Stimulation of DNA Replication in *Saccharomyces cerevisiae* by a Glutamine- and Proline-rich Transcriptional Activation Domain*

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Glutamine-rich Sp1 and proline-rich CTF1, two extensively studied mammalian transcription factors, bind to origins of replication in DNA tumor viruses and stimulate viral DNA replication in mammalian cells. Here it is shown that, when tethered to a plasmid-borne cellular origin of replication, the activation domains of both proteins can enhance origin function in *Saccharomyces cerevisiae*. Hydrophobic patches in Sp1 and CTF1 that mediate transcriptional activation in higher eukaryotes are also important for activation of replication in yeast. However, only the activation domain of CTF1 can enhance initiation of replication from a chromosomally embedded origin. This correlates with the ability of CTF1 to alter the local chromatin structure around the chromosomal origin of replication. The CTF1-induced chromatin remodeling occurs at multiple stages of the cell cycle. These findings strongly suggest a high degree of conservation in the mechanisms used by various types of transcription factors to stimulate viral and cellular DNA replication in eukaryotes.

Initiation of DNA replication and transcription in eukaryotic cells share several common molecular challenges. These include locating an initiation site, overcoming the inhibitory effect of the chromatin structure, assembling a multiprotein complex at the initiation site, and unwinding the duplex DNA. Thus, it is often the case that origins of replication identified to date share striking architectural similarities with transcriptional promoters. Analogous to the TATA box of a transcription promoter, the core sequence of an origin of replication serves as the binding site for an initiator protein which, in turn, nucleates the assembly of a large preinitiation complex.Auxiliary elements, which are located in the vicinity of the core sequence of an origin and contribute to the high efficiency of origin function, usually contain binding sites for those factors that also bind to transcriptional promoters and stimulate transcription (1–3). From a mechanistic perspective, it may be parsimonious for eukaryotic organisms to employ transcription factors to accelerate certain rate-limiting steps that are common to replication and transcription. From a regulatory point of view, these bifunctional transcription factors may play a pivotal role in coordinating gene expression with genome duplication during proliferation and differentiation.

A majority of transcription factors have a bipartite structure that contains a DNA-binding domain and transcriptional activation domain. The latter is generally classified with respect to its amino acid composition. These include the acidic (e.g. the herpes simplex virus VP16), the glutamine-rich (e.g. the mammalian Sp1), and the proline-rich domains (e.g. the mammalian CTF1). Similar to transcriptional activation, viral DNA replication can be stimulated by various types of transcription factors. For example, the flanking auxiliary sequences of simian virus 40 (SV40) origin contain binding sites for multiple cellular transcription factors; and the viral DNA replication can be significantly stimulated by transcription factors such as Sp1, CTF1, AP1, and GAL4-VP16 (3).

Recent studies in *Saccharomyces cerevisiae* suggest that transcription factors play a similar role in activation of chromosomal replication; and at least in the case of acidic activation domain, the same activator can stimulate cellular replication in yeast as well as viral DNA replication in mammalian cells. For instance, the extensively characterized ARS1 origin of replication contains four genetic elements (A, B1, B2, and B3). The A element is the binding site for the origin recognition complex (ORC) (4), whereas B1 provides additional ORC contact sites and other functions in activation of replication (5). The exact function of B2 remains to be established. The B3 element is a binding site for an acidic transcription factor Abf1p (6). While the A element is absolutely required for the ARS activity, the three B elements are collectively important for origin function (7). It has been shown that Abf1p function at ARS1 can be replaced by various acidic activation domains tethered to the origin via a heterologous DNA-binding domain. These include those from GAL4, p53, BRCA1, as well as the acidic activation domain of Abf1p itself (7–9). Furthermore, chromatin remodeling appears to be an important mechanism used by these acidic-type activators to stimulate chromosomal replication in budding yeast (9). The species-independent activation of replication by the acidic activators is reminiscent of their universal ability to activate transcription in a variety of eukaryotic organisms (10, 11).

In the current study, two non-acidic activation domains, glutamine-rich Sp1 and proline-rich CTF1, are examined for their ability to stimulate ARS1-dependent DNA replication in yeast. While both activation domains are capable of stimulating DNA replication from a plasmid-borne origin, only CTF1 can alter the chromatin structure and stimulate DNA replication at a chromosomally embedded origin.

