MODES OF TRANSCRIPTIONAL REGULATION

Inducible gene expression: diverse regulatory mechanisms

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Abstract | The rapid activation of gene expression in response to stimuli occurs largely through the regulation of RNA polymerase II-dependent transcription. In this Review, we discuss events that occur during the transcription cycle in eukaryotes that are important for the rapid and specific activation of gene expression in response to external stimuli. In addition to regulated recruitment of the transcription machinery to the promoter, it has now been shown that control steps can include chromatin remodelling and the release of paused polymerase. Recent work suggests that some components of signal transduction cascades also play an integral part in activating transcription at target genes.

Chromatin
A nucleoprotein structure formed of repeating nucleosomal units in which 147 base pairs of DNA are wrapped around an octamer of histone proteins consisting of an H3–H4 tetramer flanked by two H2A–H2B dimers

Co-activator
A protein that is recruited to promoters through interactions with transcriptional activators, and facilitates transcriptional activation through the recruitment of RNA polymerase II and the general transcription factors. Many co-activators also catalyse chromatin modifications that assist the kinetics of recruitment of the general transcription machinery.

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Cells must be able to rapidly respond to changes in their external environment — such as temperature or nutrient availability — to exploit and survive in new conditions. Even cells in a multicellular organism need to respond to developmental cues such as signalling molecules to determine when to divide, migrate or die. The production of new proteins in response to external stimuli results largely from rapid activation of gene transcription — this is known as inducible gene expression.

Inducible gene expression has several features that distinguish it from the expression of genes that are constitutively active (for example, housekeeping genes). Inducible genes are highly regulated and must be able to be rapidly and specifically activated in response to stimuli. Once the stimulus is removed, an inducible gene must quickly return to its basal, inactive state. Furthermore, multiple genes must often be synchronously activated in response to the same stimulus, such that the proteins required to respond to the stimulus are produced simultaneously at the appropriate relative levels. Similarly, multiple cells in an organism must respond to developmental cues in a coordinated fashion so that the appropriate morphogenetic process occurs over a broad region of cells.

Here, we discuss mechanisms of inducible gene expression used by eukaryotic cells. Although processes that occur following transcription such as protein translation are also regulated as part of inducible gene expression, we do not discuss them in this Review. We focus on the events that are important for recruitment of the transcription machinery and initiation of RNA polymerase II (Pol II)-dependent transcription. Although a traditional model of activator-dependent recruitment of Pol II and the general transcription factors (GTFs) holds true for many inducible genes, recent studies suggest that Pol II is already present and poised for transcription at many inducible genes[1–6]. Therefore, it is becoming increasingly apparent that there is an additional level of regulation that occurs during the initial stages of transcription elongation before Pol II is released into a productive transcription cycle. In addition, several recent studies suggest that some components of signal transduction cascades that lead to inducible gene expression that were once thought to function exclusively in the cytoplasm such as mitogen-activated protein kinases (MAPKs) are recruited to chromatin and are integral components of transcription complexes[7].

We use three well-characterized examples of inducible gene expression to illustrate some of the key mechanisms involved in transcription activation in response to stimuli: Gal gene induction in response to galactose in *Saccharomyces cerevisiae*, heat-shock gene induction in *Drosophila melanogaster* and osmostress regulation in *S. cerevisiae*. We first discuss the initial steps of the transcription cycle: activator-dependent recruitment of the transcriptional machinery and the role of co-activators and nucleosome-remodelling complexes in facilitating this recruitment. We then examine the events that occur following recruitment of the general transcription machinery, including promoter clearance and release of paused Pol II into productive transcription elongation. Finally, we examine the role of signalling kinases that seem to play an integral part in multiple aspects of

