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Activation of mammalian ribosomal gene transcription requires phosphorylation of the nucleolar transcription factor UBF

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ABSTRACT

The nucleolar factor UBF is phosphorylated by casein kinase II (CKII) at serine residues within the C-terminal acidic domain which is required for transcription activation. To investigate the biological significance of UBF modification, we have compared the trans-activating properties of cellular UBF and recombinant UBF expressed in *Escherichia coli*. Using a variety of assays we demonstrate that unphosphorylated UBF is transcriptionally inactive and has to be phosphorylated at multiple sites to stimulate transcription. Examination of cDNA mutants in which the serine residues within the C-terminal domain were altered by site-directed mutagenesis demonstrates that CKII-mediated phosphorylations of UBF contribute to, but are not sufficient for, transcriptional activation. Besides CKII, other cellular protein kinases phosphorylate UBF at distinct sites in a growth-dependent manner. The marked differences in the tryptic peptide maps of UBF from growing and serum-starved cells suggest that alterations in the degree of UBF phosphorylation may modulate rRNA synthetic activity in response to extracellular signals.

INTRODUCTION

UBF is an abundant nucleolar protein which belongs to the family of HMG-domain proteins, i.e. DNA binding proteins with one or more repeats of an 80 amino acid sequence motif originally found in the so called high-mobility group proteins HMG1 and HMG2 (1). In mammals, purified UBF migrates as a doublet of 97 and 94 kDa polypeptides, called UBF1 and UBF2, which differ from one another by a deletion of 37 amino acids within the second HMG box (2,3). We have shown before that the two splice variants of UBF differ in DNA binding and transcriptional activity (4). A remarkable structural feature of UBF is the presence of a C-terminal domain, the 'acidic tail', which plays an essential role in transcriptional activation (5–7). This hyperacidic C-terminus contains a number of serine residues which are phosphorylated by casein kinase II both *in vivo* and *in vitro* (6,8,9). The biological significance of UBF phosphorylation is not yet understood. Previous studies suggested—but did not unequivocally prove—that phosphorylation may alter the activity of UBF. We found that UBF prepared from exponentially growing cells stimulated transcription whereas UBF derived from stationary cells was virtually inactive (6). This result suggested that UBF phosphorylation may fluctuate according to cell growth and affect its transcriptional activity. In contrast, O'Mahony *et al.* (8) performed immunolocalization studies and showed loss of UBF from the nucleolus following serum starvation of CHO cells. Consequently, they concluded that growth inhibition results in inhibition of casein kinase II-mediated phosphorylation of serine residues within the acidic tail which then releases UBF from its target sequence and therefore inhibits rDNA transcription.

To reconcile these results, we have determined the activity of unphosphorylated versus phosphorylated UBF, and investigated the effect of serum starvation on both the intracellular distribution and the phosphorylation pattern of UBF. Our data show that (i) the intracellular localization of UBF is not altered in growing and quiescent cells, (ii) UBF has to be phosphorylated to activate transcription, (iii) CKII-mediated phosphorylations in the C-terminal domain contribute to, but are not sufficient for, transcriptional activation and (iv) serum starvation results in qualitative changes of the phosphopeptide pattern. Apparently, growth-dependent changes in rDNA transcription correlate with different phosphorylation states of UBF which in turn modulate its transcriptional activity.

MATERIALS AND METHODS

Plasmid constructs

The cDNA encoding UBF1 has been described before (6). Specific point mutations were constructed by overlap extension PCR using oligonucleotides that introduced the desired mutation, and the integrity of the PCR-generated fragments was verified by sequencing. In mUBF-at^{P-}, a construct which contains multiple point mutations in the C-terminal acidic tail, the following point mutations were introduced by oligonucleotide-directed mutagenesis: S673A, S675A, S677A, S703A, S713A, S714G, S715G, S755A and S760R. UBFΔC672 was constructed by substituting amino acid 673 for a stop codon using oligonucleotide 5′-GGCTCTAGATTACTGCAGGGTGGTCCGGD-3′ as a backward PCR-primer.

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Purification of transcription factors and RNA polymerase I

Purification of transcription factors and pol I was performed as described before (10). Briefly, 200 ml of a mixture of nuclear and cytoplasmic extracts were chromatographed on a DEAE-Sepharose CL-6B column. Fractions eluting at 280 mM KCl were fractionated on Heparin-Ultrogel A4-R by step-elution with buffer AM (20 mM Tris–HCl pH 7.9; 0.1 mM EDTA; 20% glycerol; 5 mM $MgCl_2$) containing different salt concentrations. TIF-IA and TIF-IC eluted at 200 mM KCl (H-200), RNA polymerase I at 400 mM KCl (H-400) and TIF-IB 600 mM KCl (H-600). The experiments described in this paper were performed with this crude pol I fraction (H-400) which showed a higher stimulation by UBF than more purified preparations (11). TIF-IA/TIF-IC were further purified on Q-Sepharose (10). TIF-IB was obtained by chromatography of the H-600 fraction on CM-Sepharose (12). UBF was purified from fractions eluting at 1M KCl from the DEAE-Sepharose column. After chromatography on Q-Sepharose (step elution at 500 mM KCl), and Mono-Q FPLC, it was purified to homogeneity on Bio-Rex 70.

