Cardiac hypertrophy in vivo is associated with increased expression of the ribosomal gene transcription factor UBF

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Received 25 April 2003; revised 27 June 2003; accepted 27 June 2003

First published online 8 July 2003

Edited by Lev Kisselev

Abstract The ribosomal DNA transcription-specific factor, UBF, is a key target for the regulation of ribosomal RNA synthesis and hypertrophic growth of isolated neonatal cardiomyocytes. In this study, we have examined whether UBF expression is also an important determinant of cardiac growth rates in vivo. We show that rDNA transcription, rRNA synthesis and UBF expression in left ventricular myocytes isolated from mice 1–6 weeks following transverse aortic constriction were significantly increased (2.5–3.5-fold) compared to the levels in myocytes from the left ventricle of sham-operated mice.

Key words: rDNA transcription; Upstream binding factor; Ribosome; Left ventricular hypertrophy; Cardiac hypertrophy; Pressure overload

1. Introduction

Growth of cardiac muscle cells during left ventricular hypertrophy (LVH) results from an increased accumulation of cellular protein due to an accelerated rate of protein synthesis [1]. Elevated rates of protein synthesis can be achieved by altering the translational efficiency of existing ribosomes and/or by accelerating the rate of synthesis of new ribosomes (increased translational capacity). Recent studies both in vivo and with isolated myocyte cultures suggest that the hypertrophic growth response is biphasic with an initial acute increase in translational efficiency, followed by an adaptive increase in translational capacity or rRNA synthesis during sustained increases in growth demand [2–4]. Indeed, accelerated rates of rRNA synthesis and ribosome accumulation have been demonstrated to accompany increased rates of protein synthesis in all models of cardiomyocyte hypertrophy in which it has been examined [5–7]. Furthermore, the increased rate of ribosome synthesis during α-adrenergic or contraction stimulation of neonatal cardiomyocytes is required for the increase of protein synthesis and cell size accompanying the hypertrophic response [8]. Together these studies suggest a major role for ribosome biogenesis in the regulation of cardiac growth.

For mammalian cells, a major rate-limiting step in ribosome biogenesis is the transcription of the ribosomal 45S gene (rDNA) by RNA polymerase I [9–11]. One factor that has been implicated in the regulation of ribosome biogenesis during cardiomyocyte hypertrophy is the rDNA transcription factor termed upstream binding factor or UBF (reviewed in [12–15]). In the context of cardiomyocyte growth, the cellular amount of UBF correlates strongly with the growth rate of non-dividing, cultured neonatal cardiomyocytes [5,6,12]. Moreover, direct over-expression of recombinant UBF1, in the absence of hypertrophic stimulus, is sufficient to significantly increase rDNA transcription in neonatal cardiomyocytes [16]. Conversely, inhibition of UBF accumulation via adenovirus-mediated delivery of UBF antisense RNA prevents increases in rDNA transcription, rRNA accumulation and protein synthesis during hypertrophic stimulation of neonatal cardiomyocytes [8].

One potential shortfall of the above studies is that they were performed in isolated neonatal cardiomyocyte cultures, which exhibit many important differences from fully differentiated adult cardiomyocytes in vivo. With respect to ribosome biogenesis, we have found that the rates of rDNA transcription in nuclei isolated from adult cardiomyocytes are approximately one-fifth of that found in equivalent amounts of nuclei isolated from neonatal cardiac myocytes (R. Hannan, unpublished data). This suggests that alternative regulatory mechanisms may control rDNA transcription in adult and neonatal cardiomyocytes. Thus, as a first step in addressing the role of UBF expression in the regulation of rDNA transcription and protein synthesis in vivo, we have examined UBF expression in cardiac myocytes isolated from control mice and mice undergoing transverse aortic constriction (TAC)-induced LVH.

2. Materials and methods

2.1. DNA constructs

A 755-bp rat atrial natriuretic peptide (ANP) cDNA, and a 177-bp rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA clone (GAPDH-177) used for RNase analysis have been described previously [17]. The mouse 45S rDNA external transcribed spacer (ETS) construct (pGEM4z-ETS) used to measure 45S rRNA levels

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Abbreviations: ANP, atrial natriuretic peptide; ERK1/2, extracellular signal-regulated kinases 1 and 2; ETS, external transcribed spacer; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LV, left ventricle; LVH, left ventricular hypertrophy; RV, right ventricle; SH, sham; TAC, transverse aortic constriction; UBF, upstream binding factor

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doi:10.1016/S0014-5793(03)00744-0

by RNase protection assay was prepared from a mouse genomic clone (pMENA) of the 45S gene [18]. An *Eco*RI-*Eco*RI (−640 to +155) fragment of pMENA was ligated into the *Eco*RI site of pGEM4z (Promega) in the antisense direction with respect to the T7 promoter site. Integrity of the clone was verified by sequencing.

