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Reviews

The evolutionary conservation of eukaryotic gene transcription

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Summary. The basic components required for eukaryotic gene transcription have been highly conserved in evolution. Structural and functional homology has now been documented among promoters, promoter factors, regulatory proteins, and RNA polymerases from eukaryotes as diverse as yeast and mammals. The ability of these proteins and DNA sequences to function across phylogenetic boundaries demonstrates that common molecular mechanisms underlie gene control in all eukaryotic cells, and provides the basis for powerful new approaches to the study of eukaryotic gene transcription.
Key words. Eukaryotic gene transcription; evolutionary conservation; common motifs; new experimental approaches.

Introduction

Transcription is the cellular process by which RNA is synthesized from a DNA template. Like other cellular processes such as DNA replication and RNA splicing, gene transcription is mediated by both protein and nucleic acid constituents. The basic components required for accurate, efficient and regulable eukaryotic transcription initiation include two types of DNA elements known as promoters and upstream regulatory sequences, two sets of proteins known as general promoter factors and regulatory proteins, and the RNA synthesizing enzyme termed RNA polymerase. Recent experiments indicate that many of these basic components have been structurally and functionally conserved in eukaryotes as diverse as yeast and mammals, indicating that similar molecular mechanisms probably underlie gene transcription in all eukaryotes.

The purpose of this review is to summarize the topic of the evolutionary conservation of eukaryotic gene transcription. I shall discuss, in turn, the functional conservation of promoters, general promoter factors, RNA polymerase, activator proteins, and the regulation of activator proteins. I shall then present an overview of some

of the conserved molecular motifs employed for DNA binding and transcriptional enhancement. Finally, I present a speculative discussion on new experimental approaches that exploit the function of transcriptional regulators across phylogenetic boundaries to study the mechanism of eukaryotic gene regulation. As a starting point, let us consider the basic components of the eukaryotic transcriptional apparatus.

The eukaryotic transcriptional apparatus

Eukaryotic RNA polymerase II, a complex enzyme consisting of about 10 protein subunits, synthesizes messenger RNA (mRNA) corresponding to the protein coding genes in eukaryotic cells[98]. Polymerase initiates transcription at specific chromosomal sites known as promoters, which are short DNA sequences (< 80 bp) located at the 5' end of transcribed genes (fig. 1 A). Eukaryotic polymerase does not recognize promoter sequences directly; rather, additional proteins known as general promoter factors bind first to promoter sequences and mediate promoter binding by polymerase[20, 38, 88, 93].

The general promoter factors are a set of four proteins known as transcription factors IID, IIA, IIE and IIB. These factors are thought to bind promoters in a stepwise manner[10, 88] and are apparently required for accurate and efficient transcription initiation at all promoters transcribed by polymerase II. Transcription studies in vitro have shown, for example, that TFIID binds directly to the promoter element known as the TATA-box (fig. 1A)[88]. The TATA-box, which is found in nearly all eukaryotic promoters, is located about 30 bp upstream of the transcription start site in mammalian promoters[30] and somewhat further and more variably upstream (40–120 bp) of the start site in yeast promoters[105]. TFIID, together with TFIIA, form a stable protein-DNA complex known as the pre-initiation complex on the promoter (fig. 1 A). The subsequent binding of TFIIB and TFIIE, which appear to interact with each other and with polymerase, yields a complete initiation complex (fig. 1 A)[10, 88]. When bound by these general promoter factors and polymerase, promoters are able to direct basal level gene expression in vitro and in vivo.

Though promoters themselves are sufficient to direct basal level gene expression, vigorous and regulable transcription requires the assistance of a second set of proteins known as regulatory proteins[68, 85]. One of the two classes of regulatory proteins, known as activators, stimulate transcription by binding to specific DNA sequences known as upstream regulatory elements. Upstream regulatory elements that confer positive promoter regulation (i.e. bind activators) are known as upstream promoter elements or enhancers in mammalian cells, and as upstream activator sequences (UASs) in yeast[31]. Upstream promoter elements and UASs are usually located within several hundred base pairs of linked promoters

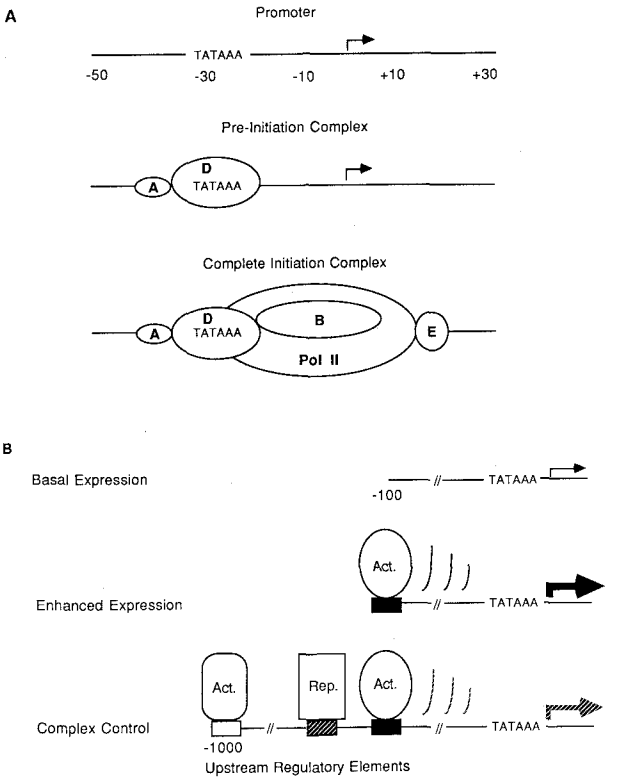

Figure 1. Proteins and DNA sequences required for eukaryotic gene expression.

