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Minireview

Yeast activator proteins and stress response: an overview ☆

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Abstract Yeast, and especially *Saccharomyces cerevisiae*, are continuously exposed to rapid and drastic changes in their external milieu. Therefore, cells must maintain their homeostasis, which is achieved through a highly coordinated gene expression involving a plethora of transcription factors, each of them performing specific functions. Here, we discuss recent advances in our understanding of the function of the yeast activator protein family of eight basic-leucine zipper *trans*-activators that have been implicated in various forms of stress response.

Keywords: Stress; *YAP1*; *YAP2*; *YAP4*; *YAP8*; Remaining Yaps; *Saccharomyces cerevisiae*

1. Introduction

The cellular stress response is evolutionarily conserved in all living organisms, a major role being attributed to the induced heat-shock proteins and other molecules that confer stress protection. The molecular responses elicited by the cells dictate whether the organism adapts, survives, or, if injured beyond repair, undergoes death. The regulation of stress response includes transcriptional, post-transcriptional and post-translational mechanisms, the former being the most extensively studied.

Transcriptional regulation is mediated by a pre-existing transcriptional activator, the heat-shock factor (HSF), which binds to arrays of a 5-bp heat-shock element (HSE; nGAAn) present upstream of all heat-shock genes. Consequently, heat-shock proteins are induced, the majority acting as molecular chaperones in protein refolding and protecting them from degradation and aggregation. The HSF is a modular protein consisting of a helix–turn–helix class DNA-binding domain (DBD), a leucine zipper domain, required for trimerization, and a carboxy-terminal transcription activation domain [1]. Both the HSF of *Saccharomyces cerevisiae* and that of the closely related yeast *Kluveromyces lactis* contain an unique transcription activation domain amino terminal to the DBD [2,3]. Although the heat-shock response is a well-conserved mechanism throughout evolution, metazoans possess three HSFs, each playing a role in heat shock and development [4]. Notwithstanding, a great number of findings have demonstrated that gene expression under stress conditions in *S. cerevisiae* also elicits HSF-independent mechanisms, of which the Msn2p and Msn4p-mediated general stress response has been the most extensively studied [5,6]. These two partially redundant zinc-finger transcription factors govern the majority of genes involved in a plethora of stress responses. Msn2p and Msn4p bind to the stress response element (STRE), a 5-bp sequence, C4T [5]. In addition, the two basic-leucine zipper (b-ZIP) transcription factors, Yap1p and Yap2p [7,8], along with six newly identified proteins, form a family of *trans*-regulators that have been implicated in various forms of stress response [9–12]. The large amount of data from the many laboratories that work within this field of research is reviewed in great detail elsewhere [13,14] and here, we review the main aspects of the stress response in which the yeast activator protein (Yap) factors have been shown to be involved.

2. Identification of the Yap1p and Yap2p transcriptions factors

Yap1p, the first member of the family of Yaps to be described, was initially identified by its ability to bind and activate the SV-40 AP-1 recognition element (ARE: TGACTAA). Based on its ARE-binding capacity, this factor was purified as 90 kDa protein and the corresponding gene was cloned by screening a λgt11 library with a monoclonal antibody against Yap1p [15]. Subsequently, this gene was also found in multicopy transformants resistant to the iron chelators 1,10-phenanthroline and 1-nitroso-2-naphtol [16] as well as to a variety of drugs including 4-nitroquinoline-*N*-oxide, *N*-methyl-*N*′-nitro-*N*-nitrosoguanine, triaziquone, sulfomethuron methyl and cycloheximide, the locus being historically designated *PAR1*/*SNQ3*/*PDR4* [17–19]. Besides *YAP1*, a second gene, *YAP2*, conferring resistance to 1,10-phenanthroline in transformed cells overexpressing a multicopy yeast library, was also described [8]. This gene encodes a 45-kDa protein that also binds the ARE *cis*-acting element. Sequence homologies identified it as *CAD1*, due to the acquisition of cadmium resistance in cells overexpressing a multicopy genomic library [20]. Later, it was

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also shown that these cells gain resistance to cycloheximide [8,21].