2.2. Animals, microsurgery and functional determination

Three-month-old male and female mice of C57/SJL crossing were used. Animals were anesthetized with a mixture of ketamine/xylazine/atropine (8, 2 and 0.6 mg/100 g, respectively) and the pain reliever temgesic at 0.1 mg/100 g was given. After tracheal intubation and mechanical ventilation, a midline incision was made at the upper sternum. With the aid of a surgical microscope, the transverse aorta was dissected and then narrowed to a lumen size of 0.4 mm according to a method previously described [17,19]. Control mice underwent similar surgery except for the narrowing of the aorta. At the scheduled time after surgery, mice were anesthetized again as described above. A 1.4F micro-tip pressure transducer catheter (Millar Instruments) was inserted into the aorta via the right carotid artery and then advanced into the left ventricle (LV). Aortic blood pressure and left ventricular pressure were acquired digitally and averages of measurements from 10 consecutive beats were used.

2.3. Organ weights

Mice were killed by an overdose of pentobarbital. The LV, right ventricle (RV), and atria were separated and weighed. The lungs and liver were weighed, and the tibial length was measured.

2.4. Isolation of adult mouse cardiomyocytes

Cannulation of hearts was carried out via the aorta according to the Langendorff method in ice-cold Ca^{2+}-free, HEPES-buffered Krebs medium containing 10 mM 2,3-butanedione monoxime, 30 mM taurine, 5 mM creatine and 0.1 IU/ml insulin, as already described [20]. Hearts were perfused with the medium at 2 ml/min in 5-ml organ baths, kept at 37°C and gassed constantly with 95% O_2/5% CO_2. Collagenase (2 mg/ml) was added to the perfusate and the hearts perfused in a recirculating manner for 30 min. LV and RV were then gently dissected apart from the digested hearts in fresh collagenase-containing medium and the tissue filtered through coarse nylon mesh. Myocytes were purified away from fibroblasts and other cardiac non-cardiomyocytes by centrifugation at $100\times g$, and gently washed by resuspension and sedimentation in collagenase-free medium before final resuspension and sedimentation in phosphate-buffered saline. Myocytes prepared in this manner were immediately processed for RNA, DNA and protein analysis without further intervention.

2.5. RNA extraction and ribonuclease protection assays

Total RNA was isolated from adult cardiomyocytes as described previously [21] at the time points indicated. Plasmids containing rat ANP cDNA, GAPDH cDNA and a mouse 45S rDNA clone were used as templates to generate ^{32}P-labeled complementary RNA probes for hybridization and RNase protection analysis, as described [21]. Following hybridization and RNase digestion, the protected RNA hybrids were analyzed on denaturing polyacrylamide gels and quantitated on a Fuji BAS-1000 phosphorimaging system. Levels of GAPDH mRNA, simultaneously hybridized with ANP mRNA or 45S ETS rRNA, were used to correct for minor differences in RNA loading.

2.6. Protein and DNA determination

DNA determinations on cardiomyocytes isolated from LV and RV were performed by the method of Pederson [22] using herring sperm DNA as a standard. Protein levels were simultaneously determined in the same myocyte samples using the Bio-Rad DC assay kit as described previously [5,6].

2.7. SDS–PAGE and western analysis

Cell extracts (100 μg of protein) were resuspended in SDS-sample buffer, boiled and then size-fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (8% gels) and transferred to Immobilon™-P (Amersham) membranes. The blot was subsequently probed with the indicated antibodies to UBF1/2 [5,8,23,24] and extracellular signal-regulated kinase (ERK, Santa Cruz, K-23) and developed using the appropriate secondary antibodies and enhanced chemiluminescence (Amersham).

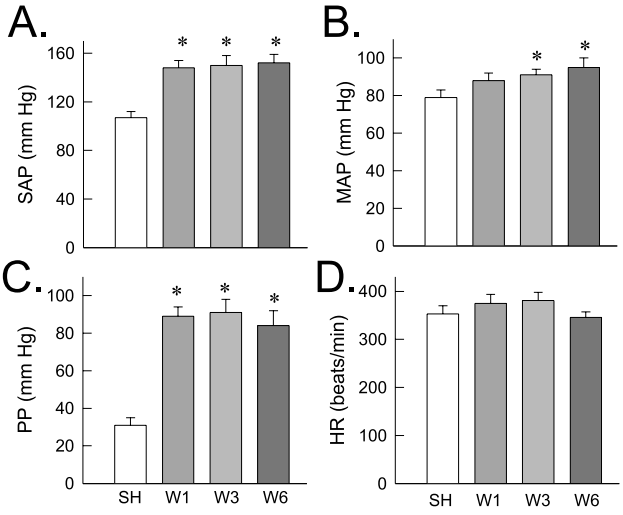

Fig. 1. Functional measurements from SH and TAC mice. Values are means ± S.E.M. SAP, proximal systolic arterial pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rates; W1–W6, week 1–6. *$P < 0.05$ vs. SH group ($n = 7$).

