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Keeping the Noise Down in ES Cells

Thomas P. Zwaka[1,*]
[1]Baylor College of Medicine, Department of Molecular and Cellular Biology and Center for Cell and Gene Therapy, One Baylor Plaza, Houston, TX 77030, USA
*Contact: tpzwaka@bcm.edu
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A new report (Szutorisz et al., 2006) suggests that embryonic stem (ES) cells adopt an unusual strategy to remain perpetually poised for differentiation. Apparently, ES cells use the proteasome to target transcriptional preinitiation complexes, thus minimizing transcriptional noise at genes that promote differentiation.

Embryonic stem (ES) cells, which are derived from the inner cell mass of the blastocyst, are becoming an increasingly popular system for study by molecular and cellular biologists. ES cells can be readily expanded in culture and can differentiate into many cell types. However, they also have special molecular features, and the list of unusual discoveries about ES cells continues to grow. For instance, ES cells in culture are less sensitive to a reduction in DNA methylation than cells of the developing embryo (Li et al., 1992). Chromatin in ES cells is also exceptionally dynamic and only loosely associated with a significant proportion of histones and other chromatin bound proteins (Meshorer et al., 2006). Moreover, recent evidence suggests that ES cells have an unusual pattern of histone modifications, called a bivalent domain, that silence key developmental genes (Bernstein et al., 2006). At the level of transcriptional control, there are also a number of surprises, such as the ability of a small core of transcription factors to "dictate" the unique pluripotent state of ES cells (Boyer et al., 2005). Even more remarkable, a handful of transcription factors can induce terminally differentiated cells to revert to an ES cell-like state (Takahashi and Yamanaka, 2006).

A new report in this issue (Szutorisz et al., 2006) reveals further surprises. These authors show that the proteasome, the principal protein degradation complex, is involved in maintaining pluripotency. The fundamental concept underlying this observation is not entirely new. Several reports have suggested the involvement of the proteasome protein complex (or parts of it) in limiting transcription and modifying chromatin elements (Collins and Tansey, 2006). The experiments in the present report are based on the observation that, although the chromatin state of ES cells is widely considered open and dynamic, ES cells do not permit transcription of a variety of genes encoding differentiation factors and other intergenic regions, such as the regions marked for early transcriptional competence. This observation led to the hypothesis that the proteasome might regulate chromatin properties to prevent ES cells from transcribing unwanted genes or intergenic regions and to prevent excessive spreading of permissive chromatin. To test this hypothesis, the authors picked the λ*5-VpreB1* locus, which is activated during early stages of B cell differentiation. When they added a chemical inhibitor of the proteasome or used siRNAs against individual components of the 20S proteasome subunit, they noticed increased transcription of some intergenic regions in this locus in ES cells. This effect is not seen in B cells, suggesting that it is specific to ES cells. Interestingly, these transcripts were not spliced properly, suggesting that the RNA had been incorrectly processed or that the wrong transcription start site had been used. Most importantly, some of these transcripts arose directly from a region known to carry markers of early transcriptional competence in ES cells. To follow up on this observation, Szutorisz and colleagues examined the transcripts more closely and identified several previously unknown transcription initiation sites that point in both directions in this locus. These results suggest that the proteasome effectively blocks transcription in this region of the genome, which is primed for expression in ES cells.

To further explore the role of the proteasome in transcription, the authors tested whether blocking its activity could have a direct effect on the binding of the transcription preinitiation complex to the λ*5-VpreB1* locus, in particular to the intergenic region that showed aberrant transcription after blocking the proteasome. Indeed, they found that key members of the preinitiation complex, including TATA binding protein (TBP), Brg1, TRAP, and even RNA polymerase II were bound to these regions only when the proteasome was inactivated. The authors then determined that parts of the proteasome were bound to the λ*5-VpreB1* locus, including the 20S subunits α4 and β4 (which are part of the "central cylinder") and the 19S protein Rpt6 and Rpn12 (which are part of the "cylinder lid"). Moreover, they found that this process was highly dynamic and, in some regions, highly dependent on proteasome activity. These results suggest that the targeting of individual components of the proteasome has different requirements at different genomic regions. In addition to this locus, the authors explored two other regions in the genome (β-*globulin* and *HoxD4*) and found similar results, suggesting that their observations may apply to many genomic regions in ES cells.

Altogether, the authors suggest a model in which two distinct tran-

scription preinitiation complexes are present in ES cells. They hypothesize that different components of the proteasome play distinct roles in the two complexes. According to their model, one of these initiation complexes is specifically recruited to regions of early transcriptional competence, where it promotes the recruitment of RNA polymerase II. This complex is associated with Rpn12, a proteasome lid protein, and Rpt3, which may act as a chaperone to form and maintain these complexes. The authors suggest that in ES cells there is a second type of preinitiation complex, which forms nonspecifically in intergenic regions, most likely due to the open chromatin environment in these regions. In contrast to the previous complex, the formation of this complex results in the recruitment of the entire active proteasome, which subsequently removes nonspecific preinitiation complexes from chromatin by protein degradation.

How does this model fit into what we currently know about pluripotency and ES cells? Clearly, the ES cell chromatin environment appears to be very permissive for gene transcription. This status is maintained by numerous means, including hyperdynamic chromatin (Meshorer et al., 2006), bivalent chromatin marks (Boyer et al., 2005), and Polycomb group proteins that suppress transcription at specific sites (Boyer et al., 2006; Lee et al., 2006). An important question is whether the existence of open chromatin that is controlled makes biological sense if one assumes that ES cells are an in vitro equivalent of cells within the inner cell mass of the preimplantation embryo? The authors suggest that the proteasome helps to maintain open chromatin at specific sites and simultaneously reduces the noise of gene transcription by actively destroying aberrant transcription preinitiation complexes from nonspecific sites. However, the developing embryo has no inherent reason to maintain pluripotent stem cells for more than a few cell divisions. As a consequence, some of the special chromatin features of ES cells, including those involving the proteasome, may be relevant only to cells that are undergoing continuous self-renewal in the petri dish. However, if this were true, it would be surprising that stable ES cell lines can exist at all. Why should such sophisticated mechanisms exist to maintain pluripotency? Perhaps ES cells are derived from cells within the embryo that are not directly geared toward differentiation but rather maintain open chromatin for a longer period of time than other cells of the inner cell mass, and only during derivation of ES cells in vitro do they acquire the seemingly endless capacity for self-renewal (Zwaka and Thomson, 2005). These unusual and unexpected findings show that we are still far from understanding what ES cells are.

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A Molecular "Zipper" for Microtubules

Masahide Kikkawa[1,*] and Zoltan Metlagel[1]
[1]Department of Cell Biology, University of Texas, Southwestern Medical Center, Dallas, TX, 75390, USA
*Contact: masahide.kikkawa@utsouthwestern.edu

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The dynamics of the microtubule cytoskeleton are controlled by microtubule-associated proteins (MAPs). In this issue, Sandblad et al. (2006) show that Mal3p, the yeast EB1 homolog, belongs to a new class of MAPs that "zipper" up the seam of the microtubule lattice.

When cells need to change their shape, microtubules—dynamic polymers that are part of the cellular cytoskeleton—polymerize and depolymerize to push against the plasma membrane from the inside of the cell. This polymerization and depolymerization has to be precisely controlled and such regulation influences spe-