The sequencing of the *YAP1* and *YAP2* genes revealed the presence of a b-ZIP-family domain in the N-terminus homologous to the true budding yeast AP-1 factor Gcn4p and to c-Jun, its mammalian counterpart. Furthermore, they also share two regions of similarity, one at the C-terminus (CR1) and an internal region (CR2) located close to the b-ZIP domain [8].

3. An extended YAP gene family

Biochemical and crystallographic analysis had previously defined the Gcn4p–DNA complex and the optimal AP-1 site (TGACTCA) [22,23]. Within the basic domain, five residues (corresponding to Asn235, Ala238, Ala239, Ser242 and Arg243 in Gcn4p) (see Fig. 1) responsible for the base-specific contacts in Gcn4p and Jun/Fos are most highly conserved [24–26]. Making use of a degenerate motif based on the sequences of a large number of basic regions in b-ZIP proteins from various organisms, the complete yeast genome was searched to identify *S. cerevisiae* b-ZIP proteins. The search revealed 14 proteins including, Gcn4p, Yap1p, Yap2p, Met28p [27], Sko1p [28] and Hac1p [29] that probably represent the complete set of budding yeast b-ZIP proteins [7]. Alignment of these sequences revealed a family of six newly identified proteins, Yap3p–Yap8p, containing conserved amino acid residues similar to those present in Yap1p and Yap2p. The features that distinguish this Yap family from Gcn4p are the amino acids that make contact with the DNA. Indeed, in position 238 a glutamine replaces an alanine and in position 242, a phenylalanine/tyrosine replaces a serine. Furthermore, there are two family-specific residues, namely, a glutamine in position 234 and an alanine in position 241 (Fig. 1). The Yap family binding site was thus subsequently characterized as TTAC/GTAA [7] for Yap1p–Yap4p. So far the corresponding binding site for Yap5p–Yap8p has not been characterized, although in the case of Yap8p, it appears to be TTAATAA [30]. We cannot, however, exclude the existence of other binding sites. Orthologues of Yap1p, but not of the remaining family members, have been found in other organisms including *Schizosaccharomyces pombe* (Pap1p) [31], *Candida albicans* (Cap1p) [32] and *K. lactis* (Klap1p) [33]. With reference to the structural similarities between the Yap family members, Yap1p shares the greatest homology with Yap2p and to a lesser extent with Yap3p; Yap4p is most homologous to Yap6p and Yap5p to Yap7p, whereas Yap8p is the least closely related family member. Approximately 15% of the genome contains one or more well-positioned consensus Yap response element (YRE) sequences within its promoter region, highlighting the potential regulatory effects of this family of transcription factors.

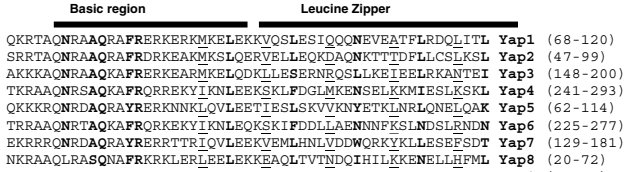

Fig. 1. Alignment of the Yap b-Zip domains. Sequences of the eight *S. cerevisiae* Yap b-ZIP domains are compared with the corresponding regions from Gcn4p and Yap-like proteins from *S. pombe* (Pap1p), *Aspergillus nidulans* (meaBp) and *C. albicans* (Cap1p). In the basic region, the residues directly interacting with base pairs are in bold face and Yap-specific residues in large font. In the leucine zipper, the conserved leucines (or other residues) d of the coiled coil (bold-face) and hydrophobic (typically) residues at position a of the coiled coil (underlined) are indicated (adapted from [10]).

4. Yap1p, the major regulon in oxidative stress response

The oxidative stress response is designated as the phenomenon by which a cell responds to alteration in its redox state due to the generation of radical oxygen species (ROS) caused by the incomplete reduction of O_2 during respiration as well as to the exposure to a variety of chemicals and metals.

