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Overexpression of the transcription factor UBF1 is sufficient to increase ribosomal DNA transcription in neonatal cardiomyocytes: Implications for cardiac hypertrophy

(transfection/RNA polymerase I/ribosome biogenesis/heart/growth)

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ABSTRACT **The accelerated protein accumulation characteristic of cardiomyocyte hypertrophy results from increased cellular protein synthetic capacity (elevated ribosome content). The rate limiting step in ribosome accumulation is transcription of the rRNA genes. During neonatal cardiomyocyte hypertrophy induced by norepinephrine or spontaneous contraction, changes in the expression of a ribosomal DNA transcription factor, UBF, correlated with increased rates of ribosome biogenesis. We hypothesized that elevated expression of UBF was part of the mechanism by which these hypertrophic stimuli effected increases in the rate of transcription from the rDNA promoter. In this study, we have examined directly the effect of overexpressing UBF on rDNA transcription in neonatal cardiomyocytes in culture. In control experiments, a novel reporter construct for rDNA transcription (pSMECAT) showed similar increases in activity in response to hypertrophic stimuli (10^{-4} M phenylephrine, 10^{-7} M endothelin, and spontaneous contraction) as did the endogenous rRNA genes. When contraction-arrested cardiomyocytes were cotransfected with pSMECAT and increasing amounts of a UBF1 expression vector; a dose-dependent (3–5 fold) increase in rDNA transcription was observed. Western blot analysis confirmed that the overexpressed, FLAG-tagged UBF accumulated in the cardiomyocyte nuclei. The observation that overexpression of UBF1 is sufficient to increase rDNA transcription in neonatal cardiomyocytes provides evidence in support of the hypothesis that the regulation of UBF is a key component of the increased ribosome biogenesis and protein accumulation associated with cardiomyocyte hypertrophy.**

Hypertrophic growth of the heart muscle cells (cardiomyocytes) is a prominent feature both of normal cardiac development and of many adult cardiovascular diseases (for review, see refs. 1 and 2). This process requires an increased protein synthetic capacity (elevated ribosome content) that is regulated at the level of transcription of the ribosomal genes (rDNA) (for review, see ref. 3). However, the molecular mechanisms by which hypertrophic stimuli regulate rDNA transcription and thus ribosome accumulation in cardiomyocytes are not known.

Increased rates of rDNA transcription can be due to alterations in the chromatin structure or to changes in the amounts and/or activities of RNA polymerase I and/or the rDNA transcription factors (4–6). Recent studies have demonstrated that different cell types use different mechanisms to regulate rDNA transcription. Thus, observations in one system cannot necessarily be extrapolated to others. For example, the down-regulation of rDNA transcription associated with the differentiation of U937 cells can, at least partially, be attributed to the direct interaction of Rb^{110} with the rDNA transcription apparatus (7). In contrast, when murine lymphosarcoma P1798 cells are exposed to hormones that repress growth, the concomitant reduction in rDNA transcription activity has been attributed to the inactivation of an RNA polymerase I-associated factor referred to as TFIC (8, 9). In the context of cardiomyocyte hypertrophy, one factor that has recently been implicated in the regulation of ribosome biogenesis is the rDNA transcription factor UBF (3, 10–13).

UBF is a nucleolar protein required for efficient transcription of the 45S rDNA promoter *in vitro* (4–6). Purified UBF consists of two proteins (UBF1, 97 kDa; UBF2, 94 kDa) that form homo- and heterodimers and bind to DNA (14–17). Interestingly, only UBF1 has been shown to activate transcription *in vitro* (18). Rat UBF binds between nucleotides −70 and −110 of the 45S promoter (19, 20) and interacts with at least one other transcription factor complex termed SL-1 (21–23). UBF is a phosphoprotein and, *in vitro*, hypophosphorylated forms of the protein are less efficient at trans-activating the 45S rDNA promoter than hyperphosphorylated forms (24, 25).

Evidence suggesting that UBF is a potential target for regulation during periods of altered rDNA transcription in cardiomyocytes stems from the strong correlation between the expression/phosphorylation of this protein and the growth status of the cells. For example, the increased rDNA transcription associated with endothelin-1 (Et-1)-induced hypertrophy of neonatal cardiomyocytes is characterized by the hyperphosphorylation of a constant amount of UBF (13). In contrast, the elevated rate of rDNA transcription associated with either adrenergic- or contraction-mediated hypertrophy is associated with 2- to 4-fold increases in the content of UBF protein and mRNA levels (11, 12). Moreover, in the absence in changes in phosphorylation, the elevated levels of UBF protein observed during hypertrophy induced by adrenergic stimulus (2- to 3-fold) and contraction (3.5- to 4.5-fold) were proportional to the increased rates of rDNA transcription observed in response to each hypertrophic stimulus (175% and 210%, respectively) (3, 11, 12). These latter results are particularly interesting in light of *in vitro* studies that have demonstrated that the addition of UBF to UBF-depleted nuclear extracts increases the efficiency of rDNA transcription (26, 27).

