Phosphorylation of Zn(II)$_2$Cys$_6$ proteins: a cause or effect of transcriptional activation?

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Abstract

Many Zn(II)$_2$Cys$_6$ transcriptional regulators exhibit changes in phosphorylation that are coincident with their roles in transcriptional activation. It is, however, unclear whether these changes occur as a cause of, or as a result of, transcriptional activation. In this paper, we explore the relationship between these two events and collate data available on the phosphorylation state of those transcriptional regulators where the relationship has not been clearly identified.

Introduction

The Zn(II)$_2$Cys$_6$ family of transcriptional regulators contains upwards of 50 members in Saccharomyces cerevisiae alone. This family of proteins, characterized by the presence of an N-terminal DNA-binding domain containing two zinc atoms bound by six cysteine residues, is involved in diverse functions, ranging from carbon metabolism (Gal4p and Rtg1p) and nitrogen metabolism (Put3p and Leu3p) to stress responsiveness (War1p and Hal9p) [1]. Like other transcriptional regulators, many Zn(II)$_2$Cys$_6$ proteins exhibit changes in phosphorylation concurrent with changes in transcriptional activation (see Table 1). It is, however, unclear whether these changes occur as a cause or as a result of activation. As with many other aspects of eukaryotic transcriptional control, Gal4p provides a paradigm for activation-related phosphorylation with which other Zn(II)$_2$Cys$_6$ family members can be compared.

Gal4p

Gal4p is responsible for the transcription of genes involved in galactose metabolism and is the archetypal Zn(II)$_2$Cys$_6$ transcription factor [2]. In the absence of galactose, the GAL genes are transcriptionally inert; DNA-bound Gal4p is physically associated with the transcriptional inhibitor Gal80p. When galactose is the sole carbon source, the inducer, Gal3p, binds galactose in an ATP-dependent manner, resulting in its association with Gal80p. This interaction relieves the repressing effect of Gal80p, allowing Gal4p to mediate transcription of the GAL genes [6,7]. Interestingly, this decrease in activity is not apparent in yeast strains in which the GAL80 gene was deleted, resulting in constitutively active Gal4p. This indicates that phosphorylation at Ser$^{699}$ is necessary for efficient induction by galactose, but not for activation in the absence of Gal80p [7]. Furthermore, Ser$^{699}$ can be phosphorylated in gal80A cells under repressing conditions, but not in cells containing Gal80p, suggesting that Gal80p can inhibit Gal4p phosphorylation [7]. This could indicate that Gal4p becomes competent for phosphorylation only when Gal80p repression is relieved.

Full Gal4p phosphorylation is wholly dependent on its DNA-binding ability [6,7]. This suggests a link between phosphorylation and other co-activators or GTFs (general transcription factors). Indeed, the appearance of Gal4p III and phosphorylation at Ser$^{699}$ are dependent on Gal11p, which is required for Mediator recruitment to the GALI promoter [6–10]. Moreover, it has been demonstrated that the cyclin-dependent kinase associated with Mediator, Srb10p, is required for phosphorylation of Ser$^{699}$ and Ser$^{837}$, while the Kin28p component solely phosphorylates Ser$^{837}$ [11].

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Table 1 | Phosphorylation sites and kinases associated with Zn(II)$_2$Cys$_6$ transcriptional activators showing concurrent phosphorylation and activation

<table>
<thead>
<tr>
<th>Protein</th>
<th>Phosphorylation site</th>
<th>Kinase</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat8p</td>
<td>Ser$_{562}$</td>
<td>Snf1p</td>
<td>Required for activation</td>
<td>[17,19]</td>
</tr>
<tr>
<td>Cat8p</td>
<td>Ser$_{697}$</td>
<td>Unknown</td>
<td>None apparent</td>
<td>[6,7]</td>
</tr>
<tr>
<td>Cat8p</td>
<td>Ser$_{699}$</td>
<td>Srb10p</td>
<td>Required for activation</td>
<td>[6,7]</td>
</tr>
<tr>
<td>Cat8p</td>
<td>Ser$_{647}$</td>
<td>Kin28p</td>
<td>None apparent</td>
<td>[6,7]</td>
</tr>
<tr>
<td>Leu3p</td>
<td>Not known</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[27]</td>
</tr>
<tr>
<td>Pdr1p</td>
<td>Not known</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[29]</td>
</tr>
<tr>
<td>Pdr3p</td>
<td>Not known</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[29]</td>
</tr>
<tr>
<td>Put3p</td>
<td>Not known</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[24,25]</td>
</tr>
<tr>
<td>Rgt1p</td>
<td>Between amino acids 750 and 760</td>
<td>Unknown</td>
<td>Required for repression</td>
<td>[15,16]</td>
</tr>
<tr>
<td>Sip4p</td>
<td>Not known</td>
<td>Snf1p</td>
<td>Required for activation</td>
<td>[20–22]</td>
</tr>
<tr>
<td>Srb10p</td>
<td>Not known</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[28]</td>
</tr>
</tbody>
</table>

