

The logic of chromatin architecture and remodelling at promoters

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The regulation of gene transcription involves a dynamic balance between packaging regulatory sequences into chromatin and allowing transcriptional regulators access to these sequences. Access is restricted by the nucleosomes, but these can be repositioned or ejected by enzymes known as nucleosome remodellers. In addition, the DNA sequence can impart stiffness or curvature to the DNA, thereby affecting the position of nucleosomes on the DNA, influencing particular promoter 'architectures'. Recent genome-wide studies in yeast suggest that constitutive and regulated genes have architectures that differ in terms of nucleosome position, turnover, remodelling requirements and transcriptional noise.

In eukaryotic cells, DNA is wrapped around histone octamers to form nucleosomes, the primary unit of chromatin structure. Nucleosomes compact the genome but also restrict the access of DNA-binding transcription factors, so there is a balance to strike between effective genome packaging and accessibility¹. But cells do more than meet this challenge — they also tailor the way that the chromatin is packaged to help regulate gene expression. This mode of regulation involves dynamic competition between nucleosomes and transcription factors for important *cis*-regulatory sequences in gene promoters. This competition is influenced by enzymes that covalently modify nucleosomes, termed 'chromatin modifiers', and enzymes that reposition, reconfigure or eject nucleosomes, termed 'chromatin remodellers'^{2–4}. Together, these factors help create promoter architectures — defined here as the density, composition and positioning of nucleosomes relative to important *cis*-regulatory sites. These factors also collaborate to alter promoter architecture to expose regulatory sites and allow activation under the appropriate conditions. Furthermore, recent genome-wide studies of nucleosome occupancy^{5–13} and new computational approaches for predicting nucleosome positioning^{14–17} have reinforced earlier notions that the biophysical properties of promoter DNA also help shape the landscape of nucleosome positioning and density at both repressed and active promoters.

Here, I consider this interplay and describe the basic logic for the regulation and remodelling of chromatin architecture at RNA polymerase II (Pol II) promoters. I review recent genome-wide studies on nucleosome occupancy and dynamics in yeast, which have revealed that a substantial minority of genes conform to two contrasting promoter architectures^{18,19} that drive either constitutive or highly regulated genes. I discuss these contrasting architectures and the different tools used to build and regulate them (Box 1) and make comparisons to promoter structures in higher eukaryotes. The underlying concepts can help us understand the regulation of these promoters, as well as 'blended' promoters, which incorporate features of both contrasting architectures.

Two contrasting promoter architectures

The locations and density of nucleosomes and nucleosome variants have been determined across whole genomes in several organisms and cell types^{5–7,9–11,20–22}. Studies in yeast suggest that some promoters (albeit a minority) can be classified into two contrasting architectural categories^{18,19}, 'open' and 'covered' (defined further later), which drive the

two broad types of gene, constitutive and highly regulated, respectively (Table 1 and Fig. 1). These two architectures are contrasting extremes, and many promoters contain a 'blend' of attributes of the two architectures, as well as more complex strategies, to achieve proper regulation. Even so, a discussion of the properties of these two contrasting promoter types is useful for understanding the logic of promoter architecture, and for understanding how and why other promoters might blend attributes of these archetypes to tune their regulation.