In this study, we have examined part of the hypothesis that alterations in UBF expression/activity alone were sufficient to increase transcription from the rDNA promoter in neonatal cardiomyocytes. To do this, we constructed a reporter for rDNA transcription (pSMECAT). We show here that the

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Abbreviations: CAT, chloramphenicol acetyltransferase; βGAL, β-galactosidase; Et-1, endothelin-1; Rb, the protein product of the retinoblastoma susceptibility gene.

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activity of pSMECAT accurately reflects the endogenous rate of rDNA transcription, meaning that chloramphenicol acetyltransferase (CAT) activity was increased 2- to 3-fold when contraction-arrested neonatal cardiomyocytes were stimulated with various hypertrophic agents. These increases are similar to those observed for the endogenous ribosomal genes after hypertrophic stimuli (3). When cardiomyocytes were cotransfected with UBF1 under the control of the cytomegalovirus promoter (pCDNA3-UBF1) and pSMECAT, CAT activity increased proportionally to the amount of pCDNA3-UBF1 cotransfected.

MATERIALS AND METHODS

Cardiomyocyte Culture. Neonatal cardiomyocytes were isolated from the ventricles of day old Sprague–Dawley rat pups and cultured as described (11, 12, 28). KCl (50 mmol/liter) was added to the media to prevent the spontaneous contraction characteristic of neonatal cardiomyocytes plated at high density (4×10^6 cells per 60-mm dish) (29).

Western Blot Analysis. Western blot analyses with a polyclonal anti-UBF antibody and a monoclonal anti-FLAG M2 antibody (catalog no. IB13026/6D1311, IBI) were carried out on nuclei isolated from cardiomyocytes as described previously (11–13). Protein and DNA determinations were performed as described (12).

Construction of rDNA Transcription Reporter Constructs. The murine ribosomal promoter fragment (30) containing the sequence from −152 to +60, relative to the site of transcription initiation (+1), was subcloned by PCR using the primers 5′-GAATTCTGAGGTCCGGTTCTTTTCG-3′ and 5′-GAATTCCTTAAATCGAAAGGGTCTC-3′. Amplification of this subclone with the primers 5′-GAATTCTGAGGTCCGGTTCTTTTCG-3′ and 5′-CCTTCCAGGTATTCTCTG-3′ resulted in a 150-bp fragment containing a G to A mutation of base −7 within the core promoter. After purification on an agarose gel, the mutated fragment was used in combination with the primer 5′-GAATTCCTTAAATCGAAAGGGTCTC-3′ to generate a complete mutant promoter, i.e., −152 to +60. Both the mutant and the *sm*all wild-type promoters (SM) were then inserted into a vector (pECAT) containing the encephalomyocarditis virus IR*E*S upstream (31) of the *Escherichia coli* CAT gene (*CAT*), resulting in pSMECAT (see Fig. 1*A*).

Construction of UBF Expression Constructs. Two UBF expression constructs containing the entire open reading frame of UBF-1 were used in this study (see Fig. 1*B*). pCDNA3-UBF1 was generated by PCR from the rat UBF1 cDNA (14) using the primer 5′-CATCCGGGATCCAATGAACGGAGAAGCCGACTG-3′, which contains a *Bam*HI linker followed by the first 20 nucleotides of the 5′ end of the UBF1 coding region, and a second primer 5′-GGACTCGAGAATTCTCAGTTGGAGTCAGAGTC-3′ that was complimentary to the last 19 nucleotides of the UBF-1 cDNA followed by an *Eco*RI linker. pCDNA3-UBF1FLAG was constructed using the same 3′ primer as used for pCDNA3UBF-1; however, the 5′ primer, 5′-CATCCGGGATCCATGGACTACAAGGACGACGATGACAAGAACGGAGAAGCCGACTG-3′ contained an additional 24 nucleotides coding for the FLAG peptide (IBI), inserted in-frame, between the start ATG and second codon of UBF1 cDNA. After PCR amplification, the products were cloned into the mammalian expression vector pCDNA-3 (Invitrogen). This vector drives expression of UBF-1 under the control of the cytomegalovirus promoter. The orientations of the inserts were confirmed by sequencing and restriction endonuclease mapping.

Transfection, CAT, and Luciferase Assays. After 2 days in culture, neonatal cardiomyocytes were cotransfected in the presence of OPTI-MEM (GIBCO/BRL) with the indicated constructs, (1–5 μg per 60-mm dish) and pSV40-βGAL (0.5 μg per 60-mm dish) (Promega) using Lipofectamine (GIBCO/BRL). Variable amounts of the basic vector, pCDNA3, was included in the transfection media so that all transfections contained the same amount of DNA. Five hours after transfection, the culture media was replaced with fresh, serum-free, defined media containing 50 mM KCl. Hypertrophic growth was initiated by treatment of the contractile arrested cardiomyocytes with phenylephrine (10^{-4} M) (12), endothelin (10^{-7} M) (13), or by allowing the cells to resume spontaneous contraction (29). After transfection (12 or 24 hr), the cells were harvested, lysates were prepared as described (32) and frozen at −80°C until assayed for CAT, luciferase, or β-galactosidase activity (βGAL) (32). The cells from 8 dishes (32×10^6 cells) were combined for each assay point. The synthesis of acetylated chloramphenicol was measured by separating acetylated [^{14}C]chloramphenicol from unmodified [^{14}C]chloramphenicol by TLC (32). The results of the CAT and luciferase assays were normalized by the results of the βGAL assays to correct for variations in the efficiency of transfection. Transfection efficiency, as assayed by an *in situ* βGAL assay (Promega), was 5–7%.

