FEBS Open Bio 2 (2012) 135–144

journal homepage: www.elsevier.com/locate/febsopenbio

Lipoma preferred partner is a mechanosensitive protein regulated by nitric oxide in the heart

Charlotte L. Hooper, Philip R. Dash, Samuel Y. Boateng *

Institute of Cardiovascular and Metabolic Research. The Schools of Biological Sciences and Pharmacy, University of Reading, Reading Berkshire, United Kingdom

ARTICLE INFO

Article history:
Received 30 April 2012
Revised 27 May 2012
Accepted 29 May 2012

Keywords:
Hypertrophy
Heart failure
Cytoskeleton
Myofilaments
Fibroblasts
Mechanosensation

ABSTRACT

Adaptor proteins play an important role in signaling pathways by providing a platform on which many other proteins can interact. Malfunction or mislocalization of these proteins may play a role in the development of disease. Lipoma preferred partner (LPP) is a nucleocytoplasmic shuttling adaptor protein. Previous work shows that LPP plays a role in the function of smooth muscle cells and in atherosclerosis. In this study we wanted to determine whether LPP has a role in the myocardium. LPP expression increased by 56% in hearts from pressure overload aortic-banded rats ($p < 0.05$ $n = 4$), but not after myocardial infarction, suggesting hemodynamic load regulates its expression. In vitro, LPP expression was 87% higher in cardiac fibroblasts than myocytes ($p < 0.05$ $n = 3$). LPP expression was downregulated in the absence of the actin cytoskeleton but not when microtubules were disassembled. We mechanically stretched cardiac fibroblasts using the Flexcell 4000 for 48 h (1 Hz, 5% maximum strain), which decreased total LPP total expression and membrane localization in subcellular fractions ($p < 0.05$, $n = 5$). However, L-NAME, an inhibitor of nitric oxide synthase (NOS), significantly upregulated LPP expression. These findings suggest that LPP is regulated by a complex interplay between NO and mechanical cues and may play a role in heart failure induced by increased hemodynamic load.
© 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

Adaptor proteins play an important role in many biological signaling pathways by providing a platform on which many other proteins can interact [1]. The interaction of these adaptor proteins mean that they can regulate both the site and duration of many important signaling pathways required for normal cell function. Equally, malfunction or mislocalization of these proteins may play a role in the development of human disease. Lipoma preferred partner (LPP) contains three LIM domains and is from a family of adaptor proteins that include thyroid hormone receptor interacting protein 6 (TRIP6) and Zyxin [2]. One of the difficulties in determining the cellular function of LPP results from the fact that there is some functional redundancy with other members of the zyxin family [3]. LPP is a nucleocytoplasmic shuttling protein located in the focal adhesions and cell–cell junctions and associates with the actin cytoskeleton [4]. The proline-rich domain of LPP enables it to form links with cytoskeletal components such as actin stress fibers and α-actinin [5]. At the focal adhesions LPP binds to the ends of actin filaments to prompt cell attachment. LPP contributes a regulatory step by controlling the rate of actin polymerization, where its LIM domains act in an inhibitory manner to impede formation of the links [6]. Such functions suggest that whilst concentrated at the focal adhesion, LPP contributes to the force exerted by the cell onto the extracellular space [7].

Mutations in the LPP gene have been associated with a group of tumors of adipose tissue [8]. These mutations result in the permanent localization of the LIM domains to the nucleus. The protein has been shown to be a nuclear-cytoplasmic shuttling protein since inhibition of the nuclear export factor CRM1 with leptomycin B causes LPP to accumulate in the nucleus [4].

LPP has been described as an oncogene in various cells [9] and has been shown to play an important role in the function of smooth muscle cells [10]. Previous work has also shown LPP to play an important role in the development of atherosclerosis in coronary vessels due to its mechanosensitivity [11]. Its activity in the heart has not been studied before because previous work had suggested that LPP was not expressed there [12].

In this paper we present evidence to show for the first time that LPP is highly expressed within the myocardium and is up regulated following heart failure induced by pressure overload but not myocardial infarction. These findings suggest that LPP may be a novel marker for hemodynamic load-induced adaptation in the heart

* Corresponding author. Address: Schools of Biological Sciences and Pharmacy, Hopkins Building, University of Reading, Whiteknights, Reading, Berkshire RG6 6UB, United Kingdom.

E-mail address: s.boateng@reading.ac.uk (S.Y. Boateng).

2211-5463/$36.00 © 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fob.2012.05.005

since its expression was much higher in the model of pressure overload. Moreover, we demonstrate that LPP is a mechanosensitive protein in cardiac fibroblasts where its expression is highest. The mechano-regulation of LPP expression is nitric oxide dependent. These data suggest that LPP is a novel mechanosensing protein in the heart regulating cell adaptation in cardiac hypertrophy and heart failure through nitric oxide signaling.

2. Materials and methods

2.1. Animals models of heart failure

Animal experiments were performed according to Institutional Animal Care of the University of Illinois at Chicago (UIC) and Use Committee and NIH guidelines. For the transverse aortic banding procedure, rats were initially anesthetized with methoxyfluorane and suture placed on ascending aorta to induce cardiac hypertrophy as previously described [13]. For myocardial infarction the left coronary artery was ligated 5 mm from the ostium with 7-0 silk suture as previously described [13]. For controls, animals were sham operated without the constriction or coronary ligation. In each case, animals were killed with end-stage heart failure 6–7 months post-surgery with carbon dioxide, and heart tissue was stored at −80 °C prior to use. In the myocardial infarcted hearts, tissue was collected from the remote region in an area away from the infarct. All surgery, hemodynamics and tissue dissections were performed at UIC and shipped to Reading on dry ice. Subsequent experiments were performed at the University of Reading.