Constitutive genes have 'open' promoters

There is dynamic competition between nucleosomes and transcription factors at many promoters. However, constitutive genes have features that favour the binding of transcription factors at the expense of nucleosomes (Fig. 1), and I term these promoters 'open' promoters. Constitutive genes typically have a large (~150-base-pair (bp)) nucleosome-depleted region (NDR) directly upstream of the transcription start site (TSS), within which key *cis*-regulatory sequences reside. This region has traditionally been termed a 'nucleosome-free' region, but there is actually a gradient of depletion, rather than a total loss, so NDR is a more appropriate term. Importantly, the NDR contains poly(dA:dT) tracts²³, DNA sequences that resist bending and so deter nucleosome formation and stability¹⁶. By contrast, AA/TT dinucleotides repeating every 10 bp (with GC dinucleotides 5 bp out of phase) impose a curvature favourable to nucleosome formation and stability, and when extending across a ~150-bp region can function as a nucleosome positioning sequence (NPS)^{14,17,24}. However, the poly(dA:dT) tract is generally considered a more important driver of translational positioning than the NPS¹⁶. Open promoters often combine these two sequence elements into a tripartite structure: a central poly(dA:dT)-rich tract, which deters nucleosome binding, flanked by two NPS elements, which help fix the positions of the two flanking nucleosomes, termed the –1 and +1 nucleosomes in yeast^{5–7,9,25} (Fig. 1). (For human genes, the –1 nucleosome is the one positioned directly upstream of the TSS.) The juxtaposition of both an excluding and a positioning element may help create an exceptionally sharp nucleosome boundary, which deters the encroachment of nucleosomes into the NDR. Importantly, the positions of the +1 and –1 nucleosomes at open promoters align quite precisely with the locations of nucleosomes predicted computationally by programs entrained with NPSs and/or poly(dA:dT) tracts^{14–17}. Indeed, the latest

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Box 1 | Chromatin concepts at promoters

The DNA sequence influences the chromatin landscape. DNA curvature or stiffness influences the positioning and density of nucleosomes. Within that landscape, specific sequences define the binding sites for sequence-specific transcription factors, the TATA box and the transcription start site.

Nucleosomes block the access of transcription factors to DNA.

Binding sites located near the middle of a nucleosome are generally inaccessible to transcription factors; those near the edge are partially accessible; and those in the linker are accessible. Inaccessibility leads to a dependence on chromatin remodelling.

Histone chaperones regulate nucleosome dynamics. Histone chaperones assist in both the deposition and the removal of promoter nucleosomes and are partly specialized to function either during or outside replication.

Chromatin remodellers alter nucleosomes. Specialized remodellers organize nucleosome arrays to promote repressed or basal chromatin states (ISWI family, NURF excepted), or incorporate histone variants to promote activation (SWR1 family), or slide and eject nucleosomes to promote DNA access (SWI/SNF family). Some remodellers are targeted or regulated by histone modifications.

'Pioneer' transcription factors typically have a binding site either in the nucleosome-depleted region or between positioned nucleosomes.

This is needed to allow initial access by a transcription factor, but additional binding sites may be hidden under nucleosomes and require a remodeller for access. Alternatively, some pioneer factors can bind sites on the nucleosome surface.

Transcription factors recruit histone modifiers. Modifiers such as acetyltransferases, methyltransferases and kinases can promote or deter the targeting or activity of chromatin remodellers on the proper nucleosome. They also promote or deter the binding of additional chromatin and transcriptional regulatory proteins.

H2A.Z resides at many promoters and positively regulates

transcription. This histone variant has a unique amino-terminal tail that is acetylated when a gene is active. H2A.Z nucleosomes may also be less stable than canonical nucleosomes, allowing ejection and helping to expose binding sites in the promoter.

The +1 nucleosome may help regulate either initiation or promoter-proximal pausing of Pol II. A common feature at inactive promoters in metazoans is the presence of a paused Pol II at the +1 nucleosome.

computational models use positioning information from the reconstitution of nucleosomes on whole genomic DNA *in vitro* to predict the positions of most nucleosomes *in vivo*¹⁵. This underscores the tremendous advances made recently in computational models for nucleosome positioning. Notably, the depth of the NDR is significantly less in human cells than in yeast¹³, perhaps because humans transcribe a much smaller fraction of their genome every cell cycle than yeast cells.

At open promoters, binding sites for transcriptional activators often reside within the NDR itself, not buried under nucleosomes far upstream²³, and their exposure in the NDR promotes activator binding and gene expression. Notably, open promoters in yeast are commonly linked to essential genes and bound by activators that are themselves essential for viability (Table 1). Two other features are correlated with open promoters: a nucleosome containing the histone H2A variant H2A.Z (termed Htz1 in yeast) at the +1 or –1 nucleosome, and a paucity of TATA sequences (discussed further later), the binding site for the TATA-binding protein (TBP) (Table 1 and Fig. 1).

