Cell Cycle 11:21, 3911–3912; November 1, 2012; © 2012 Landes Bioscience

"Remembering" tissue-specific transcription patterns through mitosis

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During cell growth, tissue-specific gene expression programs are maintained through multiple rounds of cell division. Experiments performed in the 1960s showed that RNA synthesis stops during late prophase and restarts in telophase of mitosis,[1] raising the fundamental question as to how transcriptional information is preserved through the mitotic phase of the cell cycle.

While the exact mechanisms that lead to mitotic repression of transcription are still under debate, they are known to involve pervasive phosphorylation of chromatin, chromosome condensation and premature termination of transcription.[2] Simultaneously, there is a widespread displacement of the basal transcription machinery, gene-specific transcription factors and co-factors, chromatin remodelers and modifying enzymes as well as factors that recognize and bind to specific chromatin modifications. However, evidence is emerging that select nuclear factors and histone modifications are retained on mitotic chromatin.[3,4] It has long been hypothesized that mitotic retention of nuclear factors may function to mark genes in a way that enables reassembly of transcription complexes after mitosis. This proposed mitotic memory mechanism has been dubbed "bookmarking." A small number of mitotically retained factors, including MLL and BRD4, have been shown to function as molecular bookmarks by facilitating post-mitotic transcription re-initiation of their mitotic target genes.[5-7]

The hematopoietic zinc finger transcription factor GATA1 controls the expression of virtually all erythroid-specific genes[8] and is critical for establishing and maintaining the erythroid compartment. In our recent study[9] we report that, using live-cell imaging, a small fraction of GATA1 is retained on chromatin during mitosis. We next aimed to define the genome-wide occupancy pattern of GATA1 during mitosis using ChIP-seq. To obtain highly purified mitotic cell populations for ChIP-seq analysis, we developed a novel FACS-based approach that exploits the widespread serine 10 phosphorylation of histone H3 during mitosis. The results revealed that GATA1 is preferentially retained at a subset of genes encoding key hematopoietic nuclear regulatory factors, suggesting that GATA1 bookmarking contributes to the maintenance of hematopoietic transcription patterns. This idea is further supported by our finding that genes marked by GATA1 in mitosis tend to reactivate faster than those that are not.

To test directly whether GATA1 performs a mitosis-specific function on these genes, we established a system in which GATA1 levels are nearly normal in interphase, but selectively deficient in mitosis. To this end, we generated a version of GATA1 that is destroyed in mitosis by fusing it to the mitotic destruction domain (MD) of cyclin B1. MD-GATA1 fusion constructs were introduced into GATA1-null erythroid precursor cells, which are dependent upon exogenous GATA1 for differentiation. We then measured the kinetics of post-mitotic transcription reactivation of GATA1 target genes. Genes bookmarked by GATA1 reactivated more slowly when GATA1 was degraded during mitosis, whereas non-bookmarked GATA1 target genes reactivated normally. Additionally, mitotic destruction of GATA1 also led to partial de-repression of bookmarked genes that are normally inhibited by GATA1. To our knowledge, this represents the first direct demonstration of a mitosis-specific function for any transcription factor. This approach should be superior to conventional knockout or knockdown experiments since results from the latter might be confounded by effects outside of mitosis.

Like most nuclear factors, GATA1 relies on co-factors for its ability to bind to target sites and regulate transcriptional activity. Notably, none of the examined tissue-specific GATA1 co-factors (FOG1, SCL/TAL1, Ldb1 and LMO2) were found on mitotic chromosomes, regardless of whether GATA1 was retained at these sites. However, other GATA1 co-factors might regulate GATA1 binding to mitotic chromatin. One particularly interesting candidate is the widely expressed protein Brd3, which associates with acetylated GATA1.[10] Like the closely related mitotic bookmarking factor Brd4, strong mitotic retention was observed with Brd3 (unpublished observations). Future work will examine whether Brd3 plays a role in mitotic GATA1 bookmarking.

Important questions that remain to be addressed include: (1) What distinguishes sites that are bound by GATA1 in mitosis from those that are not? (2) Do sequences that retain GATA1 during mitosis function autonomously, i.e., when integrated at heterologous genomic sites? (3) If so, do they convey rapid reactivation on a linked reporter gene, and can this approach be used to pinpoint critical DNA sequence elements and/or chromatin features that can facilitate or repress mitotic GATA1

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Submitted: 09/07/12; Accepted: 09/09/12
http://dx.doi.org/10.4161/cc.22237
Comment on: Kadauke S, et al. Cell 2012; 150:725-37; PMID:22901805; http://dx.doi.org/10.1016/j.cell.2012.06.038.

retention? While preliminary studies have not yet identified features that reliably discriminate between mitotically occupied vs. vacated sites, certain trends became apparent. For example, clustering of GATA1-binding motifs, H3K4 trimethylation and promoter-proximal location occur more frequently near mitotically maintained GATA1-binding sites.

A more general question is whether rapid post-mitotic reactivation of genes, as well as the repression of lineage- or differentiation stage-inappropriate genes, is important for lineage stability. In other words, could failure to bookmark genes facilitate lineage reprogramming? We speculate that synchronizing transcriptional reactivation after mitosis and maintaining gene repression might be a general mechanism to suppress cell-to-cell variability in gene expression, thus stabilizing cell identity. Pulsing erythroid cells that contain wild-type or mitotically unstable GATA1 with lineage reprogramming factors might be a way to test this idea directly.

In summary, this work provides new insights into the faithful propagation of transcription patterns through the cell cxycle with potential implications for lineage fidelity and cellular reprogramming. We hope that the versatile systems we have established for this study, such as the FACS-based purification of mitotic cells as well as the mitosis-specific destruction of a nuclear factor, will aid future investigation into mechanisms of mitotic bookmarking.

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