Development 140, 505-512 (2013) doi:10.1242/dev.083105
© 2013. Published by The Company of Biologists Ltd

Transcriptional repressors: multifaceted regulators of gene expression

Nicola Reynolds, Aoife O'Shaughnessy and Brian Hendrich*

Summary

Through decades of research it has been established that some chromatin-modifying proteins can repress transcription, and thus are generally termed 'repressors'. Although classic repressors undoubtedly silence transcription, genome-wide studies have shown that many repressors are associated with actively transcribed loci and that this is a widespread phenomenon. Here, we review the evidence for the presence of repressors at actively transcribed regions and assess what roles they might be playing. We propose that the modulation of expression levels by chromatin-modifying, co-repressor complexes provides transcriptional fine-tuning that drives development.

Key words: NuRD, Chromatin, co-repressor, Deacetylase, Histone, Transcription

Introduction

Every major developmental process may be regarded as being driven by changes in gene expression patterns. It is crucial that such changes, either throughout development or in response to environmental stimuli, are tightly regulated. For any given cell type, distinct transcriptional programmes must be established whereby certain genes are transcribed and others remain silent. At the same time, cells must remain responsive to changes in their environment or to developmental signals, for which the ability to rapidly change transcription patterns is essential.

The control of gene expression programmes is inextricably linked to the local state of chromatin, the form in which DNA is packaged within the cell. DNA in eukaryotic nuclei is packaged with histone proteins into nucleosomes (Kornberg, 1974). Post-translational modification of histone tails within the nucleosome may occur through the addition of a multitude of different chemical modifications, including acetylation, methylation and phosphorylation, at specific sites. These modifications influence gene expression patterns either by altering chromatin conformation, and thereby allowing or restricting access of transcription factors to that locus, or by changing interactions of transcription factors with the nucleosomes themselves (Berger, 2007; Kouzarides, 2007).

It is generally accepted that chromatin state correlates with transcriptional state; histone acetylation, for example, has most often been associated with active transcription whereas deacetylation is associated with silencing (Bannister and Kouzarides, 1996; Strahl and Allis, 2000). It therefore follows that lysine acetyltransferases (KATs), which catalyse acetylation of lysine residues, act as transcriptional activators, and lysine deacetylases (KDACs), which remove acetyl groups from acetylated lysines, act as repressors. This correlation is supported by experiments in which chromatin modifiers were found to activate or repress transcription, often in reporter gene assays (Grunstein, 1997; Pazin and Kadonaga, 1997; Wolffe, 1997; Yang and Seto, 2007). Although such a binary model of transcription factor-mediated gene expression was very important in the early stages of understanding how these proteins work at a general level, it has proven to be too simplistic to explain the full range of complexities of transcriptional control *in vivo*. Indeed, work in *Saccharomyces cerevisiae* made it clear that the correlation between acetylation state and transcriptional status is not always so clear-cut and might instead be dependent on individual activator or repressor complexes (Deckert and Struhl, 2001).

Although correlations between activators, repressors and absolute transcriptional status undoubtedly exist in many cases, recent literature has highlighted a difference between the classic viewpoint, in which transcription simply switches between off and on states, and what is likely to be a much more subtle (and therefore more biologically useful) way of modulating gene expression levels. The advent of genome-wide technologies, such as chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq), in particular, has enabled global analyses of DNA-protein interactions. These studies have revealed that many chromatin regulators that have traditionally been considered to be repressors of transcription are associated not only with silent loci, as would be predicted, but also with actively transcribed genes. In many cases, the association of what was commonly thought to be a repressor with a promoter is in fact required for full activation of gene expression. The multifaceted nature of transcriptional repressors has long been known in simple organisms such as *S. cerevisiae* and *S. pombe* (De Nadal et al., 2004; Kurdistani et al., 2002; Wang et al., 2002; Wirén et al., 2005), but growing evidence indicates that this is also true in higher organisms (Aichinger et al., 2009; Harrison et al., 2011; Murawska et al., 2011; Murawska et al., 2008; Reynolds et al., 2012a; Wang et al., 2009). Here, we summarise the growing body of evidence that proteins and protein complexes normally referred to as 'transcriptional repressors' actually function to fine-tune transcript levels, and that this transcriptional tuning, rather than all-or-nothing gene expression changes, underlies key developmental decisions during development.

Evidence for a multifaceted role for repressors

From the reasonable, although simplistic, view that repressors act to inhibit transcription, it might be expected that these complexes would only be found occupying silent regions of the genome. However, based on DNA association alone, assessed by genome-wide ChIP analyses, it is clear that lysine deacetylases are not confined to silent loci (Dovey et al., 2010; Kurdistani et al., 2002; Wang et al., 2002; Wirén et al., 2005). A comprehensive study to assess the genome-wide distribution of multiple KDACs and KATs in human T cells revealed that a surprisingly small proportion (~20%) of KDACs were found at silent loci. By contrast, up to

Wellcome Trust – Medical Research Council Stem Cell Institute, Department of Biochemistry, University of Cambridge, Cambridge CB2 1QR UK.

*Author for correspondence (brian.hendrich@cscr.cam.ac.uk)

~75% of KDAC proteins were identified at actively transcribed regions, although the proportion varied depending on the protein investigated (Wang et al., 2009). Similarly, in *Drosophila melanogaster*, the chromatin remodeller protein Mi-2 is normally associated with gene repression as part of the nucleosome remodelling and deacetylation (NuRD) co-repressor complex (Ahringer, 2000; Kehle et al., 1998). However, Mi-2 colocalises with the elongating form of RNA Polymerase II (RNA PolII) at actively transcribed genes on polytene chromosomes and is recruited to sites of transcription upon heat shock (Mathieu et al., 2012; Murawska et al., 2011; Murawska et al., 2008). Furthermore, larvae either depleted of Mi-2 or expressing a dominant ATPase-mutant form of the protein showed significantly reduced levels of heat-shock transcripts, demonstrating that Mi-2 function is important for transcription in this context (Murawska et al., 2011).

The mammalian orthologue of *Drosophila* Mi-2 is Mi2β (Chd4), a defining component of the mammalian NuRD complex. NuRD is a classic lysine deacetylase-containing co-repressor complex. In addition to Mi2β, the NuRD complex consists of a core of two lysine deacetylase proteins, Hdac1 and Hdac2, along with two chaperone proteins, Rbbp4 and Rbbp7, together with associated proteins. These accessory proteins include Gatad2a, Gatad2b, Mta1, Mta2 and/or Mta3, Cdk2ap1 and the scaffolding protein Mbd3 (Bowen et al., 2004; Le Guezennec et al., 2006; McDonel et al., 2009; Tong et al., 1998; Wade et al., 1998; Xue et al., 1998; Zhang et al., 1998). Other associated proteins, such as Sall4, Lsd1 (Kdm1a), Ikaros (Ikzf1) or Oct4 (Pou5f1), may associate with a fraction of NuRD complexes in a cell type-specific manner. NuRD has been shown to repress transcription in a wide variety of cell types and systems (Ahringer, 2000; McDonel et al., 2009). Nevertheless, a number of ChIP-seq studies have found Mi2β also to be associated with a surprisingly large number of active gene loci in different cell types (Reynolds et al., 2012b; Whyte et al., 2012; Zhang et al., 2012).

