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Ordered Recruitment: Gene-Specific Mechanism of Transcription Activation

Review

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Activators, chromatin-modifying enzymes, and basal transcription factors unite to activate genes, but are recruited in a precise order to promoters. The timing of the activation of transcription and the ordered recruitment of factors to promoters are the engines which, at the right moment and for the right length of time, drive the transcriptional regulation of each gene throughout the life of a cell.

Introduction

In the many years of investigations aimed at an understanding of how genes encode their protein products, many efforts focused initially on the identification of basal transcription factors, activators, repressors, and consensus sequences and, subsequently, on the comprehension of how these factors and their cognate binding regions within promoters maintain their cross-talk. The following "transcription scenario" emerged: (1) Activators bind one or multiple regulatory sequences; (2) In cooperation with TAF proteins, TBP (a subunit of TFIID) binds the TATA box; (3) TFIIB, which helps RNA Polymerase II to select the start, adds to the TBP complex; (4) The RNA Pol II holoenzyme, in concert with TFIIF, TFIIE, and TFIIH, associates with the promoter and forms the preinitiation complex (PIC); (5) Promoter melting and transcription initiation occur; and (6) TFIIH phosphorylates the largest RNA Pol II subunit, the Rpb1 C-terminal domain (CTD), leading to promoter clearance and progression into the elongation phase (Hampsey and Reinberg, 1999; Orphanides et al., 1996). This is, with a few variations and exceptions, the step-wise, in vitro model of transcription activation. An alternative model, however, is one in which basal transcription factors and RNA Pol II are preassembled together in a gigantic complex that associates at once with promoters (Orphanides et al., 1996). In both models, activators guide the basal transcription machinery through Mediator, a modular complex of factors that "mediate," like a regulatory bridge, the signals between activators and RNA Pol II (Hampsey and Reinberg, 1999; Myers and Kornberg, 2000; Woychik and Hampsey, 2002).

Simultaneous with the transcription activation studies, other researchers tried to explain how DNA sequences are arranged within the chromosomes. This led to the recognition of chromosomes as being organized into nucleosomes, and of nucleosomes as being formed by DNA coiled around the histone octamers (Felsenfeld and McGhee, 1986; Richmond et al., 1984; Wang, 1982). However, the functional interplay between transcription and chromatin structure was not obvious for many years, and science went on in two parallel directions. Chromatin was divided into two different categories: euchromatin, which is highly transcribed and per se permissive to the binding of transcription factors, and heterochromatin, which was thought to be silenced and never transcribed. Today, the general view has dramatically changed. We now know that heterochromatin is rich in repetitive sequences but is not at all devoid of genes. Moreover, euchromatic regions of chromosomes can be silenced by packaging in a heterochromatic form; an example is the inactive X chromosome in the female, where either the randomly selected paternal or maternal chromosome is condensed and silenced (Richards and Elgin, 2002).

Only recently has it become obvious that both gene transcription activation and chromatin structure studies are two functionally correlated fields. This conclusion has been made possible by the discovery in almost all organisms of chromatin-modifying complexes, which were recognized to be involved in modifying histone residues and nucleosome conformation, thereby leading to gene transcription activation. They can be divided into two main groups: the ATP-dependent remodeling complexes, which use energy to modify chromatin structure in a noncovalent manner, and the histone-modifying complexes, which add or remove covalent modifications from histone tails. Yeast, human, and *Drosophila* SWI/SNF belong to the class of ATP-dependent remodeling complexes. The ySwi2, hBrg1, and dBrm ATPases are their catalytic subunits, respectively. ySAGA, hPCAF, and hCBP/p300 are histone acetyltransferases (HATs) and belong to the group of histone-modifying complexes. They add acetyl groups to specific lysines within histone tails. The catalytic subunits of ySAGA and hPCAF are the HATs Gcn5 and PCAF, respectively. Although I will not discuss chromatin-modifying-complex function and structure broadly, as they have been exhaustively described in some excellent recent reviews (Berger, 2002; Featherstone, 2002; Narlikar et al., 2002), Table 1 summarizes briefly the functions of the chromatin modifying complexes and of other factors cited in this review.

The functional link between chromatin structure and transcription activation is the "histone code," which is generated by methylation or acetylation of specific arginine and lysine residues within histones H3 and H4. In fact, the transcriptional apparatus reads this histone code and, as a consequence, activates or represses the neighboring genes. At the chicken β-*globin* locus, a chromatin region spanning 53 kb, lysine 9 of H3 is methylated over the condensed transcriptionally inactive chromatin. In contrast, within the transcribed globin genes, lysine 4 methylation of H3 highly correlates with the patterns of H3 acetylation at lysines 9 and 14 (Litt et al., 2001). Coordinated methylation, acetylation, and phosphorylation of specific histone residues often promote gene transcription activation. The human methyltransferase PRMT1 specifically methylates arginine 3 of H4 in vitro. This enhances subsequent acetylation of H4

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Table 1. Eukaryotic General Transcription Factors, Activators, and Coactivators and Their Physiological Roles