Purification of recombinant UBF from *Eschericia coli*

Histidine-tagged UBF was expressed in *E.coli* BL21(DE3)pLysS cells essentially as described before (13). Following ammonium sulfate precipitation and chromatography on a Ni^{++}-NTA affinity column, UBF was further purified by Mono-Q FPLC. Contaminating proteins and the majority of C-terminally truncated UBF (p83) eluted at 300 mM KCl; UBF1 was recovered at 500 mM KCl.

In vitro transcription assays

Transcription reactions and product analysis were performed as described (11). To prevent phosphorylation by protein kinases present in the partially purified factor preparations, part of the assays was performed in the presence of 660 µM AMP-PNP or GMP-PNP instead of ATP and GTP. The template used was pMrWT (containing rDNA sequences from –170 to +155) truncated with *NdeI* to yield 378 bases run-off transcripts. A 25 µl reaction contained 10 ng of template DNA and 5 µl of a crude RNA polymerase I fraction (H-400), 3 µl of TIF-IA/TIF-IC (QS-300), 3 µl of TIF-IB (CM-400) and different amounts of UBF. Quantifications were done using a PhosphorImager (Molecular Dynamics).

DNaseI footprinting

Footprinting was performed essentially as described (14). Briefly, 0.5–1 ng of a 5′-labelled rDNA enhancer probe containing a *StuI–SalI* fragment (from –640 to –168) were incubated with increasing amounts of UBF for 15 min at 30°C in a 50 µl reaction containing 5 mM Hepes (pH 7.9), 25 mM KCl, 5 mM $MgCl_2$, 2.5 mM KF, 2.5 mM $CaCl_2$, 2% polyvinyl alcohol and 5% glycerol. DNA was digested for 1 min at room temperature by 1–10 ng of DNase I. The reaction was stopped by addition of 100 µl of 350 mM ammonium acetate, 20 mM EDTA and 10 µg/ml yeast tRNA. After phenol extraction and ethanol precipitation, the DNA fragments were resolved on a 6% polyacrylamide–8 M urea gel.

Phosphorylation and dephosphorylation of UBF *in vitro*

For dephosphorylation, 25 ng of cellular UBF was incubated in buffer AM-100 with 1 U of soluble calf intestine alkaline phosphatase (CIP) or with matrix-bound CIP (Sigma) for 15 min at 30°C. The reactions containing soluble CIP were stopped by addition of Na_3VO_4 to a final concentration of 200 µM. For phosphorylation *in vitro*, cellular UBF was first treated with CIP and then phosphorylations were performed for 15 min at 30°C in 10 µl of kinase buffer (80 mM KCl; 5 mM $MgCl_2$; 12% glycerol; 20 mM Tris–HCl, pH 7.9; 0.5 mM DTE; 0.5 mM PMSF) containing 660 µM ATP, 10 ng CKII (UBI), and 10–15 ng of UBF. One µl of the reaction mixture was assayed for transcriptional activity in the reconstituted *in vitro* transcription.

Two-dimensional gel electrophoresis

NIH3T3 cells were lysed in a buffer containing 9.5 M urea, 4% NP-40, 2% β-mercaptoethanol and 2% ampholytes. The ampholyte mixture contained 80% Pharmalyte 5-8 (Pharmacia) and 20% Biolyte 3-10 (Biorad). Protein (30–50 µg) was subjected to two-dimensional electrophoresis as described (15). After isoelectric focusing (4800 Vh), gels were equilibrated for 10 min in 125 mM Tris–HCl, (pH 6.8), 2% SDS, 10% glycerol, 10 mM DTT, 0.25% bromphenol blue. Second dimension was 6% SDS–PAGE followed by immunoblotting with α-UBF antibodies (6). The isoelectric point was determined by comparing each gel with protein standards (BioRad) run in parallel.

Immunofluorescence

The procedure for immunolocalization of UBF has been described (16). Briefly, cells were fixed in 2% formaldehyde, permeabilized with ice-cold methanol, incubated with a 1:200 dilution of the α-UBF antiserum and stained with a 1:200 dilution of affinity-purified FITC-conjugated anti-rabbit antibody (Dianova). After washing, the slides were mounted in Mowiol (Calbiochem) and examined with a Zeiss Axiophot microscope.

