assays. These results suggested that Wnt-responsiveness plays a role in MaSC behavior and provides the cells with essential signals to generate mammary glands.

To further study the effect of Wnt on MaSC, Zeng and Nusse plated isolated $Lin^{-}CD29^{hi}CD24^{+}$ cells and exposed them to purified Wnt3A, vehicle control, or the Wnt inhibitor Dkk1. In this first generation, there was no noticeable effect following Wnt3A treatment. However, subsequent serial colony formation (following colony dissociation and re-plating) was significantly increased in Wnt3A-treated cells. This effect was amplified in later serial passages. The increased clonigenicity of the Wnt3A-treated cells did not result from higher proliferation rates or from changes in apoptosis. Furthermore, $Axin^{lacZ/lacZ}$ cells exhibited even more robust repopulation as compared to wild-type cells. These findings imply that the clonigenicity of MaSC can be greatly influenced by their exposure and responsiveness to Wnt proteins. Most strikingly, the clonally propagated cells retained their full developmental potential. Wnt-treated colonies could robustly reconstitute mammary glands in cleared fat pad assays, while vehicle-treated colonies failed. Significantly, the effectiveness of Wnt-treated cells did not decrease following further passages. Furthermore, cessation of Wnt treatment prevented efficient mammary gland reconstitution, showing that MaSC require exposure to Wnt signals to initiate proper development of mammary structures. This finding has allowed the researchers to overcome the technical hurdles faced in the past, notably the inability to maintain stem cell cultures long term in their undifferentiated state.

With this paper, the study of Wnt's role in the mammary gland has come full circle: the first Wnt gene to be cloned, *Wnt1*, was identified as a prominent insertion site for mouse mammary tumor virus (Nusse and Varmus, 1982). And the current study implies a prominent role for Wnt factors in the normal physiology of the mammary gland stem cell.

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The Silence of the LADs: Dynamic Genome-Lamina Interactions during ESC Differentiation

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In a recent issue of *Molecular Cell*, Peric-Hupkes et al. (2010) use DamID to map the interactions between chromatin and the nuclear lamina (NL) in differentiating embryonic stem cells. NL-mediated locking/unlocking of genomic regions during differentiation provides an additional facet of transcription regulation.

The nuclear lamina (NL) is considered to be a principal guardian of the eukaryotic cell nucleus. It underlies the inner nuclear membrane and is comprised of a fibrous meshwork of proteins, mostly A- and B-type lamins. Lamins provide a mechanical scaffold for the nucleus, are required for communication between the nucleus and the cytoplasm, are associated both directly and indirectly with chromatin, and are involved in most nuclear activities (Prokocimer et al., 2009). The nuclear lamina of essentially all mammalian cell types contains B-type lamins. In contrast, A-type lamins are absent from the inner-cell mass (ICM) of the early mouse embryo and appear only at a later stage of development in more differentiated cells. It is therefore no surprise that embryonic stem cells (ESCs), which are derived from the ICM at the blastocyst stage, lack any lamin A expression (Mattout and Meshorer, 2010). Interestingly, in undifferentiated ESCs, lamin B is significantly more dynamic than in differentiated cell types (Bhattacharya et al., 2009), constituting a more "fluid" nuclear lamina and mirroring the hyperdynamic state of chromatin proteins in ESCs (Meshorer et al., 2006).

In human fibroblasts, Lamina Associated Domains (LADs) were previously mapped genome-wide using the DamID technique (Figure 1A). LADs are mostly

