Milestones in transcription and chromatin published in the *Journal of Biological Chemistry*

DOI 10.1074/jbc.TM118.004162

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During Herbert Tabor's tenure as Editor-in-Chief from 1971 to 2010, JBC has published many seminal papers in the fields of chromatin structure, epigenetics, and regulation of transcription in eukaryotes. As of this writing, more than 21,000 studies on gene transcription at the molecular level have been published in JBC since 1971. This brief review will attempt to highlight some of these ground-breaking discoveries and show how early studies published in JBC have influenced current research. Papers published in the Journal have reported the initial discovery of multiple forms of RNA polymerase in eukaryotes, identification and purification of essential components of the transcription machinery, and identification and mechanistic characterization of various transcriptional activators and repressors and include studies on chromatin structure and post-translational modifications of the histone proteins. The large body of literature published in the Journal has inspired current research on how chromatin organization and epigenetics impact regulation of gene expression.

The fields of transcription and chromatin structure were largely separate in 1971 when Herbert Tabor became Editor-in-Chief of JBC. During the ensuing years, studies on gene regulation in eukaryotes have focused on the fact that our genes are packaged into a nucleoprotein complex called chromatin, a complex of DNA with histone proteins and a multitude of structural proteins and enzyme complexes involved in transcription (as well as DNA synthesis, DNA repair, and recombination). The interplay between chromatin structure and how the transcription apparatus accesses genes for the productive synthesis of mRNA and various noncoding RNAs has proved to be central to the regulation of gene expression. Studies on transcription at the molecular level have been the subject of more than 21,000 publications in JBC since 1971, and searching for "transcription" and "chromatin" reveals more than 2300 JBC publications during this time (according to Web of Science). It is impossible to do justice to such a large collection of papers, so this review will reflect the author's bias toward the application of biochemical methods to understand transcriptional regulation in eukaryotes. Molecular characterization of transcriptional regulation in eukaryotes began with the identification of the three forms of RNA polymerase and the accessory factors required for basal transcription, ultimately leading to current studies on the interplay between chromatin, epigenetic mechanisms, and transcriptional regulation. This review will attempt to highlight some of the milestones in the field that have occurred under Herbert Tabor's tenure at JBC and point out how earlier studies have impacted current research being reported in JBC.

Multiple forms of DNA-dependent RNA polymerase in eukaryotes

Prokaryotes have a single species of DNA-dependent RNA polymerase that can be regulated by its association with various accessory factors, such as σ factors (1). In contrast, there are three major nuclear RNA polymerases (pol)[2] in eukaryotic cells known to be responsible for the synthesis of rRNA (RNA pol I), mRNAs and various noncoding RNAs (RNA pol II), and 5S rRNA and tRNAs, among other small noncoding RNAs (RNA pol III), respectively. Two groups, one headed by Robert Roeder (Rockefeller University, New York) and the other headed by Pierre Chambon (Strasbourg, France), made the seminal discovery that DNA-dependent RNA synthesis activity in cell-free extracts from eukaryotic cells could be chromatographically separated into three peaks, which differ in their protein subunit compositions and sensitivity to the inhibitor α-amanitin (for an early review, see Ref. 2). Although these initial findings were published in other journals (2, 3), the majority of Roeder's contributions describing the isolation of the three enzyme classes from various organisms and cell types, their biochemical and enzymatic properties, abundance (4, 5), as well as subunit compositions (6–8) were published in JBC. These ground-breaking papers set the stage for further understanding of the biochemical basis for RNA synthesis in eukaryotic cells.

Accessory factors are needed for accurate transcription

Although the biochemically isolated RNA pol species were highly active *in vitro* using either genomic DNA (4) or the syn-

This work was supported by National Institutes of Health Grants 5R01-NS062856, 5R01-EY026490, and 1R01-EY029166 and by BioMarin Pharmaceutical. This JBC Review is part of a collection honoring Herbert Tabor on the occasion of his 100th birthday. The author declares that he has no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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[2] The abbreviations used are: pol, polymerase; CTD, C-terminal domain; PIC, preinitiation complex; TBP, TATA box–binding protein; TF, transcription factor; GTF, general transcription factor; TAF, TBP-associated subunit of TFIID; HDAC, histone deacetylase; BET, bromodomain and extra-terminal domain; KMT, histone lysine methyltransferase; PHD, plant homeodomain; HAT, histone acetyltransferase.

