Chromatin and Transcription in Yeast

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ABSTRACT Understanding the mechanisms by which chromatin structure controls eukaryotic transcription has been an intense area of investigation for the past 25 years. Many of the key discoveries that created the foundation for this field came from studies of *Saccharomyces cerevisiae*, including the discovery of the role of chromatin in transcriptional silencing, as well as the discovery of chromatin-remodeling factors and histone modification activities. Since that time, studies in yeast have continued to contribute in leading ways. This review article summarizes the large body of yeast studies in this field.

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MOLECULAR biologists today take it for granted that chromatin structure plays critical roles in regulating transcription. However, there was a time, ~25 years ago, when most molecular biologists who studied gene expression were skeptical of any important role for chromatin structure. Back then, the primary evidence that chromatin might be important for gene expression came from studies showing that actively transcribed genes were more nuclease sensitive than untranscribed genes. Other studies suggested that histone acetylation might affect transcription. However, it was not possible to determine the causal relationship between these chromatin differences and transcription. At the same time, most investigators in the field of eukaryotic transcriptional regulation were focused on the exciting analysis of DNA-binding proteins, general transcription factors, and promoter regulatory elements. In general, the idea of a regulatory role for chromatin structure generated a low level of enthusiasm. Nucleosomes were viewed as a way to package DNA to fit into the nucleus, but were otherwise seen by most as static, uninteresting structures.

Over the next 10–12 years, this view changed dramatically with the demonstration that chromatin structure plays widespread, dynamic, and essential roles in the control of transcription. This change in perspective came from two broad areas of investigation. First, as the biochemistry of transcription advanced, there were improved *in vitro* systems to analyze the ability of factors to function on chromatin templates. These studies made it clear that, *in vitro*, nucleosomes are a barrier for both transcription initiation and elongation. Second, the power of yeast genetics came into play, leading to remarkable insights into the roles of chromatin structure in transcriptional control *in vivo* and showing that histones play a role in transcription *in vivo* and in identifying factors that control transcription by controlling chromatin structure and histone modifications. These advances, combined with new methods, notably chromatin immunoprecipitation and genome-wide approaches, have accelerated the rate at which we have come to understand the complex ways in which chromatin controls transcription.

This review covers how yeast studies have contributed to these dramatic advances in our understanding of eukaryotic transcription and chromatin structure. We review studies done primarily in *Saccharomyces cerevisiae*, covering research from the mid-1980s through 2010. Given the strong

conservation throughout eukaryotes, we also mention studies of larger eukaryotes where appropriate. In addition to the information reviewed in this article, please see three related chapters on silencing and heterochromatin in *S. cerevisiae* and *Schizosaccharomyces pombe*, on transcription initiation, and on transcription elongation (Hahn and Young 2011; Buratowski, S., planned YeastBook chapter; Smith, J., planned YeastBook chapter). Yeast studies of chromatin and transcription have been a powerful force in shaping our current understanding and in framing the questions for ongoing investigations.

How Yeast Has Led the Way: An Overview

The discoveries in yeast that led to understanding that chromatin structure controls transcription came from two general areas: directed studies of *S. cerevisiae* histone genes and selections and screens to find transcriptional regulatory mutants.

S. cerevisiae histone genes

In *S. cerevisiae*, two genes encode each of the four core histones, organized into four divergently transcribed gene pairs, the two encoding histones H2A and H2B, and the two encoding histones H3 and H4 (Hereford *et al.* 1979; Smith and Andresson 1983). This low copy number stands in contrast to *Drosophila* with ~100 copies (Lifton *et al.* 1978) and mice and humans with >50 copies (Marzluff *et al.* 2002). The low copy number in yeast greatly facilitated the analysis of histone function *in vivo*. For example, the initial demonstration that a histone (in this case H2B) is essential for viability came from the simple experiment of constructing two yeast strains—one with a mutation in *HTB1* and the other with a mutation in *HTB2*—crossing the two strains, and demonstrating that the double mutants were inviable (Rykowski *et al.* 1981).

S. cerevisiae also has three other types of histones, encoded by single-copy genes. Histone H1, encoded by *HHO1*, appears to play a limited role in chromatin structure and gene expression (Patterton *et al.* 1998; Levy *et al.* 2008; Schafer *et al.* 2008; Yu *et al.* 2009). An essential centromere-specific variant of histone H3, Cse4 (Meluh *et al.* 1998), plays an essential role in centromere function that will be described in Biggins, planned YeastBook chapter. Finally, an H2A variant, H2A.Z, encoded by *HTZ1* will be discussed below.

Altering histone levels changes transcription in vivo

Studies of altered levels of histone gene expression and of histone mutants provided some of the first evidence that chromatin structure is important for transcription *in vivo*. In one study, suppressors of the deleterious effect of Ty or Ty LTR insertion mutations in promoter regions were identified as mutations in *HTA1-HTB1*, one of the two loci encoding histones H2A and H2B (Clark-Adams *et al.* 1988; Fassler and Winston 1988). These mutations were shown to suppress the phenotype of the insertion mutations by altering transcription. In another study, yeast cells were depleted of histone H4 by use of the glucose-repressible *GAL1* promoter (Han and Grunstein 1988). Upon H4 depletion, the *PHO5* promoter became activated under normally repressing conditions. Both studies fit well with biochemical studies in mammalian systems that supported the idea that nucleosomes repress transcription and that activators or general transcription factors help to overcome this repression (*e.g.*, see Williamson and Felsenfeld 1978; Wasylyk and Chambon 1979; Knezetic and Luse 1986; Matsui 1987; Workman and Roeder 1987; Workman *et al.* 1991).

Given that modest changes in histone levels can have widespread effects on transcription (Norris and Osley 1987; Clark-Adams *et al.* 1988; Singh *et al.* 2010), it is not surprising that yeast histone levels are carefully regulated *in vivo*. Histone genes are transcribed in a cell-cycle-specific fashion, and this regulation is dependent upon several regulators, including Hir and Hpc proteins (Osley and Lycan 1987; Xu *et al.* 1992), Spt10 and Spt21 (Dollard *et al.* 1994), Yta7 (Gradolatto *et al.* 2008; Fillingham *et al.* 2009), Trf4/Trf5 (Reis and Campbell 2007), Asf1 (Fillingham *et al.* 2009), Rtt106 (Fillingham *et al.* 2009), and Swi4 (Eriksson *et al.* 2011). Interestingly, many of these factors (Hir, Hpc, Asf1, Rtt106) also function as histone chaperones, described in the section on histone chaperones, strongly suggesting that histone gene transcription is regulated by free histone levels. There are also post-transcriptional mechanisms that control histone levels in yeast, including dosage compensation (Moran *et al.* 1990), gene amplification (Libuda and Winston 2006), and protein stability (Gunjan and Verreault 2003; Singh *et al.* 2009; Morillo-Huesca *et al.* 2010b).

Histone mutants have revealed new facets about transcription and chromatin structure

Many classes of histone gene mutants have been isolated, resulting in a detailed genetic analysis of histone function *in vivo*. This type of analysis led to the influential discovery that the histone H4 N-terminal tail is required for transcriptional silencing (Kayne *et al.* 1988; Johnson *et al.* 1990, 1992; Megee *et al.* 1990; Park and Szostak 1990; Park *et al.* 2002). Other studies addressed specific issues regarding histone function, such as functional interactions with the chromatin-remodeling complex Swi/Snf (Prelich and Winston 1993; Hirschhorn *et al.* 1995; Kruger *et al.* 1995; Recht and Osley 1999; Duina and Winston 2004; He *et al.* 2008), histone–histone interactions (Santisteban *et al.* 1997; Glowczewski *et al.* 2000), and the requirements for N-terminal lysines (Zhang *et al.* 1998). Genome-wide expression analysis of histone mutants has provided broader understanding of the impact of specific histone mutants (*e.g.*, see Wyrick *et al.* 1999; Sabet *et al.* 2004; Dion *et al.* 2005; Parra *et al.* 2006; Parra and Wyrick 2007; Nag *et al.* 2010). Recently, large-scale studies have systematically constructed and analyzed hundreds of mutations in histone genes, providing a comprehensive data set of the histone residues that are required for normal transcription *in vivo* (Matsubara *et al.* 2007; Dai *et al.* 2008; Nakanishi *et al.* 2008; Seol *et al.* 2008; Kawano

Table 1 Mutant hunts that identified key factors in chromatin-mediated transcription in *S. cerevisiae*

Mutant hunt	Phenotype	Genes identified	Protein function
spt[a]	Suppression of Ty and LTR insertion mutations	*SPT6/SSN20/CRE2*	Histone chaperone
		SPT16/CDC68[b]	Histone chaperone, part of FACT
		HTA1/SPT11, *HTB1/SPT12*	Histones H2A, H2B
		SPT4, *SPT5*	Elongation factors; components of DSIF
		SPT10/CRE1, *SPT21*	Regulators of histone gene transcription
ada[c]	Resistance to high levels of Gal4-VP16	*GCN5/ADA4/AAS104*[d]	Histone acetyltransferase; part of the SAGA coactivator complex
		NGG1/ADA2,[e] *ADA3*	Required for Gcn5 activity within SAGA
cre[f]	Expression of *ADH2* in the presence of glucose	*SPT10/CRE1*	Regulator of histone gene transcription
		SPT6/CRE2/SSN20	Histone chaperone
swi[g]	Inability to switch mating type due to reduced *HO* transcription	*SWI1/SWI2/SWI3*	Part of the Swi/Snf chromatin-remodeling complex
snf[h]	Inability to transcribe *SUC2*; sucrose nonfermenter	*SNF2/SNF5/SNF6*	Part of the Swi/Snf chromatin-remodeling complex
sin[i]	Suppression of *swi* mutations	*SPT2/SIN1*	Transcription elongation factor
		HHT1/BUR5/SIN2	Histone H3
		SIN3/RPD1	Cofactor for Rpd3
ssn[j]	Suppression of *snf* mutations	*CYC8/SSN6/CRT8*[k]	Global repressor; recruits HDACs
		SPT6/CRE2/SSN20	Histone chaperone
bur[l]	Suppression of *SUC2* UAS deletion	*HHT1/BUR5/SIN2*	Histone H3
hir/hpc[m]	Loss of cell-cycle control of histone gene transcription	*HIR1*, *HIR2*, *HIR3*, *HPC2*	Nucleosome assembly, transcriptional regulation
rpd[n]	Suppression of *trk1*Δ	*SIN3/RPD1*	Cofactor for Rpd3
		RPD3	Histone deacetylase
rtt[o]	Reduces Ty transposition	*RTT106*	Histone chaperone
		RTT109	H3 K56 HAT

Mutant hunts that identified factors included in this chapter are listed. Several other notable yeast mutant hunts have identified key factors in transcription (*e.g.*, Nonet and Young 1989; Pinto *et al.* 1992). For each mutant hunt, we have usually cited only the publication that isolated the first mutants. The factors listed are grouped by function. Often, more factors than those listed were identified in the cited mutant hunts.

[a] Winston *et al.* (1984, 1987); Clark-Adams *et al.* (1988); Fassler and Winston (1988); Natsoulis *et al.* (1991).

[b] Spt16 was also identified as Cdc68 in a screen for start mutants (Prendergast *et al.* 1990; Rowley *et al.* 1991).

[c] Berger *et al.* (1992); Marcus *et al.* (1994).

[d] Gcn5 was initially identified as Aas104 (Penn *et al.* 1983) and later renamed GCN5 when new nomenclature was implemented for genes involved in general amino acid control. Gcn5 was initially suggested to be a coactivater in a subsequent study (Georgakopoulos and Thireos 1992) and then later shown to be a HAT (Brownell *et al.* 1996).

[e] Ada2 was also identified as Ngg1 (Brandl *et al.* 1993).

[f] Denis (1984); Denis *et al.* (1994).

[g] Stern *et al.* (1984); Breeden and Nasmyth (1987).

[h] (Carlson *et al.* (1981); Neigeborn and Carlson (1984).

[i] Sternberg *et al.* (1987).

[j] Carlson *et al.* (1984); Neigeborn *et al.* (1986).

[k] SSN6 was initially identified as CYC8 (Rothstein and Sherman 1980), and it was also identified as CRT8 (Zhou and Elledge 1992).

[l] Prelich and Winston (1993).

[m] Osley and Lycan (1987); Xu *et al.* (1992).

[n] Vidal and Gaber (1991); Vidal *et al.* (1991).

[o] Scholes *et al.* (2001).

et al. 2011). A convenient resource for information on histones and histone mutants is the Histone Systematic Mutation Database (http://baderzone.net/v2/histonedb.html) (Huang *et al.* 2009).

Mutant hunts identified the major classes of factors that control chromatin structure

Several mutant hunts in *S. cerevisiae* resulted in the identification of factors centrally involved in chromatin-mediated transcription. While the involvement of many of these factors in the regulation of chromatin structure was not initially understood, the genetic studies in yeast established that these factors play critical roles in transcription *in vivo*. Their subsequent analysis in yeast and larger eukaryotes established the gene products as playing fundamental and conserved roles in chromatin-mediated transcription. Some of the key mutant hunts leading to the identification of histones and chromatin regulators are summarized in Table 1, with more information about the gene products provided throughout this review article. These mutant studies laid the foundation for our current understanding that chromatin is controlled throughout eukaryotes by a myriad of factors that control nucleosome stability, dynamics, and histone modifications.

Analysis of chromatin structure: studies of PHO5 and other genes

Fundamental concepts concerning nucleosomes and transcription came from pioneering studies of a small set of genes

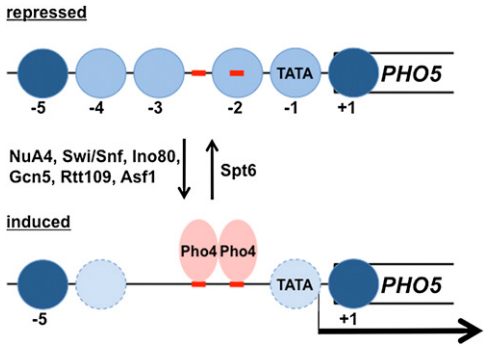

Figure 1 Regulation of the *PHO5* gene. Shown are diagrams of *PHO5* when repressed (top) and induced (bottom). In the repressed state (high phosphate), four nucleosomes (−4 to −1, shown in light blue) span the *PHO5* regulatory region, including nucleosome −2, which blocks one of the Pho4-bindings sites (red line). Several factors, shown on the left and described in the text, are required for *PHO5* induction, which results in loss of nucleosomes over the *PHO5* regulatory region, the binding of Pho4, and activation of transcription. The dotted lines around nucleosomes −4 and −1 indicate a more variable degree of loss. Upon addition of phosphate, the nucleosomes reassemble in the PHO5 regulatory region in an Spt6-dependent fashion, resulting in transcriptional repression.

in yeast. In particular, nuclease studies of the *PHO5* and *GAL1–10* genes (reviewed in Lohr 1997 and described below) established the principle that nucleosomes are found over promoters when the genes are repressed, blocking access of transcription factors to their binding sites, and that nucleosomes are altered or removed upon transcriptional induction. Subsequent studies with *PHO5* helped to establish the causal relationship between chromatin structure and transcription as well as many other aspects of chromatin-mediated transcription initiation. These studies are summarized below.

