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How eukaryotic genes are transcribed

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Summary

Regulation of eukaryotic gene expression is far more complex than one might have imagined thirty years ago. However, progress towards understanding gene regulatory mechanisms has been rapid and comprehensive, which has made the integration of detailed observations into broadly connected concepts a challenge. This review attempts to integrate the following concepts: 1) a well-defined organization of nucleosomes and modification states at most genes, 2) regulatory networks of sequence-specific transcription factors, 3) chromatin remodeling coupled to promoter assembly of the general transcription factors and RNA polymerase II, and 4) phosphorylation states of RNA polymerase II coupled to chromatin modification states during transcription. The wealth of new insights arising from the tools of biochemistry, genomics, cell biology, and genetics is providing a remarkable view into the mechanics of gene regulation.

Keywords

gene regulation; chromatin; genome-wide; pre-initiation complex; histone modification; transcription cycle; noncoding RNA

Optional Keywords

SAGA; TFIID; ubiquitylation; histone methylation; histone acetylation; RNA polymerase II CTD; nucleosomes

Introduction

Eukaryotic transcription is regulated by a large number of proteins, ranging from sequence-specific DNA binding factors to chromatin regulators to the general transcription machinery and their regulators (reviewed by Berger, 2000; Li *et al.*, 2007a; Orphanides and Reinberg, 2002; Pugh, 2000; Struhl *et al.*, 1998). Their collective function is to express a subset of genes as dictated by a complex interplay of environmental signals that is only partly understood. Classical biochemistry and cleverly devised genetic screens have led to discoveries of important components of the transcription machinery, and have provided insight into mechanisms involved in transcription by RNA Polymerase II (Pol II). Recent genome-wide expression profiling and factor location profiling have imbued our understanding of the organization of the transcription machinery and nucleosomes throughout the genome. The prevailing view of transcriptional activation is that many sequence-specific regulators interact with their cognate DNA motifs in response to cellular signals. They recruit transcriptional coactivators to alter the local chromatin environment

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and facilitate assembly of the pre-initiation complex (PIC), which is composed of the general transcription factors (GTFs) and Pol II.

The breadth of information about how genes are regulated has become sufficiently vast that it is becoming increasingly difficult to comprehend how the different aspects of transcriptional regulation fit together. This review attempts to integrate some of the major stages in gene regulation including those involving activators, chromatin remodeling and modifications, PIC assembly, and transcription elongation, which necessitates a limited depth of coverage on any one topic. To avoid "overload", a representative name of the many involved complexes is used, rather than indicating all family members and other functionally related complexes. This review draws from a number of model eukaryotic systems, but places particularly emphasis on lessons learned from the budding yeast *Saccharomyces cerevisiae*. Much of the basic mechanisms of gene control remain highly conserved in eukaryotes, and yeast has provided the most simplified route towards a basic understanding of this control. The integration present here, in the context of genome-scale inquiry, is intended to provoke new questions about how the various stages are coordinated in a genome-wide response to environmental signals.

ChIP-chip and ChIP-seq identify the location and level of a protein binding anywhere in any genome

Chromatin immunoprecipitation (ChIP) has become an invaluable tool for mapping protein interactions along genomic DNA in vivo (Figure 1), and thus has been the single most informative assay in assessing the assembly of proteins on DNA in vivo. A key feature of the ChIP assay is that it preserves physiologically relevant interactions in the cell through formaldehyde crosslinking. Formaldehyde is an ideal crosslinking agent (Orlando *et al.*, 1997; Solomon and Varshavsky, 1985; Toth and Biggin, 2000) because: i) it quickly permeates the cell and traps native interactions before the cell mounts a physiological response, ii) its single-carbon crosslinker length efficiently generates protein-DNA crosslinks in vivo (protein-protein and protein-RNA crosslinks are also formed), and iii) its readily reversible crosslink is important for subsequent DNA detection methods.

Since its inception (Ren *et al.*, 2000), ChIP coupled to microarray detection (ChIP-chip, Figure 1) has proven to be a powerful tool in understanding the interplay of the transcription machinery and chromatin (Kim and Ren, 2006; Pugh and Gilmour, 2001). It can determine the occupancy level of essentially any crosslinkable and immuno-purifiable protein across an entire genome. Early ChIP-chip microarrays have had two important limitations. First, the fabrication of such microarrays has required a sequenced genome. Second, spatial resolution of binding along a genome was limited by probe length and spacing. Today, this has been largely alleviated in the highly tiled (probes every 5–40 bp) second-generation microarrays (Figure 1). Recent break-throughs in cost-effective whole-genome shotgun sequencing has also eliminated the first limitation.

Detection of genomic segments bound by a protein has recently been taken to another level of resolution by coupling the ChIP assay with massively parallel DNA sequencing, called ChIP-seq (Figure 1) (Margulies *et al.*, 2005; Schuster, 2008). The mapping of nucleosome positions across genomes was one of the first applications of ChIP-seq. In the past few years, ChIP-seq has produced whole-genome nucleosome maps for yeast (Albert *et al.*, 2007; Mavrich *et al.*, 2008a; Shivaswamy *et al.*, 2008), fly (Mavrich *et al.*, 2008b), worm (Johnson *et al.*, 2006; Valouev *et al.*, 2008), and human (Barski *et al.*, 2007; Boyle *et al.*, 2008; Schones *et al.*, 2008). Others have used ChIP-seq to map the locations of transcription factors (Jothi *et al.*, 2008; Nielsen *et al.*, 2008; Robertson *et al.*, 2007).

Some genes are packaged into repressive chromatin structures

Since genes within the eukaryotic genome are compacted within the nucleus in the form of chromatin, the transcription machinery must overcome a formidable structural barrier to access the underlying cis-regulatory elements and coding regions. Early work on the nuclear packaging of genomic DNA found that chromosomal DNA is composed of a beads-on-a-string configuration (Hewish and Burgoyne, 1973; Olins and Olins, 1974), which is thought to be the predominant form of transcriptionally-competent chromatin. Each bead is a nucleosome. The nucleosome core particle contains 147 base pairs of DNA wrapped 1.7 times around a histone octamer containing two copies of histones H2A, H2B, H3 and H4 (Luger *et al.*, 1997). In contrast to the active state, genes may be repressed through compaction of chromatin into a 30 nanometer fiber (Figure 2A).

Except in *Saccharomyces*, this closed repressive chromatin structure is often associated with methylation of histone H3 at lysine 9 (H3K9) by SUV39H (SUppressor of Variegation 3–9 Homolog) and concomitant binding of HP1 (Heterochromatin Protein 1) to that methyl mark (Figure 2A). The repressive state may also or instead be associated with methylation at H3K27 by Polycomb Repressive Complex 2 (PRC2) and the concomitant binding of PRC1, which ubiquitylates H2AK119 (Kouzarides, 2007; Levine *et al.*, 2004; Motamedi *et al.*, 2008; Trojer *et al.*, 2009). Dimethylation of H3R2, by PRMT6 (Protein aRginine MethylTransferase 6) (Iberg *et al.*, 2008), provides an additional repressive mark that serves to prevent formation of the activating trimethyl H3K4 mark (Kirmizis *et al.*, 2007). Whether and how these repressive marks and their cognate factors promote or maintain the closed state or are simply indicators of the closed state remains to be determined. Indeed small noncoding RNAs may be instrumental in establishing repressive chromatin environments (Goodrich and Kugel, 2008; Grewal and Elgin, 2007). The H3K9 and K27 methyl marks might function cooperatively or independently in repression, and may do so in part by recruiting repressive DNA methylases and histone deacetylases that remove activating histone acetylation marks.

Surprisingly, many genes in human embryonic stem cells that contain the methyl K27 repressive mark also contain a methyl mark on H3K4 that is associated with active genes (Bernstein *et al.*, 2006). These so-called bivalent genes may be in a repressed state but potentiated for rapid or well-timed activation that is key for coordinated development of multi-cellular organisms. It remains to be determined whether the active H3K4 methyl mark on bivalent genes is established co-transcriptionally or is placed by some other mechanism, and whether components that recognize the bivalent marks co-exist on the same gene. Interestingly, PRC1 which reads the repressive H3K27 methyl mark copurifies with TAFs (TATA-binding protein Associated Factors) (Saurin *et al.*, 2001), of which at least one (TAF3) reads the active H3K4 methyl mark (Vermeulen *et al.*, 2007). TAFs are components of the general transcription machinery (see below). These and other studies described below suggest that at some genes the transcription machinery may be present at repressed genes. However, in general, the transcription machinery is recruited only when the gene is to be transcribed.

Most genes have a canonical “open” organization of nucleosomes

Chromatin contains a repeating array of nucleosomes that are spaced roughly every 160–200 base pairs throughout the genome. The impact of nucleosomes on gene regulation was generally underappreciated until key experiments, nearly two decades ago, found that the presence of nucleosomes inhibited transcription initiation in vitro (Lorch *et al.*, 1987), indicating that nucleosomes are physical barriers to transcription. In addition, depletion of histones in the budding yeast *Saccharomyces* led to a global increase in transcription (Han

and Grunstein, 1988; Wyrick *et al.*, 1999), thus providing in vivo evidence that nucleosomes can repress transcription (although many other genes were also activated).

The position of nucleosomes at several model genes, such as *PHO5*, *SUC2*, *GAL1*, *HMRa*, and *RNR3*, have been mapped in *Saccharomyces* based upon the principle that nucleosomes protect the underlying DNA from digestion by exogenously added micrococcal nuclease (MNase). These early gene-specific maps provided a glimpse into how the location of nucleosomes across a gene might impact promoter access and transcription (Almer and Horz, 1986; Li and Reese, 2001; Lohr, 1997; Perez-Ortin *et al.*, 1987; Ravindra *et al.*, 1999). The study of such model genes has proven indispensable in guiding our understanding of the interplay of nucleosomes and the transcription machinery. For example, work on the *PHO5* gene demonstrated that nucleosomes regulate transcription through occlusion of the TATA and UAS (Upstream Activating Sequence) promoter elements and that nucleosome disruption is critical for activation (Lohr, 1997; Martinez-Campa *et al.*, 2004).

The recent advances in genome-wide mapping technologies have provided a much clearer picture of the genomic nucleosome landscape (reviewed by Jiang and Pugh, 2009). With the increased tiling density of microarray probes, examining the genome-wide structure of chromatin at single nucleosome resolution is becoming routine. Using high-density tiling over a portion of the yeast genome, Rando and colleagues (Yuan *et al.*, 2005) discovered that a nucleosome-free region (NFR) was a common feature of promoters (Figure 2B). The "−1" and "+1" nucleosomes reside in canonical locations upstream and immediately downstream of the NFR, respectively. Thus, nucleosomes are not stochastically dispersed along chromosomal DNA but instead are, by design, positioned at specific distances from the transcription start site (TSS) so as to regulate transcription. Whether individual nucleosomes play specific roles in regulating gene expression is not known, and will likely be an active area of investigation in the future. As "gate-keepers" of the NFR at promoters, the −1 and +1 nucleosomes are well-positioned to have significant regulatory potential.

It is remarkable that upon aligning nucleosome positions throughout the yeast, fly, and human genomes to the TSS a predominant nucleosome organization is apparent (Figure 2B). The existence of a promoter NFR and the uniform nucleosome positioning relative to the TSS, from gene-to-gene, are two features that are evolutionarily conserved. Although these salient features are remarkably similar from yeast to human, seemingly subtle species-specific differences are evident, but have important mechanistic implications for transcription initiation. For example, in yeast the TSS is tucked in the upstream border of the +1 nucleosome, suggesting that this nucleosome potentially regulates access to the TSS (Albert *et al.*, 2007). However, the +1 nucleosome in metazoans is shifted further downstream of the TSS compared to budding yeast, leaving the transcription start site accessible (Mavrich *et al.*, 2008b; Schones *et al.*, 2008). This downstream +1 nucleosome might be better situated to regulate transcription elongation. Indeed, Pol II transcription pauses at the first nucleosome. The −1, NFR, +1 arrangement provides the stage upon which sequence-specific regulators read the genome to direct transcriptional programs.