Transient transfections and *in vivo* phosphorylation

NIH3T3 cells (1.6×10^5 per 6 cm dish) were cultured for 24 h and transfected with 5 µg of pRCMV-UBF1 (4). Twenty-four hours after transfection the medium was replaced by DMEM containing 0.5% of the normal phosphate concentration and 10% dialyzed FCS. Metabolic labelling was for 16 h with 0.7 mCi/ml [^{32}P]orthophosphate. For serum starvation, the transfected cells were cultured in the presence of 0.3% FCS for 22 h before labelling. Cells were lysed in 500 µl RIPA buffer (20 mM Tris–HCl, pH 8.0; 100 mM NaCl; 0.5% sodium deoxycholate; 0.5% NP-40; 0.5% SDS; 10 mM EGTA; 20 mM KF; 1 mM sodium orthovanadate; 10 mM potassium phosphate) and UBF was immunoprecipitated with α-UBF antibodies covalently coupled to protein A–Sepharose. Immunoprecipitates were boiled in sample buffer, separated on 6% SDS–polyacrylamide gels, transferred to nitrocellulose membranes, and labelled UBF was detected by autoradiography.

Tryptic peptide mapping and phosphoamino acid analysis

Tryptic peptide maps and phosphoamino acid analyses were performed essentially as described (17). Briefly, labeled UBF was cut out from nitrocellulose membranes and digested with trypsin

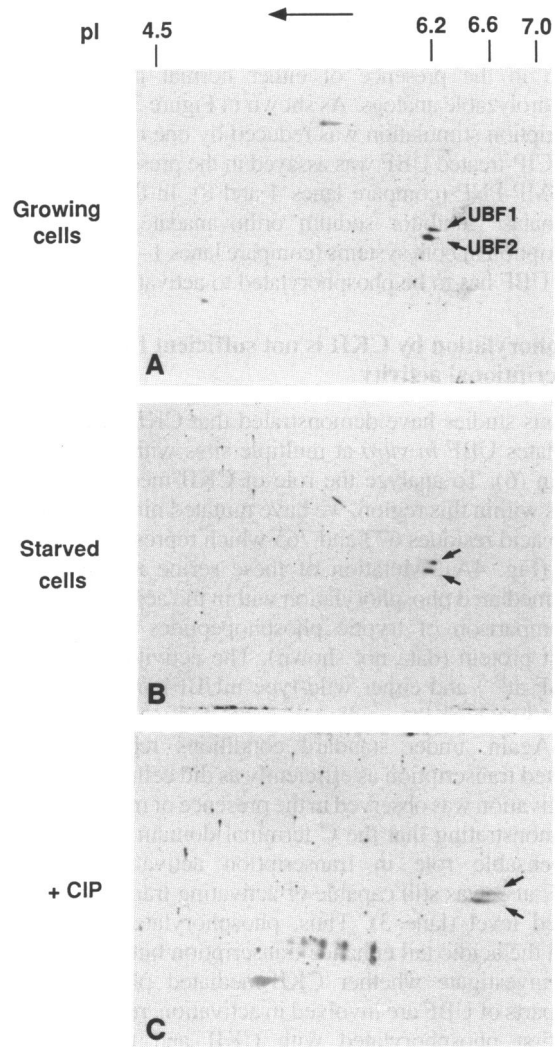

Figure 1. Two-dimensional gel electrophoresis of cellular UBF. Lysates from exponentially growing (**A**) or serum-starved (**B**) NIH3T3 cells were subjected to two-dimensional PAGE and UBF was detected by immunoblotting. (**C**) For treatment with calf intestine phosphatase (CIP), an equivalent amount of lysate from growing cells was incubated with 1 U CIP (30 min, 30°C) prior to electrophoresis. The arrow indicates the direction of IEF. The spots representing UBF1 and UBF2 are marked.

(Promega, sequencing grade) in 50 mM ammonium bicarbonate. The eluted peptides were resolved by electrophoresis for 40 min at 1000 V on cellulose thin-layer plates in pH 1.9 buffer in the first dimension, followed by ascending chromatography in isobutyric acid buffer in the second dimension. For phosphoamino acid analysis, tryptic peptides were hydrolyzed in 6 N HCl for 1 h at 110°C. Phosphoamino acids were separated by two-dimensional electrophoresis on cellulose thin-layer plates as described (17).

RESULTS

Two-dimensional gel electrophoresis of UBF from growing and serum-starved cells

UBF is heavily phosphorylated within the C-terminal domain by CKII (6). However, the functional consequence of this modification is not understood. To investigate whether UBF phosphorylation may change in response to cell growth, proteins from growing or serum-starved NIH3T3 cells were separated by two-dimensional gel electrophoresis and UBF was identified on immunoblots (Fig. 1). Surprisingly, no significant difference in the mobility of UBF could be detected in growing and serum-starved cells (Fig. 1A and B). To check whether this method can distinguish between phosphorylated and unphosphorylated UBF, the extracts were treated with alkaline phosphatase (CIP) prior to gel analysis. Indeed, CIP treatment shifts the isoelectric point of UBF from 6.2 to 6.6, indicating that dephosphorylation decreases the net charge of UBF (Fig. 1C). Since, however, the mobility of UBF from growing or quiescent cells is more or less the same, alterations in cell growth appear not to cause major changes of the phosphorylation pattern. Thus, the marked differences in the metabolic labelling of UBF observed in earlier experiments (6) were probably caused by growth-dependent changes of the intracellular pool size of inorganic phosphate or by alterations in the uptake of exogenous radioactive phosphate.