ChIP-seq experiments for components of the polycomb repressive complexes (PRCs), or the marks they leave on chromatin (e.g. trimethylation of lysine 27 of histone H3, H3K27Me3; and monoubiquitylation of lysine 119 of histone H2A, H2Aub1), have also found that PRC target genes are often expressed at intermediate or high levels (Brookes et al., 2012; Schwartz et al., 2010; Young et al., 2011). At first glance, this would appear to indicate that PRC complexes do, occasionally, co-exist with active RNA polymerase at the same genes. In apparent contradiction to this observation, an analysis of PRC target genes in *D. melanogaster* showed that PRC1-bound promoters are actually associated with stalled, but not elongating, RNA polymerase (Enderle et al., 2011). A similar set of correlations was recently reported for mammalian PRC components in mouse embryonic stem (ES) cells (Brookes et al., 2012). Using sequential ChIP (in which DNA is immunoprecipitated sequentially by antibodies recognising two different proteins to identify sequences simultaneously bound by the two proteins), Brookes et al. (Brookes et al., 2012) found that PRC components could only be immunoprecipitated at DNA associated with the poised polymerase (S5P), but not with the elongating polymerase (S2P). This indicates that PRC and the elongating polymerase do not colocalise to a given gene at the same time, but rather that ES cell cultures consist of a heterogeneous mixture of cells in which certain genes are either associated with PRC and thus transcriptionally poised, or are free of PRC influence and in the process of active elongation (Brookes et al., 2012).

Further hints of the diverse functionality of co-repressors have come from expression analyses in loss-of-function experiments. Removal of co-repressor function, either genetically or through the use of chemical inhibitors, results in gene expression changes that tend to be subtle. Rather than genes going from off to on, as might be expected if these proteins function simply to silence, loss of repressor proteins often results in a modest (up to ten times) increase in the levels of some transcripts, many of which are not normally silent in wild-type cells. Loss of co-repressor proteins usually results in similar numbers of genes decreasing in expression as increasing (Bernstein et al., 2000; Harrison et al., 2011; Lenstra et al., 2011; Reynolds et al., 2012a; Williams et al., 2011; Wirén et al., 2005; Yoshida et al., 2008; Zupkovitz et al., 2006). Although in many instances increase in transcript levels could be an indirect effect of loss of repressive activity (e.g. failure to repress a repressor), in at least some of these cases genes found to be downregulated in the mutants are bound by the repressor in wild-type cells (e.g. Reynolds et al., 2012b; Williams et al., 2011), indicating that these repressors might act to regulate different loci in opposing directions.

Repressors as modulators: mechanisms of action

As highlighted above, genome-wide analyses in yeast and in metazoans have demonstrated that repressors associate with actively transcribed loci and that their absence is associated with both increases and decreases of transcriptional output at different genes. These findings contribute to a body of compelling, yet circumstantial, evidence that repressors can play a role in activation of gene expression. The first convincing demonstration that repressor-mediated activation can regulate specific groups of genes came from work in *S. cerevisiae*. Indeed, the initial description of the Sin3 co-repressor complex identified it as a protein with dual functions: acting as both an activator and a repressor *in vivo* (Nawaz et al., 1994; Vidal and Gaber, 1991; Vidal et al., 1991). Other studies focused on the specific regulation of individual genes by a variety of repressor complexes, and these studies have perhaps been the most powerful in changing the orthodox view that repressor complexes merely inhibit transcription (summarised in Box 1). Such studies have identified clear roles for so-called repressors in activating gene expression, but how might these be mediating both activating and repressive functions? Below, we

Box 1. Repressors as activators?

The association of repressor complexes with sites of active transcription does not necessarily mean that they activate transcription, but a number of detailed studies confirm that, at least in some instances, repressors can and do activate transcription:

- Lysine deacetylase activity promotes transcription of galactose- and inositol-responsive genes in *S. cerevisiae* (Wang et al., 2002).
- Transcription induced by either osmotic or heat stress in *S. cerevisiae* requires the Sin3-Rpd3 KDAC complex for transcriptional activation to occur (De Nadal et al., 2004).
- Mi-2 is required for activation of heat-shock genes in fruit flies (Murawska et al., 2011).
- Hdac1 promotes expression of a subset of neural-specific genes in zebrafish (Harrison et al., 2011).
- Transcription of cytokine-inducible genes in mammalian cells is dependent on deacetylase activity (Chang et al., 2004; Klampfer et al., 2004; Nusinzon and Horvath, 2003; Nusinzon and Horvath, 2005; Rascle et al., 2003; Sakamoto et al., 2004; Xu et al., 2003; Zupkovitz et al., 2006).
- The NuRD complex mediates transcriptional activation during erythropoiesis in mice (Miccio et al., 2010).

summarise existing evidence for how repressors function at actively transcribed genes.

The RNA polymerase connection

In many cases, mapping protein distribution by ChIP shows that repressor complex occupancy can extend to differing extents throughout the body of a gene (Johnsson et al., 2009; Joshi and Struhl, 2005; Kurdistani et al., 2002; Mathieu et al., 2012; Miccio et al., 2010; Morey et al., 2008; Murawska et al., 2011; Murawska et al., 2008; Reynolds et al., 2012a; Reynolds et al., 2012b; Wang et al., 2002; Wang et al., 2009). This is in contrast to the more familiar pattern seen for sequence-specific transcription factors, which generally localise tightly to the promoter region of regulated genes. Consistent with a broader association across gene loci, loss of KDAC activity through mutation or chemical inhibition can result in increased acetylation of promoters, which often spreads well into coding regions (Johnsson et al., 2009; Joshi and Struhl, 2005; Keogh et al., 2005; Li et al., 2007b; Reid et al., 2004; Wang et al., 2002; Wirén et al., 2005). What factors might influence such a broad range of protein association? Often, the distribution of transcriptional repressors across genes or chromosomes closely resembles that of the transcribing RNA polymerase, suggestive of an interaction between them (Brookes et al., 2012; Mathieu et al., 2012; Murawska et al., 2008; Srinivasan et al., 2005).

Interactions between KDAC-containing repressor complexes and elongating RNA polymerase are a well-described phenomenon in yeast. At actively transcribed genes in *S. cerevisiae*, lysine deacetylase activity, in the form of the Rpd3C(S) protein complex, has been found to be recruited to gene bodies via association with dimethylated H3K36. This specific association is mediated by the Rpd3C(S) component proteins Eaf3 and Rco1. The consequence of this association is the deacetylation of histones H3 and H4 throughout the bodies of transcribed genes, which somewhat reduces elongation efficiency but, importantly, suppresses intragenic transcription initiation (Carrozza et al., 2005; Joshi and Struhl, 2005; Keogh et al., 2005; Li et al., 2007a; Li et al., 2007b; Li et al., 2009) (Fig. 1). More recent work has indicated that Rpd3C(S) can also be recruited via a direct interaction with the elongating form of RNA polymerase (Drouin et al., 2010; Govind et al., 2010).

Recently a mammalian KDAC complex with homology to *S. cerevisiae* Rpd3C(S) has been described, which, like its fungal counterpart, associates with activated genes and can specifically recognise H3K36 methylation (Jelinic et al., 2011). Furthermore, there is indirect evidence that deacetylase activity can be recruited to sites of active transcription through interaction with the elongating RNA polymerase (Wang et al., 2009). It is possible that, like in *S. cerevisiae*, the function of vertebrate co-transcriptional lysine deacetylase activity is to reset the chromatin state after the transcription machinery has passed in order to suppress spurious transcription initiation, although no evidence to support this particular function for the mammalian Rpd3C(S) orthologous protein complex has yet been found (Jelinic et al., 2011).