What creates an NFR is not fully known, although substantial evidence points to the presence of nucleosome-excluding poly-dA:dT tracts in the region (Anderson and Widom, 2001; Iyer and Struhl, 1995; Mavrich *et al.*, 2008a; Yuan *et al.*, 2005), and the presence of sequence-specific factors like Reb1 (RNA polymerase I Enhancer Binding protein 1) (Raisner *et al.*, 2005). Other sequence-specific regulators, such as Rsc3 (Remodel the Structure of Chromatin 3), can influence the nucleosome density in the NFR at some promoters (Badis *et al.*, 2008). In addition, chromatin remodeling complexes are likely to expand and contract the boundaries of the NFR, by using the energy of ATP hydrolysis to reposition nucleosomes (Whitehouse *et al.*, 2007).

Sequence-specific factors direct transcription programs from specific sets of genes

Cis-regulatory elements

Transcription programs are governed by trans-acting sequence-specific factors that control transcription by binding to cis-regulatory elements (CREs) (Table 1). Depending on their impact on transcription, these sites are collectively called upstream activating/repressing sequences (UAS/URS) in yeast or enhancers in metazoans. The number of promoters targeted by any given sequence-specific regulator ranges from a few to several hundred (or several thousand in metazoans). For example, the galactose regulator Gal4 binds to only ten promoters (Ren *et al.*, 2000), whereas Rap1 (Repressor Activator Protein 1) binds to over 300 promoters in the *Saccharomyces* genome (Buck and Lieb, 2006). The activity and subcellular localization of these factors are controlled by internal and external environmental cues, often using phosphorylation or targeted proteolysis as a molecular switch between active and inactive states.

While UAS/URSs are typically found several hundred base pairs upstream of the translation start site in *Saccharomyces* (Harbison *et al.*, 2004), other elements such as the TATA-box are present in the core promoter region and are typically 30–60 bp from the transcription start site (Table 1). Phylogenetic analysis of six *Saccharomyces* species reveals that about 20% of the 5,700 yeast genes contain a TATA-box element (Basehoar *et al.*, 2004). In metazoans, additional core promoter elements exist that interact with various components of the basal transcription machinery, such as the Initiator (Inr), downstream promoter element (DPE), motif ten element (MTE), and TFIIB recognition element (BRE) (Smale and Kadonaga, 2003; Thomas and Chiang, 2006). Some operate in lieu of a TATA box, while most promoters seem to lack any recognizable core promoter element.

Gene regulatory networks

How are sequence-specific regulators organized in the genome to allow global control of gene expression programs? The topology of regulatory circuits that underpin expression programs is best understood in yeast since the genomic binding locations for many of the ~120 sequence-specific regulators are known (Lee *et al.*, 2002). Analysis of the compendium of regulator-gene interactions identified six basic network motifs, each providing unique regulatory advantages (Figure 3). For example, the “single-input” motif ensures the concerted expression of the leucine biosynthetic genes by Leu3, whereas the “multiple-input” motif can integrate several signaling pathways to coordinate the expression of a set of genes under different conditions. The prototypical metazoan interferon beta enhancesome (Panne, 2008) may be regarded as a type of multiple-input motif. However, it is not known whether all multiple input motifs require concurrent binding of sequence-specific regulators for expression of the target gene, as in the case of the interferon beta gene. Regulation of the cell cycle by sequence-specific regulators exemplifies the “regulator chain” motif in which transcriptional events are ordered in a temporal sequence in accordance with temporal nature of the cell cycle.

The simple regulator chain motif has been expanded to construct a global pyramid-shaded hierarchical network (Yu and Gerstein, 2006). This hierarchical network describes a “chain-of-command” organization with a few master regulators at the top, which tend to have the maximal influence on global expression levels. The hierarchical regulatory structure is a decision making scheme that allows for the convergence of multiple internal and external stimuli to precisely modulate the expression of select groups of genes. For example, in yeast oxygen and heme levels activate expression of Mot3, a master regulator for aerobic growth. Mot3 activates Gcn4 (General Control Nonderepressible 4), which in turn activates Put3

(Proline Utilization 3) and Uga3 (Utilization of GAba 3), which in turn activates many genes involved in proline and nitrogen metabolism.

Sequence-specific regulators as orchestrators

While the genome-wide locations for many sequence-specific regulators have been determined in *Saccharomyces*, how these factors are specifically contributing to transcription is less clear. Whether a sequence-specific regulator acts as an activator or repressor may depend on its genomic context and what co-regulators they recruit. Sequence-specific regulators orchestrate multiple aspects of transcription through direct recruitment of: i) chromatin remodeling complexes, ii) general transcription factors, iii) chromatin modifying complexes, and iv) Pol II via the Mediator complex (Brown *et al.*, 2001; Cosma *et al.*, 1999; Garbett *et al.*, 2007; Goldmark *et al.*, 2000; Green, 2005; Larschan and Winston, 2001; Neely *et al.*, 2002; Nourani *et al.*, 2004; Park *et al.*, 2000; Yudkovsky *et al.*, 1999). Each of these aspects will be discussed below.

ATP-driven machines remodel the DNA on nucleosomes

Early work on model genes demonstrated that transcriptional activation involves the movement of nucleosomes (Almer and Horz, 1986; Li and Reese, 2001; Lohr, 1997; Perez-Ortin *et al.*, 1987; Ravindra *et al.*, 1999). However, the existence of chromatin remodeling complexes was unknown until the early 1990s (Hirschhorn *et al.*, 1992), despite the isolation of genes for key subunits (e.g. *SWI2/SNF2*) via genetic screens (Neigeborn and Carlson, 1984; Stern *et al.*, 1984). Chromatin remodeling complexes fall into four families based upon sequence conservation (Figure 4A) (Bao and Shen, 2007b): SWI/SNF, INO80/SWR1, ISWI, and CHD.

Since chromatin remodelers generally lack the intrinsic ability to target specific genes, sequence-specific regulators are likely to directly recruit these complexes to promoter regions (Figure 4B). How chromatin remodeling complexes are targeted to specific nucleosomes and their distinct roles remains an active area of investigation.

Chromatin remodeling complexes utilize the power of ATP hydrolysis to alter the structure, position, or composition, of nucleosomes (Figure 4B) (reviewed by Flaus and Owen-Hughes, 2004; Saha *et al.*, 2006; Tsukiyama, 2002). Based on numerous in vitro biochemical and single molecule optical trap studies, the current view for the mechanism of chromatin remodeling is that the DNA wound around the nucleosome forms a loop that is translocated by the mechanical power generated by these ATPase motors, resulting in the histone octamer either sliding along DNA or being altogether evicted from the DNA (reviewed by Cairns, 2007; Saha *et al.*, 2006). The mechanistic details will vary among the different families. The genomic context of such remodeling activity influences the accessibility of DNA at promoters and in some cases suppresses cryptic initiation sites within the body of the gene (Whitehouse *et al.*, 2007).

The nucleosomes surrounding promoter regions tend to be highly dynamic (Dion *et al.*, 2007), which suggests that chromatin remodeling complexes may be constitutively present and active at many promoters even when the promoter is largely quiescent. Since the positions of the −1 and +1 nucleosomes may largely influence the nucleosome architecture internal to genes (Mavrich *et al.*, 2008a), and that the −1/+1 nucleosomes control the accessibility of key promoter elements, it is likely that these nucleosomes will be prime targets of chromatin remodeling complexes (Figure 4B) (Venters and Pugh, 2009).

Conceivably, remodeling complexes that reposition nucleosomes could cause adjacent nucleosomes to reposition as well, such as through steric clashes. However, whether a

remodeling complex individually targets each nucleosome in an array, or whether targeting of a single nucleosome in an array is sufficient to move an entire array is not known. Answers to this question are key to understanding whether a remodeling complex that repositions nucleosomes on an entire gene needs to focus on a single linchpin nucleosome or needs to cover the entire domain.

SWI/SNF family and its relationship to histone acetylation

Classic genetic selections for mating-type switching deficient and sucrose non-fermenting phenotypes identified the *SWI2/SNF2* gene, which encodes the catalytic subunit of the SWI/SNF complex (Neigeborn and Carlson, 1984; Stern *et al.*, 1984). Genetic and molecular evidence with Swi2/Snf2 and Snf5 mutants later showed that the SWI/SNF complex alters the structure of chromatin through sliding and/or ejecting nucleosomes, independent of transcription (Hirschhorn *et al.*, 1992; Lorch *et al.*, 2006).

The SWI/SNF family of chromatin remodelers, which also includes the RSC (Remodels Structure of Chromatin) complex, is generally viewed as a positive regulator of transcription (although some genes are negatively regulated) (Angus-Hill *et al.*, 2001; Sudarsanam *et al.*, 2000). Location profiling by ChIP-chip finds RSC at the promoters of several hundred genes (Damelin *et al.*, 2002; Ng *et al.*, 2002), suggesting a role in transcription initiation and nucleosome organization. Consistent with this notion, RSC mutants perturb the translational setting of promoter nucleosomes (Parnell *et al.*, 2008). SWI/SNF, in particular, might have additional functions in transcription elongation (Schwabish and Struhl, 2007).

Promoter nucleosomes tend to be hyper-acetylated, and this may help retain bromodomain-containing chromatin remodeling complexes such as SWI/SNF and RSC (Figure 4B) (Hassan *et al.*, 2001; Hassan *et al.*, 2002; reviewed by Ruthenburg *et al.*, 2007). Bromodomains bind to acetylated lysines. Given that acetylation may also diminish the electrostatic histone lysine-DNA interactions, as well as disrupt higher order compaction, prior acetylation of nucleosomes might facilitate nucleosome remodeling and dismantling during gene activation. Acetylation may also be instrumental in nucleosome re-assembly directed by remodeling complexes. For example, NuA4 (NUcleosome Acetyltransferase of H4)-directed acetylation of H2A.Z is important for its deposition into promoter nucleosomes (Keogh *et al.*, 2006). H3K56 acetylation by Rtt109 (Regulator of Ty1 Transposition 109) (Schneider *et al.*, 2006), and other histone acetylation events by Hat1/HAT-B (Histone AcetylTransferase 1) (Parthun *et al.*, 1996), may be important for histone deposition. Other key acetyltransferase complexes associated with chromatin remodeling include SAGA (Spt-Ada-Gcn5 Acetyltransferase) and p300 (Sterner and Berger, 2000). As we will discuss below, SAGA has multiple roles in the transcription cycle beyond nucleosome acetylation.

In vitro transcription studies show that nucleosome acetylation by NuA4 stimulates the activity of RSC and enhances passage of Pol II through a nucleosome (Carey *et al.*, 2006). Both RSC and SWI/SNF are directed to some promoters through interactions with activators (Cosma *et al.*, 1999; Yudkovsky *et al.*, 1999). RSC also functions at many Pol I and Pol III promoters (Damelin *et al.*, 2002; Ng *et al.*, 2002), which may be the reason why RSC is essential for viability in yeast whereas SWI/SNF is not (Cairns *et al.*, 1996).

INO80/SWR1 family

The INO80/SWR1 (INOsitol requiring 80/Sick With Rat8 ts 1) family of remodelers is unique in that it contains a split ATPase domain (Bao and Shen, 2007a). The *INO80* gene was identified in a screen for genes required for activating the inositol synthetase gene (*INO1*), which synthesizes a compound required for several secondary messenger signaling pathways (Ebbert *et al.*, 1999). The INO80 complex plays a broader role in genome

regulation than many other remodeling complexes in that it participates in transcription activation, DNA repair, and resolving stalled replication forks (Shen *et al.*, 2000; Shimada *et al.*, 2008). Understanding how INO80 is targeted to sites of transcription, repair and replication will be of interest because unlike other remodeling complexes in yeast, INO80 lacks known histone recognition modules.

The SWR-C/SWR1 complex is a chromatin remodeler that alters the composition (as opposed to the position) of nucleosomes. The SWR1 complex uses ATP hydrolysis to replace H2A with H2A.Z in promoter nucleosomes (Guillemette *et al.*, 2005; Li *et al.*, 2005; Raisner *et al.*, 2005; Zhang *et al.*, 2005). H2A.Z is thought to promote transcription by destabilizing nucleosomes (Krogan *et al.*, 2004; Zhang *et al.*, 2005). Indeed, H2A.Z is associated with an open chromatin state rather than the closed state (Figure 2B), although the presence of H2A.Z does not suffice to create a transcriptionally active state. In mammalian cells, H2A.Z is required for lineage commitment by embryonic stem cells (Creyghton *et al.*, 2008), suggesting that altering the composition of chromatin with histone variants plays an important role in development, perhaps by helping commit specific genes to an activated state.

