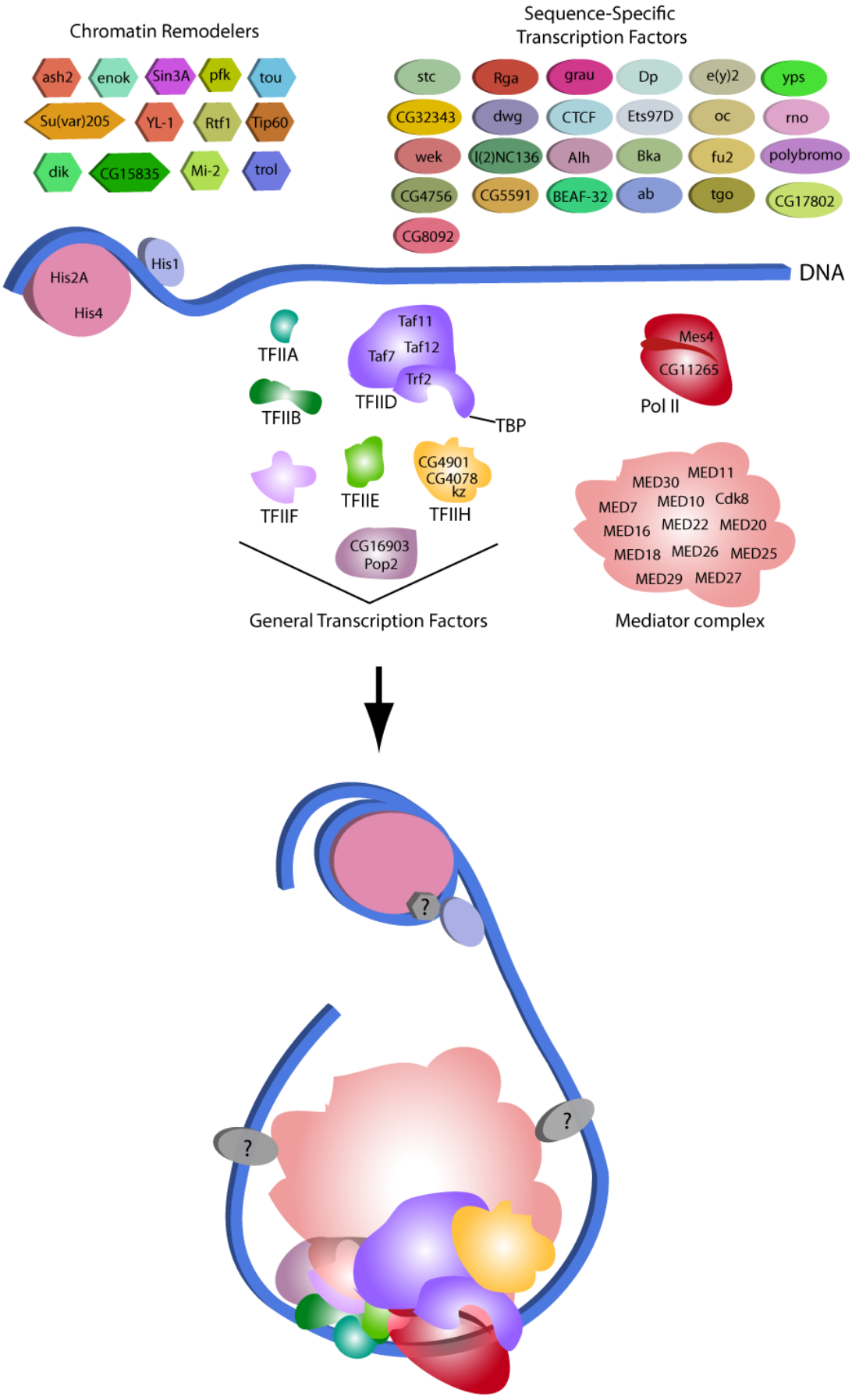
In the initially determined proteome, a large number of unspecific DNA binders was present. Possible explanations for the high number of unspecific binders are the high protein concentration used for the binding assay and the subsequently applied mild washing steps used in the experiments described. One could imagine that a Nb specific transcription factor, which possibly regulates *ball* expression, would be present in a very low amounts. This assumption is based on the fact that if the factors were Nb specific, they would be present only in a very small number of cells per embryo and only during the limited time period when Nbs are mitotically active (for review see (Maurange & Gould, 2005)). Thus, the decisive factors would represent only a minor fraction in the nuclear protein extracts used in this study. In addition, the *in vitro* DNA binding conditions of a factor to DNA oligo-nucleotides and the corresponding binding to DNA organized in chromatin might be different and additional co-factors might be required to stabilize the complexes *in vivo*. Due to this some of the key regulators could have escaped detection.

To distinguish specific from unspecific binders, the identified proteome was filtered in a pragmatic fashion (Section 2.3). A potential binding protein, which consistently binds to a certain oligo, was considered as a specific binder, whereas the identification of a factor, which was only found in a single experiment, was regarded as unspecific. In the filtered proteome, nuclear proteins with functions in the regulation of transcription were significantly overrepresented relative to the initially purified proteome (Fig. 8 and Fig. 9). The finally filtered proteome represents a pool of 296 proteins, which may include those which are biologically relevant for the

regulation of *ball* transcription. Fig. 16 summarizes the identified factors and classifies them according to their function in transcription. They include general transcription factors, polymerase subunits and proteins from the so called mediator complex (Casamassimi & Napoli, 2007; Thomas & Chiang, 2006). It was not surprising to find members of the general polymerase II transcription machinery (Thomas & Chiang, 2006) in the proteome, since the test-oligo-nucleotides encompassed the core promoter region of the *ball* gene (Section 2.3). Although it is unlikely that the entirety of the identified regulators of transcription bind the 155bp of DNA sequence in a functional manner, it is possible that stable protein complexes bound to the DNA oligo-nucleotides have been purified and thus, factors not directly associated with DNA should have been isolated as well. This proposal would explain the presence of chromatin remodelers in the proteome that are known to regulate the access of polymerase complexes to promoter regions through chromatin modifications (Williams & Tyler, 2007; Vaissiere et al., 2008).

For future studies, the groups of DNA-binders with unknown molecular functions are of particular interest together with sequence specific transcription factors and chromatin insulators such as CTCF (Yusufzai et al., 2004; Gerasimova et al., 2007). CTCF was found to bind consistently and specifically to several test oligo-nucleotides of the *ball* 5' region and not to the control oligo (Fig. 26). Further experiments will address both direct binding of candidate factors to the *ball* regulatory sequence, their expression pattern in relation to *ball* as well as their functional relevance for *ball* expression.

Figure 27: Summary of regulation of *ball* expression.
Figure shown on the next page. General transcription factors, sequence specific transcription factors and chromatin remodelers were found in a protein-DNA binding experiments to preferentially bind the *ball* regulatory region. Some of these factors might co-operate to ensure the timed expression of *ball* in a subset of cells. Chromatin remodelers could act to ensure the correct modification state of nearby chromatin, whereas specific transcription factors might recruit the general transcription machinery to the core promoter (adapted with strong modifications from Alberts et al. 1994).


Chromatin Remodelers
ash2
enok
Sin3A
pfk
tou
Su(var)205
YL-1
Rtf1
Tip60
dik
CG15835
Mi-2
trol
Sequence-Specific
Transcription Factors
stc
Rga
grau
Dp
e(y)2
yps
CG32343
dwg
CTCF
Ets97D
oc
rno
wek
l(2)NC136
Alh
Bka
fu2
polybromo
CG4756
CG5591
BEAF-32
ab
tgo
CG17802
CG8092
His2A
His4
His1
DNA
TFIIA
TFIIB
Taf11
Taf7
Taf12
Trf2
TFIID
TBP
Mes4
CG11265
Pol II
TFIIF
TFIIE
CG4901
CG4078
kz
TFIIH
CG16903
Pop2
General Transcription Factors
MED11
MED30
MED7
MED10
Cdk8
MED16
MED22
MED20
MED18
MED26
MED25
MED29
MED27
Mediator complex
?
?
?

4.3 *ball* is cell-autonomously required in undifferentiated cells

During embryogenesis, the CNS of *ball* mutant embryos shows no obvious defects (Klinge, 2006). This finding could be due to the presence of maternal *ball* mRNA which is deposited in the egg during oogenesis (Klinge, 2006). Since embryos lacking this maternal *ball* complement fail to develop, the analysis of *ball* requirement for embryonic CNS development was not possible. However, during larval development when maternally derived BALL activity is not present any longer, *ball* mRNA is also Nb specifically expressed and the *ball* protein is also present in but not restricted to Nbs (Section 2.1, Fig. 3). Consistent with its expression in larval Nbs, *ball* mutants exhibit severe degenerations of the larval brain (Klinge, 2006), indicating that *ball* is required directly or indirectly for normal larval CNS development. However, *ball* mutants also show defects in other larval organs such as the gonads (see below) and imaginal discs (Klinge, 2006). Therefore, it was necessary to demonstrate that *ball* is indeed expressed and required in Nbs, meaning that it acts in a cell autonomous fashion. The results of the MARCM study (section 2.5) show that *ball* acts cell autonomously and that the *ball* mutant Nbs lose at least one Nb-specific marker, Miranda (Fig. 23I-L), which is essential for Nb function (Ikeshima-Kataoka et al., 1997; Lee et al., 2006; Matsuzaki et al., 1998). Furthermore, the cell clones derived from *ball* mutant Nbs contain fewer cells than expected (Fig. 22E) and this loss of cells is not due to apoptosis. Thus, *ball* is not required for the survival of the Nbs and their derivatives as shown also by the presence of GMCs in *ball* mutant cell clones (Fig 23E-H). Whether the reduced number of Nb derived cells in mutant cell clones is caused by an occasional and thus undetected cell death or due to the fact that Nbs cease proliferation requires further and more detailed studies.

The loss of Miranda expression in mutant Nbs (Fig. 23I-L) unambiguously demonstrates that *ball* is required for the maintenance of Nb cell identity. Miranda is required in Nbs for the basal localization and asymmetric segregation of cell fate determinants such as Pros and Brat to the GMC daughter cell during cell division (Bello et al., 2006; Betschinger et al., 2006; Lee et al., 2006). Although further experiments are required to clarify why Miranda is not expressed in *ball* mutant Nbs, and what the consequences for these Nbs are, loss of Miranda indicates *per se* that

loss of BALL activity causes abnormal Nbs. Whether they continue to develop similar to wild-type Nbs or undergo abnormal development instead, needs to be shown.

ball is not only expressed in the nervous system, but also in both the female and male germline during larval and adult stages (Klinge, 2006). In addition to Nbs, I also addressed the function of *ball* in male GSCs (Section 2.6). Previous studies indicated that *ball* might also be required for stem cell maintenance in the male germline (Klinge, 2006). In contrast to the Nb system, GSCs represent a niche-controlled stem cell system (Gilboa & Lehmann, 2004). Nevertheless, there were surprising similarities concerning *ball* function in these two stem cell systems. First, like in Nbs (Section 2.5), *ball* is not essential for the survival of GSCs (Section 2.6). Second, both Nbs and GSCs are not maintained as functional stem cells. In contrast to the Nb system, however, loss of BALL activity resulted in GSCs differentiation, concomitant with the fact that GSCs lost their position adjacent to the stem cell niche (Fig. 25).

Wild-type GSCs occasionally undergo differentiation and are replaced by symmetric GSC division (Wallenfang et al., 2006). Unlike GSCs, there is not known natural turnover of Nbs in wild-type brains (Maurange & Gould, 2005). This could be explained by the shorter time period during which Nbs are needed in development (Maurange & Gould, 2005). This means that male GSCs proliferate for months to sustain the continuous production of male germline cells, whereas Nbs of the thoracic region cease proliferation approximately 120 hours after larval hatching in the pupal stage (Maurange & Gould, 2005). Interestingly, the loss of Miranda associated with the loss of BALL activity in *ball* mutant Nbs might be the way how wild-type Nbs exit from active mitosis (Maurange et al., 2008).

In conclusion, BALL activity is required for the maintenance of male GSCs and *ball* mutant GSCs initiate differentiation and thereby leave the stem cell niche. *ball* is also required for the maintenance of functional Nbs. Loss of *ball* in this stem cell system is manifested by a reduced number of cells in the Nb lineage, concomitant with the loss of the Nb specific expression of the functional marker gene Miranda. The apparently different phenotypes observed in the two different stem cell systems, Nbs and GSCs, might relate to similar biological process: stem cell maintenance.

5. Summary and conclusions

Stem cells divide asymmetrically and thereby generate two daughter cells with distinct developmental fates. One cell maintains the stem cell character (stem cell "self-renewal"), whereas the other cell differentiates along a given cell lineage. In different stem cell systems, the maintenance of the stem cell character is controlled by distinct external as well as internal cues and cell intrinsic mechanisms, which interpret those cues to ensure correct stem cell behavior. Recently, a conserved Serine/Threonine kinase of *Drosophila melanogaster*, encoded by the gene *bällchen* (*ball)*, which is proposed to participate in stem cell renewal, has been identified. It shows distinct expression patterns in the central nervous system (CNS) and the developing gonads and is required for the proper development of both larval brain and male germline as revealed by mutant analysis.

I first compared the expression patterns of the *ball* transcripts and the *ball* protein (BALL) in the CNS during both embryonic and larval development. *ball* transcripts are specifically expressed in neuroblasts (Nbs), the neural stem cells, which divide to produce a self-renewed Nb and a differentiating ganglion mother cells (GMCs). Like the transcripts, BALL is also enriched in Nbs but also found in GMCs and neurons. In addition, *ball* is also expressed in germline stem cells (GSCs), which reside in distinct locations of both male and female gonads. Thus the results were consistent with the proposal that *ball* plays a role in some aspects of the stem cell biology.

I next asked whether it is possible to identify *cis*-acting regulatory elements and trans-acting components that are essential for the regulation of ball expression in the CNS. *Cis*-acting regulatory elements of the *ball* gene were identified by delimitation of DNA sequences of the gene, which were sufficient to drive the expression of a reporter gene in a *ball*-like pattern in the embryonic Nbs. To identify the *trans*-acting transcriptional regulators that control *ball* expression, I used DNA sequences that are both necessary and sufficient to drive the expression pattern of the reporter gene in a DNA-protein binding assay *in vitro*. Using mass spectrometry in combination with stringent *in silico* selection criteria, a total of 296 proteins were found represent the putative *ball trans*-acting factor proteome. This protein collection includes not only components of the general transcription machinery, such as polymerases and members of the mediator complex, but also sequence specific transcriptional

regulators and chromatin remodelers. A molecular model of how the Nb-specific CNS expression of *ball* is achieved requires further studies assaying for co-expression and loss-of-function effects of the putative *trans*-acting factors on *ball* expression.

I finally asked whether *ball* activity is required in both Nbs and GSCs, whether it acts in a cell autonomous or non-autonomous fashion and in which cellular process BALL is involved. Both Nbs and GSCs are stem cells, but the processes underlying the regulation of stem cell specific fates, i.e. stem cell quiescence or stem cell self-renewal versus differentiation of their daughter cells, are either under the control of intrinsic factors (in the case of Nbs) or depend on distinct signaling events from their immediate environment, the stem cell niche (in the case of GSCs). For this part of the work, I used *ball* loss-of-function mutants. However, homozygous *ball* mutants are lethal and thus, I induced mitotic recombination events in single stem cells to generate homozygous *ball* mutant cell clones and examined the effects of loss of *ball* activity on cells in the larval CNS and the male germline. In wild-type, after GSC division, the daughter cell that does not maintain stem cell character leaves the stem cell niche and differentiates according its germline fate. I found that removal of *ball* activity from the GSCs caused a stem cell maintenance defect, meaning that both GSC daughter cells leave the stem cell niche and differentiated. Removal of *ball* activity from Nbs caused the loss of the functional Nb marker protein Miranda and resulted in fewer differentiating cells. The results show that *ball* is an essential component of the genetic system that is necessary to maintain the stem cell character of both Nbs and GSCs. Although the *ball* loss-of-function phenotypes in the two stem cell systems appear different, and stem cell self-renewal is differently controlled, the results described here are consistent with the proposal that stem cells possess a common mechanism, through which they maintain their stem cell character.

