Porin new light onto chromatin and nuclear organization

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A recent report identifies sites in the human genome that can associate with nucleoporin 93, a subunit of the nuclear pore complex. These associations are modulated by levels of global histone acetylation and highlight the dynamic nature of chromatin organization in the nucleus.

Nuclear pore complexes (NPCs) in the nuclear envelope mediate the selective exchange of macromolecules into and out of the nucleus by facilitated diffusion. The NPC is composed of multiple copies of roughly 30 distinct nucleoporins [1-3] and its structure is conserved throughout eukaryotes. Although numerous studies have examined how nucleoporins function in nucleo-cytoplasmic transport, relatively little attention has been paid to any role they might have in influencing genome organization in mammalian cells.

The nuclear periphery has customarily been associated with inactive chromatin structure and with the repression of gene expression [4-7]. Evidence for a more complex relationship between gene activity and the nuclear periphery, linking the upregulation of gene expression with the association of chromatin with the NPC, came initially from studies in budding yeast (Saccharomyces cerevisiae) and Drosophila [8,9]. Now, a recent report in Genes & Development from Pamela Silver and her colleagues (Brown et al. [10]) shows, by chromatin immunoprecipitation (ChIP), that physical associations between the nucleoporin NUP93 and specific regions of human chromosomes can be captured, and moreover that the inhibition of histone deacetylases (HDACs) leads to altered genomic associations with this nucleoporin.

Capturing nucleoporin-chromatin associations
NUP93 is the most abundant nucleoporin and is essential for NPC assembly and function [11]. It is a very stable and centrally located component of the NPC, giving confidence that any interactions between it and chromatin do indeed occur at the nuclear periphery rather than being due to free nucleoplasmic protein [12]. The Silver lab had previously shown by ChIP that various components of the yeast NPC, including Noc96, the yeast homolog of NUP93, could associate with specific regions of the yeast genome [13]. They have now extended this approach to regions of three different human chromosomes that interact with NUP93 in HeLa cells [10]. After cell fixation with dimethyl adipimidate and paraformaldehyde, lysis in detergent and then sonication, DNA sequences associating with NUP93 in the soluble extract were captured by ChIP, amplified, and hybridized to a tiling microarray covering chromosomes 5, 7 and 16. A total of 207 associated regions, significantly enriched in the ChIP material relative to control (input) chromatin, were defined. For some selected loci, their disposition at, or adjacent to, the nuclear periphery was confirmed by fluorescence in situ hybridization (FISH).

The chromosomal distribution of the NUP93-associated regions was not random. Such sites were enriched in the G-bands and depleted from the more gene-rich R-bands, consistent with the known polar organization of chromosomes in the nucleus, with G-bands tending to be concentrated at the periphery [14]. By comparison with published genome-wide distributions of histone methylation states in CD4 T cells [15], NUP93-associated regions were those typically enriched for histone H3 methylation marks that correlate with inactive chromatin (trimethylation on H3 lysine 9 (H3K9me3), lysine 27 (H3K27me3), or lysine 79 (H3K79me3)), and depleted in signatures of active genes and RNA polymerase II.

Chromatin at the nuclear periphery is generally hypoacetylated, but this can be altered by the inhibition of HDACs.
with trichostatin A (TSA) [16,17]. This treatment is sufficient to upregulate genes whose expression has been suppressed as a consequence of tethering at the nuclear periphery [7]. After treating HeLa cells for 12 hours with TSA, Brown et al. [10] found that NUP93 associations captured on the three analyzed chromosomes were substantially altered, with fewer (170) associated regions, indicating a dynamic shift in NPC-chromatin interactions in the presence of raised levels of histone acetylation.

Indeed, compared with the untreated cells, Brown et al. [10] saw an overall shift in the NUP93-associated sequences towards transcriptional start sites and regions of the genome thought to be marked with histone modifications typical of transcriptionally active regions (such as H3K4 methylation) and enriched in RNA polymerase II [15]. However, they made no analysis of chromatin structure in the TSA-treated HeLa cells, and it will be interesting to see how the histone methylation marks are altered when chromatin is hyperacetylated with TSA under the experimental conditions used in this study.

In some cases, changes in NUP93 association appear to be quite local. In one characterized example from chromosome 7, NUP93 association, previously thought to be in a purely intergenic region but actually including the 5’ end of a recently annotated transcription unit (Figure 1a), then extended out to the transcription start sites of two additional flanking genes in TSA-treated cells. Expression of one of these flanking genes was upregulated in the TSA-treated cells, but the expression of the other was unaffected.

More generally, the changes in NUP93 association caused by TSA treatment seem to be long range, as the overall chromosomal distribution of associated regions shifted away from G-bands and toward R-bands (Figure 1b). This scale of genome reorganization should be detectable at the cytological level, and indeed, relocalization of loci either towards or away from the nuclear periphery was detected by FISH in TSA-treated cells. One of the loci whose association with NUP93 was diminished by TSA treatment was the cystic fibrosis transmembrane conductance regulator (CFTR) locus at 7q31.2 (Figure 1b). This is consistent with a previous study in which CFTR was observed to move away from the nuclear periphery upon TSA treatment [18]. Such large-scale spatial reorganization of the sequences at the nuclear periphery is also consistent with the changes observed in sequences interacting with the nuclear lamina in TSA-treated Drosophila cells [5].

**Linking histone acetylation to nuclear reorganization**

What might be the mechanisms by which TSA alters the association between chromatin and the nuclear periphery? Chromatin decondensation, and hence enhanced chromatin flexibility and mobility, as a consequence of the hyperacetylation induced by TSA, might account for the localized changes in NUP93-chromatin associations. However, given the constraints on chromatin motion in human cells [19], it seems unlikely that this could account for the altered long-range landscape of NUP93-associated regions after TSA treatment. Instead, it is more likely that components of the nuclear periphery have an affinity for chromatin marked...
with specific histone modifications, histone variants, or other chromatin-associated proteins, and that TSA, by hyperacetylating chromatin and other proteins, modifies these interactions. In that case, the TSA-induced changes in NPC-chromatin association might well require passage through mitosis, as do other documented cases of large-scale nuclear reorganization with respect to the nuclear periphery [6,19,20].

As well as altering chromatin structure and organization, TSA also induces the nuclear redistribution of nuclear transport proteins and the histone acetyltransferase CBP, which can interact with NUP93 [21], and it is unclear what role these proteins might have in the altered NUP93-genome associations reported in response to TSA.

In budding yeast, Nci96 (NUP93) association is found with highly transcribed genes that contain the binding sites for a particular transcriptional regulator [13] and there are several highly transcribed genes that contain the binding sites for a particular transcriptional regulator [13] and there are several highly transcribed genes that contain the binding sites for In budding yeast, Nic96 (NUP93) association is found with highly transcribed genes that contain the binding sites for a particular transcriptional regulator [13] and there are several highly transcribed genes that contain the binding sites for a particular transcriptional regulator [13] and there are several highly transcribed genes that contain the binding sites for

Finally, it remains to be resolved whether nuclear pore associations with chromatin do have a direct role in inducing gene expression in mammalian cells. The answer is likely to be as complex as the question of whether association with other components of the nuclear periphery can silence gene expression [6,7], and indeed, given the complexities of mammalian genome organization and developmentally or physiologically induced changes in gene expression, we should not expect simple black and white answers.

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