ISWI family

In contrast to the SWI/SNF and INO80 families, the Imitation SWItch (ISWI) family of remodelers tends to negatively regulate transcription. For example, genome-wide expression profiling and DNase I sensitivity studies in *Saccharomyces* found that the ISW2 complex in concert with the histone deacetylase Rpd3 (Reduced Potassium Dependency 3) represses meiotic genes by creating a repressive nucleosome arrangement (Fazzio *et al.*, 2001). ISW2 is recruited to repressive loci by Ume6, a key sequence-specific regulator of early meiotic genes (Goldmark *et al.*, 2000). ISW2 might also cooperate with other repressive complexes, such as TUP1-SSN6 (deoxyThymidine monophosphate UPtake 1 and Suppressor of Sucrose Nonfermentor 6), to maintain repressive states (Zhang and Reese, 2004). Since ISW2 may be important in maintaining a targeted closed deacetylated state (in cooperation with Rpd3), it makes sense that these complexes lack bromodomains, as they would antagonize the generally open acetylated state that exists throughout the euchromatic genome. As a result, ISW2 may depend more on sequence-specific regulators for recruitment than on recognition of histone modifications.

CHD family

The intracellular role of the CHD (Chromatin organization modifier, Helicase, and DNA-binding domains) family of chromatin remodelers is the least understood of the four families of remodelers. Expression profiling in a Chd1 yeast mutant has shown that few genes are affected (Tran *et al.*, 2000), suggesting that Chd1 may operate in parallel pathways with other chromatin remodelers or is targeted to few genes. Biochemical purification of the SAGA complex from *S. cerevisiae* led to the identification of Chd1 as one of its components (Pray-Grant *et al.*, 2005). To what extent Chd1 co-exists with SAGA at specific genes and functions with SAGA is currently not understood. A defining feature of the CHD family is that it contains a chromodomain, which binds to methylated lysines. Indeed, Chd1 interacts with methylated H3K4 in vitro (Biswas *et al.*, 2007; Flanagan *et al.*, 2005; Pray-Grant *et al.*, 2005). However, there is disagreement between the difference in binding specificity for the yeast and human counterparts. The in vivo significance of Chd1 binding to H3K4 awaits further investigation, and its potential connection with the acetylation and de-ubiquitylation activities associated with SAGA remain largely unexplored. Since histone acetylation, chromatin remodeling, recognition of H3K4 trimethyl marks, and de-ubiquitylation are all associated with transcription, there may be some logic for these multiple activities being associated with a single SAGA complex.

General transcription factors assemble into the transcription pre-initiation complex

Mediator plays a key early role

While chromatin remodeling complexes may keep the chromatin in the promoter region dynamic, the many facets of the remodeling process may not be driven to completion until the Mediator complex and the GTFs assemble into a pre-initiation complex. Mediator is a large complex of proteins involved in the many aspects of transcription (reviewed by Biddick and Young, 2005; Kornberg, 2005). First discovered as a biochemical entity that mediated transcriptional activation in vitro (Kim *et al.*, 1994), Mediator may be recruited early during PIC assembly through direct interactions with activators (illustrated in Figure 4B) (Natarajan *et al.*, 1999). One example of how Mediator might regulate PIC assembly is through recruitment of the p300 histone acetyltransferase to promoters. This recruitment blocks PIC assembly until p300 has acetylated its targets (histones and itself), which then induces p300 to dissociate from the promoter (Black *et al.*, 2006). In essence, Mediator and p300 create a checkpoint to ensure that PIC assembly does not proceed until certain acetylation events are completed.

TBP is a highly regulated nucleator of PIC assembly in the NFR

Some thirty years ago, biochemical fractionation of crude cell extracts by the Roeder lab led to the identification of general transcription factors that accurately initiated transcription in vitro at a minimal core promoter (Thomas and Chiang, 2006). These GTFs were first isolated from mammalian cells (Matsui *et al.*, 1980) and later the corresponding factors were identified in yeast (Sayre *et al.*, 1992). The GTFs include TFIIA, -B, -D, -E, -F, and -H. In contrast to sequence-specific regulators that are targeted to a discrete set of genes, GTFs, as their name suggests, are broadly utilized by the cell at many genes and typically have minimal built-in gene specificity. These components of the basal transcription machinery function at most genes to assist in the loading and release of RNA polymerase II at the TSS.

TFIID (Transcription Factor II D) is a large multisubunit complex that contains TBP (TATA Binding Protein) and TAFs (TBP-Associated Factors). Paradoxically, TFIID is largely recruited to TATA-less “housekeeping” promoters, which represent the vast majority of all genes (Basehoar *et al.*, 2004). TBP also exists free of the TFIID complex, and is delivered to TATA-containing promoters via the SAGA complex (Sermwittayawong and Tan, 2006). TBP is removed from such promoters by the combined direct action of NC2 (Negative Cofactor 2) and the TBP-dependent ATPase Mot1 (Modifier of Transcription 1) (reviewed by Lee and Young, 1998; Pugh, 2000). TFIIA and TFIIB interact with TBP and promote PIC formation by stabilizing TBP/DNA interactions and counteracting the effects of NC2 and Mot1. At least at some genes, GTFs assemble into partial PICs that are relatively depleted of TFIIH and Pol II (Figure 5A). Partial PICs might represent regulated intermediates (for example, in the coupling of responses to heat shock and oxidative stress, in which the former causes the latter) (Zanton and Pugh, 2006). Interestingly, partial PICs can assemble in the NFR without apparent loss of the surrounding nucleosomes. Only when full PICs (containing Pol II) are assembled is the −1 nucleosome removed (Venters and Pugh, 2009).

Although TBP can replace TFIID in biochemically reconstituted transcription assays, it is the TAFs that are generally required for sequence-specific activators to promote maximal transcription (Pugh and Tjian, 1990; Reese *et al.*, 1994), suggesting that TAFs may interact with some activators. As one example, Rap1 is an activator that directly interacts with TFIID in vitro, and this interaction is important in driving transcription of the highly-transcribed ribosomal protein genes (Garbett *et al.*, 2007; Mencia *et al.*, 2002).

The physiological importance of TBP is underscored by the resources that the cell devotes to regulating its activity. Mot1 and NC2 together regulate the genomic distribution of TBP, but through different mechanisms. Mot1 might couple ATP hydrolysis to localized DNA translocation, which is then used to disrupt TBP/DNA interactions (Auble *et al.*, 1994). In this way Mot1 would act negatively in transcription. However, Mot1 also acts positively at some genes, possibly by removing improperly assembled TBP that might bind in a reverse orientation or at inappropriate sites due to TBP's intrinsic weak specificity for TATA (Muldrow *et al.*, 1999; Sprouse *et al.*, 2008b). In contrast, NC2 clamps TBP to DNA, and blocks further PIC assembly by sterically interfering with TFIIB binding (Inostroza *et al.*, 1992). Taken together, while TBP binding to DNA is intrinsically stable, its interaction in vivo may be highly dynamic (Sprouse *et al.*, 2008a). Stability appears to be driven by TFIIA and TFIIB (perhaps in cooperation with other GTFs and regulatory factors), and instability might be driven first by NC2 counteracting TFIIB (and TFIIA), which then allows Mot1 to induce dissociation. It will be interesting to learn whether loss of NC2 or Mot1, in the presence of sufficient amounts of TBP, causes TBP to accumulate at NFRs in the genome. This issue gets at whether NFRs are actually protein-free and thus capable of binding proteins, or whether they are already occupied by other unknown factors and thus inaccessible.

Atomic resolution structures of complexes containing either TFIIA or TFIIB bound to TBP-TATA DNA reveals that TFIIA binds upstream of TBP (away from the TSS) (Nikolov *et al.*, 1995; Tan *et al.*, 1996), whereas TFIIB resides mostly downstream of TBP but also straddles TBP thereby clamping the DNA on both sides of TBP. TFIIB and TFIIF are required for association of Pol II with the PIC (Figure 5B), and this is supported by the Pol II-TFIIB crystal and Pol II-TFIIF cryo EM structures (Bushnell *et al.*, 2004; Chung *et al.*, 2003).

TFIIE and TFIIH regulate transcription initiation

The TFIIE and TFIIH complexes work together to modulate the activity of Pol II and facilitate promoter clearance. Step-wise assembly of the PIC in vitro indicates that TFIIE associates after Pol II but before TFIIH (Buratowski *et al.*, 1989), although other studies indicate that TFIIE can associate with a partial PIC in the absence of Pol II (Yokomori *et al.*, 1998). Recent two-hybrid and ChIP experiments in a Mediator mutant yeast strain suggest that Mediator facilitates the incorporation of TFIIH into the PIC through direct interactions (Esnault *et al.*, 2008). TFIIE also recruits TFIIH and regulates TFIIH's helicase and kinase activities. The ATP-dependent helicase activity is important for DNA strand separation (promoter melting) so that an open promoter complex with Pol II may form (Wang *et al.*, 1992). The Kin28 (KINase 28) subunit of TFIIH mediates the transition from transcription initiation to elongation through phosphorylation of serines in the 5^{th} position of the highly repeated YSPTSPS motif in the CTD (Carboxy-Terminal Domain) of the largest Pol II subunit (Valay *et al.*, 1995). Together the GTFs function as a cohort of factors to recognize core promoters, assemble the starting platform for transcription, recruit Pol II, and facilitate the transition from Pol II initiation to elongation.

In *Saccharomyces*, the transition from transcription initiation to elongation has been suggested to be rapid, with little post-recruitment regulation except in isolated examples (Martens *et al.*, 2001; Radonjic *et al.*, 2005; Sekinger and Gross, 2001; Wade and Struhl, 2008). However, recent findings have challenged this notion. Pol II has been found to be relatively enriched in promoter regions compared to transcribed regions across the *Saccharomyces* genome (Venters and Pugh, 2009), suggesting that the conversion of the PIC into a transcription elongation complex may be at least partially rate-limiting at many yeast genes and thus targeted for regulation. This is consistent with post-recruitment regulation mechanisms identified in yeast (Zhang *et al.*, 2008). In contrast, Pol II is found more-or-less

uniformly throughout highly transcribed genes, suggesting that initiation is not rate-limiting at those genes.

Pausing of Pol II immediately after initiation is widespread

The transcription cycle involves recruitment of Pol II to promoters, then transcription initiation, elongation, and termination (Wade and Struhl, 2008). Any of these steps can potentially be rate-limiting. Historically, unexpressed genes have been thought to be rate-limited by steps leading up to Pol II recruitment (Ptashne and Gann 1997). However, this notion has recently been challenged by the finding of Pol II at most genes, from yeast to humans (Guenther *et al.*, 2007; Steinmetz *et al.*, 2006), which taken at face value suggests that the expression of many or most genes is rate-limited after Pol II recruitment (Core and Lis, 2008; Margaritis and Holstege, 2008; Price, 2008; Struhl, 2007; Wade and Struhl, 2008). This latter view cannot be entirely correct because lowly expressed genes generally have less bound Pol II than highly expressed genes. Thus, detection of Pol II at genes is not equivalent to full occupancy, and so it is likely that steps leading up to PIC assembly and steps subsequent to Pol II recruitment will both regulate the transcription cycle.

Recent studies find that Pol II is paused at the 5' end of ~10% of all *Drosophila* genes (Lee *et al.*, 2008; Muse *et al.*, 2007; Zeitlinger *et al.*, 2007), suggesting that Pol II pausing during an early elongation step is rate-limiting in the expression of many genes (Figure 5C). This pausing involves the NELF (Negative ELongation Factor) and DSIF (DRB Sensitivity Inducing Factor) complexes. Phosphorylation of NELF and DSIF by P-TEFb (Positive Transcription Elongation Factor b) promotes the release of the paused polymerase (Peterlin and Price, 2006). P-TEFb also phosphorylates the 2nd serine in the Pol II CTD repeat (in contrast to TFIIH phosphorylating the 5th serine) rendering Pol II elongation competent. In mammals, most genes are also enriched with Pol II at their 5′ ends (Guenther *et al.*, 2007), but it remains unclear whether this reflects post-recruitment regulation of a poised PIC that is not yet transcriptionally engaged (Figure 5B) and/or a paused Pol II that is transcriptionally engaged (Figure 5C).

