Nucleosome positioning and transcription: fission yeast CHD remodellers make their move

Leila Touat-Todeschini, Edwige Hiriart and André Verdel*

INSERM, U823, Université Joseph Fourier – Grenoble 1, Institut Albert Bonniot, Faculté de Médecine, La Tronche Cedex, France.

*Correspondence to: andre.verdel@ujf-grenoble.fr

The EMBO Journal (2012) 31, 4371–4372. doi:10.1038/emboj.2012.284; Published online 26 October 2012

Regularly positioned nucleosomes are a common feature of 5' ends of most eukaryotic genes. A series of three studies, Shim *et al* (2012) and Pointner *et al* (2012) in this issue of The EMBO Journal and Hennig *et al* (2012) in EMBO Reports, now show that in the fission yeast *Schizosaccharomyces pombe* this intragenic nucleosome positioning mostly requires two ATP-dependent remodellers of the CHD family, Hrp1 and Hrp3. Moreover, they suggest that Hrp1- and Hrp3-dependent nucleosome spacing contributes to the silencing of cryptic antisense transcription.

In eukaryotes, genomic DNA is packed into a nucleoprotein structure called chromatin. The nucleosome represents the basic and repetitive unit of chromatin, which consists of ~147 base pairs of DNA wrapped around an octamer of histone core proteins. Packing DNA into nucleosomes contributes to the compaction of genomic DNA into higher order chromatin states. In parallel, it makes DNA less accessible to DNA-templated processes such as transcription. This has two major consequences: first, it implies a requirement for mechanisms increasing the accessibility to nucleosomal DNA to allow transcription to occur in a proficient manner; second, it represents a powerful mean to regulate transcription by stimulating or inhibiting the remodelling of specific nucleosomes.

It has been shown in several eukaryotes that many genes have a common nucleosome organization at their 5' end, which consists of a nucleosome-free region (NFR) at the transcription start site (TSS) bordered by two arrays of well-positioned nucleosomes (Jiang and Pugh, 2009). The DNA sequence itself, either by its ability to recruit DNA-binding proteins or through its intrinsic capacity to curve more or less the DNA, contributes to some extent to this regular spacing of nucleosomes. However, it is clearly not the only element involved. ATP-dependent remodellers also play a major role (Korber, 2012).

The budding yeast *Saccharomyces cerevisiae* and its distantly related fission yeast *Schizosaccharomyces pombe* are two well-known model organisms in the chromatin field. In *S. cerevisiae*, two types of Snf2 ATP-dependent remodellers, Isw1 and Chd1, are required to maintain the regular nucleosome spacing around promoters (Gkikopoulos *et al*, 2011). Intriguingly, in *S. pombe* there are homologues of Chd1, but no apparent Isw1-type remodeller. This has brought an important question: which ATP-dependent remodeller(s) is (are) doing the job in *S. pombe*?

Three studies from Shim *et al* (2012), Pointner *et al* (2012), and Hennig *et al* (2012) now show that two CHD-type remodellers, Hrp1 and Hrp3, are required to regularly space nucleosomes at the 5' end of most genes in *S. pombe*. In addition, Pointer *et al* (2012) report that, Mit1, the third and last member of the *S. pombe* CHD remodeller family, does not play a significant role at these sites. They also examined the role of the RSC-type remodeller, Snf21, which is required for the formation of many promoter-associated NFRs in *S. cerevisiae* (Korber, 2012). Quite unexpectedly, they found that a disruption of Snf21 function did not lead to

Figure 1 Model for the positioning of nucleosomes at the 5' end of genes and the suppression of intragenic cryptic transcription in the fission yeast *S. pombe*. In *S. pombe*, as in many eukaryotes, the chromatin organization at the 5' end of most genes is well defined. It consists of a nucleosome-free region (NFR) at the promoter bordered by positioned nucleosomes (grey circles). This nucleosome positioning decreases as the distance from the promoter increases. Two CHD-type ATP-dependent remodellers, Hrp1 and Hrp3 (blue ovals), play a major role in positioning nucleosomes downstream transcription start sites (TSSs). A lack of both remodellers also leads to an increase in cryptic antisense transcription (dashed arrows), suggesting that Hrp1- and Hrp3-dependent nucleosomal positioning may silence intragenic cryptic promoters. Such silencing may act in parallel with Clr6 histone deacetylase (HDAC) complex II, a Rdp3-type HDAC complex, and Set2, the lysine 36 histone methyltransferase (KMT) (green ovals), which also participates in the suppression of such cryptic transcription.

significant changes at these NFRs in *S. pombe*. Thus, remarkably, another important distinction between the two yeasts may exist in the mechanisms generating promoter-associated NFRs.

Interestingly, Pointner and co-workers conducted a first series of *in vitro* experiments supporting a direct action of Hrp1 and Hrp3 remodellers in phasing nucleosomes. *In vitro* reconstitution of chromatin using *S. cerevisiae* extracts has been recently used successfully to form regular nucleosome arrays and NFRs at 5' ends of many genes (Zhang *et al*, 2011; Korber, 2012). Based on the fact that critical *S. pombe* remodellers are now identified and have been used in *in vitro* experiments, the time for *in vitro* reconstitution of chromatin with *S. pombe* extracts may not be so far ahead, and should then represent a powerful mean to further examine the roles of Hrp1, Hrp3, Snf21, and other possible factors in shaping chromatin at, and around, promoters.

