Basal transcription machinery: role in regulation of stress response in eukaryotes

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The holoenzyme of prokaryotic RNA polymerase consists of the core enzyme, made of two α, β, β' and ω subunits, which lacks promoter selectivity and a sigma (σ) subunit which enables the core enzyme to initiate transcription in a promoter dependent fashion. A stress sigma factor σ^s, in prokaryotes seems to regulate several stress response genes in conjunction with other stress specific regulators. Since the basic principles of transcription are conserved from simple bacteria to multicellular complex organisms, an obvious question is: what is the identity of a counterpart of σ^s, that is closest to the core polymerase and that dictates transcription of stress regulated genes in general? In this review, we discuss the logic behind the suggestion that like in prokaryotes, eukaryotes also have a common functional unit in the transcription machinery through which the stress specific transcription factors regulate rapid and highly controlled induction of gene expression associated with generalized stress response and point to some candidates that would fit the bill of the eukaryotic σ^s.

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1. Stress response

Stress can be broadly defined as any unfavourable condition. A given condition may or may not be stressful to an organism hence the stress response elicited by a given condition is dependent on the organism as well as the stressor. The stresses in general can be categorized into different groups as enlisted in table1. The physical/chemical parameters like high/low temperatures, pH, presence of toxic metal ions, osmolarity, and water content of the growth medium etc. are perceived as stress conditions by a variety of organisms. In fact, cells even respond to mechanical stress as sensed by the cell membranes/cell walls exerted internally by the turgor pressure or externally by increased atmospheric pressure. In cardiac muscle cells the differential activation of PKC (protein kinase C) pathways leads to activation of transcription factors causing differential gene expression in response to the mechanical stress perceived by the muscle cells. These differentially expressed genes have been associated with cardio myopathies and thus are of great significance (Hoshijima 2006). Even the simple bacterial cell needs to maintain the integrity of its cell envelope for survival, the trans-membrane signal-transducing protein factors monitor its perturbations and respond appropriately by modulating gene expression (Wecke *et al* 2006). While there is a large variety of stresses that living systems respond to, the most prevalent and common stress condition in nature is starvation, i.e. limiting of one or more nutrients.

Whereas, the response to heat shock as stress has been studied in great detail in a variety of systems and found to be highly conserved with respect to the heat shock proteins induced, the stresses like starvation and other environmental stressors, like competing microflora or invading pathogenic organisms, elicit complex and varied responses dependent on the organism in question. Interestingly, it has been noted in several organisms that when an organism responds to one stress, it often shows increased ability to cope with other stresses and indeed a particular stress is able to cause induction of genes required to function in response to an unrelated stress. This may be well justified from an evolutionary perspective, since in nature stresses are not encountered in isolation. Typically a cell which encounters

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Table 1. Broad categories of cellular stress

Type of stress	Parameters
Physical/chemical	Temperature, pH, presence of toxic metal ions, Osmolarity, Water level
Mechanical	Atmospheric or turgor pressure leading to mechanical stress on membranes
Starvation	Absence of one or more nutrients

one stress is likely to encounter another stress. Hence, the chance of survival might be higher if the given cell encountering a stress is prepared to cope with more than just that stress at the same time. Mechanistically, it might mean that the regulation of stress response may be linked and once the mechanism is activated by one stress, the cell might become predisposed to respond to another stress. This is indeed observed in nature and illustrated in several examples listed below.

Streptomyces coelicolor undergoing sporogenic differentiation in liquid culture also exhibits higher resistance to oxidative damage. In this case as well, a correlation between the starvation stress and the oxidative stress and their regulators has been proposed (Lee *et al 20*05). *HOG1* gene in *Saccharomyces cerevisiae* encodes a MAP kinase that controls the hyper-osmotic stress response. The homolog of *HOG1,* in *Trichoderma harzianum*, a widespread mycoparasitic fungus, when silenced, shows intermediate levels of resistance against oxidative stress (Delgado-Jarana *et al 20*06). It has been proposed that the increased susceptibility of proteins to oxidative damage may be due to some damage to the cellular components simultaneously leading to increased chaperone levels. In *Escherichia coli*, nutritional downshift in stationary phase is associated with greater tolerance to the oxidative damage (Dukan and Nystrom 1999). In mammalian kidney cells, heat shock and osmotic shock elicit overlapping response (Santos *et al* 1998). It is of course possible that some common signal(s) or intracellular phenomena are shared by osmotic and growth-limiting (associated with the stationary phase or starvation condition) and/or other stress conditions. However, an interesting alternative emerging from the recent studies is the presence of shared regulatory cascades which might result in overlapping responses by each of these apparently distinct stress conditions.

