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Many Ways of Transcriptional Regulation in Yeast

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Summary

Transcriptional regulation in eukaryotes is a complex process with many implications in the physiology of the organism. Yeast provides a unique combination of evolutive conservation of the regulatory processes and readiness of manipulation. Here I present an approximation to different levels of gene regulation. As first level, *GAL* genes respond to the presence of galactose; however, this apparently easy regulation pattern hides subtleties that have given to the discovery of many basic mechanisms of the trans-activation process. Constitutive activators, like Rap1p, provide second level of regulation. In this case, the problem lies in how a single factor can elicit different responses, from activation to repression. Two families of co-activators, histone-acetyl transferases and chromatin remodelling complexes, give further insides on how activators work and, more precisely, on the relationships between the transcriptional machinery and the chromatin. The ability to study heterologous activators in yeast increased notably our knowledge of both the functioning of these activators and of the mechanisms of transcriptional activation in yeast. Last, the paper discusses the intriguing cross-talk between different cell processes like transcriptional regulation, RNA elongation, DNA repair, recombination, and aging.

Key words: transcriptional activation, chromatin remodelling, histone acetylation, coactivators, yeast promotors

Introduction

Diversity among living beings has puzzled humanity at least since Ancient Greece. It is the central issue in understanding the differences between living and non-living beings, between animals and plants, between animal species, between animals and humans, and between humans of different sex, race or origin. It also underlies the problem of Heredity, including the overwhelming philosophical problem of how the Simple (an egg) can develop into the Complex (a living organism). The discovery, seventy years ago, that DNA was the agent of heredity solved a problem and added a new one. Kingdoms, organisms and individuals differed in their DNA content, but, if all cells of an organism have the same DNA, how can they be so different? The answer to this riddle lies in the phenomenon of Differentiation.

The problem of cell differentiation has been painstakingly investigated in recent decades. Cells are different, not because their genetic make-up is different, but because they use this common information differently. In short, they are different because they produce different proteins in different amounts. The question of how cells acquire the ability to modulate their protein expression pattern during development is the basis for modern Embryology and Developmental Biology; the mechanisms by which genes are differently expressed following the developmentally programmed pattern is a favorite issue in Molecular Biology.

Although yeast cells do not develop into multicellular organisms, they change their gene expression patterns not only as a consequence of their internal pro-

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gramme (cell cycle, sporulation *etc*.), but also in response to external signals such as environmental changes. The mechanisms involved in transcription regulation are so well preserved among eukaryotes that in many cases it is possible to combine components from many different origins (yeast, flies, mammals...) and still recover at least part of their aggregate function – although yeasts and vertebrates, for example, diverged more than half a billion years ago. Add to that the ease of genetic manipulation of yeasts, and the inescapable conclusion is that yeast has been, and will be for many years, a first-choice model for studying mechanisms of regulation of gene expression in eukaryotes.

1. Basic Activation: the *GAL4* System

The budding or baker's yeast, *Saccharomyces cerevisiae*, utilizes a variety of sugars as sources of carbon and energy. However, growing on any carbon source other than glucose or fructose requires profound changes in the expression pattern of many genes, which are normally repressed when cells grow on either of these two sugars. This switch from growing on glucose to using other sugars was one of the first regulatory issues studied in yeast (or in any other eukaryote) at a molecular level ((*1*), see also Breunig, this issue). Surprisingly, or maybe not, we still ignore many clues governing this apparently simple process.

Growing on galactose, an epimer of glucose, should have been particularly easy, given its structural similarity to glucose. However, it was not so easy: no less than 12 genes (the *GAL* genes) are required to fulfil this mission; on addition, most of them are so tightly regulated that their expression levels are below detection in the presence of glucose, regardless of the absence or presence of galactose (*1*,*2*). The understanding of this process has advanced enormously our understanding of eukaryotic regulatory proteins; still, many of their subtleties are as yet unknown.

Expression of *GAL* genes depends upon the presence of specific DNA sequences upstream from their coding region, the so-called promoter region. These sequences, called UASgal, serve as recognition sites for a DNA-binding protein, Gal4p. Transcription of *GAL* genes requires binding of Gal4p to the UASgal sites in their promoters. Since the transcription of Gal4p itself only occurs in the absence of glucose, this explains the lack of transcription of the *GAL* genes when cells grow in glucose. This dual system, a protein (the activator) able to bind to specific DNA sequences in the promoter of target genes (enhancers or UASs), constitutes the first level of transcriptional regulation for many genes (*3*); these genes are transcribed if the activator is bound to their promoters, and silenced if it is not.

