

preadipocyte to a multilocular adipocyte and, finally, a mature adipocyte) are not well defined^{7–9}. Many factors produced by surrounding tissues probably regulate adipogenesis. In addition, adipose tissue itself secretes ‘adipokines’ that have autocrine and paracrine effects, cross-talking with constituents of neighboring tissues and optimizing fat accumulation and metabolism to meet the organism’s needs.

Adipogenesis and fat deposition occur with predilection for certain sites throughout the body. One of these is around lymph nodes, where the adipocytes, particularly amenable to breakdown of lipids at those locations, may provide a ready source of energy for local lymphoid metabolic needs⁸. It follows, therefore, that there should also be mechanisms by which lymphoid tissue can replenish these much-needed fat deposits. The hypothesis that lymph itself might contain factors that induce adipogenesis has long been supported by the observation that lymphedema of the arm in individuals with breast cancer is characterized by excessive fat accumulation and that such individuals often benefit from liposuction¹⁰. Moreover, scattered reports indicate that cultured adipocytes accumulate more lipid and differentiate more readily with the addition of lymph or chylomicrons to the medium¹¹.

This is precisely what Harvey *et al.* found¹. On the basis of an observed correlation between the sites of lymph leakage in *Prox1*^{+/-} mice and the deposition of fat, coupled with the *in vitro* finding that lymph

from these mice promoted preadipocyte differentiation, the authors reasonably concluded that obesity in *Prox1*^{+/-} mice was directly related to the adipogenic effects of the lymph (Fig. 1). The possibility that the phenotype is entirely *Prox1*-specific was excluded by the finding that transgenic mice with a different defect in lymphangiogenesis caused by low levels of VEGFR-3 (ref. 12) also had increased fat deposition.

What in lymph might be adipogenic? Notably, little is known about what factors are present in lymph and how the composition changes in response to disease. Lymph is interstitial fluid that enters the lymphatic circulation and carries with it emulsified fats derived from intestinal absorption, immunocompetent leukocytes and nonabsorbed proteins that have either leaked out of the plasma or been produced in the extravascular space. Harvey *et al.* have not yet identified the relevant adipogenic factors, but undoubtedly the search is on, and their data point to the lymph fraction as a likely source.

Medical implications

The implication that obesity might be regulated by the local accumulation of factors released from the lymphatic circulation is of enormous relevance. The idea that lymph leakage due to inherited subtle defects in the lymphatic circulation might contribute to obesity represents a major paradigm shift. If it is true, considerable efforts could be exerted to identify effective

therapeutic strategies, which might include promoting lymphatic endothelial integrity, preventing release of the adipogenic factors from the lymphatics or interfering functionally with the adipogenic activity. Beyond systemic disorders of fat deposition, the effect of treating or preventing the ‘swelling’ associated with lymphedema, most commonly associated with cancer and infections (especially filariasis), would be huge. It is also worth considering the flip side of the coin: there might normally be low, physiologic levels of chyle leakage designed to promote fat deposition. In various ‘hypermetabolic’ disorders associated with cachexia, this natural source of adipogenic factors may be compromised; therapies opposite to those used for obesity treatment might be of extraordinary value.

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Out with the old, in with the new

Jason D Lieb

Transcription-coupled nucleosome turnover provides an opportunity to incorporate new nucleosomes at active genes. Swapping nucleosomes that contain silencing marks with those that are more permissive for transcription may provide a mechanism for remembering the activity state of a gene through the cell cycle.

When DNA is replicated in preparation for cell division, new nucleosomes are distributed to the newly synthesized DNA, ensuring that the mother and daughter genomes are both properly packaged into chromatin¹. In addition to replication-coupled chromatin assembly, some nucleosome deposition occurs outside of S phase in a replication-

independent manner². Rather than filling in gaps like those created by newly replicated DNA, replication-independent deposition replaces existing nucleosomes (or subunits of them) with new ones. And instead of being coupled to DNA synthesis like replication-coupled assembly, much of replication-independent deposition seems to be mechanistically coupled to RNA polymerase activity (Fig. 1). This exchange of old nucleosomes for new is therefore coupled to gene activity and provides a natural opportunity to alter chromatin on the basis of the specific transcriptional ‘experience’ of individual loci.

On page 1090 of this issue, Yoshito Mito, Jorja Henikoff and Steven Henikoff present a systematic, high-resolution study of metazoan replication-independent histone dynamics³. Their findings support the emerging view that regulation of replication-independent nucleosome dynamics is an important means of ensuring the long-term fidelity of transcriptional regulation.

It’s all about packaging...

Throughout the cell cycle and on a genomic scale, the association of histone subunits with nucleosomes and DNA is much more

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dynamic than previously assumed. Many experiments contributing to this changing view of chromatin dynamics were done in the budding yeast *Saccharomyces cerevisiae*, where nucleosomes are lost at the promoters of actively transcribed genes and can be regained quickly when those genes are shut off^{4–7}. Nucleosome occupancy is also inversely proportional to transcription rate well downstream of promoters⁶, implicating transcriptional elongation in the process of nucleosome displacement⁸. What remained unclear was whether similar nucleosome displacement occurs in organisms with genomes more complex than yeast, and if so, how this turnover is related to the deposition of histone variants and post-translational modifications.