RESULTS

pSMECAT Is an Accurate Reporter for Endogenous rDNA Transcription. The transfection efficiency of neonatal cardiomyocytes using standard techniques (e.g., $CaPO_4$, DEAE–Dextran, cationic lipids) is usually less than 10% (33). For this reason, it is not possible to directly measure the effects that UBF overexpression might have on endogenous rDNA transcription. This necessitated the utilization of a reporter plasmid (pSMECAT) that would reflect the rate of transcription of the endogenous ribosomal genes (see *Materials and Methods*; Fig. 1*A*).

The first series of experiments was designed to determine whether pSMECAT was an accurate reporter for endogenous rDNA transcription in neonatal cardiomyocytes during growth and quiescence. Neonatal cardiomyocytes were transfected with the wild-type 45S rDNA promoter construct, pSMECAT, or the mutant 45S rDNA promoter construct, pSMECAT-7, and then exposed to either vehicle or to hypertrophic stimuli as described. After 24 hr of stimulus, the cells were harvested and their relative CAT activities were measured.

Extracts prepared from contraction-arrested quiescent or stimulated (10^{-4} M phenylephrine) cardiomyocytes that had been transfected with the mutant rDNA promoter construct, pSMECAT-7, exhibited minimal CAT activity (Fig. 2*A*, lanes 1 and 2). Extracts prepared from cardiomyocytes that had been transfected with the wild-type rDNA promoter, pSMECAT, demonstrated 10-fold more CAT activity than the pSMECAT-7 transfectants (Fig. 2*A*, lane 3). These findings confirm previous studies that have demonstrated that the guanine at −7 is essential for transcription by RNA polymerase I from the 45S promoter *in vivo* (34). They also indicate that the expression of pSMECAT must to be due to transcription by RNA polymerase I rather than spurious initiation from cryptic polymerase II or III promoters.

Cardiomyocytes transfected with the wild-type promoter construct, pSMECAT, demonstrated reproducible 2- to 3-fold increases in CAT activity after stimulation with either phenylephrine or Et-1 (Fig. 2*A*, lanes 4–6; Table 1) or spontaneous contraction (Fig. 2*B*). These increases in pSMECAT activity are in close agreement with the increases in endogenous rDNA transcription observed in neonatal cardiomyocytes in response to the same hypertrophic stimuli (11–13, 29). Interestingly, treatment of cardiomyocytes with the combination of phenylephrine (10^{-4} M) and Et-1 (10^{-7} M) induced a greater transcription of pSMECAT than observed using either compound alone (Table 1). Importantly, none of the hypertrophic stimuli investigated had any significant effect on the expression

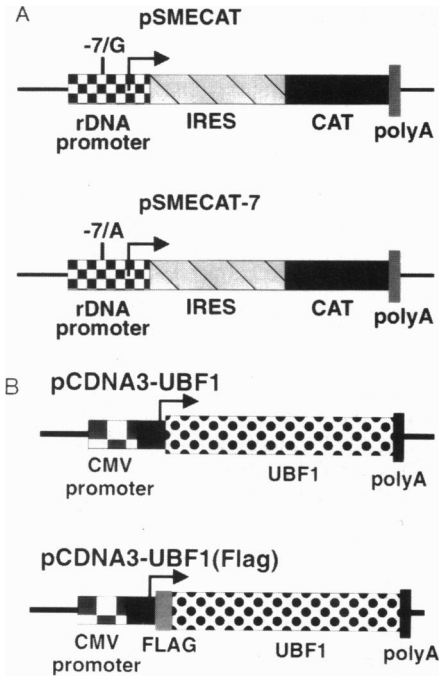

FIG. 1. Reporter constructs for 45S rDNA transcription and UBF1 expression plasmids. (*A*) Schematic representation of the 45S rDNA reporter construct, pSMECAT and pSMECAT-7. (*B*) Schematic representation of the UBF1 and UBF1-FLAG expression plasmids, pCDNA3-UBF1 and pCDNA3-UBF1(FLAG). The relevant segments of each of the vectors are indicated.

of a cotransfected reporter construct, pSV40βGAL (Table 1). Therefore, the observed increases in pSMECAT activity were not due to nonspecific affects of the growth stimuli, and pSMECAT was both quantitatively and qualitatively an accurate reporter of endogenous 45S rDNA transcription in neonatal cardiomyocytes.

