Gal4 turnover and transcription activation

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Growing evidence supports the notion that proteasome-mediated destruction of transcriptional activators can be intimately coupled to their function[1,2]. Recently, Nalley *et al.*[3] challenged this view by reporting that the prototypical yeast activator Gal4 does not dynamically associate with chromatin, but rather 'locks in' to stable promoter complexes that are resistant to competition. Here we present evidence that the assay used to reach this conclusion is unsuitable, and that promoter-bound, active Gal4 is indeed susceptible to competition *in vivo*. Our data challenge the key evidence that Nalley *et al.*[3] used to reach their conclusion, and indicate that Gal4 functions *in vivo* within the context of dynamic promoter complexes.

Studies by several groups, including ours[1,2,4–6], have reported an intimate connection between the activity of transcriptional activators such as Gal4 and Gcn4 and their destruction by ubiquitin-mediated proteolysis. This intimate connection is difficult to reconcile with the conclusion by Nalley *et al.*[3] that proteolytic turnover of Gal4 is not coupled to its function. This conclusion is based on the result of chromatin immunoprecipitation (ChIP) experiments showing that endogenous, active, Gal4 cannot be competed from the *GAL1/10* promoter by induction of a protein with the same DNA-binding specificity ('competitor'). In this case, the competitor contains the hormone-binding domain of the oestrogen receptor (ER), which allows its DNA-binding activity to be rapidly induced by the treatment of yeast with β-oestradiol. Yeast cultures expressing both the competitor and endogenous Gal4 are treated with β-oestradiol, and ChIP analysis is used to monitor the binding of the two proteins to the *GAL1/10* promoter.

We obtained reagents from the Kodadek laboratory and repeated their experiments. In the course of performing an additional control that was not included in their *Nature* paper, we observed that, in the absence of any competitor, β-oestradiol induced an up to fourfold increase in the levels of Gal4 that associated with the *GAL1/10* promoter (Fig. 1a, blue line). The unexpected ability of β-oestradiol to induce binding of endogenous Gal4 makes the competition assay difficult to interpret, as the compound is simultaneously inducing both the competitor and the species being competed.

To explore this issue further, we repeated the experiment using a different ER ligand, 4-hydroxytamoxifen (4HT). In the absence of competitor, 4HT had little effect on the association of endogenous Gal4 with its cognate promoter (Fig. 1a, red line). Consistent with the different effects of these two ligands on basal association of Gal4 with chromatin, the two compounds gave very different results in the presence of competitor (Fig. 1b). As Nalley *et al.* published[3], the addition of β-oestradiol to yeast expressing the competitor protein resulted in little if any reduction in the levels of endogenous Gal4 at the *GAL1/10* promoter, creating the impression that most promoter-bound Gal4 resisted competition (Fig. 1b, blue line). In the presence of 4HT, however, the opposite result was obtained, and ~75% of endogenous Gal4 was competed from the chromatin within 15 min of the ligand addition (Fig. 1b, red line). Notably, the loss of Gal4–chromatin association was accompanied by loading of the competitor onto the *GAL1/10* promoter (Fig. 1c), consistent with the notion that the 4HT-activated competitor can displace endogenous Gal4 from the promoter. Although the competitor protein associated with the *GAL1/10* promoter with apparently slower kinetics than endogenous Gal4 dissociated (compare red line in Fig. 1b with pink line in Fig. 1c), it is worth noting that endogenous Gal4 can bind cooperatively to several sites *in vivo*[7]. There are four Gal4-binding sites in the *GAL1/10* promoter. Thus, a single competitor bound to one of the sites could have the effect of destabilizing multiple Gal4–promoter complexes, leading to efficient displacement of endogenous Gal4 at substoichiometric levels of competitor.

On the basis of our observations, we propose that the recalcitrance of Gal4–promoter complexes originally reported by Nalley *et al.*[3] is an artefact of using β-oestradiol to stimulate the competitor. Activating the competitor with 4HT (Fig. 1b), or normalizing the β-oestradiol signal to the important 'no competitor' control (Fig. 1d), shows that Gal4 can indeed be rapidly displaced from promoter DNA *in vivo*. Their conclusion that Gal4–promoter complexes lock in and have long half-lives under activating conditions is thus unsustainable.