2.8. Statistics

Values are means ± S.E.M. Between-group differences were analyzed by one-way analysis of variance followed by Fisher's protected least significant difference test for post-hoc comparisons. Significance was determined at $P < 0.05$.

3. Results

3.1. Assessment of pressure overload

Catheterization was performed on mice with TAC or sham operation (SH) after 1, 3 or 6 weeks. Functional measurements and organ weights from SH animals did not change significantly with time at 1, 3 or 6 weeks after surgery and therefore the data were pooled to form a single SH group for clarity. TAC caused approximately 40 mm Hg increases in systolic aortic pressure (Fig. 1A) and LV systolic pressure (data not shown). The pulse pressure (Fig. 1C) was increased three-fold compared with the SH group. There was a modest rise in mean arterial pressure (Fig. 1B). Heart rates remained

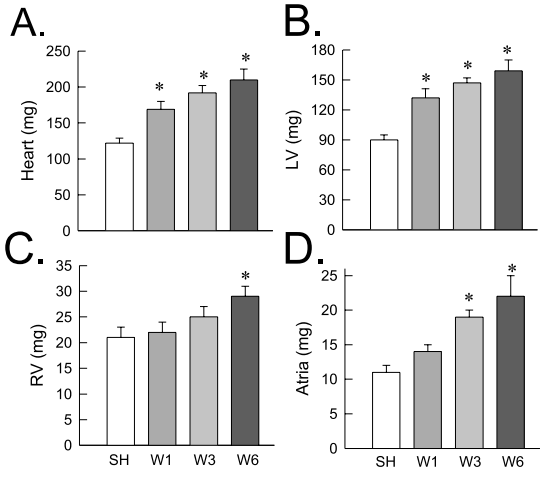

Fig. 2. Tissue weights from SH and TAC mice. Values are means ± S.E.M. W1–W6, week 1–6. *$P < 0.05$ vs. SH group ($n = 7$).

unchanged with time and were not significantly different from SH mice (Fig. 1D).

Because there were no differences in body weight and tibial length between groups, the organ weights are expressed as absolute values. TAC resulted in significant time-dependent increases in total heart weight and LV heart weight during weeks 1–6 (Fig. 2A,B). RV weights were not significantly altered in the TAC group at weeks 1 and 3 but were about 40% greater than in the SH group at week 6 following TAC (Fig. 2C). We have previously shown that hypertrophy of the RV after sustained TAC occurs in response to pulmonary congestion [25].

3.2. ANP mRNA expression

Myocytes were isolated from SH and TAC mice at the times indicated and used to prepare total RNA. RNase protection analysis was used to assess the effect of TAC on expression of the hypertrophy-induced marker gene ANP. ANP mRNA levels were three-fold higher in myocytes isolated from the LV of TAC mice compared to SH animals 3 weeks following TAC surgery (Fig. 3A,B) and remained elevated after 6 weeks (Fig. 3A). These results confirm that TAC-induced LVH is accompanied by activation of 'fetal gene program' markers as described by others [19].

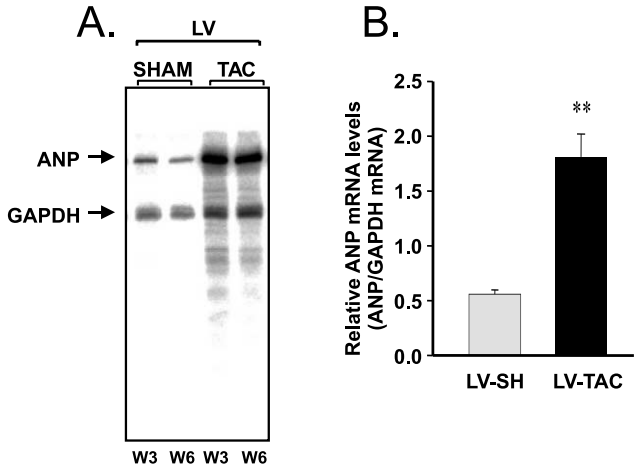

Fig. 3. Ventricular myocyte expression of ANP mRNA in SH and TAC mice. RNA was extracted from LV myocytes isolated 3 and 6 weeks (W3, W6) after SH or TAC. RNA (1 μg) was assayed by RNase protection for ANP and GAPDH mRNA. A: RNase-protected bands of ANP and GAPDH were visualized by phosphorimaging. B: Quantitative analysis of ANP mRNA expressed relative to GAPDH mRNA. Values are means ± S.E.M. of data from ventricles of SH and TAC animals at week 6. $^{**}P < 0.005$ vs. SH group ($n = 3$).

