CHROMATIN

Caught in a cage

Little is known about how higher-order chromatin structure and nuclear organization influence the transcriptional activity of genes. Reporting in *Nature Genetics*, Terumi Kohwi-Shigematsu and colleagues describe a new model for gene regulation by a nuclear protein — special AT-rich binding protein 1 (SATB1) — that casts new light on the matter.

The authors first showed, using immunostaining, that SATB1 has a cage-like network distribution in thymocyte nuclei, where it is predominantly found. The network, which also encloses heterochromatin, is mostly resistant to salt extraction and DNase I digestion, strongly suggesting that SATB1 constitutes a subnuclear structure.

To examine the functional significance of the SATB1 network, Kohwi-Shigematsu and coworkers took two complementary approaches. One involved searching for a SATB1-binding site (SBS; for SATB1-bound genomic sequence) in the *Myc* locus, which is known to be dysregulated in *Satb1*$^{-/-}$ thymocytes. Using chromatin immunoprecipitation (ChIP), the authors showed that SATB1 was bound to an upstream *Myc* sequence *in vivo*, and that binding of this SBS to the SATB1 network is essential for induction of *Myc* transcription in response to mitogen stimulation.

The second approach entailed isolating individual SBSs from thymocyte nuclei, identifying genes within 100 kb of each SBS, and determining whether their expression was altered in *Satb1*$^{-/-}$ thymocytes. Analysis of two SBSs revealed that SATB1 ablation caused gene dysregulation 4–60 kb from the SBSs, and that SATB1 can function either as a cell-type-specific activator or a repressor.

Using FISH analysis, Kohwi-Shigematsu and colleagues showed that all SBSs, identified by either approach, were localized to the bases of chromatin loops by tethering to the SATB1 network. By contrast, in the absence of SATB1, SBSs redistributed to the loop regions. So, they concluded that chromatin folding by tethering specialized DNA sequences to the SATB1 network might be a common mechanism for gene regulation by SATB1.

To explore potential effects on local chromatin structure by tethering of SBSs to the SATB1 network, the authors examined specific histone modifcations of a chromatin region containing an SBS. They carried out ChIP assays, using wild-type and *Satb1*$^{-/-}$ chromatin,

GENE REGULATION

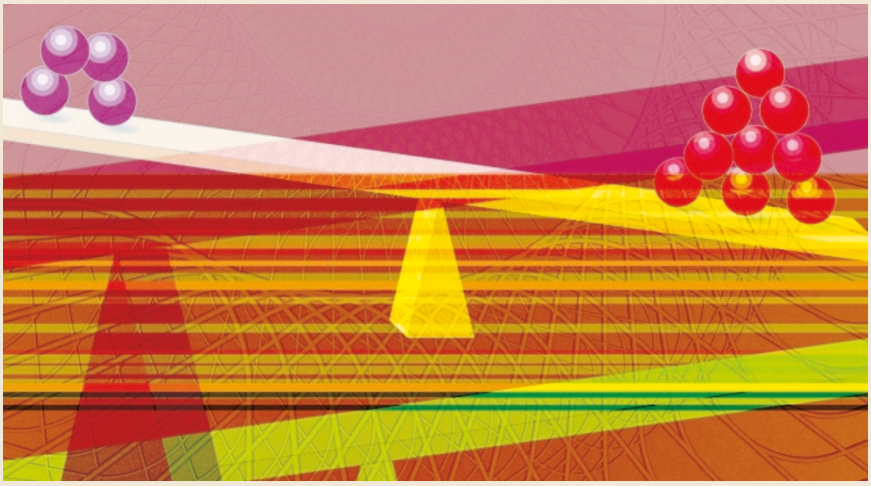
Coordinating opposites

The promoter function of the yeast forkhead transcription factors Fkh1 and Fkh2 is well established. But now, in *Science*, Jane Mellor and colleagues report a new role for Fkh1 and Fkh2 — coordinating early transcription elongation and pre-messenger RNA processing.

