Angiotensin II-induced Hypertrophy of Rat Vascular Smooth Muscle Is Associated with Increased 18 S rRNA Synthesis and Phosphorylation of the rRNA Transcription Factor, Upstream Binding Factor*

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Hypertrophy of vascular smooth muscle cells (VSMC) is an important adaptive response of hypertension. Drug intervention studies have implicated a role for angiotensin II (A-II) in the mediation of VSMC hypertrophy in vivo, and A-II is a potent hypertrophic agent for VSMC in culture. Our laboratory has previously shown that A-II-induced hypertrophy of cultured VSMC is due in part to generalized increases in protein synthesis and increased content of rRNA. The aim of the present study was to determine if A-II stimulates rRNA gene synthesis and whether the rRNA transcription factor, upstream binding factor (UBF), is involved. Nuclear run-on analysis demonstrated that A-II induced a greater than 5-fold increase in rRNA gene synthesis within 6 h of stimulation. A-II also stimulated a rapid increase in UBF phosphorylation as well as nuclear localization, but no changes in the content of UBF. Phosphoamino acid analysis showed that phosphorylation occurred only on serine residue(s). Results demonstrate that increased transcription of ribosomal DNA contributes to the A-II-induced increase in protein synthesis and VSMC hypertrophy, and suggest that an important regulatory event in this pathway may be the phosphorylation and/or nuclear localization of UBF.

It is well established that arteries from hypertensive patients (1, 2) and animals (3, 4) are thicker than those from their normotensive counterparts. The arterial medial thickening is believed to represent an important adaptive response to normalize the elevated wall stress that occurs secondary to the increased blood pressure (3). Previous studies in this and other laboratories have demonstrated that medial thickening, at least in large conduit vessels, is due in part to increased vascular smooth muscle cell (VSMC) content or mass, which occurs primarily by enlargement or hypertrophy of preexisting VSMC, with little to no change in VSMC number (5–7). As such, there has been considerable interest in identifying cellular mechanisms that mediate hypertrophic growth of vascular smooth muscle.

There is clear evidence implicating a role for angiotensin II (A-II) in mediation of VSMC hypertrophy during chronic hypertension (8). For example, angiotensin-converting enzyme inhibitors and A-II receptor antagonists have been shown to be extremely effective in inhibiting development of VSMC medial hypertrophy in a variety of hypertensive animal models (9–11). Importantly, effects of angiotensin-converting enzyme inhibitors or A-II antagonists do not appear to be due simply to blood pressure lowering, since other antihypertensive drugs were not as efficacious in blocking hypertrophy despite similar reductions in blood pressure. Consistent with in vivo studies, several laboratories, including our own, have shown that A-II stimulates increased protein synthesis and cellular hypertrophy in cultured VSMC via stimulation of angiotensin AT1 receptors (12, 13). The mechanism of this effect is not clear. Moreover, our understanding in this area has been confounded by observations implicating A-II in regulation of VSMC mitogenesis following vessel injury in vivo (14). In general, however, A-II has been shown to have a very low efficacy as a mitogen for cultured VSMC, and in cases where A-II is mitogenic, its proliferative effects seem to be mediated by autocrine factors such as platelet-derived growth factor-AA, transforming growth factor-β, and/or b-fibroblast growth factor (15–17). In contrast, the A-II-induced hypertrophy of VSMC appears to be direct (12, 18).

There has been considerable interest in identifying the mechanism and cellular signaling pathways whereby A-II stimulates VSMC hypertrophy. One approach has been to attempt to identify which of the many signal transduction pathways stimulated by A-II (e.g., Ca2+ entry into cells, increased phosphatidylinositol turnover, Na+/K+ exchange, increased activity of protein kinase C, and increased mitogen-activated protein kinase activity) (19–22) are required for the hypertrophic response, using various inhibitors of these pathways. The problem with this approach is the marginal specificity and unknown actions of many of the available signal transduction pathway inhibitors and the fact that many of these factors (e.g. intracellular calcium chelators) are known to inhibit key cellular processes required for growth, e.g. protein synthesis, even acrylamide gel electrophoresis; CKII, casein kinase II; PVDF, poly(vinylidene fluoride).
in untreated cells. An alternative approach and one our laboratory has pursued was to first identify the major structural proteins that contribute to A-II-induced hypertrophy and then study mechanisms whereby A-II stimulates their expression. To this end, we have previously demonstrated that A-II-induced hypertrophy of cultured VSMC was characterized by selective increases in the expression of a number of cellular proteins such as smooth muscle (SM) α-actin, SM α-tropomyosin, and SM myosin heavy chain (24). However, we and others have also shown that A-II-induced hypertrophy was also associated with generalized increases in protein synthesis and content, as well as increased rRNA content (12, 25). These latter results indicate that A-II-induced hypertrophy of VSMC is dependent on increases in the overall translational capacity of the cell.