2.2. Cell culture and treatments

Myocytes and fibroblasts were isolated from the cardiac ventricles of 1–2 day old Sprague–Dawley rats by sequential collagenase digestion, as previously described [14]. Myocytes were cultured PC1 medium (BioWhittaker, Walkersville, MD) for 24 h and transferred to a DMEM:M199 serum free medium. Cardiac fibroblasts were cultured in 5% serum in DMEM. Cardiac fibroblasts were cyclically stretched at 1 Hz using the Flexcell 4000 (Flexcell International). For short term experiments, cells were stretched at 10% maximum strain for 2 h. However for long term experiments, the cells were stretched at 5% maximum strain for 48 h to prevent significant cell detachment. This reduced intensity still had effects on the fibroblasts as shown by increased cross linking of actin cytoskeleton. Cells were changed to serum free medium overnight and treated with drugs prior to the cells being stretched and during stretch. Fibroblasts were treated with 10 μM vinblastine for 26 h (to dissociate microtubules) or 5 mM LNAME for 48 h (to inhibit NOS and prevent NO production). Cells were treated with cytochalasin D at 10 μM for 26 h (to dissociate polymerized actin) when combined with 2 h 10% stretch or 72 h when combined with 48 h 5% stretch. Leptomycin was used at 10 nM for 2 h to inhibit CRM1 dependent nuclear export. These drug concentrations have been shown to be specific for their respective targets.

2.3. Cellular composition and subcellular fractionation

For subcellular fractionation of myocytes, the ProteoExtract Subcellular Proteome Kit from Calbiochem was used as described previously [14]. Cellular proteins were sequentially extracted into four compartments: cytosolic, membrane/organelles, nuclei and cytoskeleton. The accuracy of the fractionation method was verified with antibodies to well documented subcellular markers (data not shown). However, we have previously documented the accuracy of this methodology [13].

2.4. Western blotting for analysis of protein expression

Neonatal rat ventricular myocytes were rinsed with warm PBS and then scraped from the silicone membranes or dishes in lysis buffer containing 1% SDS and protease inhibitor cocktail (Sigma). For whole heart protein analysis, tissue was ground in liquid nitrogen and added to lysis buffer containing 1% SDS, 50 mM NaF and protease inhibitor cocktail (Sigma). Protein samples were analyzed by Western blotting as described previously [14]. Gels were probed for LPP 1 in 2000 (Abcam), actin 1 in 1000 (Abcam), procollagen 1 in 200 (Developmental Studies Hybridoma Bank), α-smooth muscle actin 1 in 1000 (Abcam) and vimentin 1 in 1000 (Abcam). Gels were analyzed and quantified using the total protein loading from the Amido black stain as previously described [15]. The gels were scanned and analyzed using Gel Pro Analyser (mediacybernetics).

2.5. Immuno-chemistry and image analysis

After the various experimental protocols, cells for immunocytochemical staining were fixed in 4% paraformaldehyde for 5 min and then 70% ethanol for storage at −20 °C. Cells were rehydrated in PBS and then immunostained with antibodies as described previously [16]. Cells were stained for LPP, actin, procollagen, α-smooth muscle actin, tubulin and integrin-linked kinase. All primary antibodies were used at a concentration of 1 in 500 overnight. Alexa Fluor-conjugated secondary antibodies (Molecular Probes) were used to visualize the specific proteins using a Leica DMIRE2 laser scanning confocal microscope.

2.6. Statistics

For the experiments described here, at least three separate primary cultures were averaged. Each culture used about 30 neonatal hearts. All values are means ± SEM. For the animal experiments each group had between 4 and 8 animals. All values of significance were calculated using the appropriate comparisons: one way analysis of variance or the Students unpaired *t*-test. Differences among means were considered significant at $p < 0.05$. Data were analyzed using GraphPad, Minitab and SigmaStat statistical software.

3. Results

3.1. LPP distribution in normal and failing hearts

Previous work has suggested that the heart expresses very low levels of LPP compared with other tissue so we determined the expression of the protein in rat heart samples taken from adult atrium, left and right ventricles and from the neonatal ventricles. We compared the expression of LPP with another focal adhesion protein paxillin. Fig. 1a shows a Western blot of LPP from heart tissue from rat and shows that the protein was expressed in all tissues examined. Fig. 1B shows quantitation of LPP and shows that the protein is expressed in all regions of the adult heart with the atria having a two-fold higher expression of LPP compared with the ventricles, (atrium vs ventricles $p < 0.05$). The expression of LPP in neonatal ventricles was also higher compared with the adult, (neonatal heart vs adult heart, $p < 0.01$).

With high expression of LPP in neonatal hearts, we wondered whether the expression of the protein might be altered in the failing heart. We determined the expression of LPP protein in two models of heart failure, pressure overload by ascending aortic constriction (AOB) and myocardial infarction (MI) by coronary ligation. We chose these two models because our previous work has shown a significant difference in hemodynamics between the two models