While instantaneous, or rapid response to a stress may appear to be best achieved at the level of activation of functional molecules like proteins, to have the protein molecules required for a stress specific function synthesized before stress condition is encountered, is not likely to be economical or efficient. So, the cells resort to mechanisms which would enable them to synthesize the rescue operators rapidly on sensing stress. Most often this is achieved by regulating the synthesis of the required proteins at the transcriptional level. We will discuss in this review, in brief, the different factors associated with the basal transcription machinery that might contribute to the regulation of gene expression at the transcriptional level in response to a wide variety of stresses. Conceptually, a greater control over wider spectrum of genes could be achieved if the regulator is close to the core of the transcription machinery and it would enable the system to rapidly and simultaneously respond to a number of stresses at the transcriptional level.

The process of transcription is highly regulated at the level of transcription initiation. The differential transcription initiation is brought about by altered promoter selectivity by the transcription machinery. In prokaryotes, the sigma subunit of the RNA polymerase dictates specificity towards the promoter. The gene specific transcription factors and other ancillary factors further fine tune the level and specificity of expression. Extensive studies have been carried out to elucidate the mechanism of how gene specific transcription factors function both in prokaryotes and in eukaryotes, but the role of the basal transcription machinery in transcriptional regulation in stress is well studied mainly in prokaryotes hence we first briefly discuss how the prokaryotic transcription machinery regulates transcription of genes involved in stress response and then seek parallels in the complex eukaryotic system.

2. Prokaryotic transcription and role of sigma factors in stress response

In *E. coli*, the core RNA polymerase consists of five different proteins, namely two of α subunits, one each of β, β' and ω subunits. RNA polymerase transcribes genes from a specific region of the gene called promoter. In prokaryotes, the sequences at -10 and -35 regions in the promoter are important for polymerase binding for transcription. At the initiation of transcription, the holoenzyme is formed by the association of core polymerase with σ factor which helps in the promoter selection. The different sub domains of the DNA binding domain of the sigma factor contact the different regions of the conserved promoter (figure 1). Structure determination has revealed that the σ protein in the holoenzyme is in a stretched conformation which enables it to interact with these parts of the promoter (Vassylyev *et al* 2002). After the promoter recognition, the sigma factor dissociates from the core polymerase and mRNA synthesis is carried out by the core polymerase. Transcription of a gene can be regulated by the modification of core polymerase subunits, interaction with the regulatory proteins or changes in the regulatory regions of the gene. In addition to the above known modifications, studies have shown that mechanism underlying the global regulation of genes is achieved by different sigma factors alternatively associating with the core RNA polymerase.

