Centromere identity and function

Rather than being associated with specific DNA sequences, centromeric function is determined by a specialised structural organisation, the centromeric chromatin, which results from the cooperation of two specific chromatin structures: (i) centric chromatin, characterised by the presence of a centric specific histone H3 variant (CENP-A), and (ii) centromeric heterochromatin, characterised by a specific pattern of histone modifications (H3K9me3), recognised by heterochromatin associated proteins (HP1). (See Figure 1.)

CENP-A deposition determines centromere identity

Centromere identity is determined by the deposition of the centromere-specific histone H3 variant, CENP-A, which replaces canonical H3.1 in all eukaryotic centromeres. CENP-A is found exclusively at centromeres, recruits kinetochore components and is required for centromere function. How is centromere-specific deposition of CENP-A achieved? We are currently addressing this question in Drosophila (Moreno-Moreno et al., 2006). Contrary to canonical nucleosomes, deposition of CENP-A-containing nucleosomes at centromeres is not linked to DNA replication. Deposition of CENP-A nucleosomes is, however, a promiscuous process as it can also occur during DNA
replication leading to its mislocalisation throughout chromatin. Expression of CENP-A must, therefore, be tightly regulated during cell cycle progression to prevent replication-dependent deposition at non-centromeric sites during S-phase. In fact, mammalian CENP-A is only expressed during G2-phase. However, expression of the Drosophila homologue of CENP-A (CID) takes place early during S-phase. Therefore, additional mechanisms must exist to either avoid deposition of CENP-A containing nucleosomes at non-centromeric sites during DNA replication and/or to remove them afterwards. Our work shows that proteasome-mediated degradation restricts localisation of CID to centromeres by eliminating mislocalised CID as well as by regulating available CID levels. Regulating available CID levels is essential to ensure centromeric deposition of CID. Proteasome mutants show increased expression and mislocalisation of CID. (See Figure 2.) Consistent with a role on kinetochore assembly, mislocalisation of CID-nucleosomes induces cell cycle arrest. Actually, binding of CID at ectopic sites recruits kinetochore components, an effect depends on the N-terminal domain of CID. Proteasome-mediated degradation appears to be an evolutionarily conserved mechanism that regulates available CENP-A levels to favor its preferential deposition at centromeres as also in the yeasts Saccharomyces cerevisiae, the levels of CENP-A (Cse4p) are regulated by the proteasome and proteolyis-resistant mutants mislocalise throughout chromatin. Proteasome-mediated degradation of CENP-A was also reported in human cells infected with herpes simplex virus type 1 (HSV-1). How is CENP-A proteolysis regulated? In this respect, we have identified in a yeast two-hybrids screen the interaction of CID with partner of paired (Ppa), an F-box containing protein that interacts with Skp1, an evolutionarily conserved component of the SCF ubiquitin ligase complex.

Heterochromatin structure and function: the contribution of vigilin
Vigilin is a highly evolutionarily conserved protein, from yeasts to humans, which is characterised by the presence of multiple KH-domains. The KH-domain is a single-stranded nucleic-acids binding motif that, first identified in the RNA binding protein hnRNPK, has been found in a number of proteins binding single-stranded nucleic-acids. Vigilin participates in different aspects of RNA metabolism, and binds soluble and membrane-bound polyribosomes. In addition, vigilin contributes to heterochromatin structure and function. In Drosophila, vigilin (DDP1), contributes to the structure and function of centromeric heterochromatin (Huertas et al, 2004). Centromeric silencing, H3K9 methylation and HP1 deposition are affected in DDP1 mutants. Furthermore, DDP1 mutants show defects on chromosome condensation and segregation. Also S. cerevisiae, vigilin (Sctp160p) is involved in the control of cell ploidy and contributes to heterochromatin-induced silencing (Cortés et al, 1999; Marsellach et al, 2006). A sctp160 deletion relieves silencing both at telomeres and at the mating-type locus, but not ribosomal silencing. Loss of telomeric silencing is associated to a decreased Sir3p deposition but is independent of binding of Sctp160p to ribosomes. What is the mechanism underlying the contribution of vigilin to heterochromatin structure and function? Heterochromatin clusters at the nuclear envelope and binding to the nuclear envelope helps to establish heterochromatin-induced silencing. Vigilin appears to play a role in the interaction of heterochromatin structure and function: the contribution of vigilin.
erochromatin with the nuclear envelope. In yeasts (S. cerevisiae and S. pombe), Drosophila and vertebrates (chicken and humans) vigilin shows a perinuclear localisation and, in human cells, vigilin interacts with Ku70/86, an heterochromatin-associated factor involved in anchoring heterochromatin to the nuclear envelope. Moreover, in S. cerevisiae, clustering of telomeric heterochromatin at the nuclear envelope is perturbed in îscp160 cells (see Figure 3).