EXPERIMENTAL PROCEDURES

**Plasmids and Yeast Strains**—Polymerase chain reaction was used to generate DNA fragments that encode the activation domains from Sp1 (aa 149–344 and 263–542), CTF1 (aa 399–499, 399–486, and 486–

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1 The abbreviations used are: ORC, origin recognition complex; ARS, autonomously replicating sequence; MNase, micrococcal nuclease; DBD, DNA-binding domain; CEN, centromere.
RESULTS

Both Sp1 and CTF1 Activation Domains Can Stimulate ARS Replication in a Plasmid Context—It has been shown that several mammalian acidic activation domains can stimulate chromosomal replication in yeast (8, 9). To determine whether non-acidic activation domains were also capable of stimulating cellular DNA replication, the glutamine-rich domain of Sp1 and proline-rich domain of CTF1 were tethered to ARS1 via the GAL4 DNA-binding domain. These domains were chosen because: 1) they have served as paradigms for the two non-acidic types of activation domains in numerous transcriptional studies; and 2) both proteins have been shown to stimulate viral DNA replication in mammalian cells. Also included in the study as positive controls were GAL4 derivatives containing the acidic activation domains of Abf1p and the breast cancer protein BRCA1, both of which stimulate origin function in yeast (8, 9). Briefly, yeast nuclei were incubated with a limited amount of Mnase for various periods of time. The reactions were terminated and genomic DNA was isolated. For the indirect end-labeling experiment, DNA was digested with EcoRI, resolved by 1% agarose gel electrophoresis, and visualized by autoradiography. The membrane was probed with a radioactive 32P-labeled probe corresponding to either Sp1 or CTF1. Taken together with previous work on viral DNA replication, this result suggests that Sp1 and CTF1 can stimulate DNA replication from cellular origin in yeast cells as well as viral origin in mammalian cells.

Stimulation of Transcription and DNA Replication Involves Common Hydrophobic Patches in Sp1 and CTF1—The effect of the Sp1 domain on DNA replication of the ARS/CEN plasmids is in contrast to its behavior in transcriptional activation in budding yeast. Unlike the acidic and proline-rich activation domains that activate transcription in a variety of eukaryotic cells including budding yeast, the glutamine-rich domain of Sp1 has been reported to be transcriptionally inert in S. cerevisiae, at least in the case of a chromosomally embedded promoter (15–18). This raised the issue whether the Sp1 activation domain used the same amino acid residues for activation of transcription in higher eukaryotes and plasmid DNA replication in budding yeast. To test this possibility, the effects of several known mutations in the Sp1B domain on activation of replication were analyzed in the plasmid stability assay. As shown in Fig. 2A, wild-type and mutant GAL4 fusion proteins were expressed at similar levels. All mutations are clustered in a glutamine-rich hydrophobic patch (VSWYQTAQAQNA), which is critical for the Sp1B domain to activate transcription in higher eukaryotes (19, 20). M37 contains a linker substitution mutation (GAAGIRWKIP) that significantly reduces transcriptional activation of Sp1B in Drosophila and human cells. W3A and L3A contain alanine substitutions of either the single tryptophan (VSWYQTLQNL) or three leucine residues in this patch (VSWYQTAQAQNA), and both mutations diminish transcriptional activation (19, 20). As shown in Fig. 2A, none of the three GAL4-Sp1B mutants could support plasmid DNA replication in yeast. In contrast, alanine substitutions of two glutamines and an asparagine (Q/A; VSWYQTLAAL) actually resulted in a moderate increase in transcriptional activation compared with the wild-type protein (19), and interestingly, the same mutant also stimulated ARS function to a greater extent than the wild-type GAL4-Sp1B fusion protein (Q/A in Fig. 2A). In light of the evolutionary distance between human, fly, and yeast, it is remarkable that the Sp1B domain uses the same set of amino acid residues to activate transcription in higher eukaryotes and DNA replication in yeast.

In addition to the Sp1 study, the proline-rich activation domain of CTF1 was also dissected in the yeast plasmid stability assay. Previous characterization of the CTF1 activation domain (aa 399–499) reveals a hydrophobic region containing
FIG. 2. Hydrophobic patches of the glutamine- and proline-rich activation domains are required for activation of DNA replication. A, wild-type and various mutant GAL4-SP1B fusion proteins were tested in a plasmid stability assay for their ability to stimulate replication. The test plasmid used contains one GAL4-binding site as shown in Fig. 1. The expression levels of the fusion proteins were determined in an immunoblot as shown on the top, using an antibody against the hemagglutinin epitope (12CA5). B, GAL4 fusion proteins containing the full-length (aa 399–499), NH– (aa 399–486), or COOH-terminal (aa 486–499) part of the CTF1 activation domain were tested for their ability to stimulate yeast plasmid replication (hatched bars) and transcription (open bars). The test plasmid for activation of replication contains five GAL4-binding sites as used in Fig. 1. The β-galactosidase reporter construct for activation of transcription was described previously (8).

the last 14 residues (DPAGIYQAQSWYLG; aa 486–499) that are critical for CTF1-mediated transcriptional activation (21). A GAL4-CTF1 fusion protein lacking the last 14 amino acid residues of CTF1 was expressed at the same level as the wild-type protein (data not shown), but it did not stimulate either transcription or plasmid DNA replication in budding yeast (Fig. 2B). On the other hand, a GAL4 derivative containing the 14-aa hydrophobic patch alone was as potent as the full-length CTF1 activation domain in activating yeast transcription and replication (Fig. 2B). Therefore, the minimal carboxyl-terminal domain of CTF1 is both important and sufficient for stimulating transcription and replication from a plasmid-borne cellular origin in yeast.