Intracellular localization of UBF in growing and quiescent cells

Growth-dependent regulation of rRNA synthesis could involve translocation of UBF from the nucleolus to the nucleoplasm in quiescent cells as suggested by O'Mahony *et al.* (8). We therefore compared the distribution of UBF in exponentially growing NIH3T3 cells, stationary phase cells and serum-starved cells by immunofluorescent staining. Consistent with previous reports (16,18), under all conditions tested UBF was located exclusively within the nucleolus and was not released into the nucleoplasm or cytoplasm (data not shown). In mitotic chromosomes, UBF remained associated with the nucleolus organizer regions. Therefore, changes in the intracellular localization of UBF are probably not involved in growth-dependent transcriptional regulation.

Unphosphorylated UBF is transcriptionally inactive

To investigate the effect of phosphorylation on DNA binding and transcriptional activity, we used recombinant UBF1 expressed in *E.coli* which is not phosphorylated. In DNase footprinting experiments, both recombinant and cellular UBF produce an identical pattern of protected and hypersensitive sites on the rDNA enhancer, indicating that phosphorylation neither qualitatively nor quantitatively affects UBF binding (Fig. 2).

Next, we compared the transcriptional activity of cellular and recombinant UBF in a reconstituted system containing partially purified transcription factors (Fig. 3A). Since the protein fractions used contain significant amounts of CKII and other cellular protein kinases, part of the assays was performed in the presence of AMP-PNP and GMP-PNP to prevent phosphorylation of UBF during transcription. In the standard transcription system, i.e. in the presence of ATP and GTP, recombinant UBF1 stimulated transcription almost as efficiently as cellular UBF (lanes 1–3). In contrast, a marked difference in the activity of cellular and recombinant UBF was observed when the assays contained the nucleotide analogs (lanes 4–6). Again, cellular UBF stimulated transcription about 30-fold (lane 5). Recombinant UBF, on the other hand, was virtually inactive (lane 6). This observation suggests that bacterially expressed UBF can be phosphorylated by protein kinase(s) present in the reconstituted

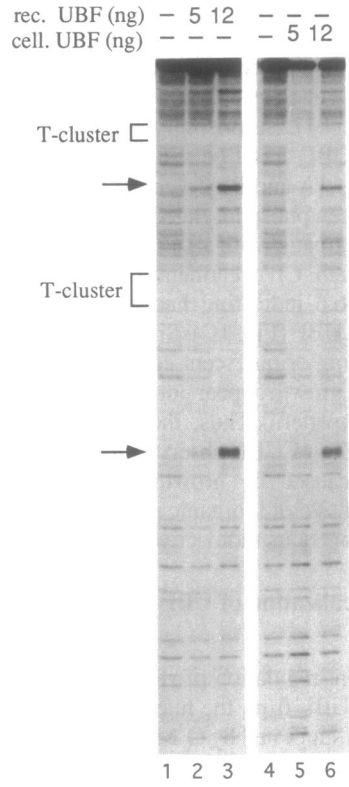

Figure 2. DNaseI footprint of recombinant and cellular UBF. A murine rDNA spacer fragment containing three enhancer repeats was labelled at the non-coding strand and incubated with 5 ng (lanes 2 and 5) or 12 ng (lanes 3 and 6) of recombinant UBF (lanes 2 and 3) or a Bio-Rex 70 fraction containing cellular UBF1 (lanes 5 and 6). Lanes 1 and 4 show the digestion pattern of naked DNA. The clusters of T-residues which flank the individual repetitive elements are marked. Hypersensitive sites are indicated by arrows.

transcription system, and that phosphorylation is required for transcription activation.

If this assumption was correct, then dephosphorylation of cellular UBF should decrease or abolish transcriptional activity. To address this issue, cellular UBF was incubated with matrix-bound CIP before assaying in the reconstituted transcription system in the presence of either normal nucleotides or the nonhydrolyzable analogs. As shown in Figure 3B, UBF-mediated transcription stimulation was reduced by one order of magnitude when CIP-treated UBF was assayed in the presence of AMP-PNP and GMP-PNP (compare lanes 4 and 8). In the presence of the phosphatase inhibitor sodium orthovanadate, UBF stimulated transcription in both systems (compare lanes 1–4 with lanes 9–12). Thus, UBF has to be phosphorylated to activate transcription.

Phosphorylation by CKII is not sufficient for transcriptional activity

Previous studies have demonstrated that CKII efficiently phosphorylates UBF *in vitro* at multiple sites within the C-terminal domain (6). To analyze the role of CKII-mediated phosphorylations within this region, we have mutated nine serines between amino acid residues 673 and 765 which represent target sites for CKII (Fig. 4A). Mutation of these serine residues abolished CKII-mediated phosphorylation within the acidic tail, as verified by comparison of tryptic phosphopeptides of wild-type and mutant protein (data not shown). The activities of this mutant (mUBF-at^{P-}) and either wild-type mUBF1 or mUBFΔC672, a mutant in which the acidic tail is deleted were compared (Fig. 4B). Again, under standard conditions recombinant UBF1 activated transcription as efficiently as did cellular UBF (lane 2). No activation was observed in the presence of mUBFΔC672 (lane 4) demonstrating that the C-terminal domain of UBF plays an indispensable role in transcription activation. Importantly, mUBF-at^{P-} was still capable of activating transcription, but at a reduced level (lane 3). Thus, phosphorylated serine residues within the acidic tail enhance transcription but are not essential.