Factor	Specific Role
General Transcription Factors (GTFs)[a]	
TBP	Binds the TATA box and induces bending of DNA (Woychik and Hampsey, 2002)
TFIID	Formed by TBP and TAF proteins. Induces PIC assembly. TAFs function also as coactivators (Woychik and Hampsey, 2002)
TFIIB	Helps PolII to select the start and bridges PolII and TBP interaction (Woychik and Hampsey, 2002)
TFIIH	Phosphorylates CTD, has helicase activity, and has a role in DNA repair (Woychik and Hampsey, 2002)
TFIIE	Promotes recruitment and regulation of TFIIH and induces PolII to start elongation (Woychik and Hampsey, 2002)
TFIIF	Stabilizes the DNA–TBP–PolII complex and leads to recruitment of TFIIE and TFIIH into the PIC (Woychik and Hampsey, 2002)
TFIIA	Binds TBP and stabilizes the TBP-DNA interaction. It may antagonize transcriptional repressors. (Hori and Carey, 1997; Lee and Young, 2000)
Activators and Repressors[b]	
NF-kB	Plays a critical role in the triggering and coordinating of immune responses (Ghosh and Karin, 2002)
ATF2-cJun	Protein complex inducing autocrine growth and primary tumor formation. Can recruit CBP/P300 and SWI/SNF (van Dam and Castellazzi, 2001)
IRF	Interferon regulatory factor. Recruits histone-acetylases to promoter target genes (Parekh and Maniatis, 1999; Taniguchi and Takaoka, 2002)
HMG-1	High mobility group proteins 1. Binds DNA and has a high affinity for bent or distorted DNA (Thomas and Travers, 2001)
HNF-1α HNF-4α	Hepatocytes nuclear factors. Induce chromatin remodeling and liver-specific gene activation (Hu and Perlmutter, 1999; Rollini et al., 1999)
E2F1 E2F2 E2F3	Bind DNA and activate gene transcription at G1/S boundary by recruiting HATs (Trimarchi and Lees, 2002)
E2F4 E2F5	Bind DNA and repress gene transcription in G0 by diminishing histone acetylation (Trimarchi and Lees, 2002)
pRB	Retinoblastoma tumor suppressor protein that forms a complex with E2F activators (Trimarchi and Lees, 2002)
p130 p107	Retinoblastoma tumor suppressor-related proteins that form complexes with E2F repressors (Trimarchi and Lees, 2002)
Pho4	Basic-helix-loop-helix protein. Activates expression of genes induced in response to phosphate starvation (Berben et al., 1990; O'Neill et al., 1996)
Swi5	Zinc finger protein, which activates many genes at the end of mitosis (Nasmyth, 1993)
SBF	Swi4/Swi6 complex, which activates many genes at G1/S boundary (Nasmyth, 1993)
Gal4	Consists of a DNA-binding and a DNA-activation domain. Can activate many yeast and higher eukaryotic genes (Ptashne and Gann, 2002)
Ume6	DNA-binding protein, which recruites Sin3-Rpd3 complex and targets histone-deacetylation (Kadosh and Struhl, 1997)
VP16	Herpes simplex virus (HSV) transcriptional activator that activates transcription initiation of many higher eukaryotic genes (Herr, 1998)
Coactivators[c]	
Mediator complex	Stimulates basal transcription by transducing the signals between activators and GTFs (Woychik and Hampsey, 2002)
AIB1	Potentiates the transcriptional activity of nuclear hormone receptors. AIB1 is amplified in a subset of human breast cancers. (Anzick et al., 1997)
PBP	Component of the multiprotein complex TRAP, DRIP, and ARC. Plays a role in the transcription activation by nuclear receptors (Zhu et al., 1997)
SWI/SNF	ATP-dependent remodeling complex. Alters the structure of chromatin and increases the accessibility of nucleosomal DNA (Narlikar et al., 2002)
Swi2	ATPase, the energy-conferring motor of yeast SWI/SNF. Hydrolizes ATP (Narlikar et al., 2002)
Brm1	Swi2 homolog, the ATPase catalytic subunit of human SWI/SNF (Narlikar et al., 2002)
SAGA	Histone acetyltransferase complex, that acetylates specific lysines within histone tails (Narlikar et al., 2002)
Gcn5	Histone acteyltransferase, the catalytic subunit of the SAGA complex (Narlikar et al., 2002)
PCAF	Histone acetyltransferase enzyme, that plays a role in transcriptional regulation, cell cycle progression, and differentiation (Narlikar et al., 2002)
CBP P300	Histone acetyltransferase, that function as global regulators of transcriptional activation (Narlikar et al., 2002)
PRMT1	Arginine methyltransferase that enhances transcriptional activation by nuclear hormone receptor (Kouzarides, 2002)
Snf1	Kinase, which has broad roles in transcriptional and metabolic responses to cellular stress (Hardie et al., 1998)
mSin3B HDAC1	Repressor complex consisting of a histone deacetylase (HDAC1) and a transcriptional repression protein (mSin3B) (Alland et al., 1997)

[a] Lead to accurate transcription initiation by RNA PolII in vitro.
[b] Bind sequence-specific enhancer elements located upstream of the core promoter. They stimulate or suppress the rate of transcription in response to physiological or developmental stimuli.
[c] Facilitate the interaction between the activators and the RNA PolII machinery.

at lysines 8 and 12 by the p300 HAT, with consequent transcription activation (Wang et al., 2001). The *Saccharomyces cerevisiae* serine kinase Snf1 specifically phosphorylates serine 10 of H3 at the *INO1* promoter. This event promotes recruitment of the Gcn5-containing HAT complex, which acetylates lysine 14 (Lo et al., 2001). It

is now clear that there is a gene-specific and timing-dependent order of events that links chromatin structure modifications and transcription activation. Chromatin-modifying enzymes mark histone residues and change nucleosome conformation, allowing the transcriptional machinery to transcribe or repress genes. In the last two to three years, some brilliant reports have shown in vivo transcription activation for a few stimulus-dependent and cell cycle-dependent genes in eukaryotes. Looking at the transcription activation mechanism of these genes, many and substantial differences emerge, and it is of great interest to compare these. By putting side-by-side the sequences of events involved in the transcriptional regulation of these "model" genes, I intend in this review to discuss that although recruitment is the general mechanism of transcription activation in eukaryotes, the ordered recruitment of factors and the recruitment timing are gene-specific events.

Ordered Recruitment of Factors and Transcription Activation Timing Are Accomplished in Different Ways for the *PHO8*, *IFN*-β, *α1-AT*, and *CATD* Genes

Phosphate deprivation, virus infection, and estrogen induction trigger *PHO8*, *IFN*-β, and *CATD* expression, respectively. Enterocyte differentiation leads to *α1-AT* expression. Various stimuli or the initiation of the differentiation program triggers transcription activation of these genes through different mechanisms. The nature of chromatin-modifying complexes and transcription factors, the ordered recruitment timing, and the functional role played by each factor occur differently at each gene.

