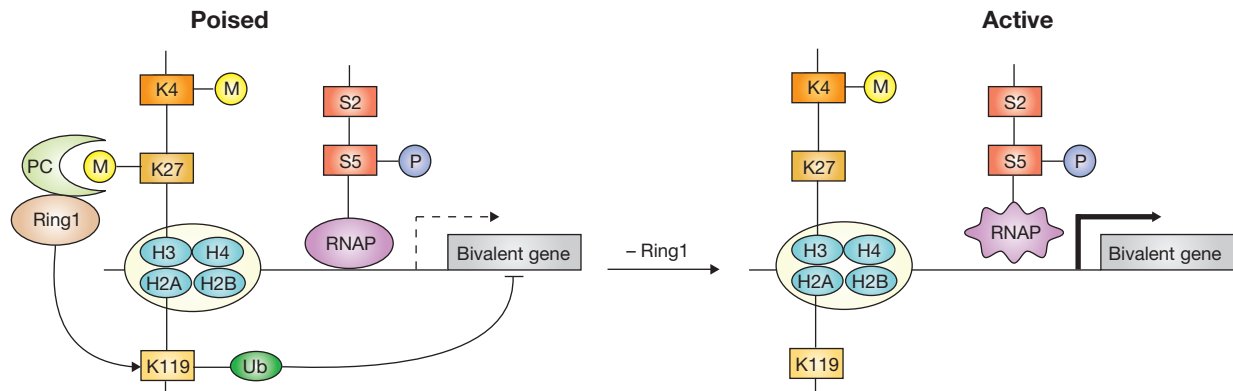


# Ring around the genes

Tanya M. Spektor and Judd C. Rice

How is RNA Polymerase II (RNAP) regulated at poised loci in embryonic stem cells? Recent work provides new insights into Ring1-mediated transcriptional control of this important subset of developmental regulatory genes.



**Figure 1** Many developmental genes in mammalian embryonic stem cells have a special bivalent histone-modification pattern that includes the activation-associated H3K4me3 and the repression-associated H3K27me3. In addition, the Polycomb repressive complex (Pc) is recruited to and binds H3K27me3 where another component of this complex, the Ring1 ubiquitin E3 ligase, monoubiquitinates H2AK119. While the serine 5 phosphorylated (S5) form of RNA polymerase II (RNAP) is poised to initiate transcription of bivalent genes, there is no elongation. Stock *et al.*<sup>1</sup> demonstrate that removal of Ring1 results in the loss of repressive histone modifications, a structural alteration in RNAP resulting in the expression of bivalent genes and, subsequently, cellular differentiation.

Embryonic stem (ES) cells derived from the inner cell mass of an early stage embryo are pluripotent: they possess an unlimited potential for self-renewal while retaining the ability to differentiate along any mature cell lineage. Once an ES cell commits to differentiate, global patterns of gene transcription shift from those involved in self-renewal to those associated with differentiation specific to the lineage chosen. Although the molecular mechanisms that control these transcription patterns in pluripotent and committed cells remain poorly understood, Pombo *et al.*<sup>1</sup>, on page 1428 of this issue, provide new insights into one of these regulatory mechanisms.

The transcriptional status of eukaryotic genes is intimately correlated with specific histone modifications located near and within the gene. The current paradigm states that transcribed genes contain only 'active' histone modifications and, when transcription is silenced, these are replaced with 'repressive' modifications: for example, promoters of actively transcribed genes are typically enriched in histone H3 that is trimethylated at lysine 4 (H3K4me3).

In contrast, repressed genes typically lack this histone modification and, instead, are enriched in histone H3 that is trimethylated at lysine 27 (H3K27me3). A corollary to this paradigm was recently discovered when a subset of developmentally regulated genes were found to contain both the active H3K4me3 and repressive H3K27me3 modifications in ES cells<sup>2,3</sup>. These findings led to the theory that these 'bivalent' genes were 'poised' for transcription and that, during differentiation, they would retain only one of these histone modifications to become either actively transcribed (H3K4me3) or remained repressed (H3K27me3), depending on the cell lineage pathway chosen.

Pombo *et al.*<sup>1</sup> further dissected the molecular mechanisms that regulate this bivalent state at specific developmental genes in mouse ES cells. They first verified that developmentally regulated genes were simultaneously enriched in several histone modifications, including active H3K4me3, repressive H3K27me3 and also the recently defined repression-associated monoubiquitination of lysine 119 on histone H2A (H2Aub1)<sup>4</sup>. To determine the role of H2Aub1 in the transcriptional regulation of these bivalent genes, the authors used an inducible knockout system to deplete cells of the Ring1 ubiquitin E3 ligase — a component of the Polycomb

repressive complex 1 (PRC1)<sup>5</sup>. Consistent with recent findings<sup>6</sup>, this resulted in the global and local reduction of H2Aub1, the activation of differentiation-associated target genes and also enhanced the rate of cellular differentiation. These new observations demonstrate that Ring1-mediated ubiquitination of histone H2A is critical in maintaining this poised bivalent transcriptional state of developmentally regulated genes in pluripotent ES cells (Fig. 1).

To examine the relationship between histone modifications and transcriptional status, the authors analysed occupancy of the different forms of RNA polymerase (RNAP) at the bivalent genes. Consistent with recent findings in human ES cells<sup>7</sup>, RNAP was found to occupy the promoters and coding regions of bivalent genes. The predominant form identified was the serine 5 phosphorylated (S5P) RNAP, which is typically associated with transcriptional initiation. In contrast, the form of RNAP associated with transcriptional elongation, phosphorylated serine 2 (S2P), was absent from the genes. Although the levels of S5P RNAP at bivalent genes were similar to actively transcribed genes, the production of full-length and spliced transcripts was low, suggesting an elongation defect and/or rapid degradation of transcripts. Regardless, these data are in agreement with

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previous findings that RNAP is present and poised for transcription in ES cells<sup>7</sup>.

Returning to the inducible Ring1 knockout system, the authors predicted that there would be a shift to the S2P form of RNAP by eliminating Ring1 and H2Aub1 to activate the expression of the bivalent genes. Surprisingly, S5P RNAP levels remained relatively unchanged when Ring1 was removed and S2P RNAP was not appreciably detected in the coding regions of the genes, despite a rather robust increase in expression of most bivalent genes (Fig. 1). The authors reason that RNAP at bivalent genes in ES cells adopts a previously uncharacterized conformation that is independent of S2P and S5P RNAP typically associated with transcription. Although this has yet to be determined, the findings reinforce the

importance of Ring1 and H2Aub1 in restraining the activation of these developmental-associated genes in ES cells. In addition, these observations strongly suggest that the changes in histone modification patterns and transcription occur early, prior to cell lineage commitment.

In summary, this report demonstrates a novel molecular mechanism of transcriptional regulation whereby the Ring1 ubiquitin E3 ligase is required to prevent aberrant activation of developmental genes in ES cells. Of course, with new answers there are new questions: for example, is it Ring1 or its ligase activity that is required for preventing transcription of bivalent genes? If it is the ligase activity, is it ubiquitination of H2A that is required or are other components of the RNAP complex also targets for this activity?

Furthermore, how does this post-translational modification, itself, function to prevent transcription by RNAP? The authors are well placed to resolve these issues using their inducible system. Regardless, their new findings provide important insights into the fundamental molecular mechanisms that maintain the pluripotent state and those that direct cell fate decisions.

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## Much HUBbub about stem-cell niches

Mark Van Doren

**Stem cells, and the microenvironment or ‘niche’ that influences them, must often reside in a particular location within a tissue to perform their function. Integrin-mediated adhesion is now shown to regulate the location of the stem-cell niche in the *Drosophila* testis.**

A critical aspect of organogenesis is the creation of the correct tissue architecture, with each cell in its proper place, making the appropriate cell–cell contacts. In many organs, it is particularly important to place a population of stem cells, and their corresponding niche, in the right location. The *Drosophila* testis and ovary provide elegant systems for studying stem-cell biology<sup>1</sup>. In the testis, sperm are made in an assembly-line fashion, starting with undifferentiated cells at the tip that mature as they travel toward the reproductive tract (Fig. 1). The undifferentiated cells are produced by a population of germline and somatic stem cells. The stem cells, together with their corresponding niche, must be located at the tip of the testis to ensure the continuous production of gametes and the proper flow of spermatogenesis through the organ. On page 1413 of this issue, Tanentzapf *et al.*<sup>2</sup> show that integrin-mediated adhesion is essential for the proper positioning of the niche within the developing testis, and for maintaining its position in the adult. Disruption of the integrin pathway causes loss of the niche

and stem cells from the testis, with catastrophic consequences for testis function.

The stem-cell niche in the *Drosophila* testis is created by a tightly clustered group of somatic cells known as the hub, around which reside the germline and somatic stem cells (Fig. 1)<sup>3</sup>. The hub acts as a signalling centre that retains the germline stem cells in an undifferentiated state<sup>4,5</sup>. When germline stem cells divide, the plane of division is regulated so that only one daughter cell remains associated with the hub and retains germline stem-cell identity<sup>6,7</sup>. The other daughter is displaced from the hub, no longer receives the signals from the niche, and enters spermatogenesis. At the same time, the somatic stem cells divide to produce support cells, which associate with each differentiating germ cell to form a spermatogenic cyst<sup>8</sup>. As the stem cells must be located at the testis tip (where undifferentiated precursors are required), the initial positioning of the hub during development, and the maintenance of its position in the adult, are critical for testis function. Therefore, one important question is how proper positioning of the hub is initially determined and maintained in the adult.

Gonad development begins in the embryo as germ cells coalesce together with specialized

somatic cells (Fig. 1). The gonad already has a male versus female identity at this time<sup>8–11</sup>, and the hub forms only in males from a subset of somatic cells in the anterior of the gonad<sup>9,12</sup>. Signalling from the germ cells to the soma functions to further restrict the number of anterior cells that take on hub-cell identity<sup>13</sup>. Prospective hub cells exhibit a pattern of gene expression that is distinct from other cells of the male gonad, including expression of several adhesion molecules (such as DE- and DN-cadherin)<sup>9</sup>. By the end of embryogenesis, these cells compact into a tight cluster similar to the adult hub, and make contact with a subset of germ cells<sup>9,14</sup>. As spermatogenesis begins early in the larval stages<sup>14</sup>, the hub may create a functioning stem-cell niche soon after it forms.

How then is the position of the hub and stem cells initially determined in the gonad, and how might this position be maintained throughout development and adult life? Tanentzapf *et al.*<sup>2</sup> have demonstrated that this depends on integrin-mediated adhesion. Integrins are cell-surface adhesion complexes that bind to the extracellular matrix (ECM) and connect to the cytoskeleton through linkers such as talin<sup>15</sup>. The authors show that a laminin-rich ECM

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