3.3. Protein and RNA content

Since over 80% of total RNA is ribosomal, RNA content was used as a measure of ribosome accumulation [14]. Adult cardiomyocytes undergo only limited, if any, DNA replication, and thus DNA content was used to normalize RNA content per cell. Myocytes were isolated from SH and TAC mice at the times indicated and used to measure total RNA, DNA and protein. Following quantitation, RNA to DNA

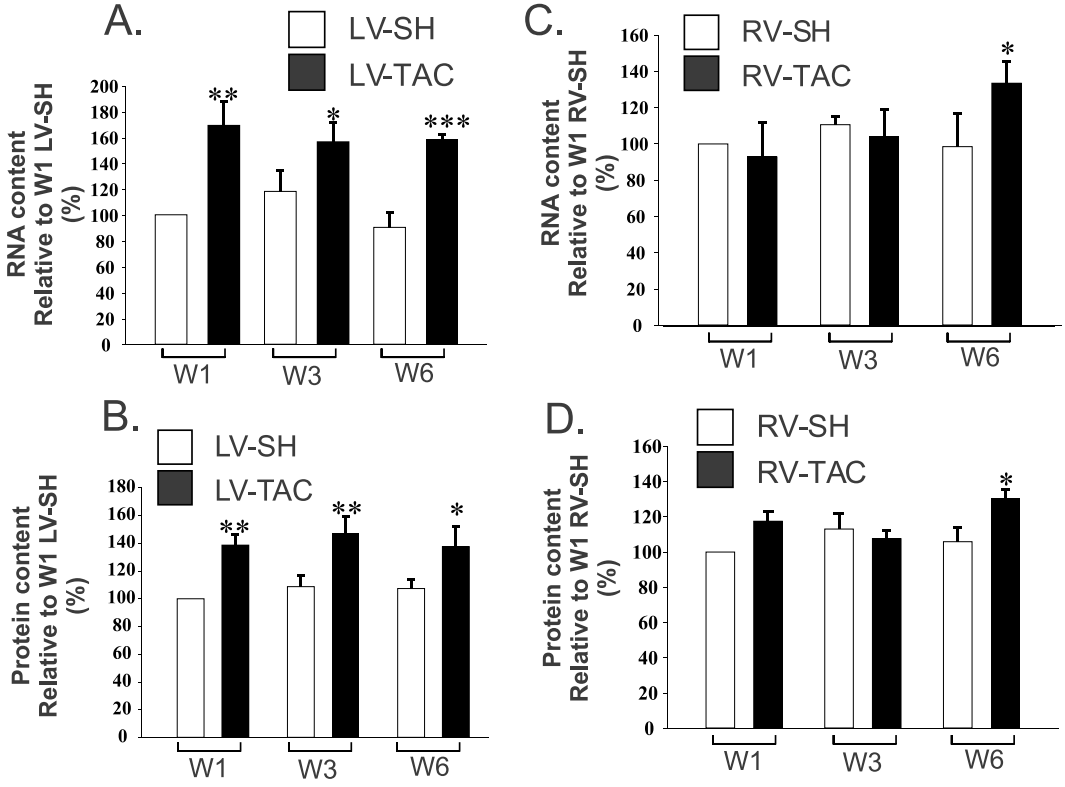

Fig. 4. Relative RNA and protein levels in ventricular myocytes isolated from SH and TAC mice. The values expressed in Table 1 were used to calculate the percentage (± S.E.M., $n = 3$–5) increase in RNA or protein content in LV or RV myocytes from TAC mice at 1, 3, and 6 weeks (W1, W3, W6), following surgery relative to the levels in LV or RV myocytes from SH mice at week 1. A,B: Increases in myocyte RNA to DNA ratios and protein to DNA ratios in LV-SH and LV-TAC mice. $^{*}P < 0.05$, $^{**}P < 0.005$, and $^{***}P < 0.0005$ vs. LV-SH at week 1. C,D: Increases in myocyte RNA and protein levels in RV-SH and RV-TAC mice. $^{*}P < 0.05$ vs. RV-SH at week 1.

