Transcription and chromatin converge: lessons from yeast genetics
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The control of transcription through the modification of chromatin has been a subject of intense study over the past year. The increasing use of genome-wide approaches to examine the role of chromatin and the complexes able to modify it is providing a global perspective that is profoundly altering our view of the transcription process.

Introduction
The genetic information of the cell is contained within a complex of DNA, histones and non-histone proteins collectively termed chromatin. Whilst providing the framework for the compaction of the entire genome into the volume of a nucleus, the nucleosome also plays a highly dynamic role in the regulation of transcription. By controlling the access of regulatory DNA to the transcription machinery and co-factors alike, the nucleosome presents a powerful barrier to unsolicited transcription. Indeed, this blockade has evolved into an exquisite regulatory tool both able to facilitate and prevent gene expression. For example, loss of nucleosomes by depletion of histone H4 results in a greater than three-fold increase (or three-fold decrease) in expression of some 15% (or 10%) of genes [1]. This result highlights the requirement for correct nucleosomal architecture in both gene activation and repression. Defining how the uniform building block of chromatin, the nucleosome — with its ubiquitous distribution over the DNA template — can itself be fine-tuned to achieve regulated transcription at specific loci has been the focus of a tremendous research effort.

Two broad classes of chromatin-modifying activities have dominated much of our progress in determining the role of chromatin in the process of transcription. These are the ATP-dependent chromatin remodeling machines typified by the SWI/SNF complex (reviewed in [2,3]), and the histone acetyltransferases (HATs) and deacetylases (reviewed in [4,5]). While ATP-dependent remodeling complexes use the energy of ATP hydrolysis to physically alter the properties of the nucleosome, the acetylation and deacetylation of the highly conserved histone amino-terminal tails has also proven to be critical in the proper and timely regulation of transcription. In this review, I attempt to set out our evolving view of the role of these complexes and the transcription machinery in the regulation of gene expression through chromatin.

Achieving activation
The primary determinant of specificity in regulated gene expression is the gene-specific activator. At the PHO5 locus, for example, the trans-activator Pho4 binds to two sites in the upstream region of the gene and the binding to one of these sites is prevented by the presence of positioned nucleosomes across the promoter [6]. Importantly, the characteristic nucleosomal perturbation observed upon induction absolutely requires the acidic activation domain of the protein [7], although not transcription of the gene per se [8].

How does the activation domain orchestrate chromatin remodeling and transcription? Acidic activation domains interact with several different classes of complex involved in the establishment of transcriptional activation. The trans-activator Gcn4, for example — which regulates genes involved in response to amino-acid starvation — has been shown to interact independently with three different classes of such complex: SWI/SNF, SAGA and Mediator [9]. Moreover, the activation domains of VP16, Gcn4, Swi5, and Hap4 are all able to elicit activator-dependent recruitment of SWI/SNF [10]. Mechanistically, however, for recruitment to be a bona fide function of the trans-activation domain, these interacting complexes must act at a stage subsequent to the DNA binding of the activator in vivo (Figure 1). Indeed, despite the fact that SAGA and SWI/SNF are necessary for chromatin remodeling at the PHO8 promoter, the activator Pho4 is able to bind in vivo to the critical UAS element in the absence of both complexes [11].

Further evidence for activators being directly responsible for the recruitment of chromatin modifiers comes from the restricted site of the modification itself. The SAGA complex, for example, has been shown to generate a local domain of H3 hyperacetylation specifically at the promoters of multiple genes such as HIS3 [12,13], PHO8, PHO5, CUP1 and GAL1 [14••]. However, is the activator the actual determinant of the localized hyperacetylation? In an in vitro system, the VP16 activation domain has been shown to direct the recruitment of both SAGA and NuA4 specifically to chromatin templates carrying the appropriate binding site as a prerequisite for transcriptional activation [15]. Importantly, a thorough in vivo investigation of the determinants of the promoter-specific acetylation at HIS3 has been performed [13]. Deletion of the binding site for Gcn4 eliminates the normal peak in acetylation at this genomic location, whilst movement of the binding site to a new position is sufficient to direct a new peak of histone acetylation to this alternative ectopic location. The Gcn4 activation domain is essential for this
targeted acetylation, which is independent of the transcriptional status of the gene [13]. Taken together, these data support a predominant role for the activator in directing recruitment of the chromatin-remodeling machines and activities necessary for the establishment of transcriptional activation (Figure 1).