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thetic polymer poly[d(A-T)] (5) as templates, these assays only measured incorporation of radioactive precursors into random RNA species. When researchers tried to use protein-free DNA and purified RNA polymerases to transcribe discrete RNAs, the experiments failed. Specific genes could be transcribed from isolated nuclei or chromatin templates, suggesting the cell-free experiments were missing accessory factors in addition to the RNA pols that are necessary for promoter recognition, initiation, and perhaps termination of transcription by each of the RNA polymerases. One landmark paper published in JBC described the specific and accurate transcription of adenovirus VA-RNA in chromatin or nuclei isolated from virus-infected cells by both endogenous and exogenous RNA pol III (9). Accurate transcription from cloned gene templates (5S rRNA genes and adenovirus VA genes) was also obtained with RNA pol III supplemented with cell-free extracts (10), strongly suggesting the requirement for cellular factors in addition to RNA pol III for accurate transcription. Such extracts were then fractionated by chromatographic methods, and distinct cellular factors were identified for both RNA pol III (11) and RNA pol II (12), but at this point these factors were simply chromatographic fractions, and further biochemical studies were needed to identify the actual protein species that comprised these factors. Similar studies on RNA pol I began with transcription of cloned rRNA genes in extracts from *Xenopus* oocyte nuclei (13) and yeast (14) and lead to the identification of distinct factors necessary for RNA pol I transcription *in vitro* in mammalian cells (15) and in yeast (16).

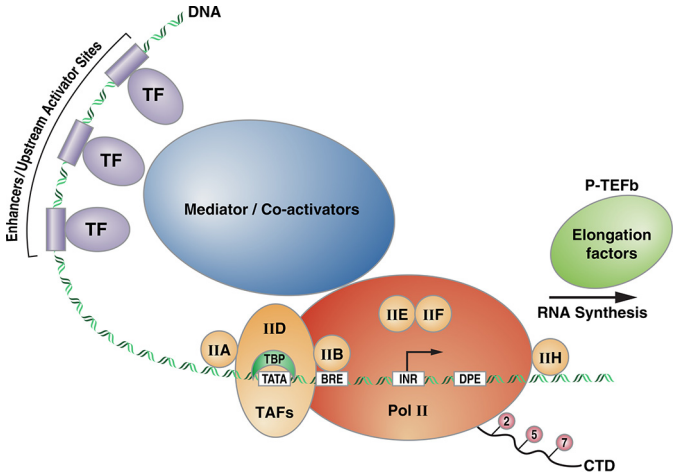

Figure 1. Schematic of a hypothetical RNA polymerase II promoter. Upstream activator sites and enhancers are bound by a variety of transcription factors, composed of DNA-binding domains (shown as *cylinders* on the DNA) and activation domains (shown as *circles*). These proteins serve to recruit co-activators, which can act on chromatin to facilitate transcription complex assembly (see below) or mediator, a large multisubunit complex that communicates with and is part of the core transcription machinery. The first step in assembly of the PIC is the association of the TBP subunit of TFIID with a TATA element, located ~30 bp upstream of the transcription start site (*arrow*). TFIID also contains TAFs that communicate with and respond to upstream activators. Other core components of the PIC are depicted (TFs IIA, IIB, etc.). *BRE* refers to a TFIIB-response element, and *INR* refers to the initiator element, which are DNA sequences found in various RNA pol II promoters. *DPE* is a downstream promoter element. TFIIH and the *P-TEFb* elongation factor both contain kinase activities that act on the CTD of RNA pol II at serine residues within heptad repeats. These phosphorylation events are associated with initiation and elongation phases of the transcription cycle (see text).