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Appendix

Table 6: *Drosophila* proteins found in three independent DNA-protein binding assays.

The numbers in each row show in how many independent experiments the respective protein was found to bind each oligo. Oligo 6 is the control oligo. The proteins are ordered alphabetically. FBgn stands for Flybase gene number.

FBgn	SYMBOL	Oligo 1	Oligo 2	Oligo 3	Oligo 4	Oligo 5	Oligo 6
FBgn0010339	128up	0	2	2	2	1	3
FBgn0020238	14-3-3epsilon	3	3	3	3	3	3
FBgn0004907	14-3-3zeta	3	3	3	2	2	1
FBgn0250848	26-29-p	3	3	3	3	3	3
FBgn0053100	4EHP	2	2	1	1	2	2
FBgn0027885	Aac11	2	2	2	0	1	3
FBgn0000011	ab	1	0	1	1	2	1
FBgn0000015	Abd-B	0	0	0	0	0	1
FBgn0000017	Abl	0	0	0	1	0	0
FBgn0015331	abs	3	3	3	3	3	3
FBgn0027620	Acf1	1	0	1	0	2	2
FBgn0028484	Ack	0	1	0	0	0	0
FBgn0000043	Act42A	2	2	2	2	3	3
FBgn0000044	Act57B	3	3	2	3	3	3
FBgn0000042	Act5C	3	3	3	3	2	3
FBgn0000045	Act79B	0	0	1	1	0	0
FBgn0000046	Act87E	3	3	3	3	3	3
FBgn0000047	Act88F	0	0	1	1	0	0
FBgn0000667	Actn	3	2	2	2	3	2
FBgn0011741	Actr13E	2	1	1	0	2	2
FBgn0037555	Ada2b	3	3	2	1	2	2
FBgn0000055	Adh	0	1	0	0	1	1
FBgn0026309	aft	1	0	0	1	0	0
FBgn0041171	ago	1	0	0	1	1	0
FBgn0087035	AGO2	1	3	0	3	3	2
FBgn0027932	Akap200	0	0	0	0	1	0
FBgn0033383	alc	0	2	0	1	0	0
FBgn0037471	Alh	3	2	1	2	3	1
FBgn0015569	alpha-Est10	1	0	0	0	0	0
FBgn0250789	alpha-Spec	3	3	3	3	3	3
FBgn0025725	alphaCop	3	3	3	3	3	3
FBgn0087040	alphaTub67C	3	3	3	3	3	3
FBgn0003884	alphaTub84B	3	3	3	3	3	3
FBgn0003886	alphaTub85E	0	0	1	1	0	0
FBgn0004372	aly	3	3	3	3	3	3
FBgn0039206	ana1	0	1	0	0	0	0
FBgn0027513	ana2	1	0	0	0	0	0
FBgn0029512	Aos1	0	0	0	0	1	2
FBgn0024833	AP-47	0	0	0	0	0	1
FBgn0026598	Apc2	0	0	0	0	0	1
FBgn0022131	aPKC	1	3	1	1	2	1

FBgn0015903	apt	3	3	3	2	3	3
FBgn0031781	Arc-p20	0	1	0	0	0	0
FBgn0032859	Arc-p34	0	0	0	0	1	0
FBgn0013749	Arf102F	0	0	0	1	0	0
FBgn0010348	Arf79F	2	0	0	0	1	1
FBgn0004908	Arf84F	1	1	0	0	0	1
FBgn0000117	arm	1	0	1	1	1	2
FBgn0011743	Arp53D	0	0	0	1	0	0
FBgn0011744	Arp66B	1	1	0	0	3	1
FBgn0030877	Arp8	1	0	1	0	2	1
FBgn0011745	Arp87C	0	0	1	0	0	0
FBgn0038369	Arpc3A	1	0	0	0	0	0
FBgn0029095	aru	1	1	2	1	1	0
FBgn0005386	ash1	0	0	0	0	1	0
FBgn0000139	ash2	2	1	2	0	3	0
FBgn0000140	asp	3	3	3	3	3	3
FBgn0010715	Atg1	1	3	1	0	2	1
FBgn0010750	atms	3	3	3	3	3	3
FBgn0010217	ATPsyn-beta	1	1	0	1	2	2
FBgn0019637	Atu	3	3	3	3	3	3
FBgn0041188	Atx2	0	0	1	0	0	1
FBgn0000146	aub	0	1	0	0	1	0
FBgn0023407	B4	0	0	0	0	1	1
FBgn0004587	B52	3	3	3	2	3	2
FBgn0025525	bab2	0	0	1	0	0	0
FBgn0031977	baf	2	1	3	3	2	3
FBgn0027889	ball	3	3	3	3	3	3
FBgn0042085	Bap170	0	0	0	0	1	1
FBgn0025716	Bap55	3	3	3	3	3	3
FBgn0025463	Bap60	3	3	3	2	3	3
FBgn0014127	barr	0	0	0	0	0	1
FBgn0015602	BEAF-32	0	3	3	1	1	1
FBgn0000171	bel	3	2	2	2	3	3
FBgn0250788	beta-Spec	3	3	3	3	3	3
FBgn0025724	beta'Cop	3	3	3	3	2	3
FBgn0008635	betaCop	3	3	3	3	3	3
FBgn0003887	betaTub56D	3	3	3	3	3	3
FBgn0003888	betaTub60D	3	3	2	2	3	2
FBgn0003889	betaTub85D	0	0	1	1	0	1
FBgn0003890	betaTub97EF	1	0	1	1	0	1
FBgn0038928	BG4	0	0	0	0	0	1
FBgn0014133	bif	3	3	3	3	3	3
FBgn0024491	Bin1	2	3	3	3	3	2
FBgn0026262	bip2	1	1	0	0	1	0
FBgn0002638	Bj1	1	0	0	0	1	1
FBgn0010520	Bka	0	1	2	1	1	0
FBgn0015907	bl	0	1	0	0	0	0
FBgn0011211	blw	3	3	3	3	3	3
FBgn0011206	bol	3	0	0	0	0	1
FBgn0023097	bon	1	1	0	0	1	0
FBgn0032105	Borr	3	3	3	3	3	3
FBgn0010300	brat	2	2	2	3	2	3
FBgn0000212	brm	1	3	1	1	2	2

FBgn0259246	brp	0	0	1	0	0	0
FBgn0037808	Bruce	2	2	3	2	2	2
FBgn0011785	BRWD3	2	2	1	2	1	2
FBgn0005666	bt	0	0	1	1	1	0
FBgn0012049	BtbVII	2	2	0	0	2	2
FBgn0025457	Bub3	2	3	3	2	3	3
FBgn0025458	BubR1	1	2	1	0	1	1
FBgn0004856	Bx42	1	0	0	0	1	0
FBgn0010292	bys	3	3	2	3	3	3
FBgn0000246	c(3)G	0	0	0	0	1	0
FBgn0015608	Ca-beta	1	0	0	0	0	0
FBgn0004551	Ca-P60A	1	0	0	1	1	0
FBgn0031114	cactin	0	1	2	1	2	0
FBgn0015610	Caf1	3	3	2	3	3	3
FBgn0013759	Caki	0	0	0	0	1	0
FBgn0038478	cal1	0	0	0	0	0	2
FBgn0000253	Cam	2	3	3	3	3	2
FBgn0033504	CAP	2	1	2	1	2	0
FBgn0039680	CAP-D2	1	2	2	2	2	2
FBgn0051989	Cap-D3	1	0	0	0	0	0
FBgn0250905	Cap-G	0	0	1	0	1	0
FBgn0000256	capu	0	0	0	0	1	0
FBgn0004878	cas	0	1	0	0	1	2
FBgn0022213	Cas	1	1	0	0	0	0
FBgn0002022	Catsup	0	0	0	0	1	0
FBgn0022943	Cbp20	1	1	1	1	0	0
FBgn0022942	Cbp80	2	3	2	2	3	3
FBgn0039396	CcapR	0	1	0	0	0	0
FBgn0010621	Cct5	1	0	0	0	1	2
FBgn0015019	Cctgamma	3	3	3	2	3	3
FBgn0025781	cdc16	3	1	1	1	2	2
FBgn0032863	cdc23	1	1	1	1	2	0
FBgn0012058	Cdc27	0	0	0	1	1	1
FBgn0004107	cdc2c	0	1	0	0	0	0
FBgn0013435	cdc2rk	2	2	2	3	3	3
FBgn0035918	Cdc6	3	3	3	3	3	3
FBgn0015618	Cdk8	2	2	1	1	1	0
FBgn0026141	Cdlc2	1	0	0	0	0	0
FBgn0032409	Ced-12	0	1	0	0	0	1
FBgn0000289	cg	3	2	3	2	3	3
FBgn0039590	CG10011	0	0	0	0	1	0
FBgn0035720	CG10077	3	3	3	3	3	3
FBgn0033951	CG10139	3	3	3	3	3	3
FBgn0035294	CG1017	1	0	0	0	0	0
FBgn0032783	CG10237	2	3	2	1	2	2
FBgn0027514	CG1024	3	3	3	3	3	2
FBgn0027512	CG10254	1	0	0	0	0	0
FBgn0032812	CG10263	1	0	0	1	0	0
FBgn0037446	CG10267	0	1	0	1	0	0
FBgn0037441	CG10284	0	1	0	0	0	0
FBgn0034654	CG10306	2	3	2	1	1	1
FBgn0032690	CG10333	3	2	3	3	3	3
FBgn0031868	CG10354	2	0	3	2	3	1

FBgn0033021	CG10417	0	0	0	0	0	1
FBgn0036277	CG10418	0	2	2	2	2	2
FBgn0033017	CG10465	0	0	0	0	0	1
FBgn0034631	CG10496	3	2	3	3	3	2
FBgn0039326	CG10562	1	0	0	0	0	0
FBgn0035608	CG10630	1	0	0	0	0	0
FBgn0032731	CG10641	1	1	0	0	1	0
FBgn0035590	CG10673	1	0	0	0	0	0
FBgn0032848	CG10722	1	0	0	0	0	1
FBgn0034420	CG10737	1	1	0	0	0	1
FBgn0036328	CG10749	2	2	0	0	1	2
FBgn0029979	CG10777	3	2	3	3	3	3
FBgn0037255	CG1078	1	0	0	0	0	0
FBgn0029666	CG10803	0	1	0	0	0	0
FBgn0038769	CG10889	3	2	3	3	2	1
FBgn0037470	CG1091	3	3	3	1	3	3
FBgn0032858	CG10949	2	1	2	0	1	2
FBgn0037379	CG10979	0	0	0	0	1	0
FBgn0036305	CG10984	2	1	0	0	0	1
FBgn0030520	CG10990	3	3	3	1	2	3
FBgn0027534	CG11006	1	0	0	0	0	0
FBgn0031208	CG11023	0	1	0	0	0	0
FBgn0031736	CG11030	1	0	1	1	1	1
FBgn0037659	CG11033	0	0	0	0	0	2
FBgn0039929	CG11076	1	0	0	0	0	0
FBgn0046222	CG1109	0	1	0	0	0	1
FBgn0027537	CG11092	3	3	3	3	3	3
FBgn0033160	CG11107	3	3	2	2	3	3
FBgn0030266	CG11122	0	0	0	1	1	0
FBgn0033169	CG11123	0	1	1	1	0	0
FBgn0037205	CG11133	0	1	1	0	0	0
FBgn0033177	CG11141	0	0	0	0	0	1
FBgn0039936	CG11148	1	0	0	0	1	3
FBgn0030499	CG11178	1	0	0	0	0	1
FBgn0031851	CG11188	3	3	3	3	3	2
FBgn0033246	CG11198	3	3	3	3	3	3
FBgn0030049	CG11265	0	0	0	0	2	0
FBgn0031883	CG11266	3	3	3	3	3	2
FBgn0035489	CG1135	3	3	2	3	2	2
FBgn0037504	CG1142	0	0	1	0	1	0
FBgn0034688	CG11474	0	0	0	0	0	1
FBgn0035397	CG11486	3	0	1	2	3	1
FBgn0039733	CG11504	0	1	0	0	0	0
FBgn0035437	CG11526	3	1	1	1	2	1
FBgn0039868	CG11563	3	3	3	3	3	3
FBgn0036847	CG11577	0	0	0	1	0	0
FBgn0035524	CG11583	3	3	3	3	3	2
FBgn0030314	CG11696	0	0	1	0	1	0
FBgn0031391	CG11723	1	0	0	0	1	0
FBgn0035296	CG11814	2	1	1	0	0	1
FBgn0039627	CG11837	1	0	2	0	1	0
FBgn0046214	CG11844	1	1	1	1	1	0
FBgn0039301	CG11875	3	3	3	3	3	2

FBgn0039274	CG11920	2	3	3	2	3	3
FBgn0031078	CG11943	3	2	2	3	3	3
FBgn0037643	CG11963	2	2	2	1	2	2
FBgn0037647	CG11968	2	1	0	0	1	1
FBgn0037655	CG11984	1	2	1	1	0	1
FBgn0040534	CG11985	1	0	1	0	0	1
FBgn0037312	CG11999	1	1	1	1	1	0
FBgn0035443	CG12010	1	0	0	0	0	0
FBgn0035276	CG12022	2	0	0	0	0	0
FBgn0032915	CG12050	2	1	3	3	3	2
FBgn0030039	CG12123	0	0	1	0	0	0
FBgn0030112	CG12124	0	0	0	0	0	1
FBgn0033473	CG12128	1	2	2	1	1	0
FBgn0036723	CG12229	0	0	2	0	1	0
FBgn0038489	CG12265	2	3	3	3	3	2
FBgn0038057	CG12267	0	0	0	0	0	2
FBgn0032620	CG12288	2	3	3	1	2	2
FBgn0036514	CG12301	3	3	3	3	3	3
FBgn0038577	CG12321	0	0	0	0	0	1
FBgn0033557	CG12325	3	2	1	2	3	1
FBgn0037489	CG1234	3	3	3	3	3	3
FBgn0038111	CG12360	1	0	0	0	0	0
FBgn0037168	CG12377	0	1	0	0	0	0
FBgn0038968	CG12499	3	3	3	3	3	3
FBgn0035171	CG12502	1	0	0	0	0	0
FBgn0250830	CG12547	1	0	0	0	1	0
FBgn0037213	CG12581	1	0	1	0	0	0
FBgn0030630	CG12608	0	0	1	0	0	0
FBgn0029952	CG12689	0	0	0	0	0	1
FBgn0031070	CG12702	1	2	1	3	3	3
FBgn0035411	CG12734	0	0	0	0	0	1
FBgn0038410	CG12785	3	3	3	3	3	3
FBgn0032050	CG13096	3	3	3	3	3	3
FBgn0032051	CG13097	3	3	3	3	3	3
FBgn0033750	CG13151	0	0	0	0	1	0
FBgn0033721	CG13159	0	0	0	1	0	0
FBgn0035526	CG1316	1	1	1	0	0	1
FBgn0033661	CG13185	1	1	3	2	3	1
FBgn0062449	CG13197	1	1	2	1	2	1
FBgn0032599	CG13277	0	0	1	2	2	0
FBgn0035692	CG13298	2	3	3	3	3	3
FBgn0033856	CG13334	1	0	0	0	0	0
FBgn0025633	CG13366	3	3	1	2	3	3
FBgn0025634	CG13367	0	0	0	0	0	1
FBgn0032031	CG13390	0	0	0	0	0	1
FBgn0034514	CG13427	1	1	1	0	0	1
FBgn0027507	CG1344	0	1	1	0	2	2
FBgn0035038	CG13588	1	0	0	0	0	0
FBgn0039177	CG13611	0	1	0	0	0	0
FBgn0039210	CG13625	0	0	0	0	1	0
FBgn0033372	CG13742	1	1	0	0	0	0
FBgn0033354	CG13745	1	1	1	0	2	1
FBgn0042092	CG13773	0	0	1	0	0	0