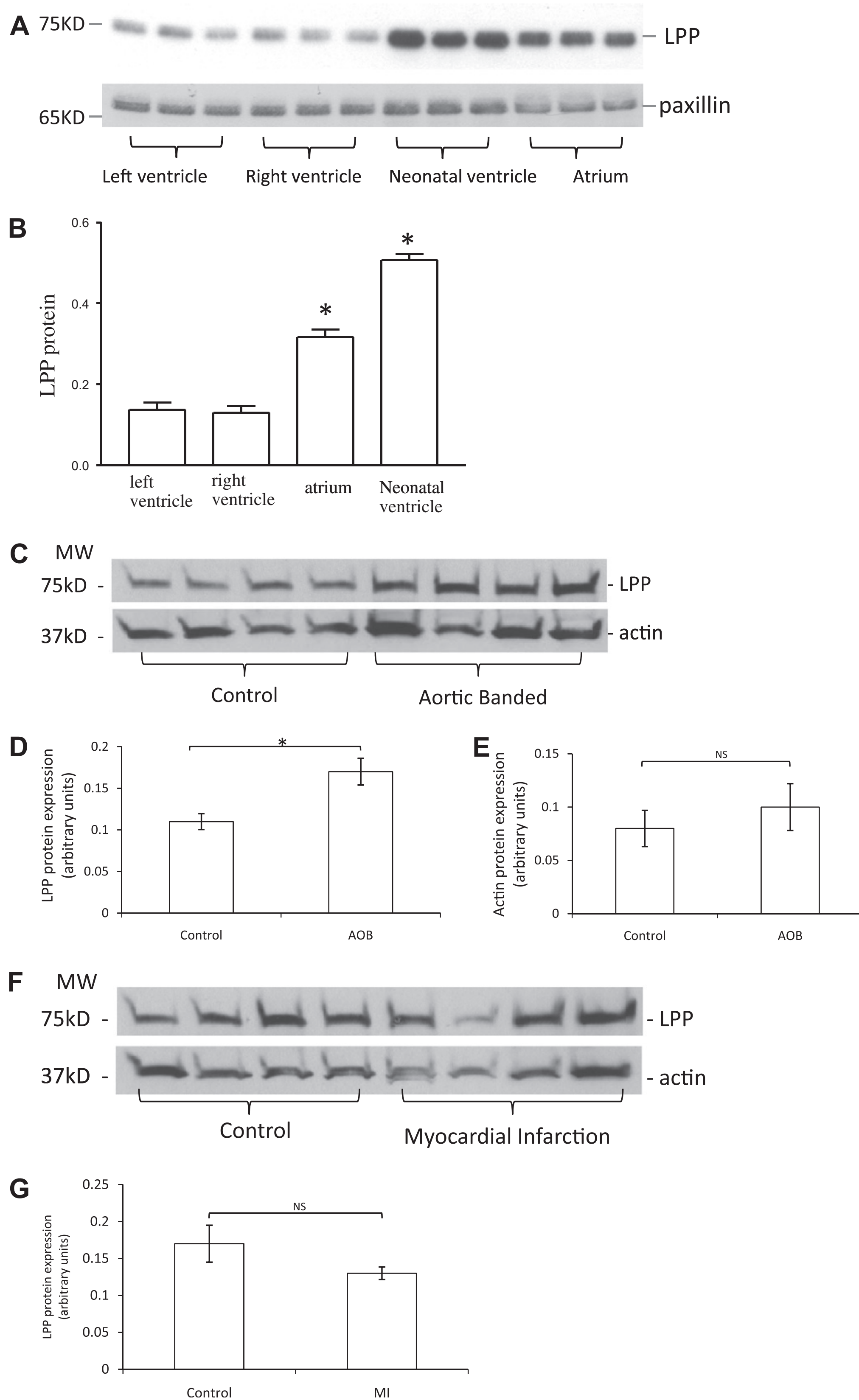

Fig. 1. (A) Western blots of LPP and paxillin protein in the adult rat left and right ventricle, adult atria and neonatal ventricle. (B) LPP protein expression in the adult rat left and right ventricle, adult atria and neonatal ventricle. LPP protein expression was three-fold higher in the neonatal ventricles than in the adult. $p < 0.05$, $n = 3$ (C) Western blots of LPP and actin protein in the left ventricular free wall of aortic banded hearts. (D) LPP protein expression is higher after aortic banding than in control hearts. $p < 0.05$, $n = 4$. (E) Actin protein expression was unchanged in the aortic banded hearts compared to controls. (F) Western blots of LPP and actin protein in the left ventricular free wall after myocardial infarction. (G) LPP protein expression was unchanged after myocardial infarction.