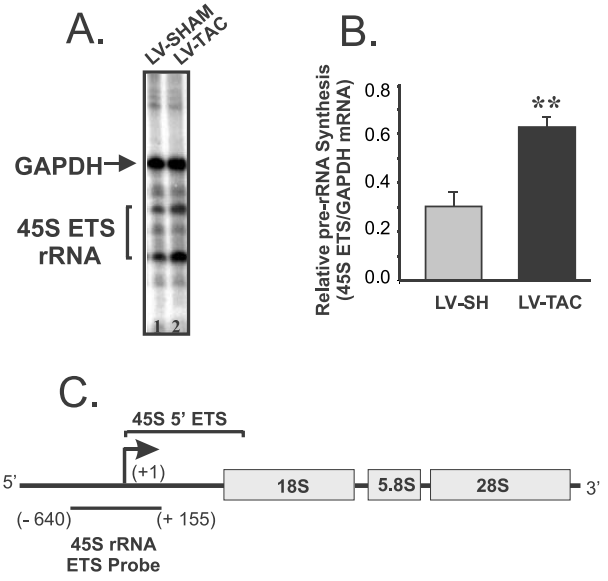

Fig. 5. Synthesis of ribosomal RNA in SH and TAC mice 1 week following surgery. RNA was extracted from LV myocytes isolated 1 week after surgery from SH and TAC mice. RNA (1 μg) was assayed by RNase protection for 45S 5′-ETS RNA and GAPDH mRNA as described in Section 2. A: RNase-protected bands of the 5′-ETS region of the 45S gene and GAPDH were visualized by phosphorimaging. B: Quantitative analysis of ribosomal RNA expressed relative to GAPDH mRNA. Values are means ± S.E.M. ($n = 3$). **$P < 0.005$ TAC vs. SH group. C: ETS probe used for the RNase protection assay protects the rapidly processed 5′-end of the 45S gene (+1 to +155). Two protected fragments with a size of ~105 and ~55 bp, respectively, can be detected on a denaturing polyacrylamide gel, resulting from rapid cleavage of a site in the 5′-ETS region [30].

and protein to DNA ratios were determined (Table 1). RNA to DNA ratios, and thus RNA content, were significantly increased (168.8 ± 18.2%, $P < 0.005$, $n = 3$) in myocytes isolated from LV of TAC mice compared to SH animals within 1 week of surgery and remained significantly elevated at 3 and 6 weeks (Fig. 4A). Total RNA content was not increased in myocytes from RV of TAC mice compared to SH mice at 1 and 3 weeks following surgery but was increased at 6 weeks (Fig. 4C), which correlated with the increase of RV mass that was observed at 6 weeks. These findings suggest that rRNA content is increased in response to pressure overload along with tissue mass. Similarly, TAC resulted in increased protein to DNA ratios (138.6 ± 7.3%, $P < 0.005$, $n = 3$), and thus protein content in LV myocytes compared to SH group 1 week following surgery, and remained elevated at weeks 3 and 6 (Fig. 4B). Protein content was also higher in the RV from TAC versus SH at 6 weeks (130.3 ± 5.5%, $P < 0.05$, $n = 3$). These results demonstrate that TAC-induced LVH is characterized by a significant increase in myocyte RNA accumulation reflecting increased rates of ribosome biogenesis, required for the accelerated accumulation of protein.

3.4. rRNA synthesis

In cardiomyocyte cultures, an important rate-limiting step in the accumulation of rRNA is the rate of transcription of the rDNA [26,27]. To confirm that the accumulation of total RNA was largely due to accelerated rDNA transcription rates, we measured rRNA synthesis in myocytes using a novel ribonuclease protection assay based on the ETS of the ribosomal 45S gene (Fig. 5C). The 28S, 5.8S and 18S rRNAs are transcribed as a single pre-rRNA transcript called the 45S pre-rRNA. Following synthesis of the 45S pre-rRNA the internal transcribed spacer and ETS regions of the 45S pre-rRNA transcript are rapidly processed and degraded to give rise to the stable 28S, 5.8S and 18S rRNA. The 5′-ETS region of the 45S pre-rRNA is degraded so proficiently (within minutes) compared to 28S, 5.8S and 18S rRNA (the latter have half-lives of several days) [9,28,29] that its accumulation can be used to measure the steady-state level of transcription of the 45S ribosomal gene. Accordingly we prepared a riboprobe that protects a portion of this ETS region and used it in a ribonuclease protection assay to measure the rate of accumulation of newly transcribed rRNA. Using this method, two main protected bands are observed for the 5′-ETS RNA (Fig. 5A) due to the presence of a major processing site within this region of the 5′-ETS (Fig. 5C) [30]. As can be seen, rRNA synthesis was significantly increased (2.1-fold) in myocytes isolated from LV of TAC mice compared to SH animals 1 week after TAC (Fig. 5A,B), and remained elevated 3 and 6 weeks after TAC (data not shown). This degree of activation is typical for the ribosomal DNA genes. Transcription of rDNA genes is regulated in the range of 1.5–4 times under a broad spectrum of in vivo and in vitro conditions [6,7,31–37]. Levels of GAPDH mRNA (upper band, Fig. 5A) were simultaneously hybridized with the 45S rDNA ETS to correct for minor differences in RNA loading.

