

HISTONE MODIFICATION REGULATED BY
SUPPRESSOR OF ZESTE 12 AND IPL1-
AURORA-LIKE KINASE IN *DROSOPHILA*

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RUBE: You like falling, do ya?

BETTY: Well, it's not the fallin', it's the jumpin'.

RUBE: I'd feel a whole lot better about the jumpin' if it weren't for the fallin'.

BETTY: Fallin's easy, you just fall. Jumping involves strength of will.

RUBE: Unless you're on a plank.

BETTY: Then it isn't your choice. But if it is, it's the best feeling in the world.

RUBE: And you don't care where you land?

BETTY: Landing is a lot like fallin, you just land.

From "Dead like me"

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LIST OF PAPERS

1. **Larsson A., Tegeling E., Chen S., Lu C-M, Stief A. and Rasmuson-Lestander Å.** (2010) Investigation of the two isoforms of SU(Z)12 shows difference in expression and interaction *in vitro* with the core components of PRC2. *Manuscript*
2. **Larsson A., Keller U., Nilsson J.A. and Rasmuson-Lestander Å.** The c-Myc to Aurora B kinase axis is conserved down to *Drosophila melanogaster*. *Manuscript*

ABSTRACT

Polycomb group (PcG) proteins are a group of genome wide silencers that are crucial for many processes during the development. In *Drosophila* PcG proteins are organised into four different complexes: PRC1, PRC2, PhoRC and PR-DUB. PRC2 consists of four core proteins: Su(z)12, E(z), Esc and Nurf. E(z) is the only known proteins with a known function, it possess a methyltransferase activity that specifically methylates lysine 27 on histone 3 (H3K27). A novel PcG gene was identified in 2001 in screen for modifiers of *zeste-white* interaction. This gene suppressed *zeste*'s repression of *white* and gave it the name *Suppressor of zeste 12 (Su(z)12)*. The *Su(z)12* gene is alternatively spliced into two transcripts; a 4.1 kb mRNA called *Su(z)12A* and a 3.7 kb mRNA called *Su(z)12B*. These transcripts are translated into two isoforms; a 95 kDa Su(z)12A protein and 100 kDa Su(z)12B protein. These isoforms show a sequence similarity of 95% and the only difference is the C-terminal end. During development these two isoforms are present at different levels. Interaction of the two isoforms with the other core components in PRC2 showed that only Su(z)12B interacts with Nurf. Also the two isoforms showed interaction with each other with the exception of a single copy of Su(z)12A that couldn't interact with Su(z)12B. Overexpression of Su(z)12B *in vivo* caused lethality and homeotic transformations.

Aurora kinases belong to a conserved family of serine/threonine kinases that are important for many processes in mitosis, such as spindle formation, chromosomal segregation and cytokinesis. Aurora kinases are overexpressed in many human cancers and inhibitors of Aurora A and Aurora B has shown to inhibit growth and induce apoptosis. There are three Aurora kinases in vertebrates; Aurora A, Aurora B and Aurora C and although they are highly similar, they have different roles and location during mitosis. Aurora B is a chromosomal passenger protein and forms the chromosomal passenger complex with INCENP, Survivin and Borealin. Depletion of Aurora B causes severe effects in mitosis and lead to large cells with several nuclei and polyploidy. The *Drosophila* homologue of Aurora B is called IpII-like-Aurora kinase (*ial*). The c-Myc transcription factor, or its relatives N-Myc and L-Myc, are also overexpressed in many, if not all human cancers. *Drosophila* has only one Myc protein, dMyc, which is encoded by the *diminutive (dm)* locus. In *Drosophila*, dMyc is mostly associated with size and growth regulation and depletion of *dm* results in endoreplication and growth arrest in early development. Previous work has shown that mammalian c-Myc induces Aurora A and Aurora B kinases. When Myc-driven lymphomas are treated with Aurora B inhibitors, cells are accumulated in G2/M phase and apoptosis is induced. Here we show that these conserved proteins have a potential connection in *Drosophila* as well since knockdown of *ial* causes severe phenotypes and leads to larger cells. When *ial* is knocked down or when dMyc is overexpressed the flies become smaller. Interestingly however transgenic flies which overexpress dMyc and knock down *ial* exhibit a different phenotype - the flies become bigger. This showing evidence that a relationship between Myc and Aurora B is evolutionary conserved down to *Drosophila*.

ABBREVIATIONS

ASX – Additional Sex Combs
CPC – Chromosomal Passenger Protein
Dm - diminutive
E(Z)- Enhancer of zeste
ESC – Extra sex comb
ESCL – Extra Sex Combs- like
Ial – ipII-*aurora*-like
INCENP – Inner Centrosomal Protein
NURF – Nucleosomal remodelling factor
PC – Polycomb
PcG – Polycomb group
PCL – Polycomb-like
PH – Polyhomeotic
PHO – Pleiohomeotic
PHOL – Pleiohomeotic-like
PhoRC – Pleiohomeotic repressive complex
PRC1 – Polycomb repressive complex 1
PRC2 – Polycomb repressive complex 2
PR-DUB - Polycomb repressive deubiquitinase
PRE – Polycomb response elements
PSC – Posterior sex comb
SFMBT – Scm-related gene containing four malignant brain tumour domains
SU(Z)12 – Suppressor of zeste 12
TRE- Trithorax response elements
TrxG – Trithorax group

BACKGROUND

DROSOPHILA AS A MODEL ORGANISM

HISTORY

The fruit fly *Drosophila melanogaster* has been used in genetic studies for over a hundred years and the first researcher to bring the fruit fly into the lab was William E. Castle at Harvard University in 1901. Two years later William H. Moenkhaus started to work with *Drosophila* at Indiana University Medical School and a couple of years later the fly ended up in Thomas Hunt Morgan's lab. Morgan discovered the first mutation *with* (darker pigmentation) in January 1910 and during 1910 a number of mutations arose in Morgan's lab like *beaded*, *miniature*, *speck*, *olive* and the famous *white* mutation. This led to a breakthrough in *Drosophila* research and opened the opportunity of studying inheritance and genetics at a whole new level. Researchers that earlier had worked with diverse model organisms now decided to focus more on the fruit fly.

In the beginning of 1912 Morgan, Bridges and Sturtevant started to construct the first genetic maps using back-crosses to measure the genetic distance. The genetic maps were established between 1919 and 1923, using data from approximately ten million flies. Since then many ground-breaking discoveries have been made in *Drosophila* generating three Nobel price awards (Kohler 1994).

WHY DROSOPHILA?

Drosophila is a useful tool in medical and biological research for many reasons. The fly is small, has a short generation time and gives many offspring (Graf, Van Schaik and Wu\rgler 1992). *Drosophila* has few chromosomes (three pairs of autosomes and one pair sex chromosomes) and gene duplicates are rare in *Drosophila*. The polytene chromosomes, that form giant chromosomes by endoreplication in the salivary glands, are very useful tools for gene localisation and immune staining (Ashburner 1989).

Drosophila belongs to the holometabolic insects, which means that it undergoes total metamorphosis (Graf et al. 1992). The life cycle consists of four stages, starting with the embryonic stage that lasts for 24 hours after egg laying. The larval stage is divided into three instars; L1, L2 and L3 and lasts for a total of 96 hours at 25°C, and after that the fly reaches the pupal stage. The only tissue that remains from the larva after pupal stage is the imaginal discs that have developed into the body parts of the adult fly. The larval stage lasts for 4-4.5 days and finally the fly hatches. The developmental time varies with variation in temperature as a lower temperature lengthens the life cycle. The mean life span of a fruit fly is approximately 45 days at 25°C under optimal conditions (Ashburner 1989).

Since *Drosophila* has been used as a model organism for over a century many useful tools has been developed. Balancer chromosomes are used to preserve recessive lethal mutations in a heterozygous state by using multiple inverted chromosomes to hinder recombination and thereby promote survival of only heterozygous flies. The insertion of the unique P-elements and the GAL4/UAS system provides opportunities to study gene expression by ectopic overexpression, knockout and mutation of genes (Graf et al. 1992).

EARLY DEVELOPMENT IN DROSOPHILA

The development in *Drosophila* is strictly regulated with a set of genes that early on hierarchically defines each segment of the embryo. The maternally contributed gene products, such as *bicoid*, establish the anterior/posterior patterning in the early embryo. When the polarity is defined in the embryo the zygotic gap genes take over and these will establish the initial segmented patterning. Mutation of a gap gene like *hunchback* or *Krüppel* causes loss of several of the segments leading to severe phenotypes. The next set of genes in the hierarchy is the pair-rule genes. The pair-rule genes define the 14 final segments and are expressed in every other segment, e.g. *even-skipped*. The anterior-posterior identity of each segment is decided by the segment polarity genes. As an example, the *engrailed* gene is expressed in the anterior part of every segment and thereby defines the anterior part of the segment. The identity of every segment is regulated and maintained by homeotic genes, so-called *Hox genes*. The *Hox genes* are highly conserved between species and in *Drosophila* they are organized in two clusters; the Antennapedia complex and the Bithorax complex. Mutations in homeotic genes can cause switches in segment identity leading to severe homeotic transformations (Lawrence 1992, Ringrose and Paro 2004).

