The factors required for the delivery of RNA polymerase II to class II promoters using naked DNA were all identified by 1998, yet their exact mechanisms of action were not fully understood in all cases, and in some instances, their precise function still remains unknown. Nonetheless, a complete understanding of the complexity of the RNA polymerase II transcription cycle necessitated the development of assays that include chromatinized DNA templates. At this time, the field was actively searching for factors that allow transcription initiation on chromatinized templates. We began studies using chromatin templates in an attempt to identify factor(s) that permit RNA polymerase II to traverse nucleosomes, i.e. that allow elongation on chromatinized DNA templates. The challenge herein was to develop an assay that directly measured the ability of transcriptionally engaged RNA polymerase II to traverse nucleosomes. This approach resulted in the isolation of FACT, a heterodimer in humans comprised of Spt16 and SSRP1. Defined functional biochemical assays corroborated genetic studies in yeast that allowed the elucidation of FACT function in vivo. Collectively, these approaches demonstrate that FACT is a factor that allows RNA polymerase II to traverse nucleosomes in vitro and in vivo by removing one H2A/H2B dimer. More recent studies using a fully defined chromatin reconstitution/transcription assay revealed that FACT activity is greatly stimulated by post-translational modification of the histone polypeptides, specifically by monoubiquitination of lysine 120 of human histone H2B.

The Discovery of FACT

A major challenge toward understanding the mechanistic aspects of transcriptional regulation is the recapitulation of this enzymatic process in vitro under physiological conditions. In the nucleus of cells, genomic DNA associates with numerous proteins to form chromatin. By its nature, DNA accessibility is highly restrictive when assembled into chromatin, which serves in a multifunctional capacity. At its most simple level, chromatin plays a role in limiting DNA accessibility for many processes, including transcription. In vitro approaches, therefore, are necessary in order to understand transcriptional regulation and its many implications. In particular, reconstitution of transcriptional machinery on chromatin-based templates is a particularly challenging problem in the field of transcriptional regulation.

Early stages of gene activation require that promoters and other cis-elements are made available to their cognate transcriptional regulators. However, the cell must also deal with chromatin during all stages of mRNA biogenesis, including transcript elongation and mRNA processing events (1). The complete reconstitution of RNAPII transcription on naked DNA with purified, well defined factors remains a prized accomplishment toward establishing the basis for transcriptional regulation. However, it was established early on that transcription initiation and transcript elongation on chromatin templates in vitro are far less efficient in comparison with naked DNA (2, 3). This discrepancy between naked and chromatinized templates suggested that specific factors are required for efficient transcription within the context of nucleosomes. In vivo, activators are required for efficient transcription although activator binding in vitro can be hampered by nucleosomes (4, 5). To determine the minimal requirements for transcriptional activation on chromatin templates, a fully reconstituted transcription system was employed (6) (supplemental Fig. S1). Physiologically spaced nucleosomes were assembled using Drosophila embryo extract, and the resultant template was purified to remove contaminating proteins from the extract and templates that were not properly assembled. The GAL4-VP16 transcriptional activator, added to the DNA prior to chromatin assembly, was utilized to obtain remodeled chromatin templates at the promoter. The isolated chromatinized DNA was then incubated with the general transcription factors (IID, IIB, IIE, IIF, and IIH (7)), RNAPII, and the co-activators TFIIA and PC4. Proteins used in this reconstituted system were either bacterially produced or highly purified to complete the highly defined system.

Although transcription on naked DNA was observed in both the reconstituted system and in HeLa nuclear extracts, only nuclear extracts supported transcription on chromatinized templates, measured by the production of an ~400-nucleotide RNA. Therefore, a biochemical complementation assay was established to identify the soluble factor(s) required for chromatin-based transcription (supplemental Fig. S1A). Old-fashioned protein purification coupled with the biochemical complementation assay described above identified two polypeptides that allowed the production of an ~400-nucleotide RNA on chromatin templates (supplemental Fig. S1B). The purified heterodimer was named FACT (facilitates chromatin
transcription) (6) and in humans consists of the highly conserved proteins Spt16 and the HMG-box containing SSRP1 (structure specific recognition protein-1) (8). Surprisingly, maximal FACT activity in vitro requires a near equimolar stoichiometry to nucleosomes (8). This observation foreshadowed the mechanistic activities of FACT as a histone chaperone (see below).

