Research Highlights

Highlights from the latest articles in epigenomics

Polycomb response elements in vertebrates

Evaluation of: Sing A, Pannell D, Karaïskakis A *et al.*: A vertebrate polycomb response element governs segmentation of the posterior hindbrain. *Cell* 138, 885–897 (2009).

The epigenetic inheritance of silent and active chromatin states during differentiation and development is determined, in part, via the polycomb (PcG)/trithorax (trxG)-mediated program. PcG and trxG genes were originally discovered in *Drosophila melanogaster* as repressors and activators of Hox genes (that specify cell identity along the anteroposterior axis of segmented animals). In general, the PcG system ensures the silencing of gene expression, whereas the trxG system facilitates activation. Multimeric complexes of PcG (e.g., Pho, PRC1 and PRC2) and trxG proteins (e.g., SWI/SNF and MLL) are thought to maintain rather than initiate patterns of gene expression. A key question in this field is: how are these complexes targeted to specific regions of chromatin? Extensive studies in *Drosophila* have characterized PcG/trxG *cis*-regulatory elements (PRE/TRE) at which PcG and trxG proteins are recruited via combinatorial signals involving multiple DNA motifs to exert their effects on nearby promoters. However, to date, corresponding PRE/TRE elements have not been identified in vertebrates [1,2].

Many mouse mutations in PcG and trxG genes alter the pattern of gene expression in segments (rhombomeres) along the anteroposterior axis of the embryonic hindbrain. Recently, Sing *et al.* postulated that one such phenotype, associated with a large inversion (called *kr*), might result from repositioning of a mouse PRE so that rather than regulating one gene (*MafB*) it was now closer to and regulating another

(*Nnat*), thereby shifting the pattern of expression of these genes to different rhombomeres [3]. The putative PRE was originally defined within a 9-kb region, and eventually localized to a 3-kb segment of DNA (PRE-*kr*^{3kb}) that was demonstrated to include some sequence hallmarks of previously defined *Drosophila* PREs (including GAGAG and Pho/YY1 binding sites). This element passed many of the *Drosophila*-based assays one would like to see in a *bona fide* PRE – it recruits PcG proteins (PRC1 and, to a lesser extent, PRC2), represses expression of a linked gene, and its activity is modulated by PcG and trxG proteins. The element also behaved (as one would predict) in transgenic experiments in a mouse background, although it did not recruit PRC2 when located at an ectopic site. This may be an important first step towards defining a vertebrate PRE, but (as discussed in [3]) it was interesting to note that PRE-*kr* was not identified in previous genome-wide profiling studies or by bioinformatics. Furthermore, in this paper, the most convincing assays for true PRE activity were from *Drosophila* assays, rather than experiments in mouse, and the two models are probably very different in their detail. We will need similar, careful analysis of many more examples of such elements to define PREs in vertebrates.

References

- 1 Schuettengruber B, Chourrout D, Vervoort M, Leblanc B, Cavalli G: Genome regulation by polycomb and trithorax proteins. *Cell* 128(4), 735–745 (2007).
- 2 Schwartz YB, Pirrotta V: Polycomb complexes and epigenetic states. *Curr. Opin. Cell Biol.* 20(3), 266–273 (2008).
- 3 Sing A, Pannell D, Karaïskakis A *et al.*: A vertebrate polycomb response element governs segmentation of the posterior hindbrain. *Cell* 138(5), 885–897 (2009).

David Garrick, Marco De Gobbi, Richard Gibbons &

Douglas R Higgs[†]

[†]Author for correspondence:

John Radcliffe Hospital, MRC Molecular Haematology Unit, The Weatherall Institute of Molecular Medicine, Headington, Oxford, OX3 9DS, UK

Tel.: +44 1865 222 393

Fax: +44 1865 222 424

doug.higgs@imm.ox.ac.uk

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

CTCF, cohesin and higher-order chromatin structure

Evaluation of: Hadjur S, Williams LM, Ryan NK *et al.*: Cohesins form chromosomal *cis*-interactions at the developmentally regulated *IFNG* locus. *Nature* 460, 410–413 (2009).