PHO5 and other phosphate-regulated genes are transcriptionally repressed in high phosphate and induced in low phosphate, depending upon the activators Pho2 and Pho4. Pioneering work on *PHO5*, by Hörz and colleagues, provided several insights into the interplay between nucleosomes and transcription factors. Their studies showed that positioned nucleosomes cover the repressed *PHO5* promoter, including one of the two Pho4-binding sites and the core promoter elements (Almer and Hörz 1986), and that these nucleosomes were disrupted upon gene activation in a Pho2/Pho4-dependent fashion (Figure 1) (Almer *et al.* 1986; Fascher *et al.* 1990). This disruption, initially measured by nuclease sensitivity, is known to result from complete eviction of the histones (Boeger *et al.* 2003; Reinke and Hörz 2003; Boeger *et al.* 2004; Korber *et al.* 2004). Additional studies have characterized the nucleosome loss in detail, providing evidence that it occurs to variable degrees within a population of induced cells, spreading from the site of Pho4 binding (Jessen *et al.* 2006). This and other studies suggest that variability in promoter nucleosome loss might contribute to cell-to-cell variability in *PHO5* transcription (Raser and O'Shea 2004; Jessen *et al.* 2006; Boeger *et al.* 2008). Analysis of *PHO5* has helped to establish the concept that a large number of chromatin regulatory factors are required to function, often in redundant fashion, in transcriptional activation, as *PHO5* regulation requires many factors described later in this review, including multiple histone-modifying enzymes (NuA4, Gcn5, and Rtt109 (Gregory *et al.* 1998; Barbaric *et al.* 2001, 2007; Nourani *et al.* 2004; Williams *et al.* 2008), nucleosome-remodeling complexes (Swi/Snf and Ino80) (Gregory *et al.* 1998; Barbaric *et al.* 2001, 2007), and histone chaperones (Asf1 and Spt6) (Adkins *et al.* 2004; Adkins and Tyler 2006; Korber *et al.* 2006).

Around the same time as the early *PHO5* studies, studies of the *GAL1–10* genes provided additional support for the concept that transcriptional activation correlated with disruption of nucleosomes. The yeast *GAL* genes are repressed in glucose and highly induced in galactose, which is dependent upon the activator Gal4. In contrast to *PHO5*, the region to which Gal4 binds in the *GAL1–10* locus—four sites spanning ~135 bp—was originally believed to be nucleosome free (Lohr 1984, 1993; Fedor *et al.* 1988; Fedor and Kornberg 1989; Cavalli and Thoma 1993). A recent study, however, showed that this region instead contains an unusual nucleosome, associated with the RSC (**r**emodels the **s**tructure of **c**hromatin) chromatin-remodeling complex, which protects a shorter fragment than canonical nucleosomes (Floer *et al.* 2010). The *GAL1–10* locus also has canonical nucleosomes positioned over the TATA region and +1 of transcription, and these nucleosomes are disrupted upon *GAL* gene activation, as shown by nuclease sensitivity studies (Lohr and Lopez 1995) and *in vivo* footprinting analysis (Selleck and Majors 1987; Axelrod *et al.* 1993).

Studies of *PHO5* have gone well beyond a correlation between chromatin and transcription, and provided early evidence that nucleosomes actually regulate transcription. For example, histone depletion activates *PHO5* even in repressing conditions (Han *et al.* 1988), whereas hyperstabilizing a *PHO5* promoter nucleosome blocks *PHO5* induction (Straka and Horz 1991). In addition, *PHO5* promoter chromatin was shown to be disrupted upon phosphate starvation even in the absence of transcription by using a *PHO5* TATA mutant (Fascher *et al.* 1993). Together, these studies suggest that chromatin changes precede, and are required for, changes in transcription at *PHO5*.

A key demonstration of the role for nucleosomes in signal processing by promoters came with the demonstration that the affinity of exposed Pho4-binding sites determines the severity of phosphate starvation required to activate expression of *PHO5*, while the affinities of all Pho4-binding sites at a promoter (both exposed and nucleosome occluded) determines the extent to which the gene can be activated (Lam *et al.* 2008). Thus, even cryptic nucleosome-occluded binding sites can contribute to the regulation of the downstream gene. Comparative studies of *PHO5* with the *PHO8* and *PHO84* genes, also regulated by Pho2 and Pho4, revealed striking differences, showing that the level of nuclear Pho4, its binding site occupancy, and the thermodynamic stability of promoter nucleosomes determine the extent to

which chromatin-remodeling activities are required for transcription at the different promoters (Gregory *et al.* 1999; Munsterkotter *et al.* 2000; Dhasarathy and Kladde 2005; Hertel *et al.* 2005; Lam *et al.* 2008; Wippo *et al.* 2009). Thus, several aspects of promoter sequence and chromatin architecture control the cofactor requirements and induction dynamics of transcriptional control.

These conclusions fit well with other studies that addressed the issue of promoter accessibility and the requirement of chromatin-remodeling factors. For example, at the *RNR3* gene, disrupting the nucleosome over the TATA element by insertion of a dA:dT tract bypassed the requirement for the Swi/Snf chromatin-remodeling complex for activation (Zhang and Reese 2007). Another example came from a genome-wide study of glucose-induced transcriptional reprogramming, which also concluded that transcription factor accessibility, rather than chromatin remodeling, determined the degree of transcriptional changes (Zawadzki *et al.* 2009).

Studies of several other yeast genes have also contributed to our understanding of how chromatin structure affects transcriptional control. These genes include *SUC2* (Hirschhorn *et al.* 1992; Matallana *et al.* 1992; Gavin and Simpson 1997), *HIS3* (Iyer and Struhl 1995), *CHA1* (Moreira and Holmberg 1998; Sabet *et al.* 2003), *RNR3* (Li and Reese 2001; Sharma *et al.* 2003; Zhang and Reese 2004, 2007; Tomar *et al.* 2009), *HIS4* (Arndt and Fink 1986; Devlin *et al.* 1991; Yu and Morse 1999; Yarragudi *et al.* 2004), and *HSP82* (Gross *et al.* 1993; Zhao *et al.* 2005). Another useful system, established by Simpson and co-workers, used an autonomous plasmid with well-positioned nucleosomes to study the effects of transcription factors and regulatory sequences on nucleosome position (*e.g.*, see Thoma *et al.* 1984; Roth *et al.* 1990; Morse *et al.* 1992). Overall, intensive study of a set of key inducible genes in yeast has established the groundwork for understanding how nucleosomes affect transcriptional regulation.

Analysis of Chromatin Structure: Genome-Wide Studies of Nucleosome Positioning in Regulatory Regions and Coding Regions

Since 2004, genome-wide approaches have been brought to bear on yeast chromatin: nucleosome occupancy has been studied using low-resolution DNA microarrays (Bernstein *et al.* 2004; Lee *et al.* 2004), high-resolution tiling oligonucleotide microarrays (Yuan *et al.* 2005; Lee *et al.* 2007b; Whitehouse *et al.* 2007; Zawadzki *et al.* 2009), and, most recently, ~4-bp resolution high-throughput sequencing (Albert *et al.* 2007; Mavrich *et al.* 2008a; Shivaswamy *et al.* 2008; Field *et al.* 2009; Kaplan *et al.* 2009; Eaton *et al.* 2010; Tirosh *et al.* 2010a,b; Tsankov *et al.* 2010; Kent *et al.* 2011). In general, yeast genes can be broken into two broad classes: "growth" genes such as those encoding ribosomal proteins and "stress" genes such as genes encoding many cell-wall proteins. These two types of genes are packaged into distinctive chromatin structures (Figure 2) and will be treated separately.

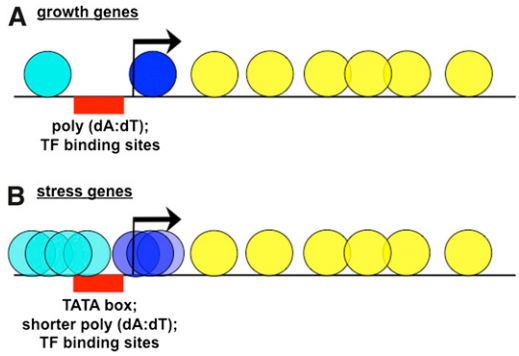

Figure 2 Chromatin structure over growth genes and stress genes. (A) Growth genes generally have a nucleosome-free region (red) that does not contains a TATA box, flanked by a −1 (turquoise) and +1 (blue) nucleosome. The +1 nucleosome contains Htz1 and the −1 nucleosome sometimes does. The downstream nucleosomes (yellow) are less well positioned. (B) Stress genes generally contain a TATA box, and the −1 and +1 nucleosome positions are less well defined, as indicated in the diagram.

Growth genes

Growth genes, also known as "housekeeping" genes, refer to genes whose expression is highest during rapid growth and that are often downregulated during stress responses. These genes encode many of the basic functions involved in rapid biomass production, such as ribosomal proteins, rRNA-processing enzymes, and glycolytic enzymes. The transcriptional machinery responsible for regulation of growth genes differs in broad ways from that involved in stress genes on the basis of the mechanism involved in TBP recruitment to promoters (Lee *et al.* 2000; Basehoar *et al.* 2004; Huisinga and Pugh 2004). In general, growth genes are regulated by TFIID rather than by SAGA, lack TATA boxes, exhibit little noise in expression levels, and are not affected by deletion of most chromatin regulatory genes (Basehoar *et al.* 2004; Newman *et al.* 2006).

Growth genes are typically characterized by a strongly nucleosome-depleted region (often called the nucleosome-free region, or NFR, but see below) found upstream of the coding region and surrounded by two well-positioned nucleosomes (Figure 2A). The NFR is the site of the majority of functional transcription factor-binding sites. These results partially explain a long-standing dilemma in the transcription field: most transcription factors, which bind short (4–10 bp, typically) sequence motifs, bind only a small fraction of their motifs in the genome. It had long been suggested that nucleosomal occlusion of a subset of sequence motifs prevents transcription factor binding to the majority of their potential binding sites. Indeed, a significant subset of sequence motifs bound by purified Leu3 *in vitro* that are not bound *in vivo* are covered by nucleosomes *in vivo* (Liu *et al.* 2006). Along with the discovery that the majority of bound sequence motifs for a given transcription factor are located in the NFR, these results support a general role for nucleosome positioning in transcription factor site occupancy.

The location of the transcriptional start site (TSS) for the majority of budding yeast genes is found ~12–13 nucleotides inside the border of the +1 nucleosome. The reason for this surprisingly stereotyped location is not currently understood. Do nucleosomes set the TSS location, or does the preinitiation complex set the +1 nucleosome location? Furthermore, we do not currently understand whether the location of the TSS inside a nucleosome creates a special need for cellular factors to enable access of transcriptional machinery to the TSS. However, it is known that the location of the TSS in different species is quite variable and located upstream of the +1 nucleosome in organisms such as *Drosophila melanogaster*, *Caenorhabditis elegans*, *Candida albicans*, and *Homo sapiens* (Tirosh *et al.* 2007; Mavrich *et al.* 2008a; Schones *et al.* 2008; Valouev *et al.* 2008; Tsankov *et al.* 2010), indicating that this architecture is not essential for eukaryotic transcriptional control.

Downstream of the well-positioned +1 nucleosome, nucleosomes become increasingly "fuzzy," meaning that the position of the nucleosome varies from cell to cell. This behavior is consistent with the "statistical positioning" model for nucleosome positioning described below. In general, nucleosome positioning over coding regions does not correlate particularly well with transcription rate except at highly transcribed genes, which exhibit relatively low nucleosome occupancy with fuzzier nucleosomes and shorter linker lengths.

Stress genes

In contrast to growth genes, stress genes are transcribed at low levels in rich media, but are induced under many different stress conditions (Gasch *et al.* 2000). Stress genes are typically regulated by the SAGA complex (rather than TFIID), have TATA boxes, are characterized by noisy or "bursty," expression, and are regulated by a wide range of chromatin-remodeling factors (Lee *et al.* 2000; Basehoar *et al.* 2004; Huisinga and Pugh 2004; Newman *et al.* 2006). In contrast to the wide, deep NFR exhibited by most growth genes, the minority class of TATA-containing stress genes exhibits more variable promoter architecture (Figure 2B). This is true across different genes (*i.e.*, various stress-responsive genes that exhibit a range of promoter packaging states) and also appears to be true across individual cells, since these promoters often are associated with delocalized nucleosomes (Ioshikhes *et al.* 2006; Albert *et al.* 2007; Field *et al.* 2008; Tirosh and Barkai 2008; Choi and Kim 2009; Weiner *et al.* 2010). Importantly, transcription factor-binding sites at TATA-containing promoters are likely to be occluded by nucleosomes, although rapid exchange of nucleosomes at some of these promoters (see below) allows binding sites to be accessed during transient time windows. This competition between nucleosomes and transcription factors might be expected to contribute to cell-to-cell variability (noise) in expression of downstream genes (Boeger *et al.* 2008; Tirosh and Barkai 2008). Furthermore, signal-induced nucleosome eviction, as seen at the *PHO5* promoter, can expose nucleosome-occluded transcription factor (TF) motifs that are not being exposed by steady-state histone dynamics in noninduced conditions.

Correlation Between Transcription and Chromatin Structure

Within the two broad types of chromatin packaging described above, changes in transcription of genes are correlated with changes in chromatin structure (Schwabish and Struhl 2004; Field *et al.* 2008; Shivaswamy *et al.* 2008; Jiang and Pugh 2009; Zawadzki *et al.* 2009; Radman-Livaja and Rando 2010; Weiner *et al.* 2010). In general, at higher transcription rates, one observes decreased occupancy ("eviction") of the −1 nucleosome, increased NFR width and depth, and closer internucleosomal spacing over coding regions. At very high transcription rates, nucleosome occupancy decreases over coding regions, and coding region nucleosomes become increasingly delocalized. This is seen by comparing highly transcribed genes to poorly transcribed genes within one growth condition, but has also been observed when genes are activated and repressed in response to changes in the environment [see above for single-gene examples and Shivaswamy *et al.* (2008) and Zawadzki *et al.* (2009) for whole-genome examples].

Many of these changes in chromatin structure are likely caused by chromatin-modulating factors (see below) recruited by transcription factors or by RNA polymerase II (RNAPII), but it is also known that RNAPII passage itself can affect nucleosomes. For example, inactivation of RNAPII activity using the *rpb1-1* conditional mutant causes an increase in occupancy of the −1 nucleosomes (Weiner *et al.* 2010). More interestingly, there is *in vitro* evidence that many RNA polymerases can transcribe through nucleosomal DNA without evicting histones by displacing the histones "backward" (Studitsky *et al.* 1994, 1997; Kulaeva *et al.* 2007, 2009, 2010; Hodges *et al.* 2009). This *in vitro* observation is consistent with the tighter nucleosome spacing observed over very highly transcribed coding regions and with the observation that eliminating RNAPII activity results in nucleosomes shifting forward into coding regions (Weiner *et al.* 2010). Importantly, even after eliminating RNAPII, many of the features (such as the gross differences in promoter nucleosome depletion) of growth and stress genes are preserved, indicating that the distinctive chromatin packaging of these genes is not simply a consequence of transcription levels during active growth.