However, things are never simple. In the absence of glucose (that is, growing in another carbon source) Gal4p is transcribed and binds to the UASgal sites, but, if there is no galactose in the medium, transcription of *GAL* genes is still abolished. This implies the existence of a secondary regulator, in this case a repressor, that would prevent Gal4p from activating transcription of *GAL* genes in the absence of galactose. This repressor was characterized as Gal80p, a protein that binds to Gal4p (not to DNA) and masks a critical part of the protein essential for activation (*1*). Two main messages can be drawn from this mechanism: gene regulation works both by activation and repression processes, and binding to DNA is not sufficient to activate transcription in all cases. Further studies demonstrated that Gal4p, like many other activator proteins, has two functional domains that are physically and genetically separable: binding to DNA, which is brought about by the DNA binding domain, and activation of transcription, which is accomplished by a different portion of the protein called the activation domain (*4*). Most activators (including Gal4p) have more than one activation domain. Apparently, Gal80p prevents functioning of the main activation domain of Gal4p, located at the very C-terminal end of the protein (*2*). In the presence of galactose, the repressive action of Gal80p is suppressed by its interaction with Gal3p, through a mechanism which is still not completely understood. Gal3p is structurally similar to the yeast galactokinase (Gal1p), but it lacks galactokinase activity. The current model is that Gal3p (and, at a lower rate, Gal1p) synthesizes some galactose derivative required for the inactivation of Gal80p and so for the induction of *GAL* genes, but the nature of such an inducer is still elusive (*2*,*5*). The complex network of positive and negative signals controlling the different *GAL* genes is depicted in Fig. 1. Of this entire network, only five genes are functional enzymes (including a permease); the rest controls their functioning.

A pivotal aspect of the regulation of *GAL* genes is the absence of Gal4p when cells grow on glucose. This is again a general problem of transcriptional regulation: who regulates the regulators? In this case, Gal4p regulation is a subset of the general system of glucose repression, which affects hundreds of genes on the yeast genome (*6*). The key factor for glucose repression of *GAL* genes is Mig1p. This is a DNA binding protein that binds to specific sequences in promoters of genes repressed by glucose, including the *GAL4* gene promoter. But, unlike Gal4p, Mig1p is a repressor, preventing transcription of genes when bound to their promoters (*6*). Mig1p requires for its repressive function two co-repressors, Ssn6p and Tup1p, which, like Gal80p, do not bind to DNA, but to Mig1p itself (*7*). The *MIG1* repression system is shown in Fig. 1. Mig1p is inactivated by phosphorylation by the SNF1 kinase complex; this complex is inactivated by autophosphorylation, which is in turn induced by the presence of glucose in the medium. The details of this inactivation of the SNF1 complex are yet unknown (Fig. 1, *6*).

Third, and equally general, question on *GAL* gene regulation is why gene transcription is facilitated by the binding of Gal4p (or, for that matter, of any transcriptional activator) to the promoter. Activation domains from Gal4p and other activators have a modular nature, in the sense that they transform any DNA binding protein (whatever its original function of origin) into a transcriptional activator when fused to it (*4*). But this is only part of the problem, because we still do not know why they do so. A partial answer to this question came from the observation that transcriptional activation by Gal4p requires the presence of another gene product, Gal11p, which acts as a co-activator, *i.e.* a factor that

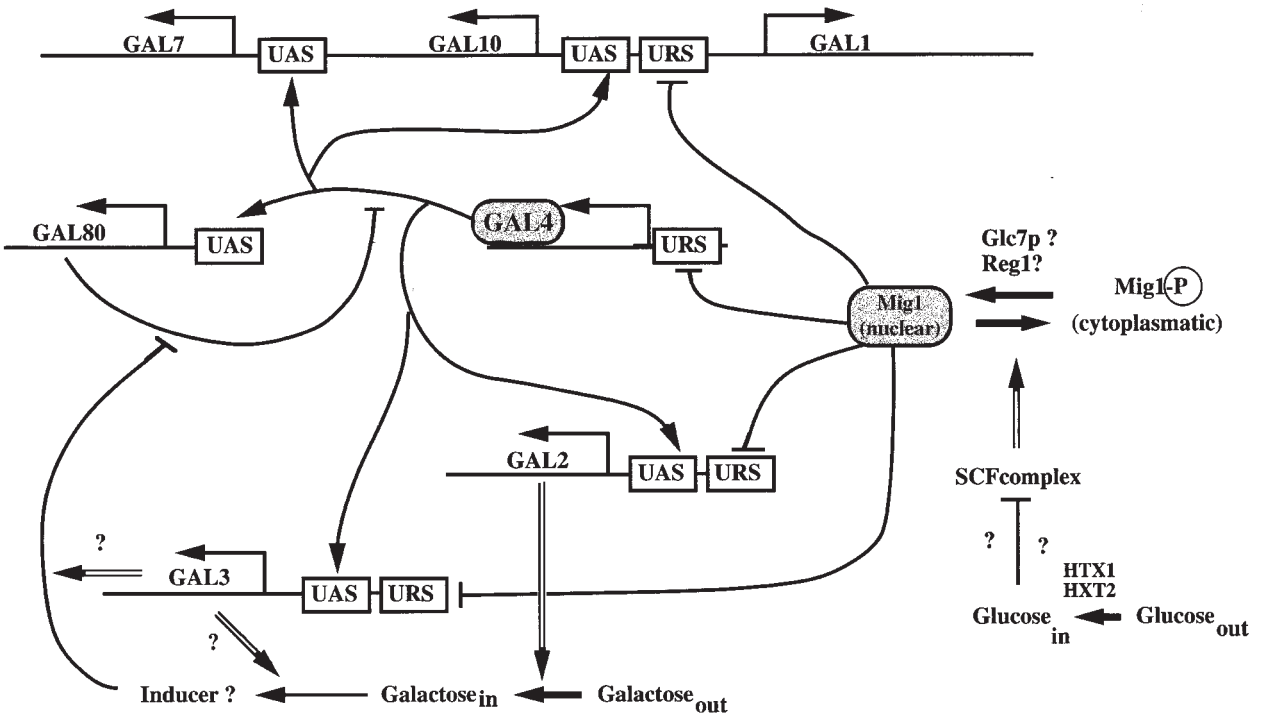

Fig. 1. Regulatory network of *GAL* genes. The scheme indicates transcriptional activation (↓), repression (⊥) and enzymatic activities (⇓). Physical transfer (*e.g.* sugar transport) and chemical modifications (*e.g.* phosphorylation) are represented by thick arrows (→). Question marks indicate steps whose peculiarities are still unknown. For example, we do not know the nature of the inducer that indicates the presence of galactose or glucose in the medium, or the exact roles of Gal3p and Glc7p