Priming for a transcriptional response

Another possible role of repressors bound to actively transcribed loci is to maintain a promoter in a state that, although not actively transcribing, is primed for transcription. This would be a logical interpretation, as many of the genes decorated by these complexes are those for which responsiveness is crucial (Box 2). Examples include signalling and developmental events in mammalian cells (Azuara et al., 2006; Bernstein et al., 2006), heat shock in insects (Murawska et al., 2011; Murawska et al., 2008) and environmental stimuli in yeast (De Nadal et al., 2004; Wang et al., 2002). Such priming allows for a more rapid response than changing bulk histone modifications and/or assembly of a new pre-initiation complex, effectively maintaining the promoter region in a state that can be switched rapidly to either fully on or completely off (Weake and Workman, 2010) (Fig. 2). This could explain the observation in T cells that, although KDAC association was often undetectable at silent genes by ChIP-seq, treatment with KDAC inhibitors resulted in a rapid (≤10 minutes) increase in acetylation at some of those same genes (Wang et al., 2009). More sensitive analysis by directed ChIP-qPCR revealed that KDACs were present at these loci at reproducible, albeit low, levels, implicating KDAC activity in maintaining transcriptional silencing. In other words, deacetylation as a true silencing event may be thought of as part of a cycle of transient association of KATs and KDACs, a mechanism that would allow rapid activation of these genes.

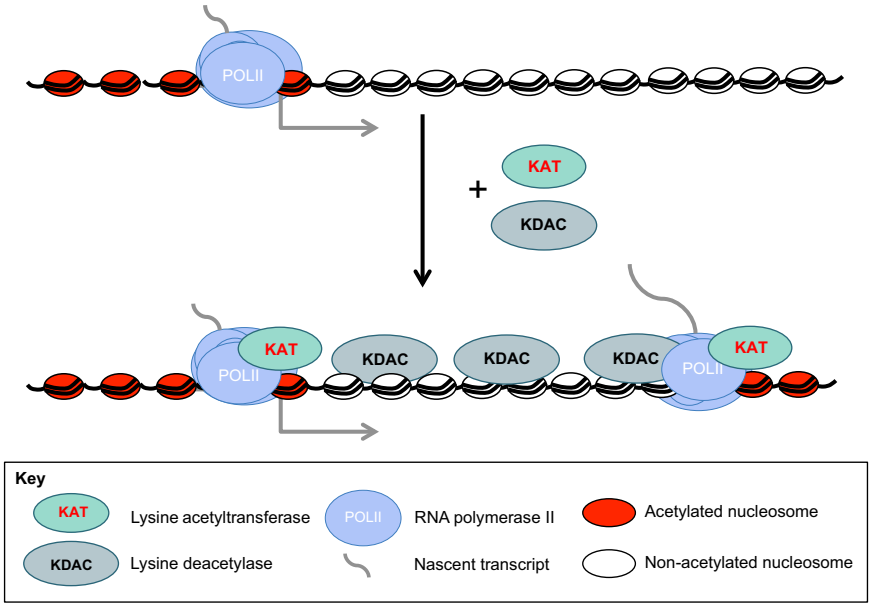

Fig. 1. Suppression of cryptic transcription by deacetylase activity. Top: Initiation of transcription by association of RNA PolII with an acetylated promoter region. Bottom: The co-transcriptional recruitment of both KAT and KDAC activity with RNA PolII results in overall deacetylation of the coding region, thus suppressing re-initiation of transcription within the transcription unit. The site of transcription initiation is indicated by grey arrows.

DEVELOPMENT

Box 2. Repressors provide transcriptional flexibility

Repressive complexes may function in a variety of ways at active sites in the genome but one overriding theme could explain their presence, even if their mode of action differs depending on circumstance: the genes decorated by these complexes are those for which responsiveness is crucial. For example:

- KDACs act as transcriptional activators in *S. cerevisiae* in response to external stimuli, such as sugar source, heat shock or osmotic stress (De Nadal et al., 2004; Wang et al., 2002).
- Mi-2 in fruit flies is recruited to sites of active transcription upon heat shock and there is impaired induction of heat-shock transcripts in the absence of functional Mi-2 (Mathieu et al., 2012; Murawska et al., 2011; Murawska et al., 2008).
- NuRD function is required for mouse ES cells to respond to differentiation signals, such as leukemia inhibitory factor (Lif) withdrawal (Reynolds et al., 2012a).
- Deacetylase activity is required for response to cytokines in mammalian cells (Nusinzon and Horvath, 2005; Zupkovitz et al., 2006).

Further evidence for the idea of transcriptional priming comes from reports of rapid switching between KAT and KDAC occupancy throughout transcription cycles at an oestrogen-inducible promoter in human breast cancer cells (Métivier et al., 2008; Métivier et al., 2003). This type of process could be envisaged to maintain either a responsive promoter or one that could have its acetylation state rapidly modified to promote or restrict transcription (Fig. 2). Furthermore, precise kinetic experiments to dissect effects on chromatin of mating-type switching in budding yeast have hinted that this model holds true for KATs, for which a partial acetylation of promoter histones allows rapid switching of transcriptional state (Desimone and Laney, 2010).

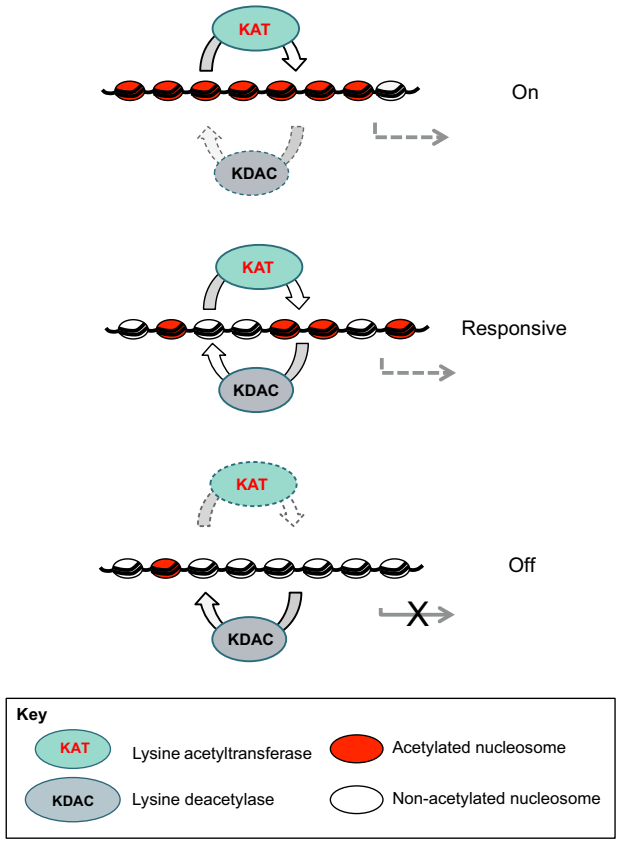

Fig. 2. Transcriptional control dictates cellular responsiveness. The priming of a promoter for transcription can occur through modulation of acetylation levels. Histone acetylation levels (red) are dependent on the cyclic action of both KATs and KDACs. If the balance between these activities is skewed towards the KAT, a promoter will be mostly acetylated and will tend towards a transcriptionally 'On' state (top). Conversely, a promoter at which KDAC activity outweighs that of the KAT will be mostly lacking in acetylation and will be in a fully 'Off' state (bottom). Promoters may be in a primed, or more 'Responsive', state when there is an equal balance between the two activities (middle), allowing a more rapid switching to either a fully on or a fully off state when required.