Overexpression of UBF-1 Increases rDNA Transcription in Cardiomyocytes. We next examined the hypothesis that overexpression of UBF-1, in the absence of hypertrophic stimuli, would be sufficient to increase transcription from the 45S rDNA promoter.

We first determined whether the transfection of primary neonatal cardiomyocyte cultures with increasing amounts of a UBF-1 expression plasmid would result in a dose-dependent accumulation of recombinant UBF-1 protein in the cardiomyocyte nuclei. To differentiate the transfected UBF-1 from the endogenous UBF-1, we constructed a plasmid [pCDNA3-UBF1(FLAG)] that would drive the expression of FLAG-tagged UBF1 (Fig. 3). Cardiomyocytes transfected for 12 hr with increasing amounts (1, 2, and 5 μg per 60-mm dish) of pCDNA3-UBF1(FLAG) exhibited a dose-dependent increase in expression of an anti-FLAG immunoreactive protein that migrated at the size predicted for UBF1-FLAG (98.5 kDa), slightly more slowly than the wild-type protein. Immunohistochemical analysis demonstrated that the FLAG-tagged UBF1 was restricted to the nucleoli of the cardiomyocytes (data not shown).

We then determined if the overexpression of UBF1 would increase transcription of a cotransfected rDNA reporter. While cotransfection of pCDNA3-UBF1 with the mutant promoter construct, pSMECAT-7, did not alter the level of CAT activity (Fig. 4*A*, lanes 1–4), the cotransfection of pCDNA3-UBF1 and the wild-type rDNA reporter resulted in a significant increase in CAT activity (Fig. 4*A*, lanes 4–8). The increases in CAT activity were proportional to the amount of UBF1 accumulated. Cotransfection of 2 and 5 μg of pCDNA3-UBF1 with pSMECAT resulted in 2.1- and 4.5-fold increases

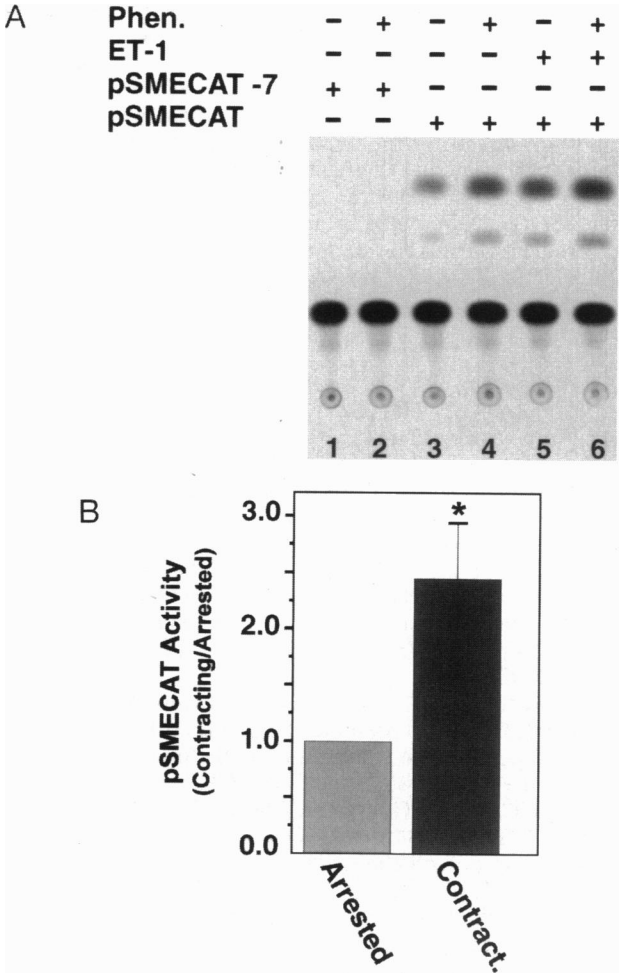

FIG. 2. Transcription from pSMECAT accurately reflects the activity of the endogenous cardiomyocyte ribosomal genes. (*A*) Phenylephrine and Et-1 stimulate transcription from pSMECAT. Cultured neonatal cardiomyocytes were transfected with the indicated constructs (2 μg per 60-mm dish) and the control vector, pSV40βGAL (0.5 μg per 60-mm dish), using lipofectamine as described. The cardiomyocytes were then treated with vehicle (lanes 1 and 3), phenylephrine (Phen., 10^{-4} M, lanes 2, 4, and 6), Et-1 (10^{-7} M, lanes 5 and 6), or a combination of phenylephrine and Et-1 (lane 6). After 24 hr, cell lysates were prepared and assayed for CAT activity and βGAL as described. (*B*) Contraction stimulates transcription from pSMECAT. Cardiomyocytes transfected with pSMECAT and pSV40βGAL as described either were contraction-arrested or allowed to spontaneously contract. After 24 hr, CAT and βGAL assays were performed and the results of the CAT assays were adjusted for the efficiency of transfection. The results are presented as the average fold increase (±SD) in pSMECAT activity in spontaneously contracting cells as compared with matched contraction arrested cells (*, $P < 0.05$; $n = 3$).