Cis-Determinants of Nucleosome Positioning

The remarkably uniform and conserved nucleosomal organization of growth gene promoters begs the question: what determines nucleosome positions throughout the genome? Are nucleosome positions primarily "encoded" in the DNA sequence (*cis*-factors), or are they a consequence of the regulatory activity of chromatin remodelers, transcription factors, and the transcription machinery (*trans*-factors)?

As befits a general packaging factor, the histone octamer has little sequence preference in the classical sense of having a binding motif. However, the constraint of having to wrap DNA tightly around a small octamer of proteins means that the energy required to bend a given genomic sequence can influence the binding affinity of the histone octamer (Kunkel and Martinson 1981; Drew and Travers 1985; Iyer and Struhl 1995; Thastrom *et al.* 1999; Sekinger *et al.* 2005; Segal and Widom 2009). Since structural properties of DNA, such as local bendability, depend on DNA sequence, one might expect that DNA sequence will at least partially contribute to nucleosome positioning. The structure of poly(dA/dT) sequences differs from the canonical double helix (Nelson *et al.* 1987) and is somewhat resistant to the distortions necessary for wrapping around nucleosomes. Conversely, sequences with AA/TT/TA dinucleotides spaced at 10-bp intervals are intrinsically bendable [or create narrow minor grooves that favor association with arginines on the histone proteins (Rohs *et al.* 2009)] and thus bind the octamer with higher affinity than random sequence (Trifonov 1980; Anselmi *et al.* 1999; Thastrom *et al.* 1999). Computational studies have shown that poly(dA:dT) sequences are enriched in NFRs, whereas AA/TT/TA dinucleotide periodicity is enriched at the location of the +1 nucleosome *in vivo*, suggesting that sequence preferences might contribute substantially to *in vivo* nucleosome positioning.

An influential demonstration of the role for sequence in dictating chromatin structure was the finding that *in vitro* reconstitution of the *HIS3* promoter into chromatin (using just histones, DNA, and buffer) recapitulates some aspects of that promoter's *in vivo* chromatin structure, most notably the promoter's NFR (Sekinger *et al.* 2005). Nuclease accessibility at the *HIS3* promoter *in vivo* increased with increasing length of poly(dA:dT) elements at the promoter and furthermore was correlated with increased transcription of the *HIS3* gene (Iyer and Struhl 1995). Conversely, *in vitro* reconstitution of the *PHO5* promoter into chromatin does not recapitulate the *in vivo* state unless yeast whole-cell extract is included, showing that only a fraction of the genome "programs" aspects of its chromatin architecture via intrinsic sequence determinants (Korber *et al.* 2004). Recently, intrinsic nucleosome affinity of yeast genomic DNA has been analyzed genome-wide via *in vitro* reconstitutions (Kaplan *et al.* 2009; Zhang *et al.* 2009). These studies find a significant role for antinucleosomal sequences [such as long poly(dA:dT) elements)] in creating a region of nucleosome depletion at promoters, indicating that promoter nucleosome depletion is indeed "programmed" by sequence to some extent. Other AT-rich sequences are also nucleosome depleted, and in fact GC% alone explains the majority of the behavior of the *in vitro* reconstitution experiments (Tillo and Hughes 2009).

While the genome clearly encodes intrinsic antinucleosomal sequences, the reconstitution experiments provide little support for a role of intrinsically bendable "pronucleosomal" sequences in nucleosome positioning. TSS-aligned averages of chromatin profiles *in vivo* reveal a strongly positioned +1 nucleosome downstream of the NFR (Yuan *et al.* 2005; Mavrich *et al.* 2008a,b; Kaplan *et al.* 2009), whereas the corresponding *in vitro* average demonstrates a strong NFR but no positioned +1 nucleosome (Kaplan *et al.* 2009; Zhang *et al.* 2009). Thus, while there is some statistical enrichment of intrinsically bendable DNA that correlates with *in vivo* nucleosome positions, this appears to play little role in the gross translational positioning of nucleosomes. Instead, it has been suggested (Mavrich *et al.* 2008a; Jiang and Pugh 2009) that the dinucleotide periodicity detected in various computational studies contributes to *rotational* positioning of nucleosomes and that, instead of sequence, *trans*-factors such as the preinitiation complex or RNA polymerase play the major role in positioning the center of the nucleosome to within ~5 bp. The direction of intrinsic curvature would then dictate the precise (1 bp) nucleosomal position and corresponding major groove helix exposure.

Altogether, we conclude that nucleosome exclusion by poly(dA:dT) sequences at promoters acts as a major force in shaping the chromatin landscape in yeast. Importantly, many types of genes have open accessible promoters despite relative depletion of these sequences, and these genes (*e.g.*, proteasome genes) appear to have promoter packaging that depends more strongly on *trans*-factors such as ATP-dependent chromatin remodelers (see below) and/or the abundant transcription factors known as general regulatory factors (GFRs). The regulatory difference between promoters with intrinsic and *trans*-regulated nucleosome depletion is currently unknown.

Cis-Determinants of Nucleosome Positioning: Statistical Positioning

A number of hypotheses have been advanced to account for the mediocre correspondence between intrinsic sequence preferences for the histone octamer and *in vivo* nucleosome positioning. While it is clear that *trans*-acting proteins are major determinants of *in vivo* nucleosome positioning, another likely contributor to the surprising order observed in budding-yeast nucleosome positions is "statistical positioning"(Kornberg 1981; Kornberg and Stryer 1988). According to this idea, even over sequences without strong nucleosome positioning behavior, nucleosomes could display uniform positioning from cell to cell if packaged into relatively short delimited stretches. One analogy for this behavior is a can of tennis balls: a single tennis ball in a can may occupy a multitude of positions, but when three balls are placed in the can, they occupy well-defined positions due to space constraints.

A great deal of genomic mapping data is consistent with predictions of the statistical positioning hypothesis. Specifically, delocalized or "fuzzy" nucleosomes are enriched distal to 5′ gene ends (Yuan *et al.* 2005), and nucleosome fuzziness increased with increasing distance into the gene body

(Mavrich *et al.* 2008a), as expected if 5′ gene ends played the role of boundaries. Furthermore, the extent of nucleosome positioning displays a nucleosome-length periodicity. In other words, a tennis ball can that is three or four balls long has well-positioned balls, but a can that is 3.5 balls long displays what appears to be a superposition of different packaging states (Vaillant *et al.* 2010). Interestingly, genes with uniform "crystalline" packaging states tend to be expressed more consistently (*i.e.*, with less noise) than genes with multiple packaging states, suggesting that variability in packaging can affect cellular heterogeneity.

One question that remains concerning statistical positioning is, what sets the boundaries of packaging units? Kornberg and Stryer initially proposed that transcription factors would be the barriers (Kornberg 1981; Kornberg and Stryer 1988). Yuan *et al.* (2005) instead suggested that the antinucleosomal poly(dA:dT)s found at so many promoters would behave as barriers, whereas Mavrich *et al.* (2008a) suggested that the +1 nucleosome is the barrier. Evidence for each of these exists, and they are not mutually exclusive. Recently, modeling of statistical positioning via a "Tonks gas" formalism suggested that positioning in coding regions was consistent with the +1 nucleosome providing the barrier, whereas the decay of positioning upstream of genes was more consistent with promoter poly(dA:dT)s forming the barrier to upstream packaging (Möbius and Gerland 2010).

Evolution of Chromatin Packaging

Recent studies in yeast suggest a broad role for chromatin organization in regulatory evolution. In now-classic studies from Brem and Kruglyak, segregants from crosses between two yeast strains (BY and RM) that differ in expression of thousands of genes were used to link individual gene expression levels to either sequence differences at the gene in question (in *cis*) or to distant loci (in *trans*). The majority of gene expression differences were associated with *trans*-acting loci (Brem *et al.* 2002), and later studies showed that most of these regulators of gene expression were chromatin-remodeling enzymes (Brem *et al.* 2002; Lee *et al.* 2006). Conversely, many transcriptional differences between *S. cerevisiae* and *Saccharomyces paradoxus* are due to linked *cis*-polymorphisms, and these polymorphisms are predicted to affect nucleosome occupancy (Tirosh and Barkai 2008; Tirosh *et al.* 2009, 2010a). These results point toward a major role for changes in chromatin structure in the evolution of gene regulation.

The overall extent to which poly(dA:dT) elements are utilized in an organism's genome has varied over evolution. Most fungi examined to date exhibit widespread nucleosome-depleted poly(dA:dT) stretches throughout the genome, but the genomes of *Debaryomyces hansenii* and *S. pombe* have many fewer long poly(dA:dT) stretches and consequently have shorter NFRs (Lantermann *et al.* 2010; Tsankov *et al.* 2010). Poly(dA:dT) appears to play a role in promoter chromatin architecture in *C. elegans* (Valouev *et al.* 2008), but, conversely, promoters in *D. melanogaster* and in mammals are typically GC-rich and are predicted to form stable nucleosomes (Tillo *et al.* 2010). Beyond global changes in AT% at promoters, individual poly(dA:dT) sequences exhibit relatively rapid length changes over evolutionary time (Vinces *et al.* 2009), resulting in poly(dA:dT) expansion/contraction playing a major role in changing gene expression levels across species. Poly(dA:dT) gain and loss often occurs coherently at specific types of genes, and this gain/loss is generally associated with the expected changes in promoter packaging; *i.e.*, genes that gain/lose Poly(dA:dT) stretches in a given species become more/less nucleosome-depleted, respectively (Tirosh *et al.* 2007, 2010a; Field *et al.* 2009; Vinces *et al.* 2009; Tsankov *et al.* 2010). This can often be observed occurring in a coherent fashion in large groups of genes that are linked to the specific physiology of the organism in question. For example, some fungal species (such as *C. albicans*) rely primarily on respiration for energy production, whereas other species (*S. cerevisiae*) will preferentially ferment carbon sources before switching to respiration. In species that primarily respire, the mitochondrial ribosomal (mRP) genes are coregulated with growth genes such as the cytosolic ribosomal proteins, whereas for respirofermentative species, the mRP genes are instead coregulated with stress genes (Ihmels *et al.* 2005). This change in regulatory strategy is accompanied by a sequence-programmed change in chromatin architecture with enrichment of AT-rich sequences upstream of the mRP genes specifically in respiratory species, resulting in wide and deep NFRs in these species (Ihmels *et al.* 2005; Field *et al.* 2009; Tsankov *et al.* 2010). Gain and loss of poly(dA:dT) stretches occur at more localized points in the Ascomycota phylogeny as well, as, for example, in *Yarrowia lipolytica*, whose genome carries many more introns than most fungal genomes and programs splicing genes as growth genes via poly(dA:dT) enrichment (Tsankov *et al.* 2010).

Trans-Determinants of Nucleosome Positioning: General Regulatory Factors

A large number of protein complexes play roles in nucleosome positioning and occupancy, most of which will be discussed below. The key role for *trans*-acting factors as a general class can be appreciated in a recent study showing that ATP-dependent reconstitution of the yeast genome into nucleosomes using yeast extracts resulted in successful establishment not only of NFRs, but also of +1 nucleosomes and of average nucleosome spacing (Zhang *et al.* 2011).

Here, we briefly cover the abundant sequence-specific DNA-binding proteins known as GRFs, since these are the *trans*-factors most simply considered in conjunction with *cis*-determinants of the chromatin state. Early studies identified the GRFs Abf1 and Rap1 as important in the chromatin structure of the *HIS4* promoter (Arndt and Fink 1986; Devlin *et al.* 1991; Yu and Morse 1999; Yarragudi *et al.* 2004), More recently, analysis of genome-wide *in vivo* nucleosome

maps revealed that, in addition to poly(dA:dT), other sequence motifs are associated with nucleosome depletion *in vivo*, and these correspond to the DNA-binding sites for GRFs such as Abf1, Reb1, and Rap1 (Lee *et al.* 2007b; Yarragudi *et al.* (2007); Badis *et al.* 2008; Kaplan *et al.* 2009; Tsankov *et al.* 2010). These sequences are not nucleosome-depleted in *in vitro* nucleosome reconstitutions (Kaplan *et al.* 2009), and this fact has allowed automated determination of GRFs in multiple species by identification of short sequence motifs that are highly nucleosome-depleted *in vivo* but not *in vitro* (Tsankov *et al.* 2010).

Three types of experiment show that GRFs play a causal role in establishing a subset of NFRs, and sometimes in a combinatorial fashion. First, a pioneering experiment from the Madhani laboratory showed that insertion of a short poly(A) stretch adjacent to a Reb1-binding site was sufficient to establish an NFR in a heterologous location (Raisner *et al.* 2005). Second, genetic inactivation of GRF function using temperature-sensitive alleles showed that loss of Abf1, Reb1, and Rap1 (Badis *et al.* 2008; Hartley and Madhani 2009; Ganapathi *et al.* 2011) lead to increased nucleosome occupancy over the relevant sequence motif *in vivo*. Third, a recent study clearly demonstrated that the NFR at the *CLN2* promoter is determined by redundant GRF-binding sites as loss of multiple binding sites for different GRFs was required to abolish the NFR (Bai *et al.* 2011). This study also suggested that many NFRs in yeast involve multiple GRFs (Bai *et al.* 2011). The mechanism of GRF action in nucleosome eviction is currently unclear. These GRFs are highly abundant factors with strong DNA-binding activity, so they could evict nucleosomes simply via competition with dynamic histone proteins. Alternatively, it has been suggested (Hartley and Madhani 2009) that GRFs might recruit the RSC chromatin remodeling complex (see below), resulting in nucleosome eviction. Questions remain as to the biological rationale behind establishment of an NFR via intrinsic sequence determinants *vs.* establishment of NFRs by GRFs. A hint is provided by the observation that certain types of genes, such as proteasome genes, exhibit GRF-driven open promoters in many species without strong enrichment of poly(dA:dT) (Tsankov *et al.* 2010). Unlike other genes with open promoters that are repressed under stress conditions, proteasome genes are upregulated during stress responses (Gasch *et al.* 2000), suggesting that perhaps global control of poly(dA:dT) access during stress is circumvented at GRF-regulated promoters.

Histone Variant H2A.Z

H2A.Z is a histone variant that is widespread throughout eukaryotic chromatin. Several studies have suggested that H2A.Z controls transcription, DNA repair, genome stability, and the control of antisense transcription (for recent reviews, see Zlatanova and Thakar 2008; Marques *et al.* 2010). H2A.Z is highly conserved among eukaryotes (~90% amino acid identity), and it has ~60% amino acid identity with H2A. While it is not essential for viability in *S. cerevisiae* (gene name *HTZ1*) (Santisteban *et al.* 2000) or in *S. pombe* (gene name *pht1*$^+$) (Carr *et al.* 1994), it is essential in several other organisms. Many studies have examined nucleosome structure and function when H2A.Z replaces H2A, but we still lack a clear understanding of precisely how this substitution affects nucleosome stability and interactions.