Pol II CTD serine 5 phosphorylation during initiation sets off a cascade of events that regulate the nascent RNA and the underlying chromatin

The Pol II CTD coordinates events during the transcription cycle by recruiting proteins involved in histone modifications, elongation, termination, and mRNA processing (reviewed by Egloff and Murphy, 2008; Meinhart *et al.*, 2005; Phatnani and Greenleaf, 2006). Numerous proteins recognize and bind to a specific phosphorylation pattern of the CTD (Figure 6), which dynamically changes during the transcription cycle. The number of YSPTSPS heptapeptide repeats of the CTD vary from 26 in *Saccharomyces* to 52 in human. Since there are five potential phosphorylation sites in each CTD repeat, a CTD code has been suggested (Buratowski, 2003). Only serine phosphorylation at the 5th and 2nd position have been well characterized. Recently, serine 7 was also discovered to be phosphorylated in human cells, and has been implicated in transcription of small nucleolar RNAs (Egloff *et al.*, 2007). Whether serine 7 has a role in other Pol II genes remains unclear.

During PIC assembly, a dephosphorylated Pol II is recruited to promoters through interactions with TFIIB and Mediator (Myers *et al.*, 1998). One of first steps in transcription initiation is the phosphorylation of serine 5 of the CTD by Kin28 (Komarnitsky *et al.*, 2000). This Pol II mark recruits a number of factors including the mRNA capping enzyme, an RNA surveillance complex called Nrd1-Nab3 (Nuclear pre-mRNA Down-regulation 1 and Nuclear polyAdenylated RNA-Binding 3), and the PAF (Polymerase II-Associated Factor) complex (Figure 6 and Figure 7A) (Hampsey and Reinberg, 2003; Komarnitsky *et al.*, 2000;

Ng *et al.*, 2003b; Vasiljeva *et al.*, 2008). The 7-methyl guanylyl cap protects the nascent RNA from degradation, and marks the mRNA for transport to the cytoplasm and ultimately translation. The Nrd1-Nab3 complex may track with Pol II and the RNA to ascertain whether the emerging RNA contains short Nrd1 binding motifs (e.g. GUA and UCUU, (Carroll *et al.*, 2004)), which may be evolutionarily depleted in mRNAs, but present in inappropriately-transcribed and thus nonfunctional regions of the genome. The Nrd1-Nab3 complex promotes termination of these latter transcripts, and directs them through the nuclear exosome pathway for degradation.

The PAF complex connects serine 5 phosphorylation of the CTD to a network of histone modifications. The multitude of interactions with serine 5 phosphorylated CTD may explain the highly repetitive nature of the heptad motif. While each complex could, in principle, interact with a subset of phosphorylated repeats there is very little known about the arrangement of factors on the CTD. The PAF complex, in conjunction with the Bur1/2 (Bypass UAS Requirement 1 and 2) kinase, engages the serine 5 phosphorylated CTD and promotes recruitment of the Rad6 (RADiation sensitive 6) and Bre1 (BREfeldin A sensitivity 1) E2 and E3 ubiquitin ligases (Figure 7A), to set off a series of histone modification crosstalks, known as the histone code.

Pol II phosphorylation is connected to writers, readers, and erasers of the histone code

The chromatin landscape is peppered with numerous posttranslational modifications (or marks) to histone tails (reviewed by Berger, 2007; Kouzarides, 2007; Lee and Shilatifard, 2007; Millar and Grunstein, 2006; Shilatifard, 2008; Weake and Workman, 2008). Histone tails consist of 20–40 amino-terminal amino acids of each histone, which extend from the globular nucleosome core into the surrounding solvent. The best studied of the histone marks include acetylation, methylation, and ubiquitylation. These marks have been proposed to function by i) directly changing the structure of chromatin by altering electrostatic or internucleosomal contacts, and/or ii) providing docking sites for proteins (“readers”). For each mark written, there is a corresponding complex that is capable of erasing it, consistent with the dynamic nature of chromatin marks (Bannister and Kouzarides, 2005; Klose and Zhang, 2007; Shi and Whetstine, 2007). Some histone modifications, such as methylated H3K9/27, are stably maintained through multiple cell divisions and reflect epigenetic processes where the modifications become self-propagating (reviewed by Bibikova *et al.*, 2008; Lunyak and Rosenfeld, 2008). Whether such modifications are the cause or consequence of epigenetic inheritance remains to be demonstrated.

From Pol II CTD serine-5 phosphorylation to H3K4 methylation

It is becoming increasingly clear that individual histone marks influence the writing and erasing of other marks, creating a network of histone crosstalk during transcription. Recent reports support a model wherein transcription initiation and elongation accompanies a dynamic cycle of coupled histone modifications, perhaps exemplified by the events associated with the ubiquitylation of H2BK123 (Figure 7A and B). Once Ser5 phosphorylation of the Pol II CTD has recruited the PAF and Bur1/2 complexes, then at least Bur1 phosphorylates Rad6 (Wood *et al.*, 2005), and this stimulates the Rad6 E2 ligase activity to mono-ubiquitylate Bre1. Bre1 then passes the ubiquitin onto H2BK123 as an E3 ligase (Kao *et al.*, 2004; Laribee *et al.*, 2005; Ng *et al.*, 2003a; Robzyk *et al.*, 2000; Wood *et al.*, 2003, 2005). H2BK123ub then directs trimethylation of H3K4 by COMPASS/Set1 (Complex Of Proteins ASsociated with Set1/*Su(var)3–9, Enhancer of zeste, Trithorax 1*) and K79 by Dot1 (Disruptor Of Telomeric silencing 1) (Briggs *et al.*, 2002; Dover *et al.*, 2002; Nakanishi *et al.*, 2008; Sun and Allis, 2002; Wood *et al.*, 2003).

Trimethylation of H3K4 also requires acetylation at H3K14 (Nakanishi *et al.*, 2008), which presumably occurs during PIC assembly or co-transcriptionally using acetyltransferases (such as Elongator) that translocate with Pol II. Since acetylation might enhance nucleosome eviction during transcription, the H3K4me3 mark might sense local acetylation levels through K14. Although it is not entirely clear what the H3K4me3 mark is doing, conceivably it might bind to "readers" such as the TAF3 subunit of TFIID, the Chd1 component of SAGA, or the Yng1 (Yeast homolog of mammalian Ing1 1) subunit of NuA3 to help maintain PIC assembly and the active acetylated state.

Transcription-associated H3K4me3 occurs at the beginning of genes (Liu *et al.*, 2005; Pokholok *et al.*, 2005), but ultimately transitions into dimethylation (H3K4me2) less than 1 kb downstream of the TSS (Figure 7C). The transition might occur through alterations in the specificity of the methyltransferase active site of Set1, which could be linked to the phosphorylation status of the CTD (i.e. a switch from serine 5 to serine 2 phosphorylation triggers a switch from H3K4me3 to me2), but this remains to be determined. H3K79me3, which is also linked to CTD serine-5 phosphorylation, is maintained throughout the body of the gene, and is linked to transcription and telomere silencing (Lacoste *et al.*, 2002; Pokholok *et al.*, 2005).

From CTD serine-2 phosphorylation to H3K36 methylation

As elongation proceeds from the 5′ end of the gene towards the 3′ end, a phosphorylation switch occurs on the CTD from serine-5 to serine-2 (Figure 6 and Figure 7C) (Komarnitsky *et al.*, 2000), although it is not clear to what extent one event triggers the other. Ctk1 (Carboxy-Terminal domain Kinase 1) in yeast and P-TEFb in higher eukaryotes phosphorylate serine-2 (Lee and Greenleaf, 1997; Marshall *et al.*, 1996; Sterner *et al.*, 1995; Wyce *et al.*, 2007), and thus are regulators of elongation.

The CTD serine-5 phosphorylation mark is erased by the Ssu72 (Suppressor of SUa7 2) phosphatase (and possibly other phosphatases such as Scp1 (*S. cerevisiae* CalPonin 1, which is a calcium binding protein)) (Hausmann *et al.*, 2005; Krishnamurthy *et al.*, 2004; Yeo *et al.*, 2003). Ssu72 may be responsible for many aspects of the transcription cycle: 1) It associates with TFIIB in the PIC; 2) It is required for the transition from serine-5 to serine-2 phosphorylation; and 3) It is part of the cleavage and polyadenylation complex that operates during transcription termination (Dichtl *et al.*, 2002a).

Remarkably, the CTD appears to regulate its own structural status through prolines located in the YSPTSPS heptapeptide repeat. Prolines are the only natural amino acid that can exist in structurally distinct *cis* and *trans* configurations, which result in altered paths of the CTD polypeptide chain. Studies using surface plasmon resonance demonstrate that the Ess1 (ESSential 1) proline isomerase preferentially associates with the doubly phosphorylated CTD at serines 2 and 5 (Phatnani *et al.*, 2004). The implications for regulating the cis/trans configuration of the two prolines in the CTD are unclear at present, but conceivably isomerization of the prolines could regulate dephosphorylation of the CTD or the binding of proteins that recognize the phosphorylation patterns on the CTD. Indeed, genetic studies suggest that the role of Ess1 may be to oppose the functions of the CTD kinases (Wilcox *et al.*, 2004). Given the two to four dozen YSPTSPS heptad repeats arrayed on a single complex of Pol II, with two prolines and three serine phosphorylation sites in each repeat, one can envision an extensive CTD code having positional, structural, and chemical information.

As Pol II transcribes into the body of a gene, PAF-stimulated H2BK123ub marks continue to be generated, possibly followed by H3K4me2 marking. However, H2BK123ub blocks nucleosomal association of Ctk1 and subsequent serine 2 phosphorylation of the CTD

(Wyce *et al.*, 2007), which represents the elongation state of Pol II. Current models suggest that the SAGA complex may travel with Pol II and promote deubiquitylation of H2BK123ub through its Ubp8 (UBiquitin-specific processing Protease 8) subunit (Henry *et al.*, 2003), although not all genes may partake in this process (Shukla *et al.*, 2006). Removal of the H2BK123ub mark by Ubp8 may allow turnover of the H3K4 methyl mark via demethylases (and perhaps resetting the chromatin state for a new round of transcription), but this remains to be tested. Alternatively, removal of the H3K4 mark might occur through dynamic histone subunit exchange with unmethylated copies. Importantly, loss of the H2BK123ub mark allows Ctk1 to phosphorylate serine-2 of the CTD.

Not only does H2BK123 ubiquitylation/deubiquitylation serve as a regulatory switch in a network of histone modifications (Weake and Workman, 2008), but H2BK123ub also regulates nucleosome dynamics during elongation. For instance, loss of H2B ubiquitylation and deletion of *SPT16* (SuPpressor of Ty 16) results in lower levels of histone occupancy at the *GAL1* locus, suggesting that H2BK123ub and FACT (FAcilitates Chromatin Transcription) (via the Spt16 subunit) regulate nucleosome reassembly in the wake of elongating Pol II (Fleming *et al.*, 2008).

Serine-2 phosphorylation triggers a second wave of communication between the CTD and chromatin, ultimately impinging upon H3K36 methylation. Whereas the PAF complex may have been the connector from the serine-5 phosphorylation mark, the H3K36 methylase Set2 appears to be the connector in this second phase. Set2 binds to the CTD phosphorylated at serine-2 (Figure 6 and 8A) (Kizer *et al.*, 2005; Li *et al.*, 2003; Li *et al.*, 2002; Xiao *et al.*, 2003), and catalyzes di and trimethylation of H3K36. Whether di versus trimethylation of K36 serve distinct purposes is not known. Nonetheless, H3K36me2 appears to be a mark of actively transcribed genes (Rao *et al.*, 2005), although its function may be to repress transcription that might arise promiscuously or cryptically in the ORF.

Isomerization of prolines 30 and 38 in the H3 tail by Fpr4 (FKBP Proline Rotamase 4) negatively regulates Set2 methylation at neighboring K36 (Nelson *et al.*, 2006), suggesting that the secondary structure of the H3 tail provides an additional dimension of regulation to transcription, as was seen in proline isomerization of the Pol II CTD. The RPD3S (RPD3-Small) histone deacetylase complex then binds to H3K36me2 (Figure 8A) through combinatorial domain recognition (Li *et al.*, 2007b; Li *et al.*, 2009; Rao *et al.*, 2005). RPD3S then deacetylates nucleosomes which returns the nucleosomes back to their transcriptionally impervious state, and thus suppressing cryptic transcription that might initiate at inappropriate sites (Carrozza *et al.*, 2005; Li *et al.*, 2007c).