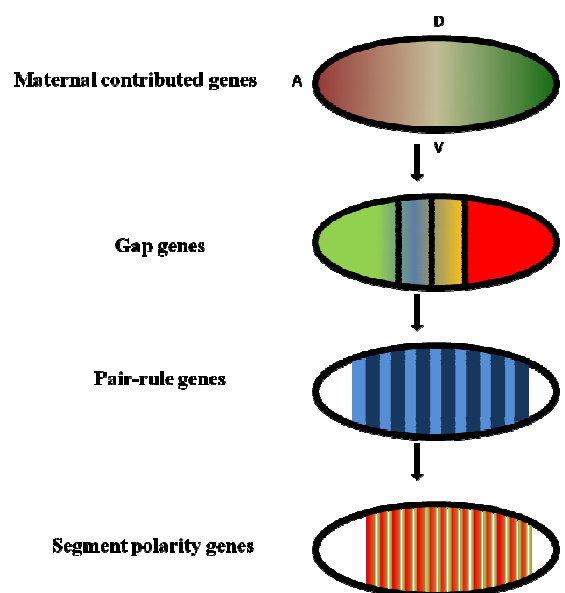


Figure 1. Some developmental genes in the *Drosophila* embryo.

CHROMATIN

EPIGENETICS

During development maintenance of silencing of specific genes is crucial for correct gene expression and cell identity. Every cell in the body has identical DNA but they face different fates in the cell differentiation process. Therefore, a system that can maintain the cell identity, keep the same gene expression pattern from mother cell to daughter cell, is important. The mechanism that helps the cell to pass the same gene expression pattern from generation to generation is called epigenetics. Epigenetics means heritable modification of gene expression without changing the actual DNA sequence. Such modifications can consist of methylation, acetylation or phosphorylation of histones that leads to chromatin remodelling and thereby changing the state of a gene. Polycomb group (PcG) genes and trithorax group (TrxG) genes are important players of the epigenetic system, where they work as silencers and activators in an antagonistic way (Breiling, Sessa and Orlando 2007, Ng and Gurdon 2008).

CHROMATIN COMPOSITION

The chromatin consists of DNA wrapped around histones and their associated proteins. 146 bp of the DNA is wrapped around every nucleosome unit and each nucleosome consists of two molecules each of the four core histones H2A, H2B, H3 and H4, and one molecule of the linker histone H1. These histones, together with the DNA, form a structure that resembles “beads-on-a-string” fibers. Chromatin remodelling is crucial for regulation of gene expression as the state of chromatin can decide the active or inactive state of genes (Francis 2009).

Approximately 30% of the *Drosophila* chromatin consists of heterochromatin that is specifically located at the centromeric regions and at telomeres, both regions where the DNA usually is transcriptionally inactive. These regions have high amounts of repetitive DNA and low gene density; however, there are genes that are essential for different biological functions located in heterochromatin. Also, the heterochromatic genes are usually bigger than euchromatic genes and contain larger introns.

Euchromatin is located between the heterochromatic arms on the chromosomes and consists of more transcriptionally active genes and has a higher gene density. Genes become inactivated when relocated into heterochromatin from euchromatin by a mechanism called position effect variegation (PEV). This effect can be suppressed by mutations in Suppressor of variegation (Su(var)) genes and enhanced by mutations in Enhancer of variegation (E(var)) genes (Schulze and Wallrath 2007). Euchromatic genes can also become relocated into telomeres causing their inactivation, this phenomena is called telomeric position effect (TPE) (Doheny, Mottus and Grigliatti 2008).

CHROMATIN REMODELLING

Chromatin remodeling decides whether or not a gene is in its active state and in order to guide this mechanism there are different epigenetic marks. These marks are usually modifications of the histones and occur at the tails and cores of side chains of specific residues (figure 2) (Mendenhall and Bernstein 2008).

In *Drosophila* ubiquitination of lysine 118 on H2A (H2AK119) is associated with transcriptional inactivation while ubiquitination of lysine 117 on H2B (H2BK117) is an activation mark (Müller and Verrijzer 2009, Scheuermann et al. 2010).

Methylation marks can be both activators and silencers; trimethylation of lysine 27 on histone 3 (H3K27) is a silencing mark in *Drosophila*, humans and mice while methylation of lysine 4 on histone 3 (H3K4) is an activation mark (Schuettengruber et al. 2007). Trimethylation of H1K26, H3K9 and H4K20 is also associated with gene repression (Kerppola 2009) and methylation of H3K36 and H3K79 are activation marks (Breiling et al. 2007).

Acetylation of histones can occur on several lysines on all histones and the acetylation process is regulated by histone acetylases (HATs) and histones deacetylases (HDACs). Acetylation is an activation mark often seen in promoter regions while transcriptionally inactive chromatin is hypoacetylated (Breiling et al. 2007). Acetylation of lysine 14 of histone 3 (H3K14Ac) is an activation mark that is associated with transcriptionally active chromatin (Sparmann and van Lohuizen 2006).

Phosphorylation of serine 10 of histone 3 (H3Ser10) is preferably done by both Aurora A and Aurora B. In addition Aurora B also phosphorylates Serine 28 on histone 3. This phosphorylation is associated with chromosome condensation; however in *Drosophila* chromosome condensation appears to be normal even when H3Ser10 phosphorylation is gone (Rea et al. 2000). The exact function of H3Ser10 phosphorylation is unclear, but when Aurora B phosphorylates H3Ser10, Heterochromatin Protein 1 (HP1) dissociates from heterochromatin (Fu et al. 2007).

All chromatic regions are not exclusively repressed or activated but can sometimes be subject to both. For example some sites that carry the H3K27me3 repression mark can also show H3K4me3 that promotes gene activation. These sites are defined as bivalent domains (Ku et al. 2008). The chromatin state can be varied and the H3K27me3 mark is connected with both repressed and balanced states. In this case a balanced state means that the gene expression is balanced between activation and inactivation. However, in a fully active state this methylation mark is gone and this lysine residue is acetylated. Chromatin can also be in a void state where neither activation nor repressing marks are seen (Schwartz et al. 2010).

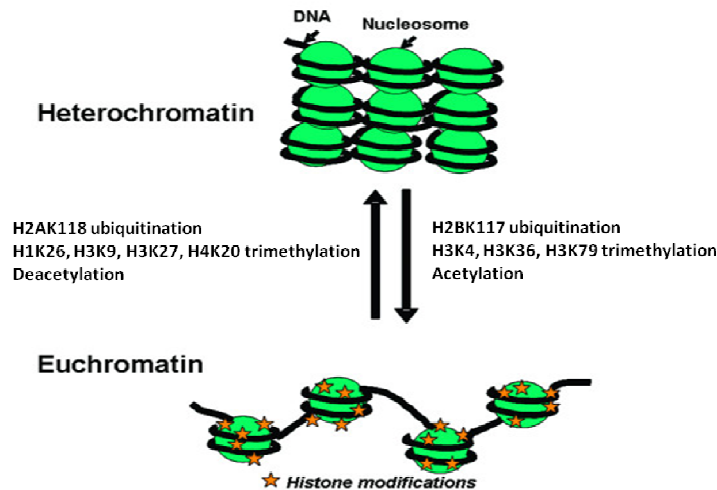


Figure 2. Chromatin remodeling marks decides the active or inactive state of genes. Modified from Adcock et al. 2006.

POLYCOMB GROUP GENES

POLYCOMB GROUP GENES

The first Polycomb group (PcG) gene, *Polycomb (Pc)*, was discovered in *Drosophila melanogaster* in 1947 by Lewis. The name Polycomb refers to its mutant phenotype where additional sex combs are present on the second and third leg pairs of males. It was later shown that this phenotype was caused by a loss of repression of the *Hox* gene *Sex combs reduced*, causing a weak homeotic transformation and this led to the conclusion that PcG genes act as silencers of *Hox* genes. The next PcG gene discovered, *extra sex combs (esc)* showed the same phenotype, and these additional sex combs became the trademark for a new family of gene silencers (Grimaud, Nègre and Cavalli 2006, Schulze and Wallrath 2007).

To date 18 PcG genes have been identified in *Drosophila* and they have been found to be involved in many biological processes, such as cell cycle control, X-inactivation, cancer, cell fate decision and stem cell differentiation, as they are important genome wide silencers (Ringrose and Paro 2004, Morey and Helin 2010). The PcG proteins mediate their repression by binding to trimethylated lysine 27 on histone 3 (H3K27me3) which is strongly associated with PcG silencing (Simon and Kingston 2009).