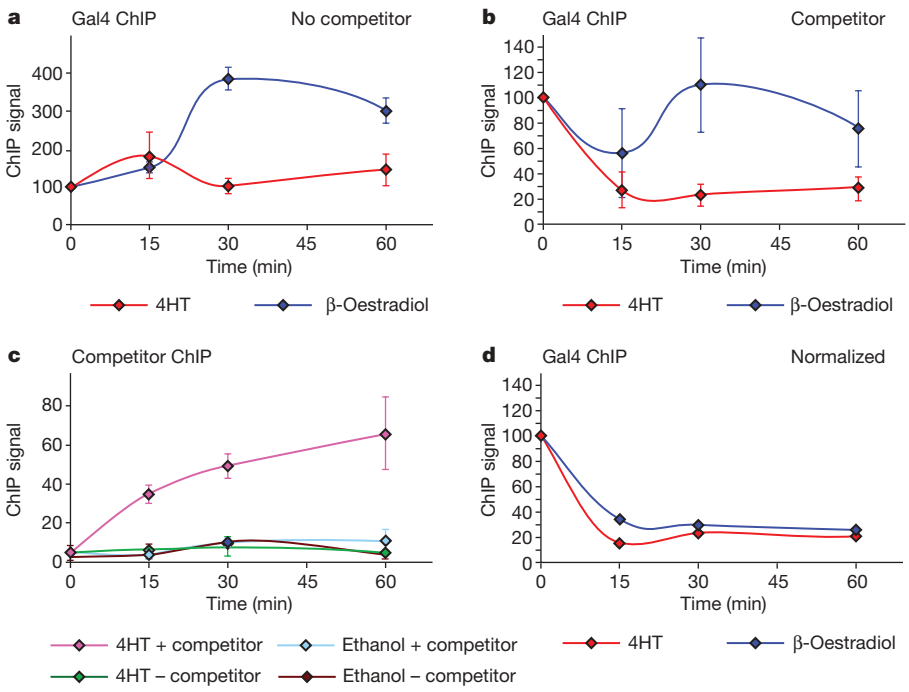

Figure 1 | Activation of a Gal4 competitor with β-oestradiol versus 4HT. a, Wild-type yeast were induced with 2% galactose for 60 min and β-oestradiol or 4HT was added. At indicated times, the occupancy of endogenous Gal4 on the *GAL1/10* promoter was determined by ChIP. ChIP signal is normalized to that at time zero. **b**, As in **a**, except that experiment was performed in yeast expressing the Myc–G4–ER–VP16 competitor (supplied by T. Kodadek[3]). **c**, As in the 4HT experiment in **b**, except that ChIP was used to monitor association of the Myc–G4–ER–VP16 competitor with the *GAL1/10* promoter. The corresponding non-competitor controls are also shown. To calculate the percentage binding in this case, ChIP signals were normalized to those from a Myc–G4–ER–VP16 ChIP (60-min time point) performed in the absence of endogenous Gal4, which corresponds to the total amount of competitor that can bind in this assay. **d**, ChIP signals from β-oestradiol or 4HT experiments in **b** normalized to the relevant 'no competitor' control in **a**. Error bars are s.e.m. ($n = 3$).

METHODS

Yeast (BY4741) with or without competitor (Myc–G4–ER–VP16)[3] were grown in complete synthetic medium (CSM) (2% raffinose) and Gal4 was induced by transferring yeast to media containing 2% galactose for 1 h. Yeast were then treated with 1 μM 17-β oestradiol (Sigma) or 100 μM 4-hydroxytamoxifen (Sigma) for the indicated times. ChIP was preformed[5] using either the Gal4-TA C-10 (anti-GAL4; Santa Cruz) or AB1 (anti-Myc; Calbiochem) antibodies. DNA enrichment was calculated as described[8] using *ACT1* as the reference locus.

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Proteasome-mediated turnover of some[1,2], but clearly not all[3,4], transcriptional activators is important for their activity. To facilitate the analysis of activator–promoter complex lifetime *in vivo*, a parameter relevant to this issue, we developed a competition chromatin immunoprecipitation (ChIP) assay in which binding of a native transactivator to its cognate promoters is challenged by a ligand-activated competitor protein with the same DNA-binding specificity. We applied this technique to the yeast Gal4 system[5] and concluded that under non-inducing conditions (raffinose media) Gal4–promoter complexes exchange rapidly, but under inducing conditions (galactose media) the activator–promoter complexes are long-lived. Collins *et al.*[6] report that, surprisingly, the addition of oestradiol to yeast lacking Myc–G4–ER–VP16 increased the amount of DNA co-immunoprecipitated with native Gal4.

This is a control we had not done, but have subsequently repeated and agree that this is the case (S.A.J. wishes to note that he had requested this control and it erroneously was not done). We thank Collins *et al.*[6] for pointing out this omission. They go on to show that inducing competitor protein activity with 4-hydroxytamoxifen (4HT) results in a significant loss in the intensity of the ChIP signal owing to native Gal4, but that this ligand does not affect the intensity of these ChIP signals in the absence of competitor. They also show significant association of the competitor protein with the promoter, although with a different time course than Gal4 dissociation. We agree that these data indicate that a large fraction of Gal4–promoter complexes are kinetically labile *in vivo* under these (4HT-containing media) conditions. It is important to note that this odd effect of steroid is not a general problem in the application of this technology to the measurement of other activator–promoter half-lives[4].

However, our ChIP data tracking association of the competitor protein do not support the conclusion of a rapidly exchanging Gal4–DNA complex *in vivo* in the presence of β-oestradiol rather than 4HT. There is no indication that these data are compromised by unanticipated effects of β-oestradiol. Under inducing conditions, much lower levels of association of the competitor protein with *GAL* promoters were observed when Gal4 was present than in *Δgal4* cells when β-oestradiol was used to trigger the competition. These data argue for the presence of a stable, functional Gal4–promoter complex in the presence of galactose and under the particular conditions used in our study[5]. It may be that the stability of Gal4–promoter complexes are somehow affected by steroid receptor ligands, which would explain the different results observed by ourselves and Collins *et al.*[6] for the association of the competitor protein in our respective experiments.

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