Nucleosome remodeling and histone acetylation have been investigated at the upstream and proximal *S. cerevisiae PHO8* promoter regions after phosphate starvation (Figure 1A). *PHO8* is activated through the ordered action of Pho4, the SAGA histone acetyltransferase, and the ySWI/SNF remodeling complexes (Gregory et al., 1999). The first event to activate *PHO8* transcription is the binding of Pho4 activator to its consensus sequence (the UASp2 element) within the promoter while the nucleosomes are still packaged. The binding of Pho4 recruits SAGA, which acetylates the nucleosomes of the promoter; this acetylation is transient, and a peak of acetylation has been recognized only in the absence of subsequent remodeling. The nucleosomes that are marked by SAGA activity are subsequently remodeled by SWI/SNF (Gregory et al., 1999; Reinke et al., 2001).

Similar to *PHO8*, at *IFN*-β, which is a mammalian gene, histone acetylation occurs before remodeling by SWI/SNF. The *IFN*-β enhancer is a key regulatory sequence and is located within the promoter between two nucleosomes involved in transcription activation. Nucleosome I lies upstream of the start site of transcription; nucleosome II, however, masks the TATA box and the start. Upon virus infection, three transcription factors, NF-κB, IRF, and ATF-2/cJun, bind cooperatively with the architectural protein HMGI(Y) to the enhancer and form the *IFN*-β enhanceosome (Figure 1B) (Maniatis et al., 1998; Munshi et al., 1999). This enhanceosome induces Gcn5 complex recruitment, resulting in acetylation of both nucleosomes I and II. This event is followed by the association of Pol II, which is in a complex with the HAT protein CBP. The Pol II/CBP complex, in turn, recruits hSWI/SNF, which remodels both nucleosomes and allows association of TBP with the TATA box. TBP binding induces bending of the DNA and sliding of nucleosome II. Finally, this chromatin conformation of the *IFN*-β promoter allows TFIID recruitment, PIC assembly, and transcription initiation (Agalioti et al., 2000; Lomvardas and Thanos, 2001).

The temporal recruitment of chromatin-modifying complexes and transcription factors has been studied at the *CATD* and *α1-AT* promoters. At the *CATD* promoter, transcription factors and coactivators associate in a cyclic fashion, and transcription starts 45 min after estrogen induction. At the *α1-AT* promoter, however, PIC components are assembled 3 days before Pol II recruitment and 5.5 days before the gene is expressed.

Following estrogen induction, the estrogen receptor α (ERα)-dependent transcription complex is assembled on the *CATD* promoter in a cyclic fashion (Figure 1D). Fifteen minutes after 17β-estradiol (E2) induction, and in cooperation with the coactivators AIB1, PBP, and p300, ERα binds to the promoter and forms the ERα transcription complex. Simultaneously, due to the p300 HAT activity, a wave of histone acetylation occurs. All these events are closely followed by the association of Pol II with the promoter. After Pol II binding, dissociation of p300 occurs without its further reassociation in the next cycles of reinitiation. This leads to the recruitment of another HAT complex, CBP, and its associated factor PCAF (45 min after E2 induction). Finally, phosphorylation of the Pol II CTD takes place, transcription initiation starts, and at the same time ERα, AIB1, and PBP are released. The timing of the association of all these factors is remarkable. ERα, AIB1, and PBP remain stably associated with the promoter for 30 min (45 min after E2 induction) and dissociate exactly when transcription initiation starts. They then reassociate with the promoter at the onset of reinitiation (90 min after E2 induction). In contrast, p300 binds to the promoter in the initial cycle of transcription activation and not in the subsequent reinitiation cycles (Shang et al., 2000).

Recruitment timing is also remarkable at the *α1-AT* promoter. In fact, its transcription activation program is achieved in 5.5 days. Two nucleosomes span the *α1-AT* promoter region. Whereas nucleosome 1 (NUC1) covers a region far from the start site and masks the HNF-1α and HNF-4α activator binding sites, nucleosome 2 (NUC2) masks the TATA box and the start (Figure 1C). The first event in the transcription activation program here is the association of the activator HNF-1α, TBP, and TFIIB with the promoter. At this stage, the nucleosomes are still packaged, but the chromatin is permissive for these associations. Two days later, the TFIID components TAFII250 and TAFII30, TFIIH, Mediator, and Pol II are recruited to the promoter. Interestingly, the phosphorylated form of the CTD is stably associated with the promoter starting from day 4, long before transcription starts. This argues against the generally accepted view that CTD phosphorylation is sufficient to convert PIC in the elongation complex. At day 4 of the differentiation program, the activator HNF-4α binds to the promoter, and CBP/PCAF and hBrm are recruited. These complexes cause acetylation and remodeling of