A Shown are the basic components of a eukaryotic promoter based on in vitro studies with the adenovirus major late promoter[10]. The AT-rich TATA-box (TATAAA) is located about 30 bp upstream of the transcription start site (arrow). Binding of the general promoter factor TFIID (D) to the TATA-box, perhaps in concert with TFIIA (A), constitutes the formation of a pre-initiation complex. Stepwise addition of TFIIB (B), TFIIE (E) and RNA polymerase (Pol II) affords a complete initiation complex. The general transcription factors and polymerase schematized here are believed to be required for accurate and efficient expression of all genes transcribed by RNA polymerase II.

B Represented here are three promoters linked to various upstream regulatory sequences. The binding of an activator protein (Act.) to its cognate upstream regulatory element confers enhanced expression (bold arrow) upon the linked promoter that otherwise mediates only basal level expression (light arrow). Many eukaryotic promoters are subject to complex control (stipled arrow), depicted here by the combination of two different activators and a repressor (rep.) bound to multiple sites present upstream. Activators and repressors alter promoter efficiency by modulating the TATA-based promoter reaction, although all three promoters are shown without bound promoter factors and polymerase to emphasize the fact that the mechanistic details of enhancement and repression remain obscure.

(fig. 1 B), whereas mammalian enhancers may reside as far as several thousand bases upstream or downstream from a given promoter. Though these DNA sequences may function slightly differently, one may collectively view them as members of a single class of upstream regulatory elements that function in the context of a bound activator to stimulate promoter activity (fig. 1 B)[86, 87]. Activator proteins, bound to upstream regulatory elements, are believed to stimulate the TATA-based reaction by interacting with a protein component of the transcriptional apparatus such a general promoter factor (TFIID, A, B, E) or with RNA polymerase, although the mecha-

nistic details of transcriptional enhancement remain to be elucidated.

A single upstream regulatory element is sufficient for promoter stimulation although, in most cases, multiple types of elements (bound by distinct activators) are found upstream of eukaryotic genes. Moreover, certain promoter regions contain a distinct class of upstream regulatory elements, known as operators, that function to decrease the rate of gene transcription. The activity of operators is mediated by regulatory proteins known as repressors, which appear to exert their effects either by antagonizing the efficacy of an activator or by decreasing the TATA-based reaction directly[44]. Complex control in eukaryotes is afforded by combining multiple binding sites for activators and repressors upstream (or downstream) of a single promoter (fig. 1 B)[115]. In general, a typical eukaryotic promoter region is best viewed as a series of discrete DNA elements to which multiple proteins bind; poorly understood interactions between the general factors, polymerase, activators and repressors determine the precision and rate of transcription.

Conservation of promoters, promoter factors and polymerase

Promoters. It has been known for nearly a decade that promoter regions from widely divergent eukaryotes contain similar DNA elements such as the TATA-box and upstream regulatory sequences[31]. The common architecture of eukaryotic promoters and the presence of enhancer elements in both yeast and mammals provided early clues that the sequences and proteins that mediated promoter function have been conserved during evolution. Ironically, the earliest demonstration of promoter function across species boundaries came from studies on RNA splicing. Beggs et al.[4] sought to determine whether splicing of a mammalian transcript could occur in a simple eukaryote by introducing a segment of chromosomal DNA encompassing the rabbit β-globin gene into yeast. Though no splicing of the primary transcript was detected in yeast cells, expression of β-globin gene was observed, indicating that the rabbit β-globin promoter was functional in yeast. In an analogous series of experiments, it was demonstrated that the Drosophila alcohol dehydrogenase gene was also functional in yeast[109].

Recent studies on promoter conservation have shown that both the ADR2 promoter from *Saccharomyces cerevisiae* (budding yeast)[90] and the early promoter from the SV40 animal cell virus function in *Schizzosaccharomyces pombe* (fission yeast)[51]. In addition, the estrogen-inducible *Xenopus* (toad) vitellogenin A2 promoter has been shown to function in human cells[52]. Since high level promoter function requires the concerted action of proteins bound to both promoter and upstream elements (fig. 1 B), detectable promoter function across species boundaries implies that both promoter factors and regulatory factors have been conserved during evolution. Indeed, the estrogen-responsive element from the *Xenopus* vitellogenin A2 gene has been shown to function as an enhancer element in human cells, demonstrating the conservation of estrogen receptors in toads and humans[52]. Moreover, recent genetic studies with the adenovirus E3 promoter in yeast have revealed that mutations in either the TATA-box or in the upstream regulatory elements, impaired the binding of yeast factors to these sequences in vitro and reduced adenovirus E3 promoter function in vivo[54].

These data suggest that many of the proteins and DNA sequences involved in promoter function have been evolutionarily conserved, but should not be taken to mean that all aspects of promoter function are identical in eukaryotes or that every eukaryotic promoter will function across phylogenetic boundaries. In many cases, for example, the start sites for transcription initiation from a given promoter differ in yeast and animal cells. In fact transcription initiation from nearly all of the promoters described above was found to occur further downstream of the TATA-box in yeast compared to higher cells. Aberrancies observed in the transcription start sites of mRNAs from mammalian promoters in yeast cells probably reflect the fact that the TATA-box in higher eukaryotic promoters is usually closer to the start site than in yeast; perhaps owing to subtle differences in the molecular device that 'measures' the distance between the TATA-box and the transcription start site in yeast and mammals.

TATA-binding protein. Extracts prepared from mammalian cells[93], *Drosophila* embryos[100] and from yeast[60] have been shown to yield accurate transcription initiation by polymerase II from a minimal TATA-containing DNA template in vitro. The competency of extracts from several eukaryotes to support in vitro transcription, coupled with the presence of the TATA element in nearly all eukaryotic promoters has led to the investigation of TFIID conservation.