The role of Yap1p in the regulation of enzymes that protect against oxidative stress was first suggested when the *yap1* mutant was found to be hypersensitive to both H_2O_2 and t-BOOH as well as to chemicals that generate superoxide anion radicals (menadione, methylviologen and plumbagine). Such *yap1* mutants have reduced specific activities of several enzymes involved in oxygen detoxification such as superoxide dismutase, glucose-6-phosphate dehydrogenase and glutathione reductase [16]. Parallel *yap1* mutant studies further indicated sensitivity to methylglyoxal, cadmium [20,21] and cycloheximide, amongst others [21]. Later, Kuge et al. [34] gave the first and clear clue towards the role of Yap1p in this response mechanism through the identification of the Yap1p target, *TRX2*, showing that its induction by H_2O_2, t-BOOH, diamide and diethylmaleate (DEM) is Yap1p-dependent and mediated by two YREs present in its promoter. Furthermore, they also demonstrated that the *yap1*-deleted strain is hypersensitive to diamide and DEM. The identification of the second Yap1p target, *GSH1*, further established its role in cadmium detoxification pathways [35]. Subsequently, several other Yap1p-dependent genes involved in cadmium tolerance have been identified [36,37]. Studies by global analysis have also added a growing number of different Yap1p targets involved in the detoxification of ROSs [38,39]. One can find, among the proteins encoded by these genes, most cellular antioxidant defenses as well as those involved in thiol redox control (for details see [14]). Although several unpublished data have indicated a slight induction of *YAP1* upon exposure to stress, the control over Yap1p-mediated gene regulation is accomplished through its cellular localization. Kuge et al. [34] demonstrated that nuclear retention of Yap1p is mediated by the cysteine-rich domain located at the C-terminus of the protein (c-CRD). Removal of this region generates a constitutively nuclear, and hence, active protein. Furthermore, three conserved cysteine residues (C598, C620 and C629) were identified as important for this post-translational regulation. Yan et al. [40] then showed that nuclear export of Yap1p is mediated by the exportin, Crm1p, binding to the Yap1p nuclear export signal (NES) which overlaps with the c-CRD. Later, Delaunay et al. [41] demonstrated, in vivo, that two cysteines, C303 from the N-terminal CRD (n-CRD) and C598 from the C-terminal CRD (Fig. 2A), are

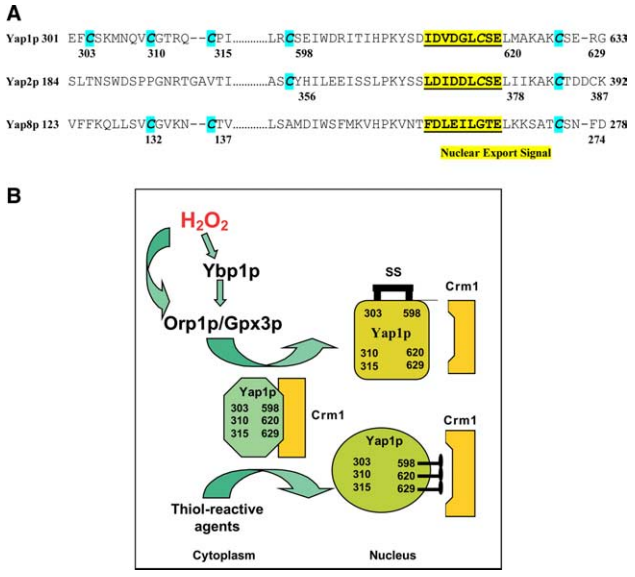

Fig. 2. (A) Alignment of the conserved cysteine residues of Yap1p, Yap2p and Yap8p. NES is underlined. (B) Schematic illustration of the two Yap1p redox centers. Under non-oxidizing conditions, Yap1p is exported from the nucleus via the exportin Crm1p. Upon H2O2, the formation of the C303–C598 disulfide bond in Yap1p via Gpx3p/Ybp1p masks the NES and Yap1p is retained in the nucleus, activating the target genes. Under thiol reactive agents, this mechanism does not operate and the drug binds directly to the Yap1p (adapted from [44,46]).

