Modulation of polymerase II composition: A possible mode of transcriptional regulation of stress response in eukaryotes

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Regulation of stress response in prokaryotes is mainly achieved at the transcriptional initiation level. Prokaryotes use alternative holoenzymes, consisting of the core polymerase associated with different sigma factors, which confer on it altered specificity of transcriptional initiation. Stress response being probably one of the most inevitable features of life, it would be interesting to find if eukaryotes also use a similar strategy at this level of regulation. Since the yeast *Saccharomyces cerevisiae* is a model system for studying many different phenomena in eukaryotes we review the transcriptional regulation of stress in this system. Based on published observations in the literature and our own studies, we have analysed the regulation of stress response in the yeast *S. cerevisiae*. Two of the core subunits of the yeast RNA polymerase II, which show altered stoichiometry within the polymerase under different conditions appear to be involved specifically in regulating the stress response. In a very broad sense then, the altered subunit composition of the core polymerase or a different holoenzyme, appears to correlate with gene expression specific to stress response in *S. cerevisiae* and probably reflects the scenario in other eukaryotes.

1. The universality of stress

Stress response is a universal phenomenon and from the simplest of prokaryotes to the most complex eukaryotes, every living system shows a response to stress conditions that is controlled by its genetic makeup. The stress conditions include all unfavourable conditions under which the organism has to adjust its growth rate according to external stimuli. In spite of the variety in which the different stress conditions appear, there is a surprisingly high number of specific proteins that show increase in level of expression irrespective of the stress condition. These are collectively called the heat shock proteins due to the fact that this class of proteins was first identified in response to heat shock in *Drosophila* (Tissieres 1974; Ashburner and Bonner 1979). The functional heterogeneity among the heat shock proteins is comparable to the stress conditions eliciting them. Most of them are indeed proteins with functions involved in coping with stress, while the rest are regulators of the response at various steps.

Among the regulators of stress response in prokaryotes, a majority are transcriptional regulators. The prokaryotes have been studied in great detail with respect to the mechanisms of transcriptional regulation and in most systems studied, this appears to be achieved by modulation of the holoenzyme, the RNA polymerase itself. In *Escherichia coli* and *Bacillus subtilis*, as is evident from the other specialized reviews in this issue (Nakahigashi *et al* 1998; Schumann *et al* 1998), there are stress specific sigma factors that replace the house keeping sigma factor in the holoenzyme. This altered holoenzyme can specifically transcribe genes required for coping with the stress being encountered.

2. The yeast paradigm

Although stress proteins are highly conserved among living systems, the mechanisms of regulation of the corresponding gene expression appear quite different. In the following paragraphs, we would like to recapitulate

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the current status of understanding of regulation of stress response in one of the most versatile eukaryotic model systems, namely *Saccharomyces cerevisiae.*

Among the eukaryotic systems, an ideal system to monitor stress response would be the one which allows ease of genetic and molecular manipulation at the whole organism level and which exhibits enough phenotypic variation so as to allow its study under various stresses. The fungi in general show varied responses to nutritional starvation stress. Under nutritional starvation, the yeast *S. cerevisiae* changes to one of two cell-types, the pseudohyphal cells or spores (figure 1). *S. cerevisiae* exists in nature predominantly in the diploid form, which can exhibit both these cell types.

The pseudohyphal form is seen as chains of elongated cells appearing like hyphae that are able to spread even through solid substrate giving the parent yeast cell the ability to move its progeny away from the focus of stress (Gimeno and Fink 1994). The pseudohyphal morphogenesis has actually been studied in various different fungi collectively called the dimorphic fungi exhibiting two major cellular forms viz., hyphal or yeast. Pseudohyphal form is seen mostly in response to starvation but many other stresses such as pH or temperature change also elicit it in certain fungi (Gow 1995).