Functional conservation of TFIID was examined directly by determining whether the yeast TATA-binding protein could substitute for mammalian TFIID in an in vitro transcription reaction containing RNA polymerase and the three other required mammalian core factors (TFIIA, B and E). It was found that TFIID strongly supported accurate in vitro transcription from the adenovirus promoter in extracts lacking mammalian TFIID[11,13]. The yeast protein also appeared to promote formation of the pre-initiation complex since agents that prevent mammalian TFIID DNA binding also blocked yeast TFIID interaction with the DNA template. The binding of yeast TFIID was stimulated by mammalian TFIIA in a manner similar to that observed for mammalian TATA-binding protein, suggesting a conservation of protein-protein contacts between yeast TFIID and mammalian TFIIA. The functional conservation of the TATA-binding protein provides strong evidence that at

least one general promoter factor has been evolutionarily conserved from yeast to mammals.

RNA polymerase. The activity of RNA polymerase II is potently inhibited by a compound known as α-amanitin. The sensitivity of polymerase to α-amanitin has facilitated the isolation of α-amanitin resistant variants of polymerase in *Drosophila*[28] and other organisms, subsequently providing the basis for the isolation of a molecular clone of the α-amanitin resistant polymerase subunit[29]. Molecular analysis of the α-amanitin resistant polymerase clone revealed that α-aminitin sensitivity maps to the largest of the 10 polymerase II subunits. The *Drosophila* polymerase clone has allowed the isolation of homologs from yeast, mouse, hamster and human, providing direct evidence that the largest polymerase subunit has been conserved throughout eukaryotic evolution[92]. Each of the eukaryotic clones also displayed homology to the β' subunit of bacterial RNA polymerase, indicating that sequences constituting eukaryotic polymerase may have been acquired from the prokaryotic enzyme[2].

Two notable features of the largest polymerase subunit are common to all known eukaryotic isolates. The first of these is a highly basic 30 amino acid segment located approximately 350 residues from the amino terminus[92]. This basic region may act to contribute general DNA affinity to polymerase, an electrostatic property presumed to be essential for juxtaposition of the enzyme with the negatively charged DNA phosphate backbone during mRNA synthesis. In fact, biochemists have long exploited the affinity of RNA polymerase for anionic resins such as phosphocellulose as a step in the purification of the enzyme.

A second common feature is a seven amino acid repeat that comprises the carboxy terminus and whose consensus sequence (Tyr-Ser-Pro-Thr-Ser-Pro-Ser) is repeated 26 times in yeast, 44 times in hamster and 52 times in human[1,72]. The presence of the heptapeptide 'tail' was unexpected since repeated stretches of amino acid sequences are most often associated with structural proteins (e.g. keratin, tropomyosin and myosin), though subsequent experiments have clearly established its functional importance. First, specific transcription initiation by polymerase in vitro is inhibited either by deletions of the heptapeptide or by pre-incubating polymerase with a heptapeptide-specific monoclonal antibody[17]. More compelling, deletions in the yeast polymerase that reduce the tail by more than 40% result in cold sensitive strains that grow slowly, and deletions that shorten the tail by more than 60% are lethal[1,72].

Although the mechanistic role of the heptapeptide repeat remains a mystery, one hypothesis postulates that it functions as a molecular 'plow', acting to remove nucleosomes during mRNA synthesis. Alternatively, the heptapeptide repeat may interact with either a general promoter factor or with a regulatory protein, and thus may play a direct role in transcriptional regulation[1]. In any case, the functional conservation of the heptapeptide during evolution was dramatized by the finding that a yeast strain carrying a deletion in the gene encoding the largest polymerase subunit could be complemented by a chimeric yeast polymerase gene fused to sequences encoding the heptapeptide from hamster[1]. The extent to which the remaining nine polymerase subunits display functional conservation awaits sequence information.

Conservation of activators

Eukaryotic transcriptional activators including steroid receptors, CCAAT-binding proteins, the products of oncogenes, animal virus gene products, heat shock transcription factors and many yeast activators bind to upstream regulatory sequences and enhance transcription from linked promoters (fig. 1 B). Recent evidence indicates that all of these activators can function across phylogenetic boundaries.

Steroid receptors. Steroid hormones coordinate complex events in mammals including development, differentiation and physiological responses to diverse stimuli. Steroid hormone action is mediated by soluble intracellular proteins known as steroid receptors[114] which, in the presence of the hormone, bind enhancer elements[14,76,95] located near regulated promoters and stimulate promoter activity by increasing the rate of transcription initiation. Molecular clones encoding glucocorticoid, mineralicorticoid, progesterone, estrogen, vitamin D, thyroid, retinoic acid and related receptors have been isolated[3,19,27]. The overall genetic composition of all of these receptors is similar, implying that steroid receptors constitute a related family of hormone-inducible regulatory proteins. The apparent relatedness of these molecules suggests that each may have arisen from a common progenitor early in the evolution of eukaryotic cells. In support of this notion, investigators have identified steroid binding activities in extracts prepared from various fungi[12].

The presence of putative steroid receptors in yeast as well as the similar architecture of yeast and animal cell promoters lead two groups to investigate whether mammalian steroid receptors could function in fungi. To test this hypothesis, cDNAs encoding the glucocorticoid or the estrogen receptor were introduced into yeast cells to allow expression of the steroid receptor protein. The ability of the steroid receptor to bind DNA and enhance transcription was easily assayed since the enhancer element had been placed upstream of a well-characterized yeast promoter linked to the lacZ gene. Thus, receptor-dependent increases in lacZ (β-galactosidase) expression would provide the experimental evidence (fig. 2).