oxidized in response to H_2O_2, forming an intramolecular disulfide bond that masks the NES, thus compromising the binding of Crm1p and leading to its activation through nuclear retention. Furthermore, in vitro work performed by Wood et al. [42] has revealed that an additional intramolecular disulfide bond, namely between C310 and C629, is formed between the n-CRD and the c-CRD upon exposure to H_2O_2. These results hence indicate that Yap1p activity is regulated by post-translational mechanisms and raise the question of whether Yap1p oxidation occurs directly by the hydroperoxides or whether there is an intermediary molecule that may allow for its oxidation. Elegant experiments provided by the group of Toledano and co-workers [43] have demonstrated that Yap1p is not directly oxidized by H_2O_2. Rather, a glutathione peroxidase (GPx)-like protein (Gpx3p/Orp1p) fulfils the sensor function, transducing the oxidation signal to Yap1p through the creation of an intermolecular disulfide bond between the Gpx3p Cys36 and the Yap1p Cys598 that is then resolved into the previously characterized Yap1p C303–C598 intramolecular bridge. Furthermore, another protein, Ybp1p, has also been shown to interact with Yap1p in vivo upon exposure to H_2O_2 and to act in the same pathway as Gpx3p. Although its specific role is not understood, it is clear that interaction of this protein with Yap1p is required for Yap1p oxidation by Gpx3p and its contribution is currently being assessed [44]. In contrast, response to diamide does not involve the peroxidase, Gpx3p [43], nor is the C303 from the nCRD required for Yap1p activation [41,45]. Consistent with these results, Yap1p has been described to possess a further redox center [46]. Indeed, *N*-ethylmaleimide (NEM), an electrophile, and the quinone and menadione, both an electrophile and superoxide anion generator, were shown to modify the c-CRD cysteines independently of the Gxp3p pathway and this is sufficient to drive Yap1p translocation into the nucleus. Furthermore, mass spectrometry analyses revealed that, in contrast to H_2O_2, NEM, and possibly menadione, binds directly to the Yap1p c-CRD. A schematic illustration of these interactions with the consequent activation of Yap1p is given in Fig. 2B. These sensing mechanisms appear conserved in *S. pombe* as suggested by in vitro studies that indicate that DEM interacts irreversibly with Pap1p through at least two cysteine residues (Cys523 and Cys532) [47].

5. Yap2p/Cad1p the second member of the Yap family

Yap2p (Cad1p) is capable, when overexpressed, to confer resistance to stress agents such as 1,10-phenanthroline [8], cadmium [21], cerulenin and cycloheximide [20], suggesting a role for this transcription factor in the response to toxic compounds. DNA microarray analyses [48] indicate that Yap2p regulates a set of proteins involved in the stabilization and folding of proteins in an oxidative environment. Interestingly enough *YAP2* contains in its leader two small open reading frames, which were shown to play a role in its mRNA stability [49,50]. If this has a role in stress response, it remains to be elucidated.

Although Yap2p transcriptional activity was found to be stimulated upon cadmium treatment [7], the mechanism of activation of this transcription factor needs further investigation. The strong sequence homology between Yap2p and Yap1p in the C-terminal CRD (residues 570–650 in Yap1p and 330–409 in Yap2p) was used to further provide an insight into the function of Yap2p. Domain swapping of the Yap1p c-CRD by that of Yap2p has shown that the fusion protein is regulated by cadmium and not by H_2O_2 (Azevedo, Toledano and Rodrigues-Pousada, unpublished observations). Nuclear localization of the fusion protein correlates with both activation of Yap1p-specific target genes and growth in increasing concentrations of cadmium but not of H_2O_2. These data indicate, therefore, that the specificity towards H_2O_2 and cadmium resides in the carboxyl-terminal domain of Yap1p and Yap2p, respectively. It has also been found that treatment with cadmium activates the full-length Yap2 protein promoting its subsequent re-localization into the nucleus through a mechanism involving a regulated Yap2p–Crm1p interaction. Furthermore, the nuclear localization of the protein correlates with the Yap2p-dependent transcription of its target gene, *FRM2*, encoding a protein with strong homology with nitroreductase. A GFP fusion of the Yap2p c-CRD domain (amino acids 328–409) and the Gal4p DBD, that contains both the nuclear import signal within the Gal4p DBD and the Yap2p NES, is cadmium-responsive. This fusion is localized to the cytoplasm in untreated cells and redistributes to the nucleus in response to cadmium. Considering its high degree of homology to Yap1p, the role of the cysteine residues may prove relevant to Yap2p activation, possibly in a manner analogous to that observed for Yap1p. Given that overexpression phenotypes do not necessarily reflect a true biological function and that no phenotype has yet been associated to the *yap2* mutant, a role for Yap2p remains to be deciphered.