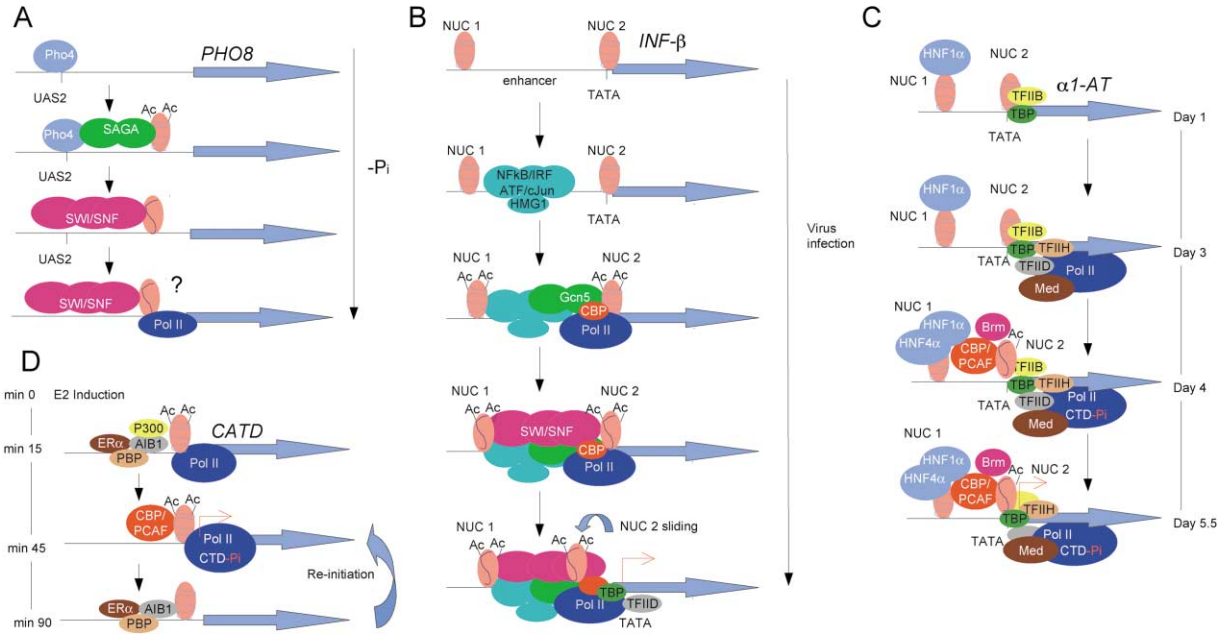

Figure 1. Ordered Recruitment of Factors to the *PHO8*, *INF*-β, *CATD*, and α*1-AT* Promoters

(A) Ordered recruitment of factors to *PHO8*. After phosphate depletion, the activator Pho4 binds to UAS2 and recruits SAGA. Transient acetylation of SAGA facilitates SWI/SNF association, with consequent nucleosome remodeling. Recruitment of Pol II and GTFs likely follow.

(B) The transcription activation mechanism of the *INF*-β gene. Upon virus infection, the enhanceosome associates with the enhancer at *INF*-β and recruits Gcn5, which then acetylates both nucleosome 1 (NUC1) and nucleosome 2 (NUC2). This event is followed by the association of the Pol II/CBP complex. Finally, SWI/SNF recruitment induces nucleosome remodeling and TBP association, which causes NUC2 sliding and transcription initiation.

(C) α*1-AT* transcription activation extends over 5.5 days from the initiation of the differentiation program. One day after the initiation of the differentiation program, HNF1-α, TFIIB, and TBP bind the packaged nucleosome 1 (NUC1) and nucleosome 2 (NUC2). Two days later, at day 3, TFIID, TFIIH, Mediator, and Pol II join the preinitiation complex. At day 4, the CTD is phosphorylated. At the same time, the activator HNF4-α, CPB/PCAF, and Brm are recruited, with consequent histone acetylation and nucleosome remodeling. At day 5.5, α*1-AT* transcription starts.

(D) *CATD* is activated upon estrogen induction. The ERα transcription complex is composed of ERα, PBP, AIB1, and p300. Fifteen minutes after estrogen induction, the ERα transcription complex is recruited to the *CATD* promoter and histones are acetylated by p300 HAT. At the same time, Pol II is recruited too and p300 leaves the promoter soon thereafter. Forty-five minutes after induction, ERα, PBP, and AIB1 dissociate from the promoter, with the consequent CBP/PCAF recruitment, CTD phosphorylation, and transcription initiation. In the following cycles of reinitiation, the ERα transcription complex reassociates with the promoter and triggers initiation of transcription without p300.

NUC2, and finally, at day 5.5, transcription starts (Soutoglou and Talianidis, 2002).

Cell Cycle-Regulated Genes: A Precise Order of Events Coupled to Cdk Activity Controls the Transcriptional Activation Mechanism

During the cell cycle, gene expression at the G0-G1, G1-S, and G2-M transitions is strictly regulated by cyclin-dependent kinases (Cdks) and by the conformational state of the chromatin. Checkpoint controls during these time windows allow some key genes to be activated and some others to be repressed. The accurate expression of many of these genes at a specific moment of the cell cycle is crucial to the triggering of several consequent events during the life of a cell. Expression of the *S. cerevisiae HO* gene is strictly cell cycle- and developmentally regulated; indeed, it occurs only in mother cells and only during late G1. Its activation program depends on Cdk1 activity and on the chromatin conformational state. Similarly, a precise order of events, occurring during the G0-G1-S phases, allows transcription of a subset of mammalian genes that are targets of the E2F family of transcription factors. E2F-dependent genes are involved in cell cycle progression and DNA replication, and they are transcriptionally regulated by Cdk and HAT activities throughout the cell cycle (Ren et al., 2002).

The E2F family of transcription factors consists of E2F-1, -2, and -3, which activate gene transcription, and E2F-4 and -5, which are transcriptional repressors. pRB, the retinoblastoma tumor suppressor protein, strongly binds to E2F-1, -2 and -3, whereas the pRB-related proteins p130 and p107 bind preferentially to E2F-4 and -5 (Humbert et al., 2000; Leone et al., 1998; Trimarchi and Lees, 2002; Wu et al., 2001). In G0, when cells are in a quiescent state, the cytoplasmic repressor E2F-4 is localized in the nucleus by the pRB-related protein p130 (Figure 2). Once there, E2F-4 recruits the repressor deacetylation complex mSin3B/HDAC1, which causes histone deacetylation and silencing of many E2F target genes, such as *Bmyb*, *Cdc2*, *E2F1*, and *cyclin A*. Furthermore, in G0, the E2F targets are also silenced because the E2F activator proteins exist in a complex with pRB and are therefore inactive (Rayman et al., 2002). In G1, when cells exit quiescence, many E2F target genes become activated due to Cdk-dependent phosphorylation. Indeed, after p130 is phosphorylated by Cdks, E2F-4 is