FBgn0033485	CG1381	3	2	3	1	3	1
FBgn0035168	CG13889	0	0	0	1	0	0
FBgn0035162	CG13900	3	3	3	3	3	3
FBgn0035277	CG13923	1	2	0	0	2	2
FBgn0039522	CG13972	0	0	0	0	1	0
FBgn0029964	CG1409	1	0	0	0	0	0
FBgn0036884	CG14098	2	2	0	1	3	0
FBgn0036219	CG14127	1	0	0	0	0	0
FBgn0031037	CG14207	2	1	0	0	1	0
FBgn0031040	CG14210	1	3	2	3	3	1
FBgn0031047	CG14213	3	3	2	2	3	3
FBgn0031052	CG14215	3	3	3	3	3	3
FBgn0031036	CG14220	1	0	0	0	2	0
FBgn0031057	CG14224	1	1	1	0	2	1
FBgn0031062	CG14230	3	3	3	2	3	3
FBgn0039462	CG14252	0	0	0	0	1	0
FBgn0031351	CG14352	2	2	2	1	2	1
FBgn0029899	CG14438	2	3	3	2	3	3
FBgn0027588	CG14476	1	1	2	2	1	2
FBgn0039413	CG14556	1	0	0	0	0	0
FBgn0037122	CG14570	0	0	0	0	0	1
FBgn0040646	CG14580	0	0	0	1	0	0
FBgn0031186	CG14614	1	2	0	0	0	0
FBgn0031191	CG14617	3	2	2	2	2	2
FBgn0037278	CG14656	2	1	2	1	3	2
FBgn0037924	CG14712	0	2	1	0	1	2
FBgn0037988	CG14740	1	0	0	0	0	0
FBgn0033316	CG14749	3	3	0	1	2	2
FBgn0023514	CG14805	3	3	3	2	3	3
FBgn0023517	CG14816	0	0	0	0	0	1
FBgn0035497	CG14995	0	0	0	0	0	1
FBgn0035541	CG15019	2	3	2	2	2	2
FBgn0030938	CG15047	1	0	0	0	0	1
FBgn0034401	CG15100	0	0	0	1	0	1
FBgn0041702	CG15107	2	2	2	1	2	2
FBgn0027580	CG1516	3	3	3	3	3	3
FBgn0032978	CG15216	0	0	0	0	0	1
FBgn0030322	CG15220	2	1	1	2	1	2
FBgn0034554	CG15227	1	0	0	0	0	0
FBgn0028918	CG15287	0	0	0	1	0	0
FBgn0031377	CG15356	0	0	1	1	0	1
FBgn0031397	CG15385	1	0	0	0	0	0
FBgn0031403	CG15387	0	1	1	1	0	1
FBgn0031460	CG15399	0	0	0	0	1	0
FBgn0039828	CG1542	3	2	3	3	3	2
FBgn0031608	CG15435	1	3	1	0	1	1
FBgn0031607	CG15440	0	0	0	0	1	0
FBgn0039712	CG15514	0	1	0	0	0	0
FBgn0039829	CG15561	0	1	0	0	1	0
FBgn0034194	CG15611	1	0	0	0	0	1
FBgn0031077	CG15618	0	0	0	0	0	1
FBgn0030474	CG15747	3	3	3	2	2	3
FBgn0029999	CG1575	3	3	3	3	3	2

FBgn0028476	CG15817	0	0	0	0	0	1
FBgn0030246	CG1582	0	1	0	0	1	0
FBgn0032136	CG15828	0	0	0	1	0	0
FBgn0033233	CG15835	0	2	1	1	0	0
FBgn0040528	CG15864	0	1	0	1	1	0
FBgn0033185	CG1603	2	3	2	2	3	1
FBgn0030468	CG1622	3	2	3	2	3	3
FBgn0030027	CG1632	0	1	0	0	1	0
FBgn0039600	CG1646	1	0	0	0	0	1
FBgn0039602	CG1647	0	0	0	0	0	1
FBgn0033449	CG1663	0	0	0	0	0	1
FBgn0033454	CG1671	2	2	0	1	2	2
FBgn0037667	CG16734	1	1	1	0	0	1
FBgn0035393	CG16753	1	2	1	2	1	1
FBgn0029941	CG1677	1	3	2	1	1	2
FBgn0032488	CG16812	3	3	3	2	3	3
FBgn0036574	CG16838	2	3	2	1	2	2
FBgn0028919	CG16865	2	1	2	1	1	1
FBgn0035073	CG16896	0	0	0	0	0	1
FBgn0040394	CG16903	3	2	2	1	2	0
FBgn0035111	CG16940	0	1	1	2	1	0
FBgn0032481	CG16972	0	1	0	0	0	0
FBgn0025621	CG16989	1	0	0	0	0	0
FBgn0033122	CG17002	1	1	1	0	1	1
FBgn0032281	CG17107	0	0	0	0	0	1
FBgn0036248	CG17153	3	3	3	2	3	3
FBgn0036440	CG17177	0	0	1	0	0	0
FBgn0030687	CG17209	0	0	0	0	0	2
FBgn0036958	CG17233	0	0	0	0	1	0
FBgn0030205	CG17255	3	3	3	3	3	3
FBgn0031497	CG17259	1	1	0	0	0	3
FBgn0033089	CG17266	2	3	3	3	3	2
FBgn0038830	CG17272	2	3	3	3	3	2
FBgn0032030	CG17293	2	3	3	1	3	2
FBgn0031443	CG17302	0	0	0	0	1	0
FBgn0035880	CG17352	1	0	0	0	0	0
FBgn0036396	CG17359	1	0	1	1	1	0
FBgn0036395	CG17361	0	1	0	1	0	0
FBgn0030293	CG1737	3	3	2	2	3	3
FBgn0030291	CG1738	0	0	1	0	0	0
FBgn0030121	CG17446	3	1	0	0	1	1
FBgn0030305	CG1749	1	0	0	0	1	1
FBgn0040010	CG17493	1	0	0	0	0	0
FBgn0039959	CG17514	3	3	3	3	3	3
FBgn0032999	CG17528	0	0	1	0	1	0
FBgn0031361	CG17652	0	0	0	0	1	0
FBgn0039997	CG17665	3	3	2	3	3	2
FBgn0037277	CG17735	1	3	1	0	2	3
FBgn0032240	CG17768	0	0	0	0	0	1
FBgn0038549	CG17802	0	1	2	1	0	1
FBgn0030061	CG1785	3	3	3	2	3	3
FBgn0034512	CG18067	1	1	1	0	2	0
FBgn0036837	CG18135	0	1	0	0	0	0

FBgn0039863	CG1815	1	0	2	1	2	2
FBgn0034403	CG18190	2	0	0	0	0	1
FBgn0030956	CG18259	3	1	3	1	1	2
FBgn0030228	CG1826	0	1	0	0	0	0
FBgn0037263	CG18271	0	0	1	0	0	1
FBgn0029525	CG18273	3	2	2	3	3	3
FBgn0030269	CG18292	1	0	0	0	1	1
FBgn0031869	CG18304	0	0	0	0	1	0
FBgn0033261	CG18316	2	3	2	3	3	3
FBgn0032979	CG1832	2	1	3	0	3	2
FBgn0033155	CG1845	1	0	2	2	3	1
FBgn0037931	CG18476	0	0	0	0	0	1
FBgn0031962	CG18591	2	3	3	2	2	1
FBgn0042111	CG18766	1	2	2	1	2	0
FBgn0042134	CG18811	3	3	3	3	3	3
FBgn0030066	CG1885	0	0	0	1	0	0
FBgn0033421	CG1888	2	3	3	0	3	1
FBgn0039585	CG1894	0	1	0	0	0	0
FBgn0033434	CG1902	0	0	0	0	0	1
FBgn0030274	CG1908	0	1	0	0	0	1
FBgn0027873	CG1957	2	3	2	1	3	3
FBgn0032876	CG1962	2	2	0	0	1	2
FBgn0037466	CG1965	1	2	0	2	1	3
FBgn0039691	CG1972	1	1	0	0	0	0
FBgn0037376	CG2051	0	0	0	0	1	0
FBgn0033400	CG2063	3	2	2	3	3	1
FBgn0037371	CG2097	3	2	3	3	3	3
FBgn0039877	CG2118	3	3	3	3	3	2
FBgn0033264	CG2158	3	3	3	3	3	3
FBgn0035213	CG2199	3	3	3	3	3	3
FBgn0035210	CG2213	0	0	0	0	1	1
FBgn0030320	CG2247	2	1	3	3	3	3
FBgn0030000	CG2260	3	3	3	3	3	2
FBgn0035205	CG2469	3	3	3	3	3	3
FBgn0024993	CG2662	2	2	0	0	1	1
FBgn0030504	CG2691	3	3	3	3	3	3
FBgn0031534	CG2774	0	0	0	0	0	1
FBgn0031266	CG2807	3	3	3	3	3	3
FBgn0029672	CG2875	2	3	3	2	2	3
FBgn0037348	CG2919	0	0	0	1	0	0
FBgn0037344	CG2926	0	1	0	0	0	0
FBgn0030177	CG2972	0	0	0	0	0	1
FBgn0029704	CG2982	3	3	3	3	3	3
FBgn0050007	CG30007	0	0	0	0	0	1
FBgn0050020	CG30020	3	3	3	3	3	3
FBgn0030142	CG3004	2	0	0	0	1	1
FBgn0050085	CG30085	3	3	3	2	3	3
FBgn0050122	CG30122	2	1	0	1	2	1
FBgn0050193	CG30193	0	0	0	0	0	1
FBgn0050349	CG30349	3	3	3	3	3	3
FBgn0050390	CG30390	2	3	3	3	3	3
FBgn0050482	CG30482	0	1	0	1	1	0
FBgn0050484	CG30484	1	0	0	0	0	1

FBgn0050492	CG30492	1	0	1	0	0	0
FBgn0024987	CG3056	2	2	0	2	1	1
FBgn0023527	CG3071	3	3	2	1	3	1
FBgn0051048	CG31048	0	1	0	0	0	1
FBgn0051211	CG31211	0	0	0	1	0	0
FBgn0051223	CG31223	0	0	0	0	1	0
FBgn0051291	CG31291	0	0	1	0	0	0
FBgn0051301	CG31301	1	0	3	1	1	1
FBgn0051327	CG31327	1	0	0	0	0	0
FBgn0051352	CG31352	3	3	2	2	3	2
FBgn0051365	CG31365	0	2	3	2	2	1
FBgn0051368	CG31368	0	1	0	0	0	1
FBgn0051453	CG31453	0	1	0	1	1	1
FBgn0051532	CG31532	2	0	2	1	1	0
FBgn0051551	CG31551	1	0	1	0	0	0
FBgn0051559	CG31559	1	1	0	0	0	0
FBgn0051712	CG31712	2	2	2	2	2	1
FBgn0051716	CG31716	0	0	0	0	0	1
FBgn0034964	CG3173	0	1	0	0	0	1
FBgn0051739	CG31739	0	0	0	0	1	0
FBgn0051759	CG31759	1	0	0	0	0	0
FBgn0051760	CG31760	1	0	0	0	0	0
FBgn0051872	CG31872	0	0	0	0	1	0
FBgn0031678	CG31918	1	1	0	0	1	0
FBgn0051938	CG31938	1	0	1	0	1	0
FBgn0029887	CG3198	0	0	1	0	0	1
FBgn0052016	CG32016	3	3	3	3	3	3
FBgn0052075	CG32075	3	3	3	3	3	3
FBgn0031545	CG3213	0	1	0	0	0	0
FBgn0052133	CG32133	0	0	0	0	1	1
FBgn0052151	CG32151	1	1	0	0	1	1
FBgn0042177	CG32164	0	0	0	1	0	0
FBgn0042178	CG32165	1	2	2	0	0	1
FBgn0052176	CG32176	1	2	1	2	2	2
FBgn0052224	CG32224	0	0	0	0	0	1
FBgn0031631	CG3225	3	2	3	1	3	2
FBgn0029882	CG3226	0	0	0	0	0	2
FBgn0052264	CG32264	0	1	0	0	0	1
FBgn0052295	CG32295	2	0	0	1	0	0
FBgn0052306	CG32306	0	0	0	1	0	1
FBgn0052343	CG32343	1	1	1	2	3	1
FBgn0052344	CG32344	3	3	3	3	3	3
FBgn0052377	CG32377	0	0	1	1	0	0
FBgn0031540	CG3238	1	0	0	0	1	0
FBgn0052394	CG32394	0	0	0	0	0	1
FBgn0052396	CG32396	0	1	0	0	1	1
FBgn0052409	CG32409	3	3	2	3	3	3
FBgn0052418	CG32418	2	3	2	3	3	2
FBgn0052479	CG32479	2	3	1	2	1	1
FBgn0029764	CG3249	1	0	1	0	3	0
FBgn0052529	CG32529	0	0	0	0	1	0
FBgn0052599	CG32599	0	0	1	0	0	1
FBgn0052606	CG32606	1	0	0	0	0	0

FBgn0052628	CG32628	0	1	0	0	0	0
FBgn0052654	CG32654	0	1	1	0	1	0
FBgn0052663	CG32663	0	0	0	0	0	1
FBgn0042083	CG3267	1	3	2	1	2	2
FBgn0052703	CG32703	0	0	0	0	1	0
FBgn0052708	CG32708	1	2	1	0	2	0
FBgn0053125	CG33125	0	1	0	0	0	1
FBgn0053217	CG33217	3	3	3	3	3	2
FBgn0053287	CG33287	0	0	0	0	1	0
FBgn0053291	CG33291	0	0	0	0	1	0
FBgn0039510	CG3339	0	1	1	1	0	0
FBgn0031240	CG3345	0	0	0	0	1	0
FBgn0053505	CG33505	3	3	3	2	3	3
FBgn0034989	CG3356	0	1	0	1	1	3
FBgn0034987	CG3363	0	1	1	1	1	0
FBgn0064122	CG33691	2	2	2	1	3	3
FBgn0064121	CG33692	1	0	0	0	0	0
FBgn0052831	CG33695	0	1	0	0	0	0
FBgn0053993	CG33993	1	0	0	0	0	0
FBgn0054053	CG34053	1	1	0	1	0	1
FBgn0083973	CG34137	0	1	0	0	0	1
FBgn0085215	CG34186	0	0	2	1	0	2
FBgn0085243	CG34214	1	1	0	2	1	0
FBgn0085379	CG34350	1	0	0	0	0	0
FBgn0031229	CG3436	2	2	3	2	3	2
FBgn0085395	CG34366	1	0	0	0	0	0
FBgn0085444	CG34415	1	1	0	1	2	2
FBgn0085446	CG34417	0	0	0	0	0	1
FBgn0085451	CG34422	2	1	0	0	2	3
FBgn0038252	CG3509	0	0	0	0	1	0
FBgn0029714	CG3527	3	2	2	1	3	2
FBgn0031492	CG3542	3	2	2	2	3	3
FBgn0029854	CG3566	1	3	0	0	1	2
FBgn0031493	CG3605	2	3	2	3	3	2
FBgn0036004	CG3654	1	0	0	0	0	0
FBgn0027521	CG3679	2	0	0	0	1	2
FBgn0034933	CG3735	3	2	3	2	3	3
FBgn0031657	CG3756	0	0	0	0	0	2
FBgn0030425	CG3775	1	0	0	1	0	1
FBgn0024989	CG3777	0	0	0	0	1	0
FBgn0034802	CG3800	0	3	2	0	0	0
FBgn0038275	CG3817	3	2	2	3	3	3
FBgn0032130	CG3838	0	0	0	0	1	0
FBgn0029867	CG3847	1	1	0	0	1	1
FBgn0031281	CG3883	2	2	1	1	2	1
FBgn0027524	CG3909	3	3	3	3	3	3
FBgn0031575	CG3980	1	2	0	0	1	1
FBgn0038473	CG3983	3	3	3	3	3	3
FBgn0038472	CG3995	2	1	2	2	3	3
FBgn0037800	CG3996	0	0	1	1	0	0
FBgn0030418	CG4004	0	1	1	1	1	1
FBgn0058057	CG40057	1	1	0	0	1	0
FBgn0058116	CG40116	2	1	0	1	0	0

