Meeting report

The dosage-compensation complex in flies and humans
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A report on the 6th EMBL Transcription Meeting, Heidelberg, Germany, 28 August-1 September 2004.

There were many exciting talks presented at the recent EMBL Transcription meeting in Heidelberg that reported recent insights into the role of chromatin-modifying protein complexes in transcriptional regulation, and four captured our interest especially. They all concern work on a ribonucleo-protein complex that has a defined biological role - namely to regulate dosage compensation in flies. This dosage compensation complex (DCC; also referred to as the MSL complex or the 'compensasome') consists of the histone acetyltransferase encoded by *males-absent-on-the-first* (*mof*), the *male-specific-lethal*-encoded proteins MSL1, MSL2 and MSL3, the Maleless (MLE) helicase and two non-coding RNAs, *RNA-on-the-X* (*roX*) *1* and *roX2*. In addition, it has been suggested that the DCC associates with the JIL1 protein kinase.

According to the prevalent model, the DCC is necessary for upregulating gene expression from the single male X chromosome precisely twofold, thus ensuring that male and female flies produce the same amount of X-linked gene products. The DCC, which does not form in females, is believed to facilitate transcription from the hyperactive X. This occurs at least in part through acetylation of histone H4 lysine 16 of the X chromosome, and this acetylation is believed to be catalyzed by the MOF histone acetyltransferase subunit. Immunostaining of the polytene chromosomes has revealed a strong and specific association between the DCC and the male X chromosome; indeed, the DCC 'paints' the X chromosome, but not the autosomes, in its entirety.

How does this peculiar localization of the DCC come about? Mutant flies lacking certain subunits of the DCC (but retaining the MSL1 and MSL2 core components) show reduced binding to the male X chromosome. In fact, instead of a chromosome-wide association, DCC binding in the mutants is restricted to 30-55 sites. This and other findings have led to a model whereby the DCC first binds to specialized 'entry sites' on the X from where it 'spreads' in an as yet undefined manner to cover the entire chromosome. This model has been around for several years but has now suddenly come under fire from two directions.

Delphine Fagegaltier from Bruce Baker's laboratory (Stanford University, USA) reported experiments showing that many large fragments derived from the X chromosome, even if they do not contain one of the reported entry sites, attract the DCC when translocated into an autosome. This strongly argues that the reported entry sites are not strictly required for DCC binding to be seeded. Furthermore, DCC was never observed to spread across the X chromosome/autosome boundary on these chromosomes. Similar results have recently been obtained in the laboratory of M. Kuroda (Oh *et al.*, *Curr Biol* 2004, **14**:481-487).

Results from experiments using fluorescence recovery after photobleaching (FRAP) and aimed at determining the dynamic nature of X-chromosome-bound DCC were reported by Peter Becker (University of Munich, Germany). The DCC turns out to be surprisingly immobile: throughout the course of the experiment, fluorescence in the bleached region was not recovered, demonstrating that the DCC is remarkably static and, once bound to the X, does not seem to want to let go. This property does not support the idea of a constant redistribution of MSL proteins that would seem to be a prerequisite for a rapid spreading mechanism.

These results are clearly incompatible with the existing entry-site/spreading model. But if there are no special entry sites on the X chromosome, why is DCC binding restricted to the X, and how does it happen that the X gets covered in its entirety? Fagegaltier suggested that 'entry sites' in fact

represent high-affinity binding sites for the DCC, which the complex can bind even when it is missing certain subunits. In addition, there could be many binding sites of lower affinity throughout the remainder of the chromosome. This scenario would be compatible both with previous work and the new findings of Fagegaltier and Becker. Only very few binding sites for the DCC have been characterized to date but no common feature has been identified, so the nature of DCC-binding sites remains mysterious.

The impact of the DCC on chromatin structure is also under investigation. John Tamkun (University of California, Santa Cruz, USA) and collaborators had previously reported that when the chromatin-remodeling ATPase known as IMITA-TION SWITCH (ISWI) is knocked out in flies, the architecture of the male X chromosome is drastically altered. Interestingly, this abnormally decompacted chromosome is rescued by disruption of the DCC. Moreover, the X chromosomes also decondense in *iswi* mutant females upon ectopic expression of MSL2, which artificially forces DCC formation. Biochemical analyses further support the notion that the DCC functionally antagonizes ISWI, at least in part, through acetylation of lysine 16 of histone H4.

At the meeting Tamkun presented new findings on the chromatin composition of the male X chromosome in *iswi* mutants: immunostaining has revealed that the chromatin of the X chromosome, but not the autosomes, lacks histone H1, suggesting that an ISWI-containing remodeling factor is involved in the assembly of H1-containing chromatin. He proposed that the reason only the X chromosome lacks H1 is that the small maternal contribution of ISWI in these flies is sufficient to ensure normal autosomal architecture, as the comparatively low levels of H4 lysine 16 acetylation on autosomes would not inhibit ISWI function. Consistent with this idea, removal of both maternal and zygotic sources of ISWI activity prevents histone H1 from loading onto all chromosomes. These results uncover a possible new role for the ISWI ATPase in the stabilization of higher-order chromatin structures by promoting genome-wide H1 incorporation. The precise relationship between dosage compensation and H1 incorporation remains to be established, however.

Different organisms achieve dosage compensation in different ways. Accordingly, one might expect the DCC to be a molecular machine that operates in flies only. In mammals, gene expression from the single X chromosome in males is not upregulated. Instead, one of the two X chromosomes in females is inactivated to achieve the same effect - equalization of X-linked gene products in the two sexes. Given the vastly different mechanisms of dosage compensation in flies and mammals, one might not expect to find subunits of the *Drosophila* DCC conserved in humans. But Asifa Akhtar (European Molecular Biology Laboratory, Heidelberg, Germany) showed that most subunits of the DCC do indeed have homologs in the human proteome and that some of

these human proteins interact *in vivo*. Human MOF (hMOF) is not able to replace its counterpart in flies, however: it is unable to rescue *mof* mutants and shows no localization to the X chromosome when expressed in flies. Instead, hMOF binds equally well to all fly chromosomes. This suggests that properties of *Drosophila* MOF that are essential for dosage compensation have not been conserved, and raises the possibility that human MOF has evolved new functions. Indeed, when Akhtar depleted hMOF from a human cell line by RNA interference (RNAi) using small interfering RNA (siRNA) she observed defects in the cell cycle and in nuclear morphology. The molecular changes underlying these phenotypes remain to be established. Understanding the function of DCC-related complexes in flies and mammals holds many challenges for the future and promises to teach us how evolution has adapted this successful molecular machine to perform different functions in each setting.