TRITHORAX GROUP GENES

The trithorax group (TrxG) proteins were identified in the beginning of the 1980s as antagonists of PcG proteins. When TrxG genes are mutated homeotic transformations caused by polycomb group mutants are suppressed. Also when the TrxG gene *trithorax* (*trx*) is mutated the expression of homeotic genes are reduced (Tie et al. 2009). At least four TrxG complexes have been identified in *Drosophila* embryo; a 2 MDa Brm complex, with the Brahma (Brm) protein and two other complexes of sizes 2 MDa and 500 kDa with the TrxG proteins Ash1 and Ash2, respectively. There is also a fourth complex of 1 MDa that contains the Trx protein, the Creb binding protein (Cbp) and the antiphosphatase Sbf 1 (Breiling et al. 2007, Ringrose and Paro 2007).

The Cbp protein mediates acetylation of H3K27 and thereby works as an antagonist of the PcG mediated H3K27 methylation (Tie et al. 2009). The Trx protein and Ash1 both contains SET domains that specifically methylate lysine 4 on histone 3 (H3K4), this mark is associated with active chromatin (Fedorova et al. 2008). Mutations in *trx* elevates the levels of H3K27me3 presenting another evidence for the antagonistic effect of TrxG proteins (Siebold et al. 2010). The TrxG gene *Brahma* shows sequence similarities to SWI1/SNF2 in yeast. SWI1/SNF2 functions as ATPase subunits in a chromatin remodelling complex that catalyses an active state by helping transcription factors and activators reach their target sites. The TrxG proteins Moira and Osa are also a part of this complex that is showing SW1/SNF2-like properties. The TrxG protein Ash1 interacts with histone acetyltransferases (HATs) that are associated with transcriptionally active euchromatin (Breiling et al. 2007)

POLYCOMB GROUP PROTEIN COMPLEXES

There are four Polycomb group protein complexes in *Drosophila* described to date; Polycomb repressive complex 1 (PRC1), Polycomb repressive complex 2 (PRC2), Pleiohomeotic repressive complex (PhoRC) and Polycomb repressive deubiquitinase (PR-DUB) (Schwartz and Pirrotta 2007, Sparmann and van Lohuizen 2006, Scheuermann et al. 2010).

PRC1

The four core proteins of PRC1 in *Drosophila* are Polycomb (PC), polyhomeotic (Ph), Posterior sex combs (Psc) and dRing. There are a number of other proteins that are associated with PRC1 like Zeste, Sex comb on midleg (Scm) and Tbp (Tata-binding protein)-associated factors like Tafs. Pc has a chromodomain that binds specifically to H3K27 trimethylation (H3K27me3). Ph contains both zinc-finger domains and a sterile alpha motif that mediates PcG binding (Morey and Helin 2010). dRing and Psc have RING domains that function as E3 ubiquitin ligase that mono-ubiquitylate lysine 119 on histone

2A (H2AK119ub) (Oktaba et al. 2008). All four core proteins are crucial for a functional PRC1 complex, although dRing and Psc alone also forms the core of the dRAF complex together with the histone lysine demethylase Kdm2 (Schwartz and Pirrotta 2007, Simon and Kingston 2009).

The same four core proteins are present in mammalian PRC1, but in both human and mouse the number of homologous genes are much higher, although they all contain the same conserved domain. Homologues of the members of PRC1 are found in zebrafish, sea urchin, frog and even nematodes have a functional homologue of PRC1 (Whitcomb et al. 2007).

PRC2

The core of PRC2 consists of four proteins; Enhancer of Zeste (E(z)), extra sex comb (Esc), Nucleosome remodelling factor (Nurf or p55) and Suppressor of zeste 12 (SU(Z)12). E(z) is the only protein with a known function, as it possesses a SET domain that specifically trimethylates H3K27. E(z) itself show low histone methyltransferase activity and the other components in the complex are crucial for this methylation (Nekrasov, Wild and Müller 2005). Both Nurf and Esc contain a WD40-domain which is a conserved domain ending with Tryptophan and Asparagine (WD) (Sparmann and van Lohuizen 2006). Esc is crucial for H3K27me₃ in the PRC2 complex, however loss of Esc does not result in severe homeotic phenotypes, as it can be replaced by a closely related protein called extra sex comb-like (ESCL) (Müller and Verrijzer 2009).

Two PRC2 complexes have been identified in *Drosophila* that both contain the four core proteins; a 600 kDa complex and a 1 MDa complex, the later also contains the histone deacetylase RPD3 and polycomb-like (PCL). These two complexes might be in equilibrium during the embryonic stage although their relationship is unclear. Rpd3 is also associated with the PRC2 complex in mammals (Tie et al. 2003).

There is a PRC2 complex in mammals that contains homologues of E(z), Su(z)12 and Esc. There are also PRC3 and PRC4 complexes that contain of isoforms of the mammalian homologue of Esc, Eed, and other proteins (Kerppola 2009). The mammalian homologue of PRC2 that consists of Suz12, Ezh2 and Eed shows, like the *Drosophila* complex, strong association with trimethylation of H3K27 (Kirmizis et al. 2004). Members of PRC2 are not only conserved among mammals, but also among frog, zebrafish, nematodes and plants (Whitcomb et al. 2007).

PHORC

The third *Drosophila* PcG complex consists of the pleiohometric protein (PHO) and its homologue pleiohometric-like (Phol). These two proteins are the only known PcG proteins that bind directly to DNA, but they are not only restricted to PcG complexes, as they are

also found in the chromatin remodelling complex INO80. The mammalian homologues of Pho are Ying Yang 1 (Yy1) and Ying Yang 2 (Yy2) that have both activating and repressing abilities. Pho has been shown to bind to silencing regions but Phol prefers to bind to active promoters (Schuettengruber et al. 2009). There is a third member of this complex called Scm-related gene containing four malignant brain tumour domains (Sfmbt). These MBT repeats bind specifically to monomethylated and dimethylated H3K9 and H4K20 (Schwartz and Pirrotta 2007, Schuettengruber et al. 2007). When both Pho and Phol are knocked down in *Drosophila* it results in lethality in the later developmental stages, but there is no absence of PcG proteins on the polytene chromosomes. This means that there have to be other ways for PcG proteins to bind to chromatin then via Pho and Phol (Schuettengruber et al. 2007).

PR-DUB

A newly identified fourth complex has been found in *Drosophila* called Polycomb repressive deubiquitinase (PR-DUB). This complex consists of Calypso and Additional sex combs (Asx) and is identified as a PcG complex, as it specifically binds PcG target genes showing overlapping binding pattern with both Pho and Ph. In the complex Calypso and Asx interact strongly and they specifically remove monoubiquitinations from H2A, but not H2B. (Scheuermann et al. 2010)

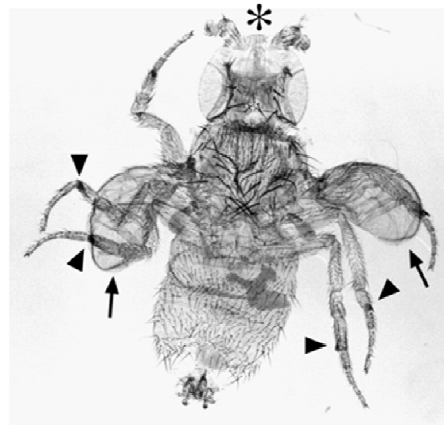
PRE

In *Drosophila*, PcG proteins act as transcriptional silencers by binding to specific DNA sequences called Polycomb Response Elements (PREs). For the TrxG protein these sequences are called Trithorax Response Elements (TREs), and, in this case, binding promotes transcriptional activation (Fujioka et al. 2008). PREs are important as epigenetic markers that can maintain gene-silencing generation after generation. The maintenance of this state is dynamic and crucial for maintaining the stem cell identity and promote differentiation. Several DNA motifs have been shown to be important for PRE function, among those are the binding sites of Pho, Zeste, GAF, Grh and Dsp1. The number of motifs that are present in the PRE varies and these motifs are not exclusive for PREs. It is hard to predict a PRE and localisation of all PREs still is not known in *Drosophila* (Ringrose and Paro 2007).

Predictions of PREs in the genome of *Drosophila* by bioinformatics estimate that approximately 150 PREs should exist. When two allelic copies of the PRE are present on homologous chromosome the effect of the PRE - whether it is repressive or activating is enhanced is called pairing-sensitive silencing (PSS) (Ringrose and Paro 2004, Fedorova et al. 2008). Until recently PREs had only been identified in flies, but now a mammalian PRE close to the *MafB* locus has been found in mouse (Sing et al. 2009).