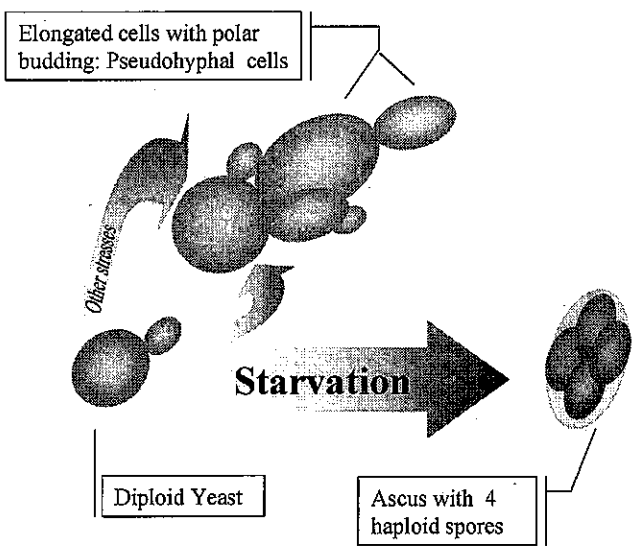

Figure 1. *S. cerevisiae* exhibits two different cell types in response to nutritional deprivation. The diploid yeast on severe carbon or nitrogen starvation undergoes meiosis and gives rise to four haploid spores encapsulated in an ascus. Under relatively milder nutritional limitation the cells show a unipolar budding pattern and elongated cell morphology. Elongated cells remaining attached to each other form what is known as pseudohyphae. The thickness of the arrows roughly indicates the severity of the stress encountered. The thin arrow labelled 'other stresses' refers to various stress conditions, which have been shown to induce pseudohyphal morphology in other dimorphic fungi and may do the same in *S. cerevisiae.*

Sporulation, on the other hand, results under extreme starvation conditions in certain fungi that can undergo meiosis and give rise to haploid spores. These are significantly more heat and starvation resistant than the vegetative diploid form and thus allow the yeast form of cells to tide-over the unfavourable situation. Sporulation is used in many other fungi as a means of coping with unfavourable conditions in nature. This response is not shown by all dimorphic fungi, which can alternate between the yeast and the hyphal form. In fact, the human pathogen *Candida albicans* is permanently 'frozen' in the diploid form (Shepherd *et al* 1985). *C. albicans* is a dimorphic fungus very well studied with respect to the regulation of its dimorphic switch. Many factors affecting the switch have been identified and many different biochemical markers expressed by the cell in response to stress during the dimorphic switch have also been analysed (Kerridge 1993). One of the recently discovered genes, *ACPR1*, which is the homologue of *STE12* transcription factor gene in *S. cerevisiae*, affects the pseudohyphal morphogenesis in both *S. cerevisiae* and *C. albicans*. Interestingly, the factor also controls the mating type gene expression in *S. cerevisiae*, which is completely lacking in *C. albicans*. Not only does *C. albicans* not undergo meiosis, and alternation in ploidy, but it also does not sporulate like *S. cerevisiae* and many other fungi (Liu *et al* 1994; Malathi *et al* 1994).

3. Regulation of stress response in *S. cerevisiae*

S. cerevisiae has been a model eukaryotic system for the studies on regulatory mechanisms of transcription and signal transduction. Since signal transduction is one of the important aspects of stress response, yeast becomes an ideal system to study the mechanisms of transcriptional regulation in response to stress. *S. cerevisiae* also turns out to be an excellent system for studying the finer regulatory mechanisms as it exhibits multiple phenotypes under stress and is amenable to genetic and molecular biological manipulations. Many of the heat shock proteins, conserved in evolution, have been cloned, sequenced and functionally characterized from *S. cerevisiae* (Mager and Ferreira 1993). The heat shock factor Hsf1p has been found to be the master regulator of the heat shock response in yeast (Sorger and Pelham 1988). It is essential for survival and unlike higher eukaryotic systems, it is the only known regulator of general heat shock response (Nover and Scharf 1997). Although it recognizes the heat shock element present in promoters of eukaryotic stress response genes, it must also control expression of some housekeeping genes since it is essential for survival under normal conditions of growth as well. The Hsf1p has been shown to act as a transcriptional activator of the heat shock response genes but the mode of interaction

between Hsf1p and the rest of the transcription machinery is not yet resolved.