RNA pol II core transcription machinery

In a remarkable series of papers, Roeder, Reinberg, and co-workers identified the core components of the mammalian RNA pol II transcription machinery (transcription factors TFIIA, TFIIB, TFIID, TFIIE, and TFIIF, known as general TFs or GTFs) and documented the order in which each of these GTFs bind an RNA pol II core promoter element (*i.e.* the DNA sequences immediately adjacent to the transcription start-sites of an mRNA-coding gene) to recruit RNA pol II and initiate transcription (17). Studies in other organisms, such as yeast by Kornberg and co-workers (18, 19) and *Drosophila* by Kadonaga and co-workers (20), lead to broadly similar conclusions but with subtle differences between species. Subsequently, additional factors were identified, such as the multisubunit factor TFIIH (21, 22). A number of studies published in JBC reported the identification of the polypeptide subunits of these TFs (23, 24), and other studies documented the interactions between the various GTFs and roles of the GTFs in assembly of the RNA pol II preinitiation complex (PIC) (25, 26). Fig. 1 provides a simplistic overview of the DNA sequence elements and protein factors involved in RNA pol II transcription. In the first-generation model for assembly of the PIC, the TATA box–binding protein (TBP) subunit of the GTF TFIID binds TATA elements located ~30 bp upstream of the transcription start-site (Fig. 1) and leads to the recruitment of the other GTFs. However, this is an idealized model that only applies to a limited number of genes because most promoters lack TATA elements, and the details of PIC assembly therefore depend upon the sequence composition of the particular promoter/gene under investigation (17). In support of this view, early studies with multiple promoter elements pointed out the different GTF requirements for basal levels of transcription (27). Investigations into the roles played by the various subunits of the GTFs in assembly of the PIC continue to be a subject of interest in the JBC. For example, JBC papers have investigated the roles played by the TBP-associated subunits of TFIID (the TAFs) in recruitment of RNA pol II and communication with other TFs (28, 29). Although core promoter elements and the GTFs (Fig. 1) were largely identified and characterized over a decade ago, recent studies reported in JBC describe new features of core promoters, such as a TFIIA recognition element (IIARE (30)). The IIARE was reported to enhance TFIIA binding and recruitment of GTFs and pol II and to enhance transcription *in vitro*, at least for TATA-containing promoters. Early studies also established that there was an energy requirement for transcription initiation by RNA pol II (31). Identification of the various steps in the transcription cycle that utilize the energy of ATP hydrolysis continues to be an active area of investigation reported in JBC (Ref. 32 and references therein).

In addition to the GTFs, another multisubunit complex was identified and shown to mediate communication between activating TFs (at enhancer and upstream activator sequences) and the GTFs and RNA pol II, hence the name "Mediator" for this complex (Fig. 1). Although Mediator was first identified in yeast by Kornberg and co-workers (33, 34), other studies in JBC have probed the role of Mediator in higher organisms and shown that Mediator facilitates recruitment of RNA pol II through the general TFs, such as TFIIB (35). Originally thought to be sepa-

rate from the GTFs and RNA pol II, Mediator is now considered to be an integral component of the PIC. Structural insights into Mediator function have recently been reviewed in JBC (36).

Preinitiation complex formation for RNA pol I- and RNA pol III-transcribed genes

Similar to findings for RNA pol II, fractionation of cell-free extracts led to the identification of TFs required for accurate transcription of rRNA by RNA pol I (15, 37) and 5S rRNA and tRNAs by RNA pol III (11). For RNA pol I, two TFs are involved, SL1 and UBF, whereas for RNA pol III, tRNA genes require TFIIIB and TFIIIC and additionally the zinc-finger protein TFIIIA for the 5S rRNA genes. Mechanistic studies on assembly of the preinitiation complexes for both RNA pol I (15, 37) and RNA pol III (38–40) were published in JBC. One of the major surprises in studies of the basal transcription machinery was the requirement for TBP for transcription by each of the RNA pols. However, TBP is localized within different multisubunit complexes for each polymerase (41). TBP is found in TFIID (for RNA pol II, Fig. 1; and the SAGA complex (42)), TFIIIB (for RNA pol III (43)), and SL1 (for RNA pol I), along with different sets of TAFs. Polymerase-specific TAFs were found to interact with other components of the transcription machinery for genes transcribed by each RNA polymerase (43–45). Another common feature of the transcription complexes for genes transcribed by each RNA pol is the stability of the TBP-containing complex (TFIID, SL1, or TFIIIB), which persists at promoter DNA (at least *in vitro*) through multiple rounds of transcription. This is likely due to the stability of TBP on both TATA-containing and TATA-less promoter elements (46) but also to the associated factors, including the TAFs and other GTFs ((47) for pol III/TFIIIB).