Both the glucocorticoid receptor[96] and the estrogen receptor[70] were shown to enhance transcription from yeast promoters linked to enhancer sequences; moreover, transcriptional activation occurred in a hormone-dependent manner. The start sites for mRNA synthesis were identi-

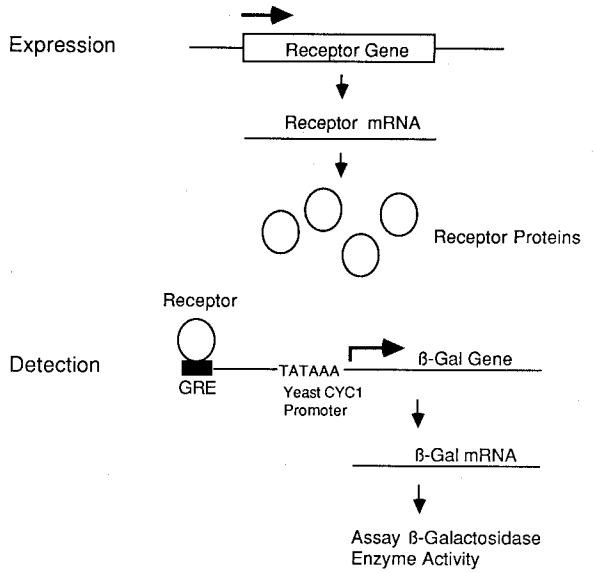

Figure 2. A simple assay for mammalian glucocorticoid receptor action in yeast.
The cDNA encoding the rat glucocorticoid receptor (receptor gene) is introduced into yeast cells, and expressed using a suitable yeast promoter. Translation of the receptor messenger RNA (receptor mRNA) affords the glucocorticoid receptor protein (shown as spheres). Receptor function is easily quantitated by measuring the expression of the lacZ gene fused to the yeast *CYC1* promoter, upstream of which is the glucocorticoid receptor enhancer element (shown as a filled square). Transcriptional activation by receptor causes an increase in β-galactosidase mRNA which, in turn, leads to an increase in the assayable β-galactosidase enzyme. Similar versions of this assay have been employed to detect enhancement by several other mammalian activators in yeast.

cal when compared to yeast promoters under the control of yeast activator proteins, indicating that mammalian steroid receptors function in yeast by faithfully interacting with a component of the yeast transcriptional machinery. The observation that mammalian steroid receptors function in yeast and in *Drosophila* (S. K. Yoshinaga and K. R. Yamamoto, manuscript in preparation) argues that the molecular mechanisms that underlie steroid-mediated enhancement arose early in evolution and imply that the family of mammalian steroid receptors may have diverged from a primordial steroid receptor. These results also suggest that steroid receptors enhance transcription by a similar mechanism in yeast and mammalian cells.

CCAAT-binding proteins. Activators that bind to upstream promoter elements containing the pentanucleotide CCAAT sequence (pronounced 'cat sequence') constitute a family of transcription factors known as the CCAAT-binding proteins[5, 68]. Members of this family include C/EBP, CTF (NF-1), CP1 and CP2 and others. The CCAAT sequence element has been found upstream of a diverse set of mammalian promoters including human α-globin, mouse α-globin, human hsp70, HSV thymidine kinase, H-2K^b, adenovirus major late promoter and origin, MSV LTR, rat γ-fibrinogen and others[16]. Although each of these factors recognizes a similar DNA element, sequence analysis of molecular clones has confirmed that separate genes encode C/EBP and CTF[57]. Molecular clones have not yet been isolated for CP-1 and CP-2, but biochemical data demonstrate that these proteins are also distinct from each other and from C/EBP and CTF. Interestingly, biochemical experiments have shown that one of the CCAAT-binding proteins, CP-1, recognizes DNA as complex composed of at least two heterologous subunits; that is, maximal DNA binding by CP-1 requires two components, CP-1A and CP-1B, that form a stable CP-1A, B protein complex in solution[16].

Regulatory elements containing the CCAAT consensus have also been identified upstream of yeast genes. The CCAAT sequence located in the *CYC1* UASs matches perfectly the consensus sequence recognized by mammalian CP-1[34]. Two yeast proteins, HAP2 and HAP3, bind to the *CYC1* UAS CCAAT element and activate the *CYC1* promoter when yeast cells are grown on non-fermentable carbon sources. Genetic and biochemical studies have shown that HAP2 and HAP3 bind DNA as a heteromeric protein complex[36]. Furthermore, yeast HAP2 and HAP3 make the same DNA contacts as the CP-1A,B complex. The existence of CCAAT regulatory sequences in yeast and mammalian cells and the heteromeric composition and similar DNA binding properties of yeast and mammalian CCAAT-binding proteins, led investigators to examine whether yeast HAP2 and HAP3 and human CP-1A and CP-1B are functionally related.

The functional interchangeability of subunits from the HAP2,3 and CP-1A,B proteins was investigated by measuring the ability of mixed chromatographic fractions from yeast and human cells to bind a DNA element containing a CCAAT consensus sequence. Remarkably, heteromeric complexes of CP-1A and HAP2 or CP-1B and HAP3 exhibited an affinity for the CCAAT sequence similar to complexes consisting of yeast HAP2,3 and mammalian CP-1A,B[15]. Thus, a CCAAT binding protein from yeast and human cells consists of heterologous protein subunits that are functionally interchangeable. No obvious physiological connection exists, however, between yeast cytochrome genes and mammalian promoters under CP-1A,B regulation. Nonetheless, the functional homology of CCAAT-binding protein subunits in yeast and mammals argues that the CCAAT pentanucleotide sequence arose as an upstream regulatory element early in eukaryotic evolution and that subunit contacts between the factors that recognize this sequence have apparently been highly conserved as well.

Oncogenic activators. Several dozen oncogenes (cancer causing genes) have been identified over the past ten years[7]. The products of these viral and cellular genes constitute a diverse class of proteins that include growth factors and growth factor receptors, G proteins, protein kinases and nuclear proteins. Oncogene products that are secreted, membrane bound or localized to the cytoplasm apparently induce neoplasia by altering normal cellular

signal transduction and metabolism. In contrast, several oncogenes that express nuclear proteins are believed to induce abnormal cell growth by altering gene expression directly.