Histone modification crosstalk may create positive feedback loops. For example, H3K4me3 and H3K14ac may reinforce each other. In this loop, SAGA binds to H3K4me3 to acetylate H3K14, and H3K14ac promotes H3K4me3 (Figure 7A and B). The SAGA complex is functionally similar to TFIID in that both have TAFs and both deliver TBP to promoters. However, SAGA is clearly involved in other capacities in transcription, in that it is associated with an acetyltransferase (Gcn5, although higher eukaryotic TAF1 is an acetyltransferase), a deubiquitinase (Ubp8), and an ATP-dependent chromatin remodeler (Chd1).

Despite such intricate crosstalk of histone modifications, neither Rad6/Bre1, Set1, Set2, Dot1, nor individual histone tails are essential for viability in budding yeast (Giaever *et al.*, 2002; Kayne *et al.*, 1988; Megee *et al.*, 1990; Morgan *et al.*, 1991; Nakanishi *et al.*, 2008). This begs the question as to why these marks exist. One plausible explanation might be that, while the marks are not essential for regulated transcription, they impart robustness and diminish stochasticity in expression (e.g., perhaps by rapidly restoring the canonical

nucleosome architecture once Pol II has passed) such that responses to activating signals can be coordinated in a population of cells. Rapid and well-timed execution of genetic programs is particularly evident in embryonic development where precisely coordinated cell division and expression is critical. The maintenance of histone modification states is particularly critical to stem cell differentiation (reviewed by Bibikova *et al.*, 2008; Lunyak and Rosenfeld, 2008).

Many questions remain regarding the purpose and interdependencies of readers, writers, and erasers of the chromatin landscape. For example, what role do the mono-, di- and tri-methylated states serve? In other words, do mono- and di- simply represent unfinished modifications or are they functionally instructive? The deubuitination activity of Ubp8 is housed in the SAGA complex, which also contains a histone acetyltransferase in Gcn5 and an ATP-dependent remodeling activity in Chd1. How then does SAGA parse these disparate activities in the same complex? Since several chromatin remodelers contain histone-binding modules that recognize acetylated and methylated lysines, what specific residues or combination of residues are recognized by these chromodomains and bromodomains?

Regulation of transcription termination

As Pol II transcription proceeds toward the 3′-end of genes serine-2 phosphorylation of the CTD predominates over serine-5 phosphorylation, thereby enhancing the recruitment (or retention) of the 3′-end RNA processing machinery (Ahn *et al.*, 2004), such as Pcf11 (Protein 1 of Cleavage and polyadenylation Factor I 11), Spt6, Rtt103, and Cft1 (Cleavage Factor Two 1) (Figure 8B) (Dichtl *et al.*, 2002b; Kim *et al.*, 2004; Licatalosi *et al.*, 2002; Yoh *et al.*, 2007). Binding of termination factors may also be dependent on the proline conformations of the CTD, since two temperature sensitive Ess1 mutants are defective in pre-mRNA 3′-end formation (Hani *et al.*, 1999).

Pcf11 is a component of the cleavage and polyadenylation factor and is involved in transcription termination. Biochemical studies in *Drosophila* demonstrate that Pcf11 terminates transcription by dismantling elongation complexes through mediating interactions between the CTD and nascent RNA (Zhang and Gilmour, 2006). Rtt103 specifically associates with phosphorylated serine-2 of the CTD, and is involved in transcription termination through interactions with the Rat1 (Ribonucleic Acid Trafficking 1) exonuclease, which degrades RNA downstream of the mRNA cleavage site (Kim *et al.*, 2004).

It is now evident that all stages of the transcription cycle from initiation, to pausing, to elongation, and finally termination, are regulated by Pol II CTD serine 5 and 2 phosphorylation and interdigitated proline isomerization. These Pol II marks control the coming and going of histone modifying complexes and pausing/termination factors. As a result, Pol II rapidly and efficiently navigates nucleosomal barriers, producing full length accurately initiated and accurately terminated transcripts.

Noncoding transcription can regulate genes

With the increasing sensitivity of RNA-sequencing and array-based technologies to study the levels of RNA transcripts, recent studies have reported detectable levels of transcription from the vast majority of the genome from yeast to human (David *et al.*, 2006; Dutrow *et al.*, 2008; Kapranov *et al.*, 2007; Mortazavi *et al.*, 2008; Nagalakshmi *et al.*, 2008; Sultan *et al.*, 2008; Wilhelm *et al.*, 2008), albeit most genes are transcribed at a relatively low level. The remarkable sensitivity of RNA detection methods also revealed the presence of antisense transcripts from coding regions (being transcribed in the opposite direction of the a “sense” transcript) and cryptic unstable transcripts (CUTs) from intergenic regions in budding and

fission yeast (Dutrow *et al.*, 2008; Neil *et al.*, 2009; Xu *et al.*, 2009). Stable antisense transcripts are generally detected in wild type strains, whereas CUTs are rapidly degraded by the exosome and thus only detectable in an exosome mutant (reviewed by Houseley *et al.*, 2006). It has been suggested that as much as 90% of Pol II initiation is biological noise (Struhl, 2007), which begs the question: Are these antisense and cryptic transcripts physiologically relevant?

Several recent studies in *S. cerevisiae* provide clues regarding the functional roles of antisense transcripts and CUTs. For example, the *SRG1* CUT generated in the sense direction upstream of the serine biosynthetic gene *SER3* regulates its transcription through a transcription-interference mechanism that abrogates utilization of the *SER3* promoter elements (Martens *et al.*, 2004). The nucleotide biosynthesis genes, *URA2* and *IMD2*, also appear to use CUTs arising upstream of the promoter to repress transcription (Davis and Ares, 2006; Kopcewicz *et al.*, 2007; Thiebaut *et al.*, 2008). The mechanistic basis for these sense-directed repressive CUTs is now being worked out. Their regulation of amino acid and nucleotide biosynthesis genes provides an opportunity for these end-product metabolites to provide feedback regulation on their respective CUTs. For example, serine binds Cha4 (Catabolism of Hydroxy Amino acids 4) which then binds to and activates the *SRG1* promoter (Martens *et al.*, 2005). At *IMD2*, high cellular guanine nucleotide levels favor Pol II initiation at upstream CUTs, which have G at their +1 transcription start sites instead of the normal A (Kuehner and Brow, 2008). Start site selection tends to be highly nucleotide concentration dependent.

GAL10 provides an example of repression via antisense transcription. The Reb1 activator appears to create an NFR near the 3′-end of the *GAL10* coding region to facilitate production of antisense transcription under *GAL10*-repressive conditions (glucose present, and galactose absent), which alters the chromatin across the *GAL10* locus (Houseley *et al.*, 2008). Specifically, *GAL10* antisense transcription leads to recruitment of the Set2 methyltransferase and the RPD3S histone deacetylase complex presumably via H3K36me2, which then maintains the chromatin in a transcriptionally impervious deacetylated state.

A somewhat different mechanism for a repressive antisense RNA occurs at *PHO84*, which codes for a phosphate uptake transporter. Under *PHO84*-repressive conditions (phosphate present), deletion or down-regulation of the nuclear exosome leads to stabilization of a *PHO84* antisense transcript, which by some unknown mechanism promotes recruitment of the HDA1 (Histone DeAcetylase 1) complex to create a deacetylated state (Camblong *et al.*, 2007). These findings suggest that the production of noncoding RNAs can repress transcription of nearby genes through altering the chromatin environment with histone deacetylases.

Antisense transcripts can also positively regulate transcription. For example, abolishing production of a transcript antisense to the *PHO5* promoter actually delays chromatin remodeling of promoter nucleosomes and recruitment of Pol II to the promoter (Uhler *et al.*, 2007). The authors propose that the act of *PHO5* antisense transcription enhances the chromatin plasticity, which positively regulates *PHO5* transcription. This notion would seem to be at odds with the mechanisms of antisense regulation of *PHO84* and *GAL10*.

Although the genome-wide distribution of antisense transcripts has been described (Perocchi *et al.*, 2007), a comprehensive functional study of CUTs is lacking. Understanding the role of more antisense transcripts and CUTs will provide additional insight into how genes are regulated using disparate and seemingly conflicting strategies to fine tune gene activity.

Is the transcription cycle basically the same at all mRNA genes?

One can understand and appreciate that each of the thousands of genes in a genome make unique contributions to cellular physiology, and thus should be uniquely regulated. However, this does not seem to be entirely the case. For certain, virtually every mRNA gene is unique to some degree in its promoter, coding, and termination regions. Although sequence-specific transcription factors provide combinatorial diversity in gene regulation, the mechanistic aspects of the regulated transcription cycle (described in this review) seem unlikely to be combinatorially controlled, although no less intricate. Perhaps the mechanistic constancy of the transcription cycle may be metaphorically related to the automobile engine. That is, engines from different manufactures pretty much run the same way. Thus, two different transcription complexes may contain protein variants that elicit each mechanistic step. But fundamentally, the transcription machinery at all mRNA genes in eukaryotes runs basically the same way. Thus, most genes have the same basic chromatin organization, and once activators are activated, they direct chromatin remodeling events, PIC assembly, and early events in transcription initiation and elongation in basically the same way.

Yet it is the intricacies of eukaryotic gene regulation that are so remarkable. Why are there so many mechanistic steps? Why can’t an activated activator bind to its target promoter and simply recruit an RNA polymerase juggernaut? Why reposition nucleosomes to cover up certain transcription factor binding sites? Why are more than 50 proteins needed for PIC assembly, when in principle Pol II should have unfettered access to nucleosome-free promoter regions? Why are there so many histone modifications, and why does the Pol II CTD need to coordinate networks of modifications that occur during transcription? Some of these questions have been debated in the literature, but most of these issues will likely remain enigmatic until the mechanistic details of eukaryotic transcriptional regulation are more fully worked out.

Archaebacteria successfully employ a simplified stripped-down version of the eukaryotic transcription machinery, and so the eukaryotic embellishment is not a fundamental constant of gene regulation. It may be widely accepted that the combination of genome duplication and evolution into diverse and/or multicellular environments that are characteristic of eukaryotes has driven the enormous number and combinatorial diversity of sequence-specific transcription factors. However, the basis of the mechanistic complexity of a regulated transcription cycle is not obvious (to us). For transcription, evolution does not have the luxury of de novo design and is forced to build on pre-existing states, which over the evolutionary history of eukaryotes is wrought with enormous changes in selective pressure caused by punctuated environmental changes. Thus, in this case, complexity might beget more complexity, leaving us with mechanisms (as illustrated here) that appear to be a Rube Goldberg design (performing a simple task using an overly complex apparatus).

