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Interaction of transcription factor YY1 with a replication-enhancing element, REE1, in an autonomously replicating human chromosome fragment

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ABSTRACT

We have previously shown that autonomous replication of human chromosome fragments is stimulated by the presence of an 18 bp sequence, REE1, which exhibits transcriptional silencer activity. The REE1 sequence is partly homologous with the serum response element (SRE) required for expression of the human c-*fos* gene. Here we have examined interaction of REE1 with human nuclear proteins using a gel retardation assay. One of the REE1–protein complexes formed showed almost the same mobility as the SRE–protein complex and complex formation was competitively inhibited by the SRE fragment. The protein complex with REE1 as well as that with SRE was found to contain the transcription factor YY1, known to bind to the SRE. These results suggest that YY1 protein may participate in stimulation of replication through its interaction with REE1.

INTRODUCTION

Initiation of replication of eukaryotic chromosomes occurs from specific regions through the actions of protein factors that recognize unique sequences. In the budding yeast *Saccharomyces cerevisiae* ~100 bp chromosomal fragments can replicate autonomously as plasmids (autonomously replicating sequence, ARS) (1,2). All the ARS fragments contain a match to an 11 bp sequence, called the ARS consensus sequence (ACS), that is essential for ARS function (3). Detailed analysis of *ars1*, which has been proven to be a chromosomal replication origin, has shown that ARS function requires element A containing the ACS, together with two out of the B1, B2 and B3 elements (4). Element A is recognized by a protein complex consisting of six protein subunits called the origin recognition complex (ORC) (5). At least some of the ORC components have been shown to be involved in transcriptional silencing at the mating type controlling locus (6). In addition, element B3 is the binding site for ARS binding factor 1 (ABF1), which is known to be a transcription factor participating in regulation of expression of many yeast genes (4,7,8). These findings suggest that certain transcription factors contribute to the initiation of yeast chromosome replication.

Structures of replication origins in higher eukaryotic chromosomes have not been well elucidated. Physical mapping of initiation sites of replication has revealed that they are restricted to regions in the range 0.5–55 kb (9). Among a number of replication origins identified in higher eukaryotic chromosomes, there are only two origins whose origin functions have been shown to require specific chromosomal regions. The human β-globin origin has been mapped within a 2 kb region upstream of the gene and deletion of the region eliminates the origin activity (10). In addition, replication from the β-globin origin requires another region ~50 kb from the origin, called the locus control region (LCR), involved in regulation of expression of globin genes (11). The replication origin for amplification of the *Drosophila* chorion gene cluster has been located within a 3 kb region in the gene cluster, with an upstream region required for efficient replication of the chorion genes (12). These findings suggest that multiple regions are required for initiation of replication and that sequence elements required for regulation of transcription might participate in replication.

Short chromosome fragments capable of autonomous replication have not yet been isolated from mammalian chromosomes. Randomly cloned human chromosome fragments of ~10 kb are capable of autonomous replication to a significant extent (13,14). By using BrdU labeling of cells shortly after transfection with plasmid DNA, we have shown that particular fragments of human chromosomes display several fold higher efficiencies of replication than others (15). One such fragment, W1-1, contains a unique 18 bp sequence, the replication-enhancing element REE1 (16). The REE1 element exhibits another activity to repress transcription from the SV40 early promoter when inserted at a site 2.7 kb upstream of the promoter. Base substitutions at various positions within REE1 impair both the replication-enhancing activity and

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the transcription silencing activity. REE1 has partial homology with the serum response element (SRE) required for activation of expression of the human c-*fos* gene (16). Since the SRE is known to bind to cellular transcription factors such as serum response factor (SRF) and YY1, we considered the possibility that REE1 may act as a binding site for protein factors that affect transcription and replication of human chromosome fragments.

In this study we show that REE1 interacts with at least two distinct protein factors present in human nuclear extracts, one being transcription factor YY1. The results suggest involvement of YY1 in stimulation of autonomous replication and repression of transcription.

MATERIALS AND METHODS

Cell culture

The cell line 293S is a suspension-adapted derivative of a human embryonic kidney cell line (293) transformed by adenovirus type 5 (17). For the present study the cells were cultured at 37°C under 5% CO_2 in DMEM supplemented with 10% fetal bovine serum.