3.5. LVH is associated with increased expression of the ribosomal gene transcription factor UBF

The level of the rDNA transcription factor UBF was determined by Western analysis of LV and RV myocyte protein extracts from SH and TAC mice. Equal amounts of total protein were fractionated by SDS–PAGE, and UBF levels

Table 1
Relative RNA and protein levels in ventricular myocytes isolated from SH and TAC mice

Tissue region		μg RNA/μg DNA		μg protein/μg DNA	
		SH	TAC	SH	TAC
LV	W1	0.56 ± 0.10	0.95 ± 0.10**	26.13 ± 2.59	36.22 ± 1.92**
	W3	0.66 ± 0.09	0.87 ± 0.08*	28.42 ± 2.15	38.58 ± 3.02**
	W6	0.51 ± 0.06	0.88 ± 0.02***	28.06 ± 1.73	35.94 ± 3.72*
RV	W1	0.59 ± 0.04	0.55 ± 0.11	24.63 ± 3.27	28.93 ± 1.41
	W3	0.65 ± 0.03	0.61 ± 0.09	27.85 ± 2.08	26.50 ± 1.09
	W6	0.58 ± 0.11	0.79 ± 0.07*	26.08 ± 2.03	32.09 ± 1.36*

RNA, protein and DNA were extracted from LV myocytes isolated 1, 3 and 6 weeks (W1, W3 and W6) after surgery from SH and TAC mice. After quantification as described in Section 2, the RNA and protein levels were expressed relative to DNA content (μg RNA/μg DNA, μg protein/μg DNA). *$P < 0.05$, **$P < 0.005$, and ***$P < 0.0005$ vs. SH group at week 1.

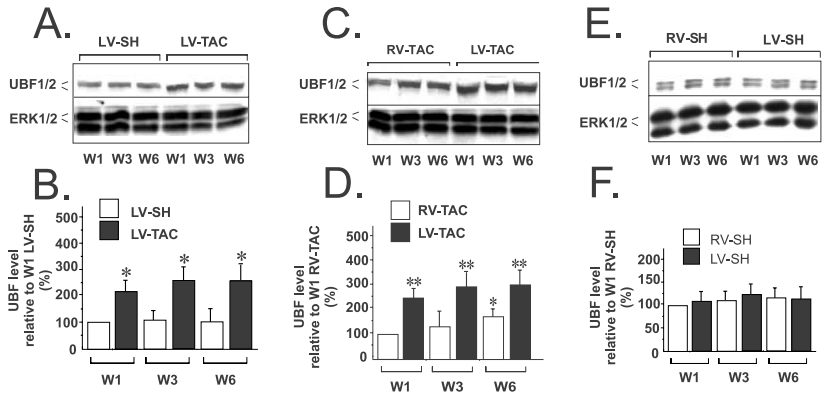

Fig. 6. UBF protein levels are increased in mouse LV myocytes following imposition of TAC. Myocyte protein was extracted from LV and RV myocytes isolated 1, 3 and 6 weeks (W1, W3, and W6) after surgery from SH and TAC mice. Equal amount of protein extracts (100 μg) were fractionated by SDS–PAGE, transferred to nylon membranes and successively probed with an antiserum against UBF1/2 and a polyclonal antibody against the mitogen-activated protein kinase, ERK1/2. A: A representative Western blot showing UBF and ERK expression in LV from SH and TAC mice 1, 3, and 6 weeks following surgery. B: UBF1/2 levels in three experiments similar to that shown in A were quantitated with the optical densitometry quantification software Scion Image for Windows (NIH, USA), normalized for ERK1/2 expression and expressed as the percentage increase (±S.E.M., $n=3$). *$P<0.05$ vs. LV-SH at W1. C: A representative Western blot showing UBF and ERK expression in LV and RV from TAC mice 1, 3, and 6 weeks following surgery. D: UBF1/2 levels in three experiments similar to that shown in C were quantitated, normalized for ERK1/2 expression and expressed as the percentage increase (±S.E.M., $n=3$). *$P<0.05$, **$P<0.001$. vs. RV-TAC at W1. E: A representative Western blot showing UBF and ERK expression in LV and RV from SH mice 1, 3, and 6 weeks following surgery. F: UBF1/2 levels in three experiments similar to that shown in E were quantitated, normalized for ERK1/2 expression and expressed as the percentage increase (±S.E.M., $n=3$).