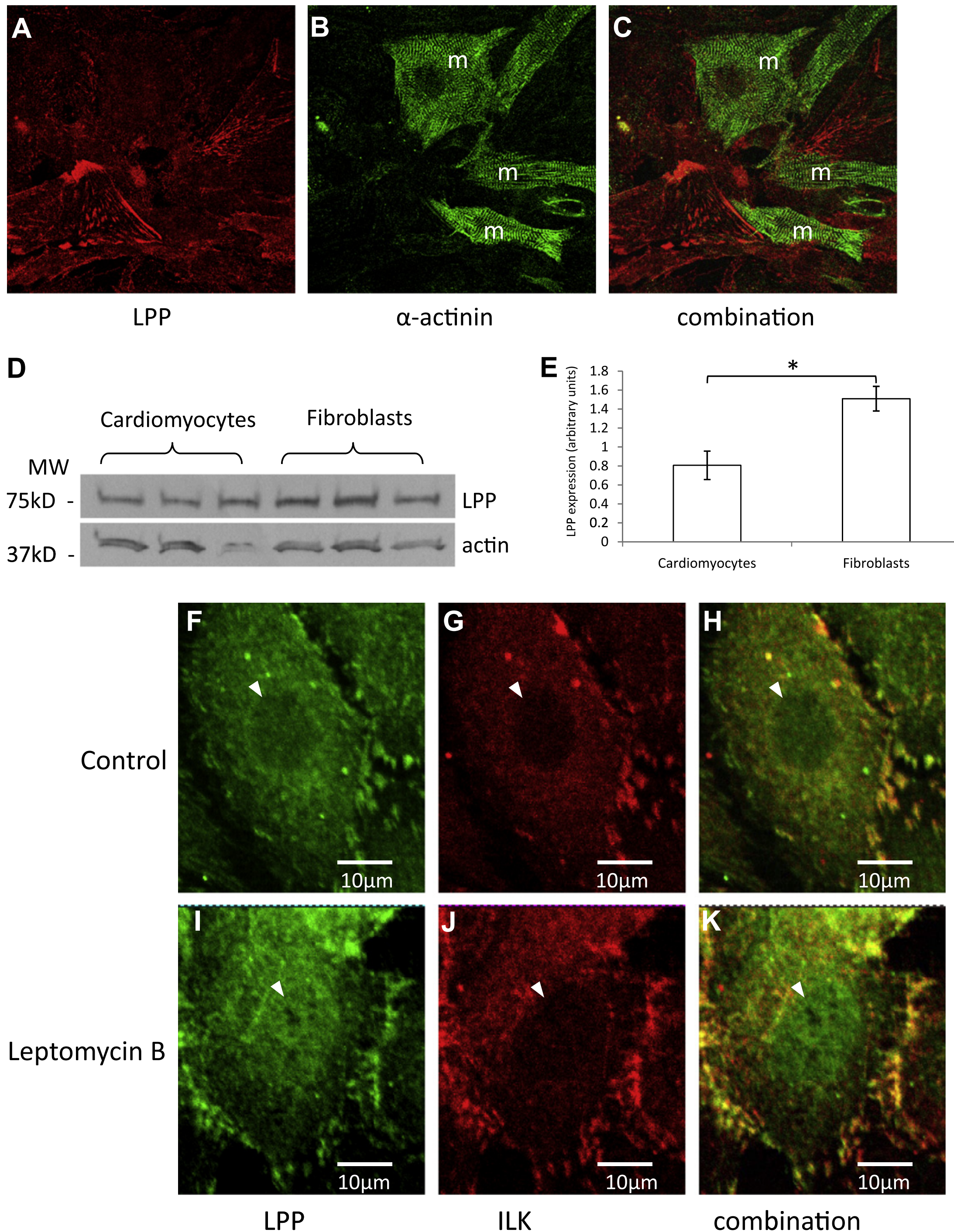

Fig. 2. Immunostaining of neonatal myocytes and fibroblasts with (A) LPP (red); (B) α-actinin (green); and (C) combination of staining of LPP (red) and α-actinin (green), myocyte (m). (D) Western blots of LPP and actin protein in myocytes and fibroblasts. (E) LPP protein expression is two-fold higher in fibroblasts than myocytes $p < 0.05$, $n = 3$. Immunostaining of fibroblasts with (F) LPP (green); (G) the focal adhesion protein ILK (red); (H) and combination staining of LPP (green) and ILK (red). White arrow shows the nucleus. Immunostaining of fibroblasts treated with 10 nM leptomycin B for 3 h to block nuclear export with (I) LPP (green); (J) the focal adhesion protein ILK (red); and combination staining of LPP (green) and ILK (red).

and has been previously published for these samples [13]. The tissue was stored in liquid nitrogen prior to being shipped to Reading. We previously found that both hearts developed significant hypertrophy with MI and AOB heart weights increasing by 28% and 47% respectively compared with sham operated controls. The contractile function (left ventricular developed pressure) of the MI hearts was significantly reduced compared with controls. However, in AOB hearts, both the left ventricular systolic pressure and end diastolic pressure were significantly elevated compared with MI. This suggests that the AOB hearts were subjected to greater hemodynamic overload. We hypothesized that LPP expression would be regulated by this additional mechanical load. The expression of LPP was compared with actin because it has been suggested that LPP expression is influenced by the actin cytoskeleton. Fig. 1D and E show that LPP is significantly increased following aortic constriction while total actin levels remained unchanged. Next we

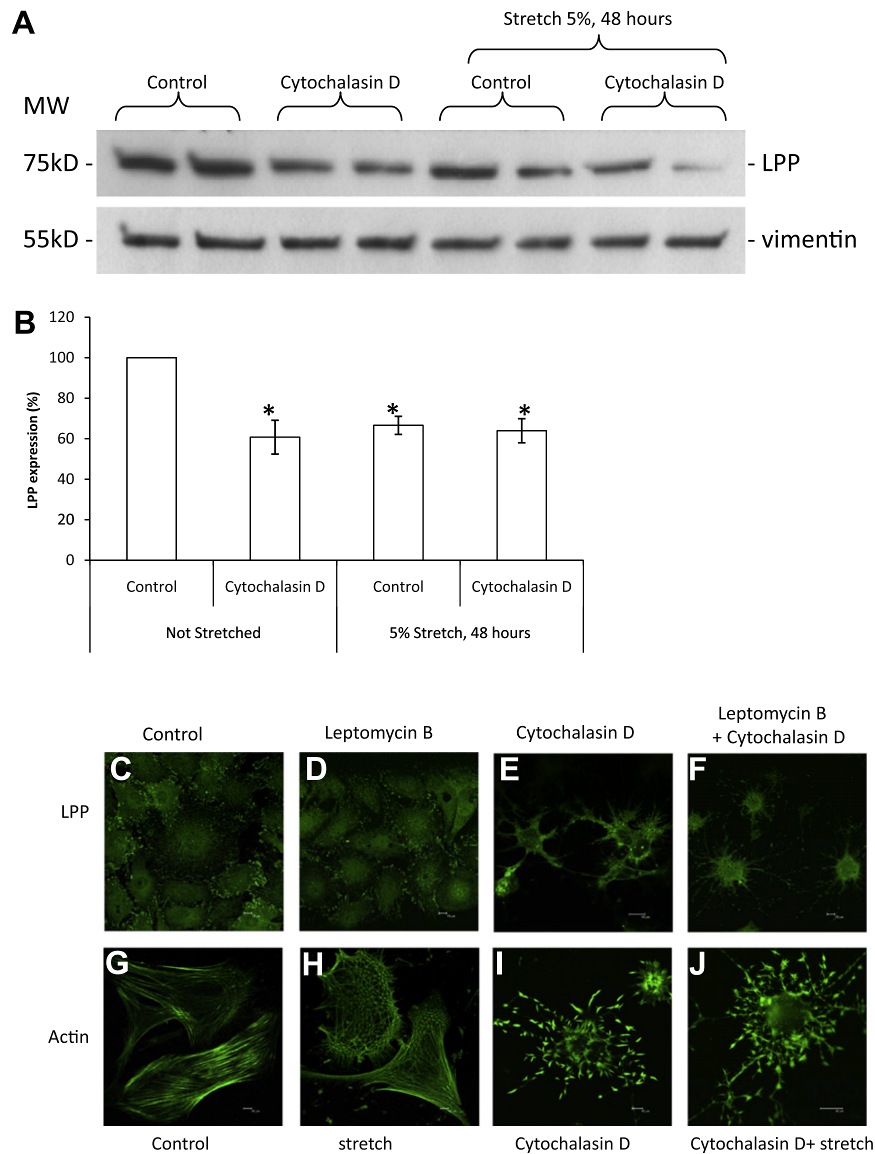

Fig. 3. (A) Western blots of LPP and vimentin protein in fibroblasts treated with 10 μM cytochalasin D for 72 h to dissociate the actin cytoskeleton, alone and in combination with 48 h mechanical stretch with 5% maximum strain at 1 Hz. (B) LPP protein expression decreased after 48 h with 5% mechanical stretch and dissociation of the actin cytoskeleton with cytochalasin D ($p < 0.05$, $n = 3$). Immunostaining of fibroblasts with LPP (green) (C) in untreated cells; (D) after inhibition of nuclear export by 10 nM leptomycin B for 3 h; (E) after dissociation of the actin cytoskeleton by 10 μM cytochalasin D for 72 h; and (F) in response to co-treatment with 10 μM cytochalasin D for 72 h and 10nM leptomycin B for the last 3 h. Immunostaining of fibroblasts with actin (green) (G) in untreated cells; (H) after exposure to 5% mechanical stretch for 48 h; (I) in response to dissociation of the actin cytoskeleton by 10 μM cytochalasin D for 72 h; and (J) co-treatment with 10 μM cytochalasin D for 72 h and 5% mechanical stretch for the last 48 h.

measured LPP following myocardial infarction and this showed no change (Fig. 1F and G).