FBgn0011824	CG4038	3	3	3	3	3	3
FBgn0058390	CG40390	0	1	0	0	1	0
FBgn0029798	CG4078	0	0	0	2	0	1
FBgn0069951	CG41072	1	0	0	0	0	0
FBgn0028474	CG4119	2	1	1	3	2	2
FBgn0084017	CG41454	0	0	0	1	0	0
FBgn0038811	CG4159	0	0	1	0	0	0
FBgn0250814	CG4169	0	0	0	0	1	0
FBgn0038300	CG4203	3	3	3	3	3	3
FBgn0250754	CG42232	3	3	3	2	3	3
FBgn0259145	CG42260	0	0	0	0	1	0
FBgn0259165	CG42270	0	1	0	0	1	1
FBgn0034598	CG4266	3	3	2	3	3	2
FBgn0034600	CG4279	0	3	2	1	2	1
FBgn0034114	CG4282	1	0	1	0	0	0
FBgn0038388	CG4287	0	1	0	0	0	0
FBgn0038795	CG4335	0	0	0	0	0	1
FBgn0032138	CG4364	3	3	3	3	3	3
FBgn0037024	CG4365	1	0	2	0	2	3
FBgn0030434	CG4400	2	1	1	1	0	2
FBgn0034132	CG4439	1	0	0	0	0	1
FBgn0031895	CG4497	1	1	2	0	2	2
FBgn0034734	CG4554	3	3	3	3	3	3
FBgn0035021	CG4622	1	0	0	0	0	2
FBgn0035036	CG4707	0	0	0	0	0	1
FBgn0033818	CG4712	1	0	0	0	0	0
FBgn0032347	CG4738	3	3	3	3	3	3
FBgn0043456	CG4747	2	1	2	1	1	1
FBgn0030788	CG4756	1	0	1	1	2	0
FBgn0032354	CG4788	1	0	0	0	0	1
FBgn0035048	CG4806	3	3	3	3	3	3
FBgn0039566	CG4849	3	3	3	3	3	3
FBgn0026083	CG4857	0	1	0	0	0	0
FBgn0034232	CG4866	2	2	3	3	3	1
FBgn0036624	CG4877	3	3	3	3	3	3
FBgn0031318	CG4887	0	1	1	0	1	1
FBgn0032194	CG4901	1	2	1	1	2	1
FBgn0028897	CG4935	0	1	2	0	2	1
FBgn0038768	CG4936	0	1	0	0	0	1
FBgn0039563	CG4951	3	3	3	3	3	3
FBgn0032364	CG4970	0	0	0	0	1	0
FBgn0039558	CG4980	1	1	0	0	1	0
FBgn0036578	CG5018	2	3	0	0	3	2
FBgn0028744	CG5033	3	3	3	3	2	3
FBgn0032471	CG5122	1	0	0	0	1	0
FBgn0037000	CG5130	1	0	0	0	0	0
FBgn0036775	CG5147	0	0	0	0	0	1
FBgn0034345	CG5174	1	0	0	0	0	0
FBgn0043457	CG5180	1	2	1	0	1	0
FBgn0031909	CG5181	0	0	0	0	0	1
FBgn0032250	CG5198	0	0	0	0	1	0
FBgn0038344	CG5205	0	0	0	0	1	0
FBgn0036987	CG5274	0	0	0	0	0	1

FBgn0038984	CG5315	0	0	0	0	1	0
FBgn0032404	CG5317	3	3	3	3	3	3
FBgn0032407	CG5325	2	0	2	2	2	0
FBgn0032248	CG5343	2	3	3	2	2	2
FBgn0032237	CG5362	0	1	0	0	1	1
FBgn0027568	CG5366	2	1	2	0	2	1
FBgn0038951	CG5380	0	0	0	0	0	2
FBgn0039153	CG5463	1	0	0	0	0	0
FBgn0039433	CG5467	1	0	0	0	1	1
FBgn0039560	CG5514	0	0	0	1	1	0
FBgn0032444	CG5525	3	3	3	2	2	3
FBgn0034908	CG5543	0	0	0	0	1	0
FBgn0036973	CG5585	3	2	3	0	3	2
FBgn0036754	CG5589	3	3	3	3	3	2
FBgn0034926	CG5591	2	0	0	0	1	0
FBgn0034920	CG5597	2	0	2	2	2	0
FBgn0032208	CG5604	1	1	2	0	1	2
FBgn0038058	CG5608	1	1	2	0	2	1
FBgn0032207	CG5640	0	0	1	1	1	1
FBgn0038046	CG5641	0	1	0	0	0	0
FBgn0036258	CG5642	3	3	2	1	2	3
FBgn0035297	CG5691	0	1	0	0	1	0
FBgn0032197	CG5694	1	1	1	0	2	1
FBgn0028471	CG5720	3	3	3	3	3	2
FBgn0034313	CG5726	3	3	3	3	3	3
FBgn0032193	CG5727	0	1	0	0	0	0
FBgn0039182	CG5728	3	3	3	2	3	3
FBgn0032454	CG5787	3	1	2	3	2	3
FBgn0032455	CG5792	3	3	3	3	3	3
FBgn0039214	CG5794	0	0	0	0	0	1
FBgn0030855	CG5800	3	3	3	3	3	2
FBgn0027617	CG5808	3	3	1	1	2	2
FBgn0030625	CG5877	3	2	2	3	3	3
FBgn0032157	CG5899	0	0	0	0	0	1
FBgn0038401	CG5916	1	1	0	0	0	0
FBgn0036548	CG5931	3	3	3	3	3	3
FBgn0038927	CG6015	0	0	2	0	1	0
FBgn0037001	CG6020	1	0	0	0	0	1
FBgn0034725	CG6044	1	0	0	0	0	0
FBgn0039491	CG6059	0	0	1	1	1	0
FBgn0039488	CG6066	0	0	0	0	0	1
FBgn0040985	CG6115	0	0	0	0	1	0
FBgn0032499	CG6116	2	3	0	1	2	1
FBgn0039152	CG6129	0	0	1	0	0	0
FBgn0038334	CG6130	0	1	0	0	0	0
FBgn0032340	CG6181	3	3	3	3	3	3
FBgn0038723	CG6195	1	0	0	0	0	1
FBgn0033859	CG6197	2	0	0	2	2	1
FBgn0039165	CG6204	0	0	0	0	1	0
FBgn0038318	CG6236	0	0	0	0	1	0
FBgn0037792	CG6241	3	2	3	3	3	3
FBgn0039474	CG6283	1	0	0	0	0	0
FBgn0036735	CG6311	3	3	3	3	3	3

FBgn0036733	CG6322	3	3	3	2	3	2
FBgn0039464	CG6330	0	1	0	0	1	1
FBgn0030648	CG6340	0	1	0	0	1	2
FBgn0036972	CG6434	0	1	0	1	0	1
FBgn0038922	CG6439	0	0	0	0	1	0
FBgn0032643	CG6453	2	2	1	3	2	2
FBgn0035923	CG6511	0	0	0	1	0	0
FBgn0033879	CG6543	1	0	0	0	0	0
FBgn0037855	CG6621	2	3	3	3	3	2
FBgn0035911	CG6638	3	2	3	3	3	2
FBgn0038301	CG6654	0	0	0	1	1	1
FBgn0036685	CG6664	2	0	2	0	1	1
FBgn0035906	CG6673	0	0	0	0	1	0
FBgn0032388	CG6686	2	0	1	2	1	2
FBgn0037878	CG6693	1	2	1	0	1	1
FBgn0039215	CG6695	1	0	1	1	1	2
FBgn0033889	CG6701	1	3	0	1	2	2
FBgn0032408	CG6712	1	3	1	0	1	3
FBgn0032298	CG6724	3	2	3	3	3	2
FBgn0033562	CG6751	3	3	3	2	2	3
FBgn0037899	CG6764	3	3	3	3	3	2
FBgn0036242	CG6793	1	2	0	0	1	0
FBgn0037182	CG6838	3	2	2	1	2	3
FBgn0036828	CG6841	3	3	2	3	3	1
FBgn0036827	CG6843	1	2	2	0	1	0
FBgn0036487	CG6876	3	3	3	2	3	3
FBgn0038293	CG6904	0	0	0	0	1	0
FBgn0035136	CG6905	3	2	2	2	3	2
FBgn0038989	CG6937	3	3	3	3	3	3
FBgn0030959	CG6961	0	2	1	1	1	1
FBgn0039229	CG6995	0	0	0	0	0	1
FBgn0039233	CG7006	2	3	3	3	3	3
FBgn0027587	CG7028	3	3	3	3	3	3
FBgn0030086	CG7033	3	3	3	1	3	2
FBgn0030091	CG7065	2	3	3	3	3	2
FBgn0031401	CG7082	1	0	0	0	0	1
FBgn0038602	CG7126	1	0	0	0	0	0
FBgn0037150	CG7133	1	0	0	0	0	0
FBgn0034422	CG7137	2	2	2	2	2	2
FBgn0027532	CG7139	1	1	0	1	3	0
FBgn0038593	CG7146	0	0	0	0	2	0
FBgn0031947	CG7154	1	3	1	2	1	3
FBgn0038583	CG7183	0	1	0	0	0	0
FBgn0038575	CG7208	0	1	0	0	0	0
FBgn0038571	CG7215	0	1	0	0	0	0
FBgn0031730	CG7236	0	0	0	0	1	0
FBgn0030081	CG7246	2	2	2	0	1	2
FBgn0036500	CG7275	3	3	2	3	3	2
FBgn0030966	CG7280	0	1	0	1	1	2
FBgn0037073	CG7338	1	0	0	0	0	0
FBgn0036188	CG7339	0	0	0	0	0	1
FBgn0036184	CG7351	2	1	1	1	3	3
FBgn0038551	CG7357	0	1	0	0	0	0

FBgn0030974	CG7358	2	3	3	3	3	3
FBgn0038258	CG7362	0	0	0	1	0	0
FBgn0035853	CG7375	1	1	0	0	1	0
FBgn0038546	CG7379	1	0	0	0	0	1
FBgn0028529	CG7516	2	2	3	2	3	2
FBgn0038108	CG7518	3	3	3	2	3	3
FBgn0037087	CG7519	1	1	0	0	1	0
FBgn0036734	CG7564	0	2	1	1	2	1
FBgn0037093	CG7597	1	1	0	0	0	1
FBgn0033548	CG7637	2	3	3	3	3	2
FBgn0038609	CG7671	2	2	2	2	3	2
FBgn0027525	CG7686	0	0	0	0	0	1
FBgn0038636	CG7698	0	2	1	1	0	1
FBgn0036686	CG7728	2	3	2	1	2	1
FBgn0033615	CG7741	0	1	0	0	1	1
FBgn0034109	CG7747	0	0	0	0	0	1
FBgn0036915	CG7757	3	3	2	2	2	1
FBgn0036124	CG7839	3	3	2	3	3	3
FBgn0033062	CG7843	0	0	1	0	0	0
FBgn0033059	CG7845	3	3	3	2	2	3
FBgn0035229	CG7852	0	0	0	1	0	0
FBgn0037549	CG7878	0	0	1	1	2	0
FBgn0035235	CG7879	0	0	0	1	0	1
FBgn0039730	CG7903	2	1	2	1	2	1
FBgn0039740	CG7928	0	0	0	0	1	0
FBgn0036505	CG7945	1	2	2	3	1	1
FBgn0039743	CG7946	3	3	3	1	2	3
FBgn0038115	CG7966	0	0	0	0	1	0
FBgn0035253	CG7971	1	0	2	1	1	1
FBgn0038244	CG7987	2	0	0	0	0	0
FBgn0038585	CG7993	3	3	3	3	3	3
FBgn0038597	CG8064	2	1	1	0	2	3
FBgn0033998	CG8092	1	1	2	0	1	1
FBgn0027567	CG8108	3	3	3	3	3	3
FBgn0030871	CG8142	3	3	3	3	3	3
FBgn0027607	CG8230	1	1	0	1	0	1
FBgn0033352	CG8232	2	3	3	2	2	2
FBgn0033897	CG8233	1	1	2	1	3	1
FBgn0033031	CG8245	0	1	0	0	1	0
FBgn0033900	CG8257	0	1	0	0	0	0
FBgn0033342	CG8258	3	3	3	2	3	3
FBgn0030851	CG8326	2	2	1	1	1	0
FBgn0035707	CG8368	3	2	2	3	3	3
FBgn0034062	CG8388	0	1	0	0	0	0
FBgn0034073	CG8414	3	3	3	3	3	3
FBgn0037670	CG8436	2	2	3	2	2	2
FBgn0034088	CG8445	0	0	0	0	0	1
FBgn0037746	CG8478	3	3	3	3	3	2
FBgn0033741	CG8545	3	3	3	3	3	3
FBgn0033742	CG8550	1	0	0	0	0	0
FBgn0035776	CG8564	0	0	0	0	0	1
FBgn0033752	CG8569	1	2	1	1	0	1
FBgn0030699	CG8578	3	3	2	3	3	2