Phosphorylation of the largest subunit of RNA pol II as a major regulatory event in transcription

The largest subunit of RNA pol II contains at its C terminus tandem repeats of the consensus sequence Tyr–Ser–Pro–Thr–Ser–Pro–Ser, first identified by Corden *et al.* (48) in a seminal paper published in another journal. Following this, a series of papers from Dahmus and co-workers published in the JBC established that mammalian mRNA synthesis is carried out by a phosphorylated form of RNA pol II (49) and that the transition from initiation to elongation is mediated by differential phosphorylation events (50). Phosphorylation within this C-terminal domain (CTD) at Ser-2, Ser-5, and Ser-7 has been associated with different stages of the transcription cycle, and numerous papers in JBC have reported identification of both the kinases and phosphatases involved (51–54). Unphosphorylated RNA pol II is recruited to the PIC; Ser-5 phosphorylation is associated with initiation of transcription, mediated by the CDK7 subunit of TFIIH (in both yeast (22, 55) and mammalian cells (54, 56)), and Ser-2 phosphorylation is associated with elongation, mediated by the positive elongation factor P-TEFb (52). Early studies in yeast suggested that Ser-7 phosphorylation was similar to Ser-5 phosphorylation both in terms of the kinase involved and its role in transcription initiation (55), but more recent work suggests that all three phosphorylation events may be required for transcription elongation (57). Recent studies have also focused on the accessory proteins that interact with P-TEFb, such as the bromodomain-containing protein BRD4 that is also involved in Ser-2 phosphorylation and transcription elongation (see below and Ref. 58). Besides RNA pol II, phosphorylation of many transcription factors has been shown to be key regulatory events (for example, cAMP-response element-binding protein (CREB) (59) and NF-κB (60) among many similar studies).

In addition to phosphorylation, the RNA pol II CTD is subject to glycosylation by *O*-GlcNAc (61), and it is reasonable to speculate that interplay between CTD modification states could be involved in transcriptional regulation (61, 62). In support of this, the *O*-GlcNAc transferase OGT has been found associated with the RNA pol II CTD as part of the PIC, and reducing OGT levels with shRNA blocks transcription (63, 64). Several transcriptional activators have also been found to be glycosylated (62); for example, *O*-GlcNAc regulates the FoxO1 transcription factor in response to glucose (65), providing insights into nutrient control of transcription (63).

Activators and repressors

Numerous studies published in JBC have identified DNA sequence elements upstream and downstream from core promoters that are required for "activated" transcription, as well as the protein factors that bind such elements. A search of Web of Science with the terms "transcription" and "activator" yields nearly 2800 such citations in the JBC since 1971, so a comprehensive review of this literature is beyond the scope of this historical perspective. Nevertheless, two highly cited examples of such studies are worth mentioning: these are the identification of antioxidant response elements that respond to the transcription factor NRF2 (NF-E2-related factor 2) (66) and the identification of the pluripotency factors OCT4 and SOX2 as regulators of the homeodomain pluripotency factor Nanog (67). These and numerous similar studies show binding of regulatory factors to their cognate DNA elements both *in vitro* and in cells, and they usually use RNA-silencing methods to demonstrate the critical role of these factors in target gene regulation. Similar to gene activation, mechanisms involved in gene repression have also been a hot topic in JBC, with more than 1400 papers since 1971. For example, Kouzarides and co-workers (68) described the methyl-CpG– binding protein 2 (MeCP2) as a link between DNA methylation and gene repression through recruitment of co-repressor complexes containing histone-modifying enzymes.