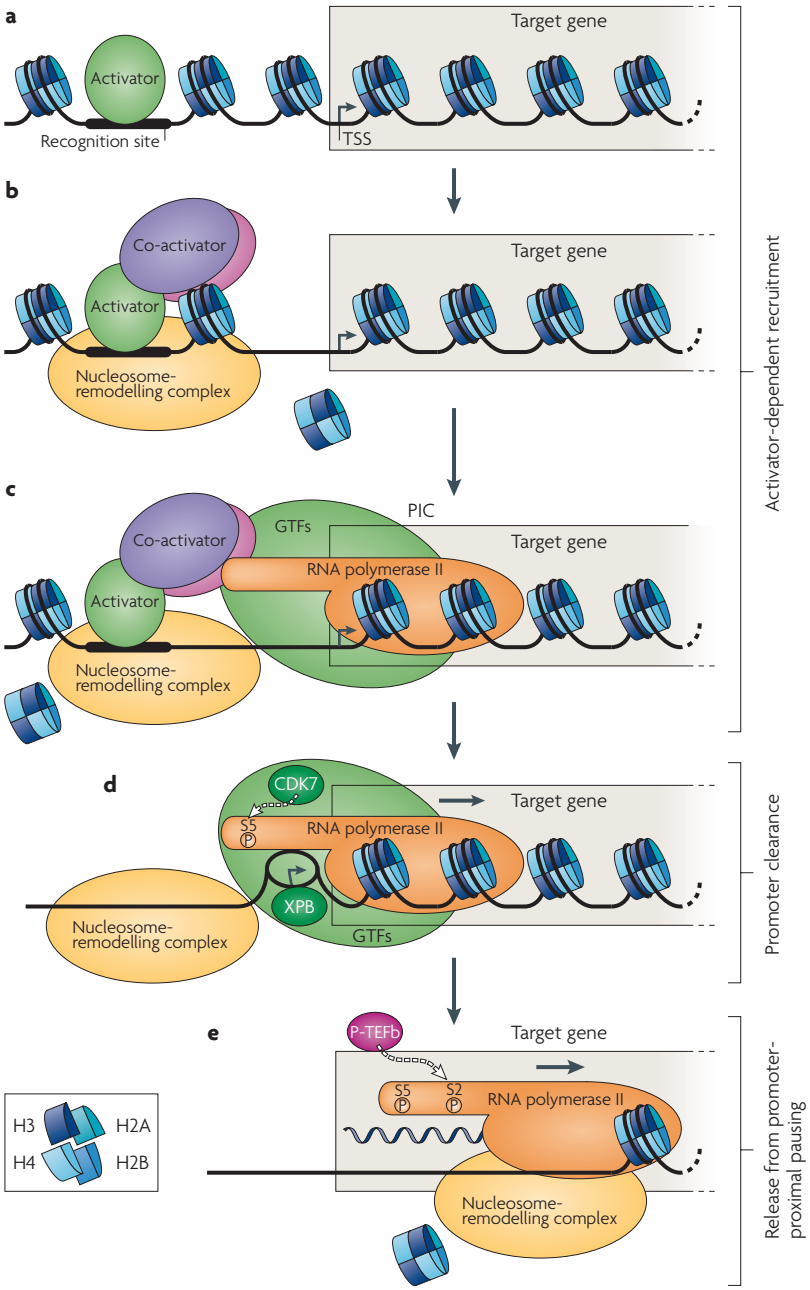


Figure 1 | **Early steps in the transcription cycle.** **a** | Promoter selection is determined by the interaction of one or more transcriptional activator(s) with specific DNA sequences (recognition sites) near target genes. Activators then recruit components of the transcription machinery to these genes through protein–protein interactions. **b** | Activation of gene expression is induced by the sequential recruitment of large multi-subunit protein co-activator complexes (shown in purple and pink) through binding to activators. Activators also recruit ATP-dependent nucleosome-remodelling complexes, which move or displace histones at the promoter, facilitating the rapid recruitment and assembly of co-activators and the general transcription machinery. **c** | Together, co-activators and nucleosome remodellers facilitate the rapid recruitment of RNA polymerase II (Pol II) and the general transcription factors (GTFs) TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH to form the pre-initiation complex (PIC) on the core promoter[9]. These first three steps (**a**–**c**) constitute activator-dependent recruitment. **d** | After PIC assembly, CDK7 in human TFIIH (Kin28 in yeast) phosphorylates the serine 5 (S5) position of the Pol II carboxy-terminal domain (CTD). At the same time, another subunit of TFIIH, the DNA helicase XPB (Rad25 in yeast), remodels the PIC, and 11–15 bases of DNA at the transcription start site (TSS) is unwound to introduce a single-stranded DNA template into the active site of Pol II[83]. Pol II then dissociates from some of the GTFs and transitions into an early elongation stage of transcription[83]. This step is often referred to as promoter escape or clearance but is not sufficient for efficient passage of Pol II into the remainder of the gene. **e** | Following promoter clearance, Pol II transcribes 20 – 40 nucleotides into the gene and halts at the promoter-proximal pause site. Efficient elongation by Pol II requires a second phosphorylation event at the S2 position of the Pol II CTD by CDK9,a subunit of human P-TEFb (Ctk1 in yeast)[8,104]. Phosphorylation of the CTD creates binding sites for proteins that are important for mRNA processing and transcription through chromatin such as the histone H3 lysine 36 (H3K36) methylase SET2 (REF. 104). Nucleosome remodellers also facilitate passage of Pol II during the elongation phase of transcription. The transcription cycle continues with elongation of the transcript by Pol II, followed by termination and re-initiation of a new round of transcription (not shown).

these initial stages of the transcription cycle. Although the mechanisms involved in the chosen examples may not always be observed in all other cases of inducible gene expression, we hope to provide a broad overview of the principles involved in inducible activation of transcription.

Activator-dependent recruitment

Gene activation involves a multistep recruitment process that consists of several potential rate-limiting steps during the initial stages of the transcription cycle (reviewed in REF. 8) (FIG. 1). During the initial steps of gene induction, transcriptional activators bind to specific DNA sequences near target genes and recruit transcriptional co-activators and components of the transcription machinery to these genes through protein–protein interactions. These steps result in formation of the pre-initiation complex (PIC) on the promoter[9,10]. For the purposes of this Review, these first three steps can be regarded as a single rate-determining process, which we refer to as activator-dependent recruitment (FIG. 1a–c). An additional level of regulation is required for polymerase to proceed to productive transcription elongation (FIG. 1d,e). Although all of the steps in the transcription cycle are subject to regulation[11], we focus in this Review on those steps that are most important for inducible gene expression: activator-dependent recruitment resulting in PIC formation; activation of the PIC and transcription initiation; and release of paused polymerase into productive elongation.

General transcription machinery
RNA polymerase II together with the general transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH.

Transcriptional activator
A sequence-specific DNA-binding protein that increases the rate of transcription by recruiting RNA polymerase II, either directly in prokaryotes or through co-activators in eukaryotes.

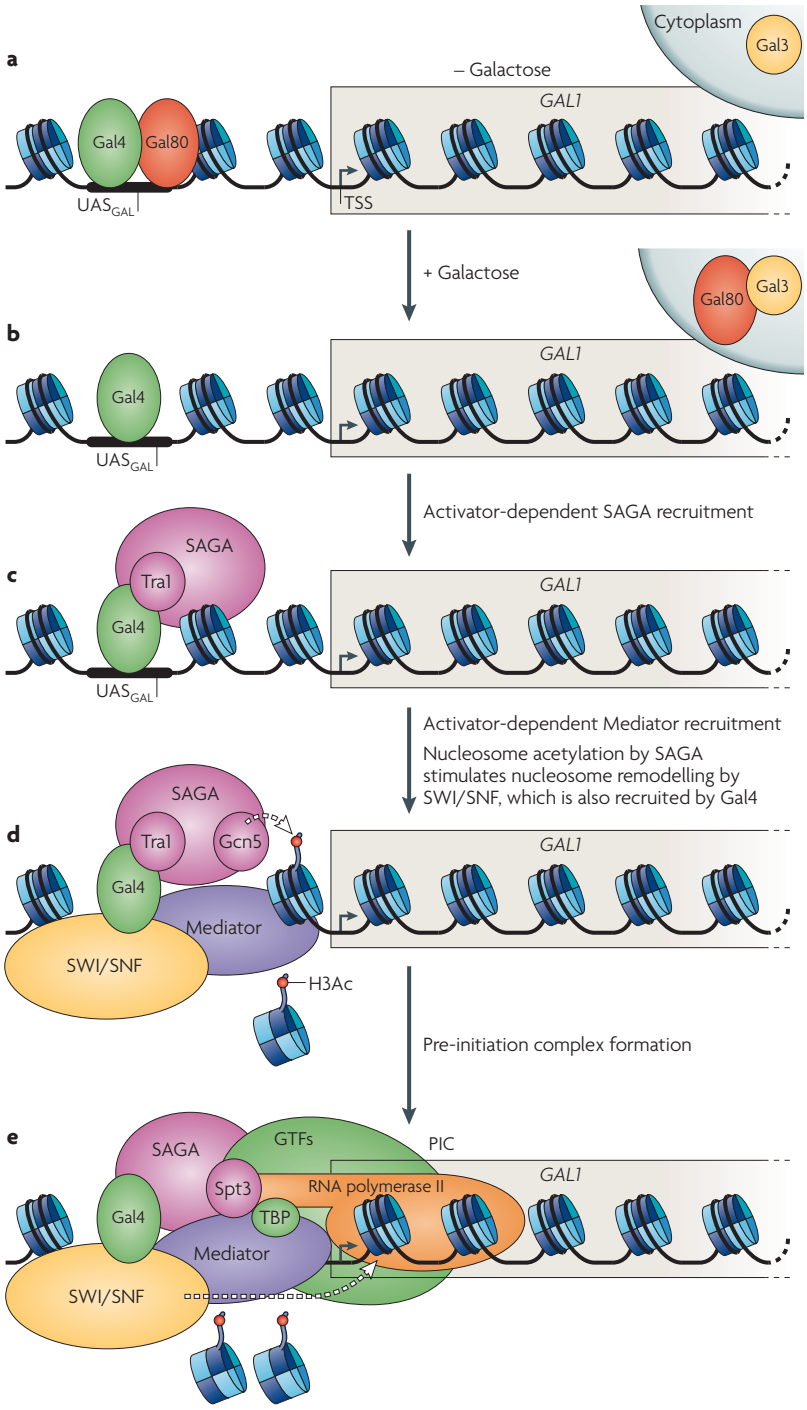


Figure 2 | **Gal4-mediated induction of Gal gene expression requires co-activators.** In yeast, in the absence of galactose, the acidic activator Gal4 is bound by its repressor Gal80 (**a**). Addition of galactose to the growth medium causes an inducer protein, Gal3, to bind and sequester Gal80 in the cytoplasm, releasing it from Gal4 (**b**). Gal4 binds target UAS$_{GAL}$ (upstream activating sequence) sites in the promoters of Gal genes such as *GAL1* and sequentially recruits co-activators, such as the acetyltransferase SAGA (**c**) and Mediator (**d**). Gal4 also recruits ATP-dependent nucleosome-remodelling complexes such as SWI/SNF that remove nucleosomes at the promoter and are stimulated by SAGA-catalysed histone acetylation.Together, SAGA and Mediator recruit RNA polymerase II and the general transcription factors (GTFs), leading to formation of the pre-initiation complex (PIC) (**e**). Nucleosome removal, catalysed by SWI/SNF, aids in the kinetics of Mediator and GTF recruitment, thereby facilitating rapid PIC formation and initiation of transcription at Gal genes. H3Ac, histone H3 acetylation; TBP, TATA-binding protein; TSS, transcription start site.