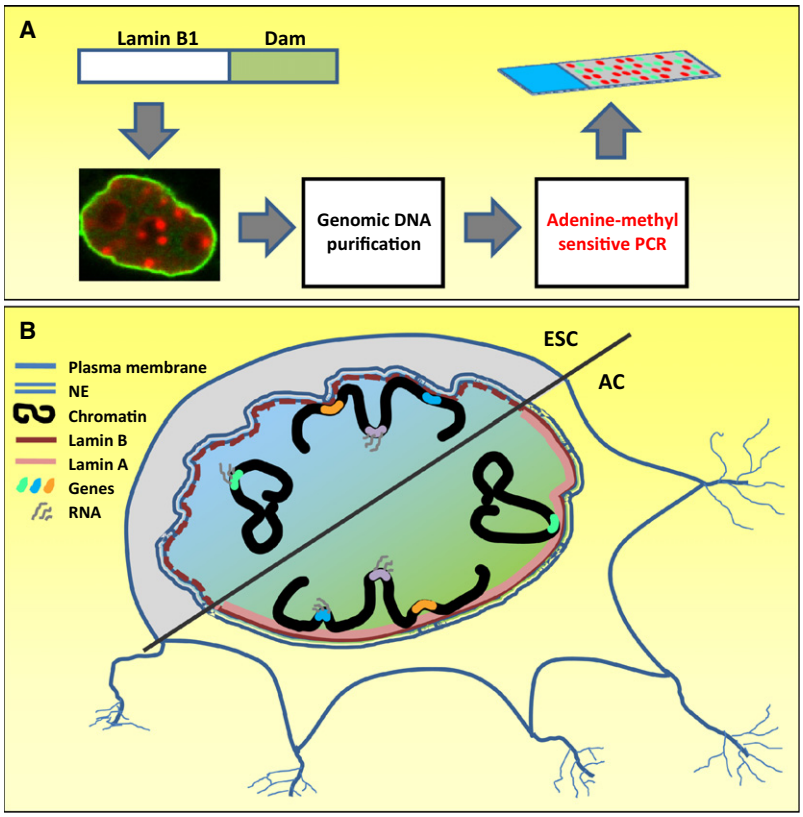

Figure 1. Tracing Interactions of Chromatin with Nuclear Lamina in Differentiating Cells

(A) In DamID, DNA adenine-methyltransferase (Dam) is fused to lamin B1 (top left) and transiently expressed, thus being tethered to the nuclear lamina (NL) and methylating NL-proximate genomic sequences (bottom left). After extracting genomic DNA (bottom middle), methyl-sensitive-PCR amplification and purification is performed (bottom right) and the product is hybridized to a genome-wide tiling microarray (top right), yielding a comprehensive gallery of NL-association maps.

(B) Divided view of cell and nucleus in neural differentiation from a pluripotent embryonic stem cell (ESC; gray cytoplasm, blue nucleoplasm) to a terminally differentiated astrocyte (AC; yellow cytoplasm, green nucleoplasm). Nuclear envelope (blue double membrane) is ill-defined in ESCs and rounds out during differentiation. The nuclear lamina (NL) protein lamin B is more dynamic in ESCs (dark red, dashed) compared to ACs (dark red, solid); lamin A is absent in ESCs and expressed in ACs (pink). Illustrated are four genes representative of different NL interactions: a housekeeping gene in an inter-LAD region (purple) is interiorly located and maintains expression levels in ESCs and throughout differentiation; a highly expressed "stemness" gene inside a LAD (green) relocates from the nuclear interior in ESCs to the NL in ACs and is repressed; two lineage-specific neural genes inside LADs (blue and orange) are peripherally located and repressed in ESCs. Both depart from the NL during differentiation and "unlock" for subsequent expression. The astrocyte-specific gene (blue) is expressed while the other neural gene (orange) remains "unlocked" but silent in ACs.

in silenced regions that are enriched with the facultative heterochromatin-related mark H3K27me3 (Guelen et al., 2008), suggesting that the nuclear interior is generally a permissive environment, while the nuclear periphery is more restrictive, for transcriptional activity. In line with this hypothesis, tethering a genomic region to the nuclear periphery was shown to reduce transcription of the tethered locus (Finlan et al., 2008; Reddy et al., 2008), although exceptions to this rule have been reported as well (Finlan et al., 2008; Kumaran and Spector, 2008).

In an elegant study published in *Molecular Cell* (Peric-Hupkes et al., 2010), the authors use DamID to map LADs in ESCs differentiating along the neural lineage. They analyzed three time points during the course of differentiation: pluripotent ESCs, multipotent neural progenitor cells (NPCs), and terminally differentiated astrocytes (ACs), as well as 3T3 mouse embryonic fibroblasts (MEFs). The authors classify roughly 40% of the genome as LADs, ranging from 40 kb to 15 Mb, and identify the DNA loci that shift spatially during differentiation. They find that LADs overlap by 73%–87% between the examined cell types, substantiating a bona fide nuclear phenomenon that is beyond the scope of a specific lineage or developmental stage.

Utilizing several previously generated data sets, the authors characterize LADs as relatively gene-poor, displaying lower expression levels than inter-LAD regions, exhibiting low levels of active chromatin marks (i.e., H3K4me3 and RNAPII) while enriched with the silenced chromatin mark H3K9me2, and replicating late in S phase, all portraying LADs as heterochromatic candidates.