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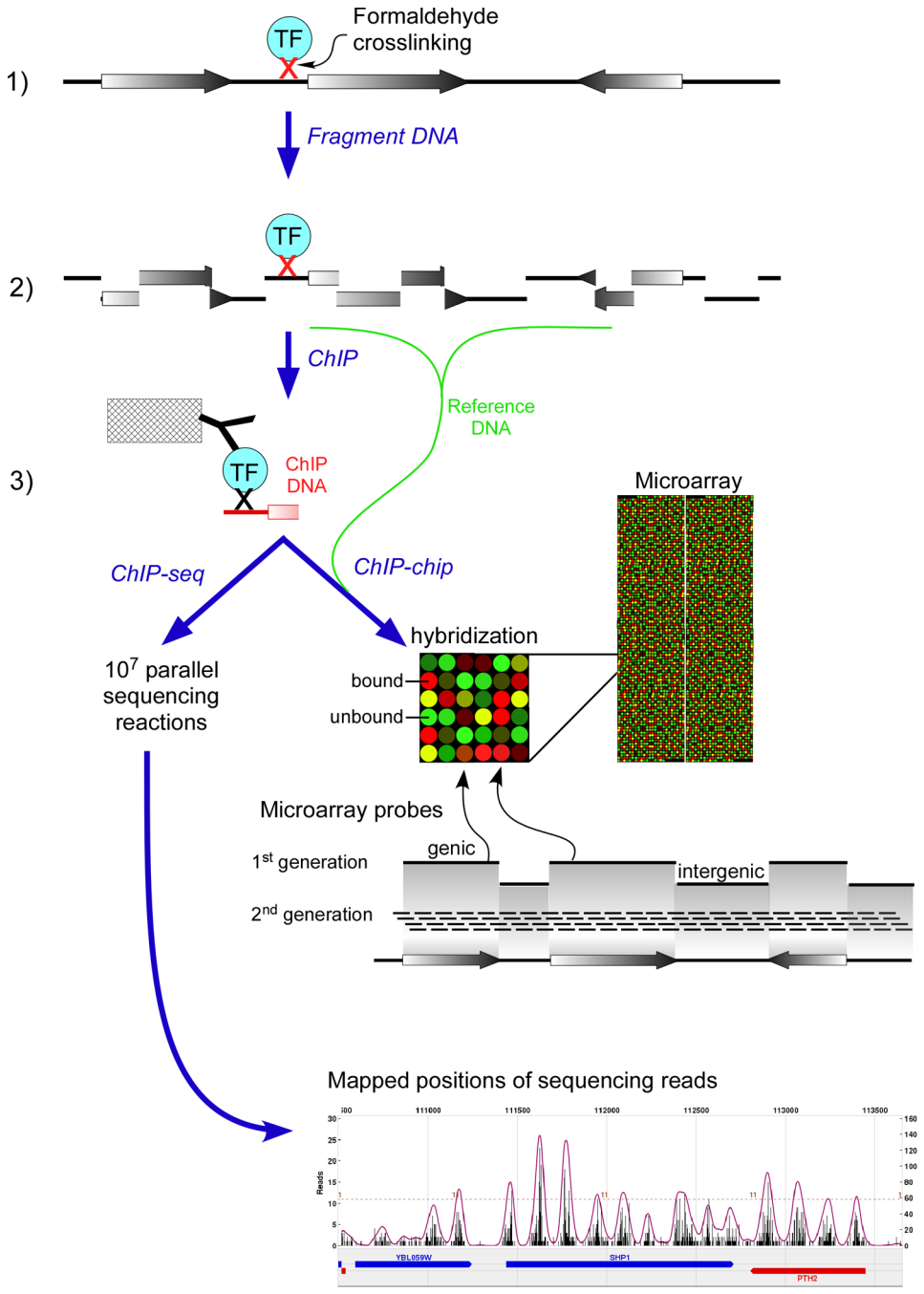

Figure 1. ChIP assays to measure protein binding across a genome

Two DNA detection methods that make use of ChIP are illustrated. 1) In both methods, formaldehyde is used to crosslink transcription factors (TF) to the genome in vivo. 2) The DNA is then fragmented. 3) The protein is immuno-purified to remove DNA that is not bound to the TF.

In ChIP-chip, the DNA crosslinked to the protein is then attached to a red dye and detected by hybridization to an array of immobilized DNA probes (microarray chip), whose sequence matches specific genomic locations. Often a reference DNA sample (illustrated in green) is co-hybridized so that probe-to-probe variation can be controlled. In ChIP-chip, the low-resolution first generation microarrays contained probes spanning all genic and/or intergenic

regions. Second generation microarrays provided higher detection resolution by tiling probes across the genome at 5 base pair spacing in yeast, and ~40 bp spacing in the larger genomes of fly and human.

In Chip-seq, each DNA molecule is directly sequenced. ChIP-seq achieves single base-pair detection resolution through sequencing, although the median DNA length of the ChIP sample preparation limits the spatial resolution that can be achieved by ChIP-seq. The sequencing read counts at each genomic coordinate are shown as a bar graph in a searchable genome browser screenshot (http://atlas.bx.psu.edu/).

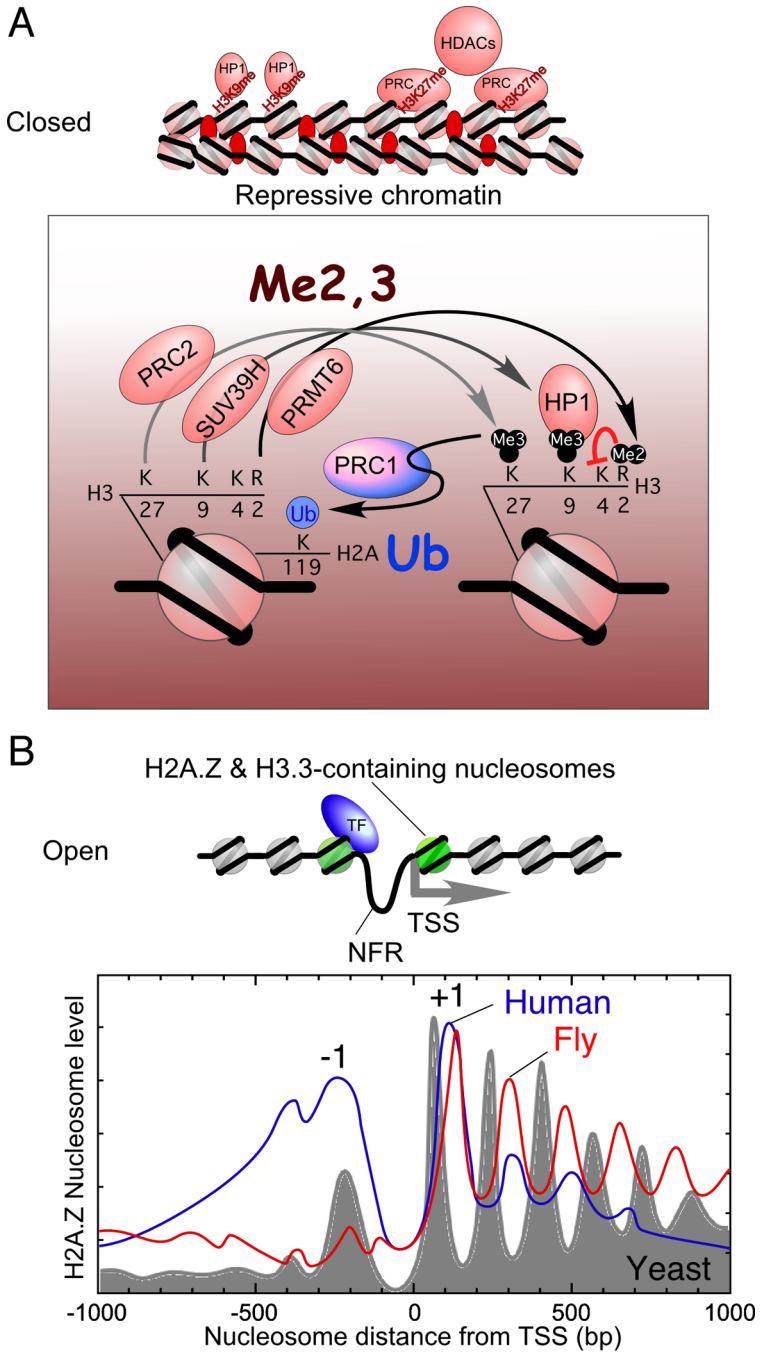

Figure 2. The organization of nucleosomes throughout the genome

(**A**) Model for closed chromatin. Repressive chromatin is shown in a closed state that is representative of the 30 nm chromatin fiber. Nucleosome-free regions (NFRs) are absent. The resident canonical histones are methylated at a number of sites including H3K9 and/or H3K27. These sites are bound by HP1 and the PRC complexes, respectively. Compaction is mediated by linker histone H1. These repressive entities recruit histone deacetylases (HDACs) to remove activating acetyl marks on histones.

(**B**) The promoters of most genes reside in an open chromatin state in which they are competent to undergo activation. Open chromatin is represented as a beads-on-a-string

configuration in which a transcription factor (e.g. Reb1 or Abf1, indicated as “TF”) binds to its cognate site, and helps to maintain the NFR as well as to promote the replacement of canonical histones H3 and H2A, with H3.3 and H2A.Z, respectively. (In yeast, H3.3 is the same as H3.) Note that this is a composite representation and may not reflect the disposition of factors at any given gene. Shown are the frequency distributions for H2A.Z-containing nucleosomes relative to the transcriptional start site (TSS) of all genes in budding yeast, fly, and human CD4+ T cells determined by ChIP-seq (Albert *et al.*, 2007; Mavrich *et al.*, 2008b; Schones *et al.*, 2008).

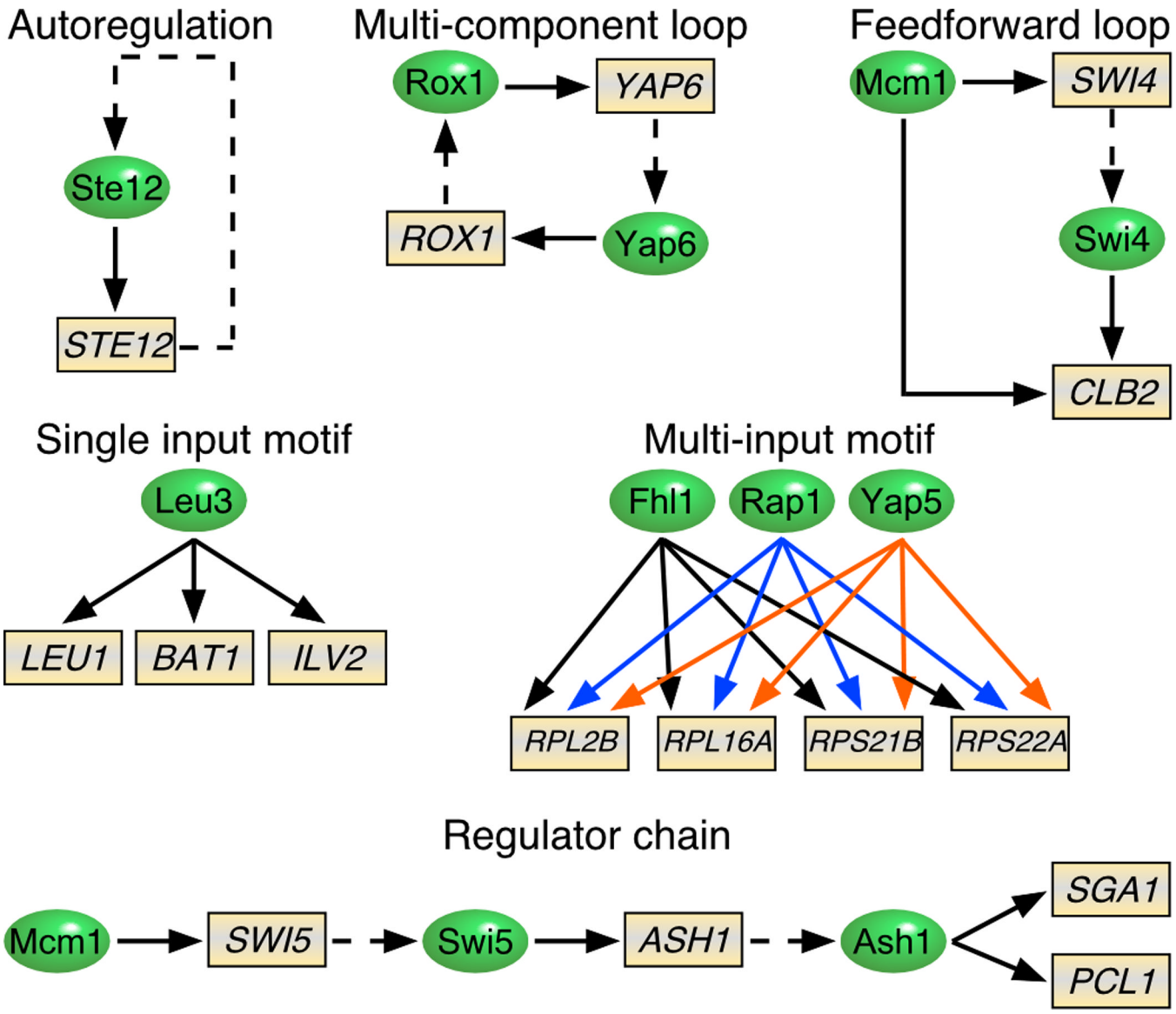

Figure 3. Regulatory networks controlled by sequence-specific transcriptional regulators

Regulatory circuits are composed of simple network motifs. Specific examples for six regulatory motifs are shown. Sequence-specific regulators and target genes are indicated by ovals and rectangles, respectively. Solid arrows denote binding of an activator to a gene promoter. Dashed arrows designate genes encoding a sequence-specific regulator. In the multi-input motif, for clarity arrows associated with the each factor are colored differently. The illustration is modified from (Lee *et al.*, 2002).