FBgn0027602	CG8611	3	3	3	3	3	3
FBgn0029629	CG8636	1	0	1	0	0	1
FBgn0033272	CG8707	1	3	2	2	1	2
FBgn0033766	CG8771	0	0	0	0	1	0
FBgn0033376	CG8777	0	0	0	0	0	1
FBgn0028473	CG8801	3	3	3	3	3	3
FBgn0033738	CG8830	1	0	0	0	0	1
FBgn0036386	CG8833	0	1	1	0	1	0
FBgn0027504	CG8878	2	1	2	2	2	1
FBgn0030833	CG8915	1	2	2	2	3	0
FBgn0030710	CG8924	1	1	1	0	1	1
FBgn0034504	CG8929	3	3	3	3	2	2
FBgn0030720	CG8939	3	3	3	3	3	3
FBgn0030680	CG8944	1	2	1	0	0	1
FBgn0030815	CG8945	0	0	0	0	0	1
FBgn0030812	CG8949	0	1	0	0	2	0
FBgn0034181	CG8963	2	2	1	1	3	2
FBgn0035336	CG9004	3	1	3	2	3	1
FBgn0036398	CG9007	2	1	1	2	3	1
FBgn0031752	CG9044	0	2	0	0	0	0
FBgn0030702	CG9056	0	0	0	0	1	0
FBgn0030809	CG9086	0	1	1	0	0	0
FBgn0031764	CG9107	3	3	2	3	3	3
FBgn0031769	CG9135	0	2	0	0	1	1
FBgn0034496	CG9143	3	3	3	3	3	3
FBgn0031775	CG9150	0	1	0	0	0	0
FBgn0032925	CG9246	3	3	3	3	3	3
FBgn0032924	CG9247	2	2	3	2	2	2
FBgn0032919	CG9253	1	2	1	0	2	1
FBgn0030672	CG9281	3	2	2	2	3	2
FBgn0036886	CG9300	3	2	3	2	3	2
FBgn0032883	CG9323	0	1	0	0	1	0
FBgn0034564	CG9344	1	1	0	0	0	1
FBgn0034572	CG9346	1	1	0	0	0	0
FBgn0036451	CG9425	1	1	0	1	0	0
FBgn0034599	CG9437	2	0	0	0	0	0
FBgn0032083	CG9541	0	1	0	1	0	1
FBgn0031822	CG9548	1	2	2	2	2	2
FBgn0037578	CG9601	1	2	0	0	0	2
FBgn0037550	CG9667	0	0	0	0	0	1
FBgn0037669	CG9740	1	3	2	1	3	1
FBgn0037270	CG9769	0	1	0	0	2	0
FBgn0037236	CG9772	1	2	0	0	1	0
FBgn0037261	CG9775	3	3	3	3	3	3
FBgn0037621	CG9797	3	3	3	2	3	3
FBgn0038146	CG9799	2	2	1	2	2	2
FBgn0037251	CG9804	1	0	0	0	1	0
FBgn0037248	CG9809	0	0	0	0	1	0
FBgn0086605	CG9853	0	0	0	1	0	0
FBgn0030738	CG9915	2	1	2	1	3	1
FBgn0034527	CG9945	2	2	2	1	0	1
FBgn0034435	CG9975	0	0	0	0	0	1
FBgn0032781	CG9987	0	1	0	0	0	0

FBgn0021760	chb	3	3	3	3	3	3
FBgn0000319	Chc	2	2	3	1	2	2
FBgn0023395	Chd3	0	0	0	1	0	1
FBgn0044324	Chro	3	3	3	3	3	3
FBgn0030171	Cht6	1	0	0	0	0	0
FBgn0028386	cic	0	0	0	0	1	0
FBgn0000317	ck	3	3	3	3	3	3
FBgn0044323	Cka	1	3	1	0	2	3
FBgn0015024	CkIalpha	3	3	3	3	3	3
FBgn0000258	CkIIalpha	3	3	3	3	3	2
FBgn0000259	CkIIbeta	3	3	3	3	3	3
FBgn0037613	Cks85A	0	1	0	0	0	0
FBgn0020503	CLIP-190	3	3	3	3	3	3
FBgn0040232	cmet	0	0	0	0	1	1
FBgn0029090	cngl	1	0	0	0	0	1
FBgn0013765	cnn	0	1	1	0	1	2
FBgn0259212	cno	2	2	1	0	1	1
FBgn0033265	coilin	1	0	2	0	3	2
FBgn0000346	comt	0	0	0	0	1	0
FBgn0010434	cora	1	0	0	0	0	0
FBgn0259173	corn	3	3	3	3	3	3
FBgn0033109	coro	3	3	3	2	3	3
FBgn0013770	Cp1	3	3	3	3	3	2
FBgn0000283	Cp190	0	0	0	0	0	1
FBgn0086690	cp309	1	1	1	2	1	0
FBgn0000360	Cp38	1	0	0	0	1	0
FBgn0034577	cpa	3	3	3	3	3	2
FBgn0011570	cpb	3	3	3	3	3	3
FBgn0024698	cpsf	1	1	1	0	0	0
FBgn0062927	CR32735	1	0	0	0	0	0
FBgn0025864	Crag	0	0	0	0	0	1
FBgn0000377	crn	3	3	1	1	2	2
FBgn0020309	crol	1	0	1	0	0	0
FBgn0001994	crp	3	3	3	3	3	2
FBgn0027057	CSN1b	0	0	0	0	1	0
FBgn0027053	CSN5	1	1	0	0	2	0
FBgn0028836	CSN7	1	2	0	0	0	1
FBgn0027841	CstF-64	0	0	0	0	1	2
FBgn0020496	CtBP	1	1	0	0	0	0
FBgn0035769	CTCF	2	2	2	1	3	0
FBgn0011760	ctp	2	3	3	3	3	3
FBgn0032956	cul-2	0	1	0	0	0	0
FBgn0033260	cul-4	3	3	2	3	3	2
FBgn0039632	cul-5	0	0	0	0	0	1
FBgn0031452	Cwc25	3	2	2	2	2	2
FBgn0004597	CycC	1	1	0	0	0	0
FBgn0004432	Cyp1	1	0	0	1	0	1
FBgn0031694	Cyp4ac2	0	1	0	0	0	0
FBgn0035141	Cypl	0	0	0	0	0	1
FBgn0022935	D19A	0	0	0	1	0	1
FBgn0022699	D19B	3	3	3	3	3	3
FBgn0033015	d4	1	1	1	0	1	0
FBgn0000413	da	0	1	0	1	1	2

FBgn0000414	Dab	0	0	0	0	1	0
FBgn0030093	dalao	1	1	0	1	1	1
FBgn0020305	dbe	0	1	0	0	1	0
FBgn0010220	Dbp45A	0	0	0	0	1	0
FBgn0004556	Dbp73D	3	3	2	2	3	3
FBgn0024804	Dbp80	3	3	2	3	3	3
FBgn0067779	dbr	0	0	0	0	1	0
FBgn0002413	dco	0	1	0	0	0	0
FBgn0034921	Dcp1	1	3	1	1	1	3
FBgn0036534	Dcp2	0	1	0	0	1	0
FBgn0027049	DDB1	3	3	3	3	3	3
FBgn0015075	Ddx1	1	0	0	1	1	1
FBgn0000426	DebB	1	2	1	0	1	0
FBgn0000427	dec-1	1	1	0	1	1	2
FBgn0036038	defl	1	2	0	1	2	1
FBgn0086251	del	1	0	0	0	0	0
FBgn0028969	deltaCOP	3	3	3	2	3	3
FBgn0039710	dgt1	0	1	0	0	0	0
FBgn0012344	Dh	0	1	0	0	0	0
FBgn0013809	Dhc16F	0	0	1	1	0	0
FBgn0013810	Dhc36C	0	0	0	1	0	0
FBgn0013811	Dhc62B	1	1	0	0	0	0
FBgn0010349	Dhc64C	2	3	3	3	3	3
FBgn0013812	Dhc93AB	0	0	1	1	0	0
FBgn0013813	Dhc98D	0	0	1	1	1	0
FBgn0011802	Dhh1	0	1	0	0	1	0
FBgn0015933	didum	3	3	3	3	3	3
FBgn0030891	dik	3	3	2	1	2	1
FBgn0024807	DIP1	1	3	1	1	0	2
FBgn0024432	Dlc90F	1	2	2	1	0	0
FBgn0030276	Dlic2	0	1	0	0	0	0
FBgn0034537	DMAP1	3	3	2	2	3	3
FBgn0015657	DnaJ-1	0	0	0	0	1	0
FBgn0011762	DNApol-alpha50	0	0	0	0	0	1
FBgn0012066	DNApol-delta	0	0	0	0	1	0
FBgn0259220	Doa	3	3	3	2	3	3
FBgn0020306	dom	3	3	3	3	3	3
FBgn0000482	dor	1	0	0	0	0	0
FBgn0000486	Dox-A2	3	3	3	1	3	3
FBgn0011763	Dp	2	1	2	0	2	1
FBgn0015929	dpa	0	0	0	0	0	1
FBgn0033083	Dpit47	1	0	0	0	0	0
FBgn0015930	dpld	3	3	3	3	3	3
FBgn0037580	DppIII	1	0	0	0	0	0
FBgn0040823	dpr6	0	0	0	0	1	0
FBgn0002183	dre4	3	3	3	3	3	3
FBgn0015664	Dref	3	3	3	2	3	3
FBgn0038145	Droj2	3	3	3	3	3	3
FBgn0020304	drongo	0	1	0	0	1	1
FBgn0026479	Drp1	3	3	3	3	3	3
FBgn0011764	Dsp1	3	3	3	3	3	2
FBgn0000520	dwg	2	2	0	2	1	1
FBgn0000541	E(bx)	3	3	3	3	3	3

FBgn0011586	e(r)	3	3	3	3	3	2
FBgn0000617	e(y)1	3	3	3	2	3	3
FBgn0000618	e(y)2	0	0	0	2	2	0
FBgn0024371	E2f2	0	0	0	0	1	0
FBgn0035624	Eaf6	1	1	0	0	1	0
FBgn0010110	east	1	0	3	1	3	3
FBgn0027066	Eb1	0	0	0	0	1	0
FBgn0023444	ebi	3	3	3	3	3	3
FBgn0000546	EcR	0	0	0	0	0	3
FBgn0000556	Ef1alpha48D	3	3	3	3	3	3
FBgn0028737	Ef1beta	2	3	2	2	2	0
FBgn0029176	Ef1gamma	3	3	3	1	2	1
FBgn0000559	Ef2b	3	2	3	3	3	3
FBgn0024556	EfTuM	1	0	0	0	0	0
FBgn0022023	eIF-3p40	2	0	1	0	3	2
FBgn0040227	eIF-3p66	2	2	1	1	2	1
FBgn0001942	eIF-4a	3	3	2	3	3	3
FBgn0015218	eIF-4E	0	2	1	0	1	0
FBgn0039726	eIF2B-alpha	0	0	0	0	1	0
FBgn0024996	eIF2B-beta	1	0	1	0	2	1
FBgn0034858	eIF2B-delta	1	0	0	0	0	1
FBgn0023512	eIF2B-epsilon	1	1	1	0	1	0
FBgn0034029	eIF2B-gamma	1	3	0	0	0	1
FBgn0037249	eIF3-S10	2	1	2	2	1	2
FBgn0034237	eIF3-S9	1	0	0	0	2	2
FBgn0037573	eIF4AIII	0	0	1	0	0	0
FBgn0023213	eIF4G	3	3	3	3	3	3
FBgn0030719	eIF5	0	1	0	0	0	0
FBgn0026259	eIF5B	0	0	0	0	0	1
FBgn0034915	eIF6	3	3	3	3	3	2
FBgn0005640	Eip63E	0	0	0	0	1	0
FBgn0000570	elav	2	0	0	0	0	0
FBgn0020443	Elf	0	0	1	0	1	0
FBgn0023212	Elongin-B	1	0	0	1	1	0
FBgn0020497	emb	1	1	2	2	2	1
FBgn0034975	enok	3	2	1	1	0	1
FBgn0035060	Eps-15	2	0	0	0	0	0
FBgn0027496	epsilonCOP	1	3	2	1	2	0
FBgn0259107	epsin-like	2	2	1	2	3	3
FBgn0004510	Ets97D	2	0	2	1	1	0
FBgn0250753	exba	0	0	0	0	0	1
FBgn0000625	eyg	0	0	0	0	0	1
FBgn0038827	Fancd2	1	1	1	1	2	2
FBgn0014163	fax	3	2	2	0	3	1
FBgn0030241	feo	3	3	3	2	3	3
FBgn0037475	Fer1	0	0	0	0	0	1
FBgn0003062	Fib	3	3	3	3	3	3
FBgn0024238	Fim	0	1	0	0	2	0
FBgn0038914	fit	0	0	2	0	0	1
FBgn0013269	FK506-bp1	3	3	3	3	3	3
FBgn0029174	FKBP59	0	0	0	0	1	0
FBgn0000659	fkh	1	2	0	0	0	2
FBgn0000662	fl(2)d	3	3	3	3	3	2

FBgn0024555	flfl	0	1	0	0	0	1
FBgn0000709	fliI	2	3	2	1	2	2
FBgn0000711	flw	2	1	1	1	3	3
FBgn0028734	Fmr1	3	3	3	3	3	3
FBgn0032773	fon	2	1	1	1	3	3
FBgn0016081	fry	2	3	3	2	3	3
FBgn0004656	fs(1)h	0	1	0	0	0	1
FBgn0000986	Fs(2)Ket	3	3	3	3	3	3
FBgn0001079	fu	1	2	0	1	1	3
FBgn0029173	fu2	0	0	0	1	2	0
FBgn0023441	fus	0	0	0	0	0	1
FBgn0259108	futsch	0	0	1	0	1	0
FBgn0001086	fzy	3	3	2	1	2	3
FBgn0001087	g	1	0	0	0	0	0
FBgn0028924	GABA-B-R1	1	0	0	0	0	0
FBgn0028968	gammaCop	3	3	3	3	3	3
FBgn0004176	gammaTub23C	1	1	0	0	0	0
FBgn0010097	gammaTub37C	3	3	2	3	3	3
FBgn0020655	Gap69C	0	3	1	0	1	1
FBgn0001091	Gapdh1	0	1	1	0	0	0
FBgn0001092	Gapdh2	0	1	0	0	2	1
FBgn0033714	garz	3	0	0	0	1	0
FBgn0031873	Gas41	2	2	1	0	2	3
FBgn0029667	Gas8	0	0	0	1	0	1
FBgn0013969	Gbp	3	3	3	2	3	2
FBgn0086736	GckIII	0	0	0	0	1	1
FBgn0004868	Gdi	0	0	0	0	0	1
FBgn0010225	Gel	0	1	0	0	0	1
FBgn0033081	geminin	0	0	0	0	0	1
FBgn0001980	gft	0	0	0	0	1	1
FBgn0250732	gfzf	3	3	3	3	3	3
FBgn0001108	Gl	3	3	3	3	3	3
FBgn0015391	glu	0	0	0	0	1	0
FBgn0004913	Gnf1	3	3	3	3	3	3
FBgn0045501	Gr22a	1	0	0	0	0	0
FBgn0045500	Gr22b	1	0	0	0	0	0
FBgn0030685	Graf	1	2	0	1	2	1
FBgn0001133	grau	1	1	2	1	0	0
FBgn0026433	Grip128	2	0	1	1	2	1
FBgn0026432	Grip163	1	1	0	0	0	0
FBgn0032705	Grip71	3	3	3	1	3	2
FBgn0026431	Grip75	3	3	3	2	3	2
FBgn0026430	Grip84	2	3	1	2	2	3
FBgn0037245	growl	3	3	3	3	3	3
FBgn0051992	gw	2	2	1	1	1	0
FBgn0014964	gypsy\env	0	0	0	0	1	0
FBgn0026575	hang	0	0	0	0	1	0
FBgn0046706	Haspin	1	2	1	0	0	1
FBgn0044055	HB\T	1	0	0	1	0	0
FBgn0039904	Hcf	3	3	3	3	3	3
FBgn0051119	HdacX	0	0	0	0	1	0
FBgn0014189	Hel25E	3	3	1	1	3	3
FBgn0022787	Hel89B	1	0	0	1	1	0

