PERSPECTIVE

Getting the right dose of repression

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In recent years, it has become apparent that eukaryotic transcriptional repression mechanisms are remarkably varied in their modes of action and effects. Repression can be established by proteins that act over a short range, or at a long distance (Mannervik et al. 1999). Some mechanisms of repression are readily reversible, but others establish a heritable state of long-term silencing (Moazed 2001). Many transcriptional repressors alter chromatin structure through histone deacetylation or methylation, and thereby affect nucleosome positioning and accessibility of the DNA to positively acting factors (Kornberg and Lorch 1999; Zhang and Reinberg 2001). Multiple steps in transcriptional initiation are also sensitive to obstruction by repressors (Maldonado et al. 1999), and individual repressors such as Ssn6–Tup1 target both chromatin and the RNA Pol II complex (Smith and Johnson 2000). The *Caenorhabditis elegans* germ line transcriptional repressor PIE-1 is a predicted RNA-binding protein that appears to act after initiation (Batchelder et al. 1999; Tenenhaus et al. 2001). This array of mechanisms makes it possible to inhibit genes in an extraordinarily wide variety of biological contexts.

We understand less about how repression might be tailored to achieve particular levels of inhibition. This is a seemingly simple matter if the goal is for a gene to be in an "off" state, although whether the repressed state is to be rapidly reversible could add a further level of complexity. But what if having an on/off switch is not enough, and instead a partial suppression of transcription is required, to attain a particular level of expression? Furthermore, what if the biological program in which this partial repression is needed also requires that other genes be inhibited more completely? Can the same or overlapping repression mechanisms be customized to have different effects at different loci, a scheme that would provide the simplest solution to the problem? Can these same mechanisms be used both to inhibit individual genes and establish a global repression over a large region?

Precisely the above situation is presented during establishment of the hermaphrodite fate in *C. elegans*. In these nematodes, whether an individual becomes a hermaphrodite (XX) or male (XO) is determined by the number of sex chromosomes present (Meyer 2000). In hermaphrodites, transcription of nearly all genes on both X chromosomes must be reduced by half, to bring their expression in line with levels that arise from a single X chromosome in males. This process is called dosage compensation, a term that refers to the various mechanisms by which species alter expression of sex chromosome genes in one sex, to compensate for the difference in chromosome number between the two sexes (Marin et al. 2000). Dosage compensation mechanisms are considerably diverse: for example, in *Drosophila*, transcription of X chromosome genes is doubled in males, but in mammals one of the two X chromosomes is inactivated in females. In *C. elegans*, in addition to a twofold global reduction in X chromosome transcription, the hermaphrodite fate also depends on specific repression of the autosomal male sex-determination gene *her-1* (Meyer 2000). In contrast to dosage compensation, this specific repression of *her-1* involves a more than 20-fold reduction in transcription. Evidence that these two distinct repression processes require some of the same proteins (Meyer 2000), has suggested that they may share some targeting or effector mechanisms.

In a recent study, the Meyer laboratory has shown that both *her-1* repression and dosage compensation are mediated through direct assembly of the same complex of proteins, referred to as the dosage compensation complex (Fig. 1; Chu et al. 2002). How can this protein complex establish such dramatically variant levels of specific and chromosome-wide repression? The dosage compensation complex also represses to different degrees when it is bound to different individual *her-1* DNA regulatory elements. Surprisingly, these repression levels do not appear to correlate with the affinity of DNA binding, suggesting that the dosage compensation complex can have significantly different effects within when it is recruited within different contexts. An important strength of these experiments is that they were performed in vivo, and have thereby provided a window into what is actually happening at these target loci. They have defined a fascinating question for further study: How does the milieu in which the dosage compensation complex is recruited influence its function?