helps activators to activate (*8*). Gal11p turned out to be an integral part of the RNApol II holoenzyme, linking activation with at least one component of the enzymatic machinery that synthesizes mRNA (*8,9*). However, this link is still controversial (see below), and the activation by Gal4p is modulated by other factors, like phosphorylation of Gal4p itself, that we do not yet understand (*10*).

2. The Hand that Rules the Cell: Rap1p

The *GAL* gene system is paradigmatic of the regulation of many genes that are only expressed when required and silenced (at a major or minor level) when they are not necessary. There is a bunch of yeast gene systems controlled in a similar (but not identical) way, including the *PHO4* system (acid phosphatases only expressed when there is a shortage of inorganic phosphate (*1*), Gregory *et al.*, this issue), the *GCN4* system (all enzymes involved in amino acid biosynthesis, only expressed upon amino acid starvation (*11*)) and the *HAP1* system (activated by the presence of oxygen (*3*)), only to mention some of the best characterized ones.

Though important for cell survival in the ever-changing environmental conditions, all these systems represent a small portion of the transcriptome, *i.e.* the collection of transcripts present in the cell under given conditions. Micro-array experiments demonstrated that the exponentially growing yeast cell contains, on average, a single transcript or less for most genes (*12*). Only three functional categories of genes escape from this low transcription pattern: cellular organization (including organelles, membrane protein and the like), energy (glycolysis and respiration, including genes regulated by *HAP1*, since the reported experiments were done in the presence of oxygen) and, above all, protein synthesis, including ribosomal proteins. These three categories make up for most of the transcriptome.

A single yeast cell contains 200,000 ribosomes, on average. Since *S. cerevisiae* can divide as fast as once every two hours, in order to maintain this figure cells need to synthesize ribosomes at a rate of 30 per second. Yeast ribosomes contain 78 different ribosomal proteins, coded by 137 genes (many genes are duplicated, coding for nearly identical proteins), plus 4 rRNA molecules; no wonder, then, that this is the most heavily transcribed set of genes in the genome (*13*). Analysis of the promoter of the ribosomal protein genes (rp genes) demonstrated that most of them contained a 14-base sequence motif that was called UASrpg (*14*). The factor binding to this sequence is Rap1p.

The cloning and characterization of Rap1p led to several surprising conclusions. Rap1p was found to bind not only to rp genes, but also to many genes codifying for glycolytic enzymes (also a heavily transcribed gene set) and, most surprisingly, to yeast telomeres, where it was required not for transcription, but for transcriptional repression, called silencing in this particular context (*15,16*). The role of Rap1p as a silencer is not exclusive to the telomeres, since two normally silent loci, *HML* and *HMR*, involved in the mating type switch, also require binding of Rap1p to their regulatory regions in order to stay silenced. This dual function of Rap1p justi-

fies its acronym (*R*epressor-*A*ctivator *P*rotein) (*15*). Fig. 2. exemplifies the panoply of genes whose regulation depends on Rap1p, either as activator or as repressor.

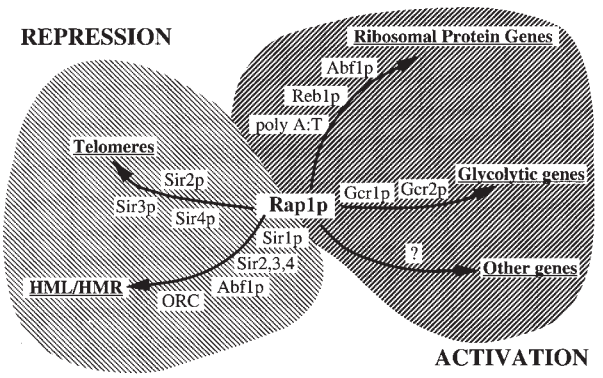

Fig. 2. Regulatory activity by Rap1p. There are at least four groups of genes regulated by Rap1p, both as activator (rp and glycolytic genes, right) and as repressor (telomeres and subtelomeric genes and HMR and HML loci, left); there are many other genes with Rap1p binding DNA sequences; it is assumed that in these genes, Rap1p acts as activator. Coactivators and DNA-binding proteins also involved in the regulation of some (or many) genes for each group are also indicated

The understanding of the dual function of Rap1p has developed a new field of understanding of how the environment of a given binding site can modulate the function of the protein that binds to it. Silencing by Rap1p requires the presence of the SIR complex, which acts as a co-repressor. This complex, made up of three subunits, Sir2p, Sir3p and Sir4p, binds to Rap1p and, most probably, also to the histones (see below) to create a unique structure on the yeast telomeres that is refractory to transcription (*17*,*18*). This is called telomere position effect, or TPE. The TPE is intimately linked to a special compartmentalisation of the telomeres inside the yeast nucleus: yeast telomeres are grouped in four o five »bouquets« physically linked to the nuclear membrane (*19*). Any alteration of this highly specific structure results in the loss of the TPE and of the Rap1p-mediated telomere silencing. Silencing of the *HMR* and *HML* loci is somewhat different, since it depends on a fourth co-repressor (Sir1p) and on the presence of a nearby ORC (Origin of Replication Complex) site, in addition to the Rap1p binding sites and the SIR complex. All these elements are thought to produce also a specific chromatin environment required for the silencing of the loci (*20*).