The authors used chromatin immunoprecipitation (ChIP) to investigate the binding of Fkh1 and Fkh2 to *CLB2*, which is part of the *CLB2* cluster of genes that is expressed in early mitosis. They found that Fkh1 and Fkh2 not only bound the upstream activating sequence, but also the coding region, which indicated that they were involved in transcriptional elongation. Next, Mellor and colleagues looked at RNA polymerase II (RNAPII) distribution on *CLB2*. In wild-type yeast strains, RNAPII accumulated at the beginning of the coding region — this was also true in strains lacking Fkh2 (*fkh2Δ*), although there was an expected marked reduction in the amount of RNAPII bound. However, in strains lacking Fkh1 (*fkh1Δ*), RNAPII accumulated towards the end of the gene, indicating that there was an elongation block involving Fkh1.

Next, the authors used 6-azauracil (6AU), to see if Fkh1 and Fkh2 had opposing effects. 6AU depletes pools of GTP and prevents RNAPII from elongating efficiently, but wild-type strains survive because another protein, Imd2, replenishes GTP pools. *fkh2Δ*'s sensitivity to 6AU, which was overcome with guanine, indicated a positive role in elongation for Fkh2. Conversely, *fkh1Δ* were resistant to 6AU and could suppress the sensitivity of *fkh2Δ*. This indicated that these Fkh factors do have opposing functions. Northern blot analysis showed that *IMD2* is a target of Fkh regulation, and association of Fkh with the coding region, which paralleled that seen for *CLB2*, was observed when *IMD2* transcription was induced by 6AU. RNAPII distribution at these loci was also Fkh-dependent.

So, how do Fkh1 and Fkh2 influence RNAPII? During elongation, serine (Ser) 5 and Ser2 of the heptad repeat (Tyr-Ser-Pro-Thr-Ser-Pro-Ser)$_{27}$ of the C-terminal repeat domain (CTD) of RNAPII are differentially phosphorylated — phosphorylation of Ser5 increases and Ser2 decreases between the 5′ and 3′ end of genes — and RNAPII then recruits activities for processing transcripts. In *fkh2Δ*, phosphorylation of Ser5 and Ser2 was more constant across the gene, indicating that release of RNAPII into elongation phase was defective and that the normal temporal control of Ser phosphorylation might be



and noticed a SATB1-dependent pattern of histone modifications confined to a 10-kb, SBS-containing region that covered genes positively regulated by SATB1. Acetylation of histone H3 at lysine (Lys) 9 and Lys14 peaked in wild type, whereas in *Satb1*$^{-/-}$ chromatin H3 methylation at Lys9 peaked. So, SATB1 mediates the histone modification pattern, which causes remodelling of the chromatin structure in a restricted region containing SATB1-dependent genes. Kohwi-Shigematsu and colleagues also reported recently that SATB1 recruits chromatin-remodelling enzymes to SBSs to regulate chromatin structure over a large distance.

The authors concluded that the SATB1 network organizes DNA sequences in a cell-type-specific manner by tethering specialized DNA sequences onto its network and providing a landing platform for chromatin-remodelling/modifying factors.

Arianne Heinrichs

References and links

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FURTHER READING Yasui, D. *et al.* SATB1 targets chromatin remodelling to regulate genes over long distances. *Nature* **419**, 641–645 (2002)

disturbed. In *fkh1Δ*, Ser5 phosphorylation was reduced in the promoter region and low throughout the coding region, and Ser2 phosphorylation was almost undetectable.

Mellor and colleagues then decided to look at pre-mRNA 3′-end formation in *fkh1Δ* strains, because increased Ser2 phosphorylation in wild-type strains is associated with pre-mRNA 3′-end processing. Indeed, they found that 3′-end formation was defective in *fkh1Δ*. Then, using a transcription run-on assay, they showed that RNAPII in *fkh1Δ* was defective in pre-mRNA 3′-end formation and predicted that "…much of the RNAPII at the 3′ end is not actively engaged in transcription, a likely consequence of the lack of Ser5 and Ser2 phosphorylation".