Gal4-mediated Gal gene induction in yeast. The expression of Gal genes, which encode products that are required for the import and metabolism of galactose, is rapidly induced when galactose is added to the growth medium of *S. cerevisiae* (reviewed in REF. 12). Activation of the Gal genes is regulated primarily through activator-dependent recruitment. Expression is initiated by the transcriptional activator Gal4, which binds to an upstream activating sequence (UAS$_{GAL}$) in the promoters of Gal genes (FIG. 2). The affinity of Gal4 binding varies among the Gal genes, thereby leading to differential levels of activation[12].

The initiation of the entire response of the Gal regulon to galactose is dependent on this transcriptional activator, Gal4. How then is Gal4 itself regulated? The regions of Gal4 that contain the DNA-binding and transcription-activation activities are separable[13]. In the absence of galactose, the acidic activation domain of Gal4 is bound tightly by an inhibitor protein Gal80. This prevents the interaction of this domain with co-activators, such as TATA-binding protein (TBP) or the SAGA acetyltransferase complex[14,15]. When galactose is added to the growth medium, an inducer protein Gal3 sequesters Gal80, alleviating repression of Gal4 and allowing it to interact with and recruit co-activators to the Gal genes[16–21]. The activation function of Gal4 is further regulated by post-translational mechanisms that include phosphorylation and ubiquitin-mediated degradation[12].

Gal gene induction requires co-activators. In Gal gene induction and many other examples of inducible gene expression, recruitment of co-activators and the transcription machinery to promoter regions is the key initial step in activating transcription. Recruitment of co-activators to Gal genes occurs in a sequential but not necessarily interdependent manner. The first co-activator to bind to Gal promoters following a shift to galactose-containing medium is SAGA, which is directly recruited by Gal4 (REFS 22–24). A few minutes following this, Mediator is recruited to Gal promoters through direct contact with Gal4 (REFS 23,25–28). Finally, Pol II and components of the general transcription machinery, including TBP, TFIIH, TFIIE and TFIIF, are recruited to Gal promoters[23]. None of these final components, including Pol II, is recruited in the absence of SAGA, which indicates that Gal4 alone is not sufficient to activate transcription[23,24]. Rather, a combination of SAGA and Mediator activities is required for the recruitment of TBP, Pol II and the remainder of the GTFs[23,24,29,30].

Although this response to galactose might seem specific to yeast and other closely related fungi, the mechanisms by which Gal4 activates gene expression must be widely conserved because Gal4 can activate UAS$_{GAL}$-specific transcription in organisms that range from *D. melanogaster* to humans[31,32]. Furthermore, SAGA, Mediator and the GTFs are highly conserved from yeast to humans (Supplementary information S1 (figure and tables)). The presence of *cis*-regulatory elements that have varying affinities for Gal4 at many different galactose-inducible genes provides a mechanism

to coordinate both the timing and relative levels of expression of the Gal genes. In higher eukaryotes, genes that are co-regulated often share common *cis*-regulatory elements. These regulatory elements can be bound by individual activators or by combinations of transcriptional activators that have varying affinities. For example, the Forkhead and Ets transcriptional activators bind together to the same DNA motif that is present upstream of a set of co-regulated genes to synergistically activate the transcription of these genes in the developing vascular endothelium[33].

Co-activators facilitate gene activation

In prokaryotes, transcriptional activators directly contact RNA polymerase[34], so why are co-activators required in eukaryotes? Many studies have shown that, in contrast to prokaryotes, most genomic DNA in a eukaryotic cell is compacted into chromatin and therefore is not directly accessible to components of the general transcription machinery (reviewed in REF. 35). Although some transcriptional activators such as the human glucocorticoid receptor can bind their target DNA sequence in a nucleosomal context[36], PIC formation and subsequent transcription are inhibited by nucleosomes *in vitro*[37–39]. Furthermore, studies in yeast suggest that inducible genes tend to have a higher density of nucleosomes covering their promoters than constitutively active genes, which have more open, nucleosome-depleted promoters (reviewed in REF. 40). In eukaryotes, co-activators and nucleosome-remodelling complexes act together to facilitate gene activation in a nucleosomal context.

Co-activators are required for PIC formation. Overcoming the nucleosomal barrier to transcription initiation requires complexes such as SAGA and Mediator. Intriguingly, complexes involved in transcription activation often possess enzymatic activities directed towards the amino-terminal tails of histone proteins in the nucleosome. SAGA, for instance, contains the histone acetyltransferase (HAT) Gcn5 (REF. 41). So, are histone-modifying activities required for recruitment of Pol II and the general transcription machinery?

Although nucleosome acetylation by activator-recruited SAGA stimulates transcription *in vitro*[42,43], surprisingly, the acetyltransferase activity of SAGA is not directly required for Pol II recruitment and PIC formation at Gal genes[22]. However, mutations in other SAGA components such as Spt3 that only modestly reduce SAGA recruitment substantially decrease PIC formation[22,24]. Therefore, importantly, the co-activator function of SAGA requires more than its enzymatic activity. Rather, both SAGA and Mediator have structural roles during inducible gene expression, forming a scaffold on which components of the general transcription machinery and Pol II can assemble. Mediator interacts directly with the unphosphorylated form of the carboxy-terminal heptapeptide repeat sequences (carboxy-terminal domain; CTD) of the RBP1 subunit of Pol II (reviewed in REF. 44). Therefore, in the case of Gal gene induction, Mediator links Gal4 and the general transcription machinery.

A HAT-independent role for other co-activators such as p300 has also been reported. Mutation of the KIX transcription factor-binding domain of mouse p300 results in severe defects in haematopoiesis but mutation of the HAT domain has little effect[45]. Furthermore, the role of human PCAF (also known as KAT2B) as a co-activator in activation of human T cell leukaemia virus type 1 long terminal repeat transcription is also independent of its HAT activity[46]. Although these examples show that some HATs can have histone acetylation-independent functions in gene activation, at some genes, histone acetylation is required for PIC formation. For example, in yeast, mutation of the HAT Gcn5 in SAGA decreases the levels of TBP and Pol II recruitment at some genes[47]. However, the findings discussed above show that, in the early stages of the transcription cycle, the structural role of co-activators is of at least equal importance to their histone-modifying activities.