3.2. LPP expression in cardiac cells

Using immunostaining of neonatal cardiac cells we wanted to determine which cells in the heart expressed LPP. Fig. 2A shows that LPP is expressed in both myocytes and non-myocytes. The expression in non-myocytes appears to be higher, mostly associated with the actin cytoskeleton. In myocytes the expression appears more punctate suggesting a strong association with the focal adhesions. We then cultured neonatal cardiac fibroblasts separately from myocytes and quantified the expression of LPP in the two cell types. Fig. 2D and E show that fibroblasts express more LPP than myocytes. For this reason, subsequent work will examine the activity of LPP in neonatal cardiac fibroblasts. Cultured fibroblasts were treated with 10 nM leptomycin B for 2 h and the cells fixed for immunochemistry. Fig. 2F–K show immunostaining for LPP and integrin linked kinase in fibroblasts following treatment with leptomycin B. Leptomycin B blocks the CRM1 nuclear export pathway and allows LPP to accumulate in the nuclear as a result of its shuttling. The staining indicates that the LPP localizes to the focal adhesions and accumulates in the nucleus following inhibition of the CRM1 nuclear export pathway.

3.3. LPP expression is dependent on mechanical stress and the actin cytoskeleton

Previous work in smooth muscle shows that LPP may be mechanosensitive so we determined whether this might also be the case in cardiac fibroblasts. We chose fibroblasts for these experiments because the expression is higher than in cardiac myocytes. In these

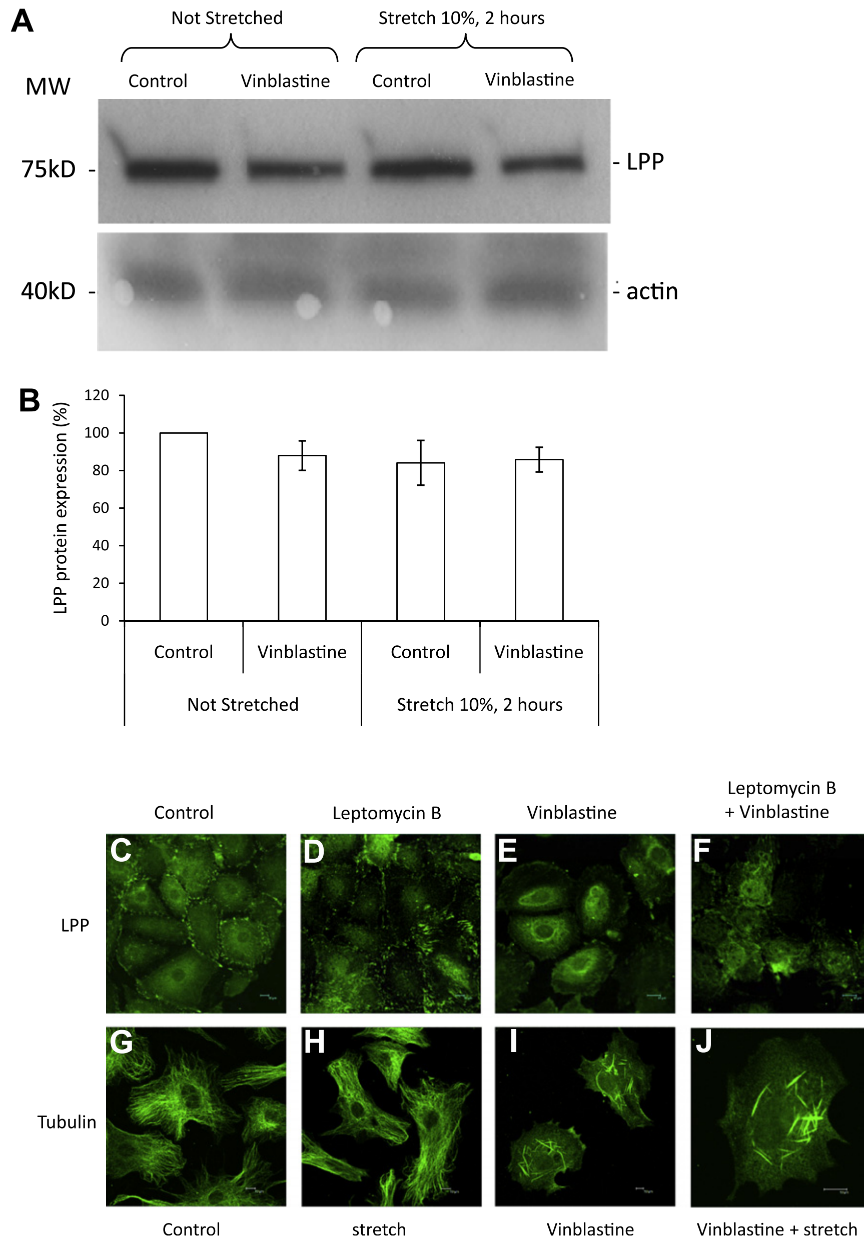

Fig. 4. (A) Western blots of LPP and actin protein in response to dissociation of the microtubules using 10 μM vinblastine for 26 h; alone and in combination with 10% mechanical stretch for 2 h. (B) LPP protein expression is unchanged in response to dissociation of the microtubules and after 2 h 10% mechanical stretch. Immunostaining of fibroblasts with LPP (green) (C) in untreated cells; (D) after inhibition of nuclear export by 10 nM leptomycin B for 3 h; (E) after dissociation of the microtubules with 10 μM vinblastine for 26 h; and (F) in response to co-treatment with 10 μM vinblastine for 26 h and 10 nM leptomycin B for the last 3 h. Immunostaining of fibroblasts for tubulin (green) (G) in untreated cells; (H) after exposure to 2 h mechanical stretch at 10% maximum strain at 1 Hz; (I) after dissociation of the microtubules with 10 μM vinblastine for 26 h; and (J) in response to co-treatment with 10 μM vinblastine for 26 h and 10% mechanical stretch for the last 2 h.

experiments, cells were mechanically stretched using the Flexcell 4000 with or without the presence of the actin cytoskeleton. The actin cytoskeleton was dissociated using cytochalasin D. LPP expression was then examined following the various treatments. Fig. 3A and B show total LPP expression following mechanical cyclic stretch with and without cytochalasin D. The data show that both cyclic stretch and cytochalasin D significantly decrease LPP expression in cardiac fibroblasts. The immunostained images for these conditions are shown in Fig. 3C–J.