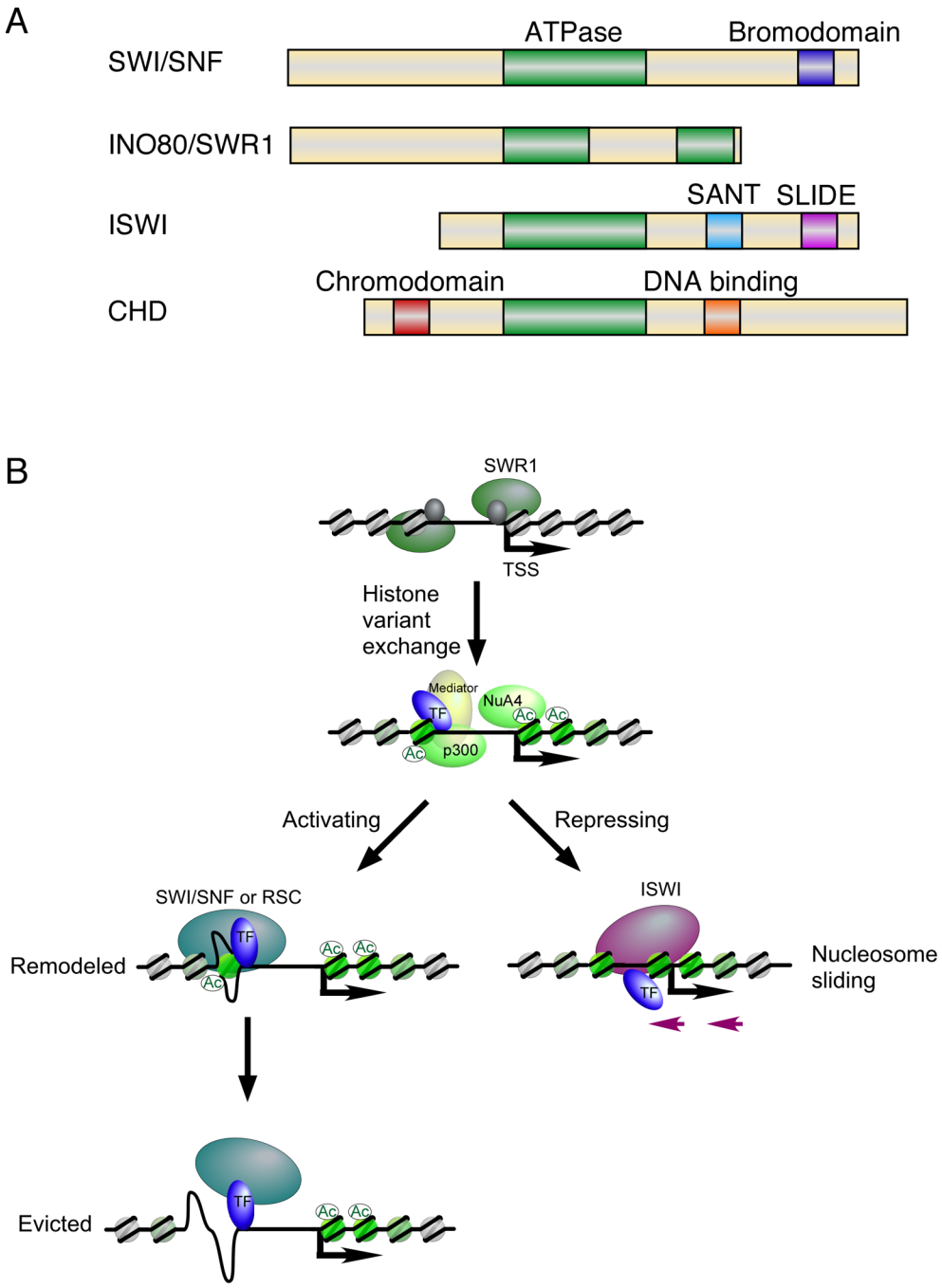

Figure 4. Chromatin remodeler families and conserved domains

(**A**) Domain organization of the ATPase subunit of chromatin remodeling complexes. All four families share an evolutionarily conserved Snf2-like ATPase domain belonging to the DEAD/H-box helicases. The catalytic subunit of the SWI/SNF family contains a bromodomain at the C-terminus that binds acetylated lysines. The INO80/SWR1 family is distinct from the other three families by having a split ATPase domain. The ATPase subunit of the ISWI family of remodelers harbors a SANT and SLIDE domain at the C-terminus, which are thought to bind histone tails and linker DNA, respectively. The CHD family

contains an N-terminal chromodomain, which binds methylated lysines, and a C-terminal DNA-binding domain. The illustration is modified from (Tsukiyama, 2002).
(**B**) In the "open" chromatin configuration, chromatin remodeling complexes use the energy of ATP hydrolysis to dissociate DNA from the histone surface so that histone variants (H2A.Z and H3.3, shown in green) may be exchanged in, or that DNA binding sites may become exposed (activating pathway) or the sites may be covered (repressing pathway). Some of this may be facilitated by histone acetylation (p300 and NuA4 examples are indicated). The presence of an NFR may allow partial assembly of the PIC prior to nucleosome remodeling or eviction. Note that this is a composite representation and may not reflect the disposition or order of remodeling at any given gene. The absence of Mediator and other components in the later steps is only for clarity.

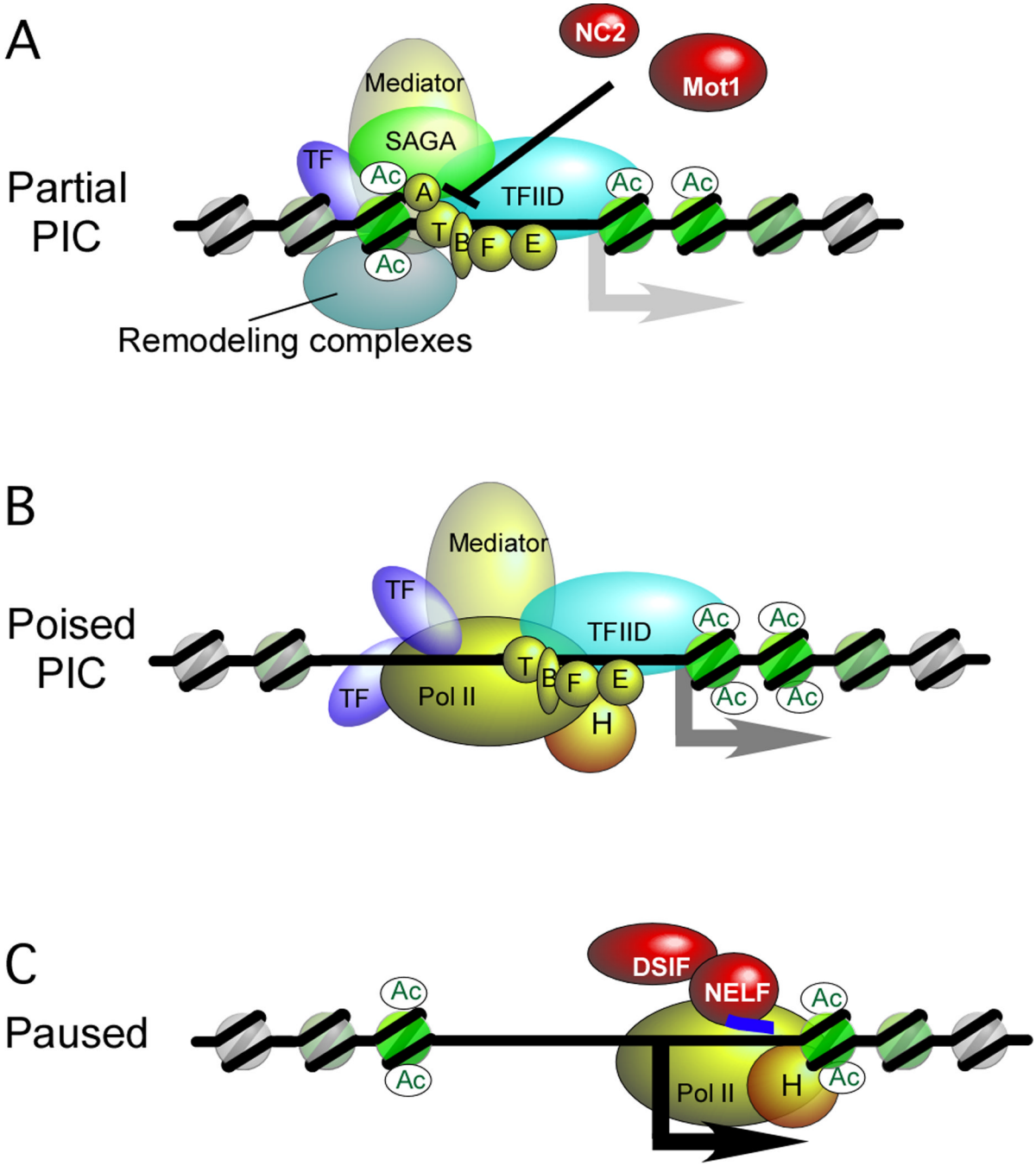

Figure 5. Assembly of the pre-initiation complex

Two forms of pre-initiation complexes and an early elongation complex are shown: A) Partial PIC (Zanton and Pugh, 2006), B) Poised PIC (Martens *et al.*, 2001; Radonjic *et al.*, 2005; Sekinger and Gross, 2001), and C) Paused Pol II complex (Lee *et al.*, 2008; Muse *et al.*, 2007; Zeitlinger *et al.*, 2007).

(**A**) A partial PIC contains GTFs assembled in the context of resident nucleosomes, but is relatively depleted of TFIIH and Pol II.

(**B**) A poised PIC contains Pol II and TFIIH in addition to the GTFs and exists in the context of an evicted -1 nucleosome. The poised PIC has not yet cleared the promoter. In vivo, such

complexes may be undergoing abortive initiation events where very short transcripts are released and degraded.

(**C**) A paused Pol II complex typically occurs 30–50 nucleotides after the TSS. Negative elongation factor (NELF) and other factors (not shown) bound to Pol II help create the paused state. The +1 nucleosomes might also contribute to pausing by creating a barrier. Many initiation and regulatory factors may be retained at the promoter after Pol II has cleared the area, which might promote subsequent rounds of transcription (not shown).

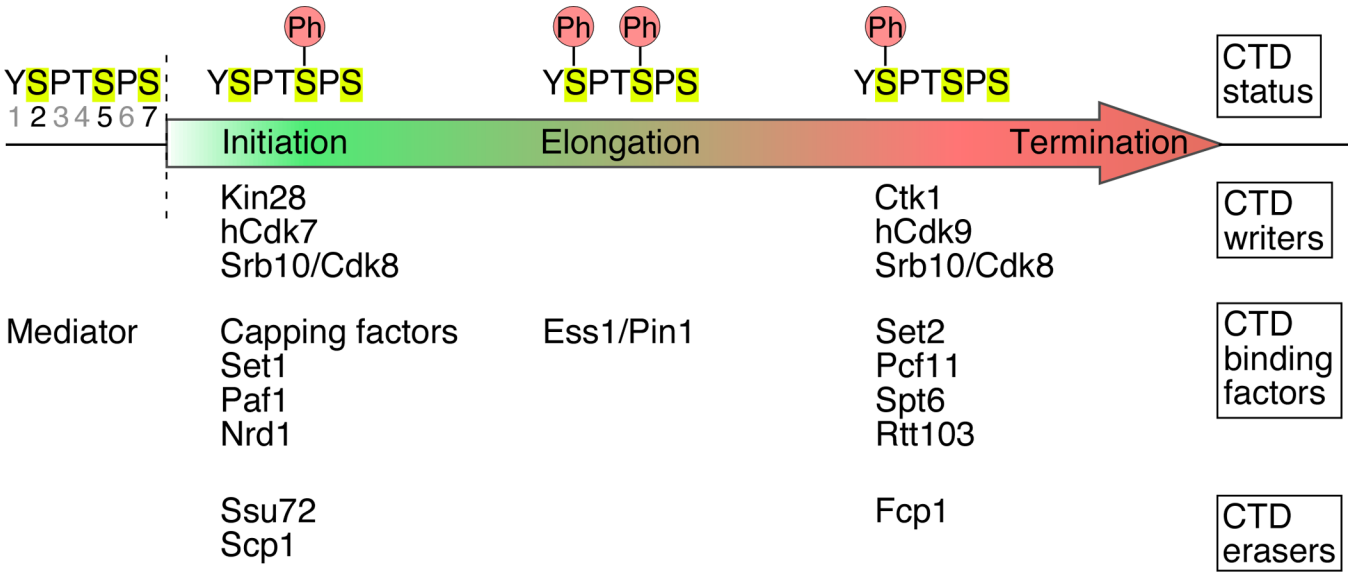

Figure 6. Writers, readers, and erasers of the Pol II CTD code

Writers (kinases), readers, and erasers (phosphatases) of the CTD code are listed under the different phosphorylation (Ph) statuses of the CTD (single amino acid code YSPTSPS). This phosphorylation status changes from the 5' end of genes to the 3' end. Y1, T4, and S7 may also be phosphorylated in vivo (Baskaran *et al.*, 1993; Egloff *et al.*, 2007; Zhang and Corden, 1991), but their function remains to be deciphered.