Oligonucleotides

The oligonucleotides used for the gel retardation assays are shown below: SREF, 5′-AGCCACAGGATGTCCATATTAGGACATCTGCGTC-3′; SRER, 5′-GCTGACGCAGATGTCCTAATATGGACATCCTGTG-3′; mSREF, 5′-AGCCACAGGATGTGGATATTACCACATCTGCGTC-3′; mSRER, 5′-GCTGACGCAGATGTGGTAATATCCACATCCTGTG-3′; RE2F, 5′-GGCCATGTCGACCATGTTGCTGTCCATGGTCCTAAGATCG-3′; RE2R, 5′-ACCCGATCTTAGGACCATGGACAGCAACATGGTCGACATG-3′; RX2F, 5′-GGCCATGTCGACCATGTTCTCGAGCATGGTCCTAAGATCG-3′; RX2R, 5′-GCCCGATCTTAGGACCATGCTCGAGAACATGGTCGACATG-3′; SX1F, 5′-CGAAACACTCGAGGCTGTCCTCGAGCCTAAATAA-3′; SX1R, 5′-ACGTTATTTAGGCTCGAGGACAGCCTCGAGTGTT-3′. Other oligonucleotides have been described previously (16).

Preparation of nuclear extracts

Human 293S cells grown in spinner flasks were collected by centrifugation at 1200 r.p.m. for 5 min in a Beckman JS-3.0 rotor and rinsed with phosphate-buffered saline (PBS) and then with hypo-buffer [20 mM HEPES, pH 7.7 at 25°C, 5 mM KCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 20 µg/ml leupeptin]. Cells suspended in an equal volume of hypo-buffer were homogenized with a Dounce homogenizer using a B pestle and incubated for 30 min on ice. The nuclei were pelleted by centrifugation and suspended in an equal volume of nuclear extraction buffer containing 25 mM Tris–HCl, pH 7.5, 0.5 M NaCl, 1 mM EDTA, 10 mM sodium bisulfate, pH 7.2, 1 mM DTT, 0.1 mM PMSF and 2 µg/ml leupeptin and stirred for 30 min. Suspensions were centrifuged at 32 000 r.p.m. at 4°C for 1 h in a Beckman type 50Ti rotor and supernatants (~7.5 mg protein/ml) were frozen in liquid nitrogen and stored at –80°C.

Immunoblotting

After polyacrylamide gel electrophoresis proteins were transferred to PVDF membranes (Millipore) at 100 mA for 90 min in blotting buffer containing 100 mM Tris, 192 mM glycine, and 20% methanol, pH 8.3, with a semi-dry transfer apparatus. The membranes were soaked in blocking solution containing 20 mM Tris–HCl, pH 7.4, and 10% skimmed milk (Difco) for 30 min and incubated with anti-YY1 antibody (18) in PBS supplemented with 0.05% Tween 20 (TPBS) for 1 h at room temperature. After washing with TPBS three times they were incubated with 1:5000 diluted peroxidase-conjugated anti-rabbit goat IgG (BioRad) in TPBS for 1 h. Unbound antibody was washed out with TPBS three times and signals were visualized with ECL detection reagent as recommended by the manufacturer (Amersham).

Gel mobility shift assay

Oligonucleotides were annealed and labeled with [^{32}P]dCTP by the large fragment (Klenow fragment) of *Escherichia coli* DNA polymerase I. Free nucleotides were removed by a spin column of Sephadex G-50. Labeled probe DNA (0.3 ng, 50 000 c.p.m.) was incubated with 15 µg nuclear extract for 1 h on ice in a binding mixture (10 µl) containing 10 mM Tris–HCl, pH 7.4, 5% glycerol, 1 mM EDTA, 1 mM DTT and 2 µg poly(dI·dC). Where indicated, non-radioactive competitor DNA was added to the binding reactions simultaneously with the labeled probe. The DNA–protein complexes formed were separated by electrophoresis in 5% polyacrylamide gels at 20 V/cm at 4°C in circulating TAE buffer (6.75 mM Tris–HCl, pH 7.9, 3.3 mM sodium acetate, pH 7.9, and 1 mM EDTA). Dried gels were placed in contact with imaging plates for 1 h and the stored images analyzed with an Image Analyzer Bas1000Mac (Fuji Film, Tokyo, Japan).

RESULTS

Human nuclear proteins that interact with REE1

A gel retardation assay was employed to examine the interaction of REE1 with human nuclear proteins. ^{32}P-Labeled human fragment W1 containing the REE1 element (from position 923 to 953 of the W1-1 fragment; Fig. 1; 16) was incubated with a nuclear extract of human 293S cells and subjected to PAGE. As shown in Figure 2, several discrete bands migrating much slower than the free probe DNA were detected (complexes R-I, R-II, R-III and R-IV in Fig. 2, lane 1). None of these bands or smears were observed with incubation of the free probe without the nuclear extract (data not shown). Addition of the non-radioactive W1 fragment as a competitor in 25- or 125-fold excess to the radioactive probe completely inhibited formation of the R-I and R-II complexes and reduced the amount of R-IV complex (Fig. 2, lanes 2–4). In contrast, formation of the R-III complex was not affected by addition of non-labeled W1 fragment. These results suggest that formation of the R-I, R-II and R-IV complexes is dependent on the sequence of the W1 fragment.