To investigate whether CKII-mediated phosphorylation in other parts of UBF are involved in activation, recombinant UBF1 was first phosphorylated with CKII and then assayed for transcriptional activity in the presence of the nucleotide analogs. To ensure that UBF was efficiently phosphorylated by CKII, the electrophoretic mobility of UBF was analyzed before and after CKII treatment. As shown in Figure 5, UBF was heavily

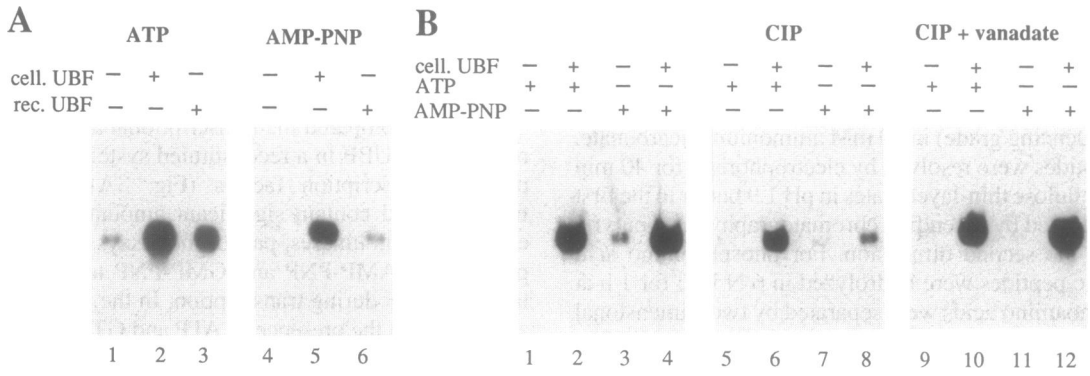

Figure 3. Comparison of the transcriptional activity of cellular and recombinant UBF. (**A**) Transcription activation by recombinant UBF requires ATP hydrolysis. Transcriptions were performed in the reconstituted UBF-responsive system in the presence of either 660 μM ATP and GTP (lanes 1–3) or 660 μM of the nucleotide analogs AMP-PNP and GMP-PNP (lanes 4–6) without UBF (lanes 1 and 4) and in the presence of 5 ng of cellular UBF (lanes 2 and 5) or 15 ng of recombinant UBF1 (lanes 3 and 6). (**B**) Dephosphorylation of cellular UBF abolishes transcription stimulation. 2.5 ng of UBF were treated with 0.2 U matrix-bound CIP in the absence (lanes 6 and 8) or presence (lanes 10 and 12) of 200 μM Na_3VO_4 and subsequently assayed in the reconstituted transcription system containing 200 μM Na_3VO_4 and 660 μM each of ATP and GTP (lanes 5 and 6, 9 and 10) or AMP-PNP and GMP-PNP (lanes 7 and 8, 11 and 12). Lanes 1–4 show the transcriptional activity with and without UBF.