Yeast genomic analysis revealed UASrpg sites in the promoters of hundreds of genes, in addition to the two above-mentioned glycolytic-enzyme and rp-gene sets (*21*). Given the high concentration of Rap1p molecules in the cell nucleus and its great affinity to its cognate DNA sites, it is most probable that all these sites are occupied *in vivo*. For some of them, it is known that their transcription depends to some extent (sometimes exclusively) on the presence of the UASrpg. For most Rap1p-driven glycolytic enzyme gene promoters, UASrpg sites are immediately adjacent to DNA sequences recognized by another activator protein, Gcr1p. Removal of either UASrpg or Gcr1p-binding sites causes a dramatic loss of transcription on the gene, indicating that both factors are required for activation in these promoters. Binding of Gcr1p to its DNA recognition sequence requires a molecule of Rap1p bound in the immediate vicinity; in these promoters, Rap1p works as a poor activator on its own (*22*). Gcr1p is not a »true« activator, either: what does the job is Gcr2p, a co-activator that binds to Gcr1p when it binds to DNA (*22*). As in the telomeres, Rap1p acts in these promoters as a milestone, defining a DNA sequence where the active complex (in this case, an activator complex) is built.

Of the known 137 rp genes, 119 of them contain Rap1p DNA binding sequences on their promoters (*14*). There is very little doubt that Rap1p itself is the main activator in these heavily-transcribed genes, which contrasts with the above-described roles of Rap1p as a repressor or as a weak activator helping other activators to bind DNA. Most rp gene promoters encompass two or more UASrpgs in tandem, a disposition that increases 10-fold or more the activation potential of UASrpgs, a phenomenon called transcriptional synergism. Synergism is a common property of many transcription factors. In most cases, like Gal4p, individual protein molecules co-operate to bind DNA, the DNA-recognizing functional unit being a homo- or heterodimer (*23*). However, this is not the case for Rap1p, which binds single and multimerized DNA sites with the same affinity (*24*). Available data indicate that synergism between adjacent Rap1p molecules is due to co-operative interaction with some components of the transcriptional machinery (perhaps a co-factor), which would be responsible for the increase in transcriptional response. This is also the favored explanation for the amazing fact that activators that never meet in nature (like activators from vertebrates or flies combined with yeast factors) can synergize to activate transcription in many cellular systems (*23*,*25*).

The ability of different transcriptional activators to interact with different cofactors and thus modulate their effects on transcriptions has another intriguing aspect. As these cofactors do not recognize DNA sequences, the question is how the same factor can interact with different cofactors in different DNA contexts. A simple situation is that a single activator molecule cannot interact with some of these cofactors (the standard explanation for synergism), but for many genes this explanation does not apply. A very suggestive mechanism is known as the allosteric effect of DNA on transcriptional activators (*26*). Following this model, some activators can bind to several versions of their DNA binding sequence, and do so by altering their structural conformation. Cofactors, as well as some components of the transcription machinery, would recognize this structural alteration, and would be able to translate this information into different levels of activation or repression. This model is well documented for mammalian steroid receptors (among other mammalian factors (*26*)), but there is evidence that a similar phenomenon applies to the different binding sites for Rap1p in yeast (*27*). A simplified version of the allosteric regulation model is shown in Fig. 3.

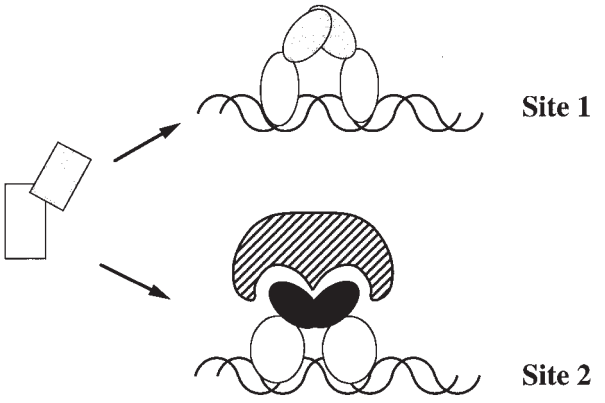

Fig. 3. Model for allosteric modulation of transcription activation by the DNA binding sequence. The model proposes that, in solution, the activator does not achieve its active conformation. Its binding to two versions of its DNA recognition sequences (sites 1 and 2) results in alternative conformations; these conformations determine its ability to bind to cofactors (coactivators or corepressors) and/or its ability to activate transcription. Although in the figure the active conformation of the activator is a dimer, this is not a necessary prerequisite

3. A Complex Saga: the SAGA Complex

The requirement for factors other than activators to activate transcription of eukaryotic promoters arose from both genetic and biochemical data (*28*). In the search for these putative coactivators (also called adaptors or mediators), the availability of sophisticated genetic tools in yeast proved decisive. Several yeast-based genetic screens were set in order to identify and then characterize genes needed for the function of activators. These screens gave in most cases apparently disparate results; in the end, they only demonstrated the interconnection of transcription with different cell functions, like DNA repair or replication.