GAL4-CTF1 Can Stimulate a Chromosomally Embedded Origin of Replication—It is known that not every ARS identified in the plasmid-based assay acts as an active origin of replication at its native chromosomal locus (22). By the same token, a GAL4 derived activator that stimulates replication in a plasmid context may not have the corresponding effect on a chromosomally embedded origin. To examine the ability of GAL4-SP1 and GAL4-CTF1 to stimulate chromosomal replication, the native chromosomal ARS1 was replaced by a modified ARS1 in which all three B elements were abolished and five GAL4-binding sites were engineered (9) (see diagram in Fig. 3). Replication intermediates encompassing the ARS1 region were analyzed by a two-dimensional gel electrophoresis assay, which separated replication intermediates initiated at ARS1 (bubble arc) from those initiated at an origin outside the genomic region being analyzed (Y arc). Using this technique, it has been shown that acidic GAL4 activators such as GAL4-ABF1 and GAL4-BRCA1 can stimulate chromosomal replication at the native ARS1 locus (8, 9).

As shown in panel A of Fig. 3, GAL4-DBD alone resulted in a very weak bubble signal (indicated by an arrow). In contrast, GAL4-CTF1 (panel B) and GAL4-ABF1 (panel D) gave rise to much stronger bubble signals. A point mutation in the ARS consensus element that destroys function of the native ARS1 (13) also obliterated the CTF1-enhanced initiation of replication at the modified ARS1 (panel C). This demonstrates that the increased bubble signal in the presence of GAL4-CTF1 indeed resulted from activation of ARS1 rather than a cryptic origin of replication in the nearby region. Curiously, neither GAL4-SP1A (panel E) nor GAL4-SP1B (panel F) could stimulate initiation of replication from the chromosomal ARS1 to an appreciable level. This is reminiscent of the behavior of the glutamine-rich activation domains in yeast transcriptional activation (18) (see below for more detailed discussion). Thus, while all three major types of mammalian activation domains stimulate plasmid DNA replication in yeast, only acidic activators and GAL4-CTF can stimulate initiation of replication from a chromosomally embedded origin.

GAL4-CTF1 Remodels the Local Chromatin Structure Around the Origin of DNA Replication—A recent study suggests that chromatin remodeling is an important mechanism used by acidic activators to enhance origin function in yeast (9). To determine whether the non-acidic domains could cause a similar effect on chromatin, nuclei of the yeast cells that expressed various GAL4 derivatives were treated with limited amounts of micrococcal nuclease (MNase), and the nuclease digestion pattern around the modified chromosomal ARS1 was examined by an indirect end-labeling assay (23). In keeping with the previous observation (9), acidic activators GAL4-ABF1 and GAL4-BRCA1 caused significant changes in the nuclease digestion pattern at the B region of ARS1 (indicated by asterisks, compare lanes 1 and 2 with 5, 6, 9, and 10 in Fig. 4). GAL4-CTF1 also induced similar alterations in chromatin structure as the acidic activators (lanes 7 and 8). In contrast, these changes in nuclease sensitivity were not detected in the presence of the glutamine-rich GAL4-SP1A (lanes 3 and 4). Therefore, the effects of the GAL4 derivatives on chromosomal replication correlate with their ability to induce chromatin reconfiguration.

The chromatin remodeling could be a cause or an effect of the enhanced initiation of replication at the chromosomal ARS1. To distinguish these two possibilities, GAL4-CTF1 expressing cells were arrested at a non-S phase period of the cell cycle: either G1 with the yeast mating pheromone a-factor or G2/M with the microtubule inhibitor nocodazole. Fluorescence activated cell sorting analysis showed that more than 95% cells were blocked at the corresponding stages of the cell cycle (data not shown). The asynchronous, G1- and G2/M-arrested cultures were compared for the MNase digestion pattern around the chromosomal ARS1 by the indirect end labeling method. As indicated in Fig. 5, the CTF1-induced changes observed in the asynchronous population (lanes 3 and 4) were also present in both G1- (lanes 5 and 6) and G2/M-arrested cells (lanes 7 and 8). This result suggests that chromatin remodeling is not merely a
The effects of various GAL4 derivatives on the local chromatin structure around the modified chromosomal ARS1. Indirect end-labeling assay was used to probe the MNase digestion pattern in the EcoRI restriction fragment encompassing the ARS1 region on chromosome IV. Nuclei were treated with MNase for 2 (lanes 1, 3, 5, 7, and 9) and 5 min (lanes 2, 4, 6, 8, and 10). The radioactive probe used was an EcoI-EcoRI fragment corresponding to one end of the genomic fragment examined (indicated by a thick bar on left). The asterisks designate the most prominent changes in nuclease digestion pattern induced by some GAL4 derivatives. The approximate positions of the GAL4-binding sites and the four ARS1 elements are indicated on the left. As a control, naked genomic DNA was digested with a limited amount of nuclease and the digestion pattern is shown in lane 11.