PCG MEDIATED SILENCING

The exact mechanism behind the PcG mediated silencing is not known, however, a probable pathway has been proposed by several studies. The Pho and Phol proteins harbour sequence-specific DNA- binding properties and these PHO-RC complexes start the silencing process by binding to a specific PRE. The complex probably works as an anchoring complex for both the PRC1 and PRC2 complexes as it has shown interaction with both (Müller and Verrijzer 2009). Pho is present at most known PREs and indicating that the binding of Pho to the PRE is important for PcG-mediated silencing, although there is evidence for other DNA binding factors to be involved, such as GAGA-factor, Pipsqueak and Zeste. Also, as mentioned earlier knockdown of *Pho* and *Phol* does not remove PcG proteins from the DNA (Schuettengruber et al. 2009, Simon and Kingston 2009). The mammalian homologue of Pho Yy1, shows interaction with the PRC2 complex and recruits PRC2 to the PRE (Oktaba et al. 2008). When PRC2 is recruited the E(z) protein trimethylates H3K27 and this mark is then spread along chromatin to cover a larger region surrounding the gene to be silenced.. Binding of the PRC1 complex can be mediated via Pc chromodomain that has a high affinity for H3K27me3, but PRC1 can bind directly to the PRE via Pho as well. The exact binding properties of the dRAF complex are not known, but it probably ubiquitinase lysine 119 at histone 2A (H2AK119ub) and dimethylates H3K36 (Müller and Verrijzer 2009, Ballestar and Esteller 2008). In mammal embryonic pluripotent cells PcG proteins are stabilising the identity of the cell and when either PRC1 or PRC2 is depleted the cells still maintain its identity proposing a redundant effect between these two complexes (Leeb et al. 2010). The exact silencing mechanism is not known, but PcG has shown direct interaction with RNA polymerase. In this case RNA polymerase is still bound to the promoter, but it does not initiate transcription. PcG proteins has also been shown to interact with cofactors, the sumoylation of the transcriptional corepressor, CtBP, is increased when PcG is present, but exactly of this effects transcription is not known (Ringrose and Paro 2004). TrxG proteins are the activators of transcription and the antagonists of PcG proteins. Genes can bind both TrxG and PcG proteins putting them into a so-called balanced state, where the gene is not completely silenced although PcG proteins are present. When the levels of TrxG proteins are elevated the gene state is switched into a fully-active state (Schwartz et al. 2010).



$Su(z)12^5 / Su(z)12^3$

Figure 3. Knock down of *Su(z)12* in flies causes homeotic transformation.

SU(z)12

A novel Polycomb group gene was identified in 2001 in a screen for modifiers of the *zeste-white* interaction. This gene suppressed *zeste*'s repression of *white* and thereby its name *suppressor of zeste 12* (*Su(z)12*). Deletion of the *Su(z)12* function causes lethality in the first or second instar for both homozygous and hemizygous conditions. Flies with less severely mutated genes can survive until the late pupae stage, but they show homeotic transformations that are characteristic of Polycomb group gene mutations; sex combs on second and third leg pairs, antenna partially transformed into legs and smaller wings, partially transformed into haltere (Figure 3). When the maternal contribution of *Su(z)12* is knocked down severe homeotic transformation occurs in the embryo's all abdominal and thoracic segments, and some of the head segments are transformed into the eighth abdominal segment (Figure 4). The homeotic transformations as well as the fact that *Su(z)12* is crucial for correct maintenance of silencing of *Ubx* identified *Su(z)12* as a Polycomb group gene (Birve et al. 2001).

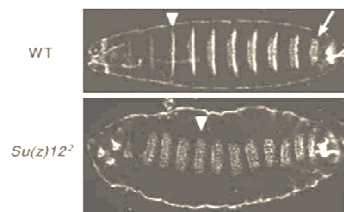


Figure 4. Knock down of maternally contributed *Su(z)12* all all abdominal and thoracic segments, and some of the head segments are transformed into the eighth abdominal segment.

SU(Z)12 is one of the four core proteins in PRC2 and also associates with PCL and RPD3 (Tie et al. 2003). The trimethylation of H3K27 is characteristic for PRC2, and SU(Z)12 is a crucial component of this complex, as trimethylation of H3K27 fails when SU(Z)12 is absent, even when the histone methyl transferase E(Z) is present. However, the mechanism behind this H3K27me3 and other functions of SU(Z)12's is not known (Chen, Birve and Rasmuson-Lestander 2008). The mammalian SUZ12 has an important role in H3K9me3, as knockdown of SUZ12 shows a significant reduction in H3K9me3 (de la Cruz et al. 2007).

The *Su(z)12* gene is alternatively spliced into two transcripts; a 4.1 kb mRNA called *Su(z)12A* and a 3.7 kb mRNA called *Su(z)12B*. These transcripts are translated into two isoforms; a 95 kDa SU(Z)12A protein and 100 kDa SU(Z)12B protein. These isoforms show a high sequence similarity of 95% and only differ in the C-terminal end (Figure 4). These two transcripts are present at different levels during *Drosophila* development.

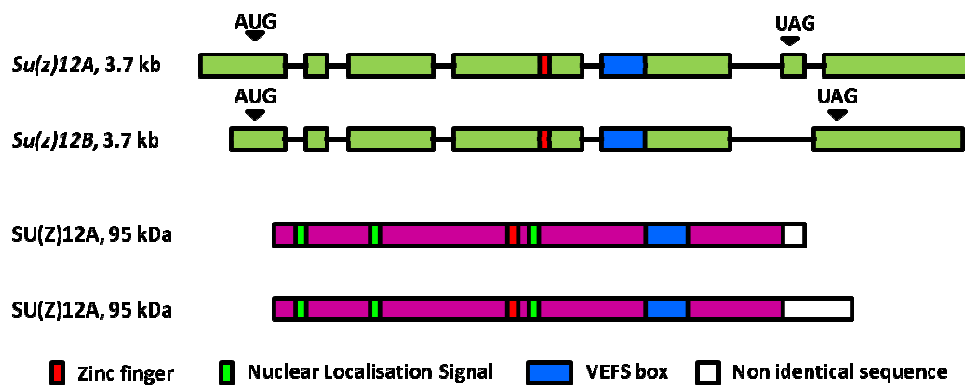


Figure 5. There are two isoforms of SU(Z)12.

PCG AND CANCER

Since PcG proteins are genome wide silencer they exert their effect on many biological processes, including regulation of factors involved in cell cycle control and cancer (Martinez and Cavalli 2006).

The first and most well-known PcG gene that is shown to be involved in cancer is *Bmi1* that is the mouse homologue of *Psc*. *BMI1* is a proto-oncogene; the encoded protein represses the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) locus. The *CDKN2A* locus encodes the tumour suppressors p16^{INK4A} that activates the retinoblastoma (RB) pathway, and p14^{Arf} which induces p53 by inhibition of its negative regulator Mdm2 (Sparmann and van Lohuizen 2006). Overexpression of *BMI1* is seen in several human cancers, such as leukaemia, mantle cell lymphoma, neuroblastoma and lung cancer. The PRC2 component EZH2 is overexpressed in metastatic melanoma and endometrium, prostate and breast cancers. The prognosis for a patient with overexpression of EZH2 in breast cancer is poor whereas overexpression of BMI1 is associated with good prognosis. The human

homologue of SU(Z)12 is also upregulated in colon, breast and liver cancers (Pietersen et al. 2008, Hormaeche and Licht 2007).

There is also a connection between PcG proteins and the oncogene *MYC* as *MYC* is inhibited by BMI1 and *MYC* autorepression is disrupted when Pc is absent (Sparmann and van Lohuizen 2006, Khan, Shover and Goodliffe 2009).

AURORA KINASES

INTRODUCTION

Aurora kinases are a group of highly conserved serine/threonine kinases that were first identified in a screen for *Drosophila melanogaster* mutants defective in spindle pole behaviour (Glover et al. 1995). They are crucial for many processes during mitosis and since mutations lead to formation of monopolar spindles, which resemble the light phenomenon, aurora borealis, the genes were named Aurora (Fu et al. 2007).

Aurora genes have been identified in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Arabidopsis* and *Xenopus*. Mammals have three paralogues of Aurora; Aurora A, Aurora B and Aurora C and they have high sequence similarity. The encoded proteins all consist of three distinct domains; the catalytic domain, the N-terminal domain and the C-terminal domain (Figure 6). The catalytic domain is highly similar between the three kinases and the C-terminal domain and N-terminal is less conserved. Despite their high sequence similarity the three Auroras have distinctly functions and locations during mitosis; this is explained by the fact that most regulatory motifs are seen in the c-terminal and N-terminal (Carmena and Earnshaw 2003, Fu et al. 2007, Bolanos-Garcia 2005). Aurora A and Aurora B also possess the KEN motif, this motif is recognition signal for Cdh1-dependent anaphase-promoting complex (APC). All three Auroras also have negative regulators in the N-terminal, the so-called Destruction boxes (D-boxes), (Bolanos-Garcia 2005). Aurora A and Aurora B are also active at different locations and time periods during mitosis (Pohl et al. 2010).