A property of many activators (including Rap1p and Gal4p) is that their excess is toxic for the cell. This phenomenon, called squelching, was first observed in cultured mammalian cells, but also extends to yeast cells (*29*). The standard explanation for this is that the excess of activator drains some key transcription factors which are then insufficient for attending the requirements of some vital promoters. This is a target for genetic screening, for a mutation of some of these limiting factors may in principle debilitate its interaction with the excessive activator and so free some of it for other necessities. Using this line of reasoning showed how a series of new factors, dubbed the *ADA* genes, were cloned (*29*,*30*).

Mutations in the *ADA* genes weakened the activation potential of some activators, including Gcn4p, but left others, like Gal4p, intact. They also impaired activated (but not basal) transcription in *in vitro* transcription experiments (*29*). But their nature remained a mystery until one of them was found to be identical to Gcn5p, the yeast homologue of the then-recently cloned *Tetrahymena* histone acetylase (*31*). The ADA proteins, including Gcn5p, are part of a large and ever growing complex, which includes, among other factors, some long-known factors cloned because they alter transcription of the yeast retro-transposon Ty, called SPTs. This complex of SPTs, ADAs and Gcn5p with histone acetylase activity is called the SAGA complex (*32*). As far as we know, it may easily surpass a million Daltons in molecular weight, including factors able to bind to the activator domains of many activators, and also proteins called TAFs, closely linked to the TATA-binding protein TBP. Since recruiting of TBP seems to be a rate-limiting step for transcription of many promoters (*33*), this provides an elegant mechanism of how binding of activators may influence transcription *via* the bridge of SAGA members. The importance of the histone acetylase activity of the SAGA complex will be discussed below.

Whereas transcriptional regulation by Rap1p or Gal4p might be considered as a nicety of the yeast system, not necessarily relevant for understanding regulation in higher eukaryotes, this argument ceases when we consider the SAGA complex. Table 1 shows several histone-acetylase complexes from yeast, *Drosophila* and humans. From these complexes, SAGA and the related ADA complex (*32*) associate with transcriptional activation. A further complex, the elongator, appears to be required for the progression of the RNApol II through the coding sequence (*34*). In vertebrates, in addition to putative SAGA or ADA homologues (TFTC and STAGA), the polypeptide CBP/p300 has some sequence homology to Ada2p, one of the ADA proteins, as well as its histone acetylase activity. CBP/p300 was characterized as required for activation mediated by the CREB factor, and activates transcription in response to the cAMP levels, much as Gal4p activates when galactose is present (not necessarily through the same mechanism). CBP/p300 is a coactivator for many mammalian activators, like nuclear receptors, c- Jun, c-Fos, c-Myb, MyoD, in addition to CREB (*35*). Apparently, this polypeptide contains many functions that in yeast are separated into several proteins.

Table 1. Chromatin modifyng complexes in yeast, *Drosophilia* and humans[a)]

		Yeast	*Drosophilia*	Human
Histone acetylation		ADA		TFTC
		SAGA		STAGA
		NuA3		
		NuA4		
		yTFIID	dTFIID	hTFIID
		Elongator		
		Hat1		hHat1
				p/CAF
				p300/CBP
Chromatin remodelling	SWI/SNF	SWI/SNF	Brahma	hBRM
				BRG
		RSC1		
		RSC2		
	ISWI	ISW1	NURF	RSF
		ISW2	CHRAC	hCHRAC
			ACF	hACF
		INO80?		

[a)] Complexes putatively homologous are shown in the same row

4. When an Irresistible Force Meets an Immovable Object: Transcription on Nucleosomes

Eukaryotic DNA is wrapped around histone octamers forming nucleosomes. This highly stable structure poses a physical constraint on all cell activities requiring handling of DNA, like repair or replication of transcription (*36*). Chromosome-organized templates are not transcribed *in vitro* unless specific factors are added to the reaction (*37*). Even the path of DNA around the histone octamer affects phenomena like binding of transcription factors (*38*) or viral integration (*39*). For many proteins, nucleosomal DNA is simply not accessible.

However, transcription occurs. The biochemical search for nuclear fractions able to allow *in vivo* transcription of nucleosomal templates gave rise to a handful of related but not identical complexes, which could in some way alter nucleosome structure on addition of ATP (*40*). In parallel, a series of yeast mutations that impaired transcription of some genes, notably those involved in the mating type switch (SWI mutants) are suppressed by some point mutations in histone genes (SIN mutations), suggesting that *SWI* genes were required for nucleosome remodeling (*41*). In the end, the two ends met: Swi2p turned out to be a chromatin-dependent ATPase and member of the so-called SWI/SNF complex, functionally related to *Drosophila* complexes able to disrupt chromatin *in vitro* (*40*). The world of chromatin remodeling complexes is already crowded, and the number of new components is still increasing. Table 1 shows different chromatin remodeling complexes from yeast, *Drosophila* and humans. This list only includes two of the main families of chromatin remodeling complexes that are thought to exist in eukaryotic cells. These families differ in their crucial component, a chromatin-dependent ATPase. These complexes are not completely redundant (actually, some of them are essential), so they are thought to fulfil different functions in the cell, though there is little doubt that some cross-function exists (*40*). Anyway, chromatin-remodeling complexes help binding of some activators, like Gal4p or the *Drosophila* GAGA factor, to nucleosomal DNA *in vitro*. A similar function is likely to occur *in vivo* (*42*,*43*).