in CAT activity, respectively (Fig. 4*B*). In contrast, cotransfection of 5 μg of pCDNA3-UBF1 had no affect on transcription from pSV40βGAL (Table 2). The average of the βGAL assays presented in Table 2 was 24.8 ± 1.9. This was not significantly different from the values obtained when the cells were transfected with either pSMECAT or pSMECAT-7 and pSV40βGAL (25.2 ± 1.7) or when the cells were cotransfected with pCDNA3-UBF1 (24.3 ± 2.0). Since the βGAL gene is transcribed by polymerase II, it was unlikely that overexpression of UBF-1 was altering cardiomyocyte transcription in a nonspecific manner. We also examined the possibility that overexpression of UBF might stimulate transcription from another gene, e.g., *ANF*, that has been shown to respond to phenylephrine (35). Treatment of cardiomyocytes with either phenylephrine (10^{-4} M) or Et-1 (10^{-7} M) induced ANF

Table 1. pSMECAT is an accurate reporter for endogenous 45S rDNA transcription

	CAT, % conversion	βGAL, milliunits	Ratio [(CAT/βGAL) × 100]	Induction*
Experiment 1				
pSMECAT-7	0.19	27.32	0.68	—
pSMECAT-7 + Phen	0.13	27.23	0.49	—
pSMECAT	2.92	28.66	10.19	1.00
pSMECAT + Phen	6.92	32.78	21.12	2.07
pSMECAT + Et-1	6.91	28.11	24.58	2.41
pSMECAT + Phen/Et-1	10.19	31.28	32.58	3.20
Experiment 2				
pSMECAT-7	0.16	25.88	0.62	—
pSMECAT-7 + Phen	0.17	28.29	0.59	—
pSMECAT	2.74	25.25	10.84	1.00
pSMECAT + Phen	6.75	30.40	22.22	2.05
pSMECAT + Et-1	7.51	29.61	25.36	2.34
pSMECAT + Phen/Et-1	10.43	32.93	31.68	2.92

Cultured neonatal cardiomyocytes were transfected with either pSMECAT-7 or pSMECAT and pSV40βGal as described. The cardiomyocytes were then treated with either vehicle, phenylephrine (Phen, 10^{-4} M) or Et-1 (10^{-7} M) or a combination of phenylephrine and Et-1 for 24 hr after which the cells were harvested and analyzed for CAT and βGAL activity as described.

*The ratio of (CAT/βGAL) × 100 derived from cardiomyocytes transfected with pSMECAT and treated with vehicle was set as 1.

expression, as demonstrated by the increased levels of luciferase activity (Fig. 4*C*). However, the cotransfection of pCDNA3-UBF1 with Np328 had no affect on transcription from the *ANF* gene.

We have demonstrated here that the engineered overexpression of UBF can drive rDNA transcription in cardiomyocytes, perhaps duplicating the mechanism of action by which adrenergic agents increase rDNA transcription in neonatal cardiomyocytes (11). Et-1 also elevates rDNA transcription in cardiomyocytes, but Et-1 appears to effect this change by increasing the phosphorylation of UBF (13). Accordingly, we carried out experiments (n = 3) to determine whether the treatment of cardiomyocytes overexpressing UBF with Et-1 would result in an even greater level of rDNA transcription, i.e., CAT activity (Fig. 5). When cardiomyocytes were treated with Et-1 (Fig. 5, lane 4) or cotransfected with 2 μg of pCDNA3-UBF1 (Fig. 5, lane 5), CAT activity increased significantly, 2.0 ± 0.12-fold and 2.1 ± 0.13-fold, respectively. When cardiomyocytes were both cotransfected with pCDNA3-UBF1 and treated with Et-1, we observed a significantly greater (3.9 ± 0.4-fold; $P < 0.05$) increase in CAT activity (Fig. 5, lane 6), suggesting that the affect of overexpressing UBF1 and treating with Et-1 was additive.

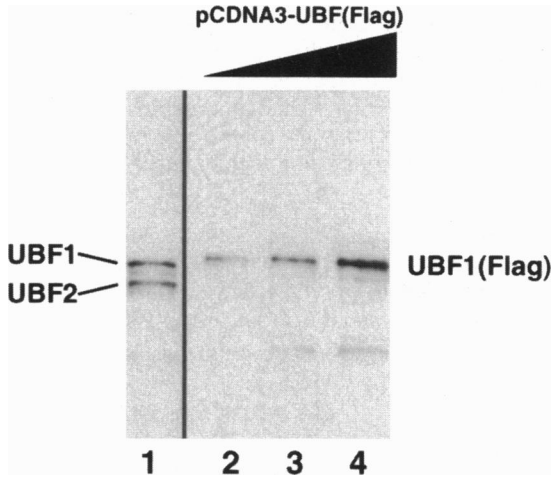

FIG. 3. Transfection of pCDNA3-UBF1(FLAG) results in a dose-dependent accumulation of UBF-1 protein in cardiomyocyte nuclei. Twelve hours after transfection with increasing amounts of pCDNA3-UBF1(FLAG) (1, 2, and 5 μg), cardiomyocyte nuclei were isolated, fractionated by SDS/PAGE, blotted to nylon membranes, and FLAG-tagged UBF was detected with a monoclonal antibody to the FLAG epitope (lanes 2–4). Nuclei of control cells transfected with the empty expression vector pCDNA3 was fractionated on a parallel lane and blotted with an anti-UBF antibody (lane 1).