Regulated genes have 'covered' promoters

At regulated genes in their repressed state, nucleosomes often cover the TSS, the regions flanking the TSS, and most of the binding sites for transcriptional activators. Such promoters are hereafter termed 'covered' promoters — a more meaningful term than 'closed' promoters, as nucleosomes cover the proximal promoter. At covered promoters, nucleosomes compete effectively with transcription factors for occupancy of key *cis*-regulatory binding sites, rendering covered promoters more reliant than open promoters on chromatin remodelling and

modifying enzymes to help 'uncover' *cis* sites and allow activity. Covered promoters typically contain NPS elements of varying strength, which help position nucleosomes over binding sites for transcription factors (Fig. 1). However, at least one binding site is typically exposed in the linker DNA between nucleosomes, or partly exposed at the nucleosome edge²³. This exposed site allows a 'pioneer' transcription factor access to the promoter, but chromatin modification and remodelling are probably required to expose the additional sites under nucleosomes³ — a two-step model for activation (Fig. 1).

A classic example of a covered promoter is that of the yeast *PHO5* gene, which has one exposed site for the activator Pho4 between two nucleosomes, and places other Pho4 sites within nucleosomes^{26,27}. Recent work on *PHO5* promoter derivatives shows the remarkable diversity of response dynamics that can be acquired by subtly varying the location and affinity of binding sites for Pho4 within *PHO5* promoter architecture²⁸. With these altered *PHO5* promoters, the affinity of the exposed Pho4 site determines the initial threshold of transcription factor (Pho4) abundance needed for promoter occupancy and initial activation — only promoters with high-affinity exposed sites are occupied and turned on with intermediate Pho4 levels — whereas the affinity of the nucleosome-occluded site(s) sets the upper end of the dynamic range of activation. By extrapolation, it is easy to imagine how combinations of sites for different activator proteins might help tune promoters to respond to multiple cellular signals. Interestingly, a subset of pioneer factors (such as the glucocorticoid receptor) do not require exposure in the linker and, instead, can bind their cognate sites on the nucleosome surface as they bind to only one face of the DNA, and can accommodate nucleosomal DNA curvature²⁹; this allows promoter binding without having prescribed nucleosome positions.

Covered promoters also differ from open promoters in some features of transcription initiation. The transcription initiation factor TBP is present at all Pol II promoters and is required for initiation at TATA-containing and TATA-less promoters. A TATA box is present at ~20–25% of yeast genes and is more highly enriched at covered promoters than open promoters, and is more enriched at highly regulated genes than constitutive genes^{30,31} (Table 1). In yeast, the distance from the TATA box to the TSS can vary from ~25 bp (observed in vertebrates) to ~125 bp. Notably, the relatively few open promoters that contain a TATA box place it quite close to the TSS — typically within 50 bp and clearly within the NDR. By contrast, TATA boxes at covered promoters are more variable in their placement, and the TATA box typically resides inside the edge of the proximal nucleosome, providing partial blockage (Fig. 1). This variable but covered placement of the TATA box at covered promoters reinforces the requirement for chromatin remodelling for TATA exposure, analogous to the remodelling requirement for transcription-factor binding-site exposure. Remarkably, at the yeast *PHO5* promoter, moving the TATA box only a few bases inside or outside the nucleosome edge greatly changes its reliance on chromatin remodelling³². Notably, TATA-containing genes use the TBP-containing complex TFIID, whereas TATA-less promoters rely on a different TBP-containing complex, termed SAGA in yeast and the pCAF/GCN5 complex in humans^{30,31}. SAGA/pCAF complexes contain multiple factors that interact with basal transcription factors, as well as histone-modifying proteins such as the histone acetyltransferase (HAT) Gcn5/pCAF³³. These activities may modify H2A.Z and other promoter proximal nucleosomes to promote nucleosome movement or ejection, promoting constitutive transcription. Notably, when considering the entire repertoire of chromatin remodelling and modifying factors (particularly in yeast), covered promoters are more reliant on chromatin remodelling factors than are open promoters^{18,19}.