Location of H2A.Z

Several genome-wide studies have shown that H2A.Z is present in approximately two-thirds of *S. cerevisiae* genes, where it is localized to the first nucleosome downstream of the NFR (the "+1" nucleosome) in the vast majority of those genes and at the upstream ("−1") nucleosome in a smaller subset of genes (Guillemette *et al.* 2005; Li *et al.* 2005; Raisner *et al.* 2005; Zhang *et al.* 2005; Millar *et al.* 2006; Albert *et al.* 2007). H2A.Z is not found at very poorly transcribed genes such as subtelomeric genes and at very highly transcribed genes. Furthermore, H2A.Z is not limited to promoter regions because some H2A.Z-containing nucleosomes are found in the bodies of genes as well. Although H2A.Z is localized to the nucleosomes adjacent to the NFR, it is not required for NFR formation (Li *et al.* 2005; Hartley and Madhani 2009; Tirosh *et al.* 2010b).

How is H2A.Z targeted to promoter nucleosomes? Part of the answer emerged when three labs showed that incorporation occurs via the SWR1 complex, named after its catalytic subunit, Swr1 (Krogan *et al.* 2003a; Kobor *et al.* 2004; Mizuguchi *et al.* 2004). Swr1 contains an ATPase/helicase domain conserved with that found in Swi/Snf chromatin-remodeling complexes (described below). The SWR1 complex contains ~12 proteins, including H2A.Z, and it catalyzes the exchange of histone H2A-H2B dimers with H2A.Z-H2B dimers in an ATP-dependent fashion (Luk *et al.* 2010). Other key members of the complex include Swc2, which specifically recognizes the carboxy-terminal tail of H2A.Z, and Bdf1, which, via its two bromodomains, binds to acetylated histones H3 and H4, helping to target H2A.Z to acetylated promoters. Mutations in the acetylase-encoding genes *GCN5* or *ESA1*, or of the lysines that are acetylated in H3 and H4, all reduce the level of H2A.Z in promoter nucleosomes (Zhang *et al.* 2005; Millar *et al.* 2006; Altaf *et al.* 2010), supporting the idea that acetylation of these histones helps to target H2A.Z to promoter nucleosomes. Conversely, the Ino80 complex carrying the Snf2 homolog Ino80 appears to carry out the reverse reaction, exchanging H2A.Z-H2B dimers for H2A-H2B dimers (Papamichos-Chronakis *et al.* 2011).

Roles for H2A.Z in transcription

Given its widespread location in most promoters, one might anticipate that H2A.Z plays important roles in transcription. However, our understanding of the function of H2A.Z in transcription is still quite cloudy. H2A.Z has been proposed to play a role in the recruitment of the coactivators Swi/Snf, Mediator, and SAGA and of the general transcription factor

TATA-binding protein (Wan *et al.* 2009; Marques *et al.* 2010). While some studies have suggested roles in activation, most genome-wide studies have shown an inverse correlation between H2A.Z levels and transcriptional levels, which has been interpreted to suggest that H2A.Z helps to poise promoters for activation (Li *et al.* 2005; Zhang *et al.* 2005; Zanton and Pugh 2006). Interestingly, in contrast to levels of H2A.Z, genome-wide studies suggest that acetylation of H2A.Z is preferentially associated with highly transcribed genes (Babiarz *et al.* 2006; Keogh *et al.* 2006; Millar *et al.* 2006). A recent study showed that H2A.Z is required for events at the promoter that impact the modification and elongation of RNAPII (Santisteban *et al.* 2011).

Gene expression analysis of *htz1*Δ mutants reveals minor effects of *HTZ1* on steady-state transcript levels, with the major effect being loss of heterochromatin boundary function (Meneghini *et al.* 2003). However, *htz1*Δ mutants have pleiotropic phenotypes: they grow poorly and are temperature sensitive (Santisteban *et al.* 2000), have membrane and ER defects (Lockshon *et al.* 2007; Copic *et al.* 2009), and show synthetic sickness/lethality with a very wide range of other chromatin-related mutants (Collins *et al.* 2007). Two recent studies have cast doubts on whether the effects observed in *htz1*Δ mutants in *S. cerevisiae* are caused by loss of H2A.Z or by the "frustrated" activity of the Swr1 complex in the absence of H2A.Z (Halley *et al.* 2010; Morillo-Huesca *et al.* 2010a). In these studies, mutations that impair Swr1 activity suppress many of the pleiotropic phenotypes caused by an *htz1*Δ. While some studies have suggested a role for H2A.Z in transcriptional memory (Brickner *et al.* 2007; Light *et al.* 2010), other studies dispute this conclusion (Halley *et al.* 2010; Kundu and Peterson 2010). Taken together, the current picture of H2A.Z is murky. Perhaps the most compelling result on H2A.Z function to date comes from *S. pombe*, where loss of H2A.Z leads to increased levels of antisense RNA at genes oriented convergently (Zofall *et al.* 2009), leading to the suggestion that this 5′-constrained histone variant serves to "inform" the cell when the 3′ end of a transcript comes from inappropriate antisense transcription. It will be interesting to see whether similar results hold in budding yeast. We anticipate that future studies will help disentangle the role of H2A.Z in transcription, genome stability, and other processes.

Chromatin-Remodeling Factors

Chromatin-remodeling factors are multi-protein complexes that use the energy of ATP hydrolysis to mobilize nucleosomes, resulting in lateral sliding (Lomvardas and Thanos 2001; Fazzio and Tsukiyama 2003) or removal from DNA (Boeger *et al.* 2004; Cairns 2005), among other activities. Eukaryotic cells contain four families of chromatin-remodeling complexes: Swi/Snf, Iswi, Chd, and Ino80 [see Clapier and Cairns (2009) for an excellent review]. In mammals, chromatin-remodeling complexes can function in tissue-specific ways to control development, and mutations that impair these complexes have been implicated in oncogenesis. In this section, we focus on some of the yeast chromatin-remodeling factors that play extensive roles in gene regulation: the closely related factors Swi/Snf and RSC and the Isw family (Isw1 and Isw2). The Swr1 complex was discussed above, and more information about the related Ino80 complex can be found in two excellent reviews (Conaway and Conaway 2009; Morrison and Shen 2009).

While it appears that ATP-dependent remodelers share a common basic mechanism—disrupting histone–DNA interactions—the different classes of complexes have very different effects on nucleosome position, stability, and composition. Swi/Snf and RSC appear to destabilize nucleosomes; the Isw family predominantly functions to slide nucleosomes laterally; and others, including Swr1 and Ino80, appear to affect H2A/H2B dimer exchange (Clapier and Cairns 2009). While this review does not focus on the mechanism of nucleosome remodeling, readers are referred to two seminal studies of RSC remodeling (Saha *et al.* 2002, 2005), as well as several other important mechanistic studies of RSC (Lorch *et al.* 1998, 2006, 2010; Zhang *et al.* 2006; Fischer *et al.* 2007; Leschziner *et al.* 2007) and other complexes (see Clapier and Cairns 2009 and references cited therein) that have provided significant insight into this topic.

Identification of the Swi/Snf and RSC complexes

The *S. cerevisiae* Swi/Snf complex was the first chromatin-remodeling complex discovered; subsequent identification of conserved complexes from other organisms established the universal nature of this type of activity throughout eukaryotes. The Swi/Snf complex and the related RSC complex are highly homologous to one another: both complexes are large ~10- to 12-subunit complexes; both have several homologous subunits such as the ATPase subunit Snf2/Sth1; and both share two components, Arp7 and Arp9. Despite their similarities, RSC is ~10-fold more abundant, and RSC is essential for viability whereas Swi/Snf is not.

The identification of Swi/Snf originated from two unrelated genetic screens for regulatory mutants. The *swi* mutants were identified in a screen for mutants unable to transcribe *HO*, a gene required for mating-type switching (Stern *et al.* 1984). Three of the genes identified, *SWI1*, *SWI2*, and *SWI3*, shared a set of pleiotropic phenotypes that suggested that they control the expression of several genes. The *snf* mutants were found in a screen for mutants unable to express the *SUC2* gene, which encodes invertase, required for utilization of sucrose as a carbon source (Neigeborn and Carlson 1984). Similar to the *swi* screen, mutations in three genes, *SNF2*, *SNF5*, and *SNF6*, caused a shared set of pleiotropic phenotypes.

Several steps led to the elucidation that the *SWI* and *SNF* genes encode members of a multi-protein complex. In one set of studies, fusions of Snf2 or Snf5 to the LexA DNA-binding domain were shown to activate transcription, but only when the other *SWI/SNF* genes were wild type (Laurent *et al.* 1991; Laurent and Carlson 1992). In other studies, *SWI* and *SNF*

genes were shown to be required for transcriptional activation at several different genes, leading to the idea that the putative Swi/Snf complex assists gene-specific activators (Peterson and Herskowitz 1992; Laurent *et al.* 1993a). Sequence analysis and biochemistry demonstrated that the Swi2/Snf2 protein is an ATPase (Laurent *et al.* 1992, 1993b). Subsequently, the Swi/Snf complex was purified from yeast and shown to contain the five gene products encoded by the *SNF2/SWI2*, *SNF5*, *SNF6*, *SWI1*, and *SWI3* genes, as well as other proteins (Cairns *et al.* 1994; Peterson *et al.* 1994). Contemporaneously, the mammalian Swi/Snf complex was also identified (Kwon *et al.* 1994).

In contrast to Swi/Snf, RSC was discovered by biochemical approaches. *STH1*, the gene encoding the RSC Swi2/Snf2-like ATPase, was identified by sequence similarity to *SNF2* (Laurent *et al.* 1992; Tsuchiya *et al.* 1992). Once the *S. cerevisiae* genome sequence was completed, homologs of other *SWI/SNF* genes were identified. Sth1 and these other homologs copurified in a complex that, like Swi/Snf, has ATP-dependent chromatin-remodeling activity (Cairns *et al.* 1996). Many of the genes encoding RSC components are essential for viability. In *S. pombe*, RSC is also essential for viability, although its composition differs substantially from that in *S. cerevisiae* (Monahan *et al.* 2008). *S. pombe* RSC has recently been shown to play a role in heterochromatin structure (Garcia *et al.* 2010).

Swi/Snf complexes have chromatin-remodeling activity

Genetic analysis first suggested that Swi/Snf might alleviate transcriptional repression caused by nucleosomes. First, it was shown that suppressors of *snf2/swi2* mutations included mutations in *HTA1-HTB1*, encoding histones H2A-H2B, and in *SPT6*, encoding a histone chaperone (Neigeborn *et al.* 1986, 1987; Clark-Adams and Winston 1987; Hirschhorn *et al.* 1992). This genetic relationship between Swi/Snf and chromatin was fortified by other results that showed that suppressors of *swi1*, *swi2*, and *swi3* mutations were in histone H3- and H4-encoding genes (Prelich and Winston 1993; Kruger *et al.* 1995). Thus, genetics suggested that the transcriptional activation defects caused by loss of Swi/Snf could be bypassed by reducing or altering nucleosome function.

The genetic results led to the model that Swi/Snf serves to overcome transcriptional repression by nucleosomes by altering histone–DNA interactions. This model was tested by an examination of the *SUC2* regulatory region, which showed that *SUC2* chromatin structure is more MNase sensitive in wild-type strains than in *snf2* and *snf5* mutants (Hirschhorn *et al.* 1992; Matallana *et al.* 1992), consistent with the idea that Swi/Snf functions to remove nucleosomes. To address the cause/effect relationship of chromatin structure with transcription, MNase sensitivity was assayed in wild-type and *snf5* mutants when the *SUC2* TATA box was mutant, abolishing *SUC2* transcription. The same MNase differences were seen as with a wild-type TATA, suggesting that Swi/Snf causes transcriptional changes, rather than the other way around (Hirschhorn *et al.* 1992).

The model that Swi/Snf directly alters chromatin structure was tested biochemically, using both yeast (Cote *et al.* 1994) and mammalian Swi/Snf complexes (Imbalzano *et al.* 1994; Kwon *et al.* 1994; Wang *et al.* 1996). These studies demonstrated that purified Swi/Snf alters nucleosome structure in an ATP-dependent fashion to help activators bind to their sites and to make the nucleosomal DNA more accessible to nuclease digestion. Thus, mutants defective for expression of two genes, *SUC2* and *HO*, led to the discovery of chromatin-remodeling complexes. Once biochemical assays were established, it became straightforward to test other purified complexes, such as RSC, which have been shown to have a similar activity (Cairns *et al.* 1996).

Regulation of transcription by Swi/Snf

The extent of transcriptional control by Swi/Snf was investigated by genome-wide transcriptional studies (Holstege *et al.* 1998; Sudarsanam *et al.* 2000). Under the growth conditions tested, Swi/Snf was shown to control the mRNA levels of 2–5% of all yeast genes. Affected genes do not fall into particular functional categories, although these data led to the discovery that Swi/Snf function is important for transcription during M phase (Krebs *et al.* 2000). More recently, analysis of the heat-shock response showed that Swi/Snf directly regulates both ribosomal protein genes and genes under the control of heat-shock factor (Shivaswamy and Iyer 2008). These microarray studies likely underestimate the extent to which Swi/Snf controls transcription, as few conditions were tested. Other studies have shown important roles for Swi/Snf in the regulation of glucose-repressed genes (Neigeborn and Carlson 1984) and genes induced during amino acid starvation (Natarajan *et al.* 1999), conditions not tested by microarrays. Expression studies under less optimal growth conditions will likely reveal other facets of Swi/Snf regulation.

In many cases, Swi/Snf functions in combination with other transcriptional regulators, with each contributing to expression. An early clue that Swi/Snf can function in a combinatorial or redundant fashion with other factors came from the discovery that *swi/snf* mutations cause lethality when combined with mutations in genes encoding members of the SAGA coactivator complex (Roberts and Winston 1997). For example, when *snf2*Δ is combined with *gcn5*Δ (*GCN5* encodes the histone acetyltransferase within SAGA), the double mutants are either inviable (Pollard and Peterson 1997) or extremely sick (Roberts and Winston 1997). There is strong evidence that both Swi/Snf chromatin-remodeling activity and Gcn5 histone-modifying activity function at an overlapping set of genes, including *SUC2* (Sudarsanam *et al.* 1999), *HO* (Cosma *et al.* 1999; Mitra *et al.* 2006), *PHO5* (Barbaric *et al.* 2007), *GAL1* (Biggar and Crabtree 1999), and Gcn4-activated genes (Govind *et al.* 2005). Other combinations also function together, such as Swi/Snf and Asf1 (Gkikopoulos *et al.* 2009).

Several studies have examined Swi/Snf recruitment and function at promoters. Recruitment likely occurs by direct

interaction with transcriptional activators (Yudkovsky *et al.* 1999). As several different activators can recruit Swi/Snf, the nature of the activator–Swi/Snf interaction is of interest; studies have shown that two or three Swi/Snf subunits can participate in recruitment (Neely *et al.* 2002; Prochasson *et al.* 2003; Ferreira *et al.* 2009). Once at a promoter, the association of Swi/Snf is stabilized by the Snf2 bromodomain (Hassan *et al.* 2001, 2002), which binds acetylated histone tails (Dhalluin *et al.* 1999); this represents an example of cooperation between Gcn5 histone acetylation and Swi/Snf and is consistent with reports that Swi/Snf association is Gcn5 dependent (Govind *et al.* 2005; Mitra *et al.* 2006). Although Swi/Snf acts at 5′ regulatory regions to remodel or evict nucleosomes, it also appears to have a role in elongation in both yeast (Schwabish and Struhl 2007) and mammalian cells (Sullivan *et al.* 2001; Corey *et al.* 2003).