**HP1 isoforms**

HP1 plays and essential role in the formation and maintenance of centromeric heterochromatin. Three HP1 isoforms exist in Drosophila (HP1a, HP1b and HP1c), which share highly conserved chromo- and chromo-shadow domains. The chromodomain is involved in specific recognition of H3K9me2,3 and the chromo-shadow domain mediates protein-protein interactions. Despite their similarities, the three HP1 isoforms show different patterns of localisation (Font et al, in preparation). In polytene chromosomes, HP1a is mostly located at centromeric heterochromatin. This localisation depends on Su(var)3-9 and, therefore, on H3K9 methylation. HP1a also localises to some euchromatic regions but, at these locations, it does not always co-localise with H3K9me2,3, nor it induces silencing. In addition, euchromatic deposition of HP1a does not depend on Su(var)3-9. On the other hand, HP1c shows a strictly euchromatic localisation that does not depend either on Su(var)3-9 but, though partially, co-localises with H3K9me3. Finally, HP1b shows a mixed localisation pattern being present both at centromeric heterochromatin and at distinct euchromatic sites (See Figure 4). Also in mammals, there are three HP1 isoforms which show a similar behaviour. These observations indicate that chromosomal deposition of HP1 is not exclusively determined by the state and type of histone methylation. Interactions with elements involved in chromatin assembly, DNA replication and transcription are likely to play an essential role in bringing HP1 isoforms to chromatin. What is, then, the role played by histone methylation? HP1 isoforms can interact with unmethylated histones, as well as with DNA and RNA, but they preferentially bind nucleosomes containing H3K9me2,3 (Font et al, in preparation), indicating that recognition of H3K9me2,3 stabilises binding of HP1 to chromatin. From these observations, a model emerges where HP1 is brought to chromatin by factors involved in various chromatin transactions and, then, lock-in at these sites by histone methylation and H3K9me2,3 recognition. Actually, complexes involved in HP1 deposition might themselves carry HMT activities.

**Telomere structure and function**

Telomeres are complex nucleo-protein structures that play important roles in tumourigenesis, senescence, genome instability and cell cycle progression. Telomeres protect chromosomes from the terminal erosion that takes place every cell division and, in addition, they constitute a reservoir of proteins known to participate in multiple cellular processes such as DNA repair and recombination. Drosophila telomeres are of an unusual class. In most eukaryotes, telomeres are composed of short repetitive guanine rich sequences and they are replicated by an
specialised ribo-nucleoprotein enzyme, the telomerase. On the other hand, though functionally equivalent to classical telomeres, Drosophila telomeres are made of successive transpositions of three retrotransposons, HeT-A, TART and TAHRE, which transpose exclusively to the end of chromosomes (See Figure 5.) This is specially interesting because retrotransposons have been classically considered deleterious, or at least parasitic, genomic elements. Drosophila telomeres constitute a good example of how retrotransposons have changed their parasitic nature into an indispensable cellular role.