Repressors as fine-tuners of gene expression and cell fate

The precise control of gene expression levels is known to be important for determining cell fate, and in many instances it is this variation in relative levels of transcripts that produces a cellular environment capable of responding to developmental cues (Box 3). Although transcriptional determination is often considered to be an all-or-nothing phenomenon, work in mouse ES cells has demonstrated that fluctuation in *Pou5f1* expression levels could lead to differentiation to three distinct cell types (Niwa et al., 2000); less than a twofold increase in *Pou5f1* expression caused differentiation to cells resembling primitive endoderm, whereas downregulation of *Pou5f1* expression induced trophectoderm formation. Thus, in self-renewing ES cells, *Pou5f1* expression levels must be very precisely controlled, most likely via interplay between positive and negative regulators of transcription (Niwa, 2007).

A relatively small degree of transcriptional variation can have significant effects on the ability of a cell to respond to developmental cues. Experimental overexpression of any of a number of pluripotency-associated transcription factors can lead to defects in the ability of ES cells to exit self-renewal (Chambers et al., 2007; Ema et al., 2008; Hall et al., 2009; Li et al., 2005; Niwa et al., 2009; Reynolds et al., 2012a; Zhang et al., 2008). Notably, most of these transcription factors show variable gene expression in self-renewing ES cell culture, with high levels of transcription often correlated with enhanced self-renewal and low expression levels associated with a propensity to differentiate (Chambers et al., 2007; Niwa et al., 2009; Reynolds et al., 2012a; Toyooka et al., 2008).

How might this fine-tuning of gene expression at the levels of both an individual cell (i.e. total transcriptional output) and cell population (i.e. degree of transcriptional heterogeneity) be controlled? This might occur by influencing the frequency of transcription initiation (defined as loading of the RNA polymerase), by transition to the elongating form or by impeding the rate of the transcribing RNA polymerase. Evidence for co-repressor proteins exerting exactly this kind of fine transcriptional control came initially from yeast, in which chromatin-modifying proteins were shown to influence transcriptional 'noise' (Huang, 2009) and absolute levels of transcription (Raser and O'Shea, 2004; Weinberger et al., 2012). More recently, it was shown that the lysine deacetylase-containing NuRD complex can influence both of these distinct aspects of transcriptional output in mammalian cells, and that this level of transcriptional control is essential to maintain the developmental responsiveness of pluripotent cells (Reynolds et al., 2012a).

DEVELOPMENT

Box 3. Modulation of transcript levels regulates cell fate

Rather than merely turning genes off, in many instances the activities of repressor complexes are important for modulating the levels of transcription, resulting in cells that are able to rapidly respond to changes in their environment, be these stresses or developmental cues:

- The expression levels of a number of pluripotency-associated genes are modulated by the NuRD complex to maintain differentiation responsiveness of mouse ES cells. This manifests as control of both transcriptional heterogeneity and dynamic range (Reynolds et al., 2012a).
- The formation of the inner cell mass and trophectoderm in pre-implantation mouse embryos is dependent upon the relative levels of the key transcription factors Pou5f1 and Cdx2 (Niwa et al., 2005; Rossant and Tam, 2009).
- Modulation of *Pou5f1* levels in mouse ES cells is crucial for maintaining the balance between self-renewal and differentiation. Forced repression of *Pou5f1* induces differentiation of ES cells towards the trophectoderm (TE) lineage, whereas upregulation results in cells becoming primitive endoderm- and mesoderm-like (Niwa et al., 2000).
- The PRC2 component proteins Jarid2 and Pcl2 (Mtf2) have been reported to be capable of fine-tuning the histone methyltransferase activity of PRC2, thus mitigating the repressive influence of PRC2 during developmental transitions (Casanova et al., 2011; Li et al., 2010; Li et al., 2011; Peng et al., 2009; Shen et al., 2009; Walker et al., 2010).

The NuRD co-repressor complex: a regulator of stem cell fate

ES cells lacking the NuRD component protein Mbd3 exhibit elevated transcript levels of genes normally associated with pluripotency, such as *Klf4*, *Klf5*, *Esrrb*, *Zfp42* and *Tbx3* (Reynolds et al., 2012a), and both Mbd3 and Mi2β are associated widely across these actively transcribed loci, as detected by ChIP-qPCR. Using culture conditions that minimise transcriptional heterogeneity in ES cells, and by sorting for cells expressing specific markers, NuRD components were shown to associate directly with actively transcribed genes, unlike the case of PRC complexes, which are found on the poised, but not fully active alleles. This is clearly not a case of a failure to silence these loci in Mbd3-null ES cells, because these genes are normally expressed in ES cells. Rather the function of NuRD is to control the dynamic range of transcription of these pluripotency-associated factors. Exactly how NuRD exerts this effect, whether through its KDAC activity, nucleosome remodelling activity, or through some other mechanism, is not yet clear.

In the absence of NuRD-mediated transcriptional modulation, ES cells express some pluripotency-associated genes at levels above a threshold such that they are no longer able to respond to differentiation cues and hence fail to exit self-renewal. Artificial reduction of transcript levels allowed for some degree of rescue of the differentiation defect in Mbd3-null ES cells, demonstrating that NuRD-mediated control of the dynamic range of transcription is essential for this developmental transition (Reynolds et al., 2012a) (Fig. 3). The fact that Mbd3 has been shown to play important roles in developmental transitions of pluripotent cells *in vivo* (Kaji et al., 2007), and that NuRD component orthologues have been shown to play important roles in a variety of different animal and plant species (Ahringer, 2000), led us to hypothesise that precise control of transcript levels by NuRD and other co-repressor proteins is essential for many developmental processes.

The curious case of Mi2β in thymocytes: one protein, two complexes

Genome-wide localisation studies in thymocytes indicated that the NuRD component Mi2β, along with the lymphoid lineage determinant transcription factor Ikaros, is widely associated with actively transcribed genes (Zhang et al., 2012). Upon deletion of Ikaros, Mi2β remained associated with Ikaros targets, which showed a local increase in chromatin remodelling resulting in transcriptional repression. Additionally Mi2β was redistributed to 'poised' non-Ikaros target genes where it induced transcriptional activation. The overall effect of these changes was to block lymphocyte maturation and activate inappropriate gene expression, resulting in progression to a leukaemic state (Zhang et al., 2012).

This multifunctional role for Mi2β is quite extraordinary. With Ikaros it associates with actively transcribed genes, whereas upon loss of Ikaros it not only silences what were once Ikaros targets, but also runs amok, turning on other genes that should remain poised for transcription. Exactly how these various influences on gene expression might work at the molecular level is far from clear. In the case of thymocyte maturation, however, it is not known whether the observed gene activation is mediated by NuRD or by Mi2β acting independently of the complex.