A provocative link between transcriptional activation and neoplasia was established by the finding that v-*jun*, the presumed oncogene of the avian sarcoma virus, apparently arose by transduction of the cellular gene that encodes a human transcriptional activator protein termed AP-1 [8]. Promoters under AP-1 regulation, including the human metallothionine gene and the SV-40 early region, contain binding sites for AP-1. Purified Jun protein binds to the same DNA sequence as AP-1 and, unexpectedly, the AP-1 binding site was found to be nearly identical to the core consensus DNA sequence recognized by GCN4, a yeast transcriptional activator involved in the coordinate regulation of amino acid biosynthetic genes [59]. Furthermore, the DNA binding domains of mammalian AP-1 and yeast GCN4 show significant protein homology suggesting that mammalian Jun may have arisen from primordial regulators whose DNA binding and transcriptional enhancement functions were conserved from yeast to mammals [108].

The DNA binding domain of Jun, when fused to the activation region of GCN4, was found to activate the expression of yeast amino acid biosynthetic genes normally under GCN4 control, indicating that Jun is capable of recognizing GCN4 binding sites upstream of yeast amino acid biosynthetic genes [104]. Mammalian Jun is also capable of activating transcription in yeast. A recombinant molecule consisting of Jun fused to the DNA binding moiety of bacterial Lex-A protein was found to stimulate expression of the yeast *CYC1* promoter fused to lexA binding sites [103]. Moreover, the AP-1 DNA regulatory element from the SV40 early promoter was shown to function as an enhancer element both in budding yeast [37] and in fission yeast [48]. Interestingly, a second yeast factor related to GCN4 also appears to recognize this site suggesting the existence of a family of AP-1/Jun-like molecules in fungi. The function of the products of mammalian oncogenes as activators in yeast appears to be a fairly general phenomenon since two other nuclear oncoproteins, Myc and Fos, also activate the *CYC1* promoter as fusions to LexA [58]. These findings suggest that one cause of neoplasia in mammals is the aberrant expression of cellular genes; moreover, the products of mammalian oncogenes may have arisen from ancestral transcriptional activators that mediate critical regulatory functions in simple eukaryotes.

Virus-encoded activators. Many animal cell viruses, like bacterial viruses, utilize the enzymes of infected cells to carry out various aspects of viral DNA replication, gene transcription and protein biosynthesis. All viruses, however, encode a small number of viral proteins needed for specialized aspects of the viral lifecycle. Virus-encoded transcriptional activators are frequently required, in concert with host cell transcription factors and RNA polymerase, to activate the expression of viral genes [49, 68].

Two viral activators, E2 from the bovine papilloma virus (BPV) and VP16 from the herpes simplex virus (HSV), have been analyzed in some detail at the molecular level. The E2 protein of BPV contains functional domains typical of gene activators including sequences that mediate DNA binding and transcriptional activation (fig. 1 B) [66]. E2 binds to specific sites near several BPV promoters and increases transcription of viral genes whose products are required in early aspects of the BPV lifecycle. Similar to the E2 protein, VP16 also contributes an enhancement domain that acts to stimulate early viral gene expression. Unlike the E2 protein, however, VP16 does not bind to enhancer elements directly, but rather interacts in a poorly understood manner with a host enhancer binding protein that occupies regulatory elements upstream of herpes virus genes under VP-16 control [107]. Nonetheless, both E2 and VP-16 are viral-encoded transcriptional activators that have co-evolved with mammalian cells to insure efficient interaction with the host transcriptional apparatus. Might the potent activators encoded by animal cell viruses activate transcription across phylogenetic boundaries?

The E2 protein was tested for enhancement activity in yeast by expressing the mammalian protein in yeast cells containing a reporter gene consisting of E2 binding sites positioned upstream of the *CYC1* promoter (as in fig. 2). It was found that E2 activated transcription of the *CYC1* promoter and that enhancement was cooperative as induction was much more efficient with two E2 sites than with a single site [55]. These experiments demonstrate that a virus-encoded activator can stimulate transcription in yeast; furthermore, E2 appears to interact with a similar protein in fungi and mammals since cooperativity of E2 action (but not E2 DNA binding) was observed in both cell types. The herpes VP-16 protein was also tested for enhancement in yeast as a fusion molecule containing VP-16 tethered to the DNA binding domain of the yeast GAL4 protein. The VP-16 molecule fused to GAL4 was found to activate the yeast *CYC1* promoter when GAL4 binding sites were present as upstream elements [91] (I. Sadowski, personal communication).

Heat shock transcription factor. The heat shock response in eukaryotic organisms is the process whereby the expression of a group of genes, the heat shock genes, is coordinately induced in response to elevated temperatures [77]. In general, thermal elevations exceeding the ambient celsius growth temperature by more than 15–20% are sufficient to trigger the heat shock response. In mammals, yeast, and *Drosophila*, thermal increases to 42 °C, 37 °C and 30 °C, respectively, elicit the heat shock response in these organisms which are normally grown at 37 °C, 30 °C and 25 °C, respectively.

The products of heat shock genes (the heat shock proteins) are believed to protect cells from elevated temperatures, in part, by binding to cellular proteins and

preventing their heat-induced denaturation[62]. The coordinate induction of heat shock genes is mediated by a phylogenetically conserved DNA element located upstream of heat shock gene promoters. The heat shock element (HSE) from mammals, yeast and *Drosophila* exhibits classical enhancer qualities, conferring heat-inducibility upon heterologous promoters[79]. The activity of HSEs is imparted by the heat shock transcription factor (HSTF), which binds specifically to the HSE consensus sequence and induces transcription from heat shock promoters in a temperature-dependent manner[75,102]. The universal existence of the heat shock response in eukaryotes and the remarkable similarity of HSEs and heat shock proteins, suggests that strong selective pressures have maintained primordial mechanisms of thermal defense.