In vitro studies showed that Swi/Snf catalyzes a stable change in nucleosome structure that persists in the absence of Swi/Snf (Owen-Hughes *et al.* 1996; Cote *et al.* 1998). However, *in vivo* studies using *snf2* and *snf5* temperature-sensitive mutants suggested a continuous need for Swi/Snf (Biggar and Crabtree 1999; Sudarsanam *et al.* 1999). This continuous requirement may be in part from a requirement for Swi/Snf for transcription elongation.

RSC plays broad roles in gene expression and chromatin structure

In contrast to Swi/Snf, RSC is involved in transcriptional regulation of genes transcribed by both RNA polymerases II and III. Several cases have shown the involvement of RSC in particular classes of RNAPII-dependent transcription (*e.g.*, see Moreira and Holmberg 1999; Bungard *et al.* 2004; Taneda and Kikuchi 2004; Govind *et al.* 2005; Mas *et al.* 2009; Erkina *et al.* 2010). Genome-wide studies show that RSC binds at hundreds of RNAPII promoters, many of which carry a specific sequence motif for the Rsc3 and Rsc30 DNA-binding subunits of RSC (Angus-Hill *et al.* 2001; Damelin *et al.* 2002; Ng *et al.* 2002b; Badis *et al.* 2008). It is not yet clear if recruitment to Rsc3/30 sequence motifs is the sole mechanism for recruitment of RSC to RNAPII promoters. At many promoters, RSC is required to maintain NFRs, which gain nucleosome occupancy upon RSC loss (Badis *et al.* 2008; Parnell *et al.* 2008; Hartley and Madhani 2009). RSC appears to have other effects on promoter chromatin as well; as mentioned earlier, RSC is required to maintain a particular chromatin structure over the *GAL1–10* regulatory region that features a partially unwound nucleosome (Floer *et al.* 2010). RSC also controls transcription elongation, with its recruitment stimulated by histone acetylation (Carey *et al.* 2006; Ginsburg *et al.* 2009; Mas *et al.* 2009). Finally, RSC also binds at several hundred RNAPIII-dependent genes (Damelin *et al.* 2002; Ng *et al.* 2002b), and loss of RSC function in conditional *sth1* degron mutants results in increased nucleosome occupancy and decreased transcription at RNAPIII genes (Parnell *et al.* 2008).

Bromodomains in Swi/Snf and RSC

Subunits of Swi/Snf and RSC, like many other chromatin-related proteins, carry bromodomains, which bind acetylated lysines (Haynes *et al.* 1992; Dhalluin *et al.* 1999; Zeng and Zhou 2002). In Swi/Snf, there is a bromodomain in Snf2, while RSC has bromodomains in Sth1, Rsc1, Rsc2, and Rsc4. The Snf2 bromodomain stabilizes interactions with acetylated lysines in histone H3 *in vitro*, and that loss of this domain has a modest effect on Swi/Snf function *in vivo* (Hassan *et al.* 2001). These results led to the notion that histone acetylation sets the stage for stable recruitment of Swi/Snf.

In RSC, bromodomain roles are more complex as Rsc1, Rsc2, and Rsc4 each have two bromodomains. Rsc1 and Rsc2 are mutually exclusive members of RSC, defining distinct forms of the complex (Cairns *et al.* 1999). Loss of either component allows viability, but *rsc1*Δ *rsc2*Δ double mutants are inviable. Mutational analysis showed that the second domain in either Rsc1 or Rsc2 is required for function. In contrast, BD#1 is required only in Rsc2 and only for a small subset of functions. As the bromodomains are not required for assembly of RSC, they are likely required for a subsequent activity, most likely binding to acetylated chromatin. At present it is unclear whether the bromodomains are required in combination with Rsc3/30 DNA binding to associate over NFRs (Badis *et al.* 2008; Hartley and Madhani 2009) or for some other type of interaction with chromatin.

In Rsc4, each bromodomain is required for function, as deletion of either one causes inviability (Kasten *et al.* 2004). Biochemical experiments showed that one of the bromodomains recognizes H3K14ac, but, surprisingly, the other bromodomain binds to an acetylated lysine on Rsc4 itself (Vandemark *et al.* 2007). Intramolecular binding of the bromodomain to acetylated Rsc4 inhibits binding of the other bromodomain to H3K14ac. Since Gcn5 acetylates both Rsc4 and H3K14, Gcn5 has both activating and repressing roles in the association of RSC with chromatin. The precise role of Rsc4 acetylation is not known, and there are only modest phenotypes when this modification is abolished (Vandemark *et al.* 2007). However, loss of Rsc4 acetylation does cause lethality when combined with mutations that abolish acetylation of histone H3 (Choi *et al.* 2008). We anticipate future studies will help decipher the importance of this form of regulation in RSC function, and we anticipate that many more nonhistone-binding partners for bromodomains await identification.

Actin-related proteins in Swi/Snf and RSC

Biochemical analysis of Swi/Snf complexes, as well as some of the other classes of chromatin-remodeling complexes (such as histone-modifying complexes), has shown that they contain either actin or actin-related proteins (Arps) (for a review see Chen and Shen 2007). The actin-related proteins are structurally similar to actin with modest conservation and ~20% amino acid identity. In *S. cerevisiae*, Swi/Snf and RSC share the actin-related proteins Arp7 and Arp9 (Cairns *et al.* 1998; Peterson *et al.* 1998). Both proteins

are important for function as deletion of either *ARP7* or *ARP9* causes lethality in one genetic background (S288C) and extremely poor growth in another (W303) (Cairns *et al.* 1998). The viability in the W303 background, along with the isolation of temperature-sensitive mutations in *ARP7* and *ARP9*, has permitted phenotypic analysis, revealing defects in transcription. Interestingly, directed mutations predicted to impair ATP hydrolysis by Arp7 and Arp9 do not cause mutant phenotypes, suggesting that this activity is not important for their function (Cairns *et al.* 1998).

The isolation of suppressors of *arp7* and *arp9* mutations led to important insights into Arp7 and Arp9 function within Swi/Snf and RSC. First, a previously unknown domain, the HSA (**h**elicases-**S**ANT-**a**ssociated) domain, was identified as a conserved domain in *SNF2*-like proteins and was shown to be required for assembly of Arp7 and Arp9 into RSC (Szerlong *et al.* 2008). Second, suppressors of *arp7* and *arp9* temperature-sensitive mutants were identified in two domains of *STH1*, called the post-HSA domain (adjacent to the HSA domain), and protrusion 1 (located in the catalytic ATPase domain). The HSA domain, the post-HSA domain, and the protrusion 1 region are all required for full ATPase activity of RSC, although activity is only down twofold in mutants that impair these domains (Szerlong *et al.* 2008). Taken together, these results suggest that the Arp7/9 module plays a role in regulation of the ATPase activity of Sth1. Consistent with this finding, Snf2 can be purified as a stable complex with Arp7 and Arp9 in *swi3*Δ mutants, and this complex has many of the activities of the complete Swi/Snf complex (Yang *et al.* 2007).

Among different chromatin-remodeling complexes, Arps play different roles. For example, in RSC, the Arps are not strongly required for ATPase activity *in vitro*; however, in Ino80, both Arp5 and Arp8 have crucial roles as either an *arp5*Δ or *arp8*Δ mutation abolishes the activity of Ino80 and an *arp8*Δ mutation causes loss of Arp4 and actin from the Ino80 complex (Shen *et al.* 2003). Analysis of the Arps in *S. pombe* Swi/Snf and RSC revealed several differences from *S. cerevisiae* (Monahan *et al.* 2008). First, there is no Arp7 in *S. pombe*; instead, *S. pombe* RSC and Swi/Snf contain Arp42 (a member of the Arp4 group) and Arp9. Second, deletion of *ARP42*, *ARP9*, or both does not cause a growth defect in rich medium. This striking difference between *S. cerevisiae* and *S. pombe* indicates that much remains to be learned about the roles of Arps in chromatin-remodeling complexes.

Isw-family remodelers

In contrast to the histone eviction function largely exhibited by Swi/Snf and Swr family remodeling complexes, Isw ATP-dependent remodelers "slide" histones along the DNA without evicting them, resulting in different consequences. For example, Isw2 is a chromatin remodeler whose major regulatory role is as a repressor. At some genes, such as *RNR3*, Isw2 contributes to repression in a redundant fashion (Zhang and Reese 2004). At the *POT1* promoter, Isw2 functions to move a nucleosome from its thermodynamically preferred sequence-directed site in the *POT1* coding region, 70 bp 5′, to a less-favored site toward the NFR, where it represses transcription by occluding the promoter region (Whitehouse and Tsukiyama 2006). Subsequent whole-genome mapping of Isw2 showed that it associates with tRNA genes as well as with ∼20% of RNA Pol II genes (Whitehouse *et al.* 2007). Comparison of genome-wide nucleosome positioning maps between wild-type and *isw2*Δ mutant cells revealed that ∼35% of Isw2-bound targets (∼400 genes) are subject to detectable Isw2-mediated chromatin remodeling. The +1 nucleosomes are shifted up to 70 bp (15 bp average) away from the NFR region in mutant cells, suggesting that in wild-type cells Isw2 inhibits transcription by positioning nucleosomes over the TSS and the NFR. Isw2-mediated cpin *isw2*Δ cells. This often occurrs at genes oriented tandemly (as opposed to convergently), suggesting a potential role for this repositioning in antisense transcriptional control. Indeed, surprisingly, Isw2-mediated repositioning of nucleosomes turns out to repress antisense noncoding transcription by positioning nucleosomes over cryptic transcription start sites in these intergenic regions (Whitehouse *et al.* 2007).

Isw1 also acts to move nucleosomes laterally although less is known about the biology of Isw1 and *isw1*Δ mutants, which have only mild phenotypes (Tsukiyama *et al.* 1999). Isw1 partners with several alternative subunits—Ioc2, Ioc3, or Ioc4—to form a variety of complexes (Vary *et al.* 2003; Mellor and Morillon 2004). Genome-wide mapping of nucleosomes in *isw1*Δ yeast identified a widespread role for Isw1 in nucleosome positioning over coding regions. In an *isw1*Δ mutant, nucleosomes throughout coding regions shift from 3′ to 5′, indicating that Isw1 plays a role in shifting nucleosomes forward (Tirosh *et al.* 2010b). Isw1-remodeled genes share no particular annotations, but tend to be enriched for H3K79me3, suggesting that Isw1 might be recruited or regulated by this modification. Functionally, the chromatin perturbations associated with loss of Isw1 are uncorrelated with changes in mRNA abundance in the mutant, but are enriched at genes previously shown to contain "cryptic" internal promoters (Li *et al.* 2007b; Cheung *et al.* 2008). Furthermore, *isw1*Δ mutants exhibit derepression of the canonical *FLO8* internal promoter (Kaplan *et al.* 2003), suggesting that a major role for Isw1 *in vivo* is to maintain repressive chromatin over coding regions to repress cryptic promoters.

Histone Modifications and Transcription Initiation

In addition to chromatin-remodeling activities, post-translational modifications of histones, particularly acetylation, play widespread roles in transcription initiation throughout eukaryotes. (Table 2 summarizes current knowledge about the different histone modifications found in yeast.) Histone acetylation has long been suspected of playing a role in transcriptional regulation (Allfrey *et al.* 1964), and once *S. cerevisiae* became a model system for studying histones, the quest was on to identify the enzymes that acetylate and

deacetylate them. However, the first HAT identified in yeast, Hat1, did not show any detectable role in transcription (Travis *et al.* 1984; Kleff *et al.* 1995; Parthun *et al.* 1996). Subsequently, however, several yeast HATs, histone deacetylases (HDACs), and other histone modification enzymes that play roles in transcription have been discovered (for reviews see Millar and Grunstein 2006; Li *et al.* 2007a; Smith and Shilatifard 2010).

Two breakthroughs opened the floodgates to studying histone acetylation in transcription initiation. First, Gcn5, a previously identified factor known to play a role in transcriptional activation (Penn *et al.* 1983; Berger *et al.* 1990; Georgakopoulos *et al.* 1995), was shown to be a HAT (Brownell *et al.* 1996). This was the first demonstration that loss of a HAT caused transcriptional changes, and it was soon followed by the identification of its mammalian counterparts, PCAF and Gcn5, in addition to other mammalian HATs. The fact that *gcn5* mutants have particular regulatory defects originally suggested that Gcn5 HAT activity might be targeted to specific promoters, although the current interpretation is that Gcn5 has widespread or even universal activity, but only a subset of promoters require acetylation for normal expression. Second, Rpd3, another previously identified transcription factor associated primarily with repression (Vidal and Gaber 1991), was shown to be an HDAC (Taunton *et al.* 1996). Together, these results established that histone acetylation plays a role in transcriptional activation *in vivo*.

The next big step was the identification of *S. cerevisiae* protein complexes that contain HAT activities. In a landmark paper, Grant *et al.* (1997) purified yeast nuclear protein complexes that contained HAT activity and identified four complexes, two of which contain Gcn5. Gcn5-containing complexes were also identified at the same time by independent studies (Pollard and Peterson 1997; Saleh *et al.* 1997). The largest and best characterized of the Gcn5-containing complexes is named SAGA (**S**pt-**A**da-**G**cn5-**A**cetyltransferase), a multiprotein, multifunctional complex that plays extensive roles in transcription initiation and elongation throughout eukaryotes (reviewed in Koutelou *et al.* 2010). SAGA acetylates histones H3 and H2B in a Gcn5-dependent fashion (Grant *et al.* 1997). It is now known that Gcn5 is present in at least two other SAGA-related complexes *in vivo* (Grant *et al.* 1997; Pray-Grant *et al.* 2002; Sterner *et al.* 2002; Wu and Winston 2002). In *S. cerevisiae*, Gcn5 controls mRNA levels of a large number of genes, albeit only a subset of those controlled by SAGA (Lee *et al.* 2000); in addition, ChIP-chip analysis showed that SAGA is localized to all active promoters at a level that correlates with their activity (Robert *et al.* 2004). Interestingly, Gcn5 also represses transcription of certain genes in both budding and fission yeast (Ricci *et al.* 2002; Helmlinger *et al.* 2008).

In addition to Gcn5, several other HATs have been identified in *S. cerevisiae*. There are three MYST-class HATs (named after the consensus sequence MYST), Sas2, Sas3, and Esa1, the last of which is the only *S. cerevisiae* HAT that is essential for viability (Reifsnyder *et al.* 1996; Smith *et al.* 1998; Clarke *et al.* 1999). Two of these HATs were found in the original identification of HAT complexes: NuA3, containing Sas3, and NuA4, containing Esa1 (Grant *et al.* 1997; Allard *et al.* 1999). The third MYST member, Sas2, which, along with Sas3, was initially identified by defects in transcriptional silencing (Reifsnyder *et al.* 1996), plays a role in counteracting silencing in yeast (Kimura *et al.* 2002; Suka *et al.* 2002). Another HAT, Rtt109, is discussed in a later section on the histone chaperone Asf1.