Specialized chromatin remodellers

Remodellers have an important role at promoters, helping to construct the initial chromatin states and catalysing transitions to alternative chromatin states (Fig. 2), using the energy from ATP hydrolysis^{2,4,34,35}. Remodellers are specialized multiprotein machines that can be classified by their main functions (Fig. 2): remodellers that belong to the ISWI

Table 1 | Attributes correlated with open or covered promoters

Promoter type	Poly(dA:dT) tracts	Many TF binding sites	TATA box	H2A.Z variant	Histone turnover	Transcriptional plasticity	Transcriptional noise	Chromatin regulation	Condition regulation	Essential genes
Open	++	–	–	++	–	–	–	–	–	++
Covered	–	++	++	–	++	++	++	+	++	–

Correlations: ++, strongly positive; +, moderately positive; –, moderately negative; – –, strongly negative. TF, transcription factor.

family (except NURF and Isw1b) help conduct chromatin assembly and organization^{36,37}, leading to the consistent spacing of nucleosomes; those in the SWI/SNF family provide access to nucleosomal DNA through nucleosome movement³⁸ or ejection³⁹; and those in the SWR1 family reconstruct nucleosomes⁴⁰ by inserting the histone variant H2A.Z into nucleosomes, thereby specializing the composition of nucleosomes. For brevity, I do not discuss the specialized functions of the CHD and INO80 families of remodellers.

ISWI remodellers organize nucleosomes

Apart from NURF and Isw1b, remodellers in the ISWI family carry out nucleosome organization^{36,37}, which often promotes repression. ISWI complexes generally remodel nucleosomes that lack acetylation (at H4K16; that is, Lys 16 of histone H4)⁴¹, confining their activity to nucleosomes at transcriptionally inactive regions. They space nucleosomes by ‘measuring’ the DNA linker between nucleosomes and sliding the nucleosome until the linker DNA reaches a fixed distance, creating nucleosome arrays of uniform spacing^{2–4,42}. In yeast, Isw2 localizes to gene 3′ ends and adjacent to transfer RNAs, and possibly to the –1 nucleosome as well^{12,43}. Chromatin organization by yeast Isw2 helps prevent antisense transcription from occurring in intergenic regions, as well as preventing ‘cryptic’ initiation by Pol II, which can occur if nucleosome density and positioning are not optimized⁴⁴.

Notably, yeast Isw2 can move nucleosomes onto unfavourable DNA sequence elements⁴⁵, which can help establish repression. The yeast *POT1* promoter is a good example of a blended promoter at which this property is illustrated; it is highly regulated but contains a poly(dA:dT) tract and *cis*-regulatory sites that in the repressed state partly overlap with a nucleosome. The omission of Isw2 causes the nucleosome to move away from its overlap with the poly(dA:dT) sequence to a location favoured by sequence alone; this movement exposes binding sites in the promoter and is correlated with partial derepression of the gene⁴⁵. This suggests that certain promoters may use ISWI remodellers to move nucleosomes to unfavourable DNA positions, which could occlude transcription-factor binding sites or the TATA box to confer repression. How might such a promoter be activated in normal cells in the presence of Isw2? Here, the modification of the H4 tail may prevent the action of Isw2 or attract an alternative remodeller (discussed later) that moves the nucleosome away from the poly(dA:dT) tract, exposing a regulatory *cis* element and allowing activation. This example illustrates how the concepts used to regulate open and covered promoters, along with action from the remodeller, can help us understand the regulation of a blended promoter.

SWI/SNF remodellers disorganize nucleosomes

Remodellers in the SWI/SNF family can both slide and eject nucleosomes, and their functions are often correlated with nucleosome disorganization and promoter activation^{2,4,39}. In keeping with this notion, histone acetylation is correlated with gene activity, and SWI/SNF remodellers have domains that bind acetylated tails, promoting their targeting or activity in promoters undergoing activation² — an area of active study beyond the scope of this Review. SWI/SNF action may be needed at open promoters to help remove nucleosomes from poly(dA:dT) sequences or at covered and blended promoters to slide or eject nucleosomes, an important area of future study. In yeast, remodellers in the SWI/SNF family are usually located at the –1 nucleosome¹², consistent with the observation that the binding sites for many condition-specific activators reside within the –1 nucleosome in regulated yeast genes. However, as these remodellers both bind and eject nucleosomes, they are not detected

at NDRs by current methods, perhaps because they remove their binding substrate, the nucleosome. So it remains possible that these remodellers help generate the NDR and regulate the occupancy and position of the –1 nucleosome. This idea is supported by the analysis of mutations in RSC, a member of the SWI/SNF family in yeast; *rsc* mutations are found to affect nucleosome density and positioning at promoters^{46–48}.