Consistent with this view, investigators have shown that a consensus HSE functions in a diverse class of eukaryotes including mammals, frogs, *Drosophila*, and yeast, implying the presence of a highly conserved heat shock transcription factor in these organisms[77]. In fact, purified yeast HSTF is identical in electrophoretic mobility to the *Drosophila* protein and binds specifically to the HSE upstream of the *Drosophila* hsp 70 gene; similarly, purified *Drosophila* HSTF binds specifically to the yeast hsp 70 heat shock element[112]. The essential cellular function of HSTF was dramatized by the recent demonstration that yeast cells carrying mutations in this transcription factor are inviable[101,111]. These and other experiments indicate that the heat shock element, heat shock transcription factor and heat shock proteins are critical in homeostasis and have been remarkably conserved during evolution.

Yeast regulatory proteins. Much is known about the proteins that regulate gene expression in yeast[32]. Perhaps the best studied of these regulatory factors is GAL4, a protein that induces the expression of a set of genes whose products are required for galactose utilization by yeast cells[45]. The functional domains of the GAL4 protein have been dissected and include sequences that mediate DNA binding, transcription activation and GAL80 association[47,65]. The GAL4 protein is active only in the presence of galactose which binds to GAL80 and triggers the dissociation of the inactive GAL4/GAL80 complex. Yeast genes under GAL4 control contain binding sites for the GAL4 protein; these upstream activator sequences can confer galactose regulability upon heterologous promoters, function without strict regard to spacing and thus display the properties of mammalian enhancers[35]. The similarities between yeast GAL4 upstream activator sequences and mammalian enhancers and the common architecture of eukaryotic promoters lead several groups to determine whether GAL4 could function in higher eukaryotic cells.

The function of GAL4 in mammalian cells was assayed by co-transfecting appropriate tissue culture lines with the yeast GAL4 gene and a suitable reporter plasmid. Reporter plasmids contained GAL4 binding sites positioned upstream of various a mammalian promoters fused to the bacterial gene encoding chloramphenicol acetyl transferase (CAT) enzyme. Significant increases in CAT activity were observed in cells co-transfected with the GAL4 protein and a GAL4 UAS-linked mammalian promoter[50,110]. Enhancement was observed from the mouse mammary tumor virus, herpes simplex virus thymidine kinase gene, rabbit β-globin gene and the adenovirus major late promoters, indicating that GAL4 interacts with a component of the general transcription apparatus required for the expression of a diverse set of mammalian and viral genes. Furthermore, GAL4 acted synergistically with several mammalian regulatory proteins such as the glucocorticoid receptor and the SV40 enhancer binding proteins, suggesting that yeast and mammalian activators probably contact a common component of the transcriptional apparatus. The GAL4 activator has also been shown to function in plant cells[63] and in *Drosophila*[21], thus providing the best evidence that gene activation is mechanistically similar in all eukaryotes.

Conservation of activator protein regulation

The gratuitous presence of a gene activator in the nucleus of a cell can lead to a dampening or 'squelching' of gene expression that presumably results from the titration of an essential component of the transcriptional apparatus[24]. Regulating the activity of gene activators is thus critical to homeostasis. Regulatory protein regulation is also essential during development. In the *Drosophila*, for example, tissue formation is known to involve a delicate hierarchy of spatially and temporally restricted patterns of gene expression; mutations in presumed gene activators can lead to profound developmental defects[97].

The potency of a given activator protein can be modulated either by varying its cellular concentration or by altering its specific activity. The specific activity of a regulatory protein can be altered by covalent modifications such as phosphorylation, or by noncovalent associations such as the binding of a ligand. Covalent modifications and noncovalent associations can be direct as in the phosphorylation of HSTF[101], or indirect as in GAL4 activation by galactose binding to GAL80[45]. In general, it appears that nature has utilized a relatively small number of mechanisms to regulate the activity of eukaryotic activators[116]. The presence of similar mechanisms of activator protein regulation in eukaryotes suggests that regulatory factors such as kinases and inhibitory proteins may have been conserved during evolution.

Mammalian steroid receptor action in yeast provides the best example of the conservation of regulatory protein regulation across phylogenetic boundaries[70,96]. Recall that receptor dependent transcriptional enhancement in yeast and animal cells occurs only in the presence of bound steroid. It is known that hormone binding triggers

glucocorticoid receptor activation or 'transformation' leading to rapid nuclear localization, DNA binding and transcriptional enhancement[115]. The unliganded, cytoplasmic form of the glucocorticoid receptor is believed to exist as a heteromeric complex with the Hsp90 protein[80,83]. According to this model, hormone binding triggers dissociation of the receptor-Hsp90 complex leading to the unmasking of receptor transcriptional regulatory activities.

If the Hsp90 model for steroid receptor regulation is correct, yeast must contain an Hsp90 homologue whose conservation is sufficient to permit formation of an inhibitory complex with the mammalian glucocorticoid receptor. In fact, studies have shown that *S. cerevisiae* expresses two Hsp90 homologues known as Hsp82 and Hsc82 (S. Lindquist, personal communication). More compelling, the mammalian Hsp90 gene can complement a yeast strain doubly deleted in Hsp82 and Hsc82, suggesting a high degree of functional homology between the mammalian Hsp90 and the yeast homologues (D. Picard, K. R. Yamamoto and S. Lindquist, unpublished results). Thus, it appears that the noncovalent regulation of the receptor by steroid ligand is mediated by a cellular component (Hsp90) that has have been conserved over a long period of eukaryotic evolution.

Evolutionary conservation of activator protein structure and function.