Dosage compensation and hermaphrodite fate: two intertwined problems

Dosage compensation is enforced by assembly of the dosage compensation complex directly on hermaphrodite X

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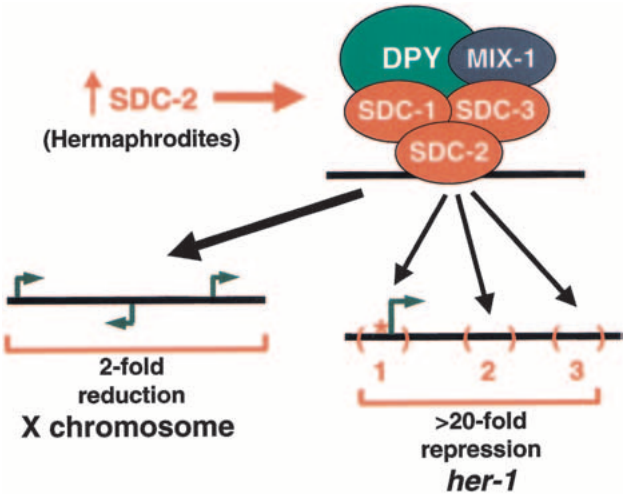

Figure 1. The dosage compensation complex is recruited directly to repress the autosomal gene *her-1*, and to decrease transcription of X chromosome genes by half. SDC-2 is expressed specifically in hermaphrodites, and coordinates all hermaphrodite-specific aspects of development by directing the dosage compensation complex to assemble on the DNA (Meyer 2000; Chu et al. 2002). This complex resembles the mitotic chromosome condensation apparatus, and includes the three SDC proteins, a series of DPY proteins, and MIX-1. By binding within three discrete regions of *her-1*, the dosage compensation complex represses its transcription more than 20-fold and thereby prevents specification of male cell fates. Region 1 is the most important of these for repression, but is bound at lowest affinity in vivo. This region includes the transcription start site and the *her-1*(gf) point mutation (red asterisk), which relieves *her-1* repression and abrogates binding of the complex to region 1. Regions 2 and 3 also contribute to repression, but to a lesser extent. The versatility with which the dosage compensation complex establishes different levels of repression at *her-1* elements suggests that to repress X chromosome genes twofold, this complex may bind at multiple discrete low-activity sites along the chromosome.

chromosomes (Meyer 2000). Identification of individual components of this complex has begun to provide a mechanistic context in which to interpret previous genetic findings. Two dosage compensation complex constituents, DPY-27 and MIX-1, are related to conserved proteins that are required for mitotic chromosome condensation and segregation in *Xenopus* and yeast, and MIX-1 is essential for mitotic chromosome segregation in *C. elegans* (Chuang et al. 1996; Lieb et al. 1998; Losada and Hirano 2001). Another component, DPY-26, is required for *C. elegans* meiotic chromosome segregation (Lieb et al. 1996). These associations with chromosome compaction mechanisms suggested that dosage compensation may also involve effects on chromatin. Recent analyses of the *Saccharomyces cerevisiae* homolog of DPY-30, which is required for dosage compensation but has additional functions, support this idea. The *S. cerevisiae* DPY-30 homolog is a critical component of the Set1 Trithorax-group protein complex, which is required for methylation of histone H3 at lysine residue 4 (Nagy et al. 2002). This conserved histone modification is found in transcriptionally competent regions (Noma et al. 2001), suggesting that it performs a regulatory function.