were determined by immunoblotting using anti-UBF antibodies. UBF exists in two forms, UBF1 and UBF2, and therefore migrates as a doublet. UBF1/2 levels per unit of total protein were significantly increased (2.5-fold) in LV extracts from TAC mice compared to the LV extracts from SH mice 1 week after surgery and remained elevated at 3 and 6 weeks (Fig. 6A,B). This level of induction is in a similar range to that observed for isolated neonatal cardiomyocyte cultures following hypertrophic stimuli [5,6]. Blots were stripped and re-probed with antibodies to ERK1/2 to confirm equal loading and quantitation of the UBF levels was normalized to the ERK1/2 signal. It has been shown that the total amount of cellular ERK1/2 in adult cardiomyocytes in vivo does not change after aortic banding, as opposed to its phosphorylation state [38]. Importantly, UBF levels were significantly higher in LV of TAC mice compared to the matched RV from the same animal, which correlates with the faster growth rate of LV following pressure overload (Fig. 6C). In contrast, no significant differences in UBF levels were observed between LV and RV of SH animals (Fig. 6E,F). Interestingly, although UBF levels were unchanged in RV extracts from TAC mice 1 and 3 weeks following surgery (Fig. 6C,D), by 6 weeks, a small but statistically significant increase was observed (Fig. 6C), which is consistent with the observation that RNA content and weight of the RV chamber from TAC mice is also elevated at this time point (see Figs. 2C and 4C). Taken together these results demonstrate that the increased UBF levels correlate with the elevated pressure load and increased growth rates and did not simply reflect tissue or regional differences in the expression of this factor in normal hearts.

4. Discussion

The development of LVH is associated with complex phenotypic changes in the cardiac cell population resulting in disturbances in cardiac electrophysiology and myocardial contractile dysfunction. Although fibrosis, cell death, proliferation and migration may all contribute to the cardiac dysfunction to some extent [39], several lines of evidence indicate that a component of the transition to heart failure is attributable to the increased LV mass itself [40,41]. At the cellular level, global accumulation of protein resulting from increased rates of protein synthesis is the primary means by which the enlargement of myocytes and subsequent increase in LV mass occurs. Thus, identification of the specific component(s) in the protein synthetic pathways that are perturbed during disease states may provide clues for the design of tools or therapies that can address the role of LV mass in heart failure.

One of the fundamental regulatory points for protein synthesis is the availability of functional ribosomes. A major rate-limiting step in the synthesis of ribosomes is rDNA transcription. In order to identify potential regulatory mechanism(s) for pathophysiological hypertrophy we have examined the molecular mechanism(s) regulating rDNA transcription during cardiac growth. Previously we identified an architectural rDNA transcription factor, UBF, whose cellular activity is regulated during hypertrophy of isolated neonatal cardiomyocytes [5–8]. Such studies suggest that increases in UBF are at least part of the mechanism by which rDNA transcription rates are modulated during cardiac hypertrophy in vivo. Such conclusions, however, assume that the molecular mechanism(s) that regulate the growth of isolated neonatal cultured cells ex vivo are the same as those responsible for ribosome biogenesis in fully differentiated adult cardiomyocytes in vivo. While this is a tempting assumption, it has been well established that rDNA transcription rates are subject to regulation at multiple levels and may differ within the same cell type depending on the stimuli (reviewed in [14]).

In this study we show for the first time that UBF is a target for regulation during hypertrophy of adult cardiomyocytes in vivo. Purified myocytes isolated from LV of mice undergoing TAC-induced LVH exhibited increased rates of rRNA synthesis and expression of UBF relative to SH mice. Moreover, UBF levels were significantly higher in LV of TAC mice com-

pared with the matched RV from the same animal, which correlates with the faster growth rate of LV following pressure overload. Interestingly, UBF level was unchanged in myocytes from the right ventricle 1 or 3 weeks after surgery of TAC mice (Fig. 6), but was moderately (but significantly) higher at 6 weeks. This increase correlates with a late increase in hypertrophy of the RV at this time point. Thus we conclude that up-regulation of UBF expression directly correlates with myocyte hypertrophic growth in vivo and that increased expression of UBF is a conserved response to hypertrophic stimuli between neonatal and adult myocytes and between species.

Acknowledgements: This work was supported by grants from the National Health and Medical Research Council of Australia and National Heart Foundation of Australia to R.D.H. (NHMRC 166900 and NHF G02M0673).