in an Spt3p-dependent manner, as part of the Srb8p–Srb11p complex [12]. Further evidence supporting this association between Srb10p, GTFs and Gal4p is witnessed in the fact that Srb10p has been shown to physically associate, both in vivo and in vitro, with a Gal4p molecule composed of the DNA-binding domain (amino acids 1–100) and activating region II (amino acids 840–881), suggesting that wild-type Gal4p can associate directly with Srb10p [13]. The fact that the Gal4p phosphorylation requires DNA binding and that the activating kinase is physically associated with Mediator, as well as with SAGA, suggests that Gal4p phosphorylation is a result of activation, rather than a cause of it. Indeed, Gal4p does not become phosphorylated until it activates transcription; yet, it does not become fully active unless Ser$_{699}$ is phosphorylated [11,14]. Thus it would appear that, while Ser$_{699}$ phosphorylation is required for galactose induction of Gal4p, phosphorylation is dependent on GTFs, possibly representing a feedback loop between initial Gal3p-based activation and the full recruitment of transcriptional machinery.

A possible interpretation of these events suggests that there are two separate mechanisms operating in the activation of Gal4p, one that feeds through Srb10p and its associated cyclin, Srb11p, as well as another that feeds through the inducer Gal3p [14]. This model is supported by the fact that srb10A and gal3A strains are individually able to grow with galactose as the sole carbon source; however, when combined, the double mutant is unable to do so, demonstrating that both pathways are required for GAL gene induction [14].

In summary, full Gal4p activation appears to require two signals: active Gal3p and active Srb10p. Based on this supposition, the following model can be proposed; under inducing conditions, Gal3p binding of Gal80p activates Gal4p to basal levels. Once the transcriptional machinery is recruited to the GAL promoters, Gal4p is then fully activated by Srb10p.

Rgt1p

Rgt1p offers a different example of Zn(II)$_2$Cys$_6$ action to the GAL system, as Rgt1p is primarily a repressor and secondarily an activator [15]. In the absence of glucose, Rgt1p represses HXT gene expression; however, it is also required for maximal HXT1 expression under high glucose conditions [15]. In glucose-deficient environments, Rgt1p is bound to the promoters of HXT1, HXT2 and HXT3. When the glucose concentration increases above 0.1%, it dissociates from these promoters, allowing transcriptional activation to proceed [15]. Rgt1p-dependent de-repression is directly proportional to its phosphorylation state; increasing the quality of the carbon source results in Rgt1p becoming increasingly phosphorylated [16]. The sites of this phosphorylation are likely to be Ser$_{753}$, Ser$_{755}$ and Ser$_{758}$, because an Rgt1p variant containing a deletion of amino acids 750–760 does not become phosphorylated, and constitutively represses HXT gene expression [16]. The kinase responsible for this phosphorylation is not currently known.

Cat8p and Sip4p

Cat8p and Sip4p both bind to the carbon-source-responsive element and regulate gluconeogenic gene expression. The genes FBP1, PCK1 and ICL1 become active when the sole carbon source is non-fermentable (e.g. glycerol or ethanol) [17–19]. Cat8p, like Gal4p, migrates as two distinct bands (Cat8p I and Cat8p II) on SDS/PAGE when harvested under non-inducing, non-repressing conditions. However, when grown on a non-fermentable carbon source, a third form appears, Cat8p III, which corresponds to the expression of...