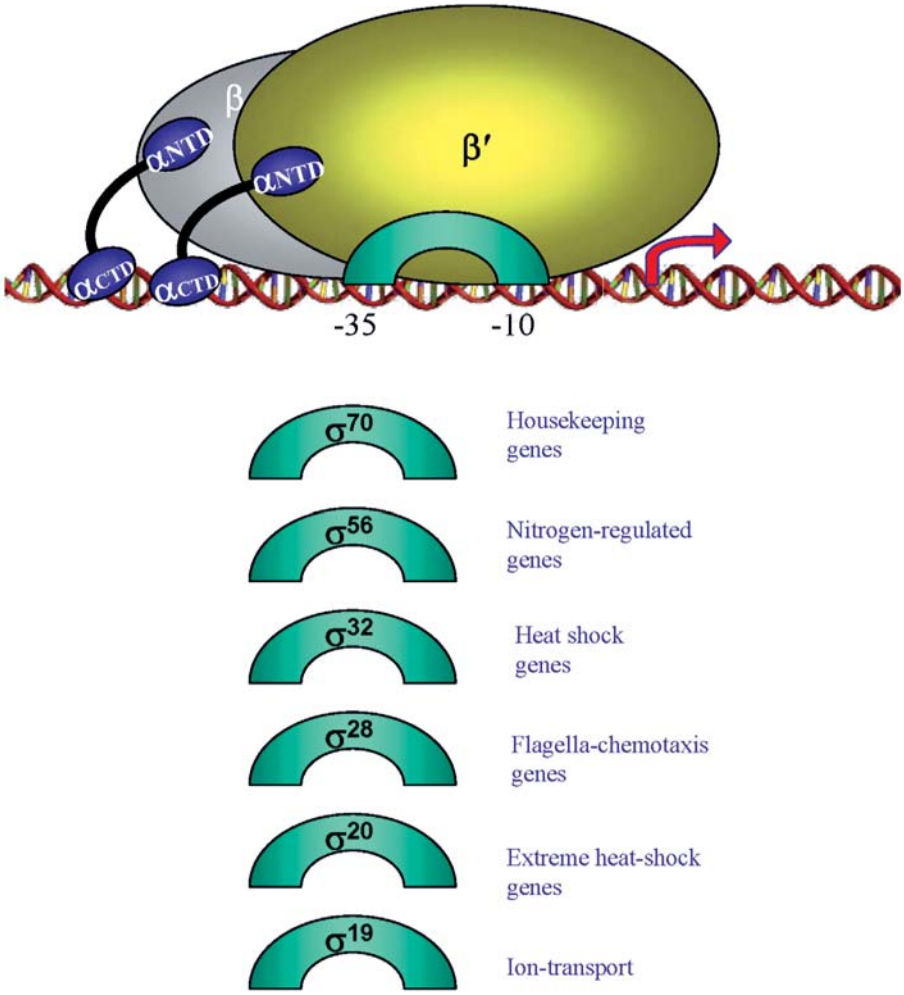

Figure 1. Prokaryotic core transcription machinery recognizes the promoter which has –10 and –35 elements. The extended conformation of the σ factor binds to these elements of the basal promoter. The N-terminal domain of each of the two α subunits, binds to the core polymerase while the C- terminal domain interacts with the upstream promoter elements. The five alternative sigma factors in *E. coli* are shown along with the genes they regulate.

To explain the role played by sigma factors in protecting the prokaryotic cell from any kind of unfavorable conditions, we shall take up the most extensively studied prokaryote *Escherichia coli* and a few other bacteria. There are at least six different sigma factors in *E. coli* identified so far which are known to associate with the core polymerase under different conditions to transcribe specific sets of genes (figure 1). The ratio of the major and alternate sigma factors determines which genes get transcribed at a given time. The major and alternative sigma factors have two distinct conserved domains which are required for binding to the core enzyme and to the basal promoter (Gruber and Gross 2003). In spite of the differences at the protein sequence level, these sigma factors show remarkable structural conservation between these domains. The differences in their abilities to compete with the housekeeping sigma factor and the promoter elements probably stem from the minor sequence variations in these domains. The choice of genes regulated by different sigma factors can be attributed to the differences in the sequences around the -10 and -35 regions and also to the number of bases separating these two sequence elements. In fact the promoter binding domain 4 of each of these σ factors interacts differentially with the extended -10 and -35 regions of the respective promoter and allows the strength of binding between the promoter and the σ factor to be altered. Promoter sequences at -10 and -35 regions recognized by these alternate sigma factors are not well known except for a few sigma factors. The σ^{70} recognizes most of the promoters which are transcribed during exponential phase of growth. A novel sigma designated as σ^{32} was isolated along with σ^{70} from heat stressed *E. coli* which was later found to regulate a subset of genes encoding proteins commonly called heat

shock proteins (Grossman *et al* 1984). These heat shock proteins have a wide variety of functions essential for survival of the organism during a variety of stress conditions (Ishihama 1990). Global transcriptional studies also show that 25% of σ^{32} regulon members are found to be present at the membrane after heat stress suggesting their involvement in protecting the cell membrane in response to the stress (Nonaka *et al* 2006).

Several stress responses are interlinked by alternative sigma factors which help RNA polymerase to choose promoters of stress genes. The σ^{32} is activated by cytoplasmic stress while σ^{E} is activated by extracytoplasmic stress. These stresses, resulting from accumulation of mis-folded or immature proteins in the cytoplasm or the cell envelope, will change the active pool of these sigma factors which are otherwise kept inactive by anti-sigma factors. The third alternative sigma factor, σ^{S} controls a set of genes required during stationary phase. There seems to be considerable amount of overlap in the induced genes during hyperosmotic, and low pH stress with σ^{S}-dependent genes (Bearson *et al* 1996; Muffler *et al* 1996). Besides performing their unique tasks in handling unique stresses, these sigma factors co-operate to respond to hyperosmotic stress. The ultimate result of the concerted effort leads to adaptation by which *E. coli* survives a variety of adverse growth conditions (Bianchi and Barney 1999).