Context matters

There is clear genetic evidence in plants that PKL, an orthologue of Mi2β, antagonises the silencing activity of PRC2 and is required for the expression of many PRC target genes (Aichinger et al., 2009). This is in direct contrast to the situation in mouse ES cells and in human leukaemic cells, in which NuRD functions to specify sites of PRC2 action, thus acting in concert with PRC2 rather than in opposition to it (Morey et al., 2008; Reynolds et al., 2012b). Nevertheless, many of the genes inappropriately activated in the Ikaros-deleted thymocyte system are normally bound by PRC2 (Zhang et al., 2012), making it conceivable that in the Ikaros-null thymocytes Mi2β functions in

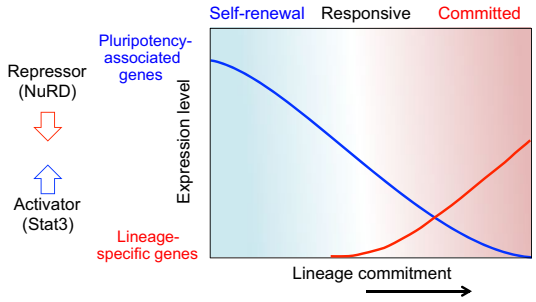

Fig. 3. Levels of gene expression control responsiveness. In mouse ES cells, the switch between a self-renewing, pluripotent state and a more differentiated one is dependent upon relative levels of distinct sets of genes. For a cell to commit to differentiation, expression levels of pluripotency-associated genes must go from high to low and those of lineage-specific genes must increase (red area of graph, 'Committed'). Similarly, when pluripotency-associated transcripts are high and lineage-specific transcripts low a cell is in a restricted, self-renewing state (blue area). At a mid-point in this process, when key transcript levels are in the mid-range, a cell is in a state in which it can be responsive to changes in the environment and move to either a lineage-committed or self-renewing state (white area). Expression levels of pluripotency-associated genes are determined by a balance between the activity of transcriptional activators (such as Stat3) and repressors (such as NuRD).

DEVELOPMENT

a NuRD-independent manner to antagonise PRC2-mediated transcriptional repression in this system as well.

The molecular mechanisms behind these examples of PRC2 antagonism, and the protein partners with which PKL or Mi2β might collaborate in this function, have not been demonstrated. One clue to this perhaps comes from an earlier study of Mi2β function in thymocyte development, in which Mi2β was found to associate with the Ep300 KAT at an enhancer independently of NuRD to activate *Cd4* expression during T-cell development (Williams et al., 2004). This is consistent with more recent ChIP-seq datasets, in which Mi2β showed significant association with enhancer sequences in ES cells (Reynolds et al., 2012b; Whyte et al., 2012). In neither of these recent studies did ChIP-seq analysis distinguish between Mi2β and NuRD; however, the more sensitive ChIP-qPCR has been used to show that NuRD components do colocalise with Mi2β at promoters (Miccio et al., 2010; Reynolds et al., 2012a; Reynolds et al., 2012b). Nevertheless, there has been a steady accumulation of evidence that Mi2β can function outside of the repressive NuRD complex to activate transcription, possibly in conjunction with Ep300.

What might be the function of Mi2β at enhancers? Whyte et al. suggested that Mi2β, as part of the NuRD complex, functions to inactivate enhancers upon gene silencing (Whyte et al., 2012). However, this model does not consider that Mi2β and NuRD can also participate in transcriptional activation or modulation. It has been reported that NuRD can exist in a cohesin-loading supercomplex in HeLa cells (Hakimi et al., 2002) and that it associates with structural maintenance of chromosomes (SMC) proteins in thymocytes (Zhang et al., 2012), which could implicate Mi2β in influencing the physical interaction between enhancer and promoter. However, analysis of genome-wide DNA-binding data in ES cells shows a significant anti-correlation between binding of Mi2β and other chromosomal proteins, such as CTCF, Rad21 and SMC proteins [using data from Reynolds et al. (Reynolds et al., 2012b) and the ES cell ChIP-seq Compendium (http://bioinformatics.cscr.cam.ac.uk/ES_Cell_ChIP-seq_compendium.html) (Martello et al., 2012)], indicating that Mi2β is probably not directly associating with these proteins in ES cells. Furthermore, evidence from *D. melanogaster* suggests that although Mi-2 does not co-purify with cohesin subunits in cultured cells (Kunert et al., 2009; Reddy et al., 2010), it can function to remove cohesin from interphase chromosomes and interacts genetically with the cohesin-loading complex protein Nipped-B (Fasulo et al., 2012). Together these data do not paint an entirely clear picture of Mi2β function at enhancers, but it would appear that Mi2β function may vary in different cell types. What is clear, however, is that Mi2β has a life of its own outside of the NuRD complex, and it is important to take this into consideration when evaluating the functions of NuRD and Mi2β in gene regulation.

Conclusions

In the 15 or so years since the initial reports that classic co-repressor function was exerted mainly via chromatin modification, our perception of transcriptional repression has evolved. Although initially repression was largely considered to be an all-or-none phenomenon, we now know that an important function of many co-repressors is in transcriptional fine-tuning. In some circumstances, co-repressor complexes appear to activate transcription directly, with a blatant disregard for the labels that we have given them. What is not so clear, although perhaps of most interest, is how a multi-protein complex with a limited range of enzymatic activities can exert opposing effects on transcription at different loci. Mechanisms for targeting co-repressor complexes to specific genes may differ according to individual loci and might influence the behaviour of the complex at a given target. How chromatin-modifying proteins are directed to their targets, and what defines the relative size of their DNA-interaction regions is likely to yield important mechanistic insights into how transcription is fine-tuned in eukaryotic cells.

In addition to repressing transcription or keeping genes poised on the verge of transcription, repressors can act to fine-tune levels of active gene expression. This modulation acts to control precisely the total transcriptional output from a given target gene. The result of this fine regulatory control is that cells are able to use gene expression not simply as a binary signal, but rather as a range of values, each of which might have different consequences for the cell. Developmental decisions are ultimately taken at the level of individual cells, which define their identities and their potential in their gene expression patterns. Thus, the major challenge will be to understand how transcriptional changes, which ultimately lead to developmental decisions, are controlled at the level of an individual cell.

Acknowledgements
We thank Sabine Dietmann for bioinformatics analyses; and Remco Loos and Paul Bertone for RNA-seq analysis. We also thank Paul Bertone for critical reading of the manuscript.

Funding
Work in the B.H. laboratory is supported by a Wellcome Trust Senior Research Fellowship in the Basic Biomedical Sciences; by the European Commission's 7th Framework Programes DisChrom and 4DCellFate; and by Wellcome Trust – Medical Research Council Stem Cell Institute.

Competing interests statement
The authors declare no competing financial interests.

References

Ahringer, J. (2000). NuRD and SIN3 histone deacetylase complexes in development. *Trends Genet.* **16**, 351-356.
Aichinger, E., Villar, C. B., Farrona, S., Reyes, J. C., Hennig, L. and Köhler, C. (2009). CHD3 proteins and polycomb group proteins antagonistically determine cell identity in Arabidopsis. *PLoS Genet.* **5**, e1000605.
Azuara, V., Perry, P., Sauer, S., Spivakov, M., Jørgensen, H. F., John, R. M., Gouti, M., Casanova, M., Warnes, G., Merkenschlager, M. et al. (2006). Chromatin signatures of pluripotent cell lines. *Nat. Cell Biol.* **8**, 532-538.
Bannister, A. J. and Kouzarides, T. (1996). The CBP co-activator is a histone acetyltransferase. *Nature* **384**, 641-643.
Berger, S. L. (2007). The complex language of chromatin regulation during transcription. *Nature* **447**, 407-412.
Bernstein, B. E., Tong, J. K. and Schreiber, S. L. (2000). Genomewide studies of histone deacetylase function in yeast. *Proc. Natl. Acad. Sci. USA* **97**, 13708-13713.
Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K. et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315-326.
Bowen, N. J., Fujita, N., Kajita, M. and Wade, P. A. (2004). Mi-2/NuRD: multiple complexes for many purposes. *Biochim. Biophys. Acta* **1677**, 52-57.
Brookes, E., de Santiago, I., Hebenstreit, D., Morris, K. J., Carroll, T., Xie, S. Q., Stock, J. K., Heidemann, M., Eick, D., Nozaki, N. et al. (2012). Polycomb associates genome-wide with a specific RNA polymerase II variant, and regulates metabolic genes in ESCs. *Cell Stem Cell* **10**, 157-170.
Carrozza, M. J., Li, B., Florens, L., Suganuma, T., Swanson, S. K., Lee, K. K., Shia, W. J., Anderson, S., Yates, J., Washburn, M. P. et al. (2005). Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell* **123**, 581-592.
Casanova, M., Preissner, T., Cerase, A., Poot, R., Yamada, D., Li, X., Appanah, R., Bezstarosti, K., Demmers, J., Koseki, H. et al. (2011). Polycomblike 2 facilitates the recruitment of PRC2 Polycomb group complexes to the inactive X chromosome and to target loci in embryonic stem cells. *Development* **138**, 1471-1482.