3.4. LPP expression is independent of the microtubule cytoskeleton

We tested to see whether dissociation of the microtubules would have a similar effect as dissociating the actin cytoskeleton. In these experiments, cells were mechanically stretched using the Flexcell 4000 with or without the presence of the microtubules. LPP expression was then examined following the various treatments. Fig. 4A and B show total LPP expression following 2 h of mechanical cyclic stretch with and without vinblastine (16 h vinblastine treatment) which dissociates the microtubules. The data show that neither short-term cyclic stretch nor the loss of microtubules alter LPP expression in cardiac fibroblasts. The immunostained images for these conditions are shown in Fig. 4C–J.

3.5. Subcellular distribution of LPP is regulated by mechanical stress

Since LPP is a nucleocytoplasmic shuttling protein we wanted to determine how its subcellular distribution was altered by different

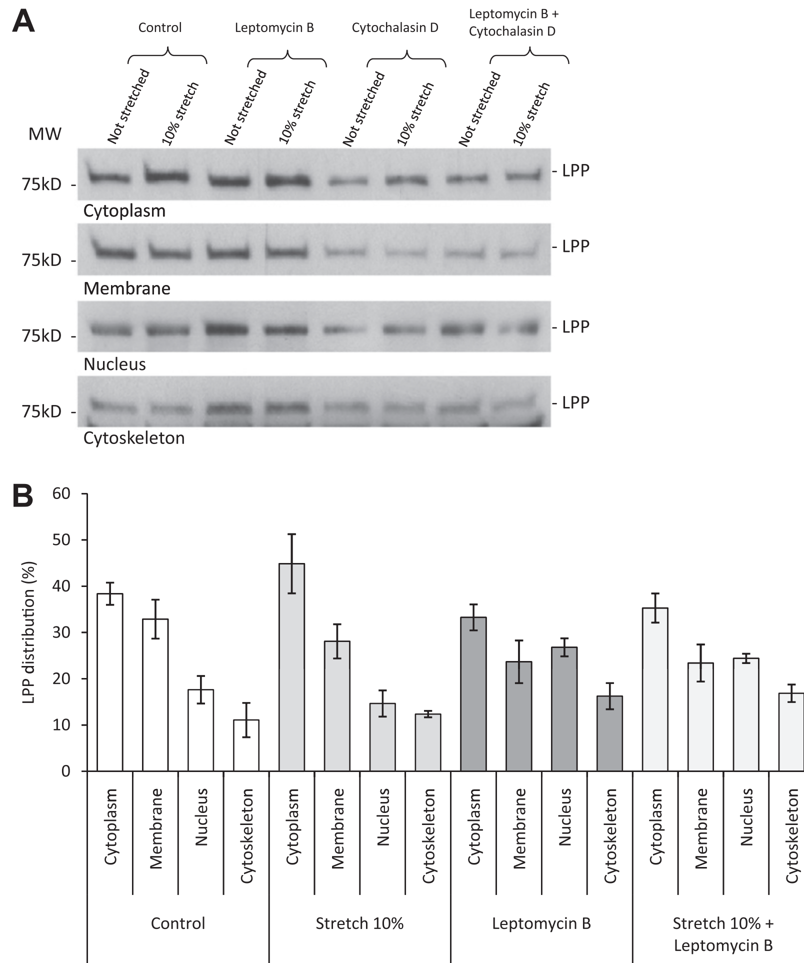

Fig. 5. (A) Western blots of LPP protein in the cytosolic, membrane, nucleus and cytoskeleton subcellular fractions in response to inhibition of nuclear export with 10 nM leptomycin B for 3 h and dissociation of the actin cytoskeleton with 10 μM cytochalasin D for 26 h; both alone and in combination with 2 h 10% mechanical stretch. (B) LPP distribution in fibroblasts. Fibroblasts were fractionated into cytosol, membrane, nucleus and cytoskeleton subcellular portions using a detergent-based method. Treatment of fibroblasts with 10 nM leptomycin B for 3 h, alone and in combination with 2 h 10% mechanical stretch, leads to increased nuclear LPP. (C) LPP protein expression in the membrane in response to leptomycin B, cytochalasin D and 10% mechanical stretch. LPP membrane localization declined in response to 2 h 10% mechanical stretch. (D) LPP protein expression in the nucleus in response to leptomycin B, cytochalasin D and 10% mechanical stretch. LPP nuclear localization increased when nuclear export was blocked by leptomycin B. (E) LPP protein expression in the cytosol in response to leptomycin B, cytochalasin D and 10% mechanical stretch. (F) LPP protein expression in the cytoskeleton in response to leptomycin B, cytochalasin D and 10% mechanical stretch. LPP cytoskeletal localization increased when nuclear export was blocked by leptomycin B, alone and in combination with 10% mechanical stretch. * Represents differences vs control where $p < 0.05$, $n = 3$ different cultures.

stimuli. Using a detergent based method we fractionated the cells into cytosol, membrane, nucleus and cytoskeletal portions for Western blotting. Fig. 5A shows a Western blot of LPP protein in the 4 fractions. Fig. 5B shows just under 40% of the protein is found in the cytosolic fraction and around 30% in the membrane in control cells. The rest is distributed between the nucleus and cytoskeletal fractions. We then determined the effect of cyclic mechanical stretch and cytochalasin D on LPP subcellular distribution. Leptomycin B was used as a positive control to test the methodology since the drug results in nuclear accumulation of LPP. Short term cyclic stretch and leptomycin B result in decreased membrane LPP (Fig. 5C). LPP levels were further reduced in all fractions following cytochalasin D treatment. In the non-cytochalasin D treatment group leptomycin B treatment resulted in increased nuclear LPP (Fig. 5D) consistent with the immunostaining in Fig. 2F and I. However, cyclic stretch alone did not result in a change of nuclear LPP. Fig. 5E shows that none of the treatments altered LPP expression in the cytosol, however leptomycin did significantly increase the protein amounts in the cytoskeleton (Fig. 5F). The increase following leptomycin treatment was abolished by the presence of cytochalasin D.