Before the biochemical isolation of FACT using the above complementation assay, genetic evidence in yeast suggested a functional role for SPT16 in transcriptional activation and chromatin modulation (9). A role for FACT in transcript elongation in vivo was established through genetic interactions with the known elongation factors TFIIS and Spt4 (6) (see below). Importantly, chromatin immunoprecipitation experiments in yeast revealed that FACT associates with active genes in vivo and co-localizes with RNA Pol II throughout the coding region (10). Moreover, FACT appears to localize just downstream of the promoter region, consistent with its necessary positioning for its function in assisting nucleosome-mediated transcript elongation (10). Similar results were observed in Drosophila, demonstrating that FACT is rapidly recruited to induced heat shock genes in a manner comparable with the elongation factors Spt5 and Spt6 (11) and to PAF, a multisubunit complex that modulates chromatin-related events downstream of transcription initiation including RNA processing (12–15). Consistent with findings in yeast, FACT localizes most prominently downstream of promoter regions, appearing at the first nucleosome in Drosophila and mammalian cells (11, 16). In the past, it was unclear how FACT is actively recruited to the correct location on transcribed genes in vivo, although recent findings suggest some mechanisms (see below).

**Mechanistic Aspects of FACT Activity**

Initially, FACT was observed to function differently from the canonical elongation factors, such as TFIIS and TFIIF (6). Several biochemical studies (described above) and genetic studies in yeast alluded to a role for FACT in histone dynamics. Early on, SPT16 was placed into the histone group of SPT genes along with DSIF (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor composed of Spt4 and Spt5) and Spt6, factors known to modulate chromatin structure (9, 12). More importantly, mutational analyses in vivo of histones themselves revealed similar phenotypes as a yeast Spt16 mutant (Cdc68) (17); based on these findings, it was speculated that Spt16 directly regulates histone dimer-tetramer interactions (17). Mechanistic studies would put this hypothesis to the test.

In a key experiment, covalent cross-linking of histone polypeptides within nucleosomes was observed to abrogate FACT-dependent transcription, suggesting that the octamer must be disassembled during active transcription (8) (Fig. 1). This experiment suggested that FACT contained nucleosome-disassembly activity, a notion strongly supported by the physical association of Spt16 with H2A/H2B dimers (8, 18) and histone H3/H4 interactions with SSRP1 (18). It was later discovered that FACT-mediated transcription through the nucleosome results in the loss of a single H2A/H2B dimer (Fig. 2A and Ref. 18). Consistently, transcription in vitro is enhanced by the removal of H2A/H2B dimers (19), and specific loss of H2A/H2B dimers has been observed during active transcription mediated by RNA Pol II in vivo (20). Moreover, FACT was observed to contain histone chaperone activity (18), suggesting that FACT also functions to re-establish disassembled nucleosomes (Fig. 2B), an assertion previously hypothesized (21). Notably, inactivation of FACT results in transcription initiation from cryptic initiation sites within the coding region of genes in yeast, implying that FACT is required for maintaining chromatin structure in vivo (10, 22). Taken collectively, a model can be formulated where FACT functionally exerts its effects on the nucleosome by removing one H2A/H2B dimer and facilitates nucleosome re-assembly after the wake of RNA Pol II transcription (Fig. 2B).

**FACT Associates with Elongation Factors and Chromatin Modulators**

Given the importance of FACT during transcription, it is not surprising that FACT displays physical and genetic interactions
with many factors implicated in transcript elongation (12). Among these are the SPT proteins Spt4, Spt5, and Spt6, all established modulators of chromatin and transcript elongation (12). Similar to FACT, Spt6 regulates chromatin structure by interacting with histones, namely histone H3, and functions as a histone chaperone (23). Moreover, mutant strains of Spt6 allow spurious initiation from within the coding regions of genes comparable with FACT in yeast, suggesting that Spt6 is important for maintaining chromatin structure. Given that FACT and Spt6 selectively interact with histone H2A/H2B and H3, respectively, these two proteins may work collectively in the maintenance of chromatin structure during active transcription (Fig. 2).