Chromatin remodelling and transcription

To understand why histone acetylation is required for the activation of some genes but not others, we need to consider another class of transcription regulators known as ATP-dependent nucleosome-remodelling complexes. Nucleosome-remodelling complexes use the energy from ATP hydrolysis to move histones or displace them from one piece of DNA onto another or onto a histone-binding protein, known as a histone chaperone (reviewed in REF. 48). Following galactose induction, Gal4 recruits the nucleosome-remodelling complex SWI/SNF to Gal gene promoters at which it rapidly removes promoter nucleosomes[28,49,50]. Intriguingly, a recent study has shown that in the absence of SWI/SNF, these promoter nucleosomes are still removed and transcription is initiated, albeit much more slowly[49]. Therefore, nucleosome removal facilitated by nucleosome-remodelling complexes is important for the swift induction of gene expression but is not necessarily essential for overall levels of induction at every inducible gene. This implies that at some inducible genes, given enough time, recruitment of co-activators and the general transcription machinery by an activator alone is sufficient to remove nucleosomes at the promoter. However, nucleosome remodelling is crucial for inducible gene expression as it facilitates the rapid activation of transcription. Furthermore, at some promoters such as at the human α1 antitrypsin gene, recruitment of nucleosome-remodelling proteins like brahma is rate-limiting for activation[51].

Is histone acetylation important for chromatin remodelling? At least in the example of the Gal genes, SWI/SNF recruitment does not depend on SAGA[28]. However, *in vitro* studies suggest that histone acetylation is important for the binding of SWI/SNF to nucleosomes and that acetylated histones are preferentially displaced by SWI/SNF[52–54]. Nucleosome-remodelling complexes such as SWI/SNF or RSC are also important for assisting Pol II transcription of a nucleosomal template[55]. Intriguingly, HAT complexes such as NuA4 and SAGA increase RSC-stimulated transcription by Pol II *in vitro*[55]. These findings support a model in which the acetylation activity of SAGA promotes SWI/SNF

Pre-initiation complex
A 44 polypeptide complex, which consists of RNA polymerase II and the general transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH. Its formation at TATA-containing core promoters is nucleated by binding of TATA-binding protein (a component of TFIID) to the TATA element in the promoter DNA sequence.

Acidic activation domain
The type of transactivation domain found in transcriptional activators such as Gal4. This domain contains a stretch of acidic amino acids and is required for interactions with co-activators.

ATP-dependent nucleosome-remodelling complex
A transcriptional regulatory complex that uses the energy obtained from ATP hydrolysis to move or displace histone octamers from DNA.

activity, therefore facilitating the rapid removal of promoter nucleosomes after galactose induction, leading to the swift activation of Gal transcription. In addition to this role in the early stages of transcription initiation, HAT complexes and nucleosome remodellers assist polymerase elongation through the coding region of Gal genes.

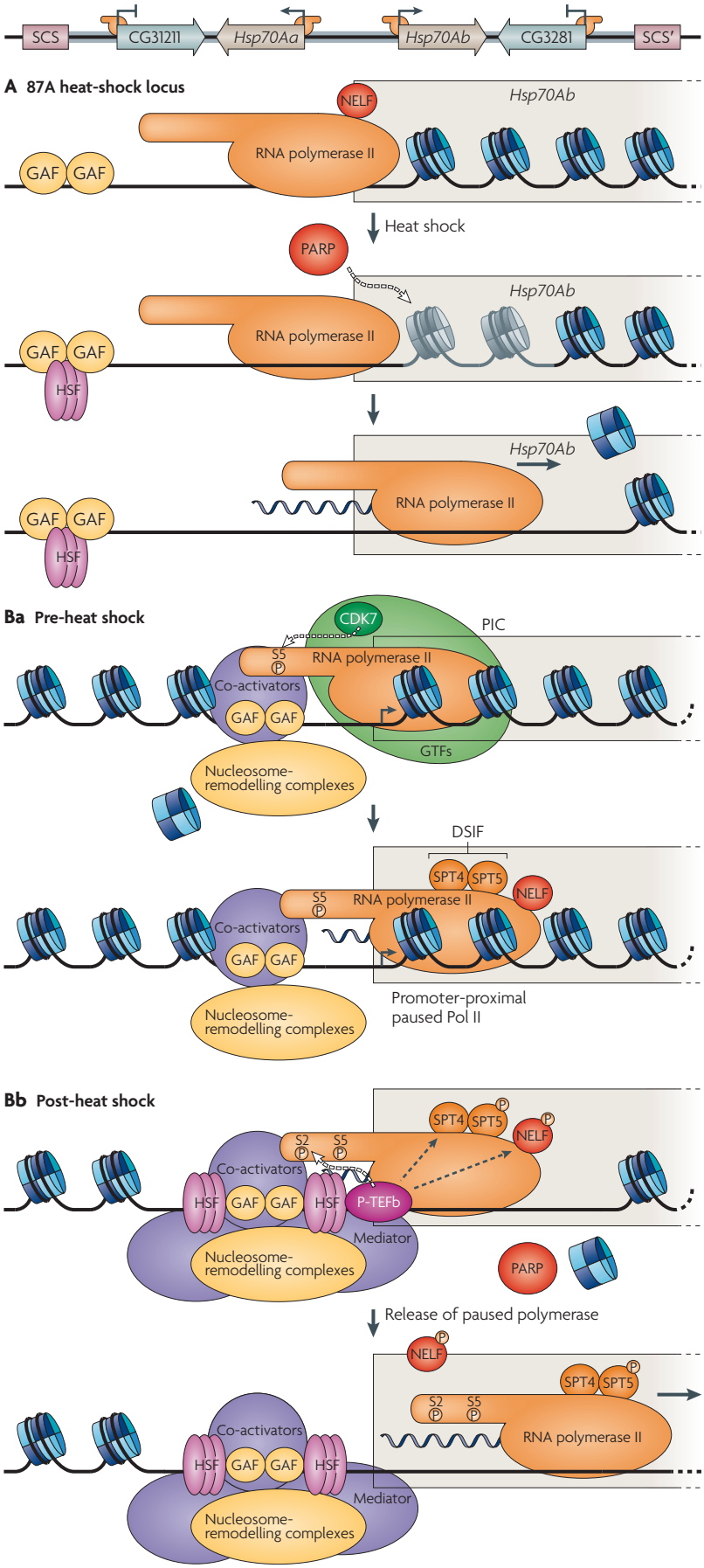
The interplay between SAGA and SWI/SNF at Gal genes is similar to that observed at the yeast *PHO5* gene under phosphate starvation conditions[56,57]. SAGA and SWI/SNF are not essential for *PHO5* activation but are important for the kinetics of activation. Therefore, the functions of a transcriptional co-activator complex such as SAGA can be separated into HAT-independent activities that are essential for PIC formation and HAT-dependent activities that are required for chromatin remodelling and the kinetics of gene induction. At some inducible genes these functions overlap, for example, the Gcn4-dependent genes in yeast[46], as HAT activity is also required for nucleosome remodelling before PIC formation.

The role of co-activators in stimulating nucleosome remodelling is not limited to HAT complexes. At some genes, Mediator is also required for the recruitment of SWI/SNF or for its chromatin remodelling activity[28,58]. However, at other genes such as yeast *CHA1*, chromatin remodelling is independent of Mediator[59]. Therefore, nucleosome remodelling can occur before or subsequent to the activities of Mediator and SAGA and, in some cases, is even required for their co-activator functions.