This correlation also holds true for other acetyltransferase complexes. NuA4, which contains the only HAT required for yeast viability Esa1 [16], acetylates histones H4/H2A [17], and can be recruited by activation domains in vitro to stimulate transcription from chromatin templates in an acetyl co-enzyme A dependent manner [15,18]. Recently, the *in vivo* targets of Esa1 and thus NuA4 have been shown to be ribosomal protein promoters, to which the complex is specifically recruited potentially through the general DNA-binding factors Rap1 and Abf1 [19••]. This defines a unique role for the only histone H4 acetylase in a discrete biological function. It is perhaps worthy of note that the only essential ATP-dependent remodeling activity, the RSC complex, causes a very similar cell-cycle progression fault to that of NuA4, although the specific targets of RSC are, as yet, undefined.

The finding that the Mediator subunit, Nut1, contains intrinsic HAT activity in an in-gel assay provides further
support for the general importance of acetylases in transcriptional activation [20•]. The Mediator is an obligatory component for transcriptional activation in vivo [21] and thus this HAT is presumably recruited to every active promoter. Similarly, the TBP (TATA-binding protein) associated factor (TAF) TAF1145 also demonstrates HAT activity [22], thus the TFIID complex itself contains an intrinsic acetylase. TFIID is not recruited to every promoter (see below), however, challenging the view of a ubiquitous role of TAFs in transcription. Whole-genome analysis of the transcriptional requirement for a particular TAF through the use of temperature-sensitive mutations has demonstrated that the lethality common to deletion of many TAFs reflects their absolute requirement for the expression of specific subsets of genes, rather than their importance at every gene [23•,24•]. Furthermore, specific promoters recruit TFIID, that is, TBP in association with TAFs, whereas others recruit TBP in a TAF-independent form. Thus TAFs are perhaps another promoter-specific determinant of transcriptional regulation. Indeed, in a manner analogous to bacterial sigma factors, environmental stress such as heat shock can dynamically alter the relative abundance of these two distinct forms of TAF-dependent and independent TBP in vivo [23•].

TAFs are also found in multiple transcription-related complexes such as SAGA [25] and SWI/SNF [26], and a subset of TAFs including TAF60, TAF17, TAF40, TAF68/61 and TAF19 have homology to specific core histones leading to the speculation that these histone-like TAFs may form a pseudo-octamer to challenge the promoter nucleosomes in the transcriptional activation process [27]. The significance of the shared TAFs is not currently understood but their presence in multiple complexes raises the possibility of shared functionality. This issue has been addressed directly for TFIID and SAGA. Although mutations in components unique to either complex cause a rather limited effect on global gene expression, the majority of genes are affected by mutations in common subunits [28•]. Furthermore, mutations of the acetyltransferase components of TFIID and SAGA, namely TAF1145 and Gcn5, demonstrate a remarkable level of redundancy, with a considerable percentage of global gene expression occurring through the function of either one or the other complex.

The plethora of different acetylation activities associated with all parts of the transcription machinery underscores the importance of acetylation in the establishment of transcription. Furthermore this suggests that redundant functions between acetylases may explain the relatively minimal phenotype that absence of individual acetylases has on the cell. However, the presence of multiple acetyltransferase complexes suggests that the biological activity of acetylation involves a controlled and finely tuned balance between specific and redundant activities. Specifically, several reports have begun to challenge the view of a purely localized role for histone acetylation in transcription. First, biochemical determination of the extent of nucleosomal acetylation in yeast suggests that, on average, some 13 lysine residues are acetylated per nucleosome [29]. This is in keeping with the level observed in the transcriptionally active domains of chromatin in higher eukaryotes. Second, genome-wide differences in acetylation have been observed for SAGA and NuA4 loss of function mutants suggesting a non-targeted role for these activities in maintaining a generally higher level of H3 and H4 acetylation respectively [13,19••,30•,31••]. Interestingly, by examination of defined chromatin regions in vivo, Grunstein and co-workers [31••] have shown that this background of H3 and H4 acetylation is balanced by the histone deacetylases (HDACs) Rpd3 and Hda1, functionally serving to reduce the basal level of transcription in a non-targeted manner. Thus, targeted specific acetylation and deacetylation of individual promoter elements occurs within a high background of global acetylation and deacetylation. Histones are thus a bona fide target of acetylase activities in vivo; it should however be reiterated that this observation does not exclude alternative targets [32].