Whereas increased rRNA synthesis is absolutely essential for sustained growth of any cell, relatively little is known regarding signal transduction pathways whereby mitogens or hypertrophic agents stimulate such changes. Studies on the regulation of rRNA synthesis in eukaryotes have led to the model that RNA polymerase I activity is regulated by post-translational modification of RNA polymerase I and/or any of the four factors associated with the polymerase, including TIF-1α, TIF-1β, TIF-1C, and upstream binding factor (UBF). Whereas little information concerning the function of the TIF family is available, considerable progress has been made on the identification and characterization of UBF. UBF has been purified to homogeneity from a number of species, and the mammalian form consists of a protein doublet of 97 and 94 kDa referred to as UBF1 and UBF2. It is a member of the high mobility group family of proteins and appears to function both as an enhancer binding protein and transcription factor (26, 27). It has also been shown to be involved in recruitment or stabilization of RNA polymerase I, as well as functioning as a transcription antirepressor by overcoming transcription inhibition caused by a repressor protein that competes with TIF-1β for DNA binding (28, 29). Although the mechanism by which UBF regulates rRNA synthesis is unclear, it has been shown that removal of the hyperacidic C-terminal tail, or phosphatase treatment, resulted in a decreased ability of UBF to transactivate rDNA transcription in vitro (30). O’Mahony et al. (31, 32) have demonstrated that serum deprivation of CHO cells resulted in decreased phosphorylation of UBF and translocation out of the nucleus. Taken together, these studies strongly suggest that phosphorylation of UBF may be important in the regulation of rRNA gene transcription in response to serum-induced cellular proliferation. However, direct proof for this is currently lacking, and the kinase-signaling pathway that mediates serum-induced changes in UBF phosphorylation has yet to be identified. Moreover, no studies have been performed that investigate the effect of a single purified growth factor on UBF phosphorylation and rRNA gene transcription.

In this report, we demonstrate that stimulation of cultured VSMC with A-II resulted in increased rRNA synthesis and a notable increase in phosphorylation and nucleolar localization of UBF. Importantly, no alterations in cellular UBF content were found. These data provide evidence that the A-II-induced phosphorylation and/or nucleolar localization of UBF may be an important regulatory event in the induction of vascular smooth muscle hypertrophy.

### EXPERIMENTAL PROCEDURES

#### Cell Culture—Rat aortic smooth muscle cells were isolated, cultured, and grown to post-confluence as described previously (12). The growth medium was then replaced with serum-free medium (SFM) for 5 days to induce quiescence as previously shown (12). Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO₂, 95% O₂ with medium changes every 2 days. All of the cells used for the experiments described herein were between the 9th and 18th passage.

Vascular smooth muscle cells that are growth-arrested in this fashion show [³H]thymidine labeling induces <5% incorporation, and no changes in cell number over extended time periods. Cells remain viable and maintain high levels of expression of multiple VSMC differentiation marker proteins including SM α-actin, SM myosin heavy chain, and SM light chain (33–35). In addition, the growth arrest state is reversible in that VSMC can be readily growth stimulated with various purified mitogens such as platelet-derived growth factor-BB or with serum (34, 35).

#### Nuclear Run-on Analysis—Nuclear run-on reactions were performed as described previously with minor modifications (36). Briefly, post-confluent growth-arrested VSMC were grown on 100-mm dishes and treated with either A-II or SFM for a specified period of time. The nuclei were harvested, resuspended in storage buffer, and frozen in liquid nitrogen.