To delimit the regions involved in formation of the R-I, R-II and R-IV complexes base substitutions were introduced into the competitor W1 fragment. Fragments X1 and X2 contained base substitutions from positions 940 to 944 and from 933 to 938 respectively and fragment SX carried a pair of substitutions from position 928 to 932 and 940 to 944 (Fig. 1B). All these substitutions have been shown to abolish the replication-enhancing activity and transcriptional silencer activity of REE1 (16). Addition of fragment X1 to the reaction mixture inhibited formation of the R-I and R-II complexes, but it did not affect R-IV complex formation (Fig. 2, lanes 5–7). In contrast, addition of fragment X2 greatly

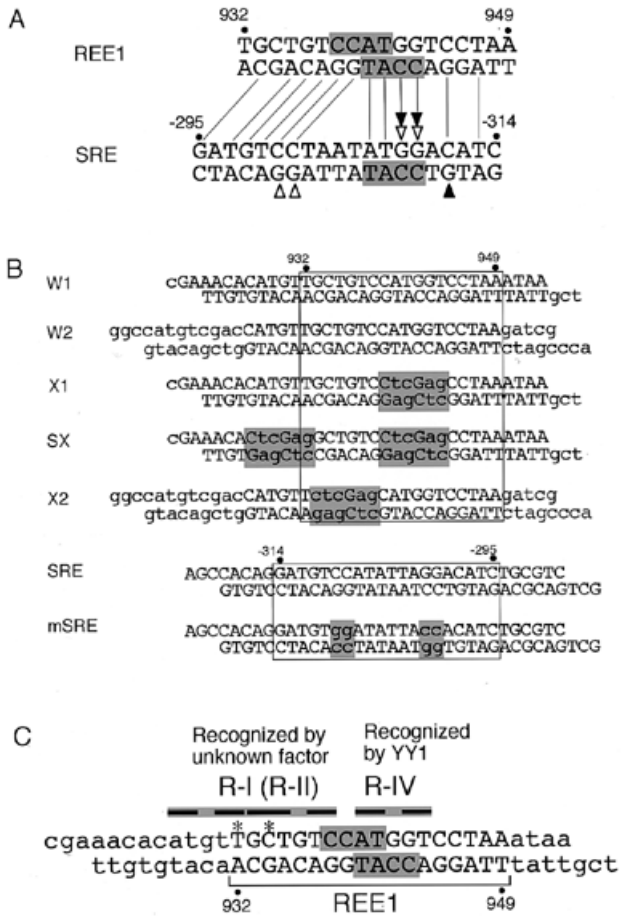

Figure 1. Nucleotide sequences of REE1 and the SRE. (**A**) Nucleotide sequences of REE1 and the SRE are presented. The numbers above the sequence indicate the positions in the human autonomously replicating fragment W1-1 (16) or in the upstream region of the mouse c-*fos* gene (36). The nucleotides homologous between REE1 and the SRE are shown by vertical lines. Shadowed bases show the core sequence for binding of YY1 protein. The filled and open triangles below the SRE sequence show bases protected from methylation by binding of YY1 and SRF respectively (37). (**B**) The nucleotide sequences of oligonucleotides used for the gel retardation assay are shown. The sequences corresponding to REE1 and the SRE are shown within thin lined squares. The lower case letters in the shaded regions are bases changed in mutant oligonucleotides. (**C**) Regions of REE1 possibly involved in interactions with protein factors as discussed below are indicated. The shaded regions indicate the CCAT motif conserved for YY1 recognition. The asterisks show bases different from those of the SRE. Bases altered by linker substitutions are indicated by thin bars above the sequence and the regions that affect formation of complexes R-I (or R-II) and R-IV are shown by shaded bars.

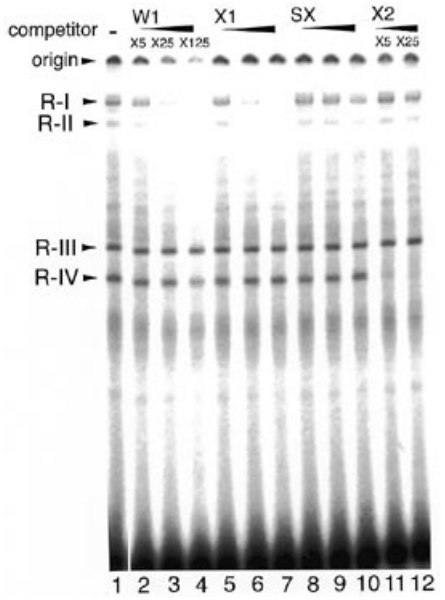

Figure 2. Interactions of REE1 with human nuclear proteins. The ^{32}P-labeled W1 fragment was incubated with the 293S nuclear extract for 1 h on ice and the protein–DNA complexes separated by polyacrylamide gel electrophoresis. Positions of complexes R-I, R-II, R-III and R-IV formed with the wild-type REE1 probe are indicated by arrowheads. Non-radioactive competitor DNA was added at 5, 25 and 125 times more than the labeled probe, as shown at the top of the panel. (–) indicates reaction without a competitor fragment.