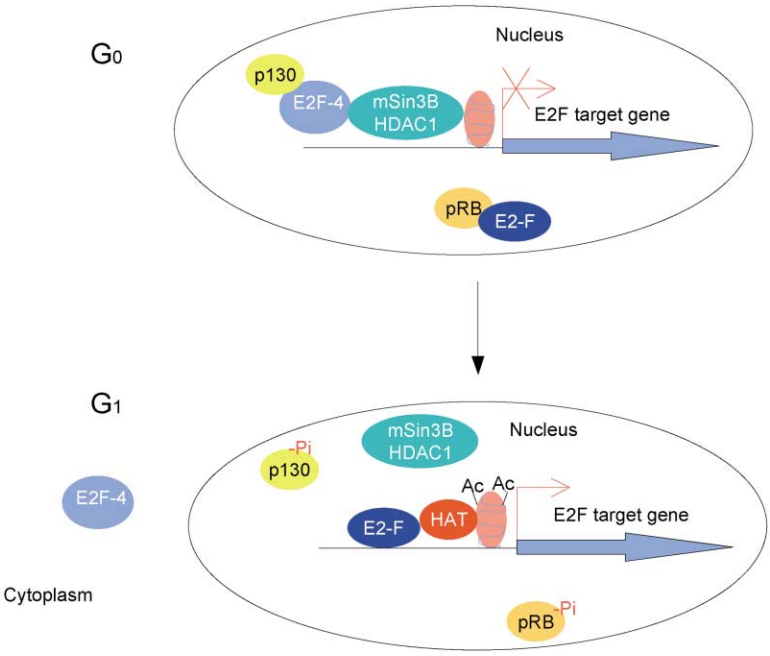

Figure 2. E2F Target Genes Are Repressed in G0 and Activated during G1

In G0, the E2F-4 repressor, localized in the nucleus by p130, recruits the deacetylation complex mSin3B/HDAC1, which represses E2F target genes. At the same time, the E2F activator proteins are in an inactive form because they are in a complex with pRB. In G1, Cdks phosphorylate p130 and pRB, allowing the release of the repressor E2F-4 and of the E2F activator proteins from p130/E2F-4 and pRB/E2F complexes, respectively. Finally, E2F-4 relocalizes to the cytoplasm, and the E2Fs can associate with target genes, recruit HAT, and activate transcription.

released from the E2F-4/p130 complex and relocalizes to the cytoplasm with the consequent dissociation of the mSin3B/HDAC1 repressor complex from the promoter. At the same time, phosphorylation of pRB by cyclinD/Cdk4 promotes the release of the E2F activators from the E2F/pRB complex. These events lead to HAT recruitment, with consequent histone H3 and H4 acetylation and transcription activation of E2F target genes (Morris et al., 2000; Rayman et al., 2002) (Figure 2).

Another cell cycle-regulated gene is the *S. cerevisiae HO*. Its transcription activation program starts during late mitosis, when Cdk1 is inactive, and ends during late G1, when Cdk1 is reactivated (Nasmyth, 1993). The *HO* promoter can be divided into two regions: a distant upstream region called URS1, which regulates mother cell expression specificity, and a more proximal region called URS2, which controls *HO* cell cycle regulation (Nasmyth, 1993). Ordered recruitment of transcription and chromatin remodeling factors to the *HO* promoter starts after cyclinB/Cdk1 degradation at the end of mitosis. Due to this event, the activator Swi5 is dephosphorylated in late anaphase, enters into the nuclei, binds to two sites within the URS1 region of *HO*, and triggers recruitment of the SWI/SNF chromatin remodeling complex to both URS1 and URS2. Thus, Swi5 and SWI/SNF, in turn, promote Mediator complex association with URS1 (Figure 3). The prior recruitment of SWI/SNF is required for subsequent recruitment of SAGA, which acetylates the nucleosomes within a 1 kb domain of the *HO* upstream region. The remodeling of nucleosomes on the *HO* promoter by SWI/SNF and SAGA permits the recruitment of a second sequence-specific transcription factor, SBF, which binds sites within the URS2 region of the *HO* promoter. SBF, in turn, recruits the Mediator complex to URS2 and the TATA box. Finally, reactivation of Cdk1 in late G1 leads to recruitment of Pol II, TFIIB, and TFIIH, with the consequent transcription initiation (Bhoite et al., 2001; Cosma et al., 2001, 1999; Krebs et al., 1999). Whereas the ordered recruitment of Swi5, SWI/SNF, SAGA, SBF, and Mediator to the *HO* promoter occurs in the absence of Cdk1 activity, recruitment of Pol II, TFIIB, and TFIIH only occurs when Cdk1 is reactivated. The observation that Pol II recruitment to the *HO* promoter is dependent on Cdk1 activity, whereas Mediator binding is independent of Cdk1, is also a characteristic of other cell cycle-regulated promoters such as *CLN1*, *CLN2*, and *PCL1* but not for housekeeping genes such as *GAL10* (Cosma et al., 2001). An obvious implication is that Mediator and RNA Pol II are recruited to cell cycle-regulated genes in two separate steps. Consistent with these results is the finding that Mediator binds first to URS1, then to URS2, and finally to the *HO* TATA box in the absence of Pol II (Figure 3). It has been postulated that the prior Mediator association with URS1 is necessary in order to bring Mediator complexes to the *HO* promoter and to have them ready to be quickly recruited to URS2 and the TATA box by SBF (Bhoite et al., 2001).

Activators and Nucleosomes: Their Interdependent Roles

Nucleosomes are modified through the action of activators, which recruit chromatin-modifying complexes; however, the activators can bind nucleosomes only at a precise moment of the transcription activation program, and only when nucleosomes are in a specific chromatin conformation. It is therefore reasonable to define a concept of interdependency among activators and nucleosomes. There is a first class of activators, which bind the packaged nucleosomes and are able to perturb chromatin structure. These recruit chromatin remodeling, chromatin acetylation, and chromatin repressor complexes by protein-protein interactions (Figure 4A). Gal4, Ume6, Pho4, the ER transcription complex, the *IFN-β* enhanceosome, E2F-4, and Swi5 have this ability (Agalioti et al., 2000; Cosma et al., 1999; Kadosh and