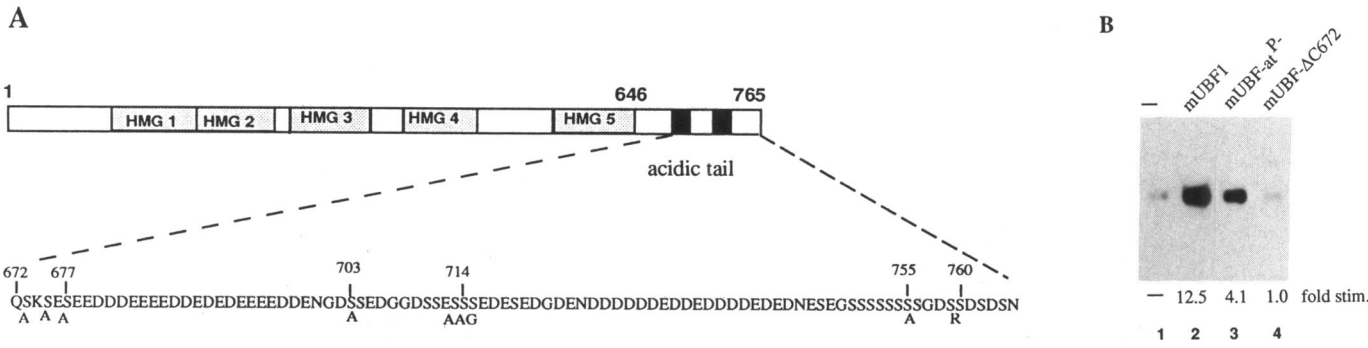

Figure 4. Phosphorylation of serine residues within the acidic tail contribute to, but are not sufficient for, transcriptional activation. (**A**) Schematic diagram of the structural domains of UBF. The sequence of the C-terminal acidic tail (amino acids 673–765) is shown. In construct mUBF-at^{P-} nine serine residues corresponding to putative casein kinase II phosphorylation target sites were mutated into the amino acids indicated. (**B**) The acidic tail plays a role in activation of transcription.Transcriptions were performed in the presence of ATP and GTP and contained either no UBF (lane 1), recombinant UBF1 (lane 2), mUBF-at^{P-} (lane 3), or the C-terminal mutant mUBFΔC672 (lane 4).

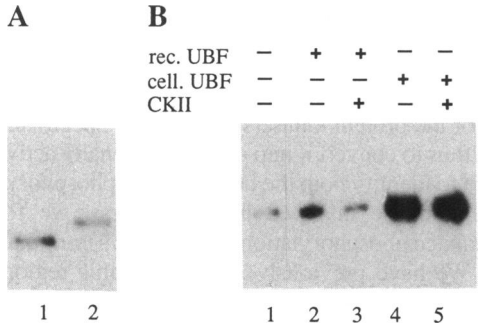

Figure 5. Phosphorylation of UBF by CKII does not restore transcriptional activity. (**A**) CKII-mediated phosphorylation alters the electrophoretic mobility of recombinant UBF1. Five ng of recombinant UBF1 were incubated in kinase buffer in the absence of CKII (lane 1) or in the presence of 5 ng of CKII (lane 2). Following electrophoresis on a 6% SDS–polyacrylamide gel UBF was visualized by immunoblotting. (**B**) Transcriptional activity of recombinant UBF1 phosphorylated with CKII. Cellular UBF or recombinant UBF1 was preincubated in the absence of CKII (lanes 2 and 4) or in the presence of 5 ng of CKII (lanes 3 and 5), and then assayed for transcriptional activity in the presence of AMP-PNP and GMP-PNP.

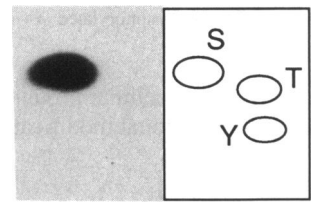

Figure 6. Phosphoamino acid analysis of UBF phosphorylated *in vivo*. Immunopurified UBF was cleaved with 6 M HCl and the phosphoamino acids were separated by two-dimensional electrophoresis. Circles mark the position of comigrating phosphoserine (S), phosphothreonine (T) and phosphotyrosine (Y) standards.

phosphorylated but failed to stimulate transcription. Cellular UBF, on the other hand, that was preincubated with CKII was not affected. The observation that phosphorylation by CKII is not sufficient to convert UBF into a transcriptionally competent form suggests that other cellular protein kinase(s) modulate its transcriptional activity.

Phosphopeptide mapping of UBF

In order to identify the sites of UBF phosphorylation, UBF was metabolically labelled with [^{32}P]orthophosphate *in vivo*, immunopurified and subjected to both phosphoamino acid analysis and tryptic peptide mapping. Phosphoamino acid analysis shows that phosphorylation of UBF *in vivo* occurs exclusively on serine residues (Fig. 6). In Figure 7, the two-dimensional peptide maps are shown. The most obvious feature is a large tryptic fragment (labelled AT) which encompasses the acidic tail. Peptide AT analyzed on a high percentage polyacrylamide gel migrates as a 9.8 kDa polypeptide, a size which corresponds to the C-terminal tryptic fragment covering amino acids 675–765 (data not shown). Because of its size and hydrophilic nature this peptide does not resolve on two-dimensional chromatograms. Peptide AT is most heavily labelled because it contains eight serine residues (amino acids 675, 677, 703, 713–715, 755 and 760) which perfectly match the CKII consensus sequence. In addition, there are nine phosphopeptide spots which map to regions outside of the acidic tail. Significantly, the phosphopeptide pattern of UBF labelled *in vivo* differs from the pattern observed after phosphorylation with CKII *in vitro* (Fig. 7C). Besides fragment AT, only two minor peptides (labelled with circles) co-localize with peptides modified by CKII *in vitro*, whereas the other CKII-directed phosphorylations *in vitro* are most likely unspecific.