**Telomere targeting**

Which are the mechanisms that target the telomeric retrotransposons exclusively to telomeres? The Gag protein of HeT-A plays a central role. Because of their essential role, it was assumed that telomeric retrotransposons would be highly evolutionary conserved among all Drosophila species. Unexpectedly, we found that, while maintaining their unique genomic organisation, telomeric retrotransposons show significant divergence with the exception of a few highly conserved motifs (Casacuberta and Pardue, 2003a and 2003b). However, in species as distant as D. melanogaster and D. virilis, the HeT-A element conserves most of its unusual characteristics while maintaining only a 21% of amino acid identity. Most important, both in D. virilis and D. melanogaster, HeT-A Gag localises to telomeres in interphase cells and, in D. melanogaster, it is required for telomere targeting of the other telomeric retrotransposons. Moreover, the HeT-A Gag protein of D. virilis also localises to telomeres when expressed in D. melanogaster cells and, vice versa, the HeT-A Gag protein of D. melanogaster localises to telomeres in D. virilis (Casacuberta et al, 2007. (See Figure 6.) These observations indicate that both the protein and the cellular determinants to correctly drive HeT-A Gag to telomeres have been conserved throughout 60 MY of evolution.

Which other cellular components might be important for telomere targeting of telomeric retrotransposons? Ten different proteins that are necessary for telomere protection have been identified in Drosophila. From these, all except one have homologues in human telomeres indicating that, despite its unique organisation, Drosophila telomeres conserved most proteins involved in telomere protection or telomere length homeostasis. Whether they play a role on telomere targeting is being analysed. Moreover, finding which are the telomere components that have adapted to the retrotransposon mechanism will help to understand how HeT-A, TART and TAHRE adapted to its cellular role and which changes were necessary to successfully achieve the unusual symbiotic collab-

![Figure 5. Structural organisation of mammalian and Drosophila telomeres.](image)

![Figure 6. The Gag protein of the telomeric retrotransposon HeT-A localises to telomeres both in distant Drosophila species such as D. melanogaster and D. virilis.](image)
oration between telomeric retrotransposons and the *Drosophila* genome.

**Structural and functional properties of Drosophila telomeric heterochromatin**

As is the rest of eukaryotes, *Drosophila* telomeres are heterochromatic and, therefore, genetically silent. However, in *Drosophila*, telomere maintenance relies on the expression of genes located at the actual telomeres. How is their expression regulated? Because of their retrotransposon nature, expression of the telomeric retrotransposons is regulated by the RNAi machinery (reviewed in Casacuberta and Pardue, 2006). Mutations in two different genes of the RNAi pathway, spn-E and aub, regulate the expression of HeT-A and TART in germ cells. Also in organisms with classical telomeres, such as *S. pombe* and *Tetrahymena*, mutations in the RNAi machinery disrupt telomeric silencing. On the other hand, in *Drosophila*, we have found that mutations in the Jil-1 kinase enhance telomeric silencing. Jil-1 phosphorylates Ser10 at the histone H3 tail, a modification that antagonises methylation of H3K9 and, therefore, HP1 binding and silencing. Does Jil-1 help to maintain expression of the telomeric retrotransposons? How is it recruited to telomeres? Jil-1 is known to interact with regulators of chromatin structure, such as Z4 and chromator. Do they play any role in the regulation of gene expression at *Drosophila* telomeres? We are investigating these questions.

**Regulation of homeotic gene expression**

In *Drosophila*, the homeotic genes are organised in two clusters, the antenapedia and bithorax complexes. The bithorax complex (BX-C) contains three homeotic genes, Ultrabithorax (ubx), Abdominal-A (Abd-A) and Abdominal-B (Abd-B), which are responsible for specifying the identity of the posterior parasegments of the fly. The expression of these genes is regulated by a complex cis-regulatory region covering 300 kb of DNA divided into nine segment/parasegment-specific subregions in which each domain controls the expression of a single homeotic gene in a particular parasegment. Early in development, gap and pair-rule genes initiate parasegment-specific patterns of homeotic gene expression which, later in development, are maintained by two ubiquitously expressed groups of proteins, the polycomb group (PcG) and the trithorax group (TrxG), responsible for maintenance of the silenced and activated states respectively. BX-C is organised in various domains, which contain positive and negative regulatory elements, not only to direct the establishment of the expression pattern (IAB initiator elements) but also to maintain it throughout development (PRE regulatory elements). There are also specialised boundary elements that delimit the different regulatory domains. (See Figure 7).