NuA4, which contains Esa1, has been extensively characterized (Allard *et al.* 1999; see Doyon and Cote 2004 for a review). In contrast to Gcn5, Esa1 acetylates H4, H2A, and H2A.Z. Interestingly, NuA4 shares subunits with three other complexes: Tra1, also in SAGA/SLIK; Arp4, also in Swi/Snf, RSC, and SWR1; and Yaf9, Swc4, and Act1, also

Table 2 Histone modifications in *S. cerevisiae*

Histone	Residue	Modification	Modification enzymes
H2A	K5	Ac	Esa1, Rpd3
	K8	Ac	Esa1, Hat1, Rpd3
	S122	P	
	T126	P	
	K126	Sumo	
	S129	P	Mec1, Tel1, Pph3
H2AZ	K3	Ac	Esa1
	K8	Ac	Esa1
	K10	Ac	Esa1
	K14	Ac	Esa1
H2B	K6/K7	Sumo	
	S10	P	Ste20
	K11	Ac	Esa1, Rpd3
	K16	Ac	Gcn5, Esa1, Rpd3, Hda1
	K123	Ub	Rad6, Ubp8
H3	R2	Me	
	K4	Me, Ac	Set1, Jhd2, Rtt109, Gcn5
	K9	Ac	Gcn5, Rpd3, Hos2, Hda1
	S10	P	Snf1
	K14	Ac	Gcn5, Rpd3, Hos2, Hda1
	K18	Ac	Gcn5, Rpd3, Hos2, Hda1
	K23	Ac	Gcn5, Rpd3, Hos2, Hda1
	K36	Me	Set2, Rph1, Jhd1
	K42	Me	
	K56	Ac	Rtt109, Hst3, Hst4
	K79	Me	Dot1
H4	S1	P	CK2
	R3	Me	
	K5	Ac	Esa1, Rpd3, Hos2
	K8	Ac	Esa1, Rpd3, Hos2
	K12	Ac	Esa1, Rpd3, Hos2
	K16	Ac	Esa1, Sas2, Sir2, Hos2, Hst1
	K20	Ac	Esa1, Sas2, Sir2, Hos2, Hst1
	K31	Me	

Most of the information for this table came from Krebs (2007). Information for Htz1 came from Babiarz *et al.* (2006), Keogh *et al.* (2006), and Millar *et al.* (2006), information for sumoylation came from Nathan *et al.* (2006), and information for H3K42 methylation came from Hyland *et al.* (2011).

in SWR1. NuA4 is involved in a multitude of activities in the nucleus, including transcription, double-strand break repair, silencing, and the cell cycle, and many of these activities correlate with Esa1 activity [see Decker *et al.* (2008) and references cited therein]. With respect to transcription, NuA4 function has been studied at several single genes such as *PHO5*, where it associates with the regulatory region under repressing conditions but plays an essential role in activation during phosphate starvation (Nourani *et al.* 2004). More globally, microarray analysis showed that the major class of NuA4-regulated genes during exponential growth is composed of those encoding ribosomal proteins (Reid *et al.* 2000). ChIP-chip analysis showed that NuA4 is localized to all active promoters at a level that correlates with their activity (Robert *et al.* 2004), indicating that NuA4 plays a fairly general role in transcriptional regulation. A smaller complex, Piccolo NuA4, that contains only three NuA4 components, including Esa1, has been identified and is believed to play a role in global histone acetylation (Boudreault *et al.* 2003).

One underappreciated aspect of histone-modifying enzyme biology is that many of these enzymes modify nonhistone substrates. For example, Gcn5 not only acetylates histones, but regulates RSC by acetylation of Rsc4 (Vandemark *et al.* 2007; Choi *et al.* 2008) and was recently shown to acetylate Swi/Snf as well (Kim *et al.* 2010). A proteome-wide screen for targets of the NuA4 HAT complex identified many nonhistone proteins and showed that one of them, Pck1, requires acetylation to have full enzymatic activity (Lin *et al.* 2009). In mammalian cells, the Gcn5 ortholog, PCAF, acetylates p53, and this modification is important for p53 function (Liu *et al.* 1999; Barlev *et al.* 2001). Similar results are observed for the histone methylases (below). Thus, it will be important in the future to separate the results of histone and nonhistone modification to understand the phenotypes of modifying enzyme mutants and to understand the logic underlying the suite of substrates affected by each enzyme.

In addition to HATs, several HDACs control transcription initiation, generally by conferring repression (for reviews see Millar and Grunstein 2006; Krebs 2007). HDACs play broad roles in transcription (Robyr *et al.* 2002) and are often recruited by the global repressor complex Cyc8/Tup1 (Davie *et al.* 2003). Studies at several genes suggest that a balance of acetylation and deacetylation activities plays a key role in normal regulation (*e.g.*, see Krebs *et al.* 1999). At some genes, HDACs play positive roles in transcription initiation (*e.g.*, Sharma *et al.* 2007), although in one case this effect is likely indirect due to negative regulation of a noncoding RNA (Bumgarner *et al.* 2009).

Histone Modifications During Transcription Elongation

In *S. cerevisiae*, a series of histone modifications occurs over transcribed regions (for recent reviews see Fuchs *et al.* 2009; Smith and Shilatifard 2010). These modifications constitute a subset of those identified in *S. pombe* and in larger eukaryotes. In this section, we will focus on the set of modifications that have been studied most extensively, including acetylation, ubiquitylation, and methylation, all associated with active transcription.

SAGA and NuA4 acetylate nucleosomes during transcription

Several studies have shown that both SAGA- and NuA4-dependent histone modifications occur across coding regions during transcription elongation. Chromatin immunoprecipitation studies show that both SAGA (Govind *et al.* 2007; Wyce *et al.* 2007) and NuA4 (Ginsburg *et al.* 2009) are associated across coding regions. One study demonstrated that SAGA stimulates levels of H3 acetylation, RNAPII levels, mRNA levels, and nucleosome eviction at *GAL1* (Govind *et al.* 2007). Another study showed that SAGA controls the level of H2B ubiquitylation; the SAGA subunit Ubp8 is an H2B deubiquitylase and is required for the recruitment of the C-terminal repeat domain (CTD) kinase Ctk1 to allow proper elongation (Wyce *et al.* 2007). Consistent with these results, H2B ubiquitylation helps to reassemble nucleosomes in the wake of RNAPII in an Spt16-dependent fashion (Fleming *et al.* 2008). In contrast to SAGA, NuA4 stimulates H4 acetylation and is required for normal elongation by RNAPII (Ginsburg *et al.* 2009), at least in part because it is required for the recruitment of RSC and subsequent nucleosome eviction (Ginsburg *et al.* 2009). This result fits well with *in vitro* analysis of NuA4–RSC interactions (Carey *et al.* 2006), which showed that RSC recruitment *in vitro* is dependent upon histone acetylation, likely due to the binding of a Rsc4 bromodomain to acetylated histones (Kasten *et al.* 2004; Carey *et al.* 2006).

Current evidence suggests that SAGA and NuA4 act in a partially redundant fashion to promote transcription elongation. Analysis of *gcn5Δ esa1* double mutants showed a significant defect in elongation *in vivo* (Ginsburg *et al.* 2009), while analysis of single mutants showed less of an effect (Govind *et al.* 2005; Ginsburg *et al.* 2009).

Histone methylation during transcription

In contrast to histone acetylation, the roles for histone lysine methylation in transcriptional control are relatively poorly understood. Trimethylation of H3K4 (H3K4me3) occurs over the 5′ nucleosomes of actively transcribed genes, with di- and mono-methylation (H3K4me2 and H3K4me) occurring more extensively across coding regions (Bernstein *et al.* 2002; Santos-Rosa *et al.* 2002; Ng *et al.* 2003b; Liu *et al.* 2005; Pokholok *et al.* 2005). H3K4 methylation is dependent upon the methyltransferase Set1 and its associated COMPASS complex (Miller *et al.* 2001; Roguev *et al.* 2001; Nagy *et al.* 2002), but it is also dependent upon several other cellular factors (for reviews see Shilatifard 2008; Fuchs *et al.* 2009). Ubiquitylation of histone H2B is required

to recruit COMPASS to actively transcribed genes (Dover *et al.* 2002; Sun and Allis 2002), as this modification is required for stable association of the COMPASS component Cps35 within the complex (Lee *et al.* 2007a). Furthermore, H2B ubiquitylation and, hence, H3K4 methylation, is dependent upon the PAF complex and, specifically, the PAF1 component Rtf1 (Ng *et al.* 2003a; Wood *et al.* 2003; Warner *et al.* 2007). The kinase Bur1 is required specifically for H3K4 trimethylation (Laribee *et al.* 2005), as well as for other histone modifications (Wood *et al.* 2005; Chu *et al.* 2006, 2007; Zhou *et al.* 2009). A large variety of histone-binding proteins, including those with PHD fingers (Shi *et al.* 2007), are regulated by H3K4 methylation, and readers are directed to recent reviews (Eissenberg and Shilatifard 2010; Smith and Shilatifard 2010) for complete lists. For example, binding of H3K4me3 by the ING homolog Yng1, in the NuA3 complex, affects H3 acetylation by the NuA3 complex, providing one of many examples of histone modification "cross talk." In another example, H3K4me2 has been shown in one study to recruit the Set3 histone deacetylases complex to *YEF3* to regulate acetylation levels across coding regions (Kim and Buratowski 2009), although another study showed that Set3 could be recruited to *ARG1* independently of H3K4 methylation (Govind *et al.* 2010).

Surprisingly, despite the many factors involved in the regulation of H3K4, and the universal occurrence of H3K4me3 at the 5′ ends of transcribed genes, the fact remains that in a *set1* mutant, where no H3K4me occurs, there are relatively few significant changes in transcription (Venkatasubrahmanyam *et al.* 2007). Indeed, while H3K4 methylation is associated with actively transcribed genes, in some cases it can be repressive (Carvin and Kladde 2004). A hint regarding the biological role for H3K4 methylation in transcriptional control comes from the above-noted role for another 5′-directed mark, H2A.Z, in control of antisense transcription (Zofall *et al.* 2009). Both repression of Ty1 by an unstable antisense RNA (Berretta *et al.* 2008) and repression of *PHO84* by an antisense transcript (Camblong *et al.* 2009) have been suggested to operate in *trans*, and in both systems repression requires Set1. We anticipate that future studies may reveal a general role for the "active mark" H3K4me3 in enabling repression of transcription by antisense transcripts. In any case, the universal occurrence and complex regulation of H3K4me3, coupled with the subtle effects of *SET1* deletion on transcription, make this system one of the most interesting mysteries in chromatin biology today.

A clearer role has been established for H3K36 methylation, which requires the methyltransferase Set2 (Strahl *et al.* 2002; Schaft *et al.* 2003). Similar to H3K4 trimethylation, H3K36 trimethylation is found over actively transcribed genes, but H3K36me3 occurs over the middle and 3′ ends of transcribed genes due to recruitment of Set2 by the elongating form of RNAPII (Krogan *et al.* 2003b; Xiao *et al.* 2003; Pokholok *et al.* 2005; Rao *et al.* 2005). Interestingly, Set2 activity also requires specific interactions with histones H3, H4, and H2B (Du *et al.* 2008; Du and Briggs 2010). H3K36me3 is not required for elongation, but rather is required to activate the histone deacetylase complex Rpd3S along transcribed chromatin, in turn leading to deacetylation of actively transcribed templates (Carrozza *et al.* 2005; Joshi and Struhl 2005; Keogh *et al.* 2005; Pokholok *et al.* 2005; Drouin *et al.* 2010; Govind *et al.* 2010). *set2* mutants are fully viable and grow well; however, the level of histone acetylation is higher than normal across transcribed regions. The major consequence of this change is the occurrence of transcription initiation at a large number of "cryptic" promoters that occur within coding regions (Carrozza *et al.* 2005; Joshi and Struhl 2005), which were first identified in *spt6* and *spt16* mutants (described below). As expected, mutations in genes encoding Rpd3S components also result in activation of a subset of cryptic promoters.

These results provide a canonical example for the mechanism behind the "context dependence" of histone modifications: H3K36me3 is a universal modification in the sense that it is deposited over all transcribed regions, yet its loss affects only a small number of genes that happen to have coding regions that include "cryptic" promoter-like sequences. Thus, H3K36me3 is perhaps the clearest case in which the seeming paradox of global deposition with localized effects has been explained in detail. We hope that future studies will shed equal illumination on examples like H3K4me3 and *HTZ1*, among others.

Less clear is the role of H3K79 methylation. The methyltransferase required for this modification, Dot1 (Feng *et al.* 2002; Ng *et al.* 2002a; van Leeuwen *et al.* 2002), was initially identified as affecting transcriptional silencing at telomeres (Singer *et al.* 1998). Similar to H3K4 methylation, K3K79 methylation is dependent upon H2B ubiquitylation (Briggs *et al.* 2002; Wood *et al.* 2003). Also as with H3K4 and H3K36 trimethylation, H3K79 methylation is essentially universal, occurring throughout coding regions, although unlike K4 and K36 methylation, K79 methylation levels exhibit very little correlation with transcription levels. The silencing defect of *dot1* mutants is believed to arise from the fact that H3K79 methylation blocks the binding of Sir proteins; loss of H3K79 methylation leads to promiscuous binding of the Sir complex throughout the genome, titrating the Sir complex away from normally silent regions (van Leeuwen *et al.* 2002). It seems unlikely that this is the only function of H3K79 methylation in transcriptional control, but our current understanding of this modification is limited.

Histone Dynamics

Much of the above discussion treats chromatin structure as essentially static in the absence of transcriptional perturbations, but this could not be further from the truth. Nucleosomes move laterally and/or are evicted in response to environmental perturbations, and even at "steady state" can be replaced multiple times in a given cell cycle. In this section we discuss histone dynamics, starting with a discussion of

dynamic responses to the environment and ending with steady-state dynamics in an unchanging environment.

Histone eviction and replacement during changes in transcription

Nucleosomes are commonly evicted from the promoters of genes during transcriptional activation. Furthermore, they are also sometimes evicted from coding regions during high levels of transcription. As described earlier, the classic paradigm for the removal of nucleosomes from promoters is the *PHO5* promoter, where four nucleosomes are removed from the promoter upon phosphate starvation and subsequent Pho4 binding (Almer *et al.* 1986; Boeger *et al.* 2003; Reinke and Horz 2003; Boeger *et al.* 2004; Korber *et al.* 2004). Similar behavior is seen at many other promoters already mentioned, including *GAL1–10* (Selleck and Majors 1987; Axelrod *et al.* 1993; Lohr and Lopez 1995), *HSP82* (Gross *et al.* 1993; Zhao *et al.* 2005), and *ARG1* (Govind *et al.* 2010). Genome-wide studies have shown this to occur at a number of stress-activated genes, most commonly those containing TATA boxes (Lee *et al.* 2004; Shivaswamy *et al.* 2008; Zawadzki *et al.* 2009). Conversely, upon repression of *PHO5*, nucleosomes are rapidly reassembled onto the promoter (Adkins and Tyler 2006). Similarly, in most cases where nucleosomes are evicted during transcription elongation, they are efficiently reassembled in the wake of elongating RNAPII. What factors are responsible for nucleosome eviction and reassembly?