Previous work has determined that a single component of the dosage compensation complex, SDC-2 (sex determination and dosage compensation), represents the critical molecular link between mechanisms that count the number of X chromosomes present, and establishment of the hermaphrodite fate. SDC-2 is expressed specifically in hermaphrodites, and it triggers assembly of the other dosage compensation components on the X chromosome (Fig. 1; Dawes et al. 1999). SDC-2 also confers the hermaphrodite fate by repressing *her-1*, which would otherwise establish male identity. SDC-2 is recruited directly to the *her-1* locus along with SDC-3, another dosage compensation complex component (Klein and Meyer 1993; Dawes et al. 1999; Chu et al. 2002). This association of SDC-2 with *her-1* has been demonstrated in vivo using a clever method. In *C. elegans*, transgenes that are introduced by DNA injection are maintained within a large extrachromosomal array that contains many tandem copies of the transgenic DNA (Mello et al. 1991). To look for recruitment of SDC-2 to the *her-1* locus, Dawes et al. (1999) created transgenic strains with extrachromosomal arrays that contained multiple copies of either *her-1* regulatory regions, or control DNA. These arrays also included bacterial *lac* operator repeats (*lacO*) and a transgene encoding the Lac repressor fused to green fluorescent protein (LacI::GFP). Because LacI binds to the *lacO* sequence, it is possible to visualize these multicopy arrays in vivo by GFP autofluorescence or antibody staining. By costaining these transgenic embryos with an SDC-2 antibody, it was determined that SDC-2 associates specifically with both the X chromosome and transgenic *her-1* regulatory sequences in living embryonic cells.

The finding that direct recruitment of SDC-2 is required for both dosage compensation and *her-1* inhibition raised an important question: Do these two seemingly different repression processes each involve recruitment of the entire dosage compensation complex, or do they involve SDC-2 and SDC-3 acting together through distinct mechanisms? The former model might seem to be the simpler from a biochemical standpoint, but genetic evidence has pointed toward the second model. Many dosage compensation complex components are not required for *her-1* repression, although in particular genetic backgrounds they may contribute to this repression (Meyer 2000; Chu et al. 2002). In addition, although some *sdc-3* mutations impair both *her-1* repression and dosage compensation, others interfere with only one of these two processes (Klein and Meyer 1993). *sdc-3* mutations that affect only dosage compensation specifically disrupt a pair of zinc finger motifs, and those that only prevent *her-1* repression have been mapped to an apparent ATP-binding domain. The latter set of *sdc-3* mutations disrupt binding of SDC-2 to the *her-1* gene, but not to the X chromosome (Dawes et al. 1999). These requirements for distinct SDC-3 functions suggested that specific repression of *her-1* and chromosome-wide repres-

sion at X might involve assembly of biochemically distinct complexes that contain SDC-2 and SDC-3 (Kuroda and Kelley 1999).

The dosage compensation complex: versatile participant in different forms of repression

New work from the Meyer laboratory argues against the above model however, by revealing the surprising finding that the entire dosage compensation complex is assembled at *her-1* regulatory regions in vivo (Fig. 1; Chu et al. 2002). The SDC-1, SDC-2, and SDC-3 proteins associate physically to form a complex, and together bind to transgenic *her-1* regulatory regions in the array assay. Each of these proteins is required for both *her-1* repression and dosage compensation. In addition, dosage compensation complex components that have not been implicated directly in *her-1* repression (MIX-1, DPY-26, DPY-27) are also localized to *her-1*. This suggests that the entire dosage compensation complex is assembled at the *her-1* gene, even though some of its components may not be essential for its repression.

Using the transgenic array colocalization assay, it was determined that the dosage compensation complex is assembled at three regions of *her-1* (Fig. 1; Chu et al. 2002). One of these regions (region 1; Fig. 1) contains the *her-1* promoter, including a previously defined point mutation [*her-1*(gf)] that partially derepresses *her-1*, and is located two base pairs before the transcription start site (Perry et al. 1994). It is reassuring that in the array assay, the *her-1*(gf) mutation eliminated binding of SDC-2 and other complex components to region 1 (Chu et al. 2002). The other two SDC protein binding regions at *her-1* (regions 2 and 3; Fig. 1) are located within an intron, and had not been identified previously in genetic studies. To test further whether these *her-1* sequences are bound by the dosage compensation machinery in vivo, Chu et al. (2002) performed chromatin immunoprecipitation (ChIP) assays on whole embryos. These experiments represent the first application of this molecular technique to the *C. elegans* system. Surprisingly, these ChIP assays detected binding of SDC-2 to regions 2 and 3, but not to region 1. Supporting the ChIP data, in the context of the full-length *her-1* regulatory region, mutation of region 1 had the least effect on SDC-2 binding in the transgenic array assay. In these experiments, it is assumed that these antibody-based detection methods are equally effective when SDC-2 is bound to each of these three sites. Because this is a reasonable assumption, these findings suggested that region 1 is a lower-affinity binding site for the dosage compensation complex in vivo, even though the effect of the *her-1*(gf) mutation indicated that it was important for *her-1* repression.