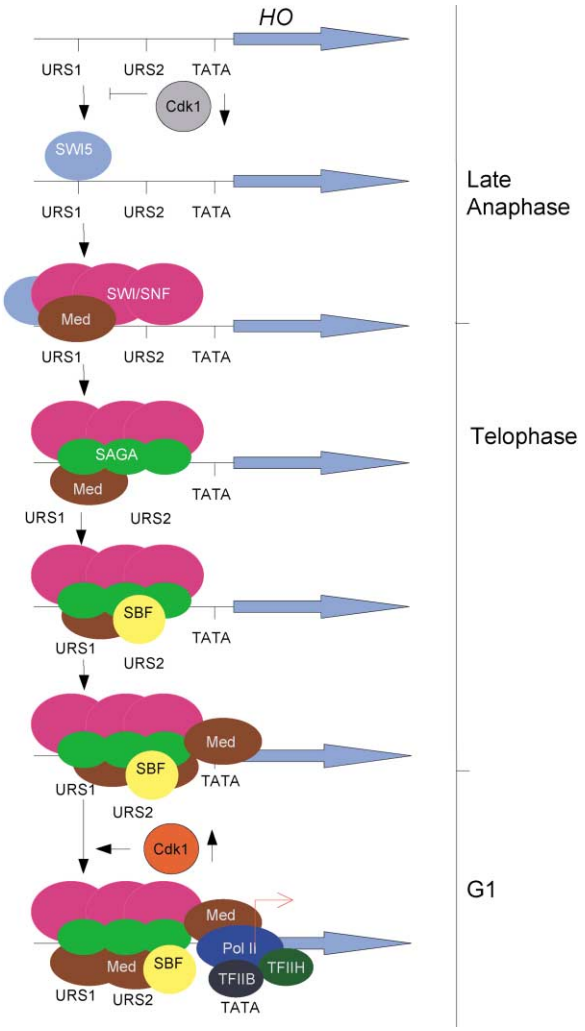

Figure 3. Transcription Factors Are Recruited at the *HO* Promoter from Late Mitosis until Late G1

Cdk1 inactivation in late anaphase promotes Swi5 nuclear entry and its association with the URS1 region of the *HO* promoter. Swi5 induces SWI/SNF recruitment, which, in turn, promotes SAGA association with the promoter. Histone acetylation and nucleosome remodeling sustain SBF binding to URS2. Mediator associates first with URS1 and then, after SBF association, with URS2 and the TATA box. Cdk1 reactivation in late G1 leads to recruitment of Pol II, TFIIB, and TFIIH.

Struhl, 1997; Rayman et al., 2002; Reinke et al., 2001; Shang et al., 2000). There is also a second class of activators, which bind to promoter regions only after nucleosomes have been conformationally changed (Figure 4B). This is the case of activators such as the E2Fs and SBF (Cosma et al., 1999; Rayman et al., 2002). The components of these two classes are not mutually interchangeable in the dependence of different promoter structures, although it is possible within the same category. Like the role of Swi5 at the *HO* promoter, Gal4 recruits SAGA and SWI/SNF to the *GAL1* promoter during late mitosis (Krebs et al., 2000). This leads one to the tempting prediction that after replacement of the Swi5 binding sites with those of Gal4, Gal4 may have the ability to activate *HO* transcription instead of Swi5.

Furthermore, activators can recruit basal transcription factors directly (Figure 4C). The Rap1-containing activator is necessary and sufficient for the recruitment of the basal transcription factor TFIID to ribosomal protein (RP) promoters (Mencia et al., 2002). Moreover, the common view that basal transcription factors and Pol II associate after nucleosomes have been remodeled is not considered as dogma anymore. The basal transcription factors TBP and TFIIB bind to the nucleosome-masked TATA box long before PIC is assembled at the α*1-AT* promoter, and long before the CBP/PCAF HAT complex in cooperation with the remodeling protein Brm is recruited to the promoter (Figure 1C) (Soutoglou and Talianidis, 2002). Furthermore, TBP causes sliding of nucleosome 2 and induces PIC assembly at the *IFN*-β promoter (Figure 1B) (Lomvardas and Thanos, 2001). That TBP has many roles was already known from previous reports. It unwinds and sharply bends DNA, and it increases transcription in the absence of the repressor Mot1, which prevents it from binding to the TATA box (Kim et al., 1993a, 1993b; Li et al., 1999; Nikolov et al., 1996). However, the novelty is that TBP binds the α*1-AT* promoter long before Pol II and that it participates in nucleosome remodeling at both the α*1-AT* and *IFN*-β promoters. Even in the absence of transcription, the heterochromatic, silenced *HMR1a* yeast promoter is permissive to the binding of TBP (Sekinger and Gross, 2001). These findings imply that packaged nucleosomes change conformation only after factor binding and chromatin-modifying enzyme recruitment occur. However, these modifications can also be induced by the action of basal transcription factors such as TBP. Nucleosomes can be seen as barriers that mask regulatory sequences. However, after the binding of key regulators, nucleosomes can be displaced, making the regulatory sequences accessible for the association of transcription factors that bind to unpackaged nucleosomes exclusively.

Are Histones Acetylated before Nucleosome Remodeling, or Vice Versa?

In the case of interphase-expressed genes and stimulus-dependent genes, histone acetylation is independent of remodeling, and HAT complexes are recruited to promoters by activators. In fact, in some cases, such as for *PHO8*, *HIS3*, and *INF*-β, histone acetylation precedes nucleosome remodeling (Agalioti et al., 2000; Gregory et al., 1999; Krebs et al., 2000; Reinke et al., 2001). In contrast, for cell cycle-dependent genes, such as yeast *HO*, *SIC1*, *PCL2*, *PCL9*, *CDC6*, *EGT2*, and *ASH1* which are expressed at the end of mitosis or in G1, the SWI/SNF remodeling complex recruits SAGA, which, in turn, acetylates histones (Krebs et al., 2000). An explanation for this has already been proposed: after mitosis, the chromatin is hypercondensed and needs first to be remodeled, and then histones can be acetylated (Fry and Peterson, 2002). This is also true for interphase genes that are forced to be expressed at the end of mitosis. In cells arrested in mitosis, *GAL1*, whose interphase expression is independent of SWI/SNF and Gcn5, is expressed after Gcn5 recruitment by SWI/SNF (Krebs et al., 2000).