As the rate of rDNA transcription is known to fluctuate according to cell growth (19), we wondered whether qualitative or quantitative changes in UBF phosphorylation are involved in growth-dependent transcriptional regulation. Therefore, the tryptic peptide pattern of UBF from growing and serum-starved cells was compared. As shown in Figure 7B, serum starvation results in marked changes of UBF phosphorylation. First, the overall incorporation of phosphate into UBF is severely reduced. Second, the relative labelling of the individual spots changes significantly. The most remarkable quantitative alterations are observed in four peptides which are most intensely labelled in UBF from growing cells and are down-regulated in resting cells, whereas the relative level of phosphorylation of the other peptides is not affected (Fig. 7A and B). Thus, although we are still ignorant of the amino acid composition of the individual tryptic peptides, the quantitative changes in the tryptic peptide maps imply that (i) the phosphorylation pattern of UBF is modulated upon

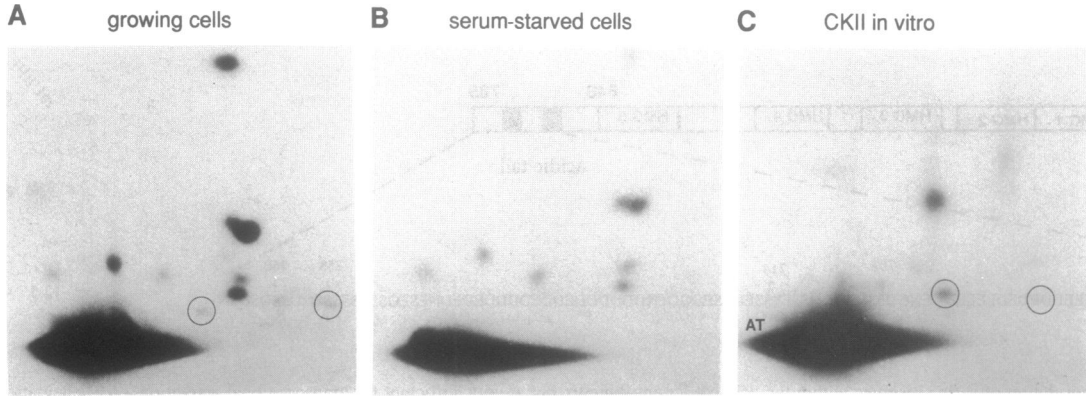

Figure 7. Two-dimensional tryptic peptide maps of UBF. NIH3T3 cells transfected with 5 μg of expression vector encoding mUBF1 were labeled for 16 h with [^{32}P]orthophosphate in the presence of 10% FCS (**A**) or, after 24 h of serum starvation, in the presence of 0.3% FCS (**B**). UBF was isolated by immunoprecipitation, digested with trypsin, and phosphopeptides were resolved by thin-layer electrophoresis (horizontal dimension) followed by ascending chromatography (vertical dimension). 'AT' marks the tryptic peptide derived from the acidic tail. Note that 10-fold more UBF was applied to chromatogram (B) compared with (A). (**C**) Tryptic peptide map of recombinant UBF1 phosphorylated *in vitro* with CKII. Encircled peptides co-localize with phosphopeptides labelled *in vivo*.

serum starvation and (ii) other cellular protein kinase(s) besides CKII contribute to posttranslational modification of UBF.

DISCUSSION

Previously we and others have shown that UBF is heavily phosphorylated at serine residues within the C-terminus (6,9). In this communication we have addressed the question whether phosphorylation of UBF is required to exert an activating function in ribosomal gene transcription. Using a partially purified *in vitro* transcription system, we provide experimental evidence that UBF-mediated transcriptional stimulation is only observed when the factor is modified by phosphorylation. UBF prepared from cultured mouse cells as well as recombinant UBF expressed in *E.coli* bind with the same affinity and specificity to the rDNA enhancer, and therefore the ability to interact with its target sites is not affected by posttranslational modification. However, the phosphorylated and unphosphorylated factors differ significantly in their ability to support rDNA transcription. In the presence of AMP-PNP the recombinant factor is virtually inactive. Similarly, dephosphorylation of cellular UBF by treatment with alkaline phosphatase eliminates UBF-directed activation of transcription. Since most, if not all, UBF-directed transcriptional activation is due to relief of repression caused by histone H1, Ku protein or other negative-acting DNA binding proteins (11,20,21), phosphorylation of UBF appears to be causally involved in antirepression and, consequently, transcription stimulation. Our data suggest that modification of UBF is required to replace inhibitory proteins from the rDNA promoter and/or to increase the affinity of TIF-IB for its target site. In this context it is interesting that a recent report has demonstrated a physical interaction between UBF and TBP, the latter one being a constituent of the TIF-IB complex (22). Their observation that the association of UBF with TBP-containing complexes is, at least *in vitro*, not limited to TIF-IB/SL1 suggests that UBF may have a function in class II transcription as well.