FBgn0011224	heph	0	0	0	0	0	1
FBgn0031107	HERC2	1	3	1	1	0	2
FBgn0014000	Hf	0	0	0	0	1	0
FBgn0051617	His1:CG31617	0	2	2	1	2	0
FBgn0051618	His2A:CG31618	2	2	2	2	1	2
FBgn0053847	His2A:CG33847	0	0	1	1	2	0
FBgn0053865	His2A:CG33865	0	0	0	0	0	1
FBgn0001197	His2Av	2	3	3	3	2	2
FBgn0001198	His2B	2	3	3	3	3	3
FBgn0001199	His3	0	1	1	1	0	1
FBgn0053866	His3:CG33866	0	2	1	0	1	2
FBgn0053871	His4:CG33871	1	0	1	1	2	0
FBgn0013981	His4r	2	3	2	2	1	3
FBgn0001203	Hk	0	0	0	0	2	0
FBgn0086441	hkl	3	3	3	3	3	3
FBgn0001565	Hlc	3	3	3	3	3	3
FBgn0015393	hoip	3	3	3	3	3	2
FBgn0017397	how	3	2	2	2	1	1
FBgn0030082	HP1b	0	0	1	0	0	2
FBgn0034453	hpo	3	3	3	0	1	1
FBgn0037382	Hpr1	1	2	0	0	0	2
FBgn0015240	Hr96	1	1	0	0	1	0
FBgn0004838	Hrb27C	3	3	3	1	3	3
FBgn0004237	Hrb87F	0	0	1	0	0	1
FBgn0037701	Hrp59	3	3	3	3	3	3
FBgn0001216	Hsc70-1	1	0	1	1	0	1
FBgn0001217	Hsc70-2	0	0	1	1	1	0
FBgn0001218	Hsc70-3	2	3	3	2	3	2
FBgn0001219	Hsc70-4	3	3	3	3	3	3
FBgn0026418	Hsc70Cb	0	1	0	0	0	2
FBgn0001222	Hsf	0	0	0	0	1	1
FBgn0001225	Hsp26	2	2	2	2	2	2
FBgn0001226	Hsp27	2	3	3	3	3	2
FBgn0001230	Hsp68	0	0	0	1	0	0
FBgn0013276	Hsp70Ab	0	0	1	0	0	0
FBgn0013277	Hsp70Ba	1	2	2	1	0	2
FBgn0051354	Hsp70Bbb	0	0	1	1	0	0
FBgn0013279	Hsp70Bc	0	0	1	1	0	0
FBgn0001233	Hsp83	3	3	3	3	3	3
FBgn0004873	hts	3	3	3	3	3	3
FBgn0002431	hyd	3	3	3	3	3	3
FBgn0037657	hyx	3	3	3	3	3	3
FBgn0024227	ial	3	3	3	3	3	3
FBgn0015247	Iap2	0	0	0	0	1	0
FBgn0041147	ida	1	1	0	0	0	0
FBgn0020414	Idgf3	0	0	0	0	0	1
FBgn0001248	Idh	0	0	0	0	0	1
FBgn0086657	ik2	2	2	0	1	1	1
FBgn0030235	Imp	3	1	2	3	3	2
FBgn0001263	inaD	0	0	0	1	0	0
FBgn0033156	Incenp	3	3	3	3	3	3
FBgn0086613	Ino80	1	0	1	1	1	1
FBgn0025582	Int6	1	0	1	0	1	2

FBgn0027108	inx2	0	0	0	1	0	0
FBgn0025366	Ip259	3	3	3	2	3	3
FBgn0011774	Irbp	2	2	2	1	3	2
FBgn0011604	Iswi	3	3	3	3	3	3
FBgn0010051	Itp-r83A	1	0	0	1	0	0
FBgn0001276	ix	2	1	0	1	1	1
FBgn0040309	Jafrac1	2	3	3	3	3	3
FBgn0011225	jar	3	3	3	3	3	3
FBgn0039350	jigr1	3	3	3	3	3	3
FBgn0024889	Kap-alpha1	1	1	2	0	2	2
FBgn0027338	Kap-alpha3	2	3	3	1	3	2
FBgn0087013	Karybeta3	0	0	1	0	0	1
FBgn0040207	kat80	1	0	0	0	0	0
FBgn0019968	Khc-73	0	0	0	1	0	0
FBgn0024887	kin17	0	0	0	0	1	0
FBgn0086902	kis	3	3	3	2	3	3
FBgn0001313	kl-2	0	0	1	1	0	0
FBgn0001314	kl-3	0	0	0	1	0	0
FBgn0030268	Klp10A	0	1	0	0	0	1
FBgn0004379	Klp67A	3	3	3	3	3	2
FBgn0004387	Klp98A	0	0	0	1	0	0
FBgn0027259	Kmn1	0	1	0	0	0	1
FBgn0051232	koko	2	3	3	2	2	2
FBgn0004167	kst	3	3	3	2	3	3
FBgn0001324	kto	1	1	2	1	2	3
FBgn0041627	Ku80	2	2	2	1	2	2
FBgn0038476	kuk	3	3	3	3	3	3
FBgn0001330	kz	2	1	1	1	3	0
FBgn0001332	L	2	0	1	0	1	1
FBgn0001491	l(1)10Bb	1	1	2	2	1	1
FBgn0001341	l(1)1Bi	3	3	3	3	3	3
FBgn0001612	l(1)dd4	1	1	0	0	0	2
FBgn0027334	l(1)G0004	3	3	3	2	3	1
FBgn0026713	l(1)G0007	0	0	0	1	0	0
FBgn0027330	l(1)G0020	0	2	1	1	2	2
FBgn0026664	l(1)G0155	2	3	2	2	2	2
FBgn0027291	l(1)G0156	0	3	1	0	1	2
FBgn0027287	l(1)G0168	1	1	0	1	0	0
FBgn0028343	l(1)G0222	2	2	2	0	1	1
FBgn0028341	l(1)G0232	1	1	0	0	0	1
FBgn0028274	l(1)G0431	2	2	1	0	1	0
FBgn0010607	l(2)05714	3	2	3	2	3	2
FBgn0022288	l(2)09851	0	1	0	1	2	0
FBgn0001986	l(2)35Df	3	3	3	3	3	3
FBgn0002121	l(2)gl	3	3	3	2	3	2
FBgn0022070	l(2)k07824	1	1	2	1	1	1
FBgn0086451	l(2)k09022	2	2	2	2	2	1
FBgn0033029	l(2)NC136	3	2	3	0	1	1
FBgn0010926	l(3)07882	3	3	2	2	3	1
FBgn0011335	l(3)j2D3	3	3	3	2	3	2
FBgn0086910	l(3)neo38	2	3	1	1	2	2
FBgn0002525	Lam	3	3	3	3	3	3
FBgn0010397	LamC	0	0	1	0	0	1

FBgn0002526	LanA	0	0	0	1	1	1
FBgn0086372	lap	0	0	0	0	1	0
FBgn0011640	lark	3	3	3	3	3	3
FBgn0040108	larp	3	3	3	3	3	3
FBgn0005654	lat	2	1	2	2	2	1
FBgn0034657	LBR	1	0	2	1	1	2
FBgn0002542	lds	3	3	3	3	3	3
FBgn0259230	lectin-22C	0	0	0	0	1	0
FBgn0034217	Lhr	0	1	1	1	1	0
FBgn0015763	lic	3	3	3	3	3	3
FBgn0020279	lig	3	3	3	3	3	3
FBgn0038035	lig3	1	1	1	2	2	3
FBgn0041203	LIMK1	1	0	0	0	0	0
FBgn0002552	lin	0	0	0	0	0	1
FBgn0015509	lin19	2	2	1	2	2	1
FBgn0250903	lmg	1	0	0	0	0	0
FBgn0005630	lola	3	3	3	3	3	3
FBgn0022238	lolal	3	3	3	3	3	3
FBgn0032515	loqs	1	0	0	0	0	0
FBgn0029688	lva	3	3	3	3	3	3
FBgn0069397	M(3)62F	0	1	0	0	0	1
FBgn0035640	mad2	2	3	3	2	2	0
FBgn0016034	mael	0	0	1	0	0	0
FBgn0002645	Map205	2	2	2	1	2	2
FBgn0010342	Map60	3	3	1	1	1	2
FBgn0033845	mars	0	2	0	0	1	0
FBgn0043884	mask	3	3	3	3	3	3
FBgn0020408	Mat89Ba	0	1	0	0	0	0
FBgn0020407	Mat89Bb	0	0	0	0	1	1
FBgn0038965	mats	1	0	0	0	1	1
FBgn0038016	MBD-R2	2	3	1	1	2	2
FBgn0086912	mbm	0	0	1	0	1	0
FBgn0026207	mbo	1	1	0	0	1	3
FBgn0005536	Mbs	2	1	0	1	0	2
FBgn0032929	Mcm10	1	0	0	0	0	0
FBgn0024332	Mcm3	0	0	0	0	0	1
FBgn0033664	MCPH1	0	0	0	0	0	1
FBgn0020240	Mcr	1	0	0	0	0	0
FBgn0010241	Mdr50	1	0	0	0	0	0
FBgn0004419	me31B	3	2	0	2	1	2
FBgn0037109	MED1	0	0	1	0	1	0
FBgn0036581	MED10	0	0	1	2	2	1
FBgn0036811	MED11	1	2	1	0	0	0
FBgn0035145	MED14	0	1	0	0	1	0
FBgn0027592	MED15	1	1	0	0	2	3
FBgn0034707	MED16	2	1	3	0	2	1
FBgn0038578	MED17	3	3	3	2	3	2
FBgn0026873	MED18	2	3	1	1	2	1
FBgn0036761	MED19	2	0	2	1	2	2
FBgn0013531	MED20	3	2	2	2	2	0
FBgn0040339	MED22	1	2	1	0	0	0
FBgn0034795	MED23	3	3	3	3	3	3
FBgn0035851	MED24	2	1	1	2	2	2

FBgn0038760	MED25	1	2	0	1	2	1
FBgn0039923	MED26	1	2	0	0	0	1
FBgn0037359	MED27	2	2	1	0	1	1
FBgn0035149	MED30	1	3	2	1	1	1
FBgn0037262	MED31	1	1	1	0	0	1
FBgn0035754	MED4	1	0	1	0	1	1
FBgn0024330	MED6	1	0	1	0	1	0
FBgn0051390	MED7	1	0	0	0	2	1
FBgn0034503	MED8	1	0	0	0	1	0
FBgn0004367	mei-41	2	0	1	0	1	0
FBgn0025874	Meics	0	1	0	0	0	0
FBgn0037207	Mes2	3	3	2	2	2	3
FBgn0034726	Mes4	1	2	0	1	0	0
FBgn0040089	meso18E	1	2	0	0	1	0
FBgn0034240	MESR4	2	1	1	0	2	1
FBgn0086783	Mhc	3	3	3	3	3	3
FBgn0026059	Mhcl	1	0	1	1	1	0
FBgn0013591	Mi-2	2	2	1	1	3	1
FBgn0053208	MICAL	3	3	3	2	3	3
FBgn0033846	mip120	0	0	0	0	0	1
FBgn0023509	mip130	0	0	1	0	1	0
FBgn0034430	mip40	1	0	0	0	0	1
FBgn0021776	mira	1	0	0	0	1	2
FBgn0004643	mit(1)15	3	3	3	1	3	2
FBgn0035889	mkg-p	0	0	0	0	1	0
FBgn0004687	Mlc-c	3	3	3	3	3	3
FBgn0002772	Mlc1	0	0	1	1	0	0
FBgn0002773	Mlc2	3	3	3	3	3	3
FBgn0037301	Mms19	0	0	0	1	0	0
FBgn0259168	mnb	0	0	0	0	0	1
FBgn0023215	Mnt	0	1	0	0	0	0
FBgn0259483	Mob4	0	0	0	0	0	1
FBgn0039581	Moca-cyp	3	3	3	3	3	3
FBgn0002780	mod	2	3	3	3	3	2
FBgn0002781	mod(mdg4)	3	3	3	3	3	2
FBgn0011661	Moe	1	0	0	0	1	1
FBgn0014340	mof	0	0	0	1	0	0
FBgn0002783	mor	2	3	3	3	3	3
FBgn0020270	mre11	0	0	0	0	1	0
FBgn0027378	MRG15	2	3	3	2	2	2
FBgn0035107	mri	0	0	0	0	1	0
FBgn0030556	mRNA-capping-enzyme	1	1	0	0	0	1
FBgn0044511	mRpS21	0	0	0	0	0	1
FBgn0044510	mRpS5	1	0	0	0	0	0
FBgn0032236	mRpS7	0	0	0	0	0	1
FBgn0036486	Msh6	0	0	0	0	1	0
FBgn0011666	msi	3	2	3	2	3	2
FBgn0026252	msk	1	2	2	2	3	2
FBgn0005617	msl-1	1	0	0	0	1	1
FBgn0005616	msl-2	1	0	0	0	0	1
FBgn0002775	msl-3	2	0	0	0	1	0
FBgn0053715	Msp-300	1	0	1	1	1	1

FBgn0027948	msps	3	3	3	3	3	3
FBgn0027951	MTA1-like	1	1	1	0	1	2
FBgn0013756	Mtor	3	3	3	3	3	3
FBgn0004177	mts	2	3	2	1	3	1
FBgn0010438	mtSSB	1	3	3	3	2	3
FBgn0002872	mu2	0	0	0	0	1	0
FBgn0002873	mud	0	0	0	0	1	0
FBgn0005655	mus209	1	0	0	0	0	1
FBgn0004698	mus210	3	1	1	3	1	2
FBgn0002901	mus304	2	1	1	1	2	2
FBgn0002905	mus308	1	0	0	0	1	0
FBgn0002906	mus309	2	2	1	1	2	2
FBgn0040347	mus81	0	0	0	0	1	0
FBgn0040299	Myo28B1	2	1	1	2	2	2
FBgn0086347	Myo31DF	3	3	3	3	3	3
FBgn0010246	Myo61F	2	3	3	3	3	3
FBgn0033379	Mys45A	3	3	3	3	3	3
FBgn0085434	NaCP60E	0	0	0	0	1	0
FBgn0004118	nAcRbeta-96A	1	0	0	0	0	0
FBgn0015268	Nap1	3	3	2	2	3	3
FBgn0031020	Nat1	1	0	0	0	1	2
FBgn0086349	nbs	1	1	0	0	1	2
FBgn0002924	ncd	3	3	3	3	3	3
FBgn0086707	ncm	1	3	2	2	3	3
FBgn0030500	Ndc80	0	1	0	0	1	0
FBgn0029970	Nek2	0	0	0	0	1	0
FBgn0027553	NELF-B	1	1	0	0	3	1
FBgn0033765	nemy	0	0	0	0	1	0
FBgn0024542	Neos	2	2	1	0	3	1
FBgn0015269	Nf1	2	2	2	1	2	3
FBgn0034243	Ngp	3	3	3	3	3	3
FBgn0029148	NHP2	2	3	3	3	3	3
FBgn0002938	ninaC	0	0	0	0	0	1
FBgn0053554	Nipped-A	3	3	3	3	3	3
FBgn0026401	Nipped-B	3	3	3	3	3	3
FBgn0027548	nito	3	3	3	2	3	3
FBgn0024321	NK7.1	1	0	0	0	0	0
FBgn0021874	Nle	0	1	1	1	1	0
FBgn0016685	Nlp	1	0	1	0	1	0
FBgn0011817	nmo	3	2	3	3	3	2
FBgn0022069	Nnp-1	3	3	3	3	3	3
FBgn0026400	Noa36	0	0	0	0	1	0
FBgn0014366	noi	3	3	1	0	2	3
FBgn0016047	nompA	0	0	0	1	0	0
FBgn0016920	nompC	0	0	0	1	0	0
FBgn0004227	nonA	3	3	3	3	3	3
FBgn0015520	nonA-l	0	0	1	0	0	0
FBgn0026196	nop5	0	1	0	1	0	1
FBgn0038964	Nop56	3	3	3	3	3	3
FBgn0023184	Nop60B	3	3	3	2	3	3
FBgn0037137	Nopp140	0	2	1	0	1	0
FBgn0085436	Not1	3	3	3	3	3	3
FBgn0013998	Nsf2	1	3	0	0	2	1