Chromatin, central to our understanding of transcriptional regulation

As noted earlier, the genetic material in eukaryotic cells is packaged with histone and nonhistone chromosomal proteins, and studies in JBC have probed virtually all aspects of chromatin organization, histone post-translational modifications, and the role of chromatin in transcriptional regulation. The basic subunit of chromatin organization is the nucleosome, consisting of 147 bp of DNA, wrapped around an octamer of the core histones, consisting of two dimers of H2A and H2B, and a tetramer of two copies of H3 and H4 (Fig. 2). Nucleosomes are joined together via linker DNA of variable length (generally

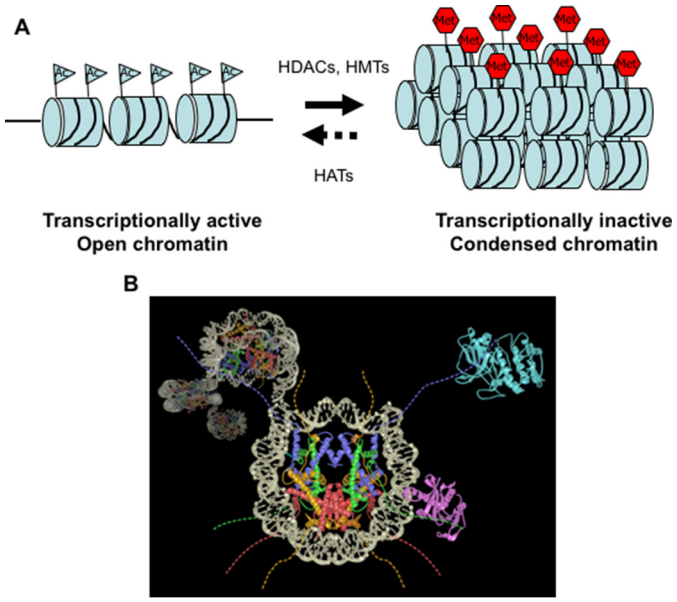

Figure 2. Histone post-translational modifications control chromatin accessibility and transcription. *A,* transcriptionally active euchromatin is associated with acetylation of the core histones, whereas transcriptionally inactive heterochromatin is associated with other histone marks, such as methylation at histone H3 lysine 9 and 27. Various HDACs, HATs, histone methylases (*HMTs*), and demethylases control the transitions between euchromatin and heterochromatin. *B,* atomic structure of the nucleosome core particle with N-terminal tails of the core histones indicated by *dashed lines* (not seen in the X-ray structure). Nucleosome core particle is shown with histone H3 in *blue*, H4 in *green*, H2A in *yellow*, and H2B in *red*. The histone tails are involved in inter-nucleosome interactions, where the N-terminal tail of histone H3 contacts the histone octamer of an adjacent nucleosome. Histone tails also provide binding sites for the readers, writers, and erasers of the histone code, involved in both gene activation and repression. The *blue* and *magenta* structures are two HATs, modeled interacting with histone tails. Adjacent nucleosomes connected by linker DNA are modeled. Images generously provided by Dr. E. Soragni (Scripps Research) and Dr. K. Luger (University of Colorado, Boulder).

40–60 bp, with variations between tissues within an organism and differences between species) along with histone H1. Early studies focused on the nucleosome and chromatin higher-order structures. Questions that were addressed in numerous JBC papers included whether (and how) DNA sequence determines nucleosome positioning (69), how histones are deposited on DNA during nucleosome assembly (70), the structure of particular genetic loci in chromatin, and the relationship between transcription and accessibility to nuclease digestion (see Ref. 71 among many others).