AURORA A

Aurora A is located at the centrosome and its activation is leading to centrosome maturation and, later, spindle formation. Aurora A has also been shown to have an important role in centrosome separation. In *Drosophila*, treatment with Aurora A RNA interference (RNAi) treatment resulted in asymmetric centrioles and in *C. elegans* the centrosomes are separated later collapsed (Carmena and Earnshaw 2003). Aurora A is also crucial for G2/M transit as RNAi knockdown of Aurora A in HeLa cells results in G2/M

arrest and leads to apoptosis (Fu et al. 2007). When Aurora A is knocked down in *Drosophila* spindle formation is altered leading to abnormally organized poles (Giet et al. 2002).

AURORA B

Aurora B forms the chromosomal passenger complex (CPC) with the Inner Centrosomal protein (INCENP), Survivin and Borealin and is thereby defined as a chromosomal passenger protein (Delacour-Larose, Vu and Molla 2009). Aurora B is localised on the centromeres from prophase until late metaphase, and it relocates to the spindle midzone from anaphase on. This relocation requires INCENP and Survivin (Andrews et al. 2003). The CPC is conserved from yeast to humans and has distinct location and many roles during mitosis like chromosome modifications, regulation and assembly of the central spindle, mitotic check-point function and cytokinesis. Aurora B is regulated in a feedback loop, as it phosphorylates INCENP and INCENP is then activating Aurora B (Vagnarelli and Earnshaw 2004, Chang et al. 2006, Ke et al. 2003). Depletion of Aurora B results in severe effects in mitosis as chromosomes become poorly condensed, unaligned and cytokinesis is delayed or incomplete. This incomplete cytokinesis leads to large cells with several nuclei and polyploidy (Adams, Carmena and Earnshaw 2001, Giet and Glover 2001).

Aurora B is conserved between species and is found in *S. cerevisiae* (Ip11), *S. pombe* (Ark1), *C. elegans* (AIR-2), *M. musculus* (AIM-1), *X. laevis* (X-Aurora B) and human (Aurora B). *Drosophila* has two Aurora kinases; Aurora A and Aurora B (Vagnarelli and Earnshaw 2004).

The *Drosophila* homologue of Aurora B; Ip11-like Aurora kinase (ial) was discovered in a cDNA screen using the mouse serine/threonine kinase gene Ayk1 as a hybridization probe (Reich et al. 1999).

AURORA C

Little is known about Aurora C and it is only found in mammals. Aurora C kinases have shown an especially high expression in testis and the protein is located at the centrosomes from anaphase to telophase (Carmena and Earnshaw 2003, Bishop and Schumacher 2002). This kinase also binds to INCENP and Survivin, and is thereby defined as a chromosomal passenger protein. Aurora C has been shown to partially rescue the knockdown phenotype of Aurora B, suggesting an overlapping function between Aurora B and Aurora C, although the binding affinity of Aurora C for INCENP is lower than that of Aurora B (Fu et al. 2007, Bolanos-Garcia 2005).

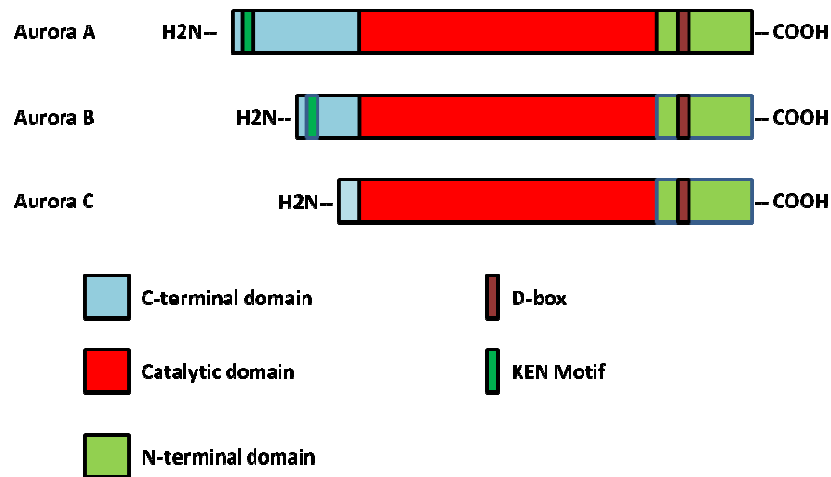


Figure 6. Aurora A, B and C have high sequence similarity.

AURORA B AND CHROMATIN

During mitosis the chromosome condensation is accompanied by phosphorylation of H1 and H3, and Aurora B is strongly correlated with these phosphorylations (Giet and Glover 2001). The hallmark of chromosome condensation is phosphorylation of Serine 10 on histone 3 (H3Ser10) and Aurora kinases specifically mediate this H3Ser10 phosphorylation (Huang et al. 2006). However, in *Drosophila* chromosome condensation appear to be normal when Aurora B is depleted and thereby H3Ser10 phosphorylation is absent (Vagnarelli and Earnshaw 2004). Both Aurora A and Aurora B can mediate H3Ser10 phosphorylation and Aurora B can also phosphorylate Serine 28 on histone 3 (H3Ser28) (Ke et al. 2003). The exact function of H3Ser10 phosphorylation is unclear, but when Aurora B phosphorylates H3Ser10, Heterochromatin Protein 1 (HP1) dissociates from heterochromatin. However, the function of HP1 in mitosis is not known (Fu et al. 2007). Aurora B is essential for phosphorylation of threonine 119 on histone 2A (H2AT119pho) in *Drosophila* (Brittle et al. 2007).

Aurora B also phosphorylates non-histone proteins, but which are correlated to chromatin, such as DNA topoisomerase II and the chromatin remodelling factor, ISWI (Andrews et al. 2003).

AURORA KINASES AND CANCER

Aurora kinases are overexpressed in many human cancers and overexpression of Aurora A leads to transformation of NIH 3T3 cells and Rat1 fibroblasts. Transplantation of these cells into nude mice leads to formation of tumours. However, overexpression of Aurora A directly in mice does not result in tumour formation, meaning that there are probably more factors involved to cause growth of tumours (Fu et al. 2007). Aurora A is localised in a region on chromosome 20q13 that is frequently amplified in tumours. When Aurora is overexpressed centromere numbers are increased leading to aneuploidy and transformation (Andrews et al. 2003).

The Aurora B gene is localised on chromosome 17p13, a region not specifically amplified in tumour. Overexpression on Aurora B is associated with colorectal cancer and low Aurora B expression is correlated to high probability of survival compared to patients with high Aurora B expression (Pohl et al. 2010). Overexpression of the other components in CPC is also seen in cancers, eg. Survivin and INCENP as well as Aurora B show high expression in head and neck squamous cell carcinoma. However, how this high expression of Survivin is correlated to the function of CPC is unclear. Overexpression of Survivin is correlated with Aurora B and high expression of both Survivin and Aurora B are correlated with manifestation of malignant tumours. When both Survivin and Aurora B are knocked down tumour formation and cell growth are inhibited (Qi et al. 2010, Ke et al. 2003). High expression of Aurora B is correlated with hepatocellular carcinoma and inhibition of Aurora B expression with the selector inhibitor AZD1152-HQPA decreased growth of tumours and specially lead to apoptosis of liver tumours (Aihara et al. 2009). This Aurora B inhibitor also showed effects in breast cancer cell lines, where inhibition of Aurora B led to apoptosis and thereby suppression tumour growth (Gully et al. 2010). ZM447439, another inhibitor of both Aurora A and Aurora B, has been shown to inhibit growth and induce apoptosis in leukaemia cells. The phenotype of HeLa cells after treatment with ZM447439 was similar to the phenotype that appear when Aurora B is knocked down, indicating that Aurora B might be the most potent therapeutic target (Ikezoe et al. 2009).

The Aurora C gene is located in a region on chromosome 19q13.4 that is highly associated with translocation, deletion and amplification in several cancers. But the exact correlation between cancer and Aurora C is not known (Ke et al. 2003).

MYC

INTRODUCTION

c-Myc belongs to a family of basic helix-loop-helix leucine-zipper (bHLH/LZ) transcription factors. This family of transcription factors are associated with basic processes like cell growth and cell proliferation, as well as triggering of apoptosis and inhibition of differentiation (de la Cova and Johnston 2006, Secombe, Pierce and Eisenman 2004). Therefore, c-Myc is a highly potent oncogene and is deregulated in approximately 70% of human cancers. Approximately 1000 genes are listed as Myc targets and they are involved in various processes, such as cell growth and cell cycle progression (Hulf et al. 2005).

c-Myc forms a hetero-dimer with another bHLH/LZ protein Max and together they act as a sequence-specific transcription factor targeting E-boxes. Max can also bind to Mad and form a complex that antagonizes Myc binding (Schreiber-Agus et al. 1997).