An element considered crucial to the understanding of chromatin structure and dynamics is the enzymatic modification of histones. Histones can be phosphorylated, acetylated, ubiquitinated, ADP-ribosylated or methylated. From all these post-translation modifications, the best known in terms of its relationship with nucleosomal structure and its interaction with different proteins is the acetylation of N-terminal lysine residues of the core histones H2A, H2B, H3 and H4 (*44*). It has long been known that transcriptionally active was more heavily acetylated than transcriptionally inactive chromatin, but a more detailed link between acetylation and activation only arose with the discovery of the SAGA and other histone acetyltransferase (HAT) complexes (*40*, Table 1). HAT and chromatin-remodeling complexes apparently reinforce each other, since mutations in some components of the SAGA complex become lethal or very serious in genetic backgrounds deprived of the SWI/SNF complex (*45*). Fig. 4 shows a multi-step model of transcriptional activation, and suggests a role for the different chromatin modifiers. The strict sequence of the concerted action of these two types of complexes is still obscure (*46*), but it is probably the same in most eukaryotic genes. An interesting property of this system is that none of the multiple complexes that contribute to transcriptional activation seem to be absolutely required for all genes: some promoters seem to be more sensitive than others to the lack of a given complex. This is true even of TAFs (see below and Fig. 4), which are thought to be at the heart of transcriptional activation machinery (*47*).

As histone acetylation promotes transcription, removal of acetyl groups from the N-terminal histone »tails« is a hallmark for transcriptional repression (*48*, *49*). Histone deacetylases (HD) also occur in complexes, and are related to several processes of transcriptional silencing (*49*). For example, one of the Sir proteins, Sir2p, is a NAD-dependent histone deacetylase (*50*). A member of the SIN family, *i.e.* suppressors of mutations on the SWI/SNF complex, Sin3p, forms a complex with the best-known yeast HD, Rpd3p (*49*). This suggests that with high levels of acetylation, chromatin-remodeling complexes are not so necessary for transcription. In addition, the glucose-repression related complex Tup1/Ssn6 binds preferentially to non-acetylated histones (*51*). In mammalian cells, some factors can act both as transcriptional activators or repressors depending on whether they bind an HAT or an HD complex. This is the case, for example, for nuclear hormone receptors, where the shift from one function to the other depends both on the ligand and on the DNA site they bind to (*52*).

5. Guest Stars: Heterologous Activators Working in Yeast

Yeast activators function when transferred to other eukaryotic systems. However, the converse is not necessarily true: although a variety of activators can be transferred from one system to another without loss of function, many transcriptional regulators from multicellular eukaryotes either do not work in yeast or have different characteristics (*53*). Many useful information about the transcriptional activation mechanisms in eukaryotes come from the study of which activators work in yeast, which ones do not, and why transcription patterns differ from one system to another.

A particularly successful field in this category is the study of nuclear receptors. Nuclear receptors form a large family, with members in all animals from *C. elegans* to vertebrates, but are absent in yeast (*54*). An especially well-known subset of this family are the vertebrate steroid hormone receptors (SHRs), which command many complex physiological responses to the presence of small ligands of the steroid family. SHRs exert their function by activating or repressing specific genes in the target cells, and were among the first mammalian activators recognized as such (*55*). A short DNA sequence, the hormone responsive element or HRE, is necessary and sufficient for conferring hormone responsiveness on any homologous or heterologous promoter; as it is the case for UASgal, HREs are functional as inverted repeats (*54*,*55*).

