CTD-like sequences are important for transcriptional activation by the proline-rich activation domain of CTF1

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Like acidic activation domains, the proline-rich activation domain of CTF1 stimulates transcription in both yeast and human cells (1). To gain an understanding of relevant structural features of this proline-rich activation domain, the minimal region sufficient for an activation response was determined by deletion analysis (Figure 1). Derivatives of the activation domain were fused to the DNA binding domain of GAL4 and assayed by introduction into a yeast strain containing a reporter CYC1-lacZ fusion gene with DNA binding sites for GAL4. The results show that a region from residues 460–479 is most important for activation since its deletion markedly reduced activation (5-fold) whereas deletion of N-terminal residues (399–459) had only a modest effect (Figure 1). Interestingly, an amino acid sequence within the minimal CTF1 activating region (SPTSPSYSPPDTSP) is very similar to the consensus sequence (SPTSPSY) of the repeat unit of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II. To more directly show the involvement of this CTD-like sequence in activation, GAL4 (1–147) was fused to the yeast CTD containing 26 tandem repeats of the SPTSPSY sequence. This domain was remarkably active in activation of the reporter gene (Figure 1). These results clearly show that SPTSPSY sequences in the CTD, as well as related sequences in the proline-rich domain of CTF1, can mediate activation in yeast.

The idea that the CTD-like sequences (**SPTSPSY**) are involved in activation is further strengthened by the results of a search for proteins containing SP motifs (2). This motif occurs frequently in transcriptional activators but less so in general proteins, suggesting its involvement in gene regulation. SP-containing transcription regulatory proteins include homeobox gene products (fushi tarazu, antennapedia, engrailed, deformed and Oct), Drosophila segmentation gene products (Krüppel and hunchback), steroid hormone receptors, yeast transcription factors (ADR1, PPR1, GAL4, HSF and SWI5), and certain products of oncogenes (fos and myc). Furthermore, the location of SP motif is restricted; it is found neither in DNA-binding domains nor in regions responsible for ligand binding or dimerization, but mostly in regions important for activation or regulation. Therefore, many activating regions could contain SP motifs. Both functional and sequence analyses suggest that the SP sequence motif is important for transcriptional activation. One interesting possibility is that phosphorylation of the SP motif plays a role in gene regulation by activators (e.g. CTF1).

The SP motif is strongly predicted, by the method of Chou and Fasman (3), to form a β-turn secondary structure. Several different approaches, including model building studies, two-dimensional NMR, and circular dichroism studies also suggest that the SP motifs in the CTD are composed of turn structures (4). Therefore, in addition to the primary sequence, the β-turn structure might be an important secondary structure in the activation domain. Recently, Johnston's laboratory has argued that the GAL4 activation domain contains antiparallel β-sheets (5, 6). However, this motif fails to explain certain characteristics of other activation domains, raising the possibility that there are several classes of activators containing distinct secondary structures. Consistent with this view, the present results with the SP-containing motif suggest a β-turn structure as an additional secondary structure important for activation.

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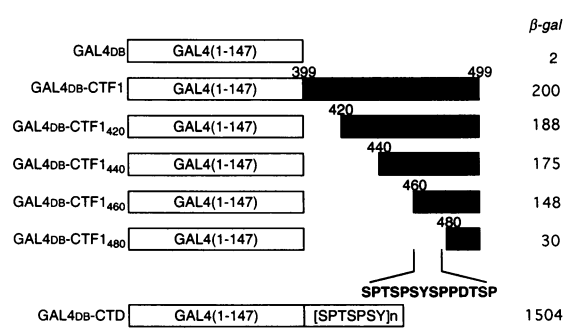

Figure 1. An SP-containing motif is important for activation by the proline-rich activation domain of CTF1. The reporter gene is a CYC1–lacZ fusion which is regulated by the binding site for the GAL4 (1). The GAL4 DNA binding domain (amino acids 1–147) is fused to the various deletion derivatives of the CTF1 proline-rich activation domain and to the 26-heptapeptide (SPTSPSY) repeat of the C-terminal domain (CTD) of the largest subunit of yeast RNA polymerase II. Deletions within the CTF1 proline-rich activation domain were made by an oligonucleotide-mediated mutagenesis method. Yeast transformants were grown and assayed for β-galactosidase activity, as described previously (1). Numbers refer to the average units of β-galactosidase activity measured in three independent cultures, which varied by <20%.

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