Enhancing elongation
Activator-induced chromatin remodeling is a necessity for the efficient recruitment of the pre-initiation complex to the promoter; however, this is itself insufficient for productive transcription. FACT (facilitates chromatin transcription) was identified as a protein complex able to overcome a nucleosome-mediated promoter proximal block to elongation [33,34]. Further biochemical characterization of the FACT complex has revealed it to be a heterodimer of two proteins Spt16 and SSRP1, the human homologues of the Saccharomyces Spt16/Cdc68 and Pob3 proteins, respectively [35•]. FACT specifically interacts with nucleosomes and H2A/H2B dimers but not with RNA Pol II by the same experimental criterion, further linking the function of this complex to chromatin. Moreover, yeast FACT has been linked to the basal transcription machinery as an interaction between Spt16/Cdc68 and TFIIE has been demonstrated [36]. Mechanistically, the ability of FACT to enhance the elongation of transcription was lost when chromatin templates containing cross-linked histones were used, which, taken together with the specific interaction of FACT with H2A/H2B dimers, is suggestive of a role for the FACT complex in catalyzing some form of nucleosome disruption [35•]. More recently, NuA3 [37], the only non-Gcn5-dependent histone H3 acetyltransferase complex, was characterized. The ‘something about
silencing' protein, Sas3, was identified as the catalytic subunit of the NuA3 complex and interestingly shown to interact with Spt16 [38]. Spt16 is an abundant and essential protein in yeast cells and as described above is a member of the yeast FACT complex (Spt16/Cdc68, Pols3). Thus FACT may also employ acetylases in modulation of chromatin for efficient transcriptional elongation.

Independently, an elegant biochemical approach aimed at identifying complexes associated directly with actively transcribing polymerase has led to the purification of Elongator [39]. Mutations in the genes for Elongator give rise to an 'adaptation phenotype' whereby the transcriptional response to a new environment is severely delayed. Detailed characterization of Elongator has identified the Elp3 subunit as a HAT, strengthening the link between elongation and histone acetylation [40]. These data connect histone acetylases with the ability to relieve the repression of elongation through chromatin, although the true in vivo targets of the catalytic domains remain to be determined.

Regulating repression
The nucleosome presents a repressive environment for the process of transcription. The view that chromatin is passively an effective barrier to the process of transcription, however, is challenged by the large numbers of genes showing unaltered expression upon nucleosome depletion [1]. One should perhaps consider the establishment of repression through nucleosomes a dedicated process in the same vein as activation. Whole-genome analysis of strains mutated for components of the SWI/SNF complex, known to be necessary for the activation of a subset of yeast genes, has identified a number of genes dependent on this complex for their repression [21,41]. Are nucleosomes also remodeled to achieve repression? Support for active positioning of nucleosomes in repression comes from work on the RSC complex, which has been shown to be necessary for the repression of transcription of the chromatin-regulated CHA1 gene [42]. Along the same line, genetic analysis of the recently purified ISWI complexes [43] indicates that the ISWI2 complex is necessary for the repression of transcription of the genes involved in the control of meiosis [44••]. This repression was shown to entail nucleosome re-positioning as a consequence of ISWI2 function, thus correlating directly its repression function with the ability to position nucleosomes in vivo. Significantly, ISWI2 is specifically recruited by the DNA-binding factor Ume6, which has previously been shown to recruit the deacetylase Rpd3 [45]. Therefore, perhaps ISWI2 is a yeast paralog of the metazoan Mi-2 complex, which contains both ATPase and HDAC activities [46]. Thus, at least in these examples, complete repression is achieved through the targeted, gene-specific and synergistic action of both deacetylase and ATP-dependent remodeling machines.