References

[1] Morgan, H., Gordon, E., Kira, Y., Chua, B., Russo, L., Peterson, C., McDermott, P. and Watson, P. (1987) Annu. Rev. Physiol. 49, 533–543.
[2] Nagatomo, Y., Carabello, B.A., Hamawaki, M., Nemoto, S., Matsuo, T. and McDermott, P.J. (1999) Am. J. Physiol. 277, H2176–H2184.
[3] Tuxworth, W.J.Jr., Wada, H., Ishibashi, Y. and McDermott, P.J. (1999) Am. J. Physiol. 277, H1273–H1282.
[4] Hannan, R., Jenkins, A., Jenkins, A. and Brandenburger, Y. (2003) Clin. Exp. Pharmacol. Physiol. (in press).
[5] Hannan, R., Luyken, J. and Rothblum, L. (1995) J. Biol. Chem. 270, 8290–8297.
[6] Hannan, R., Luyken, J. and Rothblum, L. (1996) J. Biol. Chem. 271, 3213–3220.
[7] Luyken, J., Hannan, R., Cheung, J. and Rothblum, L. (1996) Circ. Res. 78, 354–361.
[8] Brandenburger, Y., Jenkins, A., Autelitano, D. and Hannan, R. (2001) FASEB J. 15, 2051–2053.
[9] Sollner-Webb, B. and Tower, J. (1986) Annu. Rev. Biochem. 55, 801–830.
[10] Wolf, S., Sameshima, M., Liebhaber, S. and Schlessinger, D. (1980) Biochemistry 19, 3484–3490.
[11] Krauter, K., Soeiro, R. and Nadal-Ginard, B. (1979) J. Mol. Biol. 134, 727–741.
[12] Hannan, R. and Rothblum, L. (1995) Cardiovasc. Res. 30, 501–510.
[13] Grummt, I. (1999) Prog. Nucleic Acid Res. Mol. Biol. 62, 109–154.
[14] Hannan, K.M., Hannan, R.D. and Rothblum, L.I. (1998) Front. Biosci. 3, d376–d398.
[15] Moss, T. and Stefanovsky, V.Y. (1995) Prog. Nucleic Acid Res. Mol. Biol. 50, 25–66.
[16] Hannan, R., Stefanovsky, V., Taylor, L., Moss, T. and Rothblum, L. (1996) Proc. Natl. Acad. Sci. USA 93, 8750–8755.
[17] Du, X.J., Autelitano, D.J., Dilley, R.J., Wang, B., Dart, A.M. and Woodcock, E.A. (2000) Circulation 101, 71–77.
[18] Palmer, T.D., Miller, A.D., Reeder, R.H. and McStay, B. (1993) Nucleic Acids Res. 21, 3451–3457.
[19] Rockman, H.A., Ross, R.S., Harris, A.N., Knowlton, K.U., Steinhelper, M.E., Field, L.J., Ross Jr., J. and Chien, K.R. (1991) Proc. Natl. Acad. Sci. USA 88, 8277–8281.
[20] Woodcock, E.A. and Lambert, K.A. (1997) J. Mol. Cell. Cardiol. 29, 3275–3283.
[21] Autelitano, D.J. and Woodcock, E.A. (1998) J. Mol. Cell. Cardiol. 30, 1515–1523.
[22] Pederson, T. (1969) Anal. Biochem. 28, 35–46.
[23] Hannan, K., Hannan, R., Smith, S., Jefferson, L., Lun, M. and Rothblum, L. (2000) Oncogene 19, 4988–4999.
[24] Hannan, K., Kennedy, B., Cavanaugh, A., Hannan, R., Hirschler-Laszkiewicz, I., Jefferson, L. and Rothblum, L. (2000) Oncogene 19, 3487–3497.
[25] Sheridan, D.J., Autelitano, D.J., Wang, B., Percy, E., Woodcock, E.A. and Du, X.J. (2000) Cardiovasc. Res. 47, 133–141.
[26] Allo, S.N., McDermott, P.J., Carl, L.L. and Morgan, H.E. (1991) J. Biol. Chem. 266, 22003–22009.
[27] McDermott, P., Carl, L., Conner, K. and Allo, S. (1991) J. Biol. Chem. 266, 4409–4416.
[28] Financsek, I., Mizumoto, K., Mishima, Y. and Muramatsu, M. (1982) Proc. Natl. Acad. Sci. USA 79, 3092–3096.
[29] Gurney Jr., T. (1985) Nucleic Acids Res. 13, 4905–4919.
[30] Miller, K.G. and Sollner-Webb, B. (1981) Cell 27, 165–174.
[31] Castle, T., Katz, A. and Richardson, A. (1978) Mech. Ageing Dev. 8, 383–395.
[32] Grummt, I., Smith, V.A. and Grummt, F. (1976) Cell 7, 439–445.
[33] Larson, D.E., Xie, W., Glibetic, M., O'Mahony, D., Sells, B.H. and Rothblum, L.I. (1993) Proc. Natl. Acad. Sci. USA 90, 7933–7936.
[34] Loeb, J.N. and Yeung, L.L. (1975) J. Exp. Med. 142, 575–587.
[35] Nikolov, E.H., Nankova, B.B. and Dabeva, M.D. (1991) Mol. Biol. Rep. 15, 45–52.
[36] Stoykova, A.S., Dudov, K.P., Dabeva, M.D. and Hadjiolov, A.A. (1983) J. Neurochem. 41, 942–949.
[37] Zahradka, P., Larson, D.E. and Sells, B.H. (1991) Mol. Cell. Biochem. 104, 189–194.
[38] Takeishi, Y. et al. (2001) J. Mol. Cell. Cardiol. 33, 1989–2005.
[39] Francis, G.S. (1998) Curr. Opin. Cardiol. 13, 156–161.
[40] Cooper, G.4th. (1997) Annu. Rev. Med. 48, 13–23.
[41] Arnett, D.K. (2000) Curr. Hypertens. Rep. 2, 50–55.