FBgn0085424	nub	0	0	0	0	0	1
FBgn0002973	numb	0	0	0	0	0	1
FBgn0027868	Nup107	3	3	3	3	3	3
FBgn0039004	Nup133	3	3	3	3	3	3
FBgn0061200	Nup153	3	3	3	3	3	3
FBgn0021761	Nup154	0	0	0	0	0	1
FBgn0039302	Nup358	3	3	3	3	3	3
FBgn0033247	Nup44A	3	3	3	2	3	2
FBgn0034310	Nup75	3	3	3	3	3	2
FBgn0039120	Nup98	2	3	2	3	3	3
FBgn0016687	Nurf-38	3	3	3	3	3	3
FBgn0036640	nxf2	1	2	0	0	0	0
FBgn0028411	Nxt1	0	1	1	0	0	0
FBgn0004102	oc	1	0	0	0	2	0
FBgn0040295	Ogt	1	0	0	0	2	1
FBgn0015521	oho23B	2	3	3	3	3	3
FBgn0038168	omd	2	1	1	2	0	1
FBgn0028996	onecut	0	0	0	0	0	1
FBgn0022772	Orc1	3	3	1	3	2	2
FBgn0015270	Orc2	3	2	1	1	1	1
FBgn0023181	Orc4	3	3	3	2	3	2
FBgn0015271	Orc5	3	3	0	0	2	2
FBgn0023180	Orc6	0	1	0	0	0	1
FBgn0046323	Ory	2	0	0	1	1	0
FBgn0034452	Oseg6	0	1	0	0	1	0
FBgn0003015	osk	0	1	1	0	2	1
FBgn0003016	osp	0	0	0	0	1	0
FBgn0003022	Ote	3	3	1	1	2	1
FBgn0015587	p120ctn	1	1	1	0	1	0
FBgn0031437	p16-ARC	1	1	1	2	3	1
FBgn0003031	pAbp	3	3	3	3	3	2
FBgn0005648	Pabp2	2	3	1	0	2	1
FBgn0036005	pall	0	2	1	0	2	2
FBgn0026193	par-1	1	1	2	2	3	1
FBgn0026192	par-6	2	0	0	0	0	0
FBgn0010247	Parp	3	3	3	3	3	3
FBgn0039861	pasha	2	1	0	1	0	2
FBgn0028470	Patr-1	3	3	3	3	3	3
FBgn0029137	Patsas	3	2	3	2	3	1
FBgn0011692	pav	3	3	3	3	3	3
FBgn0003041	pbl	1	0	0	0	1	0
FBgn0003042	Pc	3	3	2	1	3	3
FBgn0020388	Pcaf	3	3	3	3	3	3
FBgn0020261	pcm	1	1	1	0	1	2
FBgn0017558	Pdk	1	0	0	0	0	0
FBgn0033676	pds5	1	1	2	0	2	1
FBgn0086895	pea	1	1	1	2	0	0
FBgn0003053	peb	0	0	0	0	0	1
FBgn0015527	pen	3	3	3	3	3	3
FBgn0011823	Pen	3	2	3	2	3	2
FBgn0004401	Pep	3	3	2	3	3	3
FBgn0003067	Pepck	0	0	0	0	1	0
FBgn0031799	Pez	0	2	0	0	0	0

FBgn0035405	pfk	2	2	0	1	2	0
FBgn0003074	Pgi	0	0	0	0	0	1
FBgn0014869	Pglym78	1	0	0	0	0	0
FBgn0036542	pHCl	0	0	0	2	0	0
FBgn0002521	pho	3	3	3	1	2	2
FBgn0015277	Pi3K59F	0	0	0	0	1	0
FBgn0025140	pit	3	3	3	3	3	3
FBgn0034878	pita	2	1	1	1	3	3
FBgn0016696	Pitslre	3	2	3	3	3	3
FBgn0004872	piwi	2	1	1	1	1	2
FBgn0086706	pix	0	0	0	0	0	1
FBgn0003093	Pkc98E	0	0	0	0	0	1
FBgn0020621	Pkn	0	0	0	0	0	1
FBgn0024314	Plap	2	1	0	2	1	2
FBgn0004611	Plc21C	0	1	0	0	0	0
FBgn0037737	Pnn	3	3	3	3	3	2
FBgn0029903	pod1	0	0	0	0	1	0
FBgn0011230	poe	3	3	3	2	3	3
FBgn0003124	polo	3	3	3	2	3	3
FBgn0039227	polybromo	2	2	3	2	3	1
FBgn0025739	pon	1	0	0	0	0	0
FBgn0040078	pont	3	3	3	3	3	3
FBgn0036239	Pop2	2	3	0	0	3	1
FBgn0003132	Pp1-13C	0	0	1	2	0	0
FBgn0004103	Pp1-87B	3	3	3	2	3	3
FBgn0046698	Pp1-Y2	0	0	2	2	0	0
FBgn0003134	Pp1alpha-96A	3	3	3	3	3	3
FBgn0005776	Pp2A-29B	3	3	3	1	3	3
FBgn0042693	PP2A-B'	0	0	0	0	1	1
FBgn0022768	Pp2C1	0	1	1	0	0	2
FBgn0010770	ppan	3	3	3	2	3	3
FBgn0005779	PpD6	0	0	1	0	0	0
FBgn0003137	Ppn	1	0	0	0	0	0
FBgn0030208	PPP4R2r	1	0	0	0	0	0
FBgn0082831	pps	0	1	0	0	0	0
FBgn0003139	PpV	1	0	0	0	1	0
FBgn0011474	pr-set7	0	0	0	1	0	0
FBgn0004595	pros	0	0	0	1	0	0
FBgn0015282	Pros26.4	1	0	0	0	1	3
FBgn0004066	Pros28.1	1	0	0	0	0	1
FBgn0020369	Pros45	0	1	2	1	1	3
FBgn0015283	Pros54	0	1	0	0	0	1
FBgn0026781	Prosalpha6	0	1	0	0	0	1
FBgn0026380	Prosbeta3	0	0	0	0	0	1
FBgn0033688	prp8	3	3	3	3	3	3
FBgn0005624	Psc	0	1	0	0	0	0
FBgn0004399	psq	1	2	2	1	2	2
FBgn0035770	pst	0	0	0	0	0	1
FBgn0014007	Ptp69D	1	1	0	0	1	0
FBgn0035133	Ptpmeg	2	1	1	2	1	2
FBgn0033068	Ptr	0	0	0	1	1	0
FBgn0028577	pUf68	3	3	3	2	3	3
FBgn0022361	Pur-alpha	3	3	3	2	1	2

FBgn0038538	pxt	2	0	1	0	1	0
FBgn0003177	pyd	2	0	1	0	2	0
FBgn0003178	PyK	1	0	0	1	1	1
FBgn0022986	qkr58E-1	3	3	3	3	3	3
FBgn0022985	qkr58E-2	2	3	2	2	2	3
FBgn0024733	Qm	3	3	3	3	3	3
FBgn0003187	qua	3	2	1	1	2	2
FBgn0003189	r	3	3	3	3	3	3
FBgn0045843	RacGAP84C	0	1	0	0	0	1
FBgn0020618	Rack1	3	3	3	3	3	3
FBgn0025808	Rad17	0	1	0	0	0	0
FBgn0026057	Rad21	2	1	0	0	1	1
FBgn0034728	rad50	1	1	2	0	2	2
FBgn0034646	Rae1	2	1	2	1	2	3
FBgn0020255	ran	3	2	1	1	1	1
FBgn0053139	Ranbp11	0	1	1	2	1	1
FBgn0053180	Ranbp16	3	3	3	3	3	3
FBgn0031051	Ranbp21	0	0	0	0	1	0
FBgn0037894	Ranbp9	3	3	3	3	3	2
FBgn0003346	RanGap	3	3	3	3	3	3
FBgn0003200	rap	1	0	0	0	0	0
FBgn0029840	raptor	1	0	0	0	1	0
FBgn0015799	Rbf	3	2	3	0	1	3
FBgn0030067	Rbm13	3	3	2	3	3	2
FBgn0010252	Rbp1	1	1	0	0	0	0
FBgn0030479	Rbp1-like	0	0	0	1	0	0
FBgn0243486	rdo	0	0	0	0	1	0
FBgn0029133	REG	2	2	2	2	3	1
FBgn0040075	rept	3	3	3	3	3	2
FBgn0011829	Ret	0	0	0	0	1	0
FBgn0031814	retm	1	0	0	0	2	1
FBgn0035150	Rev1	0	0	0	0	1	0
FBgn0087002	Rfabg	3	3	3	3	3	3
FBgn0032244	RfC3	3	3	3	3	3	3
FBgn0028700	RfC38	3	3	3	3	3	3
FBgn0015287	RfC40	3	3	3	3	3	3
FBgn0017550	Rga	3	2	1	1	2	1
FBgn0027376	rha	1	0	0	1	0	1
FBgn0020254	rhea	0	1	0	0	0	1
FBgn0030808	RhoGAP15B	1	0	0	0	0	0
FBgn0034249	RhoGAP54D	0	0	0	0	0	1
FBgn0036518	RhoGAP71E	0	1	0	0	0	0
FBgn0026375	RhoGAPp190	0	0	1	0	0	2
FBgn0035761	RhoGEF4	2	1	0	1	2	0
FBgn0003254	rib	2	2	0	1	2	3
FBgn0015778	rin	3	3	3	3	3	3
FBgn0014022	Rlb1	1	0	0	1	0	0
FBgn0003261	Rm62	3	3	3	2	3	3
FBgn0015477	Rme-8	1	1	0	2	1	1
FBgn0035106	rno	1	0	0	0	2	0
FBgn0037707	RnpS1	3	2	3	1	3	2
FBgn0003268	rod	2	3	3	3	3	3
FBgn0043856	roo\ORF	0	0	1	0	1	1

FBgn0005649	Rox8	3	0	0	0	0	2
FBgn0010173	RpA-70	3	3	3	3	3	3
FBgn0032906	RPA2	2	3	3	2	2	3
FBgn0039218	Rpb10	2	2	1	0	1	2
FBgn0032634	Rpb11	0	0	0	0	1	0
FBgn0033571	Rpb5	1	1	0	0	0	2
FBgn0015805	Rpd3	3	3	3	3	3	3
FBgn0003278	RpI135	0	1	0	0	0	1
FBgn0003277	RpII215	0	0	0	0	0	2
FBgn0026373	RpII33	1	1	1	0	1	2
FBgn0004463	RpIII128	0	0	0	0	0	2
FBgn0036213	RpL10Ab	3	3	3	3	3	3
FBgn0013325	RpL11	3	3	3	3	3	3
FBgn0034968	RpL12	3	2	3	3	3	2
FBgn0011272	RpL13	3	3	3	3	3	3
FBgn0037351	RpL13A	3	3	3	3	3	3
FBgn0017579	RpL14	3	3	3	3	3	3
FBgn0028697	RpL15	0	1	0	0	0	1
FBgn0029897	RpL17	3	3	3	3	3	3
FBgn0035753	RpL18	3	3	3	3	3	3
FBgn0010409	RpL18A	3	3	3	3	3	3
FBgn0002607	RpL19	3	3	3	3	3	3
FBgn0032987	RpL21	2	3	3	3	3	3
FBgn0015288	RpL22	3	3	3	3	3	3
FBgn0010078	RpL23	3	3	3	3	3	3
FBgn0026372	RpL23A	3	3	3	3	3	3
FBgn0032518	RpL24	3	3	3	3	3	3
FBgn0036825	RpL26	3	3	3	3	3	3
FBgn0039359	RpL27	2	3	3	3	3	3
FBgn0010410	RpL27A	3	3	3	3	3	3
FBgn0035422	RpL28	2	3	3	3	3	3
FBgn0016726	RpL29	2	2	2	2	2	2
FBgn0020910	RpL3	3	3	3	3	3	3
FBgn0086710	RpL30	3	3	3	3	3	3
FBgn0025286	RpL31	2	3	3	3	3	3
FBgn0002626	RpL32	1	0	2	2	1	1
FBgn0039406	RpL34a	2	2	1	1	2	2
FBgn0037686	RpL34b	1	1	3	3	1	1
FBgn0029785	RpL35	2	3	3	3	3	3
FBgn0037328	RpL35A	2	3	3	3	3	3
FBgn0002579	RpL36	3	3	3	3	3	3
FBgn0031980	RpL36A	2	3	3	3	3	3
FBgn0028696	RpL37A	3	3	3	3	3	3
FBgn0040007	RpL38	3	1	2	3	3	2
FBgn0023170	RpL39	1	1	2	3	2	2
FBgn0003279	RpL4	3	3	3	3	3	3
FBgn0003941	RpL40	2	2	3	3	2	3
FBgn0064225	RpL5	3	3	3	3	3	3
FBgn0039857	RpL6	3	3	3	3	3	3
FBgn0005593	RpL7	2	3	3	2	3	3
FBgn0014026	RpL7A	3	3	3	3	3	3
FBgn0024939	RpL8	3	3	3	3	3	3
FBgn0015756	RpL9	3	3	3	3	3	3

FBgn0000100	RpLP0	3	3	3	3	3	3
FBgn0002593	RpLP1	3	3	3	3	3	3
FBgn0003274	RpLP2	3	3	3	3	3	3
FBgn0028695	Rpn1	1	1	0	0	1	2
FBgn0028694	Rpn11	0	1	0	0	0	1
FBgn0028693	Rpn12	1	1	1	0	1	1
FBgn0028692	Rpn2	1	0	2	2	2	2
FBgn0028688	Rpn7	0	0	0	0	0	1
FBgn0028691	Rpn9	0	0	0	0	0	1
FBgn0022246	Rpp30	0	0	0	0	0	1
FBgn0027494	RpS10a	0	0	0	1	0	1
FBgn0031035	RpS10b	2	3	3	3	3	3
FBgn0033699	RpS11	2	3	3	3	3	3
FBgn0014027	RpS12	2	3	3	3	3	3
FBgn0010265	RpS13	2	3	3	3	3	3
FBgn0004403	RpS14a	2	3	3	3	3	3
FBgn0034138	RpS15	2	3	3	3	3	2
FBgn0010198	RpS15Aa	2	3	3	3	3	3
FBgn0033555	RpS15Ab	0	0	1	1	0	1
FBgn0034743	RpS16	2	3	3	3	3	3
FBgn0005533	RpS17	2	3	3	3	3	3
FBgn0010411	RpS18	2	3	3	3	3	3
FBgn0010412	RpS19a	2	3	3	3	3	3
FBgn0019936	RpS20	2	3	3	3	3	3
FBgn0033912	RpS23	2	3	3	3	3	3
FBgn0034751	RpS24	2	3	3	3	3	3
FBgn0086472	RpS25	2	3	3	3	3	3
FBgn0004413	RpS26	3	3	3	3	3	2
FBgn0039300	RpS27	3	3	3	3	3	3
FBgn0003942	RpS27A	2	3	3	2	3	3
FBgn0030136	RpS28b	3	3	3	3	3	3
FBgn0037752	RpS29	3	3	3	3	3	3
FBgn0002622	RpS3	3	3	3	3	2	3
FBgn0038834	RpS30	3	3	3	3	3	3
FBgn0017545	RpS3A	3	3	3	3	3	3
FBgn0011284	RpS4	3	3	3	3	3	3
FBgn0002590	RpS5a	3	3	3	3	3	3
FBgn0038277	RpS5b	3	2	3	3	2	3
FBgn0004922	RpS6	3	3	3	3	3	3
FBgn0039757	RpS7	2	3	3	3	3	3
FBgn0039713	RpS8	3	3	3	3	3	3
FBgn0010408	RpS9	3	3	3	3	3	3
FBgn0028687	Rpt1	1	2	1	0	1	2
FBgn0028686	Rpt3	2	0	0	1	2	1
FBgn0028685	Rpt4	1	0	0	0	1	2
FBgn0037815	Rrp46	0	2	1	0	0	1
FBgn0021995	Rs1	3	3	3	3	3	3
FBgn0011305	Rsf1	3	3	3	3	3	1
FBgn0020909	Rtc1	2	2	1	2	1	1
FBgn0034722	Rtf1	1	2	0	0	0	0
FBgn0011286	Rya-r44F	1	0	0	0	0	0
FBgn0015806	S6k	0	0	0	1	0	0
FBgn0020616	SA	3	2	1	0	3	2