So, the authors suggest that the opposing actions of Fkh1 and Fkh2 at the beginning of genes might be part of an early elongation checkpoint mechanism that has been proposed to coordinate transcription and pre-mRNA processing through phosphorylation of the CTD of RNAPII. And the evolutionary conservation of the Fkh factors indicates that this "…may reflect a general feature of gene regulation in eukaryotes".

Natalie Wilson

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ADHESION

Getting to the root of the problem

Signals that are transmitted through cell junctions can transform epithelial layers into three-dimensional structures such as hair follicles. Desmosomes, which are specialized cell junctions that make up much of the cell surfaces in mature hair follicles, contribute — in part through desmosomal cadherins — to cell–cell adhesion. Kljuic *et al.*, reporting in *Cell*, have identified desmoglein 4 and report its function in epidermal adhesion and hair-follicle differentiation.

None of the known desmosomal cadherins are expressed in the inner layers of the hair shaft despite the abundance of desmosomes here. So the authors carried out a classical genetic approach using patients with localized autosomal-recessive hypotrichosis (*LAH*), a condition in which hair is less dense, and located a candidate gene close to a desmosomal cadherin gene cluster.

Further analysis showed synteny with the mouse *lanceolate hair (lah)* mutation — which impairs hair growth — which is also near the desmosomal cadherin gene cluster. During this genomic analysis, a previously uncharacterized cadherin gene — designated desmoglein 4 (*Dsg4*) — was identified in the cluster. Desmoglein 4 was highly expressed in human and mouse skin, and colocalized with the *lah* and *LAH* linkage intervals, making *Dsg4* a candidate for both phenotypes. The authors then showed *Dsg4* to be mutated in *LAH* humans and *lah* mice. A second *lah* allele, *lah*^{J}, causes a more severe phenotype, and further sequence analysis showed that *lah*^{J}/*lah*^{J} mice are null (*lah*/*lah* mice are hypomorphic).

Dsg4 was shown to be the main desmosomal cadherin in hair follicles, and analysis of the epidermis and hair follicles from *lah*^{J}/*lah*^{J}-mutant pups showed that Dsg4 had a central role in cell–cell adhesion — desmosomes were sparse, poorly formed and often detached or torn away from their neighbours. Kljuic *et al.* then proposed, when assaying the expression of several epidermal markers, that the keratinocytes of *lah*^{J}/*lah*^{J} mice exited the basal compartment of the epidermis — in which keratinocytes proliferate — earlier or faster than normal, thereby expanding the proliferative compartment. Indeed, ectopically proliferating cells were found in the suprabasal layers, as shown by the expression of β1 integrin and the epidermal growth factor receptor (EGFR). In the context of *lah* mutants, this increased expression of β1 and EGFR correlated with enhanced keratinocyte–substrate adhesion and spreading *in vitro*.

In wild-type hair formation, keratinocytes in the lowest part of the hair follicle proliferate rapidly until they pass through a 'critical region', in which mitosis stops and the cells start to differentiate. The transition through the critical region is usually gradual, but *lah*^{J}/*lah*^{J}-mutant hair follicles abruptly stop proliferating and start differentiating. The authors propose that this abrupt transition might arise from, or be precipitated by, the defective cell–cell adhesion that impairs the transduction of signals that are important in survival and the full execution of cell-fate determination.

This is one of the first reports of a defect in a structural component — Dsg4 — of the epidermis and hair follicle that causes corresponding mouse and human phenotypes. In addition, as the authors also showed that Dsg4 functions as an autoantigen in patients with pemphigus vulgaris (an autoimmune skin disease characterized by outbreaks of blisters), the relevance of the findings extends to skin autoimmunity.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Kljuic, A. *et al.* Desmoglein 4 in hair follicle differentiation and epidermal adhesion: evidence from inherited hypotrichosis and acquired pemphigus vulgaris. *Cell* **113**, 249–260 (2003)