3.6. Nitric oxide regulates LPP expression in cardiac fibroblasts

Previous work has shown that nitric oxide (NO) levels are increased by mechanical stimuli [17] and influence gene expression in response to stretch. As a result we determined whether the changes in LPP gene expression might be influenced by NO production. Cardiac fibroblasts were stretched with or without the nitric oxide synthase (NOS) inhibitor LNAME to remove any endogenous NO production during mechanical stimulation. Cells were treated with 5 mM LNAME for 48 h and stretched cyclically at 5% maximum strain. After this treatment, cells were lysed and processed for Western blotting. The expression of LPP was measured along with other major fibroblast genes α-smooth muscle actin and pro-collagen. LPP expression decreased following long-term cyclic stretch which was rescued by LNAME treated (Fig. 6B). Cyclic stretch also decreased the expression of pro-collagen (Fig. 6C)

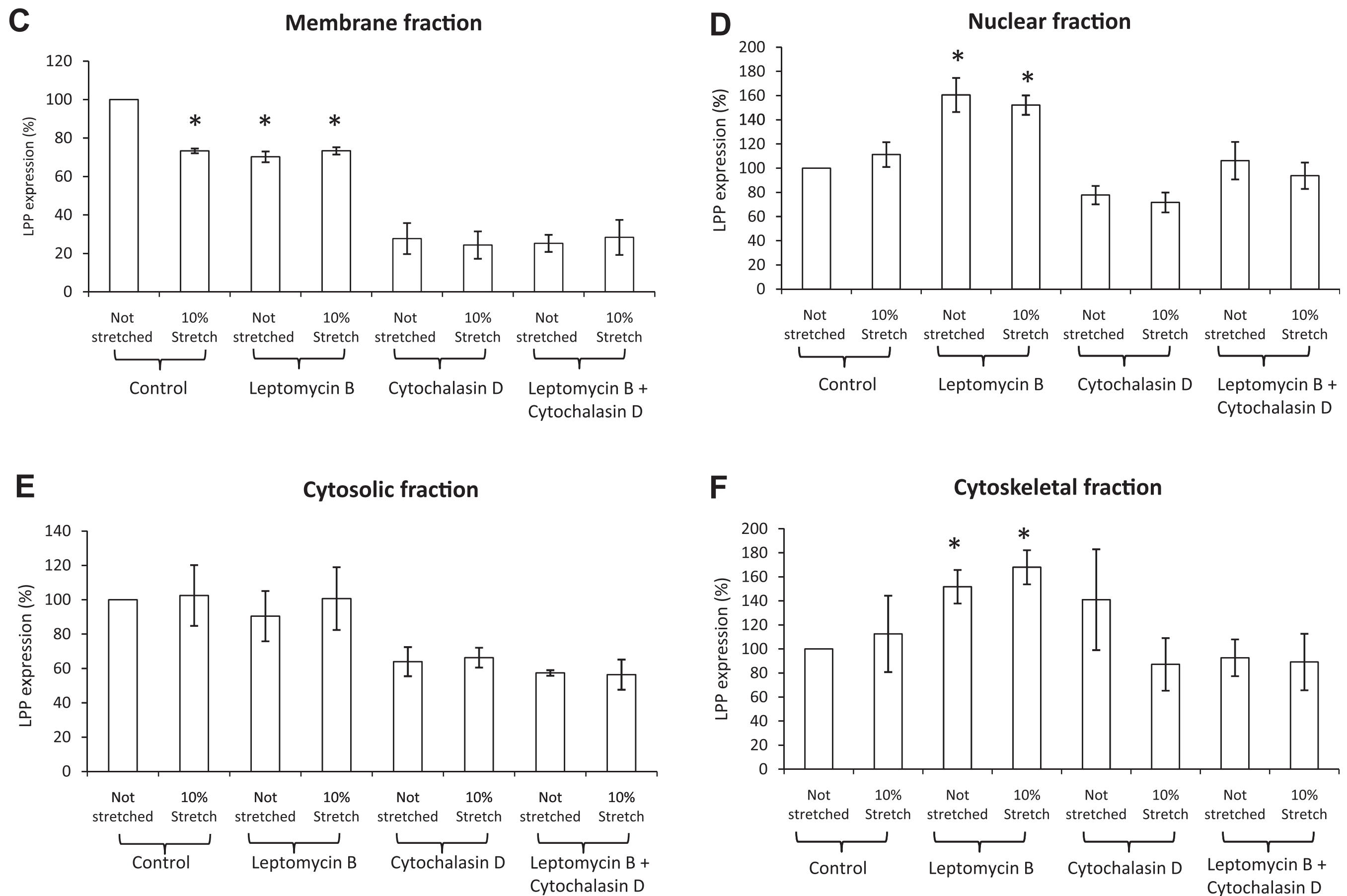

Fig. 5 (*continued*)

and α-smooth muscle actin (Fig. 6D). Unlike with LPP, inhibition of NO production did not rescue the loss of these two proteins following mechanical stress.

4. Discussion

Here, we show for the first time that LPP, an adaptor and nucleocytoplasmic shuttling protein is highly expressed within the myocardium and is regulated by nitric oxide and mechanical stress. These findings are in contrast to a previous report that suggested that the protein was poorly expressed within the heart [4]. That observation was probably because the expression in the heart was compared directly with smooth muscle where the expression of LPP is much higher. Our data here show that LPP expression is down regulated in the adult heart and re-expressed following cardiac hypertrophy induced by increased hemodynamic loading. LPP protein may therefore provide a novel marker for heart failure induced by hemodynamic overload since its expression only increased following pressure overload induced by aortic banding but not after myocardial infarction in rats. These findings strongly suggest that LPP may play an important role in mechano-dependent myocardial growth and adaptation.

