Yap8 signal transduction to the basal transcriptional machinery

1. Abstract:

Arsenicals are toxic compounds commonly present in the environment due to either the leaching of geological formations and as a result of industrial pollution. Despite their toxicity, the successfully utilization of arsenic-containing drugs as chemotherapeutic agents has increased in the last years. A limitation for such a therapy lays on the development of secondary tumours being therefore essential the investigation of arsenic-mediated toxicity and resistance. In the present project the yeast *Saccharomyces cerevisiae* will be used as a model to address both questions. The ability of these organisms to adapt to environments containing moderate concentrations of arsenic salts is dependent on the regulated activity of a key transcription factor belonging to the YAP family, namely Yap8, which in turns regulates the expression of arsenic detoxification genes. The mechanisms of Yap8 activation through a regulated nuclear accumulation and induction of its transactivation potential were already described by us. It remains to be elucidated some aspects concerning the mechanisms by which it transduces environmental signals to the basal transcription machinery. This is the aim of this project. Additionally, the characterization of the signal transduction of the whole Yap family will be also addressed.

2. State of the art

Arsenic, along with other metals and metalloids, are ubiquitous pollutants in the nature. When these compounds are present in high concentrations in the environment they can cause serious health risks. Chronic exposure to arsenic, which is mostly found in drinking water, is generally associated with an increased risk for multiple cancers, vascular diseases, developmental anomalies and neurologic disorders [1-5]. Despite their toxicity, the ability of arsenic-containing compounds, like as Trisenox® [1], to cause cell differentiation and apoptosis are being successfully used in the treatment of acute promyelocytic

leukemia and has been promising activity in other hematologic and solid tumors. However, arsenic therapy stimulates the development of secondary tumours, being therefore the investigation of the mechanisms of arsenic-mediated toxicity and carcinogenicity as well as the mechanisms by which cells respond to arsenic stress situations of major priority.

As the eukaryotic simplest organism, yeasts are ideal models to address both questions. The adaptation of an organism to any stress condition comes along a complete reprogram of gene expression, mainly through the regulation of transcription, process in which the transcription factors are key elements. The budding yeast *S. cerevisiae*, in particular, is the model of choice for studying transcriptional regulation. *S. cerevisiae* possesses a family of eight bZIP transcription factors designated Yeast AP-1 factors (YAP) [6]. Their members (Yap1 to Yap8) modulate the activation of specific genes in response to various stress conditions. Yap1p is the best-characterized member of this family and the major regulon in oxidative and multi-drug stress responses [7,8]. Yap8 (Acr1) is the YAP family member whose function is essential in the regulation of protective response towards arsenic stress, whereas Yap1p exerts a less important role [9,10]. When activated, Yap8 and Yap1 modulate induction of *ACR2*, *ACR3* and *YCF1* encoding an arsenate-reductase, a plasma membrane arsenite efflux protein and the well-known GSH-conjugate vacuolar pump, respectively, leading to the acquisition of resistance. Arsenic compounds activate Yap8 at the level of both its nucleo-cytoplasmic shuttling and the activation of its transactivation potential. Similarly to Yap1, regulation of Yap8p accumulation in the nucleus involves arsenic-sensitive Crm1-dependent nuclear export and the cysteine residues 132, 137 and 274 [9]. Recent data obtained in our laboratory indicates that at least one of these residues are modified by direct association with the drug preventing the interaction with Crm1 and targeting Yap8 to the nucleus. Under arsenic stress Yap1 is regulated in a similar manner, being only the C-terminal cysteines fundamental for its nuclear retention. The contribution of Yap1 in arsenic stress responses is not well understood. Although its deletion partially compromises transcriptional

activation of the target genes in arsenic treated cells, its deletion has only a weak effect in the resistance of cells induced with arsenate [9]. A more significant effect is observed in cells induced with arsenite indicating that Yap1p activity is mostly required in the detoxification of arsenite than in the reduction of the pentavalent arsenate to arsenite and suggesting that both Yap1 and YAP8 are independently required for the cell tolerance to arsenic compounds [9].

3. Objectives

This work is aimed at deciphering the mechanisms of Yap8 signal transduction to the basal transcriptional machinery under arsenic stress. The whole Yap family will be also investigated.

4. Detailed description

Transcriptional regulation of mRNA encoding genes in eukaryotes requires the activity of the mediator, a complex molecular machine composed by multiple subunits, which has a role in transducing the signal from specific transcription factors to the transcriptional machinery. The yeast mediator is a 20-subunit complex that is composed of 3 functionally and physically distinct modules [11]. The head and middle modules have a general role in transcription and interact with the carboxyl terminal domain (CTD) of the largest sub-unit of RNA pol II. The tail module is composed by Med2, Med3, Med15 (the putative Gal11 module), Med16 and ultimately by Med5 [12]. This domain is responsible for recognizing and binding to transcription factors. It was reported by [13] that the Gal11 module and Med16 are involved in interactions with a number of different activators including Gcn4, an AP-1 transcription factor related to the Yap family. Taking into account that nearly all activator-dependent transcription requires components of the mediator and that it is essential for transcription in nearly every promoter in yeast, it seems plausible that Yap8 and also the other members of the YAP family, recruit the basal transcriptional machinery to the promoter of their target genes by interacting with

components of the tail module of the mediator. To analyse this *med1, med2, med3, med14, med 15 and med16* knock out mutants will be subjected to phenotypic growth analysis in order to identify their requirement under various stress conditions. It will be take advantage of the two-hybrid strategy, where the YAP proteins will be fused to the Gal4p binding domain and the individual components of the mediator tail module to the Gal4p activation domain. The GFP-fragment reassembly methodology will be used as an alternative approach to detect weak interaction [14]. The positive interactions will be then validated *in vitro* by co-immunoprecipitation [15]. Yap8 will be the first protein to be analysed and further characterization of the interaction patterns of the remaining Yap proteins will enable us to verify if all of them use a common mechanism to activate transcription of their targets. As the overall structural similarity between mediators from yeast, murine and human together with the fact that almost every component of the yeast mediator has a metazoan homolog [16], the elucidation of the interaction of AP-1 transcription factors with the mediator in yeast will contribute to better understand similar mechanisms in higher eukaryotes.

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