Proteasomes beyond proteolysis: Roles in heterochromatin maintenance

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In addition to its proteolytic roles, the 26S proteasome is involved in regulating transcription and in promoting sites of active chromatin. In this report, Seo et al. provide evidence that the non-proteolytic 19S subunit of the 26S proteasome also regulates the spreading of inactive chromatin referred to as heterochromatin, suggesting further non-canonical roles of the proteasome in gene expression.

The 26S proteasome, a multisubunit complex conserved among all eukaryotes, is responsible for degrading polyubiquitinated proteins and thus regulating the levels of various proteins in cells. Although this role is fairly unambiguous, there is growing interest and controversy surrounding potential non-proteolytic roles in regulating transcription and chromatin structure. Open chromatin, or euchromatin, allows active transcription, whereas condensed heterochromatin, associated with histone deacetylation, prevents it. Evidence suggests that the 19S regulatory particle, which is an ATP-dependent protein chaperone that unfolds ubiquitinylated proteins before they enter the 20S barrel for degradation, has protein chaperone activity that is also important for active transcription. Some of these effects are due to the 19S particle directly regulating transcriptional activators, co-activators, and elongation complexes. However, the proteasome also affects transcription by regulating post-translational modifications of histones. Most reports to date regarding the non-proteolytic function of the proteasome have shown its role in promoting transcription by regulating RNA polymerase II activity. In a new paper, Seo et al. (1) delineate a role for the proteasome in controlling heterochromatin formation and regulating the borders between euchromatin and heterochromatin. This finding implies that the proteasome has a much broader role in regulating DNA-related processes than just transcription or degradation of histones.

The authors use fission yeast or Schizosaccharomyces pombe as their model system to address the role of proteasomes in heterochromatin formation. A key advantage of fission yeast as a study system compared with bakers’ yeast or more complex eukaryotes such as mammals is its well-characterized heterochromatin formation process involving small RNAs transcribed from repetitive DNA (2). These transcripts become double-stranded RNAs, which are involved in recruiting histone methylation machinery that leads to the formation of heterochromatin. At pericentric regions like those investigated in Seo et al. (1), the RNAi machinery is the primary mechanism for both heterochromatin formation and maintenance. However, alternative pathways exist that can compensate for loss of the RNAi machinery, one involving Mst2, a histone acetyltransferase (3, 4), and another involving Epe1, a H3K9 demethylase. Loss of Epe1 causes expansion and stabilization of heterochromatric regions (5, 6), and elimination of both Epe1 and Mst2 causes massive up-regulation of heterochromatin formation (3).

Previous reports of non-proteolytic roles for the proteasome have been linked to one of the six ATPases in the 19S particle, referred to in yeast as Rpt1-6. Each ATPase subunit has its unique targets with which it interacts, and one subunit may be involved in more than one process (7). To explore the roles of these ATPases further, Seo et al. (1) performed random mutagenesis on three and tested for effects using a heterochromatin formation reporter assay in which the ade6 gene is inserted into the outer repeat region of a centromere (Fig. 1A). At this location, the ade6 gene is normally assembled into heterochromatin and repressed, causing the cells to turn various shades of red due to the accumulation of an intermediate in adenine metabolism. The authors found a D249V mutation within the Rpt4 subunit that caused some of the cells to turn white, an indication of a loss of heterochromatin at this location and expression of the ade6 gene. This mutation is not in any of the conserved ATPase domains of Rpt4 and does not affect the proteolytic activity of the proteasome, as the authors showed by quantifying the levels of polyubiquitinated proteins. A different reporter system was used to examine whether spreading of heterochromatin was affected by the mutant rpt4 variant. In this assay, the ura4 gene is inserted just outside a boundary element, or IRC, on the left side of centromere 1 (Fig. 1B). Normally, cells grown in the presence of 5-fluoroorotic acid (FOA), which becomes toxic when processed by the enzyme encoded by ura4, are selected against, unless aberrant spreading of heterochromatin occurs and silences the ura4 gene. When rpt4 was mutated, some cells survived FOA treatment, confirming a role for this enzyme in controlling heterochromatin boundaries. This study indicates a surprising connection between the maintenance of heterochromatin and prevention of heterochroma-

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2 The abbreviation used is: FOA, 5-fluoroorotic acid.
Proteasomes regulate heterochromatin

The current study by Seo et al. (1) demonstrates yet another non-proteolytic function for the 26S proteasome and that proteasomes may work in conjunction with two different histone-modifying enzymes. It is not clear as to their exact interplay, but mechanisms of Rpt4’s function as possibly regulating histone modifications instead of working with factors that destabilize nucleosomes (Leo1) or read histone modifications (Bdf2) (8, 9).

The paper by Seo et al. (1) opens a new perspective in the 26S proteasome regulation of chromatin structure. Not only can the 26S proteasome modify chromatin by degrading or displacing histones, but it also may have yet another role in regulating the activity of the enzymes responsible for putting on such modifications as lysine acetylation or removing others like lysine methylation. Inevitably, researchers will need to move more clearly demonstrate that this is the case, using purified factors and well-defined biochemical assays. Although an earlier study showed that 26S proteasome does not have the activity of ATP-dependent disassembly of chromatin remodelers (10), it now appears that it may be involved in another type of chromatin remodeler, namely that of covalent histone modifications.

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Figure 1. Mutation of rpt4 affects both the maintenance and spreading of heterochromatin. A, the reporter ade6 gene is placed within the heterochromatic region (circles) of the centromere (blue arrow) to monitor for the loss of ade6 expression. As shown on the left, when heterochromatin is present, ade6 is not expressed and the cells turn red. A non-catalytic mutation of the 19S regulatory particle (rpt4-1) or loss of Epe1 (epe1Δ), a JmjC domain protein and suspected demethylase of H3K9Me3, causes a reduction in heterochromatin and re-expression of ade6. B, spreading of heterochromatin is tracked by placing the ura4 gene just outside of the centromere where heterochromatin forms. When ura4 is expressed, the cells treated with FOA die, as depicted on the right. If heterochromatin spreads as in the case of the mutant rpt4 or with the loss of epe1 (epe1Δ), then ura4 expression can be shut off and cells can survive in the presence of FOA.