In need of good neighbours: transcription factors require local DNA hypomethylation for target binding

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Whether and how DNA methylation influences the binding of transcription factors (TFs) to their corresponding DNA sequence motifs *in vivo* remains largely unresolved. In a recent publication, Schübeler and co-workers (Domcke *et al*, 2015) identify a few methylation-restricted TFs in mouse embryonic stem cells, including NRF1. The authors show that NRF1 binding to its motif can be outcompeted by *de novo* DNA methylation, suggesting that methylation-sensitive TFs rely on neighbouring motifs *in cis*—bound by pioneer TFs—to ensure local hypomethylation.

See also: S Domcke *et al* (December 2015)

Although all cells of an adult eukaryotic organism harbour the same DNA content, they constitute a multitude of different cell types. This phenotypical diversity is achieved by cell type-specific gene expression programmes; how this differential gene expression is regulated remains an active area of research. Transcription factors (TFs) play an important role in regulating gene expression by binding to their corresponding DNA sequence motifs and thereby regulating transcription of nearby genes. TF–DNA interactions have been studied extensively *in vitro*, uncovering various contributing factors such as the presence of cofactors, chromatin accessibility and DNA methylation (reviewed in Slattery *et al*, 2014). However, TFs only bind to a subset of their motifs *in vivo*. The mechanisms behind this selectivity remain largely unresolved, as it has thus far proven difficult to directly translate the knowledge

obtained in *in vitro* experiments to the *in vivo* situation (Slattery *et al*, 2014).

In a recent elegant study, Domcke, Bardet and colleagues investigate the role of DNA methylation in regulating TF binding *in vivo* (Domcke *et al*, 2015). For this, they make use of mouse embryonic stem cells (mESCs), which are the only mammalian cells that are known to survive in the absence of DNA methylation (Tsumura *et al*, 2006). DNA methylation-depleted mESCs are obtained through a CRISPR-mediated triple knockout (TKO) of the DNA methyltransferases (Dnmts). By comparing DNase I hypersensitive sites (DHS) between these TKO and wild-type (WT) cells, regions of TF binding are mapped that change in a methylation-dependent manner (Neph *et al*, 2012). Although the bulk of DHS remain unchanged, a search for known TF motifs enriched in TKO-specific DHS identifies a few methylation-sensitive TFs, most notably nuclear respiratory factor 1 (NRF1). TKO-specific NRF1 motifs contain mostly two CpGs, and NRF1 binding in TKO cells is much increased at motifs that are highly methylated in WT cells, regardless of the density of methylated CpGs in the surrounding region, suggesting that NRF1 binding to its motif is directly inhibited by methylation.

The previously described experiments all use mESCs that are cultured in medium containing serum, recapitulating the genome-wide methylation status as observed in the post-implantation epiblast (Borgel *et al*, 2010). Additionally, the authors use mESCs that are cultured in the presence of two small-molecule inhibitors (2i conditions), resulting in a more homogeneous

population of cells corresponding to the pre-implantation embryo (Ying *et al*, 2008). 2i conditions have also been shown to cause genome-wide DNA demethylation by repression of *de novo* Dnmts (Ficz *et al*, 2013), providing a physiological context of low DNA methylation in which to study NRF1 binding. In line with expectations, transfer of WT mESCs from serum-containing medium to 2i conditions results in hypomethylation of most of the previously defined TKO-specific sites as well as increased NRF1 binding to these sites. In the reverse experiment, transferring WT cells from 2i conditions back to medium containing serum causes both remethylation of methylation-dependent sites and abrogated NRF1 binding. Thus, binding of NRF1 does not protect the bound DHS against DNA methylation, as *de novo* methylation can outcompete NRF1 binding.

These findings imply that NRF1 binding depends on additional factors that are necessary to induce local hypomethylation of its motifs. Indeed, the authors perform a series of experiments using reporter constructs that confirm this hypothesis. First of all, although NRF1 can bind to its motif autonomously, this is only true in the absence of DNA methylation, as NRF1 is able to bind to its motif in a minimal sequence context exclusively upon forced demethylation. Furthermore, deletion of TF motifs for CTCF and RFX in a construct containing an NRF1 motif within an endogenous promoter sequence causes hypermethylation (Lienert *et al*, 2011) and reduced NRF1 binding. Lastly, the authors previously showed that the TF REST creates regions of low methylation at

Figure 1. NRF1 binding *in vivo* depends on the local DNA sequence context *in cis* and TFs *in trans* to ensure hypomethylation of its motif.