Snf1p mutation of the corresponding serine residue in KlCat8p. In addition, two Snf1p consensus sites are present in both Srb10p phosphorylation, and thus full activation [22].

Put3p is required for the full expression of the proline utilization genes, PUT1 and PUT2. Put3p exhibits full activation when proline is the sole nitrogen source; however, it also exhibits increases in basal activity under nitrogen-limiting conditions [23]. As nitrogen source quality decreases, Put3p exhibits increases in activity concurrent with increases in phosphorylation, showing maximal phosphorylation when proline is the sole nitrogen source [24]. Like the other Zn(II)2Cys6 transcription factors we have discussed, the apparent high-molecular-mass forms of Put3p are reduced to a single band upon phosphatase treatment [24]. Put3p also exhibits hyperphosphorylation when cells are treated with the antibiotic rapamycin, which is known to mimic nitrogen-de-repressing conditions, suggesting that Put3p could be responding to nitrogen-sensing networks [25]. In vitro, Put3p will bind proline and activate transcription when it is in an unphosphorylated form [23]. It is possible, however, that phosphorylation could increase the efficiency of both these events, although this has not yet been tested. Moreover, as the kinase and precise sites of phosphorylation have not been identified in Put3p, it is not possible to discern whether or not phosphorylation is a cause of transcriptional activation or an effect.

Leu3p is required for the transcription of the genes involved in branched chain amino acid synthesis [26]. Leu3p activation, like that for Put3p, is dependent on the presence of a low-molecular-mass metabolite, in this case α-isopropylmalate, an intermediate of leucine biosynthesis [27]. Western-blot analysis of highly purified Leu3p has demonstrated that the protein migrates as a doublet, which is reduced to a single band upon phosphatase digestion [27]. No further data are available on the relationship between Leu3p phosphorylation and activation.

**Prd1p, Pdr3p and War1p**

Prd1p, Pdr3p and War1p all control the expression of ABC transporters (ATP-binding-cassette transporters). Prd1p and Pdr3p co-regulate the expression of PDR3, SNQ2 and YOR1 and bind to the PDRE (pleiotropic drug resistance element), while War1p governs the expression of PDR12 by binding to the WARE (weak acid response element) [28,29]. Although both Pdr1p and Pdr3p have been identified as phosphoproteins [29], with Pdr3p exhibiting phosphorylation at its C-terminal half, no function has been ascribed to these events [29]. War1p (like Put3p, Leu3p and Gal4p) exhibits phosphorylation coincident with activation, yet the kinase responsible and sites of phosphorylation are not known [28].

**Conclusion**

Many Zn(II)2Cys6 transcriptional activators are known to show increases in phosphorylation coincident with transcriptional activation; however, the actual relationship between these two events is often unclear. Indeed, it is not readily apparent whether phosphorylation takes place as a cause of activation or as an effect of the activation process. The best-explored examples of this relationship are Gal4p and Sip4p, which regulate carbon metabolism, and do provide some insight as to how these events might operate. In the Sip4p and Gal4p systems, it is possible that priming for phosphorylation occurs upon initial activation: in the case of Gal4p, de-repression of Gal80p, and in the case of Sip4p, phosphorylation by Snf1p. Subsequently, full activation occurs upon recruitment of the transcriptional machinery, via phosphorylation by one of its associated kinases: an Srb10p-dependent event in the case of both Gal4p and...
Sip4p. Contrary to this assertion, only the mutation of one out of four identified phosphorylation sites in Gal4 reduced its transcriptional activity. Moreover, both Gal4p and Sip4p still exhibit band shifts when the known kinases and phosphorylation sites are ablated. It therefore seems likely that only key residues are required for priming and/or activation and subsequent residues are phosphorylated purely as a result of transcriptional activation.

Although Gal4p and Sip4p provide us with a potential model, it is possible that they share this prospective mechanism because they are both associated with carbon metabolism and repressed by glucose. Thus they are responding to similar signals and signalling pathways. It could be envisaged that genes responding to other stimuli may operate through an entirely different mechanism, potentially not interacting with the Mediator-associated Srb10p for post-priming activation. However, much more information regarding this relationship is required to make a broader assumption about the role phosphorylation plays in governance of this family of transcriptional regulators.

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References

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