By analyzing the functional effects of *her-1* transgene expression, Chu et al. (2002) were able to assess the relative importance of particular regulatory regions for *her-1* repression in vivo. In a series of site-directed mutagenesis experiments, they determined that each of the three SDC protein binding regions contributed to *her-1* repression, but that the low-affinity binding region 1 was considerably more important than regions 2 and 3, either combined or separately (Fig. 1). Apparently, the strength of repression by the dosage compensation machinery does not necessarily correlate with its binding affinity, suggesting that it can contribute to significantly different degrees of repression depending on the context within which it functions.

The observation that the dosage compensation complex is assembled at discrete DNA sequences along the *her-1* gene, and that some of these sequences act as weak repression elements, suggests that it may also bind to multiple discrete sites along the X chromosome. This model predicts that the dosage compensation complex may establish chromosome-wide repression of X by inhibiting individual genes, through either long- or short-range effects. Supporting this view, this complex binds to an X duplication in which 30% of the X chromosome is attached to an autosome, but it does not spread to adjacent autosomal sequences, suggesting that it is recruited locally by X chromosome elements (Lieb et al. 2000). The latter study was unable to detect binding of the dosage compensation complex to smaller duplications of X chromosome regions, however—an observation that appears to differ from its being assembled at small individual *her-1* fragments. Perhaps different strategies are employed to bind the dosage compensation complex in different contexts. It is consistent with this idea that sex determination-specific *sdc-3* mutants disrupt binding of SDC-2 to each of the three *her-1* target elements, but not to the X chromosome (Dawes et al. 1999; Chu et al. 2002). In addition, at the *her-1* gene a discrete recognition element that contained an essential consensus was identified within regions 2 and 3, but a larger fragment which did not contain this consensus was required for binding to region 1 (Chu et al. 2002). This consensus is also not found on the X chromosome, which is enriched with other sequence elements that might contribute to X-specific gene regulation (Lieb et al. 2000). In the future, it will be important to determine whether SDC-2 and other dosage compensation complex components bind directly to specific DNA sequences, or are recruited to their various target elements by interactions with particular DNA binding proteins.

The current findings also raise the question of how the strength of repression mediated by the dosage compensation complex is determined by the context within which it is recruited. Such contextual differences could involve interactions with other adjacent bound protein complexes. These interactions could influence possible recruitment of corepressors or other cofactors by the dosage compensation complex. Alternatively, local interactions could affect the physical accessibility of dosage compensation complex components, or result in differential modification of the complex itself. A modulation of activity by such short-range interactions is consistent with models in which information from nearby repressors and activators is integrated locally to reach an on-or-off decision (Mannervik et al. 1999). It may be important that *her-1* binding region 1 includes the transcription start site (Fig. 1), suggesting that the strong

repression associated with this element might involve interactions with transcription initiation factors, or steric interference with assembly of the initiation complex. Binding to regions 2 and 3 might interfere with transcription elongation (Fig. 1). In addition, it is an attractive model that repression in each of these contexts involves chromatin effects, as predicted from the similarity of this complex to the chromosome condensation machinery (Meyer 2000). Further investigation of these questions will lead to new fundamental insights into how transcription can be regulated, particularly into how a repressor complex can be versatile enough to establish different levels or modes of repression in different situations.

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