reduced the amount of the R-IV, but not of the R-I nor the R-II complex (Fig. 2, lanes 11–13). These results showed that proteins involved in formation of the R-IV complex are unable to interact with fragment X1, while those participating in formation of the R-I or R-II complex do not interact with X2. Fragment SX did not affect formation of any complexes (Fig. 2, lanes 8–10), suggesting that base changes from position 928 to 932 impaired formation of the R-I and R-II complexes. The results were confirmed by experiments in which ^{32}P-labeled mutant probes were incubated with the nuclear extract. The R-I and R-II complexes were formed in the presence of the labeled X1 probe, while R-IV was formed with the X2 probe (data not shown). Thus the region from position 928 to 938 appears to be required for formation of the R-I and R-II complexes, while that from position 940 to 944 is necessary for R-IV complex formation (Fig. 1C).

Interaction of REE1 and SRE with a common protein factor

Since REE1 is partly homologous with the SRE upstream of the c-*fos* gene (Fig. 1A), we tested whether proteins capable of binding to REE1 could interact with the SRE. By addition of a non-radioactive SRE fragment in excess to the reaction mixture containing the labeled REE1 probe (fragment W2; Fig. 1B) formation of R-IV was reduced, while that of R-I or R-II was not significantly affected (Fig. 3, lane 4). In contrast, addition of a mutant SRE fragment (mSRE) with base changes at putative binding sites for transcription factor YY1 and SRF (Fig. 1B) did not affect complex formation (Fig. 3, lane 5). These results suggest that a protein(s) participating in formation of the R-IV complex interacts with the SRE.

To identify the SRE–protein complex that shares sequence specificity with the R-IV complex, a ^{32}P-labeled SRE probe was incubated with nuclear extract. Among several bands shifted from the free probe a major band (complex S-I) had a mobility almost the same as that of the R-IV complex (Fig. 3, lane 6). The S-I complex was not formed with the mSRE probe (Fig. 3, lane 7), indicating a requirement for the SRE sequence. Consistent with these results, S-I complex formation was competed out by addition of non-radioactive SRE but not the mSRE fragment (Fig. 3, lanes 10 and 11). By addition of fragment W2 as a competitor to the reaction mixture containing the labeled SRE probe, formation of the S-I complex was specifically inhibited (Fig. 3, lane 8). On the

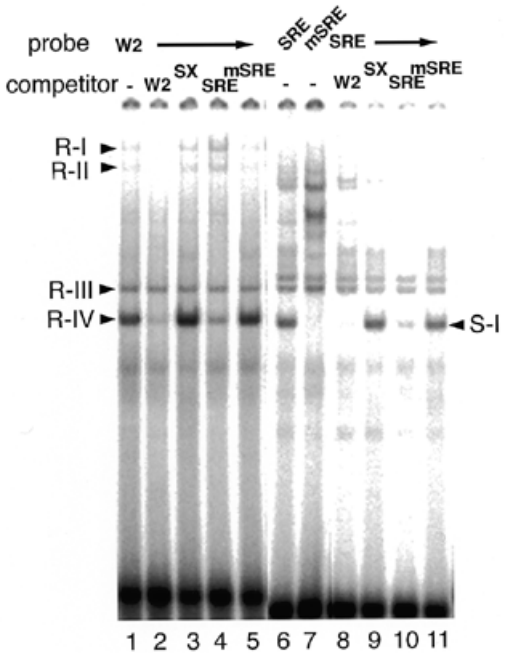

Figure 3. Competition with REE1–protein complex formation by the c-*fos* SRE fragment. A gel retardation assay was performed as described in Figure 2. The names of ^{32}P-labeled probes and the competitor DNA added to the probe at 100 times excess are shown at the top of the panel. (–) indicates reaction without a competitor fragment. The positions of complexes on the REE1 probe (R-I, R-II, R-III and R-IV) and the SRE probe (S-I) are shown by arrowheads.

other hand, it was not affected by addition of fragment SX, with base substitutions in REE1 (Fig. 3, lane 9) or the X1 fragment (data not shown). These results suggest that a common protein factor(s) recognizes both REE1 and SRE.