DISCUSSION

We have shown that a transfected reporter for 45S rDNA transcription (pSMECAT) demonstrates similar increases in activity in response to hypertrophic stimuli (10^{-4} M phenylephrine, 10^{-7} M endothelin, spontaneous contraction) as do the endogenous ribosomal genes in primary cultures of neonatal cardiomyocytes (3). In contrast, a mutant promoter with a substitution of an adenine for an guanine at −7 relative to the transcription initiation site is inactive. Thus, pSMECAT is, both quantitatively and qualitatively, an accurate reporter for 45S rDNA transcription. Because increased rDNA transcription is a consistent feature of all forms of cardiomyocyte hypertrophy (3), pSMECAT should prove to be of general use as an accurate marker of growth to investigators who need to carry out transfection experiments measuring the effects of overexpression or "knockout" of putative components of hypertrophic signaling pathways.

Most importantly, we have also demonstrated that when cardiomyocytes were cotransfected with a vector that expresses UBF-1 (pCDNA3-UBF1) and pSMECAT, transcription from the rDNA reporter was increased. Furthermore, the increase in CAT activity was proportional to the amount of UBF1 overexpressed. The observation that transcription from pSMECAT responded to manipulation of the cellular content of UBF1 suggests that within the cell, the UBF, SL-1, and the other components of the rDNA transcription apparatus may be in an equilibrium between those that are free and those that are bound to the promoter. In this model, an increase in the activity of any single component, such as UBF, would drive the formation of initiation complexes and increase the rate of rDNA transcription. It is formally possible that the overexpression of UBF1 may affect rDNA transcription indirectly. It has been demonstrated that UBF can bind to Rb[110] (7). The overexpression of UBF1 might cause the release of unidentified Rb-binding proteins, which then stimulate rDNA tran-