6. Yap4p and Yap6p in the response to osmotic stress

The fourth member of the family, Yap4p (Cin5p/Hal6p), is a 33-kDa protein and was initially characterized as a chromosome instability mutant [51]. Overexpression studies in the *ena1* mutant subsequently identified both *YAP4* (*HAL6*) and *YAP6* (*HAL7*) as genes that confer salt tolerance through a mechanism unrelated to the Na^+/Li^+ extrusion ATPase [12], whilst multicopy *YAP4* expression was shown to confer resistance to the antimalarial drugs, chloroquine, quinine and mefloquine [9,11]. Furuchi et al. [9] subsequently isolated these two genes in overexpression studies that imparted selective resistance to the chemotherapeutic agent cisplatin. Genomic DNA microarray analyses indicate a clear induction of *YAP4* and *YAP6* genes under conditions of oxidative and osmotic stress, heat, amongst others [39,52,53].

Although no further information has been obtained for *YAP6*, studies concerning the regulation of *YAP4* under conditions of hyperosmolarity have determined that not only is the null mutant osmo-sensitive but also that Msn2p-mediated *YAP4* induction occurs in a Hog1p-dependent manner through at least two STREs present in its promoter region [54]. Hyperosmotic stress leads to an arrest in cellular growth and to the altered transcription of genes involved in the stress response culminating in the adaptation of the yeast cell to the new environmental conditions. Crucial to this adaptation process is its capacity to increase the biosynthesis of glycerol, the cellular osmolyte, largely accomplished through the activity of the HOG MAP kinase pathway. Changes in external osmolarity and cell turgor activate a signal cascade that culminates with the phosphorylation of the Hog1p kinase and its translocation into the nucleus where it modulates gene expression through interaction with several transcription factors (for review [13]). The hypersensitivity of the *hog1* mutant strain to even mild hyperosmotic conditions is in part derived from a marked reduction of its intracellular glycerol content and is a clear reflection of its fundamental role in this response pathway. Indeed, studies have demonstrated that even *hog1* mutant strains can thrive in the presence of moderate hyperosmolarity when growth is performed at 37 °C, due to the concomitant activation, upon heat shock, of an alternative glycerol biosynthetic pathway via dihydroxyacetone [55]. The fact that *YAP4* overexpression clearly relieves the *hog1* osmo-sensitive phenotype has placed an added importance on the identification of its target genes (Fig. 3). Global analyses of the yeast genome and proteome are useful tools for the understanding of complex regulatory networks. Within this context, microarray analyses using the Yap4p mutant under conditions of hyperosmolarity were performed and amongst the data obtained, three genes have been validated as dependent on Yap4p to varying degrees. Two of these are involved in glycerol biosynthesis, namely *GCY1*, encoding a putative glycerol dehydrogenase, and *GPP2*, encoding a NAD-dependent glycerol-3-phosphate phosphatase. These genes show decreased induction in the *yap4* mutant strain with reduction values corresponding to 40% and 50% of the maximum levels, respectively. Although the internal glycerol content appears unaffected in the *yap4* mutant, these findings suggest that the Yap4p contribution towards its biosynthesis may be at the level of the fine-tuning of its regulation [54]. In support of this hypothesis is the fact that previous studies have demonstrated that the effects of deleting *GPP2* are only fully observed by the