FBgn0004579	salm	0	0	0	0	0	2
FBgn0029755	Sas10	3	3	3	2	2	2
FBgn0003321	sbr	1	2	2	1	2	1
FBgn0040286	SC35	2	2	2	1	1	1
FBgn0053522	scaf6	0	2	0	3	1	3
FBgn0003330	Sce	3	3	2	2	2	2
FBgn0004243	scra	3	3	3	3	2	3
FBgn0024509	sec13	2	3	3	3	3	2
FBgn0028538	sec71	0	0	0	0	0	1
FBgn0028991	seq	0	1	0	0	0	0
FBgn0003360	sesB	0	0	0	0	1	0
FBgn0030486	Set2	3	3	2	1	3	2
FBgn0025571	SF1	1	3	2	1	3	2
FBgn0040284	SF2	3	3	3	3	3	3
FBgn0032475	Sfmbt	1	1	1	3	0	3
FBgn0003392	shi	1	3	1	2	1	3
FBgn0013733	shot	3	3	3	2	3	3
FBgn0085372	sick	0	0	0	0	1	0
FBgn0028402	Sin	0	0	0	0	0	3
FBgn0022764	Sin3A	2	2	3	2	3	1
FBgn0061198	SIP1	0	0	0	1	0	0
FBgn0061197	SIP2	3	3	2	2	2	3
FBgn0052484	Sk2	0	0	0	1	1	1
FBgn0003415	skd	1	2	1	0	1	2
FBgn0025637	skpA	2	3	2	1	2	2
FBgn0043854	slam	0	1	0	0	0	0
FBgn0037810	sle	2	3	2	3	3	1
FBgn0023423	slmb	3	2	3	1	2	1
FBgn0086906	sls	3	3	3	3	3	3
FBgn0010083	SmB	3	3	3	3	3	3
FBgn0040283	SMC1	2	1	1	1	2	1
FBgn0023167	SmD3	2	3	2	2	3	3
FBgn0016070	smg	2	2	0	2	3	3
FBgn0030765	SmG	3	1	3	3	2	2
FBgn0016983	smid	2	3	3	3	3	3
FBgn0036641	Smn	1	2	2	2	1	0
FBgn0024308	Smr	3	3	3	2	3	3
FBgn0026170	smt3	1	2	1	0	1	2
FBgn0086129	snama	2	2	1	2	2	2
FBgn0003449	snf	1	1	2	0	1	0
FBgn0023169	SNF1A	1	0	0	0	1	0
FBgn0037434	snRNP2	2	2	2	2	3	1
FBgn0016940	snRNP69D	2	2	2	3	2	3
FBgn0016978	snRNP70K	3	2	2	1	0	1
FBgn0004867	sop	3	3	3	3	3	3
FBgn0015546	spel1	3	0	0	1	3	3
FBgn0016977	spen	2	2	3	2	3	3
FBgn0086683	Spf45	2	1	0	0	0	1
FBgn0010905	Spn	2	3	3	3	3	3
FBgn0028986	Spn3	0	0	0	0	0	1
FBgn0003486	spo	0	0	1	0	2	0
FBgn0028982	Spt6	1	0	0	1	0	0
FBgn0015818	Spx	1	0	2	0	1	0

FBgn0086897	sqd	1	1	0	0	0	0
FBgn0003514	sqh	0	0	1	1	1	0
FBgn0036340	SRm160	1	2	2	3	3	3
FBgn0024285	Srp54	1	2	2	2	3	2
FBgn0035947	Srp68	1	1	2	0	0	1
FBgn0038810	Srp72	2	3	1	1	3	3
FBgn0026370	SRPK	3	3	3	3	3	3
FBgn0003512	Sry-delta	0	0	0	0	0	1
FBgn0011481	Ssdp	0	1	0	0	0	0
FBgn0029157	ssh	0	0	0	0	0	1
FBgn0032723	ssp3	0	0	0	1	1	0
FBgn0010278	Ssrp	3	3	3	3	3	3
FBgn0003517	sta	3	3	3	3	3	3
FBgn0051641	stai	1	0	0	0	0	0
FBgn0016917	Stat92E	3	3	1	1	3	3
FBgn0001978	stc	2	2	0	1	0	1
FBgn0033870	stj	0	0	0	0	1	0
FBgn0046692	Stlk	0	0	0	0	1	0
FBgn0013988	Strn-Mlck	0	0	1	1	1	0
FBgn0003459	stwl	3	3	3	3	3	3
FBgn0003559	su(f)	2	2	1	0	2	3
FBgn0003612	Su(var)2-10	3	2	1	1	2	2
FBgn0026427	Su(var)2-HP2	0	0	1	1	1	0
FBgn0003607	Su(var)205	3	3	3	2	3	1
FBgn0003598	Su(var)3-7	3	3	3	2	2	3
FBgn0003600	Su(var)3-9	1	3	2	1	3	2
FBgn0020887	Su(z)12	0	0	0	0	0	1
FBgn0003545	sub	3	3	3	3	3	2
FBgn0038746	Surf6	3	2	2	3	2	2
FBgn0025355	SuUR	2	2	1	1	3	2
FBgn0003651	svp	0	0	0	0	1	0
FBgn0003676	T-cp1	3	3	3	1	2	3
FBgn0004359	T48	0	0	0	0	1	1
FBgn0026620	tacc	2	2	0	0	2	1
FBgn0010355	Taf1	3	3	3	2	2	3
FBgn0026324	Taf10b	0	0	0	1	0	0
FBgn0011291	Taf11	1	2	1	1	1	0
FBgn0011290	Taf12	0	3	1	0	1	1
FBgn0032847	Taf13	2	1	1	3	3	3
FBgn0011836	Taf2	3	3	2	1	3	2
FBgn0010280	Taf4	3	3	2	3	3	2
FBgn0010356	Taf5	0	0	1	1	0	0
FBgn0010417	Taf6	3	3	3	2	2	2
FBgn0024909	Taf7	1	3	1	1	2	1
FBgn0022724	Taf8	1	1	0	1	1	0
FBgn0030365	Tango4	2	2	1	2	1	1
FBgn0033902	Tango7	0	1	0	1	2	2
FBgn0031030	Tao-1	0	0	0	0	1	0
FBgn0003687	Tbp	1	0	1	2	2	3
FBgn0028684	Tbp-1	1	1	1	1	2	2
FBgn0025790	TBPH	1	2	2	0	1	1
FBgn0037632	Tcp-1eta	3	3	2	1	2	3
FBgn0027329	Tcp-1zeta	3	3	2	1	3	2

FBgn0037874	Tctp	0	0	0	1	1	1
FBgn0086350	tef	1	1	0	0	2	0
FBgn0045035	tefu	0	0	0	1	0	0
FBgn0004449	Ten-m	0	0	0	1	0	0
FBgn0024923	TER94	0	0	0	0	1	1
FBgn0038805	TFAM	0	1	0	1	0	0
FBgn0036513	Tfb2	1	1	0	0	1	1
FBgn0013347	TfIIA-S	2	1	2	1	2	2
FBgn0015014	tgo	0	0	0	1	2	0
FBgn0010416	TH1	2	1	1	1	1	2
FBgn0025352	Thiolase	0	0	1	0	0	0
FBgn0031390	tho2	0	1	0	0	0	1
FBgn0032988	Tif-IA	1	0	0	0	1	0
FBgn0026080	Tip60	1	1	2	0	2	1
FBgn0086899	tlk	3	3	3	2	2	3
FBgn0003721	Tm1	3	3	3	3	3	3
FBgn0004117	Tm2	3	3	3	3	3	3
FBgn0082582	tmod	3	3	3	2	3	3
FBgn0004885	tok	0	0	0	0	0	2
FBgn0030412	tomosyn	0	0	0	0	1	0
FBgn0004924	Top1	3	3	3	3	3	3
FBgn0003732	Top2	3	3	3	3	3	3
FBgn0040268	Top3alpha	0	1	0	1	1	2
FBgn0026015	Top3beta	1	0	1	1	1	1
FBgn0003733	tor	3	2	0	3	2	2
FBgn0025615	torp4a	2	3	1	3	2	2
FBgn0033636	tou	1	2	2	1	1	1
FBgn0010423	TpnC47D	2	3	1	3	2	2
FBgn0010424	TpnC73F	1	2	2	1	2	1
FBgn0003742	tra2	2	1	1	0	2	0
FBgn0041775	tral	2	3	0	0	1	2
FBgn0026761	Trap1	1	0	0	0	1	1
FBgn0038767	trem	2	1	0	0	1	1
FBgn0026758	Trf2	0	2	2	1	1	1
FBgn0015834	Trip1	1	0	0	0	0	1
FBgn0013263	Trl	0	1	0	0	1	1
FBgn0024921	Trn	3	3	3	2	3	2
FBgn0001402	trol	0	0	1	1	2	1
FBgn0032593	trpgamma	1	0	0	0	0	0
FBgn0023518	trr	2	1	1	1	0	2
FBgn0003862	trx	0	0	0	1	0	1
FBgn0040070	Trx-2	1	0	0	0	0	1
FBgn0020653	Trxr-1	1	0	0	0	0	0
FBgn0036666	TSG101	0	1	0	0	0	0
FBgn0003867	tsl	0	0	0	0	0	1
FBgn0003870	ttk	0	0	0	0	1	2
FBgn0003891	tud	1	0	0	0	0	1
FBgn0035121	Tudor-SN	0	0	0	0	0	1
FBgn0086356	tum	3	2	3	3	3	2
FBgn0003900	twi	1	0	0	0	0	0
FBgn0011725	twin	3	3	3	2	2	3
FBgn0026326	TXBP181-like	3	3	3	3	3	3
FBgn0004514	TyrR	0	1	0	0	0	0

FBgn0033210	U2A	0	0	0	0	1	1
FBgn0023143	Uba1	0	0	0	0	0	1
FBgn0029113	Uba2	1	1	0	0	0	1
FBgn0011327	Uch-L3	0	0	0	0	0	1
FBgn0027603	Ulp1	3	2	2	3	2	3
FBgn0025726	unc-13	0	0	1	0	1	0
FBgn0053519	Unc-89	0	0	0	0	1	0
FBgn0004395	unk	1	2	0	0	1	2
FBgn0004169	up	3	3	3	3	3	3
FBgn0030354	Upf1	1	1	0	0	0	1
FBgn0035633	Usp36	3	2	2	3	2	3
FBgn0003965	v	0	0	0	0	0	1
FBgn0003970	vas	3	3	2	1	3	3
FBgn0022097	Vha36	2	3	3	2	2	0
FBgn0005671	Vha55	0	1	0	0	0	0
FBgn0003977	vir	3	3	3	3	3	3
FBgn0010516	wal	2	2	0	0	0	1
FBgn0039067	wda	1	2	0	0	0	1
FBgn0005642	wdn	1	1	0	1	1	1
FBgn0040066	wds	3	3	3	3	3	3
FBgn0001990	wek	2	3	2	1	2	1
FBgn0004028	wupA	3	3	3	3	3	3
FBgn0010772	Xe7	2	2	2	2	1	0
FBgn0028554	xl6	3	2	3	2	3	2
FBgn0026751	XRCC1	1	0	0	1	0	2
FBgn0005596	yemalpha	0	0	0	0	1	0
FBgn0043842	Yeti	0	0	0	0	1	0
FBgn0032321	YL-1	3	2	1	2	3	1
FBgn0004045	Yp1	3	3	3	3	3	3
FBgn0005391	Yp2	3	3	3	3	3	3
FBgn0004047	Yp3	3	3	3	3	3	3
FBgn0022959	yps	2	3	1	1	1	1
FBgn0004049	yrt	1	1	1	1	2	1
FBgn0027616	YT521-B	0	0	0	0	0	1
FBgn0021895	ytr	3	3	3	3	3	2
FBgn0037066	Z4	3	3	3	3	3	3
FBgn0024270	ZAM\pol	1	0	0	0	0	0
FBgn0040512	zetaCOP	1	1	1	1	1	1
FBgn0022720	zf30C	1	1	0	1	1	1
FBgn0004606	zfh1	0	0	0	0	0	1
FBgn0005634	zip	3	3	3	3	3	3
FBgn0052311	zormin	1	0	1	0	0	0
FBgn0061476	zwilch	3	1	2	1	3	1

Curriculum Vitae

Personal Data

Name:	Toma Antonov Yakulov
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Education

Since 10/ 2004	**PhD in Molecular Biology** in the Department of Molecular Developmental Biology at the Max Planck Institute for Biophysical Chemistry under the supervision of Prof. Dr. Herbert Jäckle
10/ 2003 – 03/ 2005	**MSc in Molecular Biology** in the Department of Molecular Developmental Biology at the Max Planck Institute for Biophysical Chemistry under the supervision of Prof. Dr. Herbert Jäckle
10/ 2002 – 07/ 2003	**MSc in Biochemistry** at the "Sveti Kliment Ohridski" University and the National Genetic Laboratory, Sofia, Bulgaria under the supervision of Dr. Alexey Savov
10/ 1998 – 07/ 2002	**BSc in Biology** at the "Sveti Kliment Ohridski" University, Sofia, Bulgaria

Community service

11/ 2005 – 12/ 2007	Co-organizer of the International PhD Student Symposium "Horizons in Molecular Biology"
10/ 2006 – 12/ 2007	Co-organizer of the "Horizons Career Fair for Scientists"

10/ 2006 – 06/ 2007 Co-organizer of a joint retreat of PhD students from the „Molecular Biology“ graduate program from Göttingen and the graduate program of the Vienna Biocenter.

Scholarships

10/ 2004 – 10/ 2007 Scholarship of the Max Planck Society

09/ 2003 – 08/ 2004 Scholarship of the International Max Planck Research School for Molecular Biology, Göttingen

Publications

04/ 2007 Kadiyska T., Yakulov T., Kaneva R., Nedin D., Alexandrova A., Gegova A., Savov A., Mitev V., Kremensky I. Vitamin D and estrogen receptor gene polymorphisms and the risk of colorectal cancer in Bulgaria. *International Journal of Colorectal Disease, 2007 Apr; 22(4): 395-400*

Presentations

03/ 2007 Yakulov T., Jäckle H., Herzig A. Promoter analysis of Bällchen, a kinase specifically expressed in *Drosophila melanogaster* embryonic neuroblasts. *48th Annual Drosophila Research Conference*, Philadelphia, USA (poster)

08/ 2002 Yakulov T., Savov A., Laplanche J.L., Kremensky I. Met129Val prion protein polymorphism genotyping in Bulgarian and Roma populations. *5th Balkan Congress on Human Genetics*, Sofia, Bulgaria (poster)