Chambers, I., Silva, J., Colby, D., Nichols, J., Nijmeijer, B., Robertson, M., Vrana, J., Jones, K., Grotewold, L. and Smith, A. (2007). Nanog safeguards pluripotency and mediates germline development. *Nature* **450**, 1230-1234.
Chang, H. M., Paulson, M., Holko, M., Rice, C. M., Williams, B. R., Marié, I. and Levy, D. E. (2004). Induction of interferon-stimulated gene expression and antiviral responses require protein deacetylase activity. *Proc. Natl. Acad. Sci. USA* **101**, 9578-9583.
De Nadal, E., Zapater, M., Alepuz, P. M., Sumoy, L., Mas, G. and Posas, F. (2004). The MAPK Hog1 recruits Rpd3 histone deacetylase to activate osmoresponsive genes. *Nature* **427**, 370-374.
Deckert, J. and Struhl, K. (2001). Histone acetylation at promoters is differentially affected by specific activators and repressors. *Mol. Cell. Biol.* **21**, 2726-2735.
Desimone, A. M. and Laney, J. D. (2010). Corepressor-directed preacetylation of histone H3 in promoter chromatin primes rapid transcriptional switching of cell-type-specific genes in yeast. *Mol. Cell. Biol.* **30**, 3342-3356.
Dovey, O. M., Foster, C. T. and Cowley, S. M. (2010). Emphasizing the positive: A role for histone deacetylases in transcriptional activation. *Cell Cycle* **9**, 2700-2701.
Drouin, S., Laramée, L., Jacques, P. E., Forest, A., Bergeron, M. and Robert, F. (2010). DSIF and RNA polymerase II CTD phosphorylation coordinate the recruitment of Rpd3S to actively transcribed genes. *PLoS Genet.* **6**, e1001173.
Ema, M., Mori, D., Niwa, H., Hasegawa, Y., Yamanaka, Y., Hitoshi, S., Mimura, J., Kawabe, Y., Hosoya, T., Morita, M. et al. (2008). Krüppel-like factor 5 is essential for blastocyst development and the normal self-renewal of mouse ESCs. *Cell Stem Cell* **3**, 555-567.
Enderle, D., Beisel, C., Stadler, M. B., Gerstung, M., Athri, P. and Paro, R. (2011). Polycomb preferentially targets stalled promoters of coding and noncoding transcripts. *Genome Res.* **21**, 216-226.
Fasulo, B., Deuring, R., Murawska, M., Gause, M., Dorighi, K. M., Schaaf, C. A., Dorsett, D., Brehm, A. and Tamkun, J. W. (2012). The Drosophila MI-2 chromatin-remodeling factor regulates higher-order chromatin structure and cohesin dynamics in vivo. *PLoS Genet.* **8**, e1002878.
Govind, C. K., Qiu, H., Ginsburg, D. S., Ruan, C., Hofmeyer, K., Hu, C., Swaminathan, V., Workman, J. L., Li, B. and Hinnebusch, A. G. (2010). Phosphorylated Pol II CTD recruits multiple HDACs, including Rpd3C(S), for methylation-dependent deacetylation of ORF nucleosomes. *Mol. Cell* **39**, 234-246.
Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. *Nature* **389**, 349-352.
Hakimi, M. A., Bochar, D. A., Schmiesing, J. A., Dong, Y., Barak, O. G., Speicher, D. W., Yokomori, K. and Shiekhattar, R. (2002). A chromatin remodelling complex that loads cohesin onto human chromosomes. *Nature* **418**, 994-998.
Hall, J., Guo, G., Wray, J., Eyres, I., Nichols, J., Grotewold, L., Morfopoulou, S., Humphreys, P., Mansfield, W., Walker, R. et al. (2009). Oct4 and LIF/Stat3 additively induce Krüppel factors to sustain embryonic stem cell self-renewal. *Cell Stem Cell* **5**, 597-609.
Harrison, M. R., Georgiou, A. S., Spaink, H. P. and Cunliffe, V. T. (2011). The epigenetic regulator Histone Deacetylase 1 promotes transcription of a core neurogenic programme in zebrafish embryos. *BMC Genomics* **12**, 24.
Huang, S. (2009). Non-genetic heterogeneity of cells in development: more than just noise. *Development* **136**, 3853-3862.
Jelinic, P., Pellegrino, J. and David, G. (2011). A novel mammalian complex containing Sin3B mitigates histone acetylation and RNA polymerase II progression within transcribed loci. *Mol. Cell. Biol.* **31**, 54-62.
Johnsson, A., Durand-Dubief, M., Xue-Franzén, Y., Rönnerblad, M., Ekwall, K. and Wright, A. (2009). HAT-HDAC interplay modulates global histone H3K14 acetylation in gene-coding regions during stress. *EMBO Rep.* **10**, 1009-1014.
Joshi, A. A. and Struhl, K. (2005). Eaf3 chromodomain interaction with methylated H3-K36 links histone deacetylation to Pol II elongation. *Mol. Cell* **20**, 971-978.
Kaji, K., Nichols, J. and Hendrich, B. (2007). Mbd3, a component of the NuRD co-repressor complex, is required for development of pluripotent cells. *Development* **134**, 1123-1132.
Kehle, J., Beuchle, D., Treuheit, S., Christen, B., Kennison, J. A., Bienz, M. and Müller, J. (1998). dMi-2, a hunchback-interacting protein that functions in Polycomb repression. *Science* **282**, 1897-1900.
Keogh, M. C., Kurdistani, S. K., Morris, S. A., Ahn, S. H., Podolny, V., Collins, S. R., Schuldiner, M., Chin, K., Punna, T., Thompson, N. J. et al. (2005). Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell* **123**, 593-605.
Klampfer, L., Huang, J., Swaby, L. A. and Augenlicht, L. (2004). Requirement of histone deacetylase activity for signaling by STAT1. *J. Biol. Chem.* **279**, 30358-30368.
Kornberg, R. D. (1974). Chromatin structure: a repeating unit of histones and DNA. *Science* **184**, 868-871.
Kouzarides, T. (2007). Chromatin modifications and their function. *Cell* **128**, 693-705.