Activator	Source	DNA Binding	Acidic Domain	Functions In
ER	human	Zn finger	?	yeast
CP-1A,B	human	?	?	yeast[a]
Myc	human	leucine zipper	+	yeast[c]
AP-1	human	leucine zipper	+	yeast[a]
GR	rat	Zn finger	+	yeast, Drosophila
Fos	mouse	leucine zipper	+	yeast[c]
VP-16	HSV	--	+	yeast[c], hamster[c]
E2	BPV	?	+	yeast
Jun	ASV	leucine zipper	+	yeast
HSTF	yeast, Drosophila	?	+[b]	Drosophila[a], yeast[a]
GAL4	yeast	Zn finger	+	plants, Drosophila, human
Hap2,3	yeast	?	?	human[a]
GCN4	yeast	leucine zipper	+	human[c]

Shown are activator proteins from various organisms that function across phylogenetic boundaries. The estrogen receptor, glucocorticoid receptor and heat shock transcription factor are designated ER, GR and HSTF, respectively. The herpes simplex, bovine papilloma, and avian sarcoma viruses are designated as HSV, BPV and ASV, respectively. In most cases, the presence of a zinc finger or leucine zipper motif is inferred from primary sequence and biochemical data, not from structural information. Proteins for which acidic domains are thought to contribute to enhancement are indicated by a plus (+), except for HSTF in which acidity contribute by protein phosphorylation is thought to be critical (b). The function of activators in foreign organisms was assessed by enhancement in vivo, except in cases where truncated molecules were tested in vivo (c) or where DNA binding in vitro was used as the functional criterion (a). References for each of the activators can be found in the text.

Conserved motifs for binding and activation

What is the molecular basis for the functional conservation of activator proteins across phylogenetic boundaries? The answer, in part, is that the molecular motifs for DNA binding and transcriptional activation have been conserved in evolution.

DNA binding. Though a large number of activators have been identified, all available data indicates that protein-DNA interaction is mediated by three chemical attractions that include hydrogen bonding, electrostatic interactions and van der Waals forces[74]. Primary sequence and biochemical data suggest that the structures of the DNA binding domains of all known eukaryotic activators fall into three classes; these include the helix-turn-helix, the zinc finger and the leucine-zipper motifs (table)[106].

The helix-turn-helix proteins[74], which contain a DNA binding domain formed by two α-helices separated by a β-turn, constitute a diverse class of regulators that include the yeast MATα-2protein[92] and *Drosophila* and vertebrate homeo-box proteins of which there are more than 80 known members[61]. By analogy to prokaryotic activators and repressors for which crystallographic data are available, eukaryotic helix-turn-helix proteins probably recognize specific DNA elements by inserting one of the two helices into the DNA major groove. The zinc finger proteins, for which zinc coordination constitutes the major structural determinant, include the *Xenopus* transcription factor IIIA[71], the yeast GAL4 protein[46] and the mammalian steroid receptors (table)[22,113]. Residues within or adjacent to the loops or 'fingers' formed by cysteine and histidine binding to zinc are believed to mediate DNA binding[6,53]. The leucine-zipper class, first identified in the C/EBP CAAT-binding protein[56], includes several other mammalian transcription factors such as Jun and AP-1, the yeast activator GCN4, and the oncoproteins Fos and Myc (table). Leucine-zipper proteins are known to recognize DNA as dimers. Dimers of leucine-zipper proteins are believed to form through interdigitations of an evenly spaced series of leucine residues that act as a molecular 'zipper'[56].

Given that a small number of chemical forces and structural motifs are probably employed for DNA binding by all eukaryotic activator proteins, the functional conservation of the protein-DNA interactions across phylogenetic boundaries is likely explained by the similarities in these basic biophysical and structural properties.

Transcriptional enhancement. Activator proteins bind to DNA elements located near promoters and enhance transcription probably by interacting with some protein component of the transcriptional machinery[39,86,116]. Obvious candidates for the 'targets' of eukaryotic activators include histones, the general promoter factors and RNA polymerase. One study has shown that the GAL4 activator alters the DNA binding properties of TFIID, suggesting that the mechanism of enhancement by this protein may involve direct interaction with the TATA-binding protein[42]. In other studies, GCN4 has been shown to interact with RNA polymerase in vitro, possibly indicating that this protein stimulates transcription by recruiting polymerase to the promoter[9]. In no case,

however, has the mechanism of transcriptional enhancement been unequivocally established.

Though the protein(s) with which activators interact remain unknown, the regions of activators that mediate these presumed protein-protein contacts have been identified and studied in detail. Surprisingly, the activation domains of some eukaryotic regulators appear simply to consist of short polypeptide sequences that carry a net negative charge[23, 26, 40, 41, 65]. Acidic activation domains ('acid blobs'), rich in aspartic and glutamic residues, share no apparent sequence homology; in fact, random *E. coli* sequences that carry a net negative charge have also been shown to function as activation domains in yeast[64]. Thus, it appears that acidic protein sequences, perhaps with α-helical secondary structure[25], can mediate enhancement in yeast, plants, *Drosophila*, and mammals. Acid blobs do not appear to represent the only enhancement motif; in fact, several lines of evidence indicate that other sequences contribute selectively to transcriptional enhancement[9, 18, 69]. Acidic enhancement domains do, however, play an important role in transcription; moreover, the apparent plasticity of these acidic regions certainly accounts for some of the promiscuity of eukaryotic activators (table).

Perspectives-exploiting the conservation

The evolutionary conservation of all of the basic components required for eukaryotic transcription initiation suggests gene control in multicellular organisms arose by exploiting, in increasingly complex ways, the basic molecular machinery present in simple eukaryotes, rather than by discarding primordial cellular mechanisms and adopting fundamentally novel methods of transcription initiation. The functional conservation of these components across organismic boundaries provides the basis for new experimental strategies for the study of eukaryotic gene transcription.