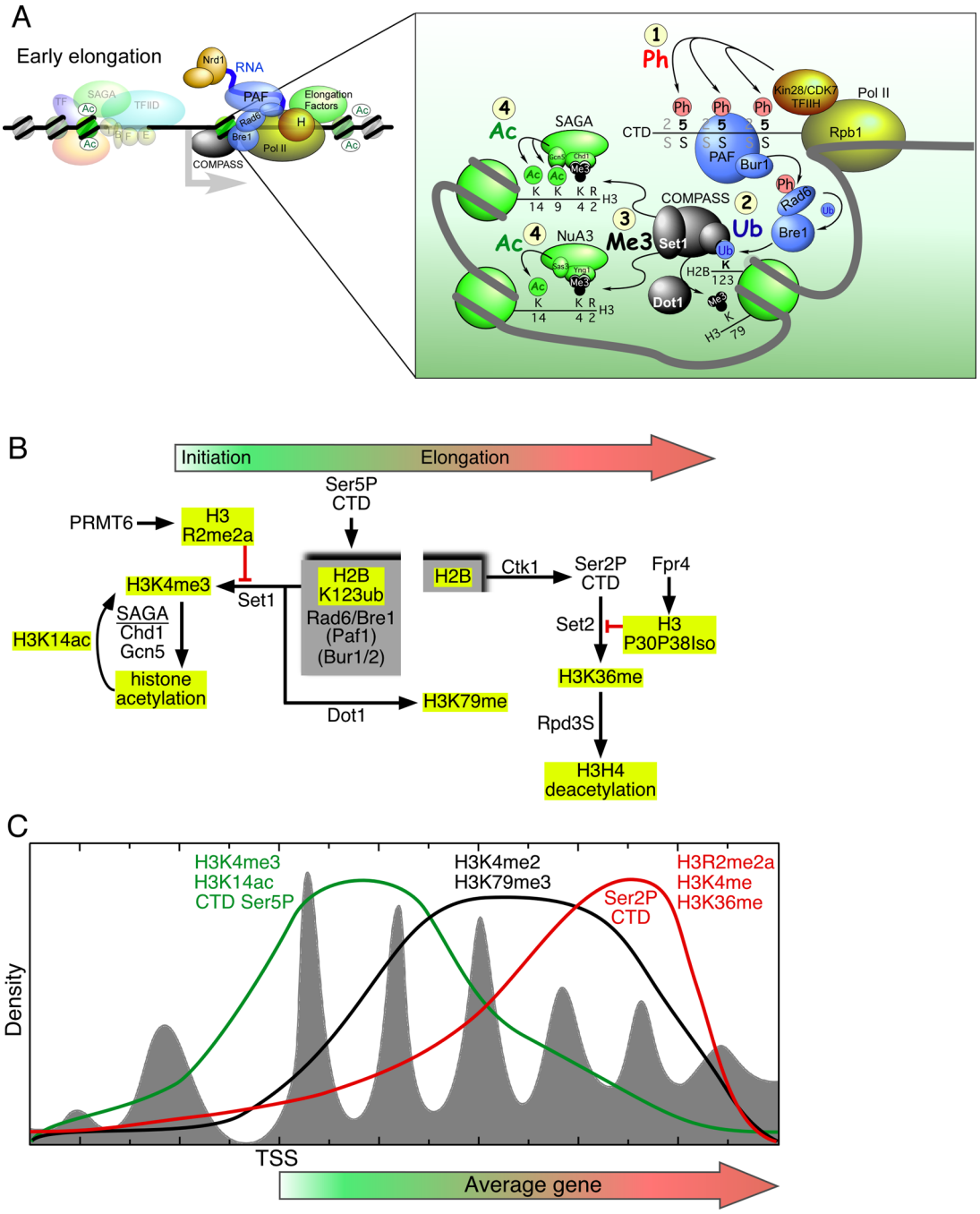

Figure 7. Histone crosstalk and the distribution of post-translational modifications across genes

(**A**) Model for early elongation. Early elongation includes promoter proximal pausing, elongation factors bound to the CTD, and a cascade of histone modifications (shown in the inset and numbered 1–4). Abbreviations include: Ph, phosphorylation; Ub, ubiquitylation; Me3, trimethylation; Ac, acetylation. See text for an explanation of the model.

(**B**) Post-translational modification network. At the hub of this histone modification network is a cycle of ubiquitylation and deubiquitylation on histone H2B, demarcated in the gray boxes. Solid arrows and red lines connect the interdependencies of post-translational modifications. The red line indicates that a particular modification blocks the subsequent

modification. Histone tail modifications are highlighted with yellow boxes. Marks generally associated with initiation are shown toward the left, whereas modifications linked to elongation are shown toward the right (with the exception of H3R2me2a, which occurs during elongation to block H3K4me3), as designated by the filled arrow at the top of the panel.

(**C**) Model for the distributions of histone modifications and phosphorylation of the Pol II CTD in relation to gene length. The nucleosome distribution relative to the TSS is shown in gray fill. The green, black, and red traces model the genome-wide distribution of the indicated histone modifications and CTD phosphorylation state.

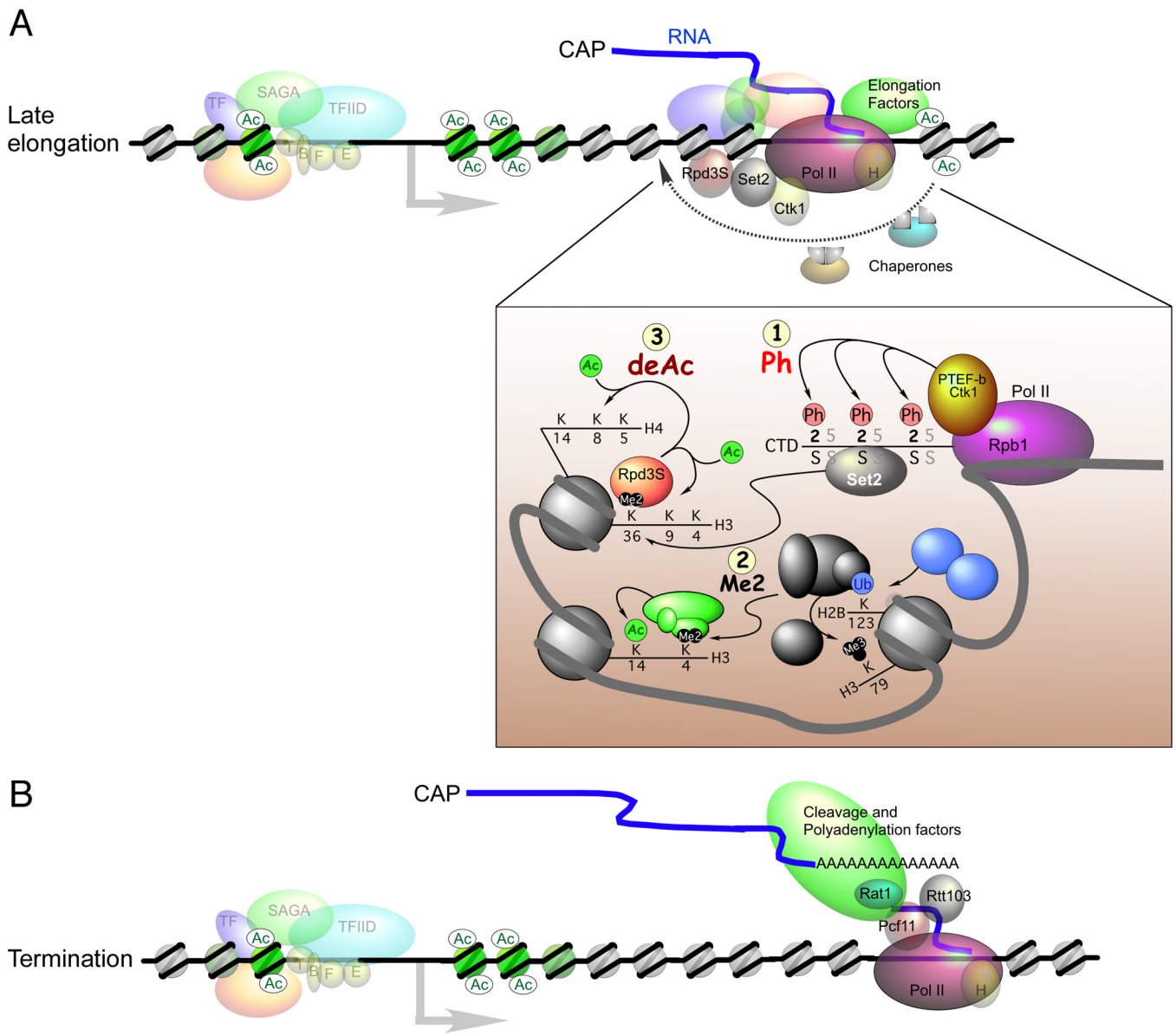

Figure 8. Models for late elongation and transcription termination

(**A**) During late stages of the transcription cycle the phosphorylation pattern on the Pol II CTD changes from serine-5 to serine-2, with the latter being recognized by a different set of proteins. The histone modifications that are most prominent during late elongation are highlighted in the inset and numbered 1–3. Also shown are the continued modifications of H3 that occur throughout the gene.

(**B**) Termination of Pol II transcription is accompanied by cleavage and polyadenylation factors that bind to the serine-2 phosphorylated form of the Pol II CTD. See text for details.

Table 1

Common DNA elements

DNA Element	Acronym meaning	Description	Bound Protein	Species specificity
Core promoter elements				
NFR	Nucleosome Free Region	~140 bp regions present at the beginning and end of genes that lack nucleosomes, rich in poly-dA:dT tracts	n.a.	Yeast and Metazoans
TSS	Transcription Start Site	First transcribed nucleotide of the RNA transcript, typically an A	Pol II	Yeast and Metazoans
TATA	--	Located ~60 bp upstream (25–30 bp in metazoans) of the transcription start site, site of PIC assembly, ~20% of genes contain a TATA box in yeast	TBP	Yeast and Metazoans
BRE	TFIIB Recognition Element	Two distinct motifs flank the site where the TATA box typically resides, helps to orient the directionality of the PIC	TFIIB	Metazoans
Inr	Initiator	Immediately adjacent to the TSS, can accurately direct initiation alone or with the TATA box	Taf1,Taf2	Metazoans
MTE	Motif Ten Element	Located 20 bp downstream of TSS, functions with Inr to enhance transcription, can substitute for TATA	n.a.	Metazoans
DPE	Downstream Promoter Element	Located 30 bp downstream of the TSS, found in many TATA-less Drosophila promoters	Taf6, Taf9	Metazoans
Upstream elements				
UAS	Upstream Activating Sequence	Recognized by activators to stimulate transcription through recruitment of coactivator complexes	Activators	Yeast
Enhancer	--	Functionally similar to the UAS but often located several thousand bp away from the corresponding promoter	Activators	Metazoans
URS	Upstream Repressing Sequence	Recognized by repressors to negatively regulate transcription	Repressors	Yeast