Nuclear run-on reactions utilized equal amounts of cellular DNA. The reaction mixture contained 0.625 mM ATP, 0.312 mM GTP, 320 μCi of [³²P]UTP (>3000 Ci/mMol), 40 mM Tris-HCl, pH 8.3, 150 mM NaCl, 7.5 mM MgCl₂, and 200 units/ml RNAse. The reaction mix was incubated for 35 min at 30 °C, then DNase (30 units) and CaCl₂ (1.25 mM) were added and incubated for 30 min. Extraction buffer (100 μl) was added and the reaction incubated for 2 h at 42 °C. The reaction was phenol/chloroform-extracted and ethanol-precipitated for 30 min. Following centrifugation, the pellet was recovered in Tris-EDTA buffer, and the incorporated counts were removed by Sephadex G-25 chromatography. Equal volumes of eluate were hybridized to a 5.8-kilobase pair insert of the human 18 S ribosomal gene which was immobilized to a nylon membrane. Hybridization was carried out for 24 h at 65 °C in 5 × SSPE, 10 × Denhard’s solution, 1% SDS, 0.5 mg/ml herring sperm DNA, 0.05% NaPPE. Blots were washed at high stringency according to the Church and Gilbert (37) protocol, dried exposed to x-ray film, and densitometric analysis was performed using a laser 2000 (Bioimage).

#### Anti-UBF Western Blot Analysis—Post-confluent growth arrested VSMC were stimulated with A-II or SFM vehicle and harvested in lysis buffer (60 mM Tris, pH 6.8, 2% SDS, 100 mM dithiothreitol) at 4, 8, and 24 h post-stimulation. The protein concentration was determined (Bio-Rad DC protein assay), and 10 μg of protein was loaded per well on a 12% SDS-PAGE Mini-Protein gel (Bio-Rad). Upon completion of the run, the proteins were transferred onto a PVDF membrane at 100 V for 1.5 h. The membrane was blocked for 1 h with 1 mg/mI bovine serum albumin and washed three times with Tris-buffered saline with 0.05% Tween 20 (TBST) buffer, before an overnight incubation with a 1:10000 dilution of anti-UBF antiserum. After extensive washing, the membrane was then incubated for 1 h with a goat anti-rabbit alkaline phosphatase-conjugated secondary antibody (Promega). Following three more washes in TBST, the protein-antibody complexes were visualized using an enzymatic detection system (5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium) according to the specifications of the manufacturer (Promega).

In Vivo Labeling and Immunoprecipitation of UBF—Post-confluent growth arrested VSMC were switched to a low phosphate SFM and labeled in vivo with [³²P]orthophosphoric acid (0.5 μCi/ml) for 4 h. In order to determine the kinetics of UBF phosphorylation, the cells were stimulated for 5, 15, and 30 min, and for 1 h with A-II (10⁻⁶ M). At the end of the stimulation, the medium was quickly removed, and the cells were rinsed twice with phosphate-buffered saline before quick freezing the cells in a bath of dry ice and ethanol. The cells were harvested in radiomune precipitation buffer (150 mM NaCl, 50 mM Tris-HCl, 1% sodium deoxycholate, 1% Nonident P-40, 0.1% SDS, pH 7.2) supplemented with protease and phosphatase inhibitors (5 μg/ml leupeptin, 5 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and 50 μM sodium fluoride) and sonicated for 15 s. After centrifugation, the total protein content of each extract was determined, and equal amounts of protein were preclared with protein A-agarose and another 1-h incubation. The lysates were centrifuged and immunoprecipitates were washed three times in radiomune precipitation buffer, resuspended in SDS-sample buffer, and analyzed on a 4% SDS-PAGE, which allowed the resolution of both isoforms of UBF. The labeled bands were visualized, quantitated, and photographed on a ImageQuant Phosphor-Imager.

#### Phosphoamino Acid Analysis—Post-confluent growth arrested VSMC were prelabeled with [³²P]orthophosphoric acid for 4 h and treated with either A-II or SFM for 30 min. UBF was immunoprecipi-
tated as described above, analyzed by 12% SDS-PAGE, and transferred to a PVDF membrane. After visualizing the immunoprecipitated UBF protein by autoradiography, the bands were cut out of the membrane and hydrolyzed in 300 μl of 5.3 M HCl at 100°C for 3 h. The membrane was then removed, and the samples were washed three times in H2O using a speed-vac concentrator and resuspended in 10 μl of pH 3.5 buffer containing 10% acetic acid and 0.5% pyridine for nonradioactive phosphotyrosine, phosphothreonine, and phosphoserine. The presence of 32P-labeled amino acids was visualized by autoradiography and matched to the ninhydrin stained phosphoamino acid standards.