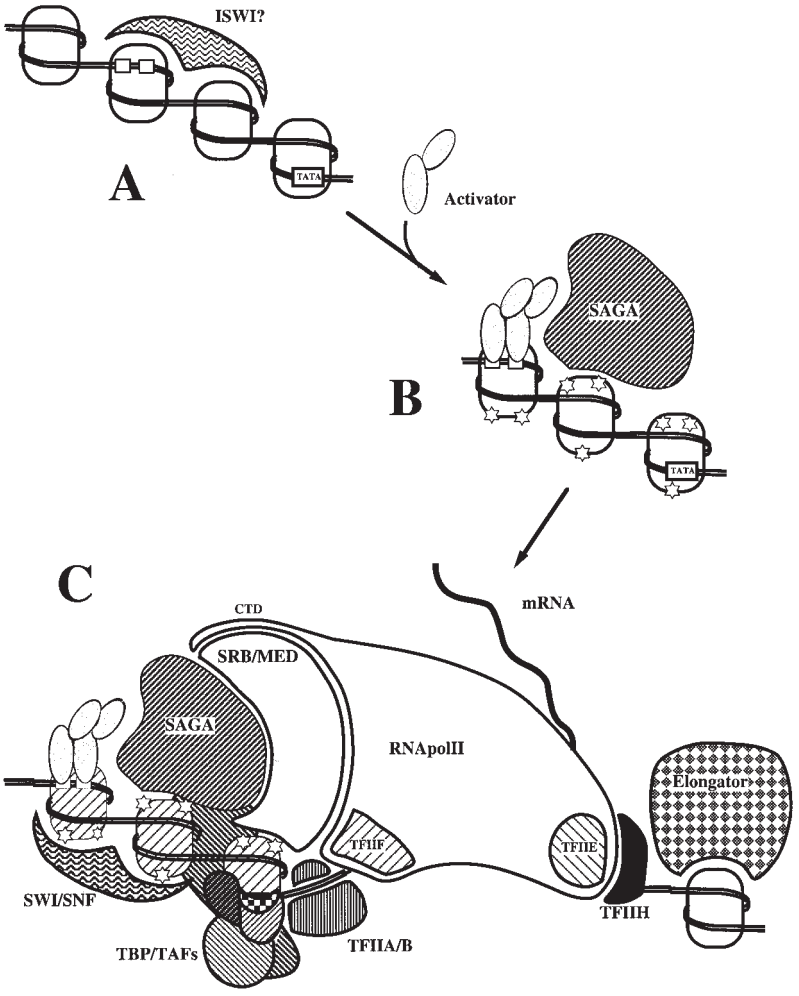

Fig. 4. Multi-step model of transcriptional activation in chromatin. A) In the absence of activator, nucleosomes adopt a closed conformation. It is likely that some ubiquitous remodeling function is required for binding of activators to their recognition sites (white boxes); this activity may well be the abundant ISWI complex. **B)** The binding of the activator helps recruiting histone-modifying complexes (here exemplified by SAGA), which acetylate histone tails (stars). **C)** Acetylated histones and the presence of the activation domain are thought to recruit further chromatin remodeling complexes (probably SWI/SNF) and destabilize chromosomes; the combined action would attract the mediator complex (SRB/MED) and would allow TBP to bind TATA boxes. TAFs, the rest of the TFIIs, and the RNApol II would bind to this open promoter (nucleosomes with stripes) and start transcription. A complex termed Elongator, structurally similar to the mediator, appears to be required for transcription to proceed

The mechanism of transcriptional induction through SHRs received an enormous boost when it was discovered that these receptors work in yeast. Ligand-free SHRs are associated with a large multiprotein complex of chaperones, and get free to bind DNA upon the addition of the ligand (*55*). Although the mechanism for this process was deduced in mammalian cells, its details were only elucidated in the yeast system. This mechanism relies upon the binding of the aporeceptor to the mammalian chaperone protein Hsp90, the role of which is covered by the yeast homologue Hsp70p. Removal of the portion of the protein that interacts with Hsp90 (or Hsp70p) results in a constitutive activator, no longer dependent on the presence of hormone. However, the lack of Hsp70p (something that can only be done in yeast) results in a partially inactive receptor, indicating that Hsp90 (and Hsp70p) contributes not only to repress the aporeceptor, but also to its correct folding (*55*). For most SHRs, the complex of the aporeceptor with chaperone proteins remains in the cytoplasm, and the addition of hormone permits the SHR to enter the nucleus. Although details differ, a similar mechanism is found for several regulatory mechanisms in yeast, such as Mig1p (*6*, Fig. 1). This demonstrates the functional similarity of transcription regulation in all eukaryotes.

Second aspect of the hormone response that has been specially favored by the transfer of the system to yeast is the study of its relationship with chromatin. Many hormone-responsive gene promoters have a distinct nucleosome positioning over their HREs. The best known case is the mouse mammary tumor virus (MMTV) promoter, where a single positioned nucleosome (called nucleosome B) covers all its five HREs, as well as the palindromic binding site for another transcription factor, NF1 (*56*). The receptor and NF1 synergize to activate the MMTV promoter, and NF1 appears to require receptor

binding to the HREs to bind its cognate site in this promoter. Interestingly, NF1 is unable to bind this site *in vitro* in the reconstituted nucleosome B, whereas the receptor can bind the HREs in the same conditions (*38*). This system can be reproduced in yeast with amazing fidelity. The MMTV promoter is functional in yeast, conserves the positioning of nucleosome B, and excludes NF1 in the absence of ligand or receptor. NF1 and the receptor are mutually required to activate transcription; in the case of NF1, this function only needs its DNA binding domain (*46*). These interactions depend on the integrity of nucleosome B: in derivatives of the MMTV promoter where nucleosome positioning is not favored, or in conditions of histone depletion (a condition only feasible in yeast), NF1 and the receptor not only do not synergize, but may simply compete for binding to DNA (*57*). NF1 is thought to have an ancillary role on MMTV activation, fixing the »open« status of nucleosome B (*46*). This mechanism may be of general importance for many mammalian and yeast promoters, since DNA-binding proteins with very little or no activation properties may help »true« activators (or repressors) only by their binding to DNA sites and their fixation of open conformation of key nucleosomes. For example, this could be the main role of Rap1p in some promoters.

The transfer of vertebrate activators to yeast has contributed decisively to the definition of activation domain. Many activation domains are of the so-called »acidic type«, including many acidic amino acids, sometimes forming part of an amphypathic α helix. However, some activation domains are rich in other amino acids, like proline (NF1) or glutamine, which work much less efficiently in yeast (*58*,*59*). However, many activators (including Rap1p and the hormone receptors) do not show any relevant feature on their activation domains, defined as the portion of the protein that renders it transcriptionally inactive when removed.