Connecting complexes
How do functionally different complexes interact with one another to build a regulated response in the control of gene expression? A precise study of the yeast HO promoter reveals the ordered recruitment of the chromatin-modifying activities known to be necessary for activation [47••]. Taken together with the complementary histone acetylation data [30•], these studies show that at the HO promoter Gcn5 (SAGA) recruitment occurs subsequent to and is dependent upon that of SWI/SNF. Interestingly, however, chromatin perturbation occurs following, or concurrently with histone acetylation by SAGA suggesting an essential function of the acetylation in the remodeling step. This conclusion is supported by the strong Gcn5 dependence of the chromatin transition of the PHO8 promoter in vivo [11]. The exact order of recruitment of the necessary complexes is likely to be a gene-specific event because the Gcn5-dependent acetylation of H3 at the GAL1, PHO8, PHO5 and CUP1 promoters is SWI/SNF-independent [14••]. Indeed, the HO promoter may demonstrate a particular order of recruitment as a result of its cell cycle conditional expression, a possibility supported by the cell stage specific SWI/SNF dependent gene expression discussed earlier [14••].

How might histone acetylation modulate successful chromatin remodeling? One possibility is that histone acetylation facilitates the action of subsequent complexes. The finding that the bromodomain of the acetyltransferase PCAF specifically binds acetylated lysine residues provides recent support for such a model [48•,49•]. Bromodomains are found exclusively in nuclear proteins, and many are members of known chromatin-modifying complexes such as Gcn5 from SAGA, Sth1 from RSC and Sfn2 from SWI/SNF. Moreover, the bromodomain of Gcn5 was shown recently to play a role in the chromatin remodeling of a hybrid promoter in vivo [50]. This conserved protein motif potentially acts as an acetylation gauge, monitoring the level of acetylation and associating with highly acetylated or specific patterns of acetylated chromatin. In this way the extent of acetylation may also define the region of chromatin remodeling.

What happens after chromatin remodeling and transcriptional activation are successfully initiated? The requirements for the maintenance of transcription are different to those of establishment. SWI/SNF, for example, appears to be required continuously to maintain elevated gene expression [51,52]. Various lines of evidence suggest, however, that the requirement for the acetylase Gcn5 may be less absolute. First, at the IFN-β promoter, the recruitment of Gcn5 and the resultant histone acetylation peaks prior to recruitment of TBP and is absent during the following 10–12 hours of robust transcription [53••]. A less dramatic but analogous result is observed at HO in yeast [30•]. Second, induction of gene expression through a number of unrelated activators such as Gal4, Hap4, Adr1 and Met4 does not result in increased H3 acetylation [54]. Although potentially as a result of the absence of a promoter-specific acetylation at these loci, I favor a model in which histone hyperacetylation is a highly transient state, unnecessary for continued gene expression (Figure 1). Indeed, it is logical for the cell to erase such promoter-specific hyperacetylation once gene activation has been achieved as
this could facilitate efficient restoration of the repressed state. Moreover, a strain deleted for the HDAC Rpd3, which increases the general level of acetylation displays a reduced kinetic of return to the repressed state of the PHO5 gene [51••]. Thus, eliminating the hyperacetylation peak after chromatin remodeling may be an important part of complete gene regulation.