Immunostaining of UBF—Vascular smooth muscle cells were grown to post-confluency in chamber wells and growth-arrested for 5 days in SFM. After the cells were treated with either A-II (10^(-6) M) or SFM vehicle, the medium was removed, and the cells were quickly washed twice with Bacto FA buffer (DIFCO phosphate buffer). Paraformaldehyde (3%) was added to the cells twice for 5 min each, followed by two washes with cold methanol for 30 min each. The cells were washed once again with FA buffer before the addition of blocking serum (1:1000 made up in FA + 3% bovine serum albumin). After blocking for 30 min, the rabbit anti-UBF antibody was added (1:80 made up in FA + 3% bovine serum albumin) and left on the cells for 1 h. After several washes with FA buffer, the cells were incubated for 1 h with the secondary antibody, rhodamine-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc). After several more washes, the cells were mounted in FA/glycerol (1:1) and viewed with a confocal microscope (Bio-Rad MRC 1000).

In Vitro Phosphorylation of UBF with Casein Kinase II—In vitro kinase reactions contained 300 μM [γ-32P]ATP (250 cpmm/μl), 7.5 μM MgCl2, 200 μg of recombinant UBF protein, 40 ng of casein kinase II (CKII) (Upstate Biotechnology Inc.), and β-glycerophosphate buffer in a final volume of 200 μl. A control reaction was also performed in which no CKII was added. At specific times, a 20-μl aliquot was removed from the reaction mix and added to a tube containing 5 μl of FA buffer for a sample to terminate the reaction. The samples were boiled 3 min, resolubilized on a 12% SDS-PAGE, and visualized by autoradiography.

In Vitro Assay of Endogenous Casein Kinase II Activity—Casein kinase II activity was determined by phosphorylation of casein using a Beckman Biomek 1000 robot. Post-confluent growth arrested cells were treated for specific times (1, 5, 10, 15, and 30 min, and 1, 2, and 3 h) with A-II or SFM vehicle. The cells were harvested in β-glycerophosphate buffer, sonicated briefly, and centrifuged for 5 min at 13,000 rpm. A 10-μl aliquot from each time point was added into a 96-well microtiter plate. The Biomek added 20 μl of buffer containing 5 μg of casein, 300 μM [γ-32P]ATP (250 cpmm/μl), and 7.5 μM MgCl2. After a 30-min incubation, the Biomek terminated the reaction by the addition of 20 μl of 350 mM H2PO4. The reactions were then spotted onto P81 paper (Whatman) and washed three times with H2PO4, and the amount of radioactivity incorporated into casein was determined by Cerenkov counting. When the reaction mixture was run on SDS-PAGE and autoradiographed, it was found that >95% of the radioactive counts were due to phosphorylation of casein.

RESULTS

A-II Increased Transcription of the 18 S Ribosomal RNA Gene—Nuclear run-on assays were performed to determine the relative rate of 18 S rRNA transcription after treatment with either A-II or the vehicle SFM. Results demonstrated that A-II increased transcription of the 18 S ribosomal gene 5.4 ± 1.3-fold (n = 6) relative to SFM-treated cells, Student’s t test; p < 0.05 (Fig. 1). This increase was transient in that no increase was observed 24 h following A-II stimulation (A-II versus SFM vehicle = 1.0 ± 0.3-fold, n = 5). The specificity of the 18 S transcription run-on reaction was shown by: 1) the lack hybridization signal to various control probes including plasmid mimic 18 S insert and a β-actin non-muscle sense cRNA; 2) loss of signal when the general (i.e. RNA polymerase I, II, and III) transcription inhibitor actinomycin D was added to the reaction mixture; and 3) persistence of the signal when the run-on assays were done in the presence of α-amanitin (80 μg/ml) which selectively inhibits RNA polymerases II and III.

FIG. 1. Nuclear run-on analysis demonstrating the effects of A-II on ribosomal RNA synthesis. Rat aortic VSMC were treated with A-II or SFM vehicle and harvested for nuclear run-on analysis. The relative rate of transcription of rRNA genes was measured using a 5.8-kilobase pair insert of the human 18 S rRNA gene as described under “Experimental Procedures.” Controls using pBR322 plasmid DNA (or sense non-muscle β-actin) showed no signal above background. Activity was resistant to treatment with α-amanitin at a concentration of 90 μg/ml, at which selectively inhibits RNA polymerases I and II. Activity was abolished by treatment with actinomycin D (40 μg/ml), an inhibitor of RNA polymerases I, II, and II (not shown). Blots were exposed to film for 3 h.