As discussed above, the nature of FACT spatial recruitment on active genes remains unclear. However, a number of recent studies have suggested a role for the chromatin-remodeling factor CHD1 in FACT localization. Several independent studies have demonstrated that FACT physically associates with CHD1 in higher organisms and yeast (24–36). In yeast, chd1 mutant alleles are sensitive to 6-AU (27), and Chd1 displays genetic interactions with numerous elongation factors (26). Similar to FACT, CHD1 localizes throughout the coding region of genes (26) and can transfer core histones onto DNA (28), perhaps functioning in concert with FACT and Spt6 in the maintenance of chromatin structure. Recently, it was identified that human CHD1 specifically recognizes histone H3 trimethylated on lysine four (H3K4me3) (29, 30). H3K4me3 correlates with the 5′-region of active genes and peaks near the start site of transcription in mammalian cells (31, 32). Therefore, FACT may associate with CHD1 bound to H3K4me3 on active genes placing it in its proper position requisite for FACT function. Indeed, FACT can associate with CHD1 when bound to H3K4me3.4 Quantitative chromatin immunoprecipitation studies have revealed a tight co-localization of FACT and H3K4me3 on active genes (16). Importantly, loss of H3K4me3 in vivo by small interfering RNA treatment resulted in reduced recruitment of FACT to active genes (34). Although CHD1/H3K4me3 may not be the exclusive mechanism for FACT recruitment during transcriptional activation, it is likely to be a major contributor to FACT localization on active genes.

In addition to the SPT group proteins and CHD1, FACT displays interactions with components of the PAF complex (25, 35), which is comprised of Paf1, Cdc73, Ctr9, Leo1, and Rtf1 subunits in yeast (25, 35–38). In human cells, the core PAF complex substitutes the Rtf1 subunit with the Ski8 protein, a factor that bridges the human PAF complex with RNA surveillance via the exosome (14). Genetic studies in yeast identified that components of the PAF complex functionally interact with FACT (35, 39), and mutant alleles of PAF subunits display sensitivity to 6-AU. A physical association between FACT and the PAF complex supports these in vivo findings (25, 35, 40). Functionally, the PAF complex regulates distinct chromatin modifications associated with active transcription, including histone H3 methylation on lysines 4 and 79 (41–43), as well as monoubiquitination of H2B on lysine 120 (15) (H2B-123 in yeast (44, 45)), a mark that facilitates H3K4me3. Given the physical and functional connections between FACT, PAF,
and H2B modification, it is likely that monoubiquitination of H2B facilitates FACT-mediated removal of H2A/H2B dimers during transcription. Consistently, a highly purified reconstituted chromatin system coupled to a highly reconstituted transcription system revealed that FACT-dependent transcript elongation is greatly enhanced by the PAF complex and the enzymes required for H2B monoubiquitination on the rate of elongation by RNAPII. Reactions were performed in the presence or absence of ubiquitin and terminated at the indicated time points. Size markers are shown on the right. Figure and legend taken from Ref. 39.

**FIGURE 3.** Histone H2B monoubiquitination on lysine 120 cooperatively stimulates ligand-dependent transcription in a FACT-dependent manner. A, *in vitro* reconstituted transcription assay. Purified PAF and RNF20/40 were added to the transcription reaction in various combinations as shown. B, transcription run-off assay showing the effect of H2B monoubiquitination on the rate of elongation by RNAPII. Reactions were performed in the presence or absence of ubiquitin and terminated at the indicated time points. Size markers are shown on the right. Figure and legend taken from Ref. 39.

Additional FACT Activities

Much of the data on FACT have indicated a clear role during transcript elongation on chromatin templates. However, studies have indicated an expanded functional role for FACT in other aspects of DNA metabolism. Some of the more notable findings include FACT's recruitment of TBP to promoters (10, 55, 56) and its involvement in DNA damage and repair (33, 63, 64). DNA replication is also challenged with limited DNA accessibility due to chromatin compaction; therefore, it is not surprising that FACT participates in this process. How FACT mechanistically facilitates this process is unclear, but FACT is both physically (57, 62) and functionally important for DNA replication (57–62). DNA replication is also challenged with limited DNA accessibility due to chromatin compaction; therefore, it is not surprising that FACT participates in this process. How FACT mechanistically facilitates this process is unclear, but FACT is both physically (57, 62) and functionally important for DNA replication (57–62).

As FACT is intimately involved in the maintenance of chromatin structure, it is easy to see how FACT impacts many aspects of RNA and DNA polymerase function. New discoveries will not only enhance our understanding of FACT-specific activities but the overall understanding of the many processes it regulates.
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