An important area of investigation in chromatin research has been histone post-translational modifications and the effects of such modifications on nucleosome and chromatin organization and gene expression, topics that have received considerable attention in the pages of JBC. These modifications include acetylation and methylation of the ϵ-amino groups of lysine residues, particularly within the ~20–30 amino acids of the N termini of histones H3 and H4, phosphorylation of serine residues, ubiquitinylation and glycosylation (Fig. 2*A*). These modifications have been proposed to constitute a "histone code" for gene activity (hypothesized by Allis and co-workers and reviewed in Ref. 72), where the enzymes responsible for modification are the "writers" of the code; proteins that bind these modified histones are the "readers," and enzymes that remove the modifications are the "erasers."

Acetylation

An early study from Allfrey and co-workers (73) documented that sodium butyrate caused histones to become highly acetylated through inhibition of histone deacetylation. Numerous subsequent studies identified the histone deacetylase (HDAC) enzymes in various organisms (the erasers of the acetylation code (74)), and inhibitors of their activity (75, 76), as well as efforts to identify the histone acetyltransferases (HATs, the writers of the acetylation code) and their substrate specificities (77–80). The identification of trichostatin A (75) and valproic acid (76) as HDAC inhibitors are two highly cited papers in the Journal, with over 1500 and over 1100 citations, respectively, as of this writing (according to Web of Science). Mechanistic studies of the HDACs have also revealed links between these enzymes and gene regulation (81, 82). Transcriptional coactivator complexes possess intrinsic histone acetyltransferase activity, providing a direct link between chromatin acetylation and transcriptional activation (83, 84). Various signaling molecules also impact histone acetylation (85). Readers of the acetyl histone code include BRD4, a protein that recognizes acetylated lysine residues and communicates with P-TEFb and RNA pol II to facilitate productive transcription elongation (for recent JBC papers, see Refs. 58, 86).

Just how chromatin structure is affected by acetylation has been intensely investigated. Biophysical studies showed that acetylation has no major effect on nucleosome structure (87); however, other studies showed that chromatin fiber solubility and sensitivity to nuclease digestion are greatly increased on histone acetylation (88). Many of these studies relied on simple methods, such as salt gradient dialysis, to reconstitute either single nucleosomes on short DNA fragments or utilized arrays of well-positioned nucleosomes. Robert Simpson's discovery that a sea urchin 5S rRNA gene contained a strong nucleosome positioning sequence provided a DNA substrate for many such studies (69, 70, 89). Acetylation has been proposed to weaken histone–DNA interactions, but studies with these defined arrays of nucleosomes showed that acetylation has more pronounced effects outside of the nucleosome core particle (90) and largely on inter-nucleosome contacts resulting in a more extended chromatin conformation (89). Recent studies have examined the roles of particular histone acetylation events in the transcription cycle. For example, O'Malley and co-workers (86) reported that H3K9 acetylation is involved in the switch between transcription initiation to elongation. Understanding just how the "histone code" regulates gene expression continues to be an active area of investigation in JBC.

Acetyl-lysine reader proteins, such as BRD4 and TRIM24, have received considerable attention due to their involvement in expression of *MYC* and other cancer-promoting oncogenes (91, 92). BRD4 is a member of the bromodomain and extra-terminal domain (BET) family and, as noted above, plays an important role in transcriptional elongation. Because of its association with cancer, BET inhibitors have been widely investigated as potential therapeutics. In one JBC study, Jung *et al.* (91) mapped the regions of the BRD4 responsible for interactions with acetylated peptides derived from histone H4 and showed that similar amino acid residues in the protein were

responsible for binding the potent BRD4 inhibitor JQ1. Studies such as this will certainly facilitate the development of BET inhibitors. Similarly, TRIM24 (tripartite motif-containing protein 24) is a reader of H3K23ac, and inhibitors are also being developed as anti-cancer agents (92).