The CCCTC-binding factor (CTCF) is a highly conserved zinc finger protein that binds DNA and appears to play an important role in transcriptional regulation. Originally identified as a transcriptional repressor [1], CTCF has also been demonstrated to have several other functions (e.g., activation, insulation, X-inactivation and parental imprinting) [2]. A great deal of work has focused on the role this protein plays in the formation of chromosomal insulators (at either enhancer blocking elements or boundary elements). These experiments were mainly transgenic studies performed on insulator sequences of very well characterized loci (e.g., β -globin and the H19/Igf2 imprinted locus) [3]. Growing evidence suggests that CTCF may also play a role in mediating long-range chromatin interactions, and that its insulation activity might depend on the role played in organizing chromatin structure [4,5]. However, at least at the β -globin locus, the functional significance of these properties *in vivo* has been questioned [5,6]. Nevertheless, the finding that, in mammals, cohesin (the protein complex essential for sister chromatid cohesion) frequently co-localizes with CTCF [7,8] strengthened the idea that these proteins may act together to promote and stabilize chromatin loops.

Recently, Hadjur *et al.* explored the potential role of CTCF and cohesin in the long-range regulation of gene expression [9]. In particular, they analyzed the

developmentally regulated human interferon- γ (*IFNG*) locus, which encodes a cytokine expressed by a subgroup of T helper cells (Th1) and not by another (Th2). CTCF and RAD21 (a component of the cohesin complex) were demonstrated by chromatin immunoprecipitation to co-localize at three conserved elements in both cell types. However, both CTCF and RAD21 were more enriched in Th1 than in Th2 cells (in which the *IFNG* locus is silenced). Moreover, it was found (by standard chromosome conformation capture [3C]) that long-range interactions between the CTCF/cohesin sites occurred only at the active *IFNG* locus in Th1. It was also reported that RAD21 was required for intrachromosomal loop formation. When RAD21 was knocked-down in Th1 cells (by short interfering RNA [siRNA]), CTCF remained bound to its sites but, in the absence of cohesin, did not maintain the long-range interactions. Furthermore, the authors concluded that chromatin looping may be important since depletion of RAD21 also resulted in a reduction of *IFNG* expression. This paper, together with another report [10], provides evidence that the cohesin complex co-operates with CTCF to physically connect distal genomic elements on the same chromosome. At the *IFNG* locus, the CTCF/cohesin interactions are not a simple static, structural feature. These sites appear to interact in a dynamically regulated manner. Considering the diverse regulatory functions of CTCF, comprehensive analysis of CTCF/cohesin sites at different loci will be important to clarify the mechanisms by which CTCF mediates chromatin interaction and if and where it promotes an insulating activity.