Because the chromatin architecture influences the level of transcription, the role of Hrp1 and Hrp3 in the control of transcription was also addressed in all three studies. They collectively show that the elimination of *hrp1* and *hrp3* genes leads to a significant increase in intragenic antisense transcription, suggesting that Hrp1 and Hrp3 nucleosome positioning may directly silence cryptic transcription. This comes in support of previous studies, which suggest that, in a similar fashion, Isw1 and Chd1 remodellers are important for maintaining low levels of cryptic transcription in *S. cerevisiae* (Owen-Hughes and Gkikopoulos, 2012). The silencing of cryptic transcription is a complex process involving several layers of regulation including histone modifications and histone exchange (Owen-Hughes and Gkikopoulos, 2012; Venkatesh *et al*, 2012). In *S. cerevisiae*, the Rpd3S histone deacetylase complex and Set2 histone methyltransferase work together with ATP-dependent remodellers to silence cryptic transcription (Smolle *et al*, 2012). The works of Shim *et al* (2012) and Hennig *et al* (2012) suggest that the *S. pombe* Rpd3S-like histone deacetylase complex, Clr6 complex-II, and the Set2 methyltransferase cooperate with Hrp1 and Hrp3 to silence

cryptic transcription. Moreover, Hennig *et al* (2012) report that the silencing mediated by Hrp1 and Hrp3 and by the histone chaperone complex FACT likely relies on different mechanisms.

Finally, an important question that has been partially addressed is the following: how then are Hrp1 and Hrp3 remodellers recruited to genes? In *S. cerevisiae*, the H3K36 methylation mark catalysed by Set2 is recognized by a subunit of the Isw1 complex, which in turn recruits the Isw1 remodeller to the body of genes (Smolle *et al*, 2012). A similar mechanism could be recruiting Hrp1 and Hrp3 to chromatin. However, the fact that Set2 and both Hrp1 and Hrp3 likely act in parallel suggests that there may be different or additional mechanisms of recruitment in *S. pombe*. Interestingly, Shim and co-workers report that Hrp3 binds to pericentromeric heterochromatin in a Swi6-dependent manner and that it interacts with Swi6. This suggests that Swi6 might directly recruit Hrp3 to heterochromatin. This interaction is unlikely responsible for Hrp1 and Hrp3 recruitment at genes, since Swi6 localizes to only few euchromatic genes (Cam *et al*, 2005; Woolcock *et al*, 2011). However, it is tempting to speculate that in other organisms a similar mode of recruitment may be involved in regulating the chromatin architecture of various genes. Indeed, Swi6 belongs to the HP1 family, which is conserved in many eukaryotes and some members of this family localize within euchromatic genes (Kwon and Workman, 2011).

Altogether, these three new studies provide an up-to-date picture of the actors involved in intragenic nucleosome positioning and silencing of cryptic transcription in *S. pombe* (Figure 1). In addition, by highlighting similarities and differences between quite diverged yeasts, they constitute a valuable source of information in defining the core mechanisms shaping chromatin structure around promoters in most eukaryotes.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Cam HP, Sugiyama T, Chen ES, Chen X, FitzGerald PC, Grewal SI (2005) Comprehensive analysis of heterochromatin- and RNAi-mediated epigenetic control of the fission yeast genome. *Nat Genet* **37**: 809–819
- Gkikopoulos T, Schofield P, Singh V, Pinskaya M, Mellor J, Smolle M, Workman JL, Barton GJ, Owen-Hughes T (2011) A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization. *Science* **333**: 1758–1760
- Hennig BP, Bendrin K, Zhou Y, Fischer T (2012) Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription. *EMBO Rep* **13**: 997–1003
- Jiang C, Pugh BF (2009) Nucleosome positioning and gene regulation: advances through genomics. *Nat Rev Genet* **10**: 161–172
- Korber P (2012) Active nucleosome positioning beyond intrinsic biophysics is revealed by *in vitro* reconstitution. *Biochem Soc Trans* **40**: 377–382
- Kwon SH, Workman JL (2011) The changing faces of HP1: From heterochromatin formation and gene silencing to euchromatic gene expression: HP1 acts as a positive regulator of transcription. *Bioessays* **33**: 280–289
- Owen-Hughes T, Gkikopoulos T (2012) Making sense of transcribing chromatin. *Curr Opin Cell Biol* **24**: 296–304
- Pointner J, Persson J, Prasad P, Norma-Axelsson U, Strålfors A, Khorosjutina O, Krietenstein N, Svensson JP, Ekwall K, Korber P (2012) CHD1 remodelers regulate nucleosome spacing *in vitro* and align nucleosomal arrays over gene coding regions in *S. pombe*. *EMBO J* **31**: 4388–4403
- Shim YS, Choi Y, Kang K, Cho K, Oh S, Lee J, Grewal SI, Lee D (2012) Hrp3 controls nucleosome positioning to suppress non-coding transcription in eu- and heterochromatin. *EMBO J* **31**: 4375–4387
- Smolle M, Venkatesh S, Gogol MM, Li H, Zhang Y, Florens L, Washburn MP, Workman JL (2012) Chromatin remodelers Isw1 and Chd1 maintain chromatin structure during transcription by preventing histone exchange. *Nat Struct Mol Biol* **19**: 884–892
- Venkatesh S, Smolle M, Li H, Gogol MM, Saint M, Kumar S, Natarajan K, Workman JL (2012) Set2 methylation of histone H3 lysine 36 suppresses histone exchange on transcribed genes. *Nature* **489**: 452–455
- Woolcock AJ, Gaidatzis D, Punga T, Buhler M (2011) Dicer associates with chromatin to repress genome activity in *Schizosaccharomyces pombe*. *Nat Struct Mol Biol* **18**: 94–99
- Zhang Z, Wippo CJ, Wal M, Ward E, Korber P, Pugh BF (2011) A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. *Science* **332**: 977–980