MYC IN DROSOPHILA

Drosophila has one *Myc* gene, dMyc, that is encoded by the *diminutive* (*dm*) locus (Pierce et al. 2008). This gene was identified as early as the 1930s and named because of its small body size. dMyc is closely related to c-Myc, as it contains a bHLH/LZ domain in the C-terminal end, the domain mediates DNA-binding. The N-terminus is less conserved but two motifs are highly conserved among vertebrates; Myc-box I (MBI) and Myc-box II (MBII). MBII, but not MBI, is found in dMyc (de la Cova and Johnston 2006). The *Drosophila* dMyc also associates with the ortholog dMax and together they stimulate transcription (Johnston et al. 1999, Orian et al. 2003).

In *Drosophila*, dMyc is mostly associated with size and growth regulation. Knock down of *dm* results in endoreplication and growth arrest in early development (Pierce et al. 2008). Knock down of dMyc in *Drosophila* results in smaller flies and smaller cells, indicating that dMyc is essential for cell growth. Cells that have higher dMyc-expression than surrounding cells out-compete them, leading to apoptosis for cells with lower expression (Secombe et al. 2004, de la Cova et al. 2004). Even though the knock down of dMyc results in smaller flies, the flies themselves seem to be normal (Wu and Johnston 2010). Transcriptional targets of dMyc are like in vertebrates characterized by the conserved E-box in the promoter region (Hulf et al. 2005). Also the ability to induce apoptosis is conserved between humans and flies (Montero, Müller and Gallant 2008).

AURORA AND MYC

Aurora B is highly expressed in aggressive forms of non-Hodgkin lymphomas, such as Burkitt lymphoma and inhibition of Aurora B by the AZD1152 inhibitor results in apoptosis by activation of the caspase pathway (Ikezoe et al. 2009).

c-Myc specifically up-regulates Aurora A and Aurora B in Myc-driven lymphomas, Aurora A by c-Myc directly binding to its E-boxes inducing transcription and Aurora B by indirect regulation. When E μ -Myc cell lines are treated with the Aurora B inhibitor AS703569, cells accumulate in G2/M phase and then apoptosis is induced. Cells accumulating higher content of DNA are also increased and phosphorylation of H3Ser10 is decreased. When mice with E μ -Myc-induced tumours were treated with AS703569, tumorigenesis was significantly delayed. Even already formed tumours were reduced greatly in size with treatment of this Aurora inhibitor. This proves that the Aurora B inhibitor AS703569 is a potent therapeutic drug for Myc-driven lymphomas and also establishes a direct correlation between c-Myc and Aurora B (den Hollander et al. 2010).

AIMS

- Do the two isoforms of Su(z)12 possess different function *in vitro* and *in vivo*?
- Is the c-Myc and Aurora B relationship conserved in *Drosophila*?

RESULTS AND DISCUSSION

The two isoforms of SU(Z)12 are differently expressed during development

The levels of the SU(Z)12 protein isoforms are clearly different at different developmental stages and the reason for this might be that they do have diverse functions during development. In the earlier stages both isoforms are present at similar levels, however during larval and pupal stages Su(z)12A is expressed at higher levels than Su(z)12B. In the adult fly the level of Su(z)12B is higher than Su(z)12A, both in male and female. Different developmental processes occur in different stages and therefore some proteins might be more important at different stages than others. Early development is very complex and strictly regulated, maybe both isoforms are expressed at the same level to secure the right developmental pattern. This theory propose a redundancy effect for Su(z)12A and Su(z)12B in early development. This redundancy effect might be gone in larvae and pupae and maybe the isoforms have other roles during these stages.

A similar scenario is seen with PRC2 component ESC and the highly similar protein ESCL. ESC is expressed during early embryonic stages, but after that only ESCL is expressed, and ESCL continues to be the only protein that is expressed during all developmental stages. These proteins show a redundancy effect as knock down of one of them does not lead to severe phenotype, this is only seen when both is knocked down. However, knock down of only one of the isoforms of SU(Z)12 is difficult hence their high sequence similarity.

The two isoforms of SU(Z)12 show differences in interaction with the core components of PRC2

The sequence difference between the two isoforms is small, however even small differences in sequence can cause conformation changes in the protein and this can affect the protein's binding properties. Since Su(z)12A does not interact with Nurf directly, while Su(z)12B does, the small difference between the two isoforms probably creates a difference in the proteins capability of binding to other proteins. However, since Su(z)12A does interact with Esc, just like Su(z)12B, this indicates that Su(z)12A might still be a part of PRC2. The direct interaction with Nurf might not be necessary for forming the PRC2 complex, since both binding to nucleosome and H3K27 methylation is possible with only SU(Z)12, E(Z) and ESC.

The isoforms might also belong to different complexes or at least not identical complexes. It is unknown if Su(z)12 is found in more complexes than PRC2, and one possibility is that one or both isoforms are participating in other complexes giving them different roles.

The two isoforms do interact with each other except for the single copy of Su(z)12A that does not bind to Su(z)12B. The reason for this can be that dimers or multimers of Su(z)12 are present in PRC2. In *Drosophila* two PRC2 complexes are found; one of 1 MDa and one of 600 kDa. Even if Rpd3 and Pcl are associated with the four core components the combined size of the subunits is far from 600 kDa and 1 MDa. So either unknown proteins are associated with PRC2, or multimers of the already known proteins are present. This means that several copies of SU(Z)12A might be crucial for binding SU(Z)12B, because when a single copy of SU(Z)12B-GST is present on the bead SU(Z)12A-His binds. There might be still unknown changes to PRC2, leading of replacement of one isoforms with another.

There could also be a competition between the two isoforms for a place in PRC2. One theory is that the presence of Su(z)12A can lead to formation of an inactive complex since it does not bind to Nurf. So there might be a balance or equilibrium between the two isoforms during development, controlling the activity of PRC2. As mentioned earlier, PRC2 still works without Nurf, but the activity is not as good as with Nurf.

Overexpression of SU(Z)12B causes lethality

The overexpression of Su(z)12B by Actin5C-GAL4 driver causes lethality and homeotic transformations. The most common phenotype is extra sex combs on the second and third leg pair of the male. Adequate expression of Su(z)12B is important for correct maintenance of silencing and even though expression of Su(z)12B is not at the same levels as Su(z)12A during larval and pupal stages it still is very important. Therefore, whether or not overexpression of Su(z)12A can cause the same severe phenotypes is very interesting. Although overexpression of PcG proteins in many cases result in the same phenotype, the comparison of the isoforms could still be interesting. Could overexpression of SU(Z)12A lead to more or less severe phenotypes?

Knock out of ial causes severe phenotypes in *Drosophila*

Aurora B has been shown to have a crucial role in mitotic progression and knock down of Aurora B results in severe phenotypes in mammals. When the *Drosophila* homologue ial is knocked down by using an UAS-ial^{RNAi} construct crossed with Actin5C-GAL4 driver, the flies die before third larval instar, this clearly shows that ial is crucial for survival. Knock out of Ial with RNAi construct crossed to eye and wing drivers, respectively, show severe phenotypes in those fly tissues with a very high penetrance. The eyes were smaller and ommatidia were missing and the wings became shriven and damaged. This clearly shows that Ial knock out causes severe damage and the probable reason for this is failure of mitotic progression or cytokinesis.

Knock out of ial with a vestigial driver showed that the cells in wing discs become larger and fewer and that their nuclei also are very large. This is confirmed by earlier studies where knock out of Ial causes endoreplication and polyploidy and thereby leading to

bigger cells. Usually these cells have several nuclei but this has not been confirmed in my study, although such result is probable, since the cells are bigger. Also, knock down of *ial* removes the phosphorylation of Serine 10 on histone 3 (H3Ser10), this is clearly shown by immunostaining in wing discs as the H3Ser10 phosphorylation is gone where *ial* is absent.

Overexpression of dMyc results in few phenotypes

When UASdm transgenic fly lines were crossed to different tissue-specific GAL4 drivers, none of the crosses resulted in visible phenotypes in the adult fly. But when dMyc was overexpressed with Actin5C-GAL4 driver, the flies died before reaching 3rd instar larval stage. Also overexpression of dMyc with the 71B-GAL4 driver resulted in a wing phenotype at 25°C and lethality at 29°C. Overexpression of dMyc with drivers that are expressed in the several tissues or in the entire fly, leads to lethality, meaning that adequate dMyc expression is essential for survival in *Drosophila*.

The c-Myc Aurora B axis is conserved in *Drosophila*

The association between c-Myc and Aurora B has been studied earlier and this showed that c-Myc induces Aurora B expression. However, when Aurora B is inhibited, so is also growth and tumorigenesis in Myc-induced lymphomas. Overexpression of c-Myc is the cause of many cancers, such as Burkitts lymphoma and therefore Aurora B is a good therapeutic target. This study presents evidence that the Aurora B and c-Myc interplay is conserved in *Drosophila*.