References

- 1 Lobanenkov VV, Nicolas RH, Adler VV *et al.*: A novel sequence-specific DNA binding protein which interacts with three regularly spaced direct repeats of the CCCTC-motif in the 5'-flanking sequence of the chicken *c-myc* gene. *Oncogene* 5(12), 1743–1753 (1990).
- 2 Filippova GN: Genetics and epigenetics of the multifunctional protein CTCF. *Curr. Top. Dev. Biol.* 80, 337–360 (2008).
- 3 Wallace JA, Felsenfeld G: We gather together: insulators and genome organization. *Curr. Opin. Genet. Dev.* 17(5), 400–407 (2007).
- 4 Murrell A, Heeson S, Reik W: Interaction between differentially methylated regions partitions the imprinted genes *Igf2* and *H19* into parent-specific chromatin loops. *Nat. Genet.* 36(8), 889–893 (2004).
- 5 Splinter E, Heath H, Kooren J *et al.*: CTCF mediates long-range chromatin looping and local histone modification in the β -globin locus. *Genes Dev.* 20(17), 2349–2354 (2006).
- 6 Bender MA, Byron R, Ragozy T, Telling A, Bulger M, Groudine M: Flanking HS-62.5 and 3' HS1, and regions upstream of the LCR, are not required for β -globin transcription. *Blood* 108(4), 1395–1401 (2006).
- 7 Parelho V, Hadjur S, Spivakov M *et al.*: Cohesins functionally associate with CTCF on mammalian chromosome arms. *Cell* 132(3), 422–433 (2008).
- 8 Wendt KS, Yoshida K, Itoh T *et al.*: Cohesin mediates transcriptional insulation by CCCTC-binding factor. *Nature* 451(7180), 796–801 (2008).
- 9 Hadjur S, Williams LM, Ryan NK *et al.*: Cohesins form chromosomal *cis*-interactions at the developmentally regulated *IFNG* locus. *Nature* 460(7253), 410–413 (2009).
- 10 Mishihiro T, Ishihara K, Hino S *et al.*: Architectural roles of multiple chromatin insulators at the human apolipoprotein gene cluster. *EMBO J.* 28(9), 1234–1245 (2009).

The regulatory interplay of CpG islands and nucleosome remodeling at mammalian primary response genes

Evaluation of: Ramirez-Carrozzi VR, Braas D, Bhatt DM *et al.*: A unifying model for the selective regulation of inducible transcription by CpG islands and nucleosome remodeling. *Cell* 138, 114–128 (2009).

The mammalian primary response genes are an important group of genes whose transcription is rapidly and directly induced by a number of external stimuli via the Toll-like receptor (TLR) and other signaling pathways. By characterizing the promoters of these genes, this paper sets out to explore the different mechanisms which exist to regulate inducible transcription in mammals [1]. A previously published simultaneous knockdown in mouse macrophages of Brg1 and Brm, the catalytic subunits of the SWI/SNF chromatin remodeling complex, revealed that the primary response genes could be divided into those whose induction was dependent or independent of the chromatin remodeling activity of SWI/SNF [2]. Here, the authors have used microarrays to expand the group of TLR-inducible genes analyzed, and compared the characteristics of SWI/SNF dependent and independent promoters among this larger set.

It was found that primary response genes that lack a CpG island are much more likely to depend on SWI/SNF, and conversely almost all primary response genes with a CpG island are SWI/SNF-independent.

Chromatin immunoprecipitation experiments suggested that the SWI/SNF independence of CpG island promoters arises since these promoters are inherently more likely to exhibit a lower and less stable nucleosome occupancy than nonisland promoters, and to adopt an active chromatin configuration (higher levels of H3K4me3, H3K9/K14ac and higher basal levels of associated RNA polymerase II) even in the uninduced state. In an *in vitro* nucleosome assembly competition assay, non-CpG island promoter sequences competed more effectively than island promoters for a limited pool of recombinant histone octamers, suggesting that nucleotide content (probably a paucity of the phased AA/TT dinucleotides known to favor stable nucleosome assembly [3]) directly reduces nucleosome stability at CpG island promoters. The authors propose that this inherent nucleosome instability in turn gives rise to the reduced nucleosome occupancy, SWI/SNF independence, and enhanced basal recruitment of Pol2, which characterize inducible CpG island promoters. Among the CpG island, SWI/SNF-independent promoter group, there was an enrichment for genes encoding transcription factors, which are known to be induced by a wide range of stimuli. The authors suggest that CpG island, SWI/SNF-independent promoters are suited to genes that require more promiscuous activation in response to diverse

stimuli. In contrast, non-CpG-island, SWI/SNF-dependent promoters were induced by relatively few stimuli. These properties may thus be important to restrict the activity of genes that require tight regulation.

This paper provides a useful framework linking primary nucleotide content to chromatin structure and the need for nucleosome remodeling among the primary response genes, and suggests that the mechanistic differences between these major promoter classes has important functional ramifications. It will be interesting to see how this framework can be extrapolated usefully beyond the primary response genes to include noninducible genes. Moreover, it will be important to investigate more conclusively how the effects of distal regulatory elements impact upon this model.