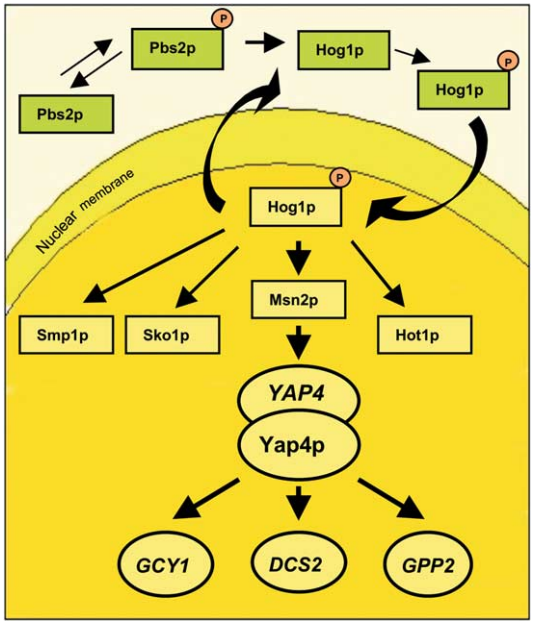

Fig. 3. Components of the HOG pathway. Upon exposure to osmotic stress, the nuclear accumulation of Hog1p activates downstream transcription factors. Msn2p is involved in the activation of *YAP4* and subsequently the encoded factor induces the targets three of which are represented (see text for further details).

concomitant deletion of its isoenzyme encoded by *GPP1* [56]. Furthermore, *DCS2*, a gene homologous to the *DCS1*-encoded decapping enzyme, shows 80% depletion in induction levels in the *yap4* mutant. Recently, *DCS1* has been described as an inhibitor of trehalase activity [57] that may have implications for osmo-response due to the role of trehalose as a superior cellular osmolyte providing protection against dehydration and desiccation [58,59]. The complete identification of the remaining *YAP4* target genes will greatly contribute towards the understanding of its functional role. Unpublished results (Nevitt, Pereira and Rodrigues-Pousada) also indicate that *YAP4* and *YAP6* are induced in response to several other stress conditions including oxidative stress, heat, and exposure to cadmium and arsenic compounds. Whilst *YAP4* regulation under oxidative stress has been shown to be dependent on Yap1p and Msn2p, mediated through the YRE and most proximal STRE, respectively, very little is known about its regulation under the remaining conditions. However, the *YAP4* promoter region contains multiple consensus HSE sites, which, together with the STREs may represent a general response switch. However, no phenotypes for the *yap4* mutant have yet been described for these stress conditions. The functional relevance of these data is not yet fully understood but it may represent a fine-tuning of regulation of gene expression under stress response.

7. Yap8p/Acr1p and its role upon arsenic conditions

The *YAP8* (*ACR1*) gene is located in chromosome XVI, in a cluster composed also by the genes *ACR2* and *ACR3* [10]. Yap8p, a 33-kDa protein, positively regulates these two genes, encoding an arsenate-reductase and a plasma membrane arsenite efflux protein, respectively, thus conferring resistance to

arsenic compounds [60–62]. Yap8p also participates in the regulation of a parallel arsenite detoxification pathway by controlling the expression of the yeast cadmium factor 1 (*YCF1*) [63].