4. Transcription machinery

The eukaryotic, in particular the yeast transcription machinery, has been analysed in great detail in the last few years (Young 1991; Struhl 1995). Compared to the prokaryotic systems the eukaryotic machinery appears to be extremely complex. The holoenzyme in prokaryotes, for instance, is comprised only of five subunits while in eukaryotes the holoenzyme is a very large complex, comprised of more than ten times as many proteins. It is this holoenzyme that is required for activator responsive transcription (Koleske and Young 1995). The requirement for the stress response specific transcriptional activator in addition to the holoenzyme has been shown but a subpopulation of holoenzyme involved in stress specific transcription has not been shown.

In spite of the increased complexity of the transcription machinery, which has introduced many more steps possible for fine-tuning of regulation, the major part of regulation of stress response is still at the transcriptional initiation level. At the initiation step several general transcription factors (GTF) are associated with the core polymerase which itself is made up of 12 subunits that get co-immunoprecipitated by antibodies directed to the largest subunit. Most GTFs assist the polymerase in specific transcription initiation from a basal promoter and then dissociate from the elongating polymerase. In addition to the GTFs, specific transcriptional regulators are involved in regulating transcription initiation levels. In the last few years, newer recruits to the eukaryotic holoenzyme have been the proteins that affect activated transcription probably by mediating interactions of the transcriptional activators and the core polymerase with its GTFs. These new recruits have been aptly termed the mediators or coactivators (Guarente 1995).

Many of these mediators were originally discovered as genes in which mutations gave rise to defects in transcription of only a small number of genes, e.g., the Gal11p was originally discovered based on its effect on transcription of genes involved in galactose metabolism (Nogi and Fukasawa 1981). Gal11p has now been shown to actually mediate transcriptional activation of many unrelated genes. The fact that these coactivators, originally identified as factors controlling transcription of only a certain group of genes, actually affect transcription of many unrelated genes has been a common observation with most of the coactivators found to date. The coactivators were earlier reported not to be associated with the core subunits of the polymerase but only recently have been shown by modified purification protocols to be interacting with the basal transcription machinery and to be components of the holoenzyme. In light of this, the two core pol II subunits, Rpb4p and Rpb7p, deserve a fresh look. These two subunits are known to dissociate relatively easily from the rest of the polymerase and affect transcription of the stress response specific genes (Choder and Young 1993). It may be that these subunits have been incorrectly defined as core subunits.

5. Are *Rpb4p* and *Rpb7p* subunits of core polymerase involved in differential gene expression?

The Rpb4p and Rpb7p subunits in yeast associate with pol II in a stoichiometry of less than one (Woychik and Young 1990). This suggests that either (i) only a fraction of all pol II molecules involved in transcription are associated with them or (ii) the subunits are only involved in the initiation step and not during elongation. In fact, earlier studies have shown that pol II lacking the two subunits is unable to function in an *in vitro* transcription initiation assay. This suggests that these subunits may be specifically involved in transcription initiation. In the same study it was also shown that addition of excess of transcriptional activator could compensate for the absence of these subunits further supporting their role in initiation (Edwards *et al* 1991). These observations also agree well with the proposal that there may be subpopulations of the holoenzymes that differ in composition and in the initiation specificity. These subunits may then be associated with only a specific fraction of the holoenzyme complexes (Goodrich *et al* 1996).