Interaction of transcription factor YY1 with REE1

The REE1 region involved in formation of the R-IV complex contains a CCAT sequence, a consensus core motif for binding of transcription factor YY1 (19). The YY1 protein, which has been shown to bind to the SRE, might be an REE1-interacting factor. In order to examine this possibility, we examined the effects of a rabbit polyclonal antibody raised against YY1 on formation of the REE1–protein complex in gel retardation experiments (18). The antibody was confirmed to react specifically with a single polypeptide with an apparent molecular mass of 68 kDa (Fig. 4A, lanes 3 and 4), consistent with the mobility of YY1 on SDS–PAGE (20). Addition of the antibody to the binding reaction mixture containing W1 or W2 probe resulted in reduced formation of R-IV but not R-I or R-II (Fig. 4B, lanes 1–3 and 7–9). No supershift band containing the REE1–protein complex and the antibody was observed. Instead, the radioactivity at the gel origin was slightly increased (Fig. 4B, lanes 2, 3 and 9). This could be due to possible aggregation of the R-IV complex in the presence of the antibody. When the antibody was added to the reaction mixture containing the SRE probe the amount of S-I complex was specifically reduced (Fig. 4B, lanes 4–6). Addition of non-YY1 antibody, such as anti-human CENP-B rabbit antibody or anti-human c-myc mouse monoclonal antibody, did not affect the results of gel retardation (data not shown). Therefore, the protein factor interacting with REE1 and SRE in common was concluded to be YY1.

Because the reduction in R-IV and S-I complexes was not accompanied by the appearance of a supershift band containing the anti-YY1 antibody, we performed an experiment to confirm the existence of YY1 in the R-IV complex. The non-labeled fragment was incubated with the extract and the products separated by gel electrophoresis. Then the proteins were transferred to a PVDF membrane and analyzed by immunostaining with the anti-YY1 antibody. As shown in Figure 4C, a single band was detected at the position corresponding to that of the R-IV complex when the W2 fragment was incubated with the extract (lane 4). The fact that this immunoreactive band was not seen in the absence of the DNA fragment excluded the possibility that the YY1 protein alone migrated to the same position (Fig. 4, lane 3). It was also not detected when a mutant SX fragment was applied instead of the W2 fragment. No immunostaining band was observed at the position of R-I or R-II, suggesting that these complexes did not contain YY1, in contrast to R-IV. The band was detected at the position of S-I with the SRE but not the mSRE fragment (Fig. 4C, lanes 5 and 7). Taking these results together with specific reduction of the R-IV and S-I complexes in the presence of the antibody (Fig. 4B), we can conclude that YY1 is a common protein factor interacting with REE1 and SRE.

DISCUSSION

We have previously identified an 18 bp sequence, REE1, that stimulates autonomous replication of human chromosome fragments. In order to elucidate the role of REE1 we examined here its interactions with human nuclear proteins and demonstrated a link with transcription factor YY1, which participates in transcriptional activation and repression of a variety of human genes (21,22).

The immunostaining experiments showed that YY1 protein is a component of both the R-IV and S-I complexes with REE1 and the SRE respectively (Fig. 4). It is not likely that the R-IV and S-I complexes contain an additional protein factor, since the mobility of complex R-IV is in good agreement with the reported mobility of the SRE fragment bound to purified YY1 protein (19; C.Obuse, unpublished results). The region of REE1 homologous with the SRE should be recognized by YY1. REE1 contains a pair of partially overlapping CCAT motifs, a core consensus motif for binding of YY1, in opposite orientations (Fig. 1). The X1 substitution, which disrupts both motifs, impairs formation of the R-IV complex, suggesting that either or both of the CCAT motifs are required for interaction with YY1. The X2 substitution, altering the first C of the CCAT motif in the top strand, does not impair interaction with YY1, perhaps suggesting that the motif on the top strand is not important for interaction with YY1. However, in the human globin locus it has been shown that an alternative consensus core motif, ACAT, efficiently binds YY1 (23). Because of the lesser importance of the first base of the CCAT motif, we cannot exclude the possibility that the motif on the top strand in REE1 is involved in the interaction with YY1.