The activity of Yap8p is not regulated at the transcriptional level. Instead, it rapidly accumulates in the nucleus upon exposure to arsenic, indicating that its translocation is a regulated mechanism [64]. Repression of *CRM1* expression retains Yap8p in the nucleus. Analogous to that observed for Yap1p [40,41], under these conditions, Yap8p is no longer actively exported to the cytoplasm, which strongly suggests that Crm1p is the protein responsible in mediating its nuclear export. Yap8p function also depends on the activation of its transactivation potential after exposure to arsenic compounds. The cysteine residues located at positions 132, 137 and 274 (Fig. 2A) are essential for Yap8p nuclear accumulation as well as for the activation of its transactivation function [64]. It remains to be elucidated whether arsenite binds these cysteines directly or whether, similarly to Yap1p [43], they are modified as a result of the alteration of the cellular redox state. However, and in contrast to this, recent results indicate not only that Yap8p is a nuclear resident protein but also that cysteines 132 and 274 do not affect its localization, although the mutant strain displays arsenic sensitive phenotype [13]. Furthermore, Yap8p was shown to interact with the *ACR3* promoter in vivo and in particular with the sequence TTAATAA [13]. It should be noted that the use of different strains, namely W303-1A and FT4, might be responsible for this contradiction. Indeed, results obtained by Veal et al. [44] have demonstrated that W303 contains a mutant allele of *YBP1*, *ybp1-1*, encoding four amino acid substitutions. The result is increased sensitivity to peroxide stress, reduced H_2O_2-induced oxidation and nuclear accumulation of Yap1p compared with cells containing the *YBP1* gene. As *YBP1* is essential for Yap1p activation under oxidative stress, one may postulate that it might also be extended to Yap8p activation. On the other hand, it is not yet clear whether arsenic causes the induction of ROS and therefore it is possible that Ybp1p also plays an important role in arsenic detoxification.

8. Remaining Yap members: Yap3p, Yap5p and Yap7p

Of the remaining Yap proteins, very little is known. In fact, for Yap3p, there is virtually no response at the level of genomic microarray analysis to multiple forms of environmental insults and cellular stress. Yap5p has been shown not to bind the TTACTAA sequence and its activation potential is not induced by aminotriazole [7]. Microarray datasets available in the Saccharomyces Genome Database [65] show that it is strongly induced under amino acid starvation, nitrogen depletion and stationary and diauxic phases. However, Northern blot analysis or real-time PCR has not yet validated these results. Similarly, with respect to *YAP7* microarray analysis shows a marked repression under nitrogen depletion and stationary phase.

9. Concluding remarks

The existence of such an extended family of AP-1-like factors has so far only been described in the yeast *S. cerevisiae*. In fact, whilst homologues for *YAP1* exist in other eukaryotes none have been found for the remaining family members. In *S. pombe*, Pap1p shares 26% identity and 41% similarity with Yap1p. However, in contrast to what has been described for *S. cerevisiae*, multiple environmental insults including osmotic, oxidative, heat shock, and nitrogen and carbon starvation have all been put into one response pathway. This MAP kinase pathway is mediated by Sty1p, which itself shares 82% identity with the *S. cerevisiae* Hog1p and is similar to mammalian p38. This and other architectural features of the Sty1 pathway make it more analogous to mammalian stress activated signaling systems and may, in part, explain the apparent lack of Yap2p–Yap8p functional homologues in these organisms.

The existing data on the Yap family members support both a degree of functional overlap between them as well as distinct physiological roles. Indeed, it has been described that the double mutant *yap1yap2* shows increased sensitivity to cadmium (Azevedo, Toledano and Rodrigues-Pousada, unpublished results) and hydrogen peroxide stress (Costa and Moradas-Ferreira, unpublished results) than the single *yap1* mutant strain. Similarly, the *yap1yap8* double mutant is more sensitive to arsenic conditions than either single mutant [64]. Whilst the existence of these multiple cross-talking signaling pathways endows *S. cerevisiae* with an added flexibility with respect to resistance, it may furthermore explain why the deletion of often key components, as exemplified by the *msn2* or *hot1* mutants, does not always give rise to a sensitive phenotype. The challenge, therefore, remains in deciphering the functional role of the remaining Yaps and to determine the interactions that occur at the protein level between each other and other cellular proteins. The construction of a yeast strain deleted for all eight *YAP* genes may provide a future insight into this protein family, in particular one that would allow for the discrimination of each of their function. Whatever the outcome, results should be obtained from well-described strains since it is becoming clear that not only do different strains have different sensitivities to the stress imposed, but also that differences at the level of the fundamental mechanisms of gene expression are starting to emerge.

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