To examine this question, Mito *et al.*³ used cultured cells from the fruit fly *Drosophila melanogaster* to study the deposition of two histones, H3 and H3.3. Histone H3 is one of four proteins that comprise the nucleosome, and H3.3 is a variant that can substitute for H3. H3.3 is deposited by a replication-independent mechanism⁹, allowing the authors to distinguish between replication-coupled and replication-independent deposition patterns by examining the ratio of H3.3 to H3 at any given genomic position.

To determine the relative amounts of H3 and H3.3, Mito *et al.* developed a new method in which recombinant H3 and H3.3 were expressed from a single plasmid in cultured cells. For a given experiment, one of the two proteins was modified so that it could be biotinylated by *Escherichia coli* biotin ligase expressed from the same plasmid. In this way, the biotin-tagged histones, along with any DNA bound to them, could be purified from the cells. The purified DNA was then analyzed by hybridization to either PCR-based or higher-resolution oligonucleotide DNA microarrays.

Mito *et al.* found that whereas H3 levels were fairly uniform, H3.3 levels varied from gene to gene. Consistent with previous studies^{10,11}, H3.3 levels were correlated with RNA polymerase density and with transcription-associated histone modifications. These observations provide additional evidence for replication-independent deposition of H3.3 and suggest that this pathway could be involved in establishing histone modifications associated with gene activity. Mito *et al.* also provide convincing evidence that nucleosome depletion occurs in promoters of active genes in animals, as previously shown in yeast (which contain only one type of H3, which is most similar to H3.3). Together, these results explicitly link tran-

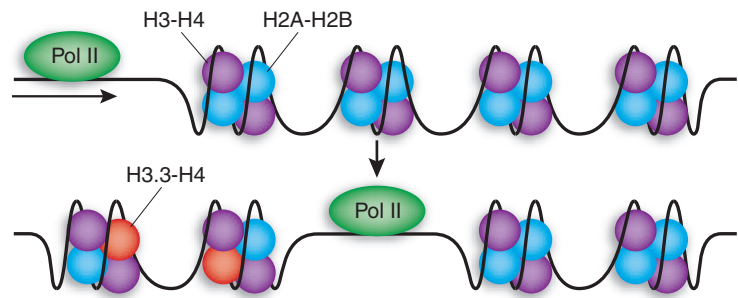


Figure 1 Replication-independent deposition of histone H3.3. Nucleosomes, made up of histone H3-H4 and H2A-H2B heterodimers, are disrupted by RNA polymerase during transcription. Upon transcription, histone H3 is gradually replaced by H3.3.

scription to nucleosome turnover and histone variant incorporation in animals, an important advance in understanding the relationship between chromatin dynamics and transcription.

The enrichment of H3.3 relative to H3 extends throughout the entire transcription unit, both upstream and downstream of annotated genes. Enrichment of H3.3 was much greater at the 5' end, with decreasing incorporation 5' to 3'. Notably, Mito *et al.* show that replacement of H3 by H3.3 was gradual rather than an all-or-none process. This allows the degree of replacement to be proportional to the degree of transcription rather than serve as a binary signal. Consistent with this relationship, the hypertranscribed male X chromosome in flies incorporates more H3.3 than do autosomes. A complementary paper by Christiane Wirbelauer, Oliver Bell and Dirk Schübeler reports findings consistent with the conclusions presented here, with the exception that Wirbelauer *et al.* reported no 5' bias in H3.3 deposition¹².

...and repackaging

Mito *et al.* hypothesize that H3.3 deposition could provide a mechanism to 'remember' the transcriptional state of a locus through the cell cycle. Replication replaces half of the existing nucleosomes at each locus, but the remaining nucleosomes, which include varying amounts of H3.3 deposited through replication-independent pathways, will remain after mitosis, presumably along with transcription-associated histone modifications. These transcription-permissive marks would promote the recruitment and activity of the transcription apparatus, and the subsequent transcriptional activity would, in turn, re-

establish the permissive chromatin.

The study by Mito *et al.* lays the groundwork for this powerful and appealing hypothesis, but there is so far no evidence that histone replacement is causative in maintaining transcriptionally active loci, and the current body of work does not explicitly address the role of this process in the context of development or differentiation. In addition, many histone modifications that are themselves mechanistically coupled to transcription could have an equally important role¹³. It will also be important in future studies to quantify the degree of H3.3 incorporation and to characterize the interplay between post-translational modifications and the process of histone variant deposition^{14,15} at the single-nucleosome level. Continued development of new tools and techniques, like those described by Mito *et al.*³, will allow these questions to be addressed.

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