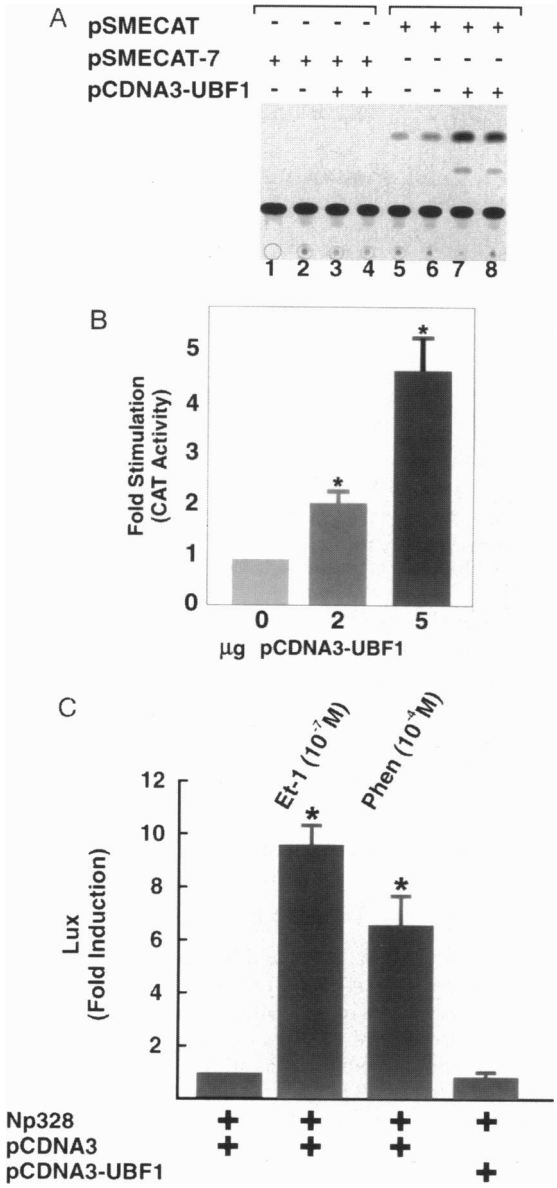

FIG. 4. Overexpression of UBF1 increases rDNA transcription but not ANF expression. (*A*) Cultured neonatal cardiomyocytes were transfected with pSV40βGAL (0.5 μg) and various combinations of pSMECAT (2 μg), pSMECAT-7 (2 μg), and pCDNA3-UBF1 (5 μg) as described. After 24 hr, cell lysates were prepared and assayed for CAT activity. (*B*) Experiments similar to those described in *A* were quantitated and the results adjusted for the efficiency of transfection. The results represent the average increase (±SD) in CAT activity in cells cotransfected with increasing amounts of pCDNA3-UBF1 (*, $P < 0.01$; $n = 5$). (*C*) Cultured neonatal cardiomyocytes were transfected with pGL3Basic (0.5 μg), a luciferase vector lacking a promoter, Np328 (0.5 μg), the ANF promoter driving luciferase expression, and pSV40βGAL (0.5 μg) and treated with vehicle, Et-1 (10^{-7} M), phenylephrine (10^{-4} M), or cotransfected with pCDNA3-UBF1 (2 μg). After 12 hr, cell lysates were prepared and assayed for luciferase activity (*, $P < 0.01$; $n = 4$).

scription by a mechanism not directly dependent upon UBF1. Even so, regardless of the mechanism(s), these results are consistent with the hypothesis that the regulation of the cellular content of UBF is part of the mechanism by which rDNA transcription is regulated during adrenergic- and contraction-induced hypertrophy of neonatal cardiomyocytes.

In contrast to the affect of phenylephrine, Et-1, a potent stimulator of rDNA transcription, does not increase the cellular content of UBF (13). Stimulation of cardiomyocytes with

Table 2. Overexpression of UBF does not affect transcription of a gene transcribed by RNA polymerase II

	CAT (% conversion)	βGAL, milliunits	Ratio [(CAT/βGAL) × 100]
pSMECAT-7	0,14	23.85	0.57
pSMECAT-7	0.25	23.47	1.05
pSMECAT-7 + UBF	0.23	27.59	0.83
pSMECAT-7 + UBF	0.25	22.19	1.14
pSMECAT	2.32	27.67	8.4
pSMECAT	2.49	25.92	9.6
pSMECAT + UBF	9.41	23.59	39.9
pSMECAT + UBF	7.88	23.73	33.2

Cultured neonatal cardiomyocytes were transfected with pSVβGal (0.5 μg) and various combinations of pSMECAT (2 μg), pSMECAT-7 (2 μg), and pCDNA3-UBF1 (UBF; 5 μg) as described. After 24 hr, the cell lysates were prepared and assayed for βGAL and CAT activity.

Et-1 results in hyperphosphorylation of UBF (13), which may be the mechanism by which Et-1 increases rDNA transcription. Interestingly, the treatment of cardiomyocytes with optimal doses of Et-1 and phenylephrine together resulted in a greater stimulation of rDNA transcription (3.1-fold) than that observed in response to either agent individually (2- and 2.4-fold, respectively). It is provoking to speculate that the ability of Et-1 to further enhance rDNA transcription in those experiments results from the hyperphosphorylation of the increased levels of UBF (24–26).

It is possible that affect of combining phenylephrine and Et-1 was only partially additive because the pathway(s) that connect these two stimuli to increased rDNA transcription overlap. Alternatively, the pathways may partially interfere with each other due to signal "cross-talk." Consistent with these models, an additive 4-fold response was observed when the cells were simultaneously transfected with pCDNA3-UBF1 (that would increase the cellular content of UBF) and stimulated with Et-1 (that would increase the activity of UBF). In this case, because the cellular content of UBF was elevated by direct overexpression, there was no potential for interference between the phenylephrine and Et-1 pathways.

In summary, these studies have demonstrated that the rate of rDNA of transcription can be increased in neonatal cardiomyocytes by the increased expression of the rDNA transcription factor UBF. Future studies on the regulation of UBF gene expression should be extremely useful in determining the second messengers and molecular signals that couple growth stimuli, such as α_1-adrenergic agents and contraction, to the activation of rDNA transcription and growth in cardiomyocytes.

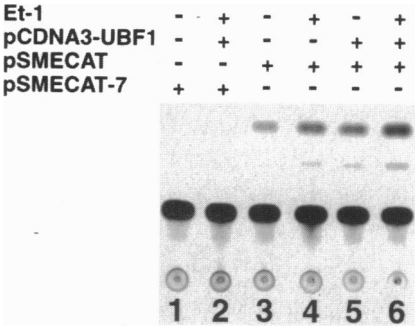

FIG. 5. The affects on rDNA transcription of overexpressing UBF1 and treating cardiomyocytes with Et-1 are additive. Cultured neonatal cardiomyocytes were transfected with pSMECAT-7 (2 μg) or pSMECAT (2 μg) and treated with vehicle (lanes 1 and 3), Et-1 (10^{-7} M, lanes 2, 4, and 6) or cotransfected with pCDNA3-UBF1 (2 μg, lanes 2, 5, and 6). After 24 hr, cell lysates were prepared and assayed for CAT activity.