Interestingly, the authors often find loci that relocate during differentiation to overlap with a single transcription unit, suggesting a focused functional basis for relocation (Figure 1B). Furthermore, most genes apparently relocate separately, not as clusters, and changes in NL-interactions are localized. Supporting this model, many loci were found to relocate according to cell type, differentiation stage, and the correlating expression levels of the harbored genes. In differentiating neural progenitor cells, neural lineage-specific genes were activated concomitantly to the departure of their loci from the NL. Following a similar rule, in postmitotic ACs, cell-cycle genes were repressed, and their loci became associated with the NL. Interestingly, the authors show that regions that separate away from the NL but remain inactive are in fact "unlocked" for subsequent activation. The opposite is also true: genes with low expression levels that relocate toward the NL can become stably silent at a later differentiation stage. Thus, the "locking/unlocking" mechanism may be another layer of nuclear architecture-related transcriptional regulation during differentiation (Figure 1B).

From a global view, the authors find that most genes maintain their pattern of NL interactions throughout differentiation. However, specific lineages are characterized by unique reorganization patterns, and these develop over time, according to the differentiation stage. Evidently, when ESCs differentiate to neural progenitors and then further to become astrocytes, notable "stemness" genes interact with the NL and downregulate, while neural genes depart from the NL and become expressed. Housekeeping and cell-cycle genes seem to change only in the AC stage.

It is noteworthy that DamID lacks the dynamic dimension of genome-lamina interaction. Once association between Lamin B-Dam fusion protein and chromatin occurs, the genomic region is tagged and subsequently scores positive as a LAD.

The hyperdynamics of Lamin B observed in undifferentiated ESCs (Bhattacharya et al., 2009) is not represented in DamID. This finding may explain the relatively small differences observed between the various differentiation stages. It may also explain the lower overall dynamic range observed in ESCs compared to the other cell types, perhaps reflecting innate heterogeneity or, as the authors suggest, less robust NL interactions in ESCs. Nevertheless, many genomic regions significantly alter their nuclear positioning, concomitant with the expression level of the harbored genes. Therefore, even if genome-lamina interactions are more dynamic than can be captured by DamID, the technique still elegantly demonstrates functional reorganization of many parts of the genome during ESC differentiation.

Similar to the case of chromatin plasticity in ESCs and its causal relationship with transcriptional promiscuity (Mattout and Meshorer, 2010), here too the authors argue causality to be an open question and entertain at least two mechanistic possibilities. Intuitively, proximity and subsequent association of LADs with the NL could result in spatial regulation of lineage specific gene expression; nevertheless, it is quite plausible that when lineage-specific transcriptional programs activate or repress a certain locus, this locus in turn recruits (or is recruited to) the NL as a spatial coregulator of expression. This speculation, however, remains to be demonstrated.

The role of lamin A in lamina-related silencing is an intriguing open question. In somatic cells, when genomic loci are silenced by their tethering to the nuclear lamina, lamin A accumulates at the tethered site (Reddy et al., 2008), possibly participating in the silencing process. Therefore, it would be interesting to test this hypothesis in ESCs, where lamin A expression is absent and where the nuclear lamina seems to be more amorphous than in differentiated cells (Mattout and Meshorer, 2010). Along these lines, DamID in the presence and absence of lamin A can yield important insights on lamin A-related regulation at a genome-wide scale. It might also be worthwhile to develop tools, which will allow controlled expression of Lamin B-Dam at short intervals. Comparing several different short expression pulses of Lamin B-Dam may provide an additional dynamic dimension. Such DamID-related experiments together with genome-wide chromosome conformation capture (Hi-C) techniques (Lieberman-Aiden et al., 2009) should provide a global three-dimensional view of nuclear architecture and its association with the nuclear lamina in the imminent future.

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Multiple, Interconvertible States of Human Pluripotent Stem Cells

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Three recent studies, including Buecker et al. (2010), in this issue of *Cell Stem Cell*, report that human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can exist in distinct but interconvertible states and describe a robust expansion of human ESCs/iPSCs that resemble mouse ESCs.

Although human and mouse embryonic stem cells (ESCs) are derived from similar developmental stages with comparable methodologies, the resulting human and mouse ESC lines show overt differences in colony morphology, proliferation rate, growth factor requirements, and cell-surface marker expression. The stark differences between human and mouse