An issue of current debate is whether DNA sequences or chromatin remodelling and modifying factors are the most important drivers of nucleosome depletion at promoters. Genome-wide nucleosome occupancy maps generated through the *in vitro* assembly of nucleosomes with yeast genomic DNA show NDRs that map to promoters, but these NDRs are not as depleted as NDRs *in vivo*¹⁵. This leaves testable roles for remodellers, modifiers and variants in NDR creation. Also, roles for SWI/SNF-family members in gene repression have been noted^{2–4}, and here it will be interesting to determine whether nucleosome disorganization promotes the binding of repressors to *cis*-acting sites in enhancers or promoters. A future challenge for studying both SWI/SNF and ISWI remodellers is to isolate *in vivo* remodelling intermediates to provide direct evidence for the involvement of the remodeller in sliding or ejecting particular nucleosomes.

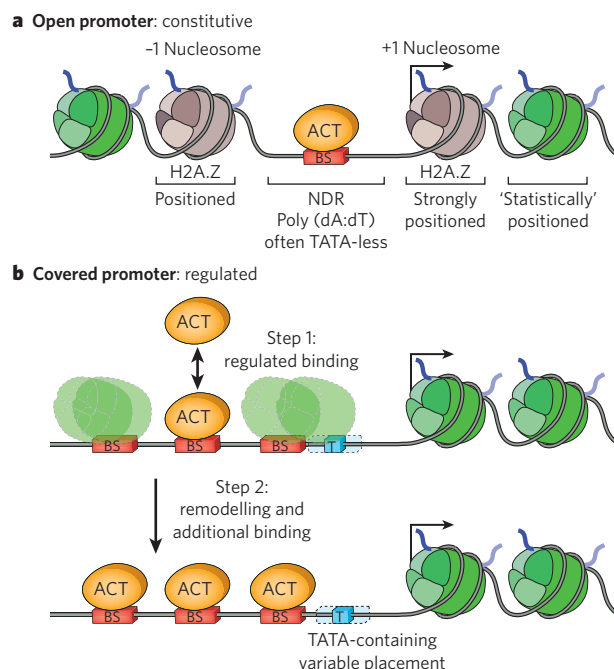


Figure 1 | Properties of open and covered promoters. **a**, Open promoters have a depleted proximal nucleosome adjacent to the transcription start site (TSS, black arrow), a feature common at constitutive genes. **b**, Covered promoters have a nucleosome adjacent to the TSS in their repressed state, a feature common at highly regulated genes. The figure depicts features more common in each contrasting promoter type, but most yeast genes blend the features shown to provide appropriate regulation. Green nucleosomes contain canonical H2A, whereas brown nucleosomes bear H2A.Z. Binding sites (BS) for transcriptional activators (ACT) are shown. These are mainly exposed for open promoters and mainly occluded by nucleosomes (in the repressed state) at covered promoters. Covered promoters typically have nucleosome positioning sequence elements of varying strength and locations that help define nucleosome positions (faded green) and promoter architecture. NDR, nucleosome-depleted region.