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1. Morgan, H., Chua, B. & Russo, L. (1992) in *The Heart and Cardiovascular System*, eds. Fozzard, H. E., Haber, E., Jennings, R., Katz, A. & Morgan, H. (Raven, New York), pp. 1505–1524.
2. Rakusan, K. (1984) in *Growth of the Heart in Health and Disease*, ed. Zak, R. (Raven, New York), pp. 131–166.
3. Hannan, R. & Rothblum, L. (1995) *Cardiovasc. Res.* **30,** 501–510.
4. Moss, T. & Stefanovsky, V. Y. (1995) *Prog. Nucleic Acids Res. Mol. Biol.* **50,** 25–66.
5. Paule, M. (1994) *Transcription: Mechanisms and Regulation* (Raven, New York).
6. Jacob, S. (1995) *Biochem. J.* **306,** 617-626.
7. Cavanaugh, A., Hempel, W., Taylor, L., Rogalsky, V., Todarov, G. & Rothblum, L. (1995) *Nature (London)* **374,** 177–180.
8. Schnapp, A., Schnapp, G., Erny, B. & Grummt, I. (1993) *Mol. Cell. Biol.* **13,** 6723–6732.
9. Gokal, P., Cavanaugh, A. & Thompson, E. (1986) *J. Biol. Chem.* **261,** 2536–2541.
10. Xie, W.-Q. & Rothblum, L. (1991) *BioTechniques* **11,** 324–329.
11. Hannan, R., Luyken, J. & Rothblum, L. I. (1995) *J. Biol. Chem.* **270,** 8290–8297.
12. Hannan, R., Luyken, J. & Rothblum, L. (1996) *J. Biol. Chem.* **271,** 3213–3220.
13. Luyken, J., Hannan, R., Cheung, J. & Rothblum, L. (1996) *Circ. Res.* **78,** 354–361.
14. O'Mahony, D. & Rothblum. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3180–3184.
15. Learned, R., Learned, T., Haltiner, M. & Tjian, R. (1986) *Cell* **45,** 847–857.
16. Kuhn, A., Voit, R., Stefanovsky, V., Evers, R., Bianchi, M. & Grummt, I. (1994) *EMBO J.* **13,** 416–424.
17. Pikaard, C., Smith, S., Reeder, R. & Rothblum, L. (1990) *Mol. Cell. Biol.* **10,** 3810–3812.
18. Smith, S., O'Mahony, D., Kinsella, B. & Rothblum, L. (1993) *Gene Expression* **3,** 229–236.
19. Bell, S., Learned, R., Jantzen, H. & Tjian, R. (1988) *Science* **241,** 1192–1197.
20. Schnapp, A., Clos, J., Hadelt, W., Schreck, R., Cvekl, A. & Grummt, I. (1990) *Nucleic Acids Res.* **18,** 1385–1393.
21. Mishima, Y., Financsek, I., Kominami, R. & Muramatsu, M. (1982) *Nucleic Acids Res.* **10,** 6659–6670.
22. Learned, R., Cordes, S. & Tjian, R. (1985) *Mol. Cell. Biol.* **5,** 1358–1369.
23. Hempel, W., Cavanaugh, A., Hannan, R. & Rothblum, L. (1996) *Mol. Cell. Biol.* **16,** 557–563.
24. O'Mahony, D., Smith, S., Xie, W.-Q. & Rothblum, L. (1992) *Nucleic Acids Res.* **20,** 1301–1308.
25. O'Mahony, D., Xie, W.-Q., Smith, S., Singer, H. & Rothblum, L. (1992) *J. Biol. Chem.* **267,** 35–38.
26. Voit, R., Schnapp, A., Kuhn, A., Rosenbauer, H., Hirshmann, P., Stunnenberg, H. G. & Grummt, I. (1992) *EMBO J.* **11,** 2211–2218.
27. Smith, S., Oriahi, E., Lowe, D., Yang-Yen, H. F., O'Mahony, D., Rose, K., Chen, C. & Rothblum, L. (1990) *Mol. Cell. Biol.* **10,** 3105–3116.
28. McDermott, P., Rothblum, L., Smith, S. & Morgan, H. (1989) *J. Biol. Chem.* **264,** 220–227.
29. Simpson, P. C., McGrath, A. & Savion, S. (1982) *Circ. Res.* **51,** 787–801.
30. Kuehn, M. & Arnheim, N. (1983) *Nucleic Acids Res.* **11,** 211–224.
31. Jang, S. K., Davies, M. V., Kaufman, R. J. & Wimmer, E. (1989) *J. Virol.* **63,** 1651–1660.
32. Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J. & Struhl, K. (1993) *Current Protocols in Molecular Biology* (Wiley, New York).
33. Kirshenbaum, L., Maclellan, W., Mazur, W., French, B. & Schneider, M. (1993) *J. Clin. Invest.* **92,** 381–387.
34. Xie, W. Q., O'Mahony, D., Smith, S., Lowe, D. & Rothblum, L. (1992) *Nucleic Acids Res.* **20,** 1587–1592.
35. Ardati, A. & Nemer, M. (1993) *EMBO J.* **12,** 5131–5139.