Is nucleosome loss sufficient to activate transcription? In some examples in yeast, nucleosome loss alone is sufficient to induce PIC formation and activate gene expression, even in the absence of an inducing signal[60,61]. Moreover, when yeast are shifted to growth media containing glucose, transcription of the Gal genes is repressed and nucleosomes rapidly reform at Gal promoters[62,63]. Therefore, at many genes, nucleosome loss correlates positively with transcription. However, several lines of evidence across different species show that a nucleosome-free region on its own is not necessarily sufficient to activate transcription. Furthermore, repression is possible in the absence of nucleosomes. In yeast, expression of the Gal genes is repressed in the presence of a combination of glucose and galactose, although Gal4 still recruits SWI/SNF and maintains a nucleosome-free promoter region[49]. Another example of this — heat shock induction in *D. melanogaster* — is discussed below,

***PARP-dependent nucleosome loss at* D. melanogaster Hsp70.** Heat shock induces a rapid loss of nucleosomes across the entire *Hsp70* locus in *D. melanogaster* that precedes and is independent of transcription[64–66] (FIG. 3a). This nucleosome loss corresponds at least partially to the diffuse appearance or puffing of the heat-shock loci on polytene chromosome spreads and is important for optimal levels of *Hsp70* expression[64] (reviewed in REF. 67). Although nucleosome loss is required for optimal transcription of the heat-shock genes at *Hsp70*, it is not sufficient to activate expression of other genes that lie in the region of nucleosome disruption[64]. Furthermore, chemicals that induce puffing and nucleosome loss at heat-shock loci such as sodium salicylate do not activate expression of the heat-shock genes[68]. Therefore, although nucleosome loss is important for rapid activation of gene expression and is sometimes required for optimal levels of gene expression, these studies show that nucleosome loss alone is not necessarily sufficient to induce activated transcription. Rather, nucleosome remodelling can be an important step in providing access to transcriptional activators and the general transcription machinery.

Figure 3 | **Heat shock induces nucleosome loss and release of paused RNA polymerase II. a** | At the top is a schematic of the *Drosophila melanogaster* 87A heat-shock locus; the example of *Hsp70Ab* is used below. Equivalent events occur at *Hsp70Aa*. The arrows at the *Hsp70Aa* and *Hsp70Ab* promoters indicate the direction of transcription through the gene. RNA polymerase II (Pol II, shown in orange) and GAGA factor (GAF or Trithorax-like) are bound at the promoters of the Hsp70 genes before heat shock. After heat shock, the transcriptional activator heat-shock factor (HSF) forms a stable trimeric complex that binds the *Hsp70* promoter[105,106]. Heat shock stimulates nucleosome loss at the *Hsp70* locus. Nucleosome loss is dependent on HSF, GAF and poly(ADP)-ribose polymerase (PARP)[64]. PARP localizes at many sites along polytene chromosomes but only catalyses formation of ADP-ribose polymers from donor NAD^+ at the heat-shock loci after induction by heat shock[107]. Nucleosome loss proceeds outwards from the 5′ end of the *Hsp70* genes ahead of Pol II, extending as far as the SCS and SCS′ boundary elements (region of loss is grey in top panel). The CG31211 and CG3281 genes are not transcribed (as shown by blunt-headed arrows at their promoters) although Pol II is bound at their promoters and nucleosomes are lost. **Ba** | Before heat-shock activation at *Hsp70*, GAF recruits co-activators, the GTFs and ATP-dependent nucleosome-remodelling complexes, thereby facilitating pre-initiation complex (PIC) formation at the promoter. At *Hsp70*, however, PIC formation is not sufficient to activate productive transcription elongation[89,108–110]. The CDK7 subunit of TFIIH phosphorylates serine 5 (S5) of the carboxy-terminal domain (CTD) and Pol II initiates transcription into the first 20–40 bases of the gene, at which it pauses at the promoter-proximal pause site. Pol II is held here by the negative elongation factor (NELF) and DRB sensitivity-inducing factor (DSIF), which is composed of SPT4 and 5 (REFS 111,112). **Bb** | Heat shock induces binding of HSF, which recruits additional co-activators and nucleosome-remodelling activities. HSF is required but is not sufficient to recruit the pause release factor P-TEFb to *Hsp70* (REF. 85). Recruitment of Mediator by HSF, which occurs independently of PIC formation, might contribute to P-TEFb binding[113]. Recruited P-TEFb phosphorylates S2 of the CTD, SPT5 and NELF. These phosphorylations cause NELF to dissociate from Pol II, releasing polymerase into productive transcription elongation. Although Pol II still pauses briefly at the promoter-proximal pause site under heat-shock conditions, the duration of these pauses are much shorter than at normal temperatures.



These findings might seem at first to contrast with results from older studies in which histone H4 was depleted in yeast using a genetic approach[69]. In the absence of H4, the yeast *PHO5* promoter was activated independently of its UAS. However, although nucleosome depletion resulted in activation of *PHO5*, the level of activation was significantly lower than that observed under physiological induction conditions. A similar result is observed for *GAL1* in which nucleosome loss activates a low level of transcription that is independent of UAS_{GAL}. In both these examples, the level of transcription observed after nucleosome loss is significantly lower than that observed under inducing conditions when nucleosomes are present. Therefore, although some transcription may occur in the absence of nucleosomes, full activation of inducible gene transcription generally requires additional factors such as transcriptional activators.

Post-recruitment initiation of transcription

Together, the activator-dependent recruitment of the general transcription machinery and chromatin remodelling at the promoter facilitate formation of the PIC. However, once Pol II and the GTFs are present in the PIC, a series of sequential chromatin modification events are required for Pol II to clear the promoter and initiate efficient transcription elongation (FIG. 4). Although loss of some histone modifications can impair overall levels of transcription activation at some inducible genes (for example, loss of acetylation by the HAT Gcn5 (REF. 70) or loss of H2B ubiquitylation by Rad6 (REF. 71)), these histone modifications are often not essential for co-activator-dependent transcription from a chromatin template *in vitro*[72]. It is likely that the *in vitro* experiments do not completely recapitulate *in vivo* regulation. In addition, it seems that many of these histone modifications are important for later stages of the transcription cycle. Therefore, it is useful to describe the chromatin modification events that occur during the early steps of PIC formation and promoter clearance to provide an overview of the role of these chromatin marks in activating efficient transcription elongation by Pol II.

Histone modifications and transcription initiation. In addition to recruiting more traditional co-activators such as SAGA and Mediator, the yeast Gal4 transcriptional activator recruits histone-modifying activities, including the ubiquitin conjugase–ligase pair Rad6–Bre1 (REFS 71,73,74) (FIG. 4). Together, Rad6 and Bre1 monoubiquitylate histone H2B[73–78]. Monoubiquitylation of H2B is a prerequisite for histone H3 lysine 4 (H3K4) di- and trimethylation by the Set1-containing complex COMPASS in yeast (reviewed in REF. 79). Studies suggest that some of the histone modification events that occur during transcription activation such as H2B ubiquitylation might also be important for steps that occur during promoter clearance and release of Pol II into productive elongation. For example, deubiquitylation of H2B mediated by a ubiquitin protease in the yeast SAGA co-activator complex Ubp8 (REFS 80,81) is required for