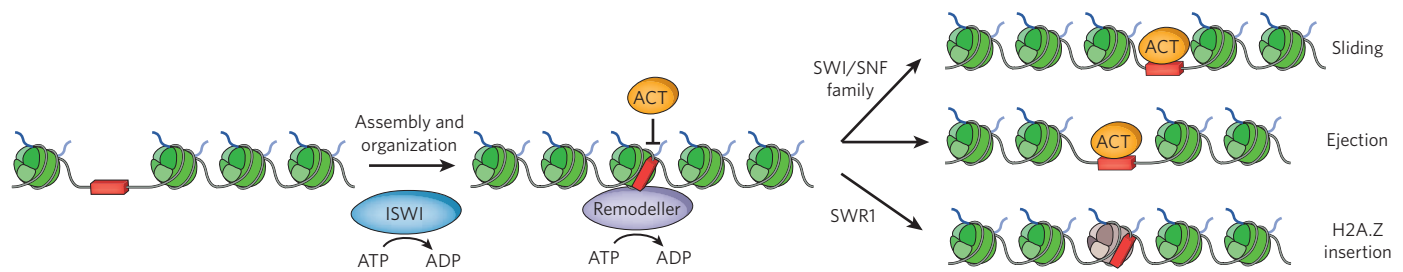


Figure 2 | Basic functions of chromatin remodellers in nucleosome dynamics. Remodellers use ATP hydrolysis to alter nucleosomes and are specialized for certain tasks. Most remodellers of the ISWI family (except NURF and Isw1b) help conduct chromatin assembly and organization and provide consistent spacing of nucleosomes. This organization can cover a binding site (red) for a transcriptional activator (ACT). SWI/SNF-family

remodellers provide access to binding sites in nucleosomal DNA, mainly through nucleosome movement or ejection. SWI/SNF-family remodellers reconstruct nucleosomes by inserting the histone variant H2A.Z into nucleosomes, specializing their composition. This can create an unstable nucleosome in certain compositional and temporal contexts, and might lead to ejection, sliding or reconstruction at promoters.

SWR1 and properties of H2A.Z nucleosomes

The histone H2A variant H2A.Z differs from canonical H2A in its amino-terminal tail sequence and also at key internal residues that may affect its interactions with itself and with the H3/H4 tetramer in the nucleosome, thereby affecting nucleosome stability. H2A.Z is assembled into particular promoter nucleosomes, replacing canonical H2A in a replication-independent manner in a reaction conducted by remodellers of the SWR1 family⁴⁰. There is variation in the placement of H2A.Z within the promoter in different organisms and in its precise role, although certain themes have emerged. In yeast, H2A.Z (Htz1) is found at most genes and mainly occupies the +1 and -1 nucleosome, with lower amounts at +2 (refs 20–22). H2A.Z is highly enriched at open TATA-less promoters²² but is not exclusive to open promoters (many blended promoters contain H2A.Z). In *Drosophila*, H2A.Z is absent at the -1 position but is highly prevalent at the +1 nucleosome, and then likewise decays into the coding region¹¹. In humans, H2A.Z is localized to the promoter, but more widely than in yeast, extending from -3 to +3 in genes with low expression¹³. In plants, H2A.Z is found in the promoter, where it promotes transcription, and is reciprocal with DNA methylation⁴⁹. Functional studies in yeast support roles for H2A.Z in promoting activation^{20–22}, and H2A.Z nucleosomes are lost from genes as transcription increases^{20,22}, a feature also found in humans, where there is profound loss at the -1 position¹³. However, the presence of acetylated H2A.Z increases with activation^{50,51}. This suggests that, in both yeast and humans, inactive or basal genes contain a high level of H2A.Z and that both acetylation and loss of H2A.Z accompanies activation. By contrast, in flies, H2A.Z occupancy at the +1 position correlates with transcription rate¹¹.

At first glance, these contrasting observations suggest very different strategies for H2A.Z. Alternatively, H2A.Z may promote activation by different mechanisms, reflecting differences in the way each organism uses the TSS and the +1 nucleosome in gene regulation. In yeast, the TSS is normally placed just inside the +1 nucleosome, whereas flies tend to have the TSS in the NDR (50–75 bp upstream of the +1 nucleosome)^{11,18}. Furthermore, repressed fly genes often have Pol II already present near the TSS, which may be transcriptionally engaged but paused at the transition to processive elongation (see below and page 186). By contrast, paused Pol II occurs much less frequently in yeast¹². H2A.Z may therefore be poised to regulate transcription initiation in yeast (TSS access), whereas in flies it may help to regulate elongation or pausing at the +1 nucleosome. Another consideration is the timing of H2A.Z placement; yeast genes place H2A.Z into genes in the repressed/basal state, whereas in flies H2A.Z may be inserted after the initial round of transcription to facilitate subsequent rounds¹¹. This may underlie the observation that H2A.Z is lost from yeast genes during activation^{20,22}, whereas H2A.Z abundance increases at the +1 position in flies¹¹. However, in both organisms the strategy is to promote transcription, although by different processes.