Different HAT complexes have nonredundant roles. Indeed, we must distinguish the effect of the acetylation

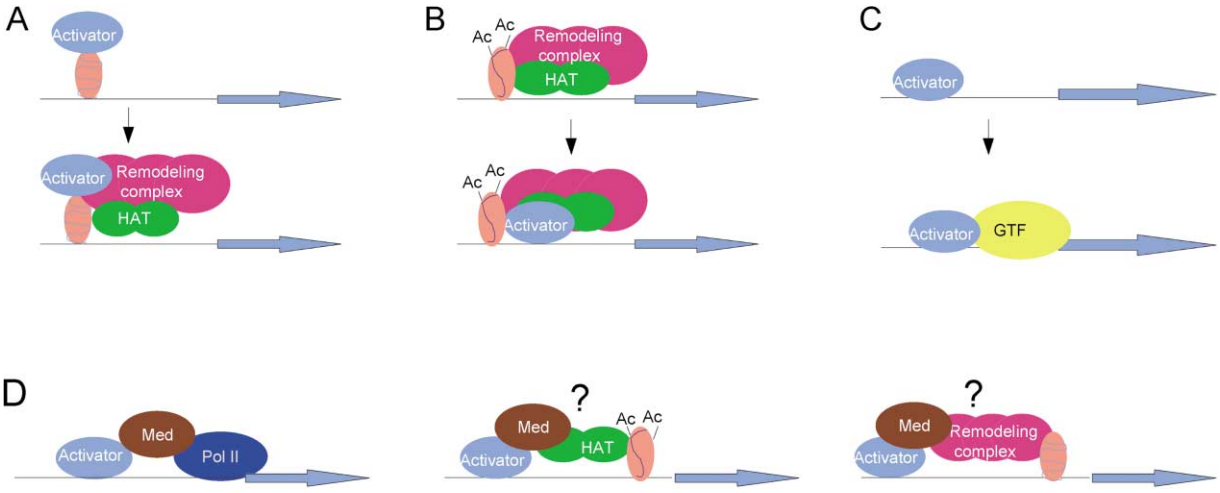

Figure 4. Activators and Mediator Complex

(A–C) Different activators have different modes of action. (A) An activator binds to packaged nucleosomes and recruits chromatin remodeling and/or HAT complexes. (B) An activator binds to promoter regions only after histone acetylation and nucleosome remodeling have occurred. (C) An activator directly recruits a general transcription factor.

(D) Mediator functions. In addition to "mediating" the signals between activators and basal transcription factors, the Mediator complex may also stabilize links between activators and HAT complexes or between activators and chromatin remodeling complexes.

due to Gcn5 or p300 from that due to CBP/PCAF. The former two proteins modify the nucleosome topological structure of the *PHO8*, *CATD*, *IFN-β*, and *HO* promoters long before PIC formation. In contrast, the latter associates simultaneously with Pol II at the *IFN-β* and *CATD* promoters, or even after Pol II at the *α1-AT* promoter (Figure 1). One can postulate two different roles for these HAT complexes. Whereas the Gcn5-containing complex and p300, recruited by activators to nucleosomes far away from the start site, would be devoted to modifying histones in order to induce the assembly of the transcription activation machinery, CBP/PCAF would perhaps directly modulate Pol II activity by modifying the nucleosomes that mask the TATA box or the start site.

Mediator: New Insights into Its Role

The association of Mediator and Pol II occurs independently at *HO* and at other cell cycle-regulated promoters such as *CLN1*, *CLN2*, and *PCL1* (Cosma et al., 2001). Moreover, that Mediator and Pol II associate in two distinct steps, and that Mediator binds to promoters before HAT complexes, has been shown for *α1-AT* and *HIS4* also. The Mediator complex, in cooperation with components of PIC, assembles with *α1-AT* before CBP/PCAF (Figure 1C) (Soutoglou and Talianidis, 2002). Reinitiation at the *HIS4* promoter is achieved by the assembly of Pol II without Mediator (Yudkovsky et al., 2000). The fact that Mediator is a complex of many different factors may imply that it performs different roles. This could be an explanation of why Mediator complexes are associated with the *HO* regulative regions upstream from the start site long before *HO* activation (Bhoite et al., 2001). Perhaps Mediator is also crucial in the mediation of interactions between activators and HAT complexes, or could help activators in recruiting or stabilizing remodeling complexes (Figure 4D). Gal11 and Sin4 are two components of yeast Mediator; the activator Swi5 possesses a Gal11 interaction domain and binds Mediator in vitro. Moreover, a *sin4* mutant suppresses a *gcn5* mutant, allowing *HO* transcription (Bhoite et al., 2001; Neely et al., 1999). A recent finding has highlighted the importance of Mediator conformation in the assembly of a transcriptionally active preinitiation complex. CRSP and ARC-L are two mammalian cofactors that share common features with the yeast Mediator. Whereas ARC-L is inactive, the other complex, CRSP, is highly active. Furthermore, one of the CRSP characteristics involves its specific activator-induced conformational changes. An activator, such as VP-16, recruits the CRSP complex and induces its conformational change, which has a deep impact on the mechanism of transcription activation. It has been proposed that this conformational change may facilitate transcription initiation by recruiting or by stabilizing other cofactors and components of the PIC, including RNA Pol II (Taatjes et al., 2002). In addition, to mediate the signals between activators and basal transcription factors, the Mediator complex has certainly many other roles that remain to be uncovered. The identification of the genes for which the association of Mediator to promoters occurs independently of Pol II may help in this direction.

Timing: Its Relevance in the Ordered Recruitment of Factors

Regulated recruitment achieved by protein–protein interactions is a general mechanism in the regulation of gene expression (Ptashne and Gann, 1997; Zaman et al., 1998). Only an accurate order of events can activate transcription of a gene; factors come onto the stage neither randomly nor all at once; on the contrary, the timing of their recruitment to promoters is defined. Transcription activation occurs in 45 min in a cyclic fashion for *CATD* or in 5.5 days for the *α1-AT* gene (Figure 1) (Soutoglou and Talianidis, 2002). What is the biological