Knock down of *Ial* with a daughterless-GAL4 driver resulted in adult flies with lower weight than wild type control. Overexpression of c-Myc with daughterless Gal4 driver also leads to lower weight in the adult fly. But when a transgenic fly that combined both Aurora B knock down and c-Myc overexpression was created, there was no longer any significant decrease in the adult weight compared to the wild type. The male's weight increased significantly and the female's weight became even bigger than the wild type. The conservation of such relationship in the fly shows the importance of this association, however the exact mechanism behind it is not known.

In *Drosophila*, c-Myc, like in human and mouse, is associated with cell growth and proliferation and prevention of important growth factors can lead to growth inhibition and mitotic arrest. When Aurora B was depleted in Eu-myc mice cells, the cells first showed polyploidy leading to apoptosis. It is likely that this happens in *Drosophila* too, however, this has to be studied further.

CONCLUSIONS

- Su(z)12A and Su(z)12B are expressed at different levels during development.
- Su(z)12A does not interact with PRC2 component Nurf, unlike Su(z)12B that binds to Nurf *in vitro*. A single copy of Su(z)12A does not interact with Su(z)12B.
- Overexpression of Su(z)12B leads to lethality. Result of overexpression of SU(Z)12A is still unknown.
- Knock out of *ial* causes severe phenotypes in the fly with tissue-specific drivers and lethality with drivers that are more widely expressed in the fly.
- Overexpression of dMyc does not result in any phenotypes with tissue-specific drivers and lethality with drivers that more widely expressed in the fly.
- The relationship between c-Myc and Aurora B is probably conserved in *Drosophila*, as knock down of *ial* and overexpression of dMyc result in flies with larger weight; this is not seen in flies with knock down of *ial* and overexpression of dMyc respectively.

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REFERENCES

- Adams, R., M. Carmena & W. Earnshaw (2001) Chromosomal passengers and the (aurora) ABCs of mitosis. *Trends Cell Biol*, 11, 49-54.
- Adcock, I., P. Ford, K. Ito & P. Barnes (2006) Epigenetics and airways disease. *Respir Res*, 7, 21.
- Aihara, A., S. Tanaka, M. Yasen, S. Matsumura, Y. Mitsunori, A. Murakata, N. Noguchi, A. Kudo, N. Nakamura, K. Ito & S. Arii (2009) The selective Aurora B kinase inhibitor AZD1152 as a novel treatment for hepatocellular carcinoma. *J Hepatol*.
- Andrews, P., E. Knatko, W. Moore & J. Swedlow (2003) Mitotic mechanics: the auroras come into view. *Curr Opin Cell Biol*, 15, 672-83.
- Ashburner, M. 1989. *Drosophila : a laboratory handbook*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.
- Ballestar, E. & M. Esteller (2008) Epigenetic gene regulation in cancer. *Adv Genet*, 61, 247-67.
- Birve, A., A. Sengupta, D. Beuchle, J. Larsson, J. Kennison, Rasmuson-Lestander A & J. Müller (2001) Su(z)12, a novel Drosophila Polycomb group gene that is conserved in vertebrates and plants. *Development*, 128, 3371-9.
- Bishop, J. & J. Schumacher (2002) Phosphorylation of the carboxyl terminus of inner centromere protein (INCENP) by the Aurora B Kinase stimulates Aurora B kinase activity. *J Biol Chem*, 277, 27577-80.
- Bolanos-Garcia, V. (2005) Aurora kinases. *Int J Biochem Cell Biol*, 37, 1572-7.
- Breiling, A., L. Sessa & V. Orlando (2007) Biology of polycomb and trithorax group proteins. *Int Rev Cytol*, 258, 83-136.
- Brittle, A., Y. Nanba, T. Ito & H. Ohkura (2007) Concerted action of Aurora B, Polo and NHK-1 kinases in centromere-specific histone 2A phosphorylation. *Exp Cell Res*, 313, 2780-5.
- Carmena, M. & W. Earnshaw (2003) The cellular geography of aurora kinases. *Nat Rev Mol Cell Biol*, 4, 842-54.
- Chang, C., S. Goulding, R. Adams, W. Earnshaw & M. Carmena (2006) Drosophila Incenp is required for cytokinesis and asymmetric cell division during development of the nervous system. *J Cell Sci*, 119, 1144-53.
- Chen, S., A. Birve & A. Rasmuson-Lestander (2008) In vivo analysis of Drosophila SU(Z)12 function. *Mol Genet Genomics*, 279, 159-70.
- de la Cova, C., M. Abril, P. Bellosta, P. Gallant & L. Johnston (2004) Drosophila myc regulates organ size by inducing cell competition. *Cell*, 117, 107-16.
- de la Cova, C. & L. Johnston (2006) Myc in model organisms: a view from the flyroom. *Semin Cancer Biol*, 16, 303-12.
- de la Cruz, C., A. Kirmizis, M. Simon, K. Isono, H. Koseki & B. Panning (2007) The polycomb group protein SUZ12 regulates histone H3 lysine 9 methylation and HP1 alpha distribution. *Chromosome Res*, 15, 299-314.
- Delacour-Larose, M., H. Vu & A. Molla (2009) Aurora B kinase, an immobile passenger! *Cell Cycle*, 8, 3600-1.
- den Hollander, J., S. Rimpi, J. Doherty, M. Rudelius, A. Buck, A. Hoellein, M. Kremer, N. Graf, M. Scheerer, M. Hall, A. Goga, N. von Bubnoff, J. Duyster, C. Peschel, J. Cleveland, J. Nilsson & U. Keller (2010) Aurora kinases A and B are up-regulated by Myc and are essential for maintenance of the malignant state. *Blood*.

Doheny, J., R. Mottus & T. Grigliatti (2008) Telomeric position effect--a third silencing mechanism in eukaryotes. *PLoS One*, 3, e3864.

Fedorova, E., N. Sadoni, I. Dahlsveen, J. Koch, E. Kremmer, D. Eick, R. Paro & D. Zink (2008) The nuclear organization of Polycomb/Trithorax group response elements in larval tissues of *Drosophila melanogaster*. *Chromosome Res*, 16, 649-73.

Francis, N. (2009) Mechanisms of epigenetic inheritance: copying of polycomb repressed chromatin. *Cell Cycle*, 8, 3513-8.

Fu, J., M. Bian, Q. Jiang & C. Zhang (2007) Roles of Aurora kinases in mitosis and tumorigenesis. *Mol Cancer Res*, 5, 1-10.

Fujioka, M., G. Yusibova, J. Zhou & J. Jaynes (2008) The DNA-binding Polycomb-group protein Pleiohomeotic maintains both active and repressed transcriptional states through a single site. *Development*, 135, 4131-9.

Giet, R. & D. Glover (2001) *Drosophila* aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J Cell Biol*, 152, 669-82.

Giet, R., D. McLean, S. Descamps, M. Lee, J. Raff, C. Prigent & D. Glover (2002) *Drosophila* Aurora A kinase is required to localize D-TACC to centrosomes and to regulate astral microtubules. *J Cell Biol*, 156, 437-51.

Glover, D., M. Leibowitz, D. McLean & H. Parry (1995) Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell*, 81, 95-105.

Graf, U., N. Van Schaik & F. E. Wu\rngler. 1992. *Drosophila genetics : a practical course*. Berlin ; New York: Springer-Verlag.

Grimaud, C., N. Nègre & G. Cavalli (2006) From genetics to epigenetics: the tale of Polycomb group and trithorax group genes. *Chromosome Res*, 14, 363-75.

Gully, C., F. Zhang, J. Chen, J. Yeung, G. Velazquez-Torres, E. Wang, S. Yeung & M. Lee (2010) Antineoplastic effects of an Aurora B kinase inhibitor in breast cancer. *Mol Cancer*, 9, 42.

Hormaeche, I. & J. Licht (2007) Chromatin modulation by oncogenic transcription factors: new complexity, new therapeutic targets. *Cancer Cell*, 11, 475-8.

Huang, X., A. Kurose, T. Tanaka, F. Traganos, W. Dai & Z. Darzynkiewicz (2006) Sequential phosphorylation of Ser-10 on histone H3 and ser-139 on histone H2AX and ATM activation during premature chromosome condensation: relationship to cell-cycle phase and apoptosis. *Cytometry A*, 69, 222-9.

Hulf, T., P. Bellosta, M. Furrer, D. Steiger, D. Svensson, A. Barbour & P. Gallant (2005) Whole-genome analysis reveals a strong positional bias of conserved dMyc-dependent E-boxes. *Mol Cell Biol*, 25, 3401-10.

Ikezoe, T., T. Takeuchi, J. Yang, Y. Adachi, C. Nishioka, M. Furihata, H. Koeffler & A. Yokoyama (2009) Analysis of Aurora B kinase in non-Hodgkin lymphoma. *Lab Invest*, 89, 1364-73.

Johnston, L., D. Prober, B. Edgar, R. Eisenman & P. Gallant (1999) *Drosophila* myc regulates cellular growth during development. *Cell*, 98, 779-90.