We have previously shown that base substitutions in REE1, including X1 and X2 used in this study, abolish replication-enhancing activity as well as transcription silencer activity of REE1 (16). Since the X2 substitution does not impair binding of YY1, interaction of an additional factor with REE1 seems to be required for the *in vivo* functions of REE1. It has been suggested

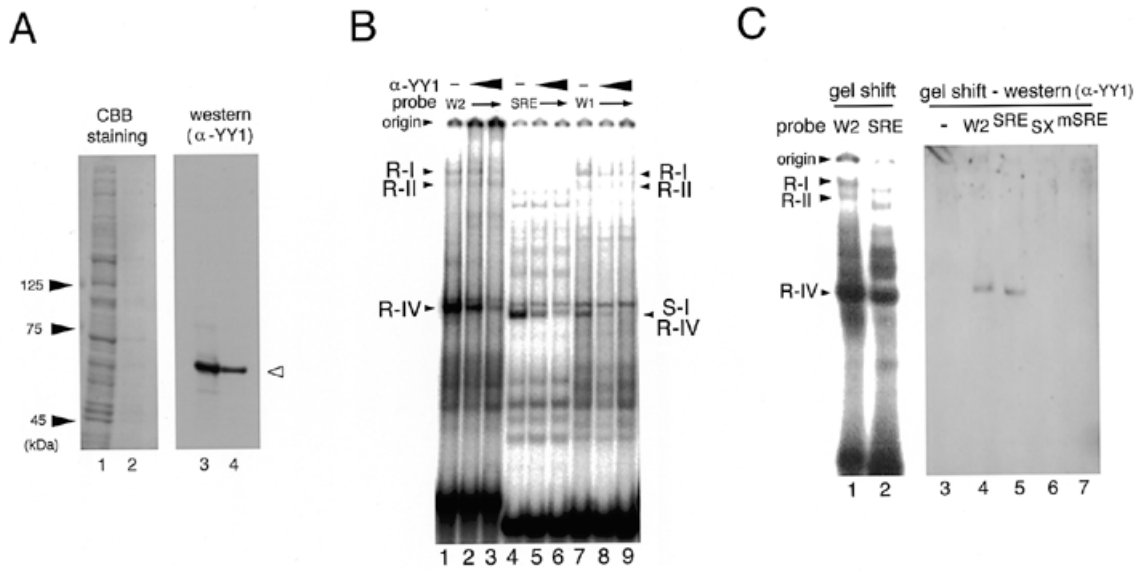

Figure 4. Identification of YY1 in complexes with REE1 and the SRE. (**A**) Human nuclear extract (7.5 µg for lanes 1 and 3 or 0.75 µg for lanes 2 and 4) was separated by 6% polyacrylamide gel electrophoresis. Proteins were either stained with Coomassie brilliant blue (lanes 1 and 2) or analyzed by Western blotting with anti-YY1 polyclonal antibody (lanes 3 and 4). The positions of molecular mass marker proteins are shown on the left. (**B**) A gel retardation assay was performed as described in Figure 2 except that 0.5 (lanes 2, 5 and 8) or 1 µl (lanes 3, 6 and 9) anti-YY1 antibody was added to the reaction mixture. The names of probes are shown above the panel. Positions of complexes R-I, R-II and R-IV are indicated at the sides of the panel. Complex R-III was not efficiently formed in this experiment. (**C**) ^{32}P-Labeled probes (lanes 1 and 2) or non-radioactive DNA (30 ng, lanes 4–7) were incubated with nuclear extract (15 µg). After gel electrophoresis, proteins incubated with non-radioactive DNA were transferred to PVDF membrane and analyzed by immunostaining with anti-YY1 antibody (lanes 3–7). The names of the oligonucleotides are shown above the panel. (–) indicates reaction without any oligonucleotide.

that transcriptional activation or repression by YY1 requires an additional protein factor that interacts with sequences adjacent to the YY1 binding site (24–26). One such factor has been shown to be SRF, which interacts with the SRE. However, the SRE sequence inserted in human fragments does not show any effect on autonomous replication activity, suggesting that the SRF is not involved in stimulation of replication (C.Obuse, unpublished results). Since the human 293S cell was made by transformation with an adenovirus fragment, it might be possible that the adenovirus gene product E1A, which has been shown to interact with YY1, may participate in stimulation by REE1. However, we have previously shown that the W1-1 fragment carrying REE1 replicated at a higher efficiency than other fragments in human IMR32 and mouse 10T1/2 cells, which were not transformed by any viral DNA (15). Thus E1A is unlikely to be involved in stimulation of replication of the human fragment by REE1.

Besides the R-IV complex containing YY1, complexes R-I and R-II were specifically made on REE1. The results of competition experiments (Fig. 2) suggest that the R-I and R-II complexes are likely to contain a common component that recognizes a portion of REE1 adjacent to the possible YY1-interacting sequence. Formation of R-I and R-II is not inhibited by addition of the SRE fragment, showing that proteins participating in complex formation do not interact with the SRE. The R-I or R-II complex might be required for a REE1-specific activity, such as stimulation of replication. Since the region involved in formation of the R-I and R-II complexes is homologous to the SRE except for bases 932 and 934 (shown by asterisks in Fig. 1C), these positions of REE1 could be required for formation of the R-I and R-II complexes. Because the entire region of REE1 is necessary for stimulation of replication as well as repression of transcription, simultaneous interactions of REE1 with YY1 and another protein factor(s) seem to be required for the *in vivo* activities of REE1. We have not as yet been able to detect a complex which depends on the entire region of REE1. Possible complexes containing components of R-I (or R-II) and R-IV might be too large to enter the gel matrix or might not be formed efficiently under our experimental conditions.