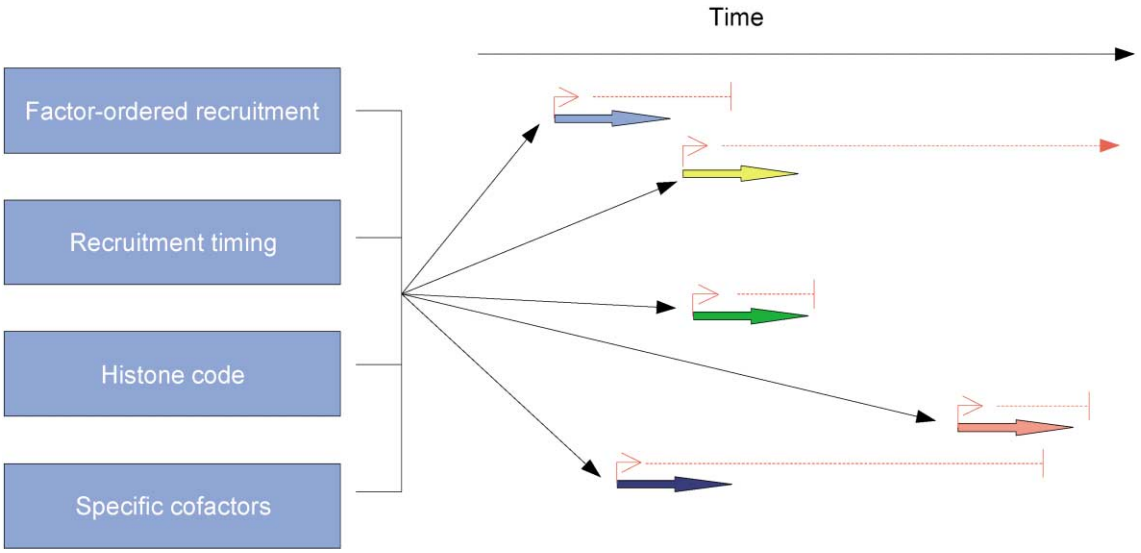

Figure 5. Events Triggering Transcription Activation

The ordered recruitment of factors, the recruitment timing, the histone code, and a selection of specific cofactors control transcription activation of each gene at the right moment and for the right length of time.

meaning of these temporal differences? *CATD* is an estrogen target gene and must be activated soon after the stimulus, while α*1-AT* is expressed when enterocytes are induced to differentiate. Differentiation is a stimulus as well, but it is a slow process with respect to hormone induction. Many genes and pathways are involved, and the transcription activation machinery activates α*1-AT* in addition to other genes throughout differentiation. Similarly, the *HO* transcription activation program is time dependent; it starts in late anaphase when the activator Swi5 binds the promoter for only 5 min and ends in late G1 when *HO* mRNA is finally produced (Figure 3) (Cosma et al., 1999). At the *PHO8* promoter, SAGA-dependent histone acetylation is a transient event, which guides SWI/SNF recruitment (Figure 1A) (Reinke et al., 2001). The transcription timing forces the activation of genes to occur at precise moments, leading to the temporal presence of their protein products during the life of a cell. Cells need the right proteins at the right moments and for the right length of time; otherwise, deregulation occurs. Probably, the ordered recruitment of factors is influenced by the speed with which a gene must be activated. However, gene transcription activation is strictly dependent upon the activation of specific signal transduction pathways (Brivanlou and Darnell, 2002). We can hypothesize that signal transduction pathways influence the timing of gene transcription activation by determining the speed with which transcription factors are activated. Finally, transcription activation timing, in turn, influences the order of recruitment of transcription factors. It is possible, in fact, that the assembly of activators, nucleosome-modifying enzymes, basal transcription factors, Mediator, and Pol II on gene promoters is determined in a precise order by the time-lapse during which the transcription activation is achieved. Timing is important to maintain the steady-state level of histone acetylation. Rapid kinetics exist with acetylation and deacetylation, which maintain the proper dynamic equilibrium of chromatin and dramatically influence gene expression and silencing (Katan-Khaykovich and Struhl, 2002). It is bizarre and cannot be accidental that genes that are activated for a long time are also regulated so differently in terms of recruitment of factors. Further experiments should shed light on the link between transcription activation timing and ordered recruitment of factors for each gene.

Is the Gene-Specific Ordered Recruitment of Factors Associated with Different Biological Needs for Each Gene Product?

According to the needs of a cell, activators recruit nucleosome-modifying enzymes and basal transcription factors, and these remain associated with regulatory promoter sequences for the right periods of time. In cooperation with p300 HAT, the ER transcription complex recruits Pol II and leaves the *CATD* promoter soon thereafter because Pol II can work without any further help (Figure 1D). Swi5 associates with the *HO* promoter for only a few minutes because it is required to trigger changes in the promoter chromatin structure by recruiting SWI/SNF and SAGA, but it is not required to maintain the new chromatin state (Figure 3). During G0–G1 transition, E2F target genes pass from a silenced state to an activated state; this is regulated through the transient action of Cdks (Figure 2). SWI/SNF associates with *IFN*-β nucleosome 2 to modify its conformation and to allow TBP association, which can promote subsequent sliding (Figure 1B). Nothing arises by chance; each event is selected and chosen, aimed at obeying the rules dictated by the different needs of the cell that are associated with differentiation, development, or specific external stimuli. For this reason, the players are often not mutually interchangeable; in the activation program of a gene, the natural selection for the involvement of one activator rather than another, or of one particular remodeling or HAT complex rather than others, can be ascribed

to the slightly different roles of each factor or complex. Depending on the need of the cell for each gene product, a particular group of key players will be chosen. Only if one factor is impaired in its function, such as in the case of mutants, will the cell adapt and decide to use other pathways to survive.

Conclusions

In eukaryotes, gene transcription is achieved through factor recruitment. Today, we can add a few more concepts to this original and innovative observation. The transcription activation mechanism depends on the ordered recruitment of factors, on the recruitment timing, on the histone code, and on the choice by the cell for specific cofactors (Figure 5). This elaborate activation program allows gene transcription at the right moment and for the right period of time. Cells have to propagate under many different physiological conditions; therefore, they need to modulate different transcription activation mechanisms according to their needs. Many questions remain still unanswered: What are the factors or the physiological events determining this gene-specific transcription activation timing? Why is the transcription activation time-lapse so variable among different eukaryotic genes? Could the transcription activation timing influence the recruitment of specific factors, instead of others, to promoters? Could specific signal transduction pathways activate key epigenetic factors, which, in turn, control the transcription activation timing of eukaryotic genes? The "model genes" described in this review represent a stimulating starting point. Further investigations focused on comprehension of the in vivo transcription regulation mechanisms of numerous other eukaryotic genes will allow us to find the link between transcription regulation control and the biological advantage for each gene product throughout the life of a cell.

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