Although the two subunits appear to form a subcomplex within the polymerase, Rpb7p is essential for survival but Rpb4p is not (McKune *et al* 1993). In fact, the absence of Rpb4p has been shown to lead to defects in various stress responses from stationary phase survival to growth at high and low temperatures in haploids (Choder 1993; Woychik and Young 1989). This suggests that the expression of stress response specific genes may be regulated by holoenzymes that are specifically associated with these subunits. Interestingly, earlier we found that the gene for the essential subunit, *RPB7* is highly conserved in evolution from archaebacteria to humans and either the yeast or the human homologue of *RPB7* when overexpressed causes alteration in morphology of yeast diploids in response to nitrogen starvation (Khazak *et al* 1995). Homozygous *RPB4* deletion diploids were found to be completely defective in sporulation (unpublished results). These observations would suggest that the Rpb4p of the subcomplex is especially required for appropriate stress response. In fact, since overexpression of Rpb7p exaggerates the stress response specific phenotypes, one could postulate that specific ratio of these subunits may be critical for proper stress response specific transcription. This may fit in well with the earlier observations that stoichiometry of Rpb4p subunit with respect to the polymerase goes up from 0·5 to 1 during

the transition from log phase to stationary phase, the most stressful phase of growth. It was earlier suggested that these two subunits affect the polymerase stability under stress (Choder and Young 1993). Our own recent results have suggested that the presence of the two subunits in a specific ratio allows correct interactions with the transcriptional activators resulting in desired transcription level of the corresponding genes (manuscript in preparation). The Rpb4p subunit alone probably has a two-fold function; (i) to stabilize the interaction of the Rpb7p subunit with the rest of the polymerase and (ii) to mediate either directly or indirectly the interactions of certain transcriptional activators with the holoenzyme. We have recently seen in our laboratory, that over-expression of the Rpb7p to various levels rescues the defects associated with *RPB4* deletion and dominant mutants of *RPB7* can be generated, which will rescue these phenotypes even when expressed from low copy number plasmids. The rescue of phenotypes associated with complete absence of the Rpb4p subunit suggests that the Rpb7p mutant protein interacts better with the components of the transcription machinery eliminating the need for Rpb4p (manuscript in preparation).

In accordance with the above idea we expected the transcriptional pattern to be different in the different *RPB7* or *RPB4* mutants and when the ratio of these subunits is altered. We have evidence now to show that the transcriptional pattern is indeed different in each of these cases (manuscript in preparation). In fact, as expected from the inability of the homozygous *rpb4Δ/rpb4Δ* diploid to sporulate, there is a dramatic reduction in the transcript levels as compared to the wild type