NRF1 can only bind autonomously to its motif when it is unmethylated (upper panel). Methylation-insensitive TFs that bind to nearby motifs *in cis* can induce local hypomethylation of the NRF1 motif (middle panel), allowing NRF1 binding (lower panel). In this study, the authors induced global hypomethylation by CRISPR-mediated TKO of Dnmts or transition from serum-containing culture conditions to 2i.

its binding sites, which become remethylated upon genetic removal of REST (Stadler *et al*, 2011). Knockout of REST leads to *de novo* methylation also at a few sites that were identified to harbour an NRF1 motif adjacent to a REST binding site, causing loss of NRF1 binding at these sites. Together, these experiments show that NRF1 binding *in vivo* depends on the local DNA sequence context *in cis* and TFs *in trans* to ensure hypomethylation of its binding motif (Fig 1).

Importantly, while mESCs are able to grow in the absence of DNA methylation, for the majority of mammalian cell types studied so far DNA methylation has been shown to be essential. It remains to be determined whether differentiated cells express a similar set of DNA methylation-sensitive TFs, and how these behave in such a context. Domcke, Bardet and colleagues show that in neuronal progenitors differentiated from mESCs as well as in several human cell lines and primary cells, NRF1 binding decreases upon increased methylation of NRF1 motifs, which is linked to lower expression of neighbouring genes as well. This suggests that at least for NRF1, methylation-sensitive binding is a general phenomenon that regulates gene expression also in other cell types. Extending these studies into other cellular contexts will

be an important step to improve our understanding of the regulation of TF binding by the chromatin context and DNA methylation.

Finally, it has been reported that certain transcription factors behave as “pioneer factors” that can interact with DNA, even in the context of condensed chromatin (Iwafuchi-Doi & Zaret, 2014). Although NRF1 is capable of forming DHS *de novo* and has therefore previously been suggested to be such a pioneer factor (Sherwood *et al*, 2014), the present study shows that NRF1 can only bind autonomously when its motif is already unmethylated. Conversely, in the presence of DNA methylation it rather acts as a “settler factor”, relying on other factors to mediate hypomethylation of its motif. The identity of these DNA methylation-insensitive pioneer factors which induce local DNA hypomethylation remains largely unknown, as well as how their binding to DNA is regulated. Addressing this question is essential to grasp the mechanisms behind gene expression regulation, and ultimately to understand the molecular principles of cellular diversity.

References

Borgel J, Guibert S, Li Y, Chiba H, Schübeler D, Sasaki H, Forné T, Weber M (2010) Targets and dynamics

of promoter DNA methylation during early mouse development. *Nat Genet* 42: 1093–1100

Domcke S, Bardet AF, Adrian Ginno P, Hartl D, Burger L, Schübeler D (2015) Competition between DNA methylation and transcription factors determines binding of NRF1. *Nature* 528: 575–579

Ficz G, Hore TA, Santos F, Lee HJ, Dean W, Arand J, Krueger F, Oxley D, Paul Y-L, Walter J, Cook SJ, Andrews S, Branco MR, Reik W (2013) FGF Signaling Inhibition in ESCs Drives Rapid Genome-wide Demethylation to the Epigenetic Ground State of Pluripotency. *Cell Stem Cell* 13: 351–359

Iwafuchi-Doi M, Zaret KS (2014) Pioneer transcription factors in cell reprogramming. *Genes Dev* 28: 2679–2692

Lienert F, Wirbelauer C, Som I, Dean A, Mohn F, Schübeler D (2011) Identification of genetic elements that autonomously determine DNA methylation states. *Nat Genet* 43: 1091–1097

Neph S, Vierstra J, Stergachis AB, Reynolds AP, Haugen E, Vernot B, Thurman RE, John S, Sandstrom R, Johnson AK, Maurano MT, Humbert R, Rynes E, Wang H, Vong S, Lee K, Bates D, Diegel M, Roach V, Dunn D *et al* (2012) An expansive human regulatory lexicon encoded in transcription factor footprints. *Nature* 489: 83–90

Sherwood RI, Hashimoto T, O'Donnell CW, Lewis S, Barkal AA, van Hoff JP, Karun V, Jaakkola T, Gifford DK (2014) Discovery of directional and nondirectional pioneer transcription factors by modeling DNase profile magnitude and shape. *Nat Biotechnol* 32: 171–178

Slattery M, Zhou T, Yang L, Dantas Machado AC, Gordân R, Rohs R (2014) Absence of a simple code: how transcription factors read the genome. *Trends Biochem Sci* 39: 381–399

Stadler MB, Murr R, Burger L, Ivanek R, Lienert F, Schöler A, van Nimwegen E, Wirbelauer C, Oakeley EJ, Gaidatzis D, Tiwari VK, Schübeler D (2011) DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature* 480: 490–495

Tsumura A, Hayakawa T, Kumaki Y, Takebayashi S, Sakaue M, Matsuoka C, Shimotohno K, Ishikawa F, Li E, Ueda HR, Nakayama J, Okano M (2006) Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. *Genes Cells* 11: 805–814

Ying Q-L, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, Cohen P, Smith A (2008) The ground state of embryonic stem cell self-renewal. *Nature* 453: 519–523