Another notable feature of nucleosomes containing H2A.Z is their differential stability, which depends on the histone H3 subtype in the assembled nucleosome. H2A.Z nucleosomes are more stable than

H2A-containing nucleosomes when co-assembled with canonical histone H3 but less stable when co-assembled into nucleosomes with the H3 variant H3.3 (ref. 52). Notably, H3.3-containing nucleosomes are inserted into genes that lose nucleosomes during transcription or chromatin remodeling⁵³ and may therefore contain H2A.Z/H3.3 hybrid nucleosomes at the +1 position after the first round of transcription; this may explain why H2A.Z levels correlate with transcription in flies. Here, the instability (and/or modification status) of the hybrid nucleosome might promote reinitiation or Pol II elongation during subsequent rounds of transcription⁴⁹. By contrast, yeast have only H3.3 (their 'canonical' H3 is the orthologue of vertebrate H3.3), making all yeast H2A.Z nucleosomes slightly unstable, perhaps underlying their depletion during activation⁴⁹. Furthermore, H2A.Z instability or acetylation may render H2A.Z nucleosomes sensitive to movement or ejection by chromatin remodellers and more fully expose the promoter. Finally, one outstanding question is how H2A.Z and SWR1 are targeted to promoter nucleosomes *in vivo*, with DNA-binding proteins and histone modifications probably both contributing^{21,22}.

Transcriptional noise

Transcriptional noise refers to the variability in the expression of single gene alleles in a cell population held under constant growth conditions: low-noise genes show uniform expression across the population, whereas high-noise genes show variable expression. Here, covered promoters have a much higher level of noise, a feature demonstrated by genome-scale studies¹⁸ and careful analysis of the yeast *PHO5* gene⁵⁴, a covered promoter. Covered promoters may be noisier than open promoters because of the relative differences in chromatin structure between their basal and active states⁵⁴. Open promoters have a pronounced NDR that encompasses and exposes their relatively few transcription-factor binding sites. The transition of open promoters from moderate constitutive expression to a higher active state may involve modest additional nucleosome depletion at the NDR, -1 or +1 site, and perhaps the binding of one additional factor. By contrast, many covered promoters require major changes in both nucleosome positioning and occupancy, requiring the extensive use of chromatin remodellers to permit full binding by transcription factors. Certain covered promoters may therefore be more bistable in nature — prone to bursts of transcription followed by periods of chromatin repression, rather than the steady reinitiation allowed by constitutively open promoters. Although chromatin plays important roles, there are other sources of noise in gene regulation, including signalling and transcription-factor regulation⁵⁵.

'Statistical' positioning and phasing

Most yeast genes, and many active genes in metazoans, have on their coding region 'phased' nucleosome arrays, in which the nucleosomes display consistent alignment and spacing relative to the physical map. These phased nucleosome arrays may help prevent the cryptic internal initiation of transcription within genes^{44,56,57}, which can produce dominant truncation derivatives. Phased arrays typically start at the

+1 nucleosome and decay slowly over distances, but it is not clear how they are formed. One possible mechanism is 'statistical' positioning, in which phased arrays emanate from a decisive element: a strongly positioned nucleosome or a nucleosome-free region⁵⁸. Either of these elements could establish a 'boundary' from which adjacent nucleosomes acquire phasing, resulting in a statistically positioned array that decays in phasing coherence over a distance.

As described earlier, open promoters may use the poly(dA:dT) stretch flanked by strong NPS elements at the -1 and +1 nucleosome to fix the position of these two nucleosomes, forming a decisive boundary element from which additional nucleosomes could, in principle, be statistically positioned in both directions without a requirement for additional flanking NPS elements²⁵. This phasing could involve the spacing function of ISWI-family remodellers⁴² acting to evenly space nucleosomes upstream and downstream of the positioned -1 and +1 nucleosomes, in open promoters and coding regions, respectively (Fig. 1).