Genetic experiments with mammalian transcription factors in yeast, for example, circumvent the prohibitive nature of similar approaches in tissue culture cells. In principle, random mutagenesis coupled with a genetic screen or selection should provide an efficient means by which to study the structure and function of any mammalian regulator whose function is assayable in yeast. In fact, Schena et al. (manuscript submitted to *Genes and Development*) have recently used chemical mutagenesis and a yeast plate assay to genetically dissect the zinc finger region of the mammalian glucocorticoid receptor. These studies have lead to the identification of a rare class of apparent 'positive control' mutants that retain DNA binding activity but fail to enhance transcription.

Yeast genetics could also be useful in dissecting consensus DNA binding sites for regulatory proteins from higher cells. One could, for example, insert DNA fragments containing random mutants of a consensus binding site upstream of a yeast reporter gene and screen yeast cells expressing the mammalian factor for inserts that fail to mediate enhancement. A similar type of experiment could be used to identify a binding site for a cloned mammalian factor for which a consensus sequence has yet to be identified such in the case of several mammalian oncogenes. An elegant experiment designed to determine the residues of a regulatory protein that mediate DNA contact involves generating second site suppressor mutants that display altered sequence recognition. It might be possible to isolate a mutant protein that recognizes altered DNA site.

Experiments across species boundaries also holds promise for identifying additional cellular factors required for transcriptional control including proteins that participate directly in enhancement such as general promoter factors and RNA polymerase, and proteins involved indirectly such as proteins that regulate the activity of activators.

Yeast genetics might facilitate the identification of mammalian transcription factors. It might be possible, for example, to isolate cDNAs encoding a desired mammalian homologue either by complementing a yeast strain bearing a mutation in a similar factor, or by isolating a yeast mutant that selectively requires the expression of mammalian protein for viability. This latter approach, recently termed 'cloning by function', embodies the distinct advantage of allowing one to clone functionally homologous proteins that show no primary sequence homology. In cases where a binding site (but no factor) is available, one should also be able to devise assays in yeast to identify these novel regulatory proteins.

In vitro transcription experiments using mammalian and *Drosophila* extracts are much more advanced than similar experiments using yeast extracts. Since it is clear that TFIID is conserved in yeast and mammals, it should be possible to combine biochemical and genetic approaches to study and purify transcription factors. Cloned yeast transcription factors will be useful in establishing the in vivo role of a given protein via gene replacement strategies, and may serve as a useful hybridization probes for isolating mammalian homologues.

All of these approaches assume and require a high degree of conservation of transcriptional regulatory proteins in eukaryotic cells. This discussion is not intended to suggest, however, that all aspects of transcriptional regulation are identical in all eukaryotic organisms. It is already clear from mammalian steroid receptor experiments in yeast, for example, that subtle differences in receptor function exist between yeast and mammals. Furthermore, the apparent ability of random sequences bearing a net negative charge to function as enhancement domains provides the basis for potential artifacts in interpreting the physiological relevance of a given mammalian protein to activate yeast gene transcription. Nonetheless, cautious genetic and biochemical experimentation across phylogenetic boundaries provides the

basis for powerful new approaches to the study of eukaryotic gene transcription.

I have largely excluded discussions of the prokaryotic transcription literature in this review. The reader should be aware, however, that many the conceptual themes presented here derive from experiments with bacterial promoters and activators[43, 67, 74, 82, 84, 89, 117]. Furthermore, while fundamental differences clearly exist between prokaryotic and eukaryotic transcription, the general chemical principles that appear to govern regulation in complex organisms probably apply to all living cells.

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Research Articles

Drosophila melanogaster does not dealkylate [^{14}C]sitosterol[1]

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Summary. Drosophila melanogaster was unable to dealkylate and convert [^{14}C]sitosterol to cholesterol and no evidence was found for conversion of [^{14}C]desmosterol to cholesterol. Therefore, *D. melanogaster* is incapable of dealkylating and converting C_{28} and C_{29} phytosterols to cholesterol.
Key words. Drosophila melanogaster; sterol metabolism; phytosterols; dealkylation; desmosterol; sitosterol; radiolabeled sterols.

Many insects can dealkylate the C-24 alkyl group from the side chain of dietary sterols and so convert phytosterols to cholesterol. Generally, those insects that can dealkylate grow about equally well on either sitosterol or cholesterol as well as on a number of other C_{28} and C_{29} phytosterols, such as campesterol (C_{28}) or stigmasterol (C_{29})[2]. Extensive biochemical studies using radiolabeled sterols supported by extensive sterol analyses, e.g. by gas-liquid chromatography (GLC) and mass spectrometry (MS), have verified that desmosterol is a common intermediate in the dealkylation and conversion to cholesterol of all phytosterols studied[2–5]. Consequently, desmosterol, as a dietary sterol, is readily converted to cholesterol in those insects that can dealkylate and supports normal growth and development[2,6]. In a study on sterol utilization by *Drosophila melanogaster*, Cooke and Sang concluded, based on the ability of various sterols to support development, that the *tu bw; st su-tu* strain of this species could dealkylate C-24 alkyl groups from the side chain and also convert phytosterols to cholesterol[7]. However, it was reported that phytosterols such as sitosterol (C_{29}) were superior to cholesterol (C_{27}) in supporting growth and development, and that desmosterol (C_{27}) very poorly supported growth[6]. The only analysis of dietary or insect sterols presented by these investigators was by thin-layer chromatography (TLC) which does not satisfactorily resolve cholesterol from other neutral sterols. Recently, in an investigation of ecdysteroid biosynthesis in *D. melanogaster*, several dietary sterols were utilized in aseptic media, and it was concluded that the C_{28} sterol, ergosterol, apparently was dealkylated by the insect, but no analyses of insect neutral sterols