The general stress response sigma factor enables the cells to respond to the growth-limiting stresses and also protects the organism from any further stress. σ^{S} of *E. coli* and σ^{B} of *Bacillus subtilis* and other gram positive species are among the well studied general stress response sigma factors. σ^{B} regulates over 200 genes in response to stresses like heat, oxidative conditions, acidic pH, salt etc (Wecke *et al* 2006). Its homologs in various species of bacteria regulate virulence, adherent biofilm formation in response to varied stress stimuli. The role of *B. subtilis* σ^{B} in cell envelope response is well understood. The integrity of cell wall or envelope of bacteria is crucial for survival of the organism as they encounter many unfavorable chemicals in normal habitat, for example, soil, intestines etc. Studies using *Bacillus licheniformis*, a close relative of *B. subtilis*, have revealed that even though there is conservation of sigma factors, presence or absence of additional trans-acting proteins makes sigma factor respond differently to the cell envelope stress. Unlike *B. subtilis*, σ^{B} dependent promoter activation is not seen even in the presence of functional *B. licheniformis* σ^{B} homolog under cell envelope stress (Wecke *et al* 2006).

Study of the functional homolog of *E. coli* σ^{S}, σ^{B} in *Streptomyces coelicolor*, has shown that σ^{B} induces a wide variety of defense proteins, sigma factors to overcome the osmotic shock as well as oxidative stress. It induces itself and also its two paralogs, which work in cascade to ensure proper and efficient sporulation of *S. coelicolor*. Thus σ^{B} plays an important role in maintaining proper differentiation of the organism and to counter different stress conditions (Lee *et al 20*05). In some of the gram positive pathogenic organisms like *Listeria monocytogenes,* and *S. aureus,* pathogenesis appears to be tightly associated with stress response and several virulence factor genes are controlled by the stress sigma factor σ^{B} (Schaik and Abee 2005). Thus, the mechanistic link in regulating responses to apparently unrelated stresses as well as conditions conducive for expression of virulence factors etc., in prokaryotes, lies in the shared regulators which are part of the basal transcription machinery.

3. Eukaryotic transcription

Unlike prokaryotes, the process of transcription in eukaryotes is much more complex in keeping with the fact that transcriptional machinery has to function with the larger genomes that are packaged into higher order chromatin structure. In addition, in most multi-cellular organisms, the temporal, spatial and tissue specific regulation of gene expression is crucial. All these factors contribute to the increase in complexity of the transcription machinery. Although eukaryotic transcriptional machinery consists of a larger number of protein complexes than that of prokaryotes, the general principles of transcription and its regulation are conserved. The task of eukaryotic transcription is shared by three different RNA polymerases I, II and III, which synthesize different classes of RNA. Among these the RNA polymerase II (Pol II), which catalyzes the transcription of all protein coding genes, has been studied in greater detail. In yeast as well as humans, the pol II is composed of 12 subunits, designated Rpb1 to Rpb12. Several of these subunits (Rpb5, Rpb6, Rpb8, Rpb10 and Rpb12) are shared with the other two polymerases. In addition, Rpb1, Rpb2, Rpb3/Rpb11 and Rpb6 are homologous to bacterial core RNA polymerase subunits β', β, α and ω respectively (Hampsey 1998). Rpb9 is important in active site selection (Hampsey 1998) and recently it has also shown to play a role in transcription coupled repair. Rpb4 and Rpb7 form a separate sub-complex of the pol II in *S. cerevisiae* that has been shown to have a variety of roles (*see* below).

A large number of transcription factors and several protein complexes assist the polymerase in its function. Six general transcription factors, TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH are important for accurate initiation of transcription by RNA polymerase (reviewed in detail by Thomas and Chang 2006). TFIID is one of the primary factors which recruits on promoter and helps in further assembly of other general transcription factors and pol II to form pre-initiation complex (PIC). This multi-subunit complex recognizes several elements of eukaryotic promoter (figure 2): the

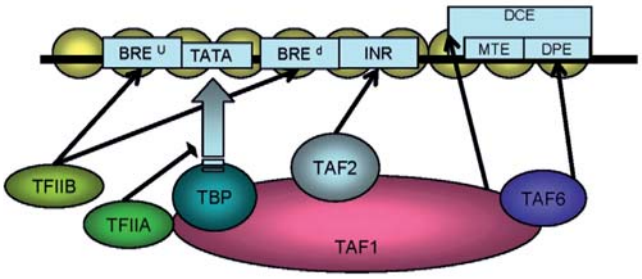

Figure 2. The eukaryotic promoter is much more complex with several different elements, each of which interacts with a different component of the core transcription machinery. The components of the TFIID complex, the TBP and the TBP associated factors (TAFs) interact with the TATA element and with various other elements in the basal promoter respectively. TFIIB interacts with the B responsive elements flanking the TATA box while TFIIA affects the TBP interaction with the TATA element (indicated by a blunt arrow). This suggests that unlike the single sigma factor in the prokaryotic polymerase, several protein factors in the basal transcription machinery interact with the basal promoter in the eukaryotes. Only the factors known to interact with the promoter elements or known to affect directly the interactions of other factors are depicted.