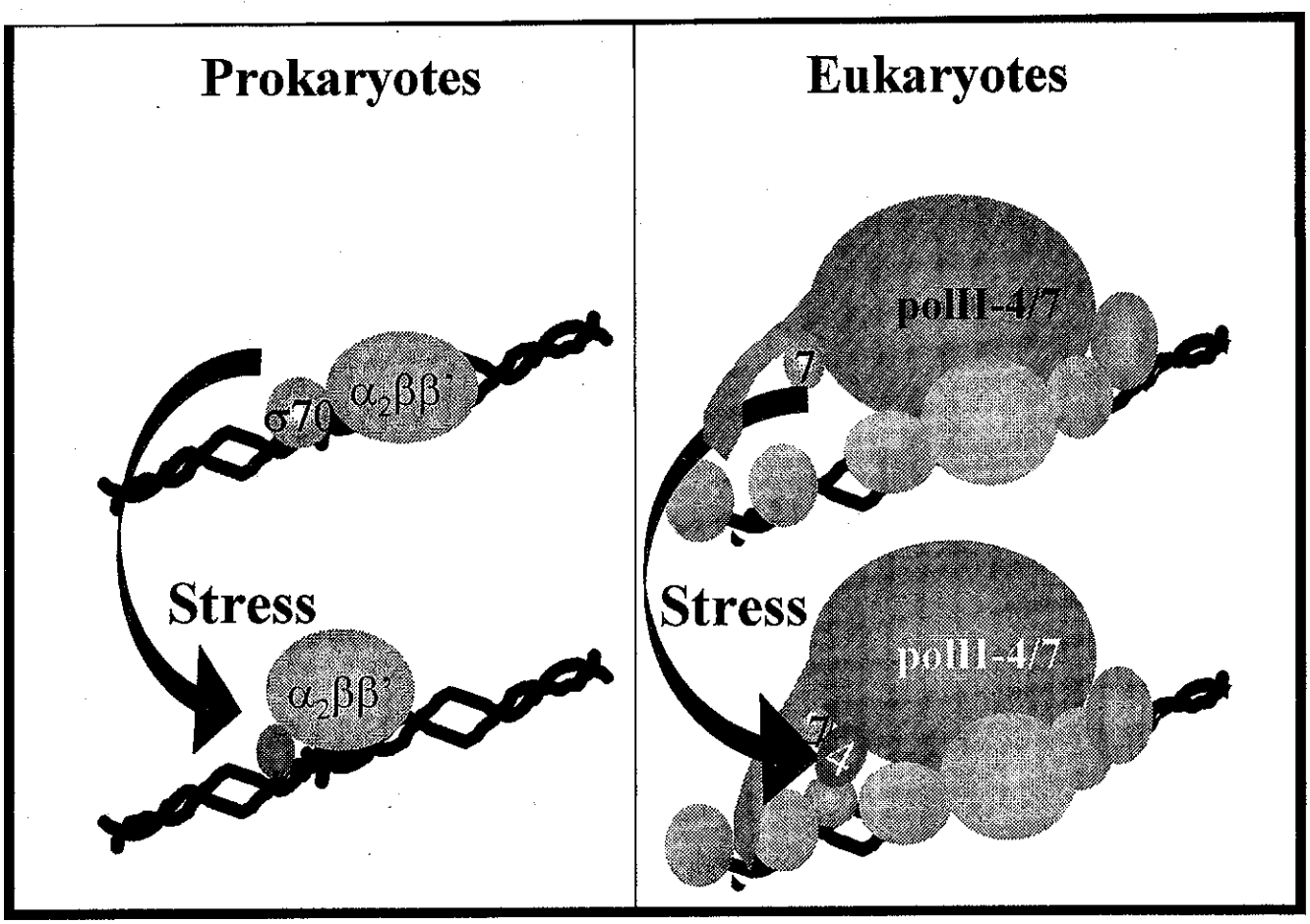

Figure 2. The transcription machinery is altered in composition during stress. The left panel shows the prokaryotic scenario. The holoenzyme is known to have altered composition in response to stress; the σ^{70} is replaced with the stress specific sigma factor shown as a red oval. The right panel shows a schematic representation of a hypothetical model based on our own recent observations with the yeast system and published information in literature. We propose that even in eukaryotes a holoenzyme with different core subunit composition and initiation specificity is employed to achieve stress specific gene expression. Although the polymerase devoid of Rpb4p/Rpb7p subunits (pol II-4/7) is shown with an extension like the CTD (the C Terminal Domain of the largest subunit of pol II from all eukaryotes), actual contact between the subunits and the CTD is not implied.

sporulating diploid. These observations support the hypothesis about the role of Rpb4p in transcription proposed above.

6. Future scope

The ideas presented here suggest that subpopulations of the yeast polymerase exist that differ even with respect to the core subunit composition (figure 2). Considering some of the differences seen between the holoenzymes of yeast and higher eukaryotes, it remains to be seen whether a similar phenomenon is seen in other eukaryotes as well and if the stress response genes are under the regulation of a specific holoenzyme. As in the case of Hsf1p, the Rpb4p/Rpb7p subcomplex appears to be required not only for up-regulation of stress response genes, but also for normal growth. The possibility that the effect on stress response specific gene activation is a result of a general defect in activated transcription has also not been ruled out.

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