

Is HP1 an RNA detector that functions both in repression and activation?

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Heterochromatin is defined as regions of compact chromatin that persist throughout the cell cycle (Heitz, 1928). The earliest cytological observations of heterochromatin were followed by ribonucleotide labeling experiments that showed it to be transcriptionally inert relative to the more typical euchromatic regions that decondense during interphase. Genetic studies of rearrangements that place euchromatic genes next to blocks of heterochromatin also pointed out the repressive nature of heterochromatin (Grigliatti, 1991; and references therein). The discovery of the heterochromatin-enriched protein heterochromatin protein 1 (HP1)* by Elgin and co-workers in the mid-1980s suggested that the distinct cytological features of this chromatin may be related to its unique nucleoprotein composition (James and Elgin, 1986; James et al., 1989). HP1 immunostaining on polytene chromosomes from *Drosophila* larval salivary glands was used to show enrichment of the protein in pericentric heterochromatin. Since that initial discovery, HP1 homologues have been found in species ranging from fission yeast to humans where it is associated with gene silencing (Eissenberg and Elgin, 2000; and references therein). A number of euchromatic sites of localization were also reported in this original study. It has been generally assumed that these sites might constitute euchromatic sites of transcriptional repression by HP1. Indeed, several genes located at one of these sites (cytological region 31) have increased transcript levels in mutants for HP1 (Hwang et al., 2001).

In this issue, Piacentini et al. (2003) report the unexpected association of HP1 with transcriptionally active regions of euchromatin. Moreover, this article shows recruitment of HP1 to ecdysone-activated puffs and to the well known heat shock-induced puffs of polytene chromosomes. The heat shock loci have served as models for transcriptional activation and the accompanying changes in chromatin organization. The finding that HP1 is recruited to transcriptionally activated

puffs flies in the face of current thinking about HP1 function as a transcriptional repressor. Wakimoto and co-workers showed a transcriptional activating function for HP1 in the expression of genes located in heterochromatin (Hearn et al., 1991), but this is the first report of a requirement for HP1 for proper expression of euchromatic genes. This association of HP1 with puffs is shown to be functionally relevant; both hsp70 transcript and protein levels are reduced in loss of function mutants for HP1 and elevated in stocks carrying two extra copies of the HP1-encoding gene as a transgene.

Interestingly, several lines of evidence support a role for RNA in the recruitment of HP1 to puffs. Chromatin immunoprecipitation experiments suggest an association of HP1 with hsp70 gene coding sequences rather than their promoters. Protein-protein interactions with the heat shock transcription factor HSF are not likely to be involved; HP1 is not recruited to ectopic binding sites for HSF unless transcription is initiated from those sites. HP1 is also not recruited to puffs induced by sodium salicylate without accompanying transcription. Finally, HP1 is released from puffs by treating the heat shock-induced chromosomes with RNase.

RNA has also been implicated in targeting of HP1 to heterochromatin. Its association with centric heterochromatin in mammalian cells is RNase sensitive (Maison et al., 2002). Recent studies in *S. pombe* also implicate RNA in HP1 targeting to centric heterochromatin. Double-stranded interference RNA produced from centromeric transposons direct a lysine 9-specific histone H3 methyltransferase activity to centromeres that provides a chromatin binding site for HP1 (Reinhart and Bartel, 2002; Volpe et al., 2002). This histone modification has been strongly implicated in HP1 association with chromatin, and binding of HP1 to this modification requires its conserved chromodomain (Bannister et al., 2001; Jacobs et al., 2001; Lachner et al., 2001; Schotta et al., 2002).

Interestingly, Piacentini et al. (2003) also show failure of HP1 mutants lacking the chromodomain to associate with puffs. This is of interest because an earlier piece of work by Becker and co-workers showed the chromodomain of the MOF histone H4 acetyltransferase to confer RNA-binding activity in the hyperactivation of the X chromosome in *Drosophila* males (Akhtar et al., 2000). This suggests dual roles for the HP1 chromodomain in HP1 targeting, one involving binding to histones and another involving binding to RNA.

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*Abbreviation used in this paper: HP1, heterochromatin protein 1.

These dual binding activities for the chromodomain carry intriguing implications for both how HP1 is recruited to chromosomes and how it might specify distinct activities at distinct sites. HP1 is now known to be a highly interactive protein; it is capable of interacting with a host of proteins with a range of nuclear activities (Kellum, 2003; and references therein). Do RNA sense strands recruit transcriptional activators to sites of active transcription through HP1, while RNA double strands specify targeting of a separate class of HP1 repressing complexes to heterochromatic regions? If sense RNA strands are capable of recruiting HP1 to euchromatic sites, why is recruitment largely restricted to sites of intense transcriptional activity? Does HP1 affect different aspects of transcriptional regulation at active versus repressed sites, or does it have reciprocal effects on a single process? These are just some of the interesting questions prompted by these findings.

It is generally assumed that HP1 forms a repressive chromatin structure that affects transcription initiation rates at repressed sites. Nuclease hypersensitivity mapping studies on transgenes inserted into heterochromatin support this assumption (Wallrath and Elgin, 1995; Cryderman et al., 1999). However, the majority of studies of HP1 regulation have relied on phenotypic assays (such as position effect variegation) or measurements of steady state mRNA levels. One exception to this is the use of a nuclear run-on assay showing HP1 regulation of transcription initiation rates at cenH transposons (Volpe et al., 2002). The finding by this study (Piacentini et al., 2003) that HP1 is associated with gene coding rather than promoter sequences suggests alternative mechanisms for HP1 in regulating gene expression. For example, it could function in the elongation phase of transcription or even in stabilizing mRNAs. The effects reported for HP1 gain and loss of function mutants on heat shock gene mRNA and protein levels in this study are complex and may suggest roles for HP1 in regulating gene expression after the initiation of transcription. At 3 h after heat shock induction, transcript and protein levels were decreased in loss of function mutants but increased in gain of function mutants. The reverse was observed 7 h after heat shock. The authors speculate that the inverse effects of each class of mutation at the different time points are a result of an autoregulatory feedback mechanism that is known to respond to heat shock protein levels in heat shock recovery. It is conceivable that HP1 functions in this autoregulatory mechanism, and that HP1 levels affect the speed of recovery from heat shock activation. Such a role may be more akin to the more generally held view for HP1 function as a transcriptional repressor.

Regardless of the precise role for HP1 at the heat shock-induced puffs, the finding that it is associated with transcriptionally active regions and that this association is RNA dependent prompts questions that will certainly add a new

dimension to our understanding of HP1 targeting and function.

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