Paused Pol II and nucleosome positioning

Pol II pauses at the edge of the first nucleosome downstream of the TSS at many genes in humans⁵⁹ (see page 186), and these promoters often lack a nucleosome at the -1 position (the NDR in humans). This raises the possibility that the +1 nucleosome is involved in pausing, either by physically blocking progression or by regulating the presence or activity of factors that help Pol II overcome this pause. In human cells, at inactive genes the +1 nucleosome resides ~10 bp downstream of the TSS, whereas at active genes the +1 nucleosome resides ~40 bp downstream of the TSS, suggesting that the +1 nucleosome slides as part of the transition to processive elongation¹³. The modification state of the +1 nucleosome is highly regulated; for example, it has high levels of histone H3 trimethylated at Lys 4 (H3K4me3), which binds the PHD domain in the Taf3 subunit of TFIID, and also has acetylation, which binds the Bdf1/2 subunits of TFIID⁶⁰. In principle, TFIID, the +1 nucleosome and Pol II could form a functional unit within which initiation and elongation are regulated. In humans, the phasing of nucleosomes in the coding region is largely confined to genes with active Pol II¹³. One possibility is that in humans this functional unit serves as a boundary around which nucleosomes are subsequently positioned after Pol II pauses, perhaps by statistical positioning and ISWI function, although this remains to be tested. Experiments to address the roles of remodellers in regulating the +1 nucleosome in higher eukaryotes are also eagerly awaited.

Promoter nucleosomes have high turnover

One model of inactive promoters posits that they are static entities with immobile nucleosomes. However, if this is true, it might be difficult for certain pioneer transcription factors to find their sites in promoters, especially if their sites reside within the edge of a nucleosome. Recent results in yeast have shown that promoters have 'hot' nucleosomes; that is, nucleosomes with high turnover rates in both their repressed and active states^{61,62}. Remarkably, promoter nucleosomes are hotter than those in coding regions, even at active genes with transcribing Pol II. This suggests that the intense focus of chromatin modifying and remodelling enzymes (and H2A.Z) near the future TSS greatly promotes nucleosome turnover, allowing the inspection of promoters by transcription factors at a tuned rate. Acetylation at H3K56 has been shown to be strongly correlated with hot nucleosomes⁶². One attractive possibility is that histone modifications such as H3K56ac attract SWI/SNF-family remodellers to eject nucleosomes^{61,62}. Finally, histone chaperones have been shown to assist both the replication-independent histone deposition machinery and remodellers during histone eviction^{39,63}. Indeed, the coordinated action of remodellers and chaperones is probably at the forefront of histone eviction and assembly dynamics.

Concluding remarks

Over the past five years, genome-wide and gene-specific studies have revealed important concepts for the regulation of promoter architecture (Box 1). Among the recent advances is a greater understanding of how DNA sequences help define nucleosome positions, and how chromatin

modification and remodelling machineries optimize both the initial repressed (or basal) state and the transition to the active state. Here, the notion that promoters are poised in the repressed state is becoming better appreciated, with nucleosome positioning and depletion, histone variants, histone modifications and (in metazoans) the presence of a paused Pol II all being prominent aspects of this poised state. Furthermore, these poised, repressed architectures are now understood as dynamic structures themselves, with high nucleosome turnover. Indeed, it is the precise nature of the poised state that sets the requirements for the transition to the active state. Finally, the effect of distal enhancer proteins on promoter dynamics is an important area of current and future study. The transitions of promoters between their repressed and active states have been studied for decades, but our recent ability to simultaneously monitor many aspects of promoter architecture, modification and composition at high temporal and spatial resolution will doubtless reveal welcome detail in these individual steps, and this can also be extended to enhancers. In addition, genetic experiments are required to determine the dependency relationships — to see which factors, histone modifications and nucleosome movements are truly instructing the next transition. Understanding these relationships will greatly enhance our understanding of the logic and orchestration of promoter regulation. ■

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Acknowledgements I thank T. Parnell and C. Clapier for comments and assistance with figures. I am grateful for support from the US National Institutes of Health (grant GM60415) and the Howard Hughes Medical Institute.

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