Ke, Y., Z. Dou, J. Zhang & X. Yao (2003) Function and regulation of Aurora/Ipl1p kinase family in cell division. *Cell Res*, 13, 69-81.

Kerppola, T. (2009) Polycomb group complexes--many combinations, many functions. *Trends Cell Biol*, 19, 692-704.

Khan, A., W. Shover & J. Goodliffe (2009) Su(z)2 antagonizes auto-repression of Myc in *Drosophila*, increasing Myc levels and subsequent trans-activation. *PLoS One*, 4, e5076.

Kirmizis, A., S. Bartley, A. Kuzmichev, R. Margueron, D. Reinberg, R. Green & P. Farnham (2004) Silencing of human polycomb target genes is associated with methylation of histone H3 Lys 27. *Genes Dev*, 18, 1592-605.

Kohler, R. E. 1994. *Lords of the Fly : Drosophila genetics and the experimental life*. Chicago: Univ. Chicago P.

Ku, M., R. Koche, E. Rheinbay, E. Mendenhall, M. Endoh, T. Mikkelsen, A. Presser, C. Nusbaum, X. Xie, A. Chi, M. Adli, S. Kasif, L. Ptaszek, C. Cowan, E. Lander, H. Koseki & B. Bernstein (2008) Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet*, 4, e1000242.

Lawrence, P. A. 1992. *The making of a fly : the genetics of animal design*. Oxford: Blackwell Scientific.

Leeb, M., D. Pasini, M. Novatchkova, M. Jaritz, K. Helin & A. Wutz (2010) Polycomb complexes act redundantly to repress genomic repeats and genes. *Genes Dev*, 24, 265-76.

Martinez, A. & G. Cavalli (2006) The role of polycomb group proteins in cell cycle regulation during development. *Cell Cycle*, 5, 1189-97.

Mendenhall, E. & B. Bernstein (2008) Chromatin state maps: new technologies, new insights. *Curr Opin Genet Dev*, 18, 109-15.

Montero, L., N. Müller & P. Gallant (2008) Induction of apoptosis by Drosophila Myc. *Genesis*, 46, 104-11.

Morey, L. & K. Helin (2010) Polycomb group protein-mediated repression of transcription. *Trends Biochem Sci*.

Müller, J. & P. Verrijzer (2009) Biochemical mechanisms of gene regulation by polycomb group protein complexes. *Curr Opin Genet Dev*, 19, 150-8.

Nekrasov, M., B. Wild & J. Müller (2005) Nucleosome binding and histone methyltransferase activity of Drosophila PRC2. *EMBO Rep*, 6, 348-53.

Ng, R. & J. Gurdon (2008) Epigenetic inheritance of cell differentiation status. *Cell Cycle*, 7, 1173-7.

Oktaba, K., L. Gutiérrez, J. Gagneur, C. Girardot, A. Sengupta, E. Furlong & J. Müller (2008) Dynamic regulation by polycomb group protein complexes controls pattern formation and the cell cycle in Drosophila. *Dev Cell*, 15, 877-89.

Orian, A., B. van Steensel, J. Delrow, H. Bussemaker, L. Li, T. Sawado, E. Williams, L. Loo, S. Cowley, C. Yost, S. Pierce, B. Edgar, S. Parkhurst & R. Eisenman (2003) Genomic binding by the Drosophila Myc, Max, Mad/Mnt transcription factor network. *Genes Dev*, 17, 1101-14.

Pierce, S., C. Yost, S. Anderson, E. Flynn, J. Delrow & R. Eisenman (2008) Drosophila growth and development in the absence of dMyc and dMnt. *Dev Biol*, 315, 303-16.

Pietersen, A., H. Horlings, M. Hauptmann, A. Langerød, A. Ajouaou, P. Cornelissen-Steijger, L. Wessels, J. Jonkers, M. van de Vijver & M. van Lohuizen (2008) EZH2 and BMI1 inversely correlate with prognosis and TP53 mutation in breast cancer. *Breast Cancer Res*, 10, R109.

Pohl, A., M. Azuma, W. Zhang, D. Yang, Y. Ning, T. Winder, K. Danenberg & H. Lenz (2010) Pharmacogenetic profiling of Aurora kinase B is associated with overall survival in metastatic colorectal cancer. *Pharmacogenomics J*.

Qi, G., Y. Kudo, T. Ando, T. Tsunematsu, N. Shimizu, S. Siriwardena, M. Yoshida, M. Keikhaee, I. Ogawa & T. Takata (2010) Nuclear Survivin expression is correlated with malignant behaviors of head and neck cancer together with Aurora-B. *Oral Oncol*, 46, 263-70.

Rea, S., F. Eisenhaber, D. O'Carroll, B. Strahl, Z. Sun, M. Schmid, S. Opravil, K. Mechtler, C. Ponting, C. Allis & T. Jenuwein (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature*, 406, 593-9.

Reich, A., A. Yanai, S. Mesilaty-Gross, A. Chen-Moses, R. Wides & B. Motro (1999) Cloning, mapping, and expression of ial, a novel Drosophila member of the Ipl1/aurora mitotic control kinase family. *DNA Cell Biol*, 18, 593-603.

Ringrose, L. & R. Paro (2004) Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu Rev Genet*, 38, 413-43.

--- (2007) Polycomb/Trithorax response elements and epigenetic memory of cell identity. *Development*, 134, 223-32.

Scheuermann, J., A. de Ayala Alonso, K. Oktaba, N. Ly-Hartig, R. McGinty, S. Fraterman, M. Wilm, T. Muir & J. Müller (2010) Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature*, 465, 243-7.

Schreiber-Agus, N., D. Stein, K. Chen, J. Goltz, L. Stevens & R. DePinho (1997) Drosophila Myc is oncogenic in mammalian cells and plays a role in the diminutive phenotype. *Proc Natl Acad Sci U S A*, 94, 1235-40.

Schuettengruber, B., D. Chourrout, M. Vervoort, B. Leblanc & G. Cavalli (2007) Genome regulation by polycomb and trithorax proteins. *Cell*, 128, 735-45.

Schuettengruber, B., M. Ganapathi, B. Leblanc, M. Portoso, R. Jaschek, B. Tolhuis, M. van Lohuizen, A. Tanay & G. Cavalli (2009) Functional anatomy of polycomb and trithorax chromatin landscapes in Drosophila embryos. *PLoS Biol*, 7, e13.

Schulze, S. & L. Wallrath (2007) Gene regulation by chromatin structure: paradigms established in Drosophila melanogaster. *Annu Rev Entomol*, 52, 171-92.

Schwartz, Y., T. Kahn, P. Stenberg, K. Ohno, R. Bourgon & V. Pirrotta (2010) Alternative epigenetic chromatin states of polycomb target genes. *PLoS Genet*, 6, e1000805.

Schwartz, Y. & V. Pirrotta (2007) Polycomb silencing mechanisms and the management of genomic programmes. *Nat Rev Genet*, 8, 9-22.

Secombe, J., S. Pierce & R. Eisenman (2004) Myc: a weapon of mass destruction. *Cell*, 117, 153-6.

Siebold, A., R. Banerjee, F. Tie, D. Kiss, J. Moskowitz & P. Harte (2010) Polycomb Repressive Complex 2 and Trithorax modulate Drosophila longevity and stress resistance. *Proc Natl Acad Sci U S A*, 107, 169-74.

Simon, J. & R. Kingston (2009) Mechanisms of polycomb gene silencing: knowns and unknowns. *Nat Rev Mol Cell Biol*, 10, 697-708.

Sing, A., D. Pannell, A. Karaiskakis, K. Sturgeon, M. Djabali, J. Ellis, H. Lipshitz & S. Cordes (2009) A vertebrate Polycomb response element governs segmentation of the posterior hindbrain. *Cell*, 138, 885-97.

Sparmann, A. & M. van Lohuizen (2006) Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer*, 6, 846-56.

Tie, F., R. Banerjee, C. Stratton, J. Prasad-Sinha, V. Stepanik, A. Zlobin, M. Diaz, P. Scacheri & P. Harte (2009) CBP-mediated acetylation of histone H3 lysine 27 antagonizes Drosophila Polycomb silencing. *Development*, 136, 3131-41.

Tie, F., J. Prasad-Sinha, A. Birve, A. Rasmuson-Lestander & P. Harte (2003) A 1-megadalton ESC/E(Z) complex from Drosophila that contains polycomblike and RPD3. *Mol Cell Biol*, 23, 3352-62.

Vagnarelli, P. & W. Earnshaw (2004) Chromosomal passengers: the four-dimensional regulation of mitotic events. *Chromosoma*, 113, 211-22.

Whitcomb, S., A. Basu, C. Allis & E. Bernstein (2007) Polycomb Group proteins: an evolutionary perspective. *Trends Genet*, 23, 494-502.

Wu, D. & L. Johnston (2010) Control of wing size and proportions by Drosophila myc. *Genetics*, 184, 199-211.