Steady-state histone dynamics

Even in the absence of environmental perturbation, nucleosomes are not static entities. In bulk, it has long been known that histones are among the most stably bound proteins in the cell. In mammals, fluorescence recovery after photobleaching studies show that most DNA-associated proteins exchange with the free pool of protein with half-lives on the order of seconds, but histones have a recovery time on the order of 30+ min (Kimura and Cook 2001). However, pioneering work in Drosophila showed that specific histone isoforms are exchanged within a cell cycle. The H3 isoform H3.3 is replaced throughout the cell cycle (Ahmad and Henikoff 2002), whereas the H3.1 isoform is incorporated into DNA only during replication.

In yeast, which does not have separate H3.1 and H3.3 isoforms (yeast H3 most closely resembles H3.3), discerning replication-independent histone dynamics ("turnover") has been more difficult. Nonetheless, by using pulse-chase approaches, several investigators have been able to follow the incorporation of new histone molecules in cells prevented from going through genomic replication. Briefly, an epitope-tagged histone (H3 in several studies, H2B in one) is driven by an inducible promoter (Schermer *et al.* 2005). Yeast are arrested in the cell cycle to prevent replication, then HA-H3 (for example) is induced, and at varying times after induction HA-H3 mapping is carried out to identify loci undergoing exchange with the free histone pool (Dion *et al.* 2007; Jamai *et al.* 2007; Rufiange *et al.* 2007).

These studies provide multiple insights into steady-state dynamic behavior of histone molecules. First, H3 turnover is rapid over promoters and other intergenic regions (such as replication origins), but very slow (less than one exchange per cell cycle) over most coding regions, despite ongoing transcription. Second, H3 turnover over coding regions can occur, but only at very high transcription rates (see below). Third, whereas H3 replacement is quite heterogeneous over the genome, H2B replacement was observed to be rapid at both promoters and coding regions, with the only "cold" genomic loci observed in a small-scale study being the heterochromatic subtelomeric regions (Jamai *et al.* 2007).

Histone dynamics: mechanism

What is the mechanistic basis for histone dynamics? First, it is worth noting that there are likely a large number of mechanisms at play in nucleosome eviction, with different mechanisms acting at different genomic loci. Conversely, it is likely that fewer nucleosome deposition activities exist and that they act more globally to fill in gaps in chromatin structure left after eviction events.

At promoters, nucleosomes are evicted by two major classes of factor: the ATP-dependent remodelers such as Swi/Snf and RSC, described above (and which will not be further treated here), and transcription factors. Regarding the latter, while nucleosomes typically prevent transcription factor association with their binding sites, under certain circumstances, transcription factors alone can disrupt a nucleosome (Workman and Kingston 1992). Mechanistically, it has been observed that DNA located near the entry/exit points on the octamer surface transiently unwraps from the octamer (Anderson and Widom 2000, 2001; Anderson *et al.* 2002; Poirier *et al.* 2008), and thus transcription factors that bind to sites located in these regions can trap the partially open nucleosome state. Interestingly, promoters with more transcription factor binding sites tend to exhibit more rapid H3/H4 replacement than do promoters with fewer transcription factor binding sites (Dion *et al.* 2007; Field *et al.* 2008), consistent with a transcription factor–nucleosome competition model.

Over coding regions, RNAPII is the most likely candidate for nucleosome eviction. However, as detailed above, *in vitro*, RNAPII is capable of transiting a nucleosome without dissociating the octamer from DNA (Kulaeva *et al.* 2009, 2010), and, *in vivo*, most coding regions exhibit little H3/H4 turnover (Dion *et al.* 2007). Interestingly, histone replacement over coding regions is more rapid at stress genes than at growth genes after correcting for RNAPII abundance (Dion *et al.* 2007; Jamai *et al.* 2007; Rufiange *et al.* 2007), which may be related to the fact that transcription occurs at stress genes in "bursts" rather than via evenly spaced polymerases. Furthermore, *in vitro*, it has been shown that after one round of transcription the original nucleosome loses an H2A/H2B dimer, and running a second polymerase into this hexameric nucleosome results in complete histone removal (Kulaeva *et al.* 2007, 2009, 2010; Jin *et al.* 2010).

Together, these results support a model in which RNA polymerase passage through chromatin leaves behind a number of "damaged" hexameric nucleosomes, with nucleosome dynamics then depending on the subsequent race between hexamer repair and a second polymerase. At highly transcribed, or "bursty," genes, the rapid occurrence of a second polymerase causes H3/H4 eviction. In support of this model, Strubin and colleagues observed that mutants in the putative H2A/H2B chaperone Spt16 exhibit increased H3/H4 eviction over genes, suggesting that Spt16 plays a key role in repairing hexameric nucleosomes that have lost an H2A/H2B (Jamai *et al.* 2009). Other evidence, however, suggests that Spt16 functions on whole nucleosomes rather than just H2A/H2B (Xin *et al.* 2009). Similarly, the histone chaperone Vps75 biochemically resembles the H2A chaperone Nap1 and binds H2A/H2B *in vitro*, yet *vps75*Δ mutants exhibit increased H3/H4 turnover (Kaplan *et al.* 2008; Selth *et al.* 2009), revealing yet another link between octamer integrity and H3/H4 dynamics.

While there may be many different ways to evict a nucleosome, all share in common the fact that histone molecules must be transferred to some type of acceptor—the histone chaperones. In multiple studies, yeast lacking various histone chaperones exhibit slowed H3/H4 turnover dynamics. This has been observed globally for Asf1 (Rufiange *et al.* 2007; Kaplan *et al.* 2008), Rtt106 (Imbeault *et al.* 2008), and the CAF-1 and Hir complexes (Rosa *et al.* 2010), although *spt16* mutants, as cited above, do show increased turnover (Jamai *et al.* 2009). Similarly, in *spt6* mutants there is slow histone redeposition at *PHO5* (Adkins and Tyler 2006), and nucleosomes are generally depleted from highly transcribed genes (Ivanovska *et al.* 2011). It is important to note that steady-state turnover studies report on both eviction and replacement and thus do not distinguish between these two processes, but mutants that preferentially affect histone incorporation over eviction are expected to exhibit decreased nucleosome occupancy (Fillingham *et al.* 2009; Ivanovska *et al.* 2011), while the converse will be true of mutants that preferentially act in histone eviction. Furthermore, in activation/repression paradigms (*e.g.*, *PHO5* induction) the two processes can be disentangled. Interestingly, it is often inferred from histone occupancy studies that histone chaperone mutants affect the kinetics of both processes to similar extents.

Histone dynamics: consequences

What is the biological role of histone replacement? Mutants that affect histone chaperones typically have pleiotropic phenotypic effects, as would be expected from factors that play roles in global chromatin dynamics. One fairly common feature of mutants that affect global histone dynamics is that expression of the histone genes is altered. As noted above, the HIR histone chaperone complex was originally isolated as a regulator of the H3/H4 promoter. Thus, it is important to be aware that the phenotypes described below may reflect the effects of changing histone levels, rather than histone dynamics *per se*.

Histone dynamics are intimately related to epigenetic silencing. Heterochromatic genes are protected from rapid histone turnover and, in fact, are the only tested loci that do not exhibit H2A/H2B replacement (Jamai *et al.* 2007). Furthermore, the boundary elements that constrain the spreading of heterochromatin complexes (Valenzuela and Kamakaka 2006) exhibit rapid histone replacement (Dion *et al.* 2007), which has been speculated to play a mechanistic role in boundary function. If a spreading chromatin state is being constantly erased via turnover, then this will prevent further spread. As might be expected from the above observations, mutants in many histone turnover factors, from Rtt109 to Asf1, exhibit silencing defects.

Histone dynamics are also intimately related to suppression of retrotransposons, a fact highlighted by the identification of numerous histone turnover factors such as Spt6, Spt16, Rtt106, and Rtt109 in the *SPT* and *RTT* screens, as described above. Finally, histone replacement plays a significant role in the kinetics of gene induction/repression. As an example, various mutants that delay nucleosome eviction from the *PHO5* promoter upon phosphate starvation, such as *asf1*Δ or *rtt109*Δ, display delayed mRNA expression as well (Adkins *et al.* 2004; Korber *et al.* 2006; Williams *et al.* 2008).

Histone Chaperones

Several factors, referred to as histone chaperones, that are believed to play essential roles in the removal and replacement of histones from promoters and transcribed regions have been identified (for reviews see Williams and Tyler 2007; Eitoku *et al.* 2008; Park and Luger 2008; Das *et al.* 2010; Avvakumov *et al.* 2011). These factors interact with nucleosomes *in vitro*; associate with chromatin *in vivo*; and facilitate histone deposition, exchange, or eviction from chromatin. While most of these factors are conserved throughout eukaryotes, they were originally found in yeast from mutant hunts that initially had no obvious connection to chromatin. Interestingly, many of these chaperones appear to play multiple roles in transcriptional control (Table 1) and, furthermore, most show genetic interactions with each other (*e.g.*, see Malone *et al.* 1991; Swanson and Winston 1992; Kaufman *et al.* 1998; Sutton *et al.* 2001; Formosa *et al.* 2002; Takahata *et al.* 2009). In this section, our understanding of many of these chaperones will be summarized.

Asf1 and its roles as a histone chaperone in histone acetylation and its interactions with the HIR and CAF chaperones

One of the best-characterized chaperones is Asf1 (**a**nti-**s**ilencing **f**actor), which plays a prominent role in transcription initiation and elongation, as well as DNA replication and repair (Eitoku *et al.* 2008). Asf1 was first identified in yeast in high-copy-number screens for effects on silencing (Le *et al.* 1997; Singer *et al.* 1998). Subsequently, it was identified in Drosophila and mammalian cells and shown to be a histone

chaperone (Tyler *et al.* 1999; Munakata *et al.* 2000). Asf1 is associated with promoter regions in yeast (Schwabish and Struhl 2006) and is required for the eviction of promoter nucleosomes upon induction of the *PHO5* gene (Adkins *et al.* 2004; Korber *et al.* 2006), but not for their reassembly upon repression. MNase studies of an *asf1*Δ mutant suggest that Asf1 is globally required for nucleosome eviction (Adkins and Tyler 2004). Furthermore, Asf1 is also associated with several coding regions in yeast where it was shown to be required for the eviction of histone H3, but not H2B (Schwabish and Struhl 2006).

Biochemical and structural studies of Asf1 have provided a detailed understanding of its chaperone function. Biochemical studies showed that Asf1 interacts with a region of H3 that is required for H3–H3 interactions in an H3-H4 tetramer (Munakata *et al.* 2000; Mousson *et al.* 2005) and binds to an H3-H4 heterodimer (English *et al.* 2005). This finding was surprising, as it had been commonly believed that the H3-H4 intermediate in nucleosome assembly and disassembly was a tetramer (for a review see Akey and Luger 2003), although more recent studies have provided evidence for tetramer splitting *in vivo* (Xu *et al.* 2010; Katan-Khaykovich and Struhl 2011). In the meantime, structural studies showed that the amino-terminal 155 amino acids of Asf1, which are sufficient for function *in vivo*, form an immunoglobulin-like structure and provide evidence for regions that bind to histone H3 and to the histone chaperone Hir1 (Daganzo *et al.* 2003; Mousson *et al.* 2005). The structure of this region bound to an H3-H4 heterodimer, combined with genetic studies, revealed interactions between Asf1 and the histones H3 and H4 that suggests that Asf1 might split the H3-H4 tetramer by binding to H3 and altering the conformation of the carboxy-terminus of H4 to stabilize the Asf1–H3-H4 interaction (English *et al.* 2006). Taken together, these studies have provided the most in-depth view of the function of a histone chaperone.

Asf1 also forms a complex with Rtt109, a histone acetyltransferase for H3K56, and Asf1 is required for Rtt109-catalyzed H3K56 acetylation (Recht *et al.* 2006; Schneider *et al.* 2006; Collins *et al.* 2007; Driscoll *et al.* 2007; Han *et al.* 2007; Tsubota *et al.* 2007). This finding fits well with the observation that regions of high H3/H4 turnover are enriched for K56 acetylation, and *asf1*Δ mutants have slower H3/H4 turnover (Rufiange *et al.* 2007; Kaplan *et al.* 2008). At the *PHO5* gene, H3K56 acetylation increases upon induction (Williams *et al.* 2008), presumably by increased exchange at the *PHO5* promoter, as H3K56 acetylation occurs only on free histones and not on nucleosomes (Tsubota *et al.* 2007). H3K56 is located near the entry/exit points of DNA on the histone octamer, and K56-acetylated nucleosomes have been suggested to be less stable than unacetylated nucleosomes. Consistent with this idea, H3K56 acetylation is required for nucleosome eviction and induction at *PHO5* (Williams *et al.* 2008). Furthermore, several phenotypes of *asf1*Δ mutants—including slow growth, slow *PHO5* induction, and hydroxyurea sensitivity—are partially suppressed in an H3K56Q mutant, which mimics the acetylated state (Recht *et al.* 2006). Overall, these studies show that H3K56 acetylation is a significant component of the role of Asf1 in transcriptional control. Its role in transcription elongation remains to be determined.

Asf1 has been genetically and biochemically tied to two other chaperone complexes, HIR and CAF-1. The four members of the HIR complex were initially identified by mutations defective for transcription of histone genes (Osley and Lycan 1987; Xu *et al.* 1992). The HIR complex functions in several chromatin-related processes including chromatin assembly (Sharp *et al.* 2001; Green *et al.* 2005; Prochasson *et al.* 2005), kinetochore function (Sharp *et al.* 2002), and transcription elongation (Formosa *et al.* 2002; Nourani *et al.* 2006). HIRA, the human homolog of yeast Hir1 and Hir2 (Hall *et al.* 2001), is also a histone chaperone (Ray-Gallet *et al.* 2002), and Hira of *S. pombe* is required for both heterochromatin formation and repression of antisense transcription (Blackwell *et al.* 2004; Anderson *et al.* 2009; Yamane *et al.* 2011).

The CAF-1 complex was originally identified from Hela cells as an activity that assembles nucleosomes onto replicating DNA (Stillman 1986; Smith and Stillman 1989). In yeast, CAF-1 is also able to assemble nucleosomes *in vitro* (Kaufman *et al.* 1997). Somewhat surprisingly, deletions of any of the three yeast genes encoding CAF-1 subunits results in only mild phenotypes (Kaufman *et al.* 1997). Genetic connections among Asf1, HIR, and CAF-1 emerged from several studies in yeast that showed that these factors cooperate in control of transcription, silencing, and kinetochore function (Kaufman *et al.* 1998; Sharp *et al.* 2001, 2002; Sutton *et al.* 2001). Furthermore, biochemical and structural studies from *S. cerevisiae*, *S. pombe*, and mammalian cells have provided strong evidence for physical interactions between these complexes (Sutton *et al.* 2001; Mello *et al.* 2002; Daganzo *et al.* 2003; Tagami *et al.* 2004; Tang *et al.* 2006; Malay *et al.* 2008). Evidence from mammalian cells (Tagami *et al.* 2004) and *S. pombe* (Malay *et al.* 2008) suggests that Asf1 exists in independent complexes with either the HIR or CAF-1 complex, as binding of either HIR or CAF-1 to Asf1 is mutually exclusive (Malay *et al.* 2008). Interestingly, CAF-1 also physically interacts with another histone chaperone, Rtt106 (Huang *et al.* 2005, 2007). More recent studies have shown that Rtt106 plays a general role in both regulation of histone gene transcription (Fillingham *et al.* 2009) and general transcription initiation (Imbeault *et al.* 2008).