Kunert, N., Wagner, E., Murawska, M., Klinker, H., Kremmer, E. and Brehm, A. (2009). dMec: a novel Mi-2 chromatin remodelling complex involved in transcriptional repression. *EMBO J.* **28**, 533-544.
Kurdistani, S. K., Robyr, D., Tavazoie, S. and Grunstein, M. (2002). Genome-wide binding map of the histone deacetylase Rpd3 in yeast. *Nat. Genet.* **31**, 248-254.
Le Guezennec, X., Vermeulen, M., Brinkman, A. B., Hoeijmakers, W. A., Cohen, A., Lasonder, E. and Stunnenberg, H. G. (2006). MBD2/NuRD and MBD3/NuRD, two distinct complexes with different biochemical and functional properties. *Mol. Cell. Biol.* **26**, 843-851.
Lenstra, T. L., Benschop, J. J., Kim, T., Schulze, J. M., Brabers, N. A., Margaritis, T., van de Pasch, L. A., van Heesch, S. A., Brok, M. O., Groot Koerkamp, M. J. et al. (2011). The specificity and topology of chromatin interaction pathways in yeast. *Mol. Cell* **42**, 536-549.
Li, Y., McClintick, J., Zhong, L., Edenberg, H. J., Yoder, M. C. and Chan, R. J. (2005). Murine embryonic stem cell differentiation is promoted by SOCS-3 and inhibited by the zinc finger transcription factor Klf4. *Blood* **105**, 635-637.
Li, B., Gogol, M., Carey, M., Lee, D., Seidel, C. and Workman, J. L. (2007a). Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. *Science* **316**, 1050-1054.
Li, B., Gogol, M., Carey, M., Pattenden, S. G., Seidel, C. and Workman, J. L. (2007b). Infrequently transcribed long genes depend on the Set2/Rpd3S pathway for accurate transcription. *Genes Dev.* **21**, 1422-1430.
Li, B., Jackson, J., Simon, M. D., Fleharty, B., Gogol, M., Seidel, C., Workman, J. L. and Shilatifard, A. (2009). Histone H3 lysine 36 dimethylation (H3K36me2) is sufficient to recruit the Rpd3s histone deacetylase complex and to repress spurious transcription. *J. Biol. Chem.* **284**, 7970-7976.
Li, G., Margueron, R., Ku, M., Chambon, P., Bernstein, B. E. and Reinberg, D. (2010). Jarid2 and PRC2, partners in regulating gene expression. *Genes Dev.* **24**, 368-380.
Li, X., Isono, K., Yamada, D., Endo, T. A., Endoh, M., Shinga, J., Mizutani-Koseki, Y., Otte, A. P., Casanova, M., Kitamura, H. et al. (2011). Mammalian polycomb-like Pcl2/Mtf2 is a novel regulatory component of PRC2 that can differentially modulate polycomb activity both at the Hox gene cluster and at Cdkn2a genes. *Mol. Cell. Biol.* **31**, 351-364.
Martello, G., Sugimoto, T., Diamanti, E., Joshi, A., Hannah, R., Ohtsuka, S., Göttgens, B., Niwa, H. and Smith, A. (2012). Esrrb is a pivotal target of the gsk3/tcf3 axis regulating embryonic stem cell self-renewal. *Cell Stem Cell* **11**, 491-504.
Mathieu, E. L., Finkernagel, F., Murawska, M., Scharfe, M., Jarek, M. and Brehm, A. (2012). Recruitment of the ATP-dependent chromatin remodeler dMi-2 to the transcribed region of active heat shock genes. *Nucleic Acids Res.* **40**, 4879-4891.
McDonel, P., Costello, I. and Hendrich, B. (2009). Keeping things quiet: roles of NuRD and Sin3 co-repressor complexes during mammalian development. *Int. J. Biochem. Cell Biol.* **41**, 108-116.
Métivier, R., Penot, G., Hübner, M. R., Reid, G., Brand, H., Kos, M. and Gannon, F. (2003). Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. *Cell* **115**, 751-763.
Métivier, R., Gallais, R., Tiffoche, C., Le Péron, C., Jurkowska, R. Z., Carmouche, R. P., Ibberson, D., Barath, P., Demay, F., Reid, G. et al. (2008). Cyclical DNA methylation of a transcriptionally active promoter. *Nature* **452**, 45-50.
Miccio, A., Wang, Y., Hong, W., Gregory, G. D., Wang, H., Yu, X., Choi, J. K., Shelat, S., Tong, W., Poncz, M. et al. (2010). NuRD mediates activating and repressive functions of GATA-1 and FOG-1 during blood development. *EMBO J.* **29**, 442-456.
Morey, L., Brenner, C., Fazi, F., Villa, R., Gutierrez, A., Buschbeck, M., Nervi, C., Minucci, S., Fuks, F. and Di Croce, L. (2008). MBD3, a component of the NuRD complex, facilitates chromatin alteration and deposition of epigenetic marks. *Mol. Cell. Biol.* **28**, 5912-5923.
Murawska, M., Kunert, N., van Vugt, J., Längst, G., Kremmer, E., Logie, C. and Brehm, A. (2008). dCHD3, a novel ATP-dependent chromatin remodeler associated with sites of active transcription. *Mol. Cell. Biol.* **28**, 2745-2757.
Murawska, M., Hassler, M., Renkawitz-Pohl, R., Ladurner, A. and Brehm, A. (2011). Stress-induced PARP activation mediates recruitment of Drosophila Mi-2 to promote heat shock gene expression. *PLoS Genet.* **7**, e1002206.
Nawaz, Z., Baniahmad, C., Burris, T. P., Stillman, D. J., O'Malley, B. W. and Tsai, M. J. (1994). The yeast SIN3 gene product negatively regulates the activity of the human progesterone receptor and positively regulates the activities of GAL4 and the HAP1 activator. *Mol. Gen. Genet.* **245**, 724-733.
Niwa, H. (2007). How is pluripotency determined and maintained? *Development* **134**, 635-646.
Niwa, H., Miyazaki, J. and Smith, A. G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.* **24**, 372-376.
Niwa, H., Toyooka, Y., Shimosato, D., Strumpf, D., Takahashi, K., Yagi, R. and Rossant, J. (2005). Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell* **123**, 917-929.