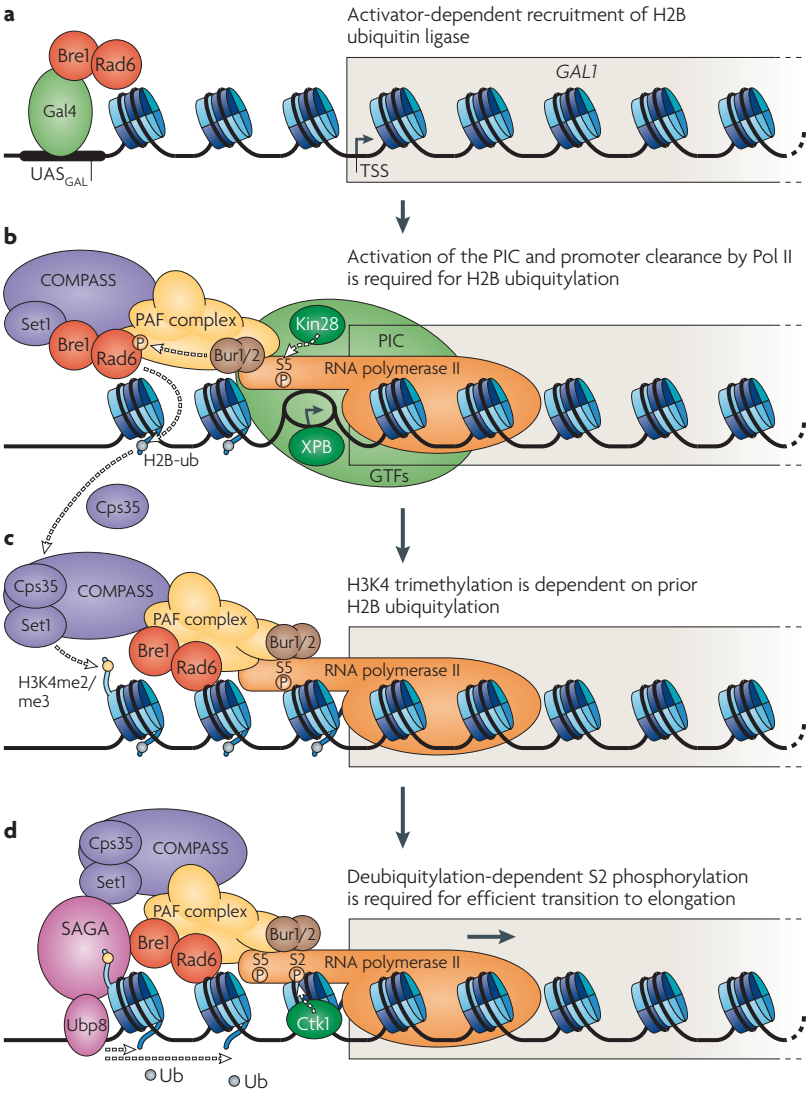


Figure 4 | **Sequential chromatin modification events occur during the early stages of transcription initiation at inducible genes. a** | The ubiquitin ligase–conjugase pair Bre1–Rad6 are directly recruited to yeast genes such as *GAL1* by the Gal4 activator[71,73,74] (shown) or to human genes such as *MDM2* by p53 (REF. 114) (not shown), but require additional factors to monoubiquitylate H2B. **b** | Once the pre-initiation complex (PIC) has been established through the action of co-activator complexes, such as the acetyltransferase SAGA and Mediator, a DNA helicase within TFIIH (XPB in humans and Rad25 in yeast) unwinds DNA at the promoter to introduce a single-stranded template into the active site of RNA polymerase II (Pol II). Another subunit of TFIIH, Kin28 in yeast (CDK7 in humans), phosphorylates serine 5 (S5) of the carboxy-terminal domain (CTD) of RNA polymerase II (Pol II), releasing Pol II from the promoter. Both phosphorylation of S5 and initiation of transcription, together with the PAF and BUR complexes that are important for this phase of transcription[115], are required for Rad6–Bre1 catalysed monoubiquitylation of H2B in yeast and humans[73–78]. Recent work has shown that the human RAD6–BRE1 pair requires PAF-mediated transcription *in vitro* for effective H2B ubiquitylation activity[72]. Phosphorylation of Rad6 by the BUR complex might also directly stimulate its ubiquitin conjugase activity. **c** | During promoter clearance by Pol II, the general transcription factors separate from polymerase, which then transcribes a short region into the gene. During or preceding this stage of transcription, H2B ubiquitylation recruits the Cps35 subunit of the COMPASS complex, which is required for the methyltransferase Set1 to di- and trimethylate histone H3 lysine 4 (H3K4me2/me3) (REFS 79,116). **d** | An additional phosphorylation at S2 of the CTD of Pol II by yeast Ctk1 then occurs and is important for efficient transition of Pol II into the elongation stage of transcription. Deubiquitylation of H2B mediated by the Ubp8 subunit of yeast SAGA is important at some genes for Ctk1 activity[82] and for optimal levels of transcriptional activation[80,81]. TSS, transcription start site; Ub, ubiquitin.

recruitment of the serine 2 CTD kinase Ctk1. In the absence of Ubp8, Ctk1 is not recruited to elongating Pol II, and the subsequent recruitment of the H3K36 methyltransferase Set2 is inhibited[82]. Phosphorylation of Pol II S2 by Ctk1 (P-TEFb/CDK9 in *D. melanogaster* and humans) is a critical rate-limiting step in releasing paused Pol II into productive elongation at certain inducible genes in higher eukaryotes and this is discussed below.

Release of paused Pol II. The activator-dependent model of gene activation assumes that PIC formation is sufficient to activate gene expression. This assumption was historically supported by evidence showing that pre-formation of a PIC on a DNA template before chromatin assembly facilitates transcription initiation *in vitro*[37–39]. Furthermore, many well-studied inducible genes such as the yeast Gal genes behave as the activator-dependent recruitment model would predict. However, in other examples of inducible gene expression such as the heat-shock genes in *D. melanogaster* neither PIC formation nor nucleosome loss is sufficient to activate transcription. Additional factors are required to release Pol II from the PIC into productive transcription elongation (reviewed in REF. 83). Therefore, the rate-limiting step during the transcription cycle can differ between various inducible genes.

Heat-shock regulation by release of paused Pol II. Early studies of the *D. melanogaster Hsp70* genes showed that, before heat-shock induction, Pol II is already associated with the promoter and has transcribed 20–40 bases downstream of the transcription start site at which it remains in an arrested state[84] (FIG. 3Ba). How is this paused polymerase released into productive transcription elongation? For *Hsp70*, release requires recruitment and activation of the specific transcriptional activator heat-shock factor (HSF). HSF recruitment alone, however, is not sufficient to activate transcription[68]. Instead, HSF is required for recruitment of a second factor, P-TEFb, a heterodimer composed of CDK9 and cyclin T[85] (FIG. 3Bb). P-TEFb recruitment has been found to be the crucial step for release because P-TEFb that is artificially tethered to the *Hsp70* promoter is sufficient to activate transcription in the absence of heat shock[85]. Evidence suggests that in addition to phosphorylating S2 of the Pol II CTD, P-TEFb phosphorylates negative elongation factor (NELF), and SPT5 in DRB sensitivity-inducing factor (DSIF). Phosphorylation relieves the inhibitory effects of these factors on the polymerase, therefore releasing Pol II into productive transcription elongation[83,86].