GAL4-CTF1 can remodel chromatin in the absence of ORC binding. A, indirect end-labeling assay was used to compare the effect of GAL4-CTF1 on chromatin structure in the presence (5×G/-B) and absence (5×G/-A/-B) of the ORC-binding site. B, the same nuclease-digested genomic DNA as used in A was analyzed in a nucleosome array assay. The blot was first probed with a 200-base pair fragment covering the ARS1 region (top), subsequently stripped, and reprobed with a SalI-HindIII fragment located 6 kilobases upstream of the chromosomal ARS1 (bottom). The two radioactive probes are indicated as thick bars at the right. In both panels, nuclei were treated with MNase for 2 (lanes 1, 4, and 7), 5 (lanes 2, 5, and 8), and 10 min (lanes 3, 6, and 9).

Sp1 and CTF1 Enhance Yeast DNA Replication

DISCUSSION

Both Sp1 and CTF1 are known to stimulate viral DNA replication in mammalian cells. In the current study, it is shown that the activation domains of both proteins are capable of enhancing function of a plasmid-borne cellular origin of replication. Interestingly, both activation domains appear to employ common hydrophobic patches to stimulate transcription and DNA replication. Together with previous studies of acidic activators, this work clearly indicates a high degree of conservation in the mechanisms used by transcription factors to stimulate eukaryotic DNA replication. Furthermore, it supports the notion that activation of replication and transcription may share similar mechanistic pathways.

The study also reveals some intriguing differences among
the three different types of transcriptional activation domains. In particular, the glutamine-rich domains of Sp1 can only stimulate DNA replication in a plasmid, but not in a chromosomal context. In contrast, the acidic activators and the minimal activation domain of CTF1 can function in both contexts. Furthermore, acidic activators and GAL4-CTF1, but not GAL4-Sp1, can induce changes of the local chromatin structure around the chromosomal origin of replication. These differences may reflect distinct mechanisms used by these activators to stimulate replication. Alternatively, it could merely be due to different affinities of these domains for a common target(s). In the case of acidic activators and GAL4-CTF1, chromatin remodeling appears to be an important mechanism for activation of chromosomal DNA replication. How does the glutamine-rich Sp1 stimulate plasmid DNA replication in yeast remains to be understood. Likewise, it is not known why GAL4-Sp1 fails to induce chromatin remodeling and stimulate replication in the chromosomal context. In light of the differences in chromatin structure between plasmid and chromosome, it is conceivable that the interaction between Sp1 and its putative target, which suffices in activation of plasmid DNA replication, may not be strong enough to withstand the negative effect imposed by the more compacted chromatin structure on chromosome.

The behavior of the Sp1 glutamine-rich domains in activation of yeast DNA replication bears obvious similarity to that in yeast transcriptional activation. In contrast to acidic activators and GAL4-CTF that can activate transcription in both budding yeast and higher eukaryotes, glutamine-rich domains are known to be transcriptionally inactive in budding yeast (15, 16). However, a recent study by Xiao and Jeang (18) suggests that, while glutamine-rich domains do not function in a chromosomally embedded promoter in budding yeast, they can significantly stimulate transcription in a plasmid such as a 2-micron based vector. It is reasonable to propose that the lack of Sp1-mediated activation of transcription and replication on the chromosome can be attributed to the same cause, namely, the inability of the glutamine-rich domain to overcome the chromosomal repression. It remains to be determined whether glutamine-rich activators such as Sp1 could stimulate yeast replication or transcription on chromosomes when other transcription factors are present in the same origin/promoter region.

The minimal region in CTF1 that mediates chromatin remodeling and activation of DNA replication is mapped to the last 14 amino acid residues of the activation domain of the protein. Interestingly, the same domain has been shown to be responsible for the transforming growth factor-β responsive transcriptional activation by CTF1 in mammalian cells (21). Furthermore, this domain of CTF1 is also important for the physical interaction between CTF1 and histone H3 (21). While the exact target for CTF1-dependent chromatin remodeling and activation of replication in yeast remains to be elucidated, it is tempting to speculate that the histone H3-CTF1 interaction may cause alteration of the local nucleosome positioning, which in turn may facilitate assembly of a preinitiation complex at the origin of replication. The powerful genetic tools offered by the yeast system should allow us to test this hypothesis and to identify other potential targets that mediate CTF1 function in activation of DNA replication.

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