Niwa, H., Ogawa, K., Shimosato, D. and Adachi, K. (2009). A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature* **460**, 118-122.
Nusinzon, I. and Horvath, C. M. (2003). Interferon-stimulated transcription and innate antiviral immunity require deacetylase activity and histone deacetylase 1. *Proc. Natl. Acad. Sci. USA* **100**, 14742-14747.
Nusinzon, I. and Horvath, C. M. (2005). Histone deacetylases as transcriptional activators? Role reversal in inducible gene regulation. *Sci. STKE* **2005**, re11.
Pazin, M. J. and Kadonaga, J. T. (1997). What's up and down with histone deacetylation and transcription? *Cell* **89**, 325-328.
Peng, J. C., Valouev, A., Swigut, T., Zhang, J., Zhao, Y., Sidow, A. and Wysocka, J. (2009). Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells. *Cell* **139**, 1290-1302.
Rascle, A., Johnston, J. A. and Amati, B. (2003). Deacetylase activity is required for recruitment of the basal transcription machinery and transactivation by STAT5. *Mol. Cell. Biol.* **23**, 4162-4173.
Raser, J. M. and O'Shea, E. K. (2004). Control of stochasticity in eukaryotic gene expression. *Science* **304**, 1811-1814.
Reddy, B. A., Bajpe, P. K., Bassett, A., Moshkin, Y. M., Kozhevnikova, E., Bezstarosti, K., Demmers, J. A., Travers, A. A. and Verrijzer, C. P. (2010). Drosophila transcription factor Tramtrack69 binds MEP1 to recruit the chromatin remodeler NuRD. *Mol. Cell. Biol.* **30**, 5234-5244.
Reid, J. L., Moqtaderi, Z. and Struhl, K. (2004). Eaf3 regulates the global pattern of histone acetylation in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **24**, 757-764.
Reynolds, N., Latos, P., Hynes-Allen, A., Loos, R., Leaford, D., O'Shaughnessy, A., Mosaku, O., Signolet, J., Brennecke, P., Kalkan, T. et al. (2012a). NuRD suppresses pluripotency gene expression to promote transcriptional heterogeneity and lineage commitment. *Cell Stem Cell* **10**, 583-594.
Reynolds, N., Salmon-Divon, M., Dvinge, H., Hynes-Allen, A., Balasooriya, G., Leaford, D., Behrens, A., Bertone, P. and Hendrich, B. (2012b). NuRD-mediated deacetylation of H3K27 facilitates recruitment of Polycomb Repressive Complex 2 to direct gene repression. *EMBO J.* **31**, 593-605.
Rossant, J. and Tam, P. P. (2009). Blastocyst lineage formation, early embryonic asymmetries and axis patterning in the mouse. *Development* **136**, 701-713.
Sakamoto, S., Potla, R. and Larner, A. C. (2004). Histone deacetylase activity is required to recruit RNA polymerase II to the promoters of selected interferon-stimulated early response genes. *J. Biol. Chem.* **279**, 40362-40367.
Schwartz, Y. B., Kahn, T. G., Stenberg, P., Ohno, K., Bourgon, R. and Pirrotta, V. (2010). Alternative epigenetic chromatin states of polycomb target genes. *PLoS Genet.* **6**, e1000805.
Shen, X., Kim, W., Fujiwara, Y., Simon, M. D., Liu, Y., Mysliwiec, M. R., Yuan, G. C., Lee, Y. and Orkin, S. H. (2009). Jumonji modulates polycomb activity and self-renewal versus differentiation of stem cells. *Cell* **139**, 1303-1314.
Srinivasan, S., Armstrong, J. A., Deuring, R., Dahlsveen, I. K., McNeill, H. and Tamkun, J. W. (2005). The Drosophila trithorax group protein Kismet facilitates an early step in transcriptional elongation by RNA Polymerase II. *Development* **132**, 1623-1635.
Strahl, B. D. and Allis, C. D. (2000). The language of covalent histone modifications. *Nature* **403**, 41-45.
Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E. and Schreiber, S. L. (1998). Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. *Nature* **395**, 917-921.
Toyooka, Y., Shimosato, D., Murakami, K., Takahashi, K. and Niwa, H. (2008). Identification and characterization of subpopulations in undifferentiated ES cell culture. *Development* **135**, 909-918.
Vidal, M. and Gaber, R. F. (1991). RPD3 encodes a second factor required to achieve maximum positive and negative transcriptional states in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **11**, 6317-6327.
Vidal, M., Strich, R., Esposito, R. E. and Gaber, R. F. (1991). RPD1 (SIN3/UME4) is required for maximal activation and repression of diverse yeast genes. *Mol. Cell. Biol.* **11**, 6306-6316.
Wade, P. A., Jones, P. L., Vermaak, D. and Wolffe, A. P. (1998). A multiple subunit Mi-2 histone deacetylase from Xenopus laevis cofractionates with an associated Snf2 superfamily ATPase. *Curr. Biol.* **8**, 843-848.
Walker, E., Chang, W. Y., Hunkapiller, J., Cagney, G., Garcha, K., Torchia, J., Krogan, N. J., Reiter, J. F. and Stanford, W. L. (2010). Polycomb-like 2 associates with PRC2 and regulates transcriptional networks during mouse embryonic stem cell self-renewal and differentiation. *Cell Stem Cell* **6**, 153-166.
Wang, A., Kurdistani, S. K. and Grunstein, M. (2002). Requirement of Hos2 histone deacetylase for gene activity in yeast. *Science* **298**, 1412-1414.
Wang, Z., Zang, C., Cui, K., Schones, D. E., Barski, A., Peng, W. and Zhao, K. (2009). Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* **138**, 1019-1031.
Weake, V. M. and Workman, J. L. (2010). Inducible gene expression: diverse regulatory mechanisms. *Nat. Rev. Genet.* **11**, 426-437.
Weinberger, L., Voichek, Y., Tirosh, I., Hornung, G., Amit, I. and Barkai, N. (2012). Expression noise and acetylation profiles distinguish HDAC functions. *Mol. Cell* **47**, 193-202.
Whyte, W. A., Bilodeau, S., Orlando, D. A., Hoke, H. A., Frampton, G. M., Foster, C. T., Cowley, S. M. and Young, R. A. (2012). Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. *Nature* **482**, 221-225.
Williams, C. J., Naito, T., Arco, P. G., Seavitt, J. R., Cashman, S. M., De Souza, B., Qi, X., Keables, P., Von Andrian, U. H. and Georgopoulos, K. (2004). The chromatin remodeler Mi-2beta is required for CD4 expression and T cell development. *Immunity* **20**, 719-733.
Williams, K., Christensen, J., Pedersen, M. T., Johansen, J. V., Cloos, P. A., Rappsilber, J. and Helin, K. (2011). TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity. *Nature* **473**, 343-348.
Wirén, M., Silverstein, R. A., Sinha, I., Walfridsson, J., Lee, H. M., Laurenson, P., Pillus, L., Robyr, D., Grunstein, M. and Ekwall, K. (2005). Genomewide analysis of nucleosome density histone acetylation and HDAC function in fission yeast. *EMBO J.* **24**, 2906-2918.
Wolffe, A. P. (1997). Transcriptional control. Sinful repression. *Nature* **387**, 16-17.
Xu, M., Nie, L., Kim, S. H. and Sun, X. H. (2003). STAT5-induced Id-1 transcription involves recruitment of HDAC1 and deacetylation of C/EBPbeta. *EMBO J.* **22**, 893-904.
Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Côté, J. and Wang, W. (1998). NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. *Mol. Cell* **2**, 851-861.
Yang, X. J. and Seto, E. (2007). HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene* **26**, 5310-5318.
Yoshida, T., Hazan, I., Zhang, J., Ng, S. Y., Naito, T., Snippert, H. J., Heller, E. J., Qi, X., Lawton, L. N., Williams, C. J. et al. (2008). The role of the chromatin remodeler Mi-2beta in hematopoietic stem cell self-renewal and multilineage differentiation. *Genes Dev.* **22**, 1174-1189.
Young, M. D., Willson, T. A., Wakefield, M. J., Trounson, E., Hilton, D. J., Blewitt, M. E., Oshlack, A. and Majewski, I. J. (2011). ChIP-seq analysis reveals distinct H3K27me3 profiles that correlate with transcriptional activity. *Nucleic Acids Res.* **39**, 7415-7427.
Zhang, Y., LeRoy, G., Seelig, H.-P., Lane, W. S. and Reinberg, D. (1998). The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. *Cell* **95**, 279-289.
Zhang, X., Zhang, J., Wang, T., Esteban, M. A. and Pei, D. (2008). Esrrb activates Oct4 transcription and sustains self-renewal and pluripotency in embryonic stem cells. *J. Biol. Chem.* **283**, 35825-35833.
Zhang, J., Jackson, A. F., Naito, T., Dose, M., Seavitt, J., Liu, F., Heller, E. J., Kashiwagi, M., Yoshida, T., Gounari, F. et al. (2012). Harnessing of the nucleosome-remodeling-deacetylase complex controls lymphocyte development and prevents leukemogenesis. *Nat. Immunol.* **13**, 86-94.
Zupkovitz, G., Tischler, J., Posch, M., Sadzak, I., Ramsauer, K., Egger, G., Grausenburger, R., Schweifer, N., Chiocca, S., Decker, T. et al. (2006). Negative and positive regulation of gene expression by mouse histone deacetylase 1. *Mol. Cell. Biol.* **26**, 7913-7928.