PolII pausing is a key regulatory step for many genes. Recent studies suggest that regulation of promoter-proximal pausing may be a widespread phenomenon. Chromatin immunoprecipitation studies have shown that many developmental control and environmental response genes in *D. melanogaster* have paused Pol II at their promoters before activation[1,2]. Similarly, it had previously been found that approximately 20%

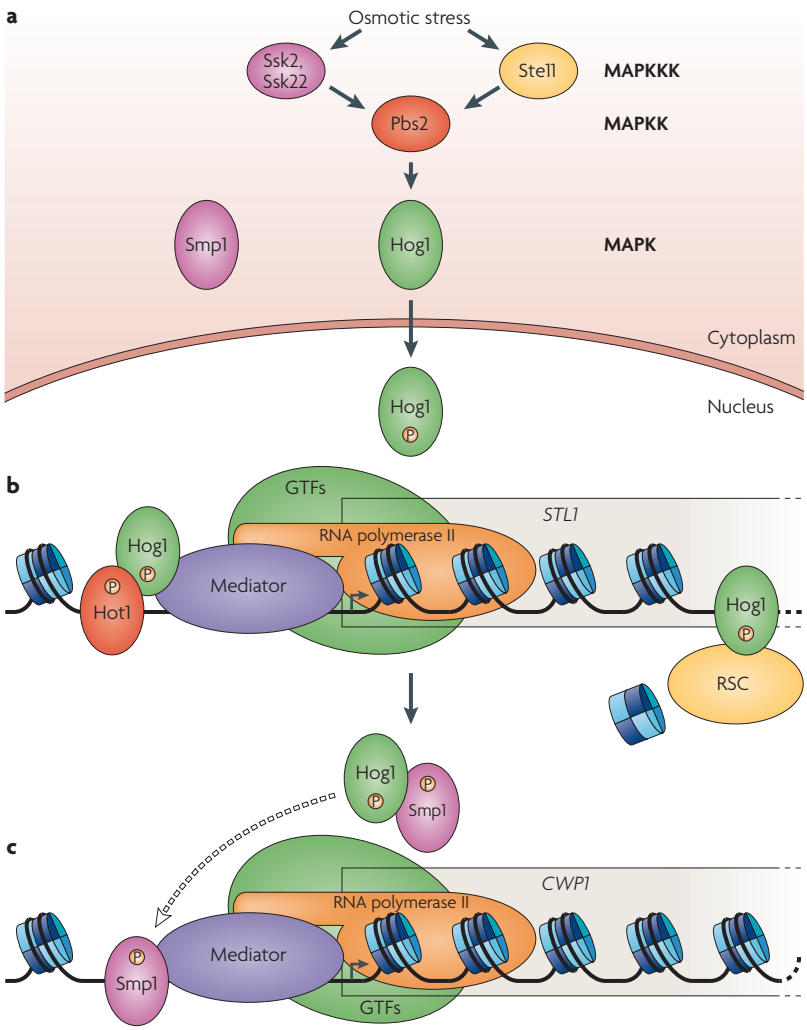


Figure 5 | **The Hog1 MAPK induces osmostress gene expression through different mechanisms. a** | Osmotic stress in yeast induces a mitogen-activated protein kinase (MAPK) cascade in which the MAPKKKs Ssk2, Ssk22 and Ste11 converge to phosphorylate and activate the MAPKK Pbs2. Phosphorylated Pbs2 activates the high osmolarity glycerol response 1 (Hog1) MAPK, which then translocates to the nucleus to activate osmotic stress-response gene expression. **b** | Hog1 is recruited to the promoters of osmostress-response genes such as *STL1* by interactions with its substrate, the transcriptional activator high-osmolarity-induced transcription 1 (Hot1) (REF. 93). Hog1 functions as a transcriptional activator at *STL1*, interacting directly with Mediator and components of the general transcription machinery. Hog1 also recruits the RSC nucleosome-remodelling complex to the coding region of *STL1*, at which it assists the passage of RNA polymerase II (Pol II) during transcription elongation. **c** | Hog1 phosphorylates transcriptional activators such as Smp1 (REF. 91), and this phosphorylation is important for retention of Smp1 in the nucleus. Phosphorylated Smp1 then recruits additional co-activators and the general transcription machinery to genes, including *CWP1* and also *STL1*, to initiate Pol II-dependent transcription. P, phosphorylation.

of actively transcribing genes in *D. melanogaster* cell culture nuclei have a paused polymerase, which can be released by sarkosyl or high salt conditions in nuclear run-on assays[87]. Furthermore, these observations are not restricted to *D. melanogaster*, as Pol II and marks of active transcription such as trimethylated H3K4 are also observed at the promoters of genes that are not being actively transcribed in human embryonic stem cells[4–6] and in differentiated cells[3,4].

Why would Pol II be present at the promoters of genes that are not being actively transcribed? It has been speculated that regulated release of paused polymerase might facilitate a faster response to developmental signals than activator-dependent recruitment alone, allowing rapid changes in developmental gene expression during embryogenesis[1]. There is also strong evidence supporting a role for polymerase pausing in synchronous gene activation: the coordinate expression of genes in many cells in a given tissue. Genes that are activated in a synchronous rather than stochastic pattern during *D. melanogaster* embryogenesis tend to have paused Pol II present at their promoters before activation[88]. Synchronous activation of inducible gene expression across different cells in higher eukaryotes ensures that morphogenetic changes in an organism such as cell division and migration occur in a coordinated fashion. Other studies suggest that pausing may allow time for the mRNA processing machinery to correctly assemble and cap nascent RNAs[83]. Whatever the reasons, the mechanisms involved in the regulation of pausing seem to be more widely used than one might expect given the specialized nature of heat-shock gene expression. Genome-wide studies in *D. melanogaster* indicate that NELF associates with more than 2000 genes, suggesting that its role in regulating promoter-proximal pausing is not confined to stress-regulated genes such as the heat-shock loci[89]. Furthermore, a recent study has shown that more than one-third of *D. melanogaster* genes show a form of polymerase stalling that might be partly caused by the nucleotide composition and stability of the initial RNA–DNA hybrid[90].

Signalling kinases and transcription

Whether inducible gene expression is regulated at the level of activator-dependent recruitment or the release of paused polymerase, genes must be activated quickly and specifically in response to the appropriate extracellular signal. Various mechanisms are used to regulate the activity of transcriptional activators. Two examples have already been discussed in this Review: release of Gal80 repressor binding to Gal4 and heat-shock-dependent trimerization of HSF. The post-translational modification of transcriptional activators is another example of a mechanism used to induce gene expression. In signal transduction cascades such as the MAPK system, tiers of protein kinases phosphorylate kinases that are further downstream. These phosphorylation cascades result in the phosphorylation of target transcriptional activators and the subsequent induction of gene expression. Several recent studies suggest that, surprisingly, components of these MAPK signal transduction cascades may have additional roles in regulating transcription through direct interactions with co-activators and the general transcription machinery.

Hog1-mediated response to osmostress. The *S. cerevisiae* stress-activated kinase high osmolarity glycerol response 1 (Hog1) responds to increases in extracellular osmolarity and is required to activate the expression of several genes that are needed to respond to this stress[7].

In response to osmotic stress, two different signalling pathways converge to phosphorylate and activate the MAPK Pbs2, which then phosphorylates Hog1 (REF. 7) (FIG. 5a). Hog1 phosphorylation induces its nuclear localization, allowing it to interact with and phosphorylate several transcriptional activators, including high osmolarity-induced transcription 1 (Hot1) (FIG. 5b) and Smp1 (FIG. 5c). Hog1-dependent phosphorylation of Smp1 is required for stress-induced gene expression[91,92] but Hog1-mediated phosphorylation of Hot1 is not[93]. Instead, Hog1 is recruited to Hot1-regulated promoters through the interaction of the kinase with its substrate, Hot1 (REF. 94). Furthermore, although the kinase activity of Hog1 is required for binding to chromatin and transcriptional activation[94], Hog1-dependent phosphorylation of Hot1 is not[93]. These findings suggest that the kinase activity of Hog1 may recruit it, through interactions with transcriptional activators such as Hot1, to the promoters of stress-response genes, at which it might then play additional parts in transcriptional activation.