Spt6 and FACT: factors controlling transcriptional integrity

Spt6 and FACT are two conserved chaperones that interact directly with nucleosomes to modulate transcription and chromatin structure. We have grouped them here as *spt6* and *spt16* mutants share many mutant phenotypes (*e.g.*, Malone *et al.* 1991; Kaplan *et al.* 2003; Mason and Struhl 2003; Cheung *et al.* 2008), although they do not associate

with chromatin in an identical pattern across the genome (Mayer *et al.* 2010).

Spt6 Spt6 was originally identified in *S. cerevisiae* by several mutant hunts (Table 1). Spt6 is essential for viability in *S. cerevisiae* (Clark-Adams and Winston 1987; Neigeborn *et al.* 1987) [athough not in *C. albicans* (Al-Rawi *et al.* 2010) or *S. pombe* (Kiely *et al.* 2011)]. Spt6 also plays critical or essential roles in mammalian cells (Yoh *et al.* 2007, 2008), zebrafish (Keegan *et al.* 2002; Kok *et al.* 2007; Serluca 2008), *Drosophila* (Formosa *et al.* 2002; Ardehali *et al.* 2009), and nematodes (Nishiwaki *et al.* 1993). In addition to chromatin structure and transcription, Spt6 functions in recombination (Malagon and Aguilera 2001), mRNA surveillance and export (Andrulis *et al.* 2002; Estruch *et al.* 2009), and histone modifications (Carrozza *et al.* 2005; Chu *et al.* 2007; Youdell *et al.* 2008). Thus, Spt6 appears to play roles in most chromatin-mediated processes.

Spt6 forms a heterodimeric complex with another protein, Spn1/Iws1 (Fischbeck *et al.* 2002; Krogan *et al.* 2002; Lindstrom *et al.* 2003). This interaction, believed to be dynamic and to govern the ability of Spt6 to interact with nucleosomes (McDonald *et al.* 2010), is required for several steps in transcription—from initiation (Zhang *et al.* 2008) to histone modifications, RNA processing, and mRNA export (Yoh *et al.* 2007, 2008). Structural analyses of Spn1 and the Spn1–Spt6 complex have recently been described (Diebold *et al.* 2010a; McDonald *et al.* 2010; Pujari *et al.* 2010). Although these two proteins appear to interact, they do not have the same pattern of association across the yeast genome (Mayer *et al.* 2010).

Spt6 is a large protein (1451 amino acids in *S. cerevisiae*) with multiple domains that suggest interactions with DNA, RNA, and several proteins in addition to Spn1 (Doherty *et al.* 1996; Johnson *et al.* 2008; Dengl *et al.* 2009; Close *et al.* 2011). Among its domains are tandem SH2 domains at its carboxy-terminal end, the only SH2 domains in *S. cerevisiae* (Maclennan and Shaw 1993; Dengl *et al.* 2009; Diebold *et al.* 2010b; Sun *et al.* 2010; Close *et al.* 2011; Liu *et al.* 2011). The SH2 domains are required for Spt6 to interact with the CTD of Rpb1 of RNAPII, for normal levels of Spt6 recruitment to chromatin *in vivo*, and for wild-type function (Dengl *et al.* 2009; Diebold *et al.* 2010b; Mayer *et al.* 2010; Sun *et al.* 2010; Close *et al.* 2011; Liu *et al.* 2011).

The control of chromatin structure by Spt6 is likely direct, as *in vitro* studies have demonstrated direct interactions of Spt6 with histones (Bortvin and Winston 1996; Winkler *et al.* 2000) and nucleosomes (McDonald *et al.* 2010) and that Spt6 can assemble nucleosomes *in vitro* (Bortvin and Winston 1996). To bind nucleosomes, Spt6 requires the HMG protein, Nhp6 (McDonald *et al.* 2010), similar to FACT (see below). The region of Spt6 required for interaction with nucleosomes is in the amino-terminal region and overlaps with the region required for Spt6–Spn1 interactions (McDonald *et al.* 2010). *In vivo*, Spt6 is required to maintain a normal level of nucleosomes across highly transcribed coding regions (Kaplan *et al.* 2003; Ivanovska *et al.* 2011). The Spt6–chromatin connection is also supported by genetic interactions: *spt6* mutations suppress the loss of the Swi/Snf chromatin-remodeling complex (Neigeborn *et al.* 1986, 1987; Bortvin and Winston 1996), and *spt6* mutations themselves are suppressed by elevated levels of histone H3 (Bortvin and Winston 1996). Spt6 also affects histone modification, as it is required for normal levels of H3K36 di- and trimethylation (Carrozza *et al.* 2005; Chu *et al.* 2007; Youdell *et al.* 2008), although the effects of Spt6 on histone modifications could be an indirect consequence of its effects on chromatin structure (Youdell *et al.* 2008). Taken together, these results suggest that direct Spt6–histone interactions control chromatin structure *in vivo*.

The consequences of Spt6-dependent chromatin effects on transcription are broad, varied, and to a large degree remain to be understood. Genetic and biochemical studies have suggested that Spt6 controls transcription initiation, elongation, and 3′ end formation. However, although Spt6 associates with coding regions genome-wide (Mayer *et al.* 2010; Ivanovska *et al.* 2011), there is little understanding of what makes transcription of some genes Spt6 dependent and others not (Ivanovska *et al.* 2011). With respect to initiation, *spt6* mutants are defective for nucleosome reassembly or positioning over some promoter regions during transcriptional repression (Adkins and Tyler 2006; Jensen *et al.* 2008; Ivanovska *et al.* 2011), and *spt6* mutations suppress some promoter insertions or deletions (Winston *et al.* 1984; Prelich and Winston 1993). Elongation is also controlled by Spt6 on the basis of both *in vitro* (Endoh *et al.* 2004) and *in vivo* (Ardehali *et al.* 2009) studies. A role in elongation is supported by the finding that Spt6 interacts directly with the elongating form of RNAPII (Endoh *et al.* 2004; Yoh *et al.* 2007), that Spt6 facilitates elongation on a chromatin-free template *in vitro* (Endoh *et al.* 2004), and that Spt6 localizes across coding regions *in vivo*, with the level of Spt6 association corresponding to the level of transcription (Andrulis *et al.* 2000; Kaplan *et al.* 2000, 2005; Krogan *et al.* 2002; Mayer *et al.* 2010; Ivanovska *et al.* 2011).

One of the key roles for Spt6 during elongation is to repress cryptic promoters within coding regions (Kaplan *et al.* 2003; Cheung *et al.* 2008). In *spt6* mutants, a genome-wide assay revealed that cryptic initiation occurs at ~1000 genes (Cheung *et al.* 2008). This level of cryptic initiation is likely an underestimate of the true level, as this study looked only at coding strands, and the method of detection would have found cryptic initiation only in genes transcribed at low levels (Cheung *et al.* 2008; Lickwar *et al.* 2009). Cryptic initiation has also been observed in several other mutants, including *spt16* and *set2* (Kaplan *et al.* 2003; Mason and Struhl 2003; Carrozza *et al.* 2005; Prather *et al.* 2005; Nourani *et al.* 2006; Li *et al.* 2007b; Xiao *et al.* 2007; Cheung *et al.* 2008; Imbeault *et al.* 2008), with *spt6* and *spt16* mutants having the strongest effects (Cheung *et al.* 2008).

There are clearly multiple mechanisms that normally repress cryptic initiation in wild-type cells. One mechanism

operates by maintaining a deacetylated state for nucleosomes across coding regions. As detailed above, this state is dependent both on the elongating form of RNAPII and H3K36 methylation for the recruitment and the activation of the Rpd3S HDAC complex, which deacetylates histones (Carrozza *et al.* 2005; Joshi and Struhl 2005; Keogh *et al.* 2005; Drouin *et al.* 2010; Govind *et al.* 2010). Impairing this deacetylation results in cryptic promoters being more permissive for initiation (Govind *et al.* 2007, 2010; Ginsburg *et al.* 2009). However, there may be other mechanisms for repression of cryptic promoters, as many mutants that allow cryptic initiation have normal levels of H3K36 methylation (Cheung *et al.* 2008). In *spt6* mutants, cryptic initiation may be caused by multiple reasons in addition to loss of H3K36 methylation, including an inability to reassemble nucleosomes in the wake of elongating RNAPII (Kaplan *et al.* 2003) and defective recruitment of two factors, Spt2 and Elf1, that each contribute to the repression of cryptic promoters (Prather *et al.* 2005; Nourani *et al.* 2006).

FACT The *S. cerevisiae* FACT complex is composed of two proteins, Spt16 and Pob3, that act, along with the HMG protein Nhp6, as a histone chaperone during transcription (Brewster *et al.* 2001; Formosa *et al.* 2001). The FACT complex was also discovered in mammalian cells by a biochemical assay for factors that allow transcription elongation *in vitro* across a chromatin template (Orphanides *et al.* 1998). The purified mammalian FACT complex was shown to contain two proteins, Spt16 and SSRP, a bipartite protein that resembles both Pob3 and Nhp6 (Orphanides *et al.* 1999). Spt16 was initially identified in yeast by mutant screens for cell division cycle mutations that arrest at G1 (Prendergast *et al.* 1990; Rowley *et al.* 1991), for genes that, when overexpressed, cause an Spt^- phenotype (Malone *et al.* 1991), and as an activator of the *SWI4* gene (Lycan *et al.* 1994). Pob3 was initially identified biochemically as a protein that strongly interacts with DNA polymerase α, a biochemical screen that also found Spt16 (Wittmeyer *et al.* 1999).

FACT functions in both transcription initiation and elongation. The basis for the purification and characterization of mammalian FACT, transcription *in vitro* along a chromatin template (Orphanides *et al.* 1998; Belotserkovskaya *et al.* 2003), and *in vivo* studies in flies (Saunders *et al.* 2003) led to the idea that FACT is devoted to transcription elongation. This view was reinforced by studies in yeast that showed that Spt16 physically interacts with other elongation factors (Krogan *et al.* 2002; Squazzo *et al.* 2002; Simic *et al.* 2003), that Spt16 is localized across coding regions (Mason and Struhl 2003; Kim *et al.* 2004; Mayer *et al.* 2010), and that FACT has genetic interactions suggesting a role in reassembling nucleosomes in the wake of RNAPII elongation (Formosa *et al.* 2002). An additional study identified a histone H3 mutant that alters the pattern of Spt16 association across transcribed regions in an allele-specific fashion, suggesting that a direct H3–Spt16 interaction is important for its recruitment during elongation (Duina *et al.* 2007). However, there is substantial evidence that FACT also functions in initiation, as FACT regulates TBP, TFIIB, and RNAPII binding over promoter regions (Mason and Struhl 2003; Biswas *et al.* 2005; Ransom *et al.* 2009), and *spt16* mutations display genetic interactions with *spt15* (TBP), *toa2* (TFIIA), and *spt3* (SAGA) mutations, all of which affect initiation (Biswas *et al.* 2005). At the *PHO5* promoter, FACT is required to remove H2A-H2B dimers under derepressing conditions, presumably a step required prior to Asf1-dependent removal of H3-H4 tetramers (Adkins and Tyler 2004; Ransom *et al.* 2009). The most direct evidence for FACT functioning in initiation comes from studies of *HO* (Takahata *et al.* 2009). In this case, during the process of *HO* induction, FACT becomes physically associated with a specific region of the *HO* regulatory region, URS2, where it acts with Asf1 to promote nucleosome loss, a requirement for coactivator recruitment to this region. This is the first and only demonstration to date of FACT physical association with a regulatory region.

Several biochemical studies have addressed the mechanism by which FACT functions as a histone chaperone. These studies identified regions in both yeast and mammalian FACT components that interact directly with multiple histones and nucleosomes (Orphanides *et al.* 1999; Belotserkovskaya *et al.* 2003; Vandemark *et al.* 2006; Stuwe *et al.* 2008). One study suggests that there are redundant interactions of different histones with both Spt16 and Pob3 that contribute to nucleosome binding by FACT (Vandemark *et al.* 2006). With respect to mechanism, *in vitro* binding and transcription studies of mammalian FACT suggested that FACT removes one H2A-H2B dimer during elongation to facilitate the passage of RNAPII (Orphanides *et al.* 1999; Belotserkovskaya *et al.* 2003). However, other studies disagree with this model. One study, using DNaseI sensitivity as an assay for nucleosome structure, suggested that FACT decreases histone–DNA interactions in many locations around the nucleosome (Rhoades *et al.* 2004). A more recent study takes this analysis further, using restriction enzyme accessibility as an assay, and provides evidence that FACT relaxes histone–DNA interactions in many places around a nucleosome (Xin *et al.* 2009). In this study, H2A-H2B dimer loss was a variable consequence of nucleosomal reorganization by FACT and was not required for increased accessibility to DNA *in vitro*, nor did it occur *in vivo* upon transcriptional activation (Xin *et al.* 2009). From these results, the authors propose that FACT reversibly destabilizes nucleosomes to facilitate the passage of RNAPII. Such an activity can also account for the roles of FACT in initiation and other chromatin-related processes, as well as for the observations of the role of FACT in histone recycling (Jamai *et al.* 2009) and free histone levels (Morillo-Huesca *et al.* 2010b). Given the *in vitro* (Belotserkovskaya *et al.* 2003) and *in vivo* (Ransom *et al.* 2009) evidence that FACT may promote H2A-H2B dimer removal under some conditions, however, the consequences of FACT activity may be

varied, influenced by the myriad of other chaperones that exist and that may have gene- or sequence-specific roles.

Perspectives

Much of our understanding of how chromatin structure controls transcription has come from pioneering studies in yeast. We believe that yeast studies will continue in a leadership role in helping to unravel the roles of histone modifications, histone exchange, chromatin-remodeling complexes, and histone chaperones in the control of transcription. While many genome-wide studies have already provided detailed descriptive analysis, the future will provide new information, as there will be more characterization of mutants and different growth conditions by genome-wide approaches, as well as comparative studies in other yeasts (*e.g.*, Tsankov *et al.* 2010).

One emerging field in which yeast will likely play a leading role is in the elucidation of roles of noncoding RNAs (ncRNAs) in regulating transcription and chromatin structure. Studies in both *S. cerevisiae* and *S. pombe* have already identified regulatory roles for several ncRNAs that occur by a diversity of mechanisms (for reviews see (Berretta and Morillon 2009; Winston 2009). In some cases, ncRNAs control chromatin structure (Hirota *et al.* 2008; Hainer *et al.* 2011; Thebault *et al.* 2011) or histone modifications (Houseley *et al.* 2008; Camblong *et al.* 2009; Pinskaya *et al.* 2009). Other types of control will likely emerge from other recent studies (*e.g.*, Hongay *et al.* 2006; Bumgarner *et al.* 2009).

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