A-II Stimulated an Increase in Phosphorylation of UBF, but Did Not Alter UBF Content—Previous studies have shown that the degree of phosphorylation of UBF is significantly reduced when CHO cells are serum-deprived (32). To determine whether A-II treatment resulted in increased phosphorylation of UBF in VSMC, prelabeled cells were stimulated with A-II or SFM for various times and immunoprecipitated with an anti-UBF antibody. Results demonstrated basal phosphorylation of UBF in growth-arrested cells in SFM (Fig. 2). This result was not unexpected since constitutive rRNA synthesis is presumably required for the positive protein balance and cell viability of VSMC under the conditions of our experiments. However, A-II induced a marked increase in phosphorylation of UBF that was detectable within 15 min, peaked at 30 min (223 ± 34% above SFM, Student’s t test; p < 0.05, n = 4), and remained elevated for at least 1 h.

To determine if the concentration of exogenous A-II had an effect on the magnitude of UBF phosphorylation, VSMC were stimulated with different concentrations of A-II. Since the results from the preceding studies on the kinetics of UBF phosphorylation showed maximal UBF phosphorylation at 30 min, this time point was examined. Results demonstrated that the largest increase in phosphorylation of UBF (241%) was observed at an A-II concentration of 10^-8 M (Fig. 3).

To test whether the A-II induced increase in 18 S rRNA synthesis and/or UBF phosphorylation were associated with increases in content of UBF, Western blot analyses were performed using an anti-UBF antibody at 1, 2, and 24 h following A-II or vehicle treatment. Two immunoreactive proteins were detected corresponding to the 94- and 97-kDa UBF isoforms (Fig. 4). Densitometric analysis of the blots showed no significant difference in UBF protein content between A-II and SFM groups at any time point examined (n = 4).

Phosphorylation of UBF Occurred Exclusively on Serine Residues—The majority of cellular phosphoproteins are phosphorylated on either tyrosine, threonine, or serine residues. To determine the amino acid(s) that were phosphorylated in the basal (SFM) state, and in response to A-II, in vivo labeled UBF bands were cut out of the PVDF membrane and acid-hydrolyzed. Phosphoamino acid analysis revealed that, in both the SFM- and A-II-stimulated conditions, phosphorylation occurred exclusively on serine residue(s) (Fig. 5). This experiment does not reveal how many serines are phosphorylated nor does it indicate if the phosphorylated residues are the same in the basal (SFM) state and following A-II treatment.

A-II Increased Accumulation of UBF within the Nucleus—To determine if A-II affected the intracellular distribution of UBF, post-confluent growth arrested VSMC were stimulated with A-II and fixed with 3% formaldehyde for immunostaining.
Thus, A-II appears to be a potent activator for UBF translocation into the nucleolus of VSMC. Casein Kinase II (CKII) is a ubiquitous protein kinase that is intrinsically involved in the regulation of serum-induced 
proliferation and increased rDNA transcription in CHO cells. Importantly, however, this study is the first to demonstrate such an effect in response to a known ligand (A-II), rather than serum. Moreover, an important distinction between our study and previous work is that A-II induces hypertrophy not hyperplasia under the conditions of our experiments (12). As such, this is the first investigation to provide evidence for the involvement of UBF phosphorylation and/or nucleolar localization in the hypertrophic response of vascular smooth muscle A-II treatment.

A-II-induced hypertrophy of vascular smooth muscle cells in culture has been shown to be associated with a generalized increase in protein synthesis as well as selective increases in synthesis of cell-specific proteins such as SM α-actin and SM myosin heavy chain (23, 24). The former observation suggests that at least part of the hypertrophic effect of A-II is due to an alteration in the translational capacity and/or activity of the cell. A-II stimulation has been shown to increase RNA content (12, 25), an obvious requisite step for increasing the translational capacity of the cell. Results from nuclear run-on assays in the present study demonstrate that A-II induces a transient yet marked increase in 18 S rRNA synthesis, as well as increased phosphorylation and nucleolar localization of the rRNA transcription factor UBF. The preceding are three cellular processes that might be expected to serve as a regulatory event for the enhanced protein synthesis characteristic of VSMC hyper-

Fig. 2. Phosphorimage illustrating the kinetics of UBF phosphorylation in response to A-II. Post-confluent growth arrested VSMC were prelabeled with [32P]orthophosphoric acid and treated with A-II (10^{-8} M) or vehicle (SFM). The cells were harvested and lysates immunoprecipitated with an antibody to UBF. The immunoprecipitates were analyzed on a 4% SDS-PAGE, and labeled bands were visualized and quantitated on a PhosphorImager (6-h exposure). Increased phosphorylation above SFM was detectable within 15 min and persisted up to 1 h after A-II treatment.