Intriguingly, tethering of a Hog1–LexA fusion protein to a stress-responsive promoter is sufficient to activate gene expression after stress[93]. Moreover, recombinant Hog1 physically associates with subunits of Pol II, TFIIH and Mediator independently of transcriptional activators such as Hot1 (REF. 93). These findings suggest that Hog1 recruits Mediator and the general transcription machinery to stress-responsive promoters during osmotic stress, and is required for PIC formation[93]. This unexpected role for a signalling kinase in activator-dependent recruitment is not restricted to this example from yeast. In humans, progesterone induces recruitment of both the MAPK ERK1 and its target MSK1 to target genes[95]. Similar to the Hog1–Hot1 example, ERK1 recruitment to FOS serum response element-containing genes requires the transcriptional activator ELK1 (REF. 96). Furthermore, an example from a recent study in human cells that involves gene repression rather than activation showed that the protein kinase ERK2 (also known as MAPK1) phosphorylates transcription factors, exhibits sequence-specific DNA binding and represses the expression of interferon γ-responsive genes independently of its kinase activity[97]. In addition to Hog1, many other kinases in yeast localize to chromatin, suggesting that the presence of signalling kinases at the promoters of target genes might be a widespread mechanism used by signal transduction pathways.

Interestingly, the role of these MAPKs might not be limited to PIC formation. Hog1 also interacts with the elongating form of Pol II and is found on the coding regions of osmostress-response genes following osmotic shock[98–100]. Recent work suggests that Hog1 recruits the RSC nucleosome-remodelling complex to the coding regions of osmostress-response genes and this facilitates efficient transcription by Pol II[101]. This suggests that signalling kinases might have additional roles in the regulation of transcription elongation, confirming their function as integral components of the transcription cycle. Recent work suggests that the presence of the MAPKs at the promoters of their target genes might facilitate a rapid switch between activation and repression in response to stimuli. In human pancreatic β-cells, ERK1 and 2, p38, JNK and their upstream effector kinase RSK bind together to the same region of the insulin promoter in the presence of the proinflammatory cytokine interleukin-1β (IL-1β)[102]. These kinases promote activation or repression in the presence of IL-1β, depending on whether the cells are in fasting or hyperglycaemic conditions. Therefore, the presence of signalling kinases at the promoters of target genes, which act as integral components of the transcription machinery, allows rapid activation or repression of transcription in response to extracellular signals.

Conclusions

Regulation of inducible gene expression can occur at multiple stages of the transcription cycle. At some genes such as the Gal genes in yeast the key regulatory step occurs during activator-dependent recruitment of the transcription machinery. By contrast, Pol II is already present on the promoters of other genes such as the heat-shock genes in *D. melanogaster*. At these genes, the key regulatory step in inducing gene expression occurs at the release of Pol II into productive transcription elongation. In addition, we are beginning to understand that there is an overlapping relationship between signal transduction pathways and inducible gene expression, as components of some signal transduction pathways are observed to function as co-activators; for example, the Hog1 kinase in the response to osmotic stress.

Certain fundamental principles still hold true for all inducible genes despite differences in the relative importance of each transcriptional stage to inducible gene activation. First, inducible genes must be specifically activated in response to stimuli. Whether or not the rate-limiting step occurs at the level of paused polymerase, PIC formation must occur as the initial step in transcriptional activation to specify the appropriate start site for transcription, as identified by sequence-specific transcriptional activators and DNA-binding proteins in the transcriptional machinery. Following PIC formation and release of Pol II from the promoter, additional factors are required to facilitate the switch from an initiation mode of Pol II into transcription elongation.

The widespread dependence of *D. melanogaster* genes on NELF[89] and higher than expected occurrence of stalled polymerase molecules in *D. melanogaster*[90] suggest that regulation at the level of polymerase pausing might be a general feature of inducible gene expression. Even at the Gal genes, which seem to be regulated primarily at the level of activator-dependent recruitment, processes important for release of paused polymerase are regulated. For example, deubiquitylation of H2B by SAGA is required for the yeast equivalent of P-TEFb Ctk1 to phosphorylate S2 of the Pol II CTD[82]. Perhaps the only differences in terms of regulation between the two classes of inducible genes typified by the Gal and heat-shock genes are the relative kinetics and contribution of these two regulatory steps to gene activation. At the Gal genes, SAGA is required for Ctk1-mediated phosphorylation of the Pol II CTD and is directly recruited by the transcriptional activator Gal4 during the first stages of

gene induction. Therefore, no additional activators are required for Ctk1 recruitment and/or activity at Gal. By contrast, two different activators are required for establishment of PIC formation and release of paused Pol II at the heat-shock genes; GAF is sufficient for PIC formation whereas activated HSF is required for P-TEFb recruitment and release of paused Pol II.

The relative use of these two regulatory mechanisms of inducible gene expression is not yet understood on a genome-wide scale. One key question remaining in the field is how release of paused Pol II is activated at particular genes but not at others. Does binding of additional transcriptional activators genes regulate the specificity of release of paused Pol II at most genes? Or are regulation of the recruitment and/or activity of additional co-activators, such as SAGA and/or Mediator, involved? It remains to be determined whether there is a role for as yet unidentified co-activators in the release of paused Pol II. Furthermore, it is possible that components of signal transduction cascades such as MAPKs might play additional parts in regulating this release.

A second major question is why the rate-limiting step during gene activation differs among genes. One appealing hypothesis is that the release of pausing allows the same gene to be activated simultaneously across many different cells in response to a signal[88]. In contrast to the release of paused Pol II, which is essentially dependent on a single factor (P-TEFb), multiple processes must occur during activator-dependent recruitment before transcription can be initiated. Therefore, if genes are to be expressed coordinately in response to developmental cues or stress, regulating the release of paused Pol II provides a rapid mechanism by which this can be achieved in multiple cells simultaneously.

Owing to space constraints, we have not discussed events in transcription that occur following the release of paused Pol II, including elongation, mRNA splicing and processing, termination and nuclear export. Many studies suggest that these events occur co-transcriptionally[11], and therefore inducible gene expression is likely also to be regulated at these steps. Further studies examining both the kinetics of gene activation and the sequential localization of proteins to activated genes will help identify factors that have more subtle roles in gene activation. In addition, many of the co-activator complexes involved in inducible gene expression have tissue-specific forms in mammalian cells[103], suggesting that an additional level of complexity is present in the regulation of inducible gene expression in mammals.

The multiple layers of regulatory control that govern inducible gene expression provide a myriad of ways in which cells can control their responses to external stimuli. By regulating different stages during the transcription cycle, cells can regulate both the speed and coordination of their response to particular signals. Integrating the components of signal transduction cascades directly with the core transcriptional machinery provides another mechanism to coordinate the activation of transcription. Together, these regulatory mechanisms provide organisms that range from yeast to humans with the tools their cells need to respond effectively to a rapidly changing environment.

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Competing interests statement
The authors declare no competing financial interests.

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