6. The Cell Wide Web: Interactions between Transcription Activation, RNA Elongation, Recombination, DNA Repair and Aging

Generally speaking, transcriptional regulation consists of the modulation of an enzymatic activity, RNA polymerase II (RNA pol II). Yeast RNA pol II is composed of 12 subunits, which are conserved through all eukaryotes (*28*). This multiprotein complex is able to transcribe DNA into RNA with great accuracy on its own, but it does not distinguish between true promoters and non-coding DNA sequences. Promoter recognition depends on the general transcription factors TBP, TFIIB, TFIIE, TFIIF and TFIIH, some of which are composed of several polypeptides (*28*). TBP, the TATA-box binding protein, forms a huge complex by its interaction with several proteins, the TAFs. A unique feature of the largest RNApol II sub-unit, an extended and repetitive C-terminal domain, CTD, also serves as anchorage point for an even larger complex, the so-called Srb/Mediator complex (*28*, see Fig. 4).

The presence of two large multiprotein complexes closely linked to the transcription machinery, the TAF complex and the Srb/Mediator complex, prompted the search for some of their components as targets for transcriptional activation and repression functions. Some components of these complexes were previously characterized coactivators, like Gal11p, a member of the Srb/Mediator complex. At least five TAFs are also part of the SAGA complex, and another TAF, Swp29p, is part of the SWI/SNF complex. Mutations in several TAFs or Srb/Mediators result in specific loss of activation for certain activators, but not for others (much like SAGA mutations), indicating that different activators (different activation domains) interact with different components of the transcriptional machinery.

At least part of the transcriptional machinery, including some of the components involved in transcriptional regulation, appears to have important roles in other cell functions. Components of TFIIH are essential for nucleotide excision repair (NER); their lack of function increases mutation rate and, in multicellular organisms, including humans, premature aging (*28*). Histone acetylation appears to be also important for the fast repairing of mismatches occurring or detected upon mRNA synthesis (*60*). Stalled RNApol II molecules appear to be strongly recombinogenic; a suppressor of this effect is actually a member of the Srb/mediator complex, Med3p (*61*).

The relationship between transcription and DNA metabolism extends also to transcriptional silencing. The NAD-dependent histone deacetylase Sir2p is essential for the maintenance of the rRNA repeats in the nucleolus (*62*), whereas Sir4p and Sir3p are required for double-strand brake (DBS) repair (*63*). Conversely, a factor long associated with DBS repair, Ku, appears to be required for the maintenance of telomere structure and telomere silencing in *S. cerevisiae* (*63*). Lately, glutamine- and proline-rich activation domains, supposedly non-acting in yeast, have been shown to stimulate DNA replication (*59*).

The relationship between transcription factors and aging exists not only in mammals (*28*,*64*), but also in yeast. An individual mother cell produces a finite number of daughter cells before senescing, number of which is determined genetically (*64*). Short lifespans are linked to lack of control of homologous recombination between rDNA repeats, which is strongly inhibited by Sir2p (*65*). However, the SIR complex as such (including Sir2p, Sir3p and Sir4p) appears also to contribute to the stability of rDNA repeats, by a different and still unknown mechanism (*65*).

Perhaps the most important lesson of the recent findings about transcription factors is that the cell uses a complex set of protein factors to perform an even more complex set of functions. This provides a beautiful example of functional economy, but complicates the study of these factors. From this point of view, transcriptional regulation is just another aspect of DNA metabolism, like DNA replication or repair, and many of the enzymatic activities required for any of these functions are likely to be required for the rest. There are chances that the network of common activities between these functions is going to increase, along with our knowledge of how eukaryotic cells function. The study of these functions in yeast provides a unique insight into mammalian processes where transcription, replication and DNA repair converge, such as development, aging and cancer.

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Mnogostruki načini regulacije transkripcije u kvascu

Sažetak

Regulacija transkripcije eukariota je složeni proces s mnogim implikacijama na fiziologiju organizma. Kvasac je jedinstvena kombinacija evolutivne konzervativnosti regulatornih procesa i mogućnosti njihove manipulacije. U radu su prikazani različiti stupnjevi regulacije gena. U prvom stupnju regulacije, *GAL* geni odgovaraju na prisutnost galaktoze. Međutim, taj prividno lagani sustav regulacije skriva pojedinosti koje su omogućile otkriće mnogih osnovnih mehanizama trans-aktivacijskog procesa. Konstitutivni aktivatori, kao Rap1p, omogućuju drugi stupanj regulacije. U tom slučaju problem je kako jedan faktor može uzrokovati različite odgovore, od aktivacije do represije. Dvije skupine koaktivatora, histonacetiltransferaza i kompleksi koji dovode do promjene strukture kromatina, omogućuju daljnji uvid o tome kako aktivatori djeluju, odnosno točnije uvid u vezu transkripcije i strukture kromatina. Mogućnost da se proučavaju heterologni aktivatori u kvascu bitno je pridonjelo našem razumijevanju djelovanja tih aktivatora, kao i razumijevanju mehanizama aktivacije transkripcije u kvascu. Konačno, u radu se govori o zamršenim međusobnim odnosima između različitih procesa u stanici, kao što su regulacija transkripcije, elongacija RNA, popravak DNA, rekombinacija i starenje.