References

- 1 Ramirez-Carrozzi VR, Braas D, Bhatt DM *et al.*: A unifying model for the selective regulation of inducible transcription by CpG islands and nucleosome remodeling. *Cell* 138, 114–128 (2009).
- 2 Ramirez-Carrozzi VR, Nazarian AA, Li CC *et al.*: Selective and antagonistic functions of SWI/SNF and Mi-2 β nucleosome remodeling complexes during an inflammatory response. *Genes Dev.* 20(3), 282–296 (2006).
- 3 Segal E, Fondufe-Mittendorf Y, Chen L *et al.*: A genomic code for nucleosome positioning. *Nature* 442(7104), 772–778 (2006).

XNP/ATRX at sites of nucleosome replacement

Evaluation of: Schneiderman JL, Sakai A, Goldstein S, Ahmad K: The XNP remodeler targets dynamic chromatin in *Drosophila*. *Proc. Natl Acad. Sci. USA* 106, 14472–14477 (2009).

Members of the SNF2 family are generally regarded as chromatin remodelers

that use the energy of ATP binding and hydrolysis to manipulate histone–DNA contacts in nucleosomes [1]. However, for factors such as ATRX that perform poorly in the standard remodeling assays, there are few clues as to their function [2]. When ATRX is mutated in humans it gives rise to a severe form of syndromal mental retardation [3]. One feature of this is the presence

of α -thalassemia, a mild anemia, resulting from downregulation of α -globin expression. To date it is unknown how ATRX is targeted or what role it plays in transcription. Valuable clues are suggested by recent research in the Ahmad laboratory on XNP, the *Drosophila* homolog of ATRX [4].

XNP was observed by indirect immunofluorescence to localize to transcribed

genes and, intriguingly, to a focus that high-resolution mapping revealed to be a 50-kb satellite block of TAGA repeats. On finding that the latter had a low histone density, they hypothesized that this repeat might be difficult to wrap around a histone octamer and may therefore be a site of nucleosome instability. In common with transcribed genes, such a site might be associated with nucleosome replacement throughout the cell cycle. The H3.3 histone variant is used for replication-independent nucleosome replacement, and by pulse-labeling larvae with green fluorescent protein-labeled H3.3 they indeed demonstrated that the XNP focus was a major site of H3.3 deposition [4]. It is therefore apparent that what the XNP targets

(transcribed genes and TAGA repeat) have in common is rapid nucleosome replacement. In *Drosophila*, disruption of XNP did not appear to affect H3.3 deposition at these loci; therefore, although XNP appears to target sites of rapid nucleosome turnover, it is obviously not required for this process in this species. However, it seems highly likely that a penchant for such loci is an intrinsic property of this protein and the next steps will be to explore the biological function of XNP/ATRX at sites of nucleosome turnover.

References

- 1 Kingston RE, Bunker CA, Imbalzano AN: Repression and activation by multiprotein complexes that alter chromatin structure. *Genes Dev.* 10, 905–920 (1996).
- 2 Xue Y, Gibbons R, Yan Z *et al.*: The ATRX syndrome protein forms a chromatin-remodeling complex with Daxx and localizes in promyelocytic leukemia nuclear bodies. *Proc. Natl Acad. Sci. USA* 100(19), 10635–10640 (2003).
- 3 Gibbons RJ, Picketts DJ, Villard L, Higgs DR: Mutations in a putative global transcriptional regulator cause X-linked mental retardation with α -thalassemia (ATR-X syndrome). *Cell* 80, 837–845 (1995).
- 4 Schneiderman JI, Sakai A, Goldstein S, Ahmad L: The XNP remodeler targets dynamic chromatin in *Drosophila*. *Proc. Natl Acad. Sci. USA* 104(34), 14472–14477 (2009).