How could binding of YY1 to REE1 participate in stimulation of autonomous replication as well as repression of transcription? It has been shown that binding of YY1 induces bending of DNA (19). Possible alteration of DNA structures around REE1 could affect interactions of DNA with proteins involved in assembly of replication initiation complexes as well as those involved in initiation of transcription. YY1 may directly interact with factors involved in initiation of replication, as reported for those in initiation of transcription (24,25,27). Alternatively, since it has been reported that autonomous replication of a chromosome fragment is inhibited by transcription through the fragment (28), it is possible that repression of transcription by interaction of YY1 with REE1 would reduce this inhibitory effect. We have detected a putative promoter located near the REE1 element on the native 10 kb chromosome fragment and found transcription from the promoter to be enhanced by disruption of REE1 (C.Obuse, unpublished results). However, deletion of the putative promoter region did not affect the replication-enhancing activity of REE1 (16). Thus it is likely that interactions of protein factors, including YY1, with REE1 rather than the resulting repression of transcription mediate enhancement of replication.

It has been shown that fragments derived from the *DHFR* origin region of Chinese hamster ovary cells do not replicate autonomously significantly differently from non-origin fragments (29). On the

other hand, we have found that the chromosomal replication origins in a 320 kb region of human chromosome 10 are associated with fragments exhibiting higher autonomous replication activity than other fragments (Y.Ogawa and H.Masukata, unpublished observations). The difference in autonomous replication efficiency in the latter study was at most 3-fold, which might not have been distinguished in the former study. It has been shown that multiple regions distantly located from each other are required for efficient replication from distinct origins in higher eukaryotic chromosomes (11,12). A fragment carrying one such region would replicate more efficiently than other fragments. The REE1 element that stimulates autonomous replication may be a possible element involved in initiation of chromosomal replication. It would be necessary to examine possible involvement of REE1 in initiation of replication from a specific region of the chromosome. Among various replication origins discovered on mammalian chromosomes, a requirement for specific regions has been shown for the human β-globin replication origin. The LCR located >50 kb from the origin is required for origin activity (11) as well as for regulation of expression of the globin genes. Since the LCR contains a number of putative YY1 recognition sites, YY1 is assumed to be one of many proteins involved in transcriptional regulation of the locus (23). Although sequence elements of the LCR required for replication from the β-globin origin have not been elucidated, binding of YY1 to the LCR, as observed for REE1, may participate in initiation of replication from the β-globin origin.

The budding yeast ORC, which binds to the essential ARS consensus element, is considered to be crucial for initiation of replication (5), while transcription factors such as ABF1 that bind to a replication-enhancing element have only an auxiliary role. Recently genes homologous to components of the ORC have been identified in various eukaryotic cells (30–32). It has been shown that the *Xenopus* ORC complex is essential for replication of sperm DNA in *Xenopus* egg extracts (33). Because initiation occurs at random sites on the chromosome DNA in *Xenopus* eggs and early embryos (34), the *Xenopus* ORC complex itself may not recognize highly specific nucleotide sequences. On the other hand, replication in *Xenopus* somatic cells is initiated from specific regions of the chromosome (35). These observations suggest that recognition of specific regions of the chromosome by a certain factor other than the ORC complex might be involved in initiation of replication in somatic cells. One major difference between embryonic and somatic cells is the presence and absence of transcription. Activation or repression of specific regions of somatic chromosomes is achieved by interaction of transcription factors with specific recognition sequences on the chromosome. Activation of specific chromosome regions for initiation of replication may utilize a mechanism overlapping with that for regulation of transcription. Stimulation of replication through transcription factors such as YY1 may increase the frequency of initiation from specific regions of the chromosome. Replication-enhancing elements that stimulate initiation of replication could play a more important role in higher eukaryotic cells than in yeast cells.

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REFERENCES

1 Struhl,K., Stinchcomb,D.T., Scherer,S. and Davis,R.W. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 1035–1039.
2 Hsiao,C.-L. and Carbon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3829–3833.
3 Broach,J.R., Li,Y.-Y., Feldman,J., Jayaram,M., Abraham,J., Nasmyth,K.A. and Hicks,J.B. (1983) *Cold Spring Harbor Symp. Quant. Biol*., **47**, 1165–1173.
4 Marahrens,Y. and Stillman,B. (1992) *Science*, **255**, 817–823.
5 Bell,S.P. and Stillman,B. (1992) *Nature*, **357**, 128–134.
6 Bell,S.P., Kobayashi,R. and Stillman,B. (1993) *Science*, **262**, 1844–1849.
7 Buchman,A.R., Kimmerly,W.J., Rine,J. and Kornberg,R.D. (1988) *Mol. Cell. Biol.*, **8**, 210–225.
8 Shore,D., Stillman,D.J., Brand,A.H. and Nasmyth,K.A. (1987) *EMBO J.*, **6**, 461–467.
9 Huberman,J.A. (1995) *Cell*, **82**, 535–542.
10 Kitsberg,D., Selig,S., Keshet,I. and Cedar,H. (1993) *Nature*, **366**, 588–590.
11 Aladjem,M.I., Groudine,M., Brody,L.L., Dieken.E.S., Fournier,R.E.K., Wahl.G.M. and Epner,E.M. (1995) *Science*, **270**, 815–819.
12 Delidakis,C. and Kafatos,C. (1989) *EMBO J.*, **8**, 891–901.
13 Krysan,P.J., Haase,S.B. and Calos,M.P. (1989) *Mol. Cell. Biol.*, **9**, 1026–1033.
14 Heinzel,S.S., Krysan,P.J., Tran,C.T. and Calos,M.P. (1991) *Mol. Cell. Biol.*, **11**, 2263–2272.
15 Masukata,H., Satoh,H., Obuse,C. and Okazaki,T. (1993) *Mol. Biol. Cell*, **4**, 1121–1132.
16 Obuse,C., Okuno,Y., Okazaki,T. and Masukata,H. (1996) *Mol. Biol. Cell*, **7**, 43–55.
17 Stillman,B.W. and Gluzman,Y. (1985) *Mol. Cell. Biol.*, **5**, 2051–2060.
18 Nemoto,Y., Terajima,M., Shoji,W. and Obinata,M. (1996) *J. Biol. Chem.*, **271**, 13542–13548.
19 Natesan,S. and Gilman,M.Z. (1993) *Genes Dev.*, **7**, 2497–2509.
20 Shi,Y., Seto,E., Chang,L. and Shenk,T. (1991) *Cell*, **67**, 377–388.
21 Shrivastava,A. and Calame,K. (1994) *Nucleic Acids Res.*, **22**, 5151–5155.
22 Hyde-DeRuyscher,R.P., Jennings,E. and Shenk,T. (1995) *Nucleic Acids Res.*, **23**, 4457–4465.
23 Yant,S.R., Zhu,W., Millinoff,D., Slightom,J.L., Goodman,M. and Gumucio,D.L. (1995) *Nucleic Acids Res.*, **23**, 4353–4362.
24 Seto,E., Lewis,B. and Shenk,T. (1993) *Nature*, **365**, 462–464.
25 Shrivastava,A., Saleque,S., Kalpana.G.V., Artandi,S., Goff,S.P. and Calam,K. (1993) *Science*, **262**, 1889–1892.
26 Usheva,A. and Shenk,T. (1994) *Cell*, **76**, 1115–1121.
27 Lee,J.S., Galvin,K.M., See,R.H., Eckner,R. Livingston,D., Moran,E. and Shi,Y. (1995) *Genes Dev.*, **9**, 1188–1198.
28 Haase,S.B., Heinzel,S.S. and Calos,M.P. (1994) *Mol. Cell. Biol.*, **14**, 2516–2524.
29 Burhans,W.C., Vassilev,L.T., Caddle,M.S., Heintz,N.H. and DePamphilis,M.L. (1990) *Cell*, **62**, 955–965.
30 Gossen,M., Pak,D.T.S., Hansen,S.K., Acharya,J.K. and Botchan,M. (1995) *Science*, **270**, 1674–1677.
31 Gavin,K.A., Hidaka,M. and Stillman,B. (1996) *Science*, **270**, 1667–1671.
32 Carpenter,P.B., Mueller,P.R. and Dunphy,W.G. (1996) *Nature*, **379**, 357–360.
33 Rowles,A., Chong,J.P.J., Brown,L., Howell,M., Evan.G.I. and Blow,J.J. (1996) *Cell*, **87**, 287–296.
34 Hyrien,O. and Mechali,M. (1993) *EMBO J.*, **12**, 4511–4520.
35 Hyrien,O., Maric,C. and Mechali,M. (1995) *Science*, **270**, 994–997.
36 Treisman,R. (1985) *Cell*, **42**, 889–902.
37 Gualberto,A., LePage,D., Pons,G., Mader,S.L., Park,K., Atchison,M.L. and Walsh,K. (1992) *Mol. Cell. Biol.*, **12**, 4209–4214.