TATA box, an A/T rich sequence present approximately 25 to 30 nucleotides upstream of transcription start site, is recognized by TATA binding protein (TBP); initiator (INR), a pyrimidine rich sequence, is recognized by TAF1/TAF2 subunits of TFIID, and promoter element called downstream promoter element (DPE), is recognized by TAF6 and TAF9 subunits of human TFIID (Shao *et al* 2005). Another transcription factor which makes contact with the promoter is TFIIB which recognizes two TFIIB-recognition elements (BRE) present upstream (BRE^u) and downstream (BRE^d) of TATA box (figure 2). In addition to these elements, another consensus sequence is present between +18 to +29, called MTE (Motif Ten Element) which is shown to enhance PolII mediated transcription in conjunction with INR (Lim *et al* 2004) but its cognate protein factor is not known.

Besides these general transcription factors, several cofactors are required for transcription initiation. These cofactors can be divided into two classes. The first class includes factors or enzymes required for modification of chromatin. The second class contains factors which are important for interaction with RNA pol II and general transcription factors. The proteins belonging to this class are collectively called mediator complex because they serve as a bridge between activator and the basal transcriptional machinery (Chadick and Asturias 2005). Mediator complex is composed of ~ 21 subunits which can be divided in four sub-modules. The head module, composed of Med6, Med8, Med11, Med17 (Srb4), Med18 (Srb5), Med19 (Rox3), Med20 (Srb2), and Med22 (Srb6), is thought to have a general role in transcription and interacts with the CTD of RNA pol II (Lee and Kim 1998). The second module, the middle domain, which is composed of Med1, Med4, Med5 (Nut1), Med7, Med9, Med10, Med14 (Rgr1), and Med21 (Srb7), interacts with the CTD of RNA pol II similar to the head module. The subunits of the tail module, composed of Med2, Med3 (pgd1), Med15 (Gal11), and Med16 (Sin4), have been identified by genetic methods. This module is presumably responsible for recognizing and binding to activators (Bhoite *et al* 2001). A fourth distinct sub-complex (also called Cdk8 sub-complex) that includes mediator subunits Cdk8 (Srb10), CycC (Srb11), Med12 (Srb8), and Med13 (Srb9) has been implicated in negative regulation of transcription (Hampsey 1998). A kinase defective mutant of Cdk8 showed, on genome wide transcription profiling, up-regulation of significant subset of genes (Holstege *et al* 1998). Recent studies have shown that the Ras/PKA pathway can modulate mediator activity (Chang *et al* 2004) suggesting that mediator can have direct signaling-processor role.

3.1 *Does the eukaryotic transcription machinery have sigma analogs involved in stress response?*

From the above discussion it is clear that there is no single protein in eukaryotes that functions in identical manner as the σ subunit of the prokaryotic polymerase, in that a protein enables the polymerase to interact directly with the specific sequence on the DNA. Considering the complexity of the transcription machinery in the eukaryotes one could envision that the functionality of the σ^{70} factor is taken over by a complex of proteins e.g. TFIID, a complex made up of TBP and its associated factors (TAFs), which allows the association of the polymerase with the promoters of most of the housekeeping genes. The gene specific transcription factors that regulate activities of specific genes or subsets of the TFIID controlled genes can function over and above the TFIID. This is similar to several gene specific transcription regulators that function in prokaryotes in conjunction with the sigma factors in modulating specific gene transcription. What follows the above argument is that if we look for factors which can function similar to σ^S subunit during stress response, three factors in eukaryotic transcription machinery, discussed below, come to the fore.