Fig. 3. The concentration dependence of A-II on phosphorylation of UBF. Prelabelled VSMC were stimulated for 30 min with different concentrations of A-II or the vehicle SFM. UBF was immunoprecipitated from cell lysates and run on 12% SDS-PAGE mini-gel. Labeled proteins from the immunoprecipitated material were visualized with a PhosphorImager (6-h exposure).

Fig. 4. Western blot analysis of UBF1 and UBF2 in A-II- and vehicle-treated VSMC. Post-confluent VSMC were growth-arrested in SFM and stimulated for 4, 8, and 24 h with A-II (10^{-8} M) or SFM vehicle. Cells were harvested, and equal amounts of cellular protein were analyzed by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with a rabbit anti-UBF antibody. Two immunoreactive proteins corresponding to UBF1 and UBF2 were detected. No reactivity was observed when the membrane was immunoblotted with control rabbit serum.

Fig. 5. Phosphoamino acid analysis of UBF from A-II- and SFM-treated cells. UBF immunoprecipitates were transferred onto a PVDF membrane, and the labeled bands were cut out and acid hydrolyzed. The sample was mixed with phosphothreonine (P-Thr), phosphoserine (P-Ser), and phosphotyrosine (P-Tyr), spotted on a thin layer cellulose plate (origin), and electrophoresed toward the anode. The dashed circles indicate the migration of the phosphorylated standards.

Confocal images of cells stained for UBF following a 15-min treatment with either A-II or SFM are shown in Fig. 6. In the absence of A-II, UBF was found to be evenly dispersed throughout the nucleus (Fig. 6a). In contrast, treatment with A-II led to almost exclusive localization of UBF within the nucleolus (Fig. 6b). This nucleolar concentration of UBF in response to A-II is compatible with previous reports which describe accumulation of UBF within the nucleus during transcriptionally active periods of the cell cycle (38). Thus, A-II appears to be a potent activator for UBF translocation into the nucleolus of VSMC.
The kinase responsible for phosphorylation of UBF in vivo has not been determined. Casein kinase II is an attractive candidate for the UBF kinase for several reasons. First, CKII phosphorylates UBF obtained from a variety of sources. Second, this kinase has been shown to be associated with RNA polymerase I in the nucleolus and to phosphorylate several nuclear proteins (39–41). Third, the activity of CKII has been reported to fluctuate with the growth rate of cells, and mirror the activity of rDNA transcription (42). Results of the present studies show that although CKII is able to phosphorylate recombinant UBF in vitro, the activity of CKII in vivo is unchanged after A-II treatment. This does not necessarily eliminate a possible role for CKII-mediated phosphorylation of UBF in vivo, however, our results suggest that CKII is not the A-II-inducible kinase responsible for increased UBF phosphorylation. CKII may, however, be important as a kinase that is constitutively active, or its regulatory activity may be dependent upon localization of UBF. We are currently investigating whether CKII is actually the kinase that phosphorylates UBF in vivo by comparative two-dimensional phosphotryptic mapping of UBF phosphorylated in vivo (SFM and A-II) versus purified recombinant UBF phosphorylated in vitro with CKII.

The distribution of UBF at various stages of the cell cycle and in response to serum stimulation has been studied to investigate the relationship between UBF localization and rDNA...
transcription (32, 38). In all cases, little or no UBF labeling was observed outside the nucleus. In addition, there is a lack of direct evidence that cleolar translocation into the nucleolus. The mechanisms revealed that maximal phosphorylation of UBF did not occur 
precede its transcription regulatory activity. We have shown likely that translocation of UBF inside the nucleolus should 
initiation complex at the nuclear organization region, it seems 
UBF activation. Since UBF forms part of the transcription 
ationization data may provide some insight into the mechanism of 
avtivation and/or localization of UBF.

mitogenesis. However, the present studies are the first to dem- 
strate such effects utilizing a single well defined agonist which induces hypertrophic, rather than mitogenic, growth. 
Further studies are required to determine the signaling path-
way that mediates phosphorylation of UBF and to determine 
the mechanisms whereby phosphorylation of UBF might regu-
late RNA synthesis in vivo.

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