**The contribution of chromatin structure and nuclear organisation**

Chromatin has an essential contribution to the regulation of homeotic gene expression. Regulatory elements involved either in initiation or maintenance are characterised by a similar pattern of histone modifications but they show a different accessibility to nucleases (Pérez-Lluch et al, submitted). At BX-C, IABs are accessible to nucleases only during early embryo development but, on the contrary, PREs are sensitive to nucleases at all stages of embryo development and independently of the silencing activity of the PRE suggesting that these regulatory regions are depleted of nucleosomes in both the ON and OFF transcriptional states. Chromatin-mediated interactions, and nuclear organisation, play an important role in the regulation of homeotic gene expression. We have identified a new PRE in the iab-6 cis-regulatory domain of BX-C and we have shown that it interacts with the Abd-B promoter in cells that do not express the gene (Pérez-Lluch et al, submitted). Moreover, 3C analyses showed that different PREs, which regulate the expression of the different homeotic genes of BX-C, are in close proximity to each other within the nucleus indicating the formation of chromatin loops. These loops would be stabilised by chromatin-chromatin interactions between the regulatory elements implicated in maintenance of the silenced state. These results suggest a role of three-dimensional chromatin folding in the mechanism through which PRE-tethered PcG protein complexes act over long distances.

![Figure 7. Structural organisation of the bithorax complex (BX-C) of Drosophila.](image)
GAGA and dSAP18 contribute to Fab-7 function

One of the best-characterised cis-regulatory elements of BX-C is the Fab-7 element. Fab-7 participates in the regulation of the expression of the homeotic gene Abd-B and contains two different functional elements, a PRE and a boundary region. Fab-7 function requires the contribution of GAGA, a trxG protein previously identified as a transcriptional activator of the homeotic genes (Canudas et al., 2005). On the other hand, GAGA interacts with dSAP18, a component of the Sin3/HDAC co-repressor complex (Espinás et al., 2000), and this interaction also contributes to the regulation of Fab-7 function (Canudas et al., 2005). dSAP18 appears to have a more general contribution to the regulation of gene expression in Drosophila. Using Drosophila expression microarrays, changes in gene expression in null dsap18 mutant embryos were determined. These analyses show that most of the genes exhibiting altered expression in the mutant background are related to insect immunity. Our preliminary data points to an implication of dSAP18 in the regulation of genes controlled by the Toll pathway (Costa et al., in preparation). Moreover, dSAP18 associates to both chromosomes and the nuclear matrix, and forms a complex with the Drosophila homologue of pinin, a protein factor involved in mRNA splicing (Costa et al., 2006), suggesting a role for dSAP18 in linking RNA processing and chromatin regulation events. On the other hand, GAGA is a general transcriptional regulator that plays multiple functions in Drosophila, which are likely regulated through post-translational modifications (Bonet et al., 2005).
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Project Coordinator: F. Miguel Beato

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Project Coordinator: Ferran Azorín

Cromatina y expresión génica: análisis a nivel molecular del silenciamiento de loci euromáticos  
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Project coordinator: Alejandro Vaquero

COLLABORATIONS  
Determination of the chromosomal distribution of chromatin binding proteins in Drosophila polytene chromosomes  
Sergio Pimpinelli (University of Rome, Italy)

Telomere structure and function in Drosophila  
Mary-Lou Pardue (Department of Biology, MIT, USA)

Development of mammalian episomal-vectors  
Hans Joachim Lipps (University of Witten, Germany)