3.1.1 *SAGA complex:* The **SAGA** complex, **S**PT3-**A**DA2-**G**CN4-histone Acetyltransferase is known to contain the TBP and some of the TBP associated factors along with other components that enable the complex to take part in histone acetylation required for chromatin remodeling. This 1.8MDa multi-subunit complex comprises of many distinct classes of proteins: (i) the Ada proteins (Ada1,

Ada2, Ada3, Gcn5 and Ada5); (ii) Spt proteins (Spt3, Spt7, Spt8 and Spt20); (iii) a subset of TBP-associated factors (TAFs: Taf5, Taf6, Taf9, Taf10 and Taf12; and (iv) Tra1, an ataxia telangiectasia mutated (ATM)-related protein, which plays an important role in activator recruitment (Brown *et al* 2001). Several components of the TFIID complex are also part of this complex. Gcn5 is the catalytic histone acetyltransferase (HAT) subunit of the SAGA complex but this activity is not always required for all gene activation events regulated by this complex. SAGA complex is extensively studied in *S. cerevisiae* and most of the knowledge about its structure, function and regulation has come from this model system. The primary function of this complex is to help in the delivery of TBP onto promoters. Moreover, SAGA also acts as an adapter in making contacts with other complexes of transcription machinery (Larschan and Winston 2001; Barbaric *et al* 2003) to form proper PIC similar to σ factor in bacteria. Higher organisms also have complexes similar to SAGA complex, *e.g.*, TFTC (TBP free TAF- containing complex), PCAF (p300 and CBP associated factor) and STAGA are human homologs of SAGA complex (Brand *et al* 1999; Ogryzko *et al* 1998; Martinez *et al* 1998; Martinez *et al* 2001). However, detailed information about their role in these organisms is lacking. Studies done in *S. cerevisiae* have shown that SAGA complex is mainly associated with promoters which contain TATA box consensus sequence (A/T)A(A/T)(A/G) (Basehoar *et al* 2004). Furthermore it has been shown that the SAGA complex regulates approximately 10% of the genes in the yeast genome (Huisinga and Pugh; 2004). These genes are highly induced by a variety of environmental stresses such as heat, starvation etc. In another study, this group, using chromatin immuno-precipitation (ChIP) assay conducted on genome-wide scale (ChIP on chip), has shown that the SAGA complex assembly occurs on the promoters which are activated during heat stress in response to heat shock (Zanton and Pugh; 2004). Their study also suggests that SAGA assembly always correlates with gene activation. A SAGA