The possible role of LPP as a mechanosensor in the myocardium is highly plausible since the protein has been shown to be mechanosensitive in smooth muscle cells. In smooth muscle cells LPP was shown to be important for cytoskeletal organization and its expression was regulated by the mechanical environment [11]. This suggests that LPP may provide a direct link between cytoskeletal remodeling and mechanical overload. LPP was shown to interact with ETV5 in endometrial carcinomas and together act as a sensor of the extracellular environment [18]. The activities of the protein in heart have not been previously investigated but its highly regulated expression there suggests an important role in myocardial function. At any given time, there is little LPP in the nucleus in cardiac cells as seen by our immunochemistry or subcellular fractions. However, blocking nuclear export with leptomyocin results in the rapid nuclear accumulation of the protein in cardiac fibroblasts within a few minutes. This would suggest a highly regulated but very short nuclear retention time, raising the possibility that the LPP could also act as a nucleocytoplasmic courier for other signaling proteins. This hypothesis is supported by the fact that LPP interacts with another focal adhesion protein called LASP-1 which has no nuclear localization signal of its own, but still localizes to the nucleus [19]. Altered LPP expression in cardiac hypertrophy along with changes in its subcellular distribution following stretch suggest a possible role in mechanically-linked growth and gene expression. The presence of LPP in the focal adhesions places it in an area of force transmission between the cell membrane and extracellular matrix.

It has been suggested that LPP may act as a co-activator allowing co-localization of other proteins to promoter sites. This role is supported by the fact that LPP binds and alters the activity of the transcription factor PEA3 [20], however it does not directly regulate its activity. LPP has been shown to bind to a large variety of molecules, facilitated by interactions with LIM and PDZ domains. It interacts with a variety of upstream components of the ERK signaling pathway, resulting in NF-κB activation and the concomitant changes in gene expression [21]. This could allow LPP to provide a link between the extracellular mechanical environment and cellu-

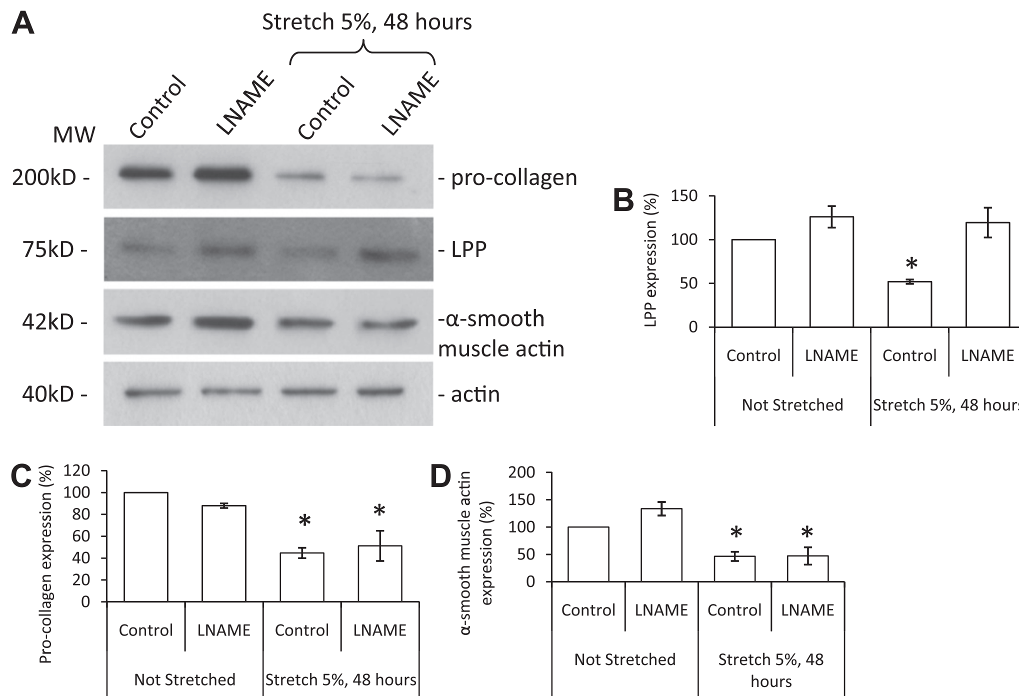

Fig. 6. (A) Western blots of pro-collagen, LPP, α-smooth muscle actin and actin protein expression in response to inhibition of NO synthesis using 5 mM LNAME for 48 h, alone and in combination with exposure to mechanical stretch for 48 h at 5% maximum strain at 1 Hz. (B) LPP protein expression in response to inhibition of NO synthesis using 5 mM LNAME for 48 h; alone and in combination with mechanical stretch for 48 h at 5% maximum strain at 1 Hz. (C) Pro-collagen protein expression in response to inhibition of NO synthesis using 5 mM LNAME for 48 h, alone and in combination with exposure to mechanical stretch for 48 h at 5% maximum strain at 1 Hz. (D) α-Smooth muscle actin protein expression in response to inhibition of NO synthesis using 5 mM LNAME for 48 h, alone and in combination with mechanical stretch for 48 h at 5% maximum strain at 1 Hz. * Represents differences vs control where $p < 0.05$, $n = 3$ cultures.

lar growth through these major signaling pathways. LPP is a member of a family of LIM domain containing proteins that include zyxin and TRIP6. Knockdown of LPP may be compensated for by the activity of TRIP6 and zyxin, which are also focal adhesion nucleo-cytoplasmic shuttling proteins [3]. This functional redundancy is supported by the fact that neither LPP nor zyxin knockout mice have a discernible phenotype. However, knockdown of LPP did result in reduced alpha actinin and another binding partner palladin [3]. Clearly, multiple knockouts are needed to determine the function of these potentially important proteins.

Our findings suggest that LPP expression is regulated by both the mechanical environment and nitric oxide. Nitric oxide is up-regulated in cardiac hypertrophy and failure as well as by mechanical stimuli [17]. This complex interplay between nitric oxide and mechanical cues could explain the difference in LPP expression between the aortic banded and myocardial infarct models of heart failure. All forms of heart failure eventually lead to increased mechanical stress but the hemodynamