PERSPECTIVES

MOLECULAR BIOLOGY

An expanded view of transcription

A new method expands chromatin to provide detailed images of transcription sites

By **Timothy J. Stasevich**[1] *and* **Hiroshi Kimura**[2]

How promoters and enhancers activate genes, and how they communicate to facilitate transcription, are unknown. The main transcriptional RNA polymerase, called RNA polymerase II (RNA Pol II), was originally thought to travel along a linear stretch of DNA. However, evidence gathered over the years paints a more complex picture, in which enhancer and promoter DNA recruit RNA Pol II and genes to larger superstructures, sometimes referred to as transcription factories, hubs, or condensates (*1*–*4*). The precise organization of these superstructures remains elusive, leaving many questions unanswered. On page 92 of this issue, Pownall *et al.* (*5*) describe a new form of expansion microscopy they call ChromExM, which they used to expand chromatin in zebrafish embryos to visualize transcription at high resolution. This expanded view provides evidence that promoters and enhancers come together during gene activation, only to be "kicked" apart soon after.

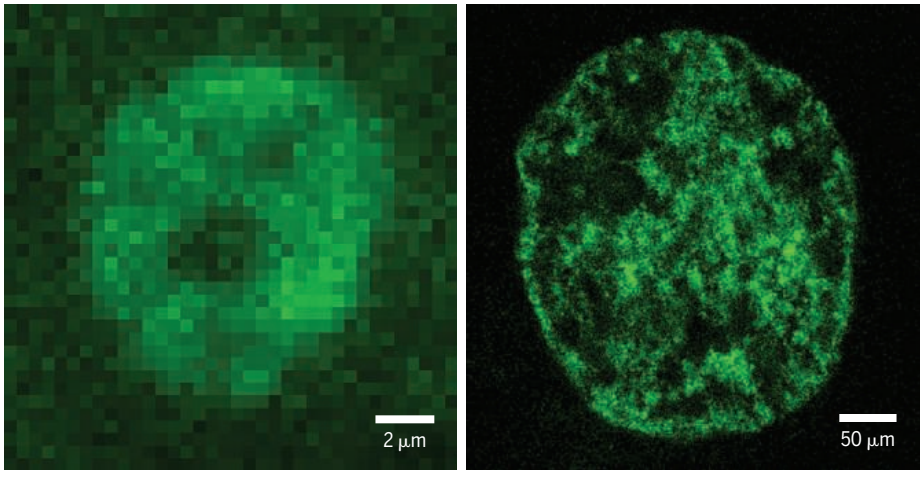
ChromExM modifies PanExM (*6*)—a technique to iteratively expand the full proteome using an entangled hydrogel mesh—to specifically expand chromatin 16-fold. For this, ChromExM uses a mesh that is optimized for catching and fluorescently labeling DNA-bound biomolecules such as histones, the major protein constituent of chromatin. Confocal imaging was used to visualize the labels, achieving a net spatial resolution of ~15 nm compared with the standard optical resolution of ~200 nm (see the figure). Pownall *et al.* also used stimulated emission depletion super-resolution microscopy to further zoom in on fluorescently labeled histones in the expanded zebrafish embryos, achieving a resolution of ~3 nm.

Chromatin forms a complex polymer, so it was unclear if chromatin architecture would be preserved after expansion. Although one previous study suggested that isotropic expansion is possible (*7*), other studies have demonstrated substantial artifacts after expansion (*8*). To test the ability of ChromExM to preserve chromatin architecture, Pownall *et al.* developed a method to optically etch equidistant parallel lines into chromatin. The lines remained essentially equidistant and parallel after expansion. They also confirmed that finer chromatin ultrastructure is preserved after expansion by comparing in vitro clusters of nucleosomes, which are basic units of chromatin consisting of DNA and histones, imaged with electron microscopy and ChromExM.

During the maternal–zygotic transition stages, when zygotic transcription is activated in a subset of genes, Pownall *et al.* observed that highly expressed microRNA gene clusters had hundreds of active RNA Pol II molecules arranged in a line. The authors also saw similar strings of RNA Pol II at nonspecific loci scattered across the nuclei. They interpreted each string as RNA Pol II molecules arranged in single file, generating nascent RNAs from a single gene. These strings are reminiscent of the RNA Pol II convoys that are predicted from models of transcription imaging in live cells (*9*). Previously, nascent RNAs have been observed arranged along string-like loops, but only in extremely long genes (*10*). Occasionally the strings observed by Pownall *et al.* were branched, suggesting multiple intertwined genes, indicative of a transcription factory or hub. In total, there were around 800 RNA Pol II strings per nucleus. Pownall *et al.* also observed ~30 larger "macroclusters" of RNA Pol II per nucleus, some connected to RNA Pol II strings, similar to the large and transient RNA Pol II clusters observed in living cells (*3, 11*).

Intriguingly, the macroclusters were sometimes filled with clusters of the transcription factor Nanog, but those clusters were physically distinct from the RNA Pol II clusters, suggesting that they bind to different sections of DNA. Pownall *et al.* hypothesized that those DNA elements were promoters and enhancers that were in close proximity before active RNA production by elongation. To test this hypothesis, they blocked elongation using an RNA Pol II inhibitor. This led to a significant reduction in the number of RNA Pol II strings, a closer association between Nanog and RNA Pol II, and an increase in RNA Pol II at promoters. These data indicate that there is an increase in transient enhancer–promoter contact when transcription factors cluster together but transcription is not activated (*12*).

This expanded view of transcription provides further support for the "kiss and run" model (*3*), where promoters and enhancers make transient contact to activate



A fluorescent probe for DNA is used to visualize the nuclei from two different zebrafish embryos. The embryo on the right has undergone the ChromExM expansion process, providing a much more detailed view.

IMAGES: M. POWNALL *ET AL.*

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genes. Furthermore, given that elongation inhibition led to an increase in promoter–enhancer association (kissing), Pownall *et al.* refine the model to "kiss and kick" to emphasize the active role elongation seems to be playing in separating promoters and enhancers. If elongation truly kicks apart enhancers and promoters, then the kick provides a simple explanation for why transcription would occur in well-defined bursts. The existence of this kick is supported by other observations. Phosphorylation of RNA Pol II at the onset of elongation can convert the association of RNA Pol II from transcription initiation factor condensates to splicing factor condensates, which are required for the processing of transcripts to mRNA (*4*). Indeed, transcription coactivators on enhancers and elongating RNA Pol II are physically separated (*13, 14*).

Looking ahead, it will be important to devise more direct tests of the kiss and kick model by imaging specific promoters and enhancers during ChromExM. This could be done by combining ChromExM with techniques for in situ labeling of endogenous DNA elements. This experiment would help establish whether the macroclusters observed by Pownall *et al.* correspond to a specific set of genes or if they represent a stage that all activated genes pass through.

Other questions should also be addressed. For example, can the RNA Pol II strings be seen in most genes without ChromExM, and what are their dynamics in live cells? It is also unknown how these findings relate to the three-dimensional organization of the genome, including chromatin domains and their stability, and whether these features are specific to developing embryos. In contrast to the early embryo—which has a rapid cell cycle and thus a robust transcription program that is executed in a short space of time—enhancer–promoter contact might not be so critical in somatic cells (*15*). Ultimately, it is exciting to see experiments beginning to converge on a unified model for transcriptional activation. ■

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PLANT BIOLOGY

Maintaining asymmetry in cell division

Promoting asymmetric division through microtubule dynamics establishes cell fate

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In multicellular organisms, cell division contributes to the generation of specialized cells. In walled organisms such as plants, the orientation of the division plane by which two daughter cells separate is of special importance because cells are embedded in a matrix and cannot relocate. To define the future cell division zone before mitosis, plants have evolved a specific structure called the preprophase band (PPB), which is composed of microtubules. In the context of asymmetric cell division, a polar domain is established at the plasma membrane to allow the differentiation of one of the daughter cells while maintaining a stem cell population. How plant cells establish the polar domain remains enigmatic. On page 54 of this issue, Muroyama *et al.* (*1*) reveal that a polarized destabilization of microtubules demarcates a region of the plant cell as incompatible with microtubule PPB establishment, thereby orienting asymmetric cell division and guiding the development of stomata (pores for gas exchange).

During leaf development, stomata are produced in the surface cell layer by iterative asymmetric cell divisions. This process involves the conversion of a subset of protodermal cells into meristemoid mother cells that divide asymmetrically to produce two daughter cells of unequal volumes. The small daughter cell becomes a meristemoid, which ultimately produces guard cells surrounding the stomatal pore while the larger daughter cell [the stomatal lineage ground cell (SLGC)] further divides asymmetrically. The daughter cells of the SLGC eventually differentiate into puzzle-shaped epidermal cells (pavement cells) while maintaining a controlled amount of meristemoids (*2*). During this process, a plasma membrane–associated polarity crescent defined by BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL) and BREVIS RADIX family (BRXf) proteins is formed and maintained throughout asymmetric cell division (*3*). The orientation of the subsequent asymmetric cell division, defined by the BASL-BRXf polarity crescent, is tightly controlled to enforce the "one-cell spacing rule," which ensures that stomata do not directly touch, thus optimizing gas exchange during photosynthesis while minimizing water loss (*4*).

To address the long-standing question of how the orientation of asymmetric divisions is determined and maintained, Muroyama *et al.* used stomatal lineage formation in *Arabidopsis thaliana* as a model. They showed that the cortical division zone (a landmark for the site of future division) and the microtubule PPB cannot form within the BASL-BRXf polarity crescent. This result agrees with earlier reports showing that the localization of BASL-BRXf is polarized before PPB formation when cortical division zone components are initially recruited to the cortex (*5–7*). Within asymmetrically dividing cells in the leaf epidermis, the default state for cell division orientation appears to be the shortest wall (requiring the least energy consumption). However, when the shortest path conflicts with the one-cell spacing rule, these rules need to be broken. The authors report that the polarized BASL-BRXf domain is required to override default division patterns during formative asymmetric divisions and that this is altered in *basl* mutants. Reciprocally, the establishment of the microtubule PPB is required for stomatal patterning. In a mutant that no longer forms the microtubule PPB, the division plane often bisects the BASL-BRXf polarity crescent, leading to aberrant cell fate in the resulting daughter cells.

How does the BASL-BRXf polar domain influence PPB positioning? Using time-lapse imaging in planta, Muroyama *et al.* demonstrate that the BASL-BRXf polar domain locally depletes cortical microtubules from the plasma membrane. There, the BASL-BRXf polar domain induces microtubule plus-end depolymerization rather than physically preventing microtubule polymerization into the domain per se. Based on these results, the authors conclude that polarization-mediated destabilization of the

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