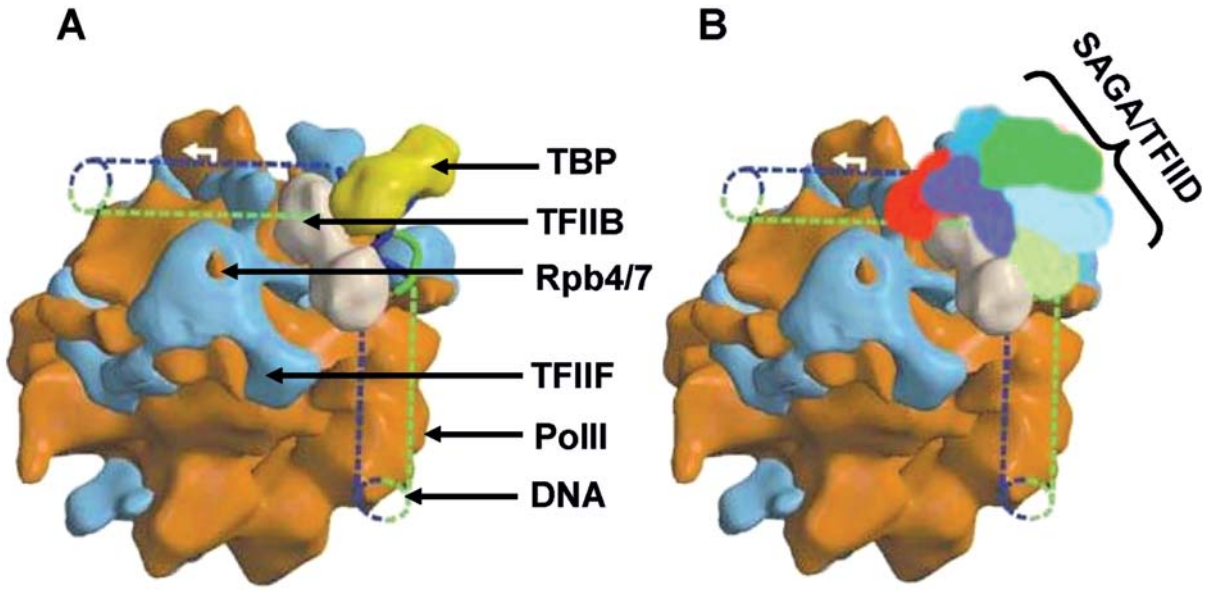

Figure 3. (**A)** Model of the structure of a minimal transcription complex. RNAPII–IIF–IIB–TBP and DNA constitute the minimal complex that is capable of promoter-directed initiation, and therefore the catalytic core of the eukaryotic transcription machinery. The trajectory of DNA along the RNAPII surface (predicted using the distribution of IIF density in the RNAPII–IIF complex), along with the TATA box to transcription start site spacing of a typical eukaryotic promoter, the expected location of IIB on the surface of RNAPII and the X-ray structure of the TBP–IIB–DNA complex lead to a model for the organization of the catalytic core of the eukaryotic transcription machinery. In the deduced structure of the complex TFIIF interacts with the polymerase in an extended conformation and almost completely eclipses the 4/7 sub-complex. The DNA being transcribed is bent at almost right angle with interaction of the TBP. The bent white arrow indicates the position of the transcription start site. The X-ray structure of the TBP–IIB–DNA complex was filtered to 15Å resolution for inclusion in the model (reprinted with permission from Asturias 2004). (**B)** With TBP at the core, the SAGA/TFIID complex is formed by several interacting proteins that are either common or unique to the respective complex. The coloured patches overlapping the TBP in the structure in **A,** represent the TBP associated factors making up the TFIID/SAGA. As described in the text, SAGA complex might replace the TFIID on the TATA containing promoters specifically upstream of the stress regulated genes. The TFIIF and the Rpb4/7 sub-complex of the polII are known to affect stress response and may affect interactions of the polymerase with downstream stress specific components. For more details, see text.

complex component Spt3 has also shown to be important for induction of adequate response during nitrogen starvation (Laprade *et al* 2002). Though SAGA complex of eukaryotic transcriptional machinery does not have any protein factor which shows sequence similarities with σ factor, several functions like ability to make contact with promoter DNA and with different components of transcriptional machinery for efficient and rapid induction of genes required during stress response, make SAGA complex a potential functional analogue of σ^s.

3.1.2 *TFIIF:* This is a general transcription factor, initially identified by its association with RNA polymerase II and its requirement in transcription initiation. TFIIF is a hetero-tetramer complex composed of 2 large (TFIIFα/RAP74 in human and Tfg1 in *S. cerevisiae*) and two small (TFIIFβ/RAP30 in human and Tfg2 in *S. cerevisiae*) subunits (Flores *et al* 1990). Besides these two conserved subunits, *S. cerevisiae* also contains one smaller nonessential subunit, designated as Tfg3 (Henry *et al* 1992, 1994). Not only is Tfg3 present in TFIIF, it is also a part of other complexes like TFIID (thus also designated as Taf14) and SWI/SNF chromatin remodeling complexes (Cairns *et al* 1996). It has been reported that TFIIF complex is able to interact with TFIIA (Langelier *et al* 2001) and TFIIB (Ha *et al* 1993; Kimura and Ishihama 2004). Furthermore, genetic studies show that TFIIF functionally interacts with TFIIS, a general transcription factor required for efficient elongation by RNA pol II (Fish *et al* 2006).

There are several reasons which justify why TFIIF should be regarded functionally analogous to the σ subunit of bacterial RNA polymerase. Firstly, both RAP74 and RAP30 show limited sequence homology with σ^{70} (Garrett *et al* 1992; McCracken and Greenblatt 1991; Sopta *et al* 1989; Hamsey 1998). Secondly, Human TFIIF complex can bind to *E. coli* RNA polymerase and can be displaced by σ^{70} (Hamsey 1998; McCracken and Greenblatt 1991). Thirdly, the distribution of Tfg2 in RNA pol II and TFIIF complex resembles the σ factor distribution in bacterial holoenzyme as shown in figure3A (Chung *et al* 2003). Furthermore, TFIIF is involved in recruitment of polymerase to form the PIC and for its stability, as shown in case of the σ factor. All these features of TFIIF make it a potential σ factor analog in eukaryotes. Recent studies using Tfg3 subunit of TFIIF in *S. pombe* have shown that *tfg3*$^-$ mutation is associated with stress sensitive phenotypes like temperature sensitivity, reduced cell growth during osmotic and heavy metal stress. Enhanced interaction of Tfg3 with isolated TFIID during elevated temperature further supports role of this subunit of TFIIF under stress (Kimura and Ishihama 2004).