Histone methylation is linked to both gene activation and repression

Methylation of histone lysine residues is associated with either gene activation (for example, H3K4me3 (trimethylation of histone H3 lysine 4) at or near transcription start sites) or gene repression (H3K9 and K27 di- and trimethylation). Studies published in JBC have focused on both the enzymes responsible for these methylation events (the methyltransferases) and the activators and repressors that recognize histone methylation states to effect gene activation or repression (93–97). In the case of H3K4me3 at active promoter elements, this mark serves to recruit the nucleosome remodeling factor NURF (see below and Ref. 98). Methylated H3K9(me2/3) is recognized by heterochromatin proteins, such as HP1, leading to recruitment of corepressor complexes (97). Components of PRC2 (polycomb-repressive complex 2) recognize methylated H3K27 leading to transcriptional repression (99). Methylation at other residues of the core histones, such as H3K36 and H3K79, is linked to the elongation phase of the transcription cycle (100). Thus, "readers" of the histone code discriminate between repressive and activating histone methylation marks to either repress transcription or to recruit co-activators or chromatin remodeling factors. Understanding the cross-talk between the various histone modification states and their role in the transcription cycle continues to be a subject of great interest in the Journal. For example, Gates *et al.* (86) showed that although H3K4me3 is involved in transcription initiation, H3K9ac mediates the switch from the initiation to elongation phases by promoting release of paused pol II by recruitment of an elongation complex.

Similar to studies with inhibitors of acetyl-lysine readers (91, 92), both the histone lysine methyltransferases (KMT) and methylated histone readers have been the subject of anti-cancer drug development efforts (101, 102). In one recent study, Coussens *et al.* (101) used nucleosome substrates to screen compound libraries for inhibitors of the KMT NSD2, which is overexpressed or mutated in a variety of human cancers. Active molecules were found to bind the KMT nuclear receptor-binding SET domain. This assay platform will enable future oncology drug development efforts where either chromatin-modifying enzymes or the readers of such modifications are the targets. As for the KMTs, readers of methylated histone marks are often mutated or overexpressed in cancer and other diseases. One such class of readers of H3K4me3 is the plant homeodomain (PHD) zinc finger proteins (such as Ing2 and the mix lineage leukemia proteins), and a study in JBC identified macrocyclic calixarenes as potent inhibitors to disrupt binding of PHD fingers to H3K4me3 *in vitro* and *in vivo* (102). Such studies will also facilitate future oncology drug development efforts.

Phosphorylation, glycosylation, and ubiquitinylation of histones

A paper describing phosphorylation of a particular subtype of histone H2A (encoded by the H2A gene family member X, H2AX), at serine 139 upon DNA damage, is one of the highest cited JBC papers of all time, with 3095 citations as of this writing (according to Web of Science). This modification, called γ-H2AX, has been linked to various cellular processes, including apoptosis (103), and a kinase responsible for this modification has been identified as ATM (for ataxia telangiectasia mutate, (104)). Regions of chromatin containing γ-H2AX are likely more open to the DNA repair machinery thus providing a link between this histone modification and chromatin structure. In addition to γ-H2AX, another important histone phosphorylation event is H3S10p, which is coupled to mitotic chromosome condensation (105). The kinases and phosphatases that regulate this modification have also been identified (106). In addition, histone H3 can be phosphorylated at tyrosine 41 as well as threonine 45, and a recent study in JBC reported that these modifications regulate DNA accessibility in the nucleosome (107). Another modification of histone H3 is *O*-GlcNAc glycosylation at Thr-28, which is cell cycle-regulated. This modification also regulates mitotic phosphorylation at Ser-10, providing another example of cross-talk between histone modification states (63, 108).

Histone H2B can be modified by conjugation of the 76-amino acid protein ubiquitin to lysine residues. Whereas most cellular functions of ubiquitin are involved in protein stability and turnover through the proteasome (109), H2B ubiquitinylation is required for cell cycle progression, telomere gene silencing, and transcriptional repression (109). An important JBC paper showed that ubiquitinylation of H2B at lysine 123 is the signal for H3 methylation, leading to gene silencing at yeast telomeres (110). Another small protein modification of histones is sumoylation (by the small ubiquitin-like modifier SUMO), where this modification of histone H4 regulates chromatin compaction (111). H4 sumoylation weakens internucleosome interactions leading to more open chromatin, and hence it may be involved in transcriptional activation. Sumoylation can also occur on other chromatin-associated proteins, and a recent study from Barton and co-workers (92) described the cross-talk between histone acetylation and sumoylation of the acetyl/methyl reader protein TRIM24. As noted above, TRIM24 is aberrantly expressed in many cancers, so the link between TRIM24 sumoylation and chromatin association may be vital to understand the role of TRIM24 in oncogenesis.