Conclusions

The past year has seen our understanding of the molecular determinants of gene expression develop on a genomic scale. Acetylases have been identified in almost every stage of transcriptional control whereas remodeling machines have expanded from positive factors in activation to important regulators of repression through nucleosome positioning and histone deacetylation. This global perspective has served to underscore the importance of chromatin in transcription but also highlights areas where our understanding is less than complete. For instance, a typical DNA binding factor might recognize some 6–9 bp of sequence, to which it can be shown to bind with a high degree of specificity. One would predict that such a factor would bind with approximately the frequency of a restriction site, that is, many hundreds of times throughout the genome. By using a combination of whole-genome analysis and chromatin immunoprecipitation, however, the sites to which the Gal4 protein actually binds in vivo have been determined. Amazingly, despite the huge number of potential binding sites genome wide, this protein was found to be present at just 10 promoters in vivo [55••]. This leads to a question: how can an activator select for these sites when presented with the entire genome? Certainly the presence of chromatin will play a defining role in determining site selection in vivo [56]. The mechanisms by which this is achieved are still to be elucidated and yet are the keys to the control of regulatory networks in eukaryotes.

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References and recommended reading

Papers of particular interest, published within the annual period of review, will be highlighted as:
* of special interest
** of outstanding interest


Furthermore, it is shown in [23] that this distribution can be dynamically altered in response to heat shock.


Using whole-genome array technology the effects of mutations in the SAGA and the TFIIID complexes are analyzed. Surprisingly, the majority of genes can be expressed through the HAT activity of one or other complex.


Using chromatin immunoprecipitation, the active recruitment of the Gcn5 acetylase, as a prerequisite to chromatin remodeling and gene activation, is demonstrated.


Careful ChIP analyses of large chromosomal regions are here used to demonstrate a non-targeted role of Gcn5 and Esa1 acetylases, and the Rpd3 and Hda1 deacetylases in the regulation of histone acetylation levels. Furthermore, a functional significance to these balancing activities is provided.

32. Kouzarides T: Histone acetyltransferases in the regulation of histone acetylation levels. Furthermore, this paper demonstrates targeting of the ISW2 complex by Ume6 linking the repression function to histone deacetylation.

33. Orphanides G, Wu WH, Lane WS, Hampsey M, Reinberg D: The Elp3 subunit of the Elongator is shown to be a HAT. Mutations that abolish this activity also ablate the function of the complex. This suggests a role for acetylases in elongation.


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35. Orphanides G, Wu WH, Lane WS, Hampsey M, Reinberg D: The Elp3 subunit of the Elongator is shown to be a HAT. Mutations that abolish this activity also ablate the function of the complex. This suggests a role for acetylases in elongation.

36. Kang SW, Kuzuhara T, Horikoshi M: Hda1 deacetylases in the regulation of histone acetylation levels. Furthermore, this paper demonstrates targeting of the ISW2 complex by Ume6 linking the repression function to histone deacetylation.


The structure of the double bromodomain of TAF7,250, the mammalian homologue of yeast TAF1,45, and member of the TFIID complex, is solved to 2.1 Å resolution. Importantly, the structure aligns perfectly with the spacing of diacetylated tail of histone H4, strongly suggesting a role for this domain in chromatin binding and recognition of a particular acetylation status.


Structural demonstration that the bromodomain specifically interacts with acetylated lysine residues, such as those found in histone tails. Strong evidence for the function of the bromodomain.


The ISW2 complex is shown to regulate the repression of meiotic genes and, most significantly, this repression is associated with the active positioning of nucleosomes in vivo. Furthermore, this paper demonstrates targeting of the ISW2 complex by Ume6 linking the repression function to histone deacetylation.


A beautiful data set showing the ordered recruitment of the SWI/SNF and SAGA complexes to the HO promoter in yeast.


Structural demonstration that the bromodomain specifically interacts with acetylated lysine residues, such as those found in histone tails. Strong evidence for the function of the bromodomain.


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52. Sudarsanam P, Cao Y, Wu L, Laurent B C, Winston F: The nucleosome remodeling complex, Sin3/Swi, is required for the maintenance of transcription in vivo and is partially redundant with the histone acetyltransferase, Gcn5.


ChIP analysis used to present a clear demonstration of the order of recruitment for the various transcription complexes that collectively form the enhancosome. TBP recruitment, Gcn5 recruitment, histone acetylation and transcription are linked together in one combined data set.


Whole-genome analysis is here used to show that of the hundreds of potential sites available for the activator, on the basis of sequence identity, in fact only 10 (for G4A4) are actually bound. A striking result.