3.1.3 *RPB4:* Rpb4 is the fourth largest subunit of RNA polymerase II and is one of the two non essential subunits in *S. cerevisiae* (Woychik and Young 1989) but Rpb4 homolog of *S. pombe* has been found to be essential for cell viability and is more similar in structure and function to those of higher eukaryotes than that of *S. cerevisiae* (Sakurai *et al* 1999). Several reports suggest that the absence of RPB4 leads to slow growth, temperature sensitivity and poor efficiency of survival during stationary phase (Woychik and Young 1989; Choder and Young 1993; Rosenheck and Choder 1998; Maillet *et al* 1999). It was later observed that cell wall integrity defects are also associated with *rpb4Δ* strain (Bourbonnais *et al* 2001). Furthermore, yeast cell lacking Rpb4 is defective in exhibition of two starvation specific phenotypes, e. g. sporulation and predisposition to forming pseudohyphal cells (Pillai *et al* 2003; Sampath *et al* 2003). Most of these phenotypic defects were involved in inability of the mutant cells to cope with the variety of stresses tested. Earlier work from our laboratory (Sharma and Sadhale 1999) had shown that the pseudohyphal phenotype of *rpb4Δ* cells was exaggerated when the levels of Rpb7, the interacting partner of Rpb4, were increased. Interestingly, homologs of the RPB7 gene from other eukaryotes showed different extent of pseudohyphal exaggeration indicating that the minor differences in the protein sequence might contribute to this phenotypic difference through protein-protein interactions (Khazak *et al* 1995; Singh *et al* 2004). Whole genome expression analysis done by our group to characterize this mutant showed that during permissive condition, this mutant affects only small subset of the genome, but in non permissive conditions like temperature stress, this mutant can affect differential expression of a large number of genes (Pillai *et al* 2003). In conclusion, these results suggest that Rpb4 of RNA polymerase II also acts during stress conditions similar to σ^s subunit of bacterial polymerase. Recent crystal structure of yeast RNA polymerase containing 12 subunits has suggested that the Rpb4/Rpb7 sub-complex is present near the clamp region of the polymerase. In addition to this, this sub-complex is also closely associated with TFIIF (figure 3A). Though Rpb4 also does not share any sequence similarity with σ subunit of bacterial polymerase, its several features discussed below, make it a worthy candidate to be called a functional analog of bacterial σ subunit. Firstly, Rpb4 and its partner Rpb7, present in the polymerase, result in closed clamp conformation of RNA polymerase as seen in crystal structure of bacterial RNA polymerase holoenzyme containing core polymerase with σ factor (Bushnell and Kornberg 2003; Armache *et al* 2003). Secondly, this subunit of polymerase has been speculated to play an important role in initiation by functioning as scaffold for further assembly of the components of PIC as shown in case of σ factor. The Rpb4 protein also interacts with the CTD phosphatase Fcp1 (Kimura *et al* 2002) and is reported to be defective in transcriptional activation (Pillai *et al* 2001). Since CTD

modification has direct bearing on the ability of polymerase to respond to transcription activation, Rpb4 in effect has an obvious role in modulating transcriptional activation crucial for stress responsive gene expression (Sampath and Sadhale 2005). Thus, for the first time a core subunit of the RNA polII has been shown to distinctly affect two specific stress responses like the stress sigma subunits of the prokaryotic cells.

4. Concluding remarks

It is observed that in nature living organisms encounter several stresses together. The mechanisms evolved in defense of these stresses indeed appear to be linked such that the organisms presented with one stress condition also show alacrity in responding to other apparently unrelated stresses. Mechanistically this has been achieved in prokaryotic systems by having promoter elements of several stress response genes being contacted by single stress sigma factor that determines the promoter selectivity of the transcription machinery. In eukaryotes since the responses to the variety of stresses are much more complex and varied the stress response regulatory system also has correspondingly increased in complexity. Strictly speaking sigma factor homologue does not exist in eukaryotes in that there is no single protein that allows the transcription machinery to be recruited at the promoter in eukaryotes. The components of the transcription machinery instead that are sufficient to recruit the polymerase at the promoter through DNA binding can be visualized as functionally analogous to sigma factors. The single protein sigma factor in the basal transcription machinery has been replaced by the multi-component factors and is functionally best represented by the TFIID/TFIIB among the GTFs on the housekeeping genes while SAGA complex (in place of the TFIID), the TFIIF general transcription factor as well as the Rpb4-7 sub-complex of core subunit of the polII might play a significant role in the transcription of the stress regulated genes. All of these factors are conserved in evolution to a great extent and interestingly, they also are physically located in the transcription machinery in such a way as to be able to interact with each other (Figure 3B). That the stress response regulatory machinery should be close to the core of the transcription machinery to allow concerted co-regulation of genes involved in response to diverse stresses, is the theme that appears to be conserved in evolution.

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