Chromatin remodeling complexes

Numerous studies published in JBC have concerned chromatin-remodeling complexes, multisubunit complexes, some of which utilize the energy of ATP hydrolysis to catalyze the movement or displacement of nucleosomes. Chromatin remodeling is an essential process for both preinitiation complex assembly and transcription initiation and elongation (112, 113). Although these complexes were first identified in yeast, mammalian remodeling complexes have been identified (113–115), and their mechanisms of action have been intensively investi-

gated (115, 116). For example, the NURF complex has been shown to slide the histone octamer along the DNA in steps of 10 bp or one helical turn of the DNA on the surface of the octamer (116). ATP-dependent chromatin assembly factors such as Asf1 (anti-silencing factor 1) in yeast (117) and RSF (remodeling and spacing factor) in higher organisms (115) have been shown to evict nucleosomes from gene promoters allowing active transcription complexes to form. One other important aspect of chromatin remodeling is the incorporation of histone variants into nucleosomes. For example, the ATP-dependent remodeler SWR1 is responsible for the exchange of canonical H2A–H2B dimers with dimers containing the H2A variant H2A.Z. Studies have shown that histone acetylation facilitates SWR1-mediated histone dimer exchange (118). Nucleosomes containing H2A.Z are located at promoters, which are susceptible to eviction on transcriptional activation. Structural studies have indeed shown that oligonucleosomes containing H2A.Z are destabilized compared with canonical nucleosomes (119), likely allowing for eviction by other remodeling complexes. Mechanistic studies of chromatin-remodeling complexes continue to be of interest to the JBC. A recent study by Formosa and co-workers (120) described the role of the high-mobility group B (HMGB) domain in nucleosome assembly and reorganization by FACT (facilitates chromatin transcription), which is an ATP-independent chromatin remodeler. High-mobility group domains are minor-groove DNA-binding domains that bend DNA, and this study showed that both histone and DNA binding are involved in chromatin remodeling.

In vitro reconstitution of active chromatin templates

Various viral, yeast, and mammalian promoter elements have been reconstituted in defined systems to analyze the requirements for active transcription by RNA pol II (115, 121). In these systems, chromatin assembly on a defined DNA sequence is mediated by one of the ATP-dependent chromatin assembly complexes containing a histone chaperone, the core histones, a transcriptional activator (such as the artificial activator Gal4–VP16), and the basal transcription factors discussed above (or unfractionated cell-free extracts). One finding of importance is that acetyl-CoA is required for preinitiation complex assembly in these *in vitro* systems (122, 123), providing further evidence that protein acetylation is required for active transcription. Mass spectrometry was used in one study to identify histone H3K9, H3K27, H3K36, and H3K37 as sites of p300-catalyzed acetylation in promoter-proximal nucleosomes in such a reconstituted system (123). Chromatin reconstitution from defined components continues to be an area of active investigation, and a recent report from Kadonaga and co-workers (124) described the refinement of such a system for ATP-dependent assembly of chromatin using a histone chaperone (*Drosophila* nucleoplasmin–like protein (dNLP)), an ATP-remodeling enzyme (imitation switch (ISWI)), core histones, and various DNA substrates. This experimental resource will benefit future detailed mechanistic studies on the relationship between chromatin and transcription. While studies on the role of chromatin in transcriptional regulation have been ongoing for nearly 5 decades, this remains an area of great interest to the readers of JBC. Stay tuned for more!

Acknowledgments—I thank E. Soragni and K. Luger for images in Fig. 2 and C. Goodman for editorial assistance.

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