Under standard conditions, we find no difference in the activity of cellular UBF, recombinant UBF or phosphatase-treated UBF. However, strong differences are observed if ATP is replaced by the nonhydrolyzable analog AMP-PNP. Apparently, the partially purified reconstituted transcription system contains sufficient amounts of the protein kinase(s) required to specifically modify UBF and thus to convert it into a transcriptionally active form. In an attempt to identify both the target sites of phosphorylation and the protein kinase involved in this process, we focused on CKII-mediated phosphorylations within the C-terminal acidic tail of UBF. We have previously shown that this region contains multiple CKII consensus sites and that UBF is phosphorylated *in vitro* by a cellular protein kinase which by several criteria resembles CKII (6). Moreover, a C-terminally truncated version of UBF binds efficiently to DNA but does neither relieve histone H1-mediated transcriptional repression nor mediates transcription activation indicating that the tail plays an essential role in UBF function (4,6,7). To elucidate the biological significance of CKII-directed serine phosphoesterifications in UBF, we have altered any of the serine residues within the tail that is a likely target site for CKII, and analyzed the effect of serine substitutions on UBF function. We found that mutation of the serine residues within the tail decreased, but did not eliminate, transcriptional activity. The moderate inhibition observed, together with the failure to restore transcriptional activity of recombinant UBF by CKII, demonstrates that phosphorylation of these serines contributes to the efficiency of UBF-directed transcription activation, but *per se* is not sufficient to convert UBF into a transcription-competent form. Consistent with this result we found that recombinant UBF phosphorylated with CKII *in vitro* is transcriptionally inactive. Apparently, phosphoesterification by CKII which accounts for the majority of UBF modifications does not affect transcriptional activity. This implies that phosphorylations by yet to be identified protein kinase(s) play a more important role in modulating the transacting function of UBF.

This view is supported by analysis of two-dimensional phosphopeptide maps of cellular UBF. The majority of UBF phosphorylations is found in one large tryptic peptide which corresponds to the acidic tail. However, there are several additional phosphopeptides that do not originate from the tail and were not phosphorylated by CKII. Although we are still ignorant of the nature of the individual peptides and the cellular protein kinase(s) that act in concert with CKII, the fact that the phosphopeptide pattern is different in growing and resting cells

strongly suggests that changes in the pattern of UBF phosphorylation serve a regulatory function. Apparently very subtle changes in the phosphorylation pattern have a pronounced effect on UBF activity. Probably hierarchical phosphorylation reactions similar to those described for a number of transcription factors directing class II gene transcription, appear to be involved in adapting class I gene transcription to cell proliferation, too.

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REFERENCES

1 Jantzen, H.-M., Admon, A., Bell, S. P., and Tjian,R. (1990) *Nature* **344,** 830–836.
2 O'Mahony, D.J. and Rothblum, L. I. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3180–3184.
3 Hisatake, K., Nishimura, T., Maeda, Y., Hanada, K., Song, C.-Z., and Muramatsu, M. (1991) *Nucleic Acids Res.* **19,** 4631–4637.
4 Kuhn, A., Voit, R., Stefanovsky, V., Evers, R., Bianchi, M., and Grummt, I. (1994) *EMBO J.* **13,** 416–424.
5 McStay, B., Frazier, M. W., and Reeder, R. H. (1991) *Genes Dev.* **5,** 1957–1968.
6 Voit, R., Schnapp, A., Kuhn, A., Rosenbauer, H., Hirschmann, P., Stunnenberg, H. G., and Grummt, I. (1992) *EMBO J.* **11,** 2211–2218.
7 Jantzen, H.-M., Chow, A. M., King, D. S., and Tjian, R. (1992) *Genes Dev.* **6,** 1950–1963.
8 O'Mahony, D. J., Xie, W. Q., Smith, D. S., Singer, H. A., and Rothblum, L. I. (1992) *J. Biol. Chem.* **267,** 35–38.
9 O'Mahony, D. J., Smith, D. S., Xie, W. Q., and Rothblum, L. I. (1992) *Nucleic Acids Res.* **20,** 1301–1308.
10 Schnapp, A. and Grummt, I. (1991) *J. Biol. Chem.* **266,** 24588–24595.
11 Kuhn, A. and Grummt, I. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7340–7344.
12 Eberhard, D., Tora, L., Egly, J.-M., and Grummt, I. (1993) *Nucleic Acids Res.* **18,** 4180–4186.
13 Schnapp, G., Santori, F., Carles, C., Riva, M., and Grummt, I. (1994) *EMBO J.*,**13,** 190–199.
14 Bell, S. P., Learned, R. M., Jantzen, H.-M., and Tjian,R. (1988) *Science* **241,** 1192–1197.
15 O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
16 Zatsepina, O. V., Voit, R., Grummt, I., Spring, H., Semenov, M. V., and Trendelenburg, M. F. (1993) *Chromosoma* **102,** 599–611.
17 Boyle, W. J., van der Geer, P., and Hunter, T. (1991) *Methods Enzymol.* **201,** 110–149.
18 Roussel, P., Masson, A. C., Géraud, G., and Hernandez-Verdun, D. (1993) *J. Cell Sci.* **104,** 327–337.
19 Grummt, I. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 727–731.
20 Kuhn, A., Stefanofsky, V., and Grummt, I. (1993) *Nucleic Acids Res.* **21,** 2057–2063.
21 Brou, C., Kuhn, A., Staub, A., Chaudhary, S., Grummt, I., Davidson, I., and Tora, L. (1993) *Nucleic Acids Res.* **21,** 4011–4018.
22 Kwon, H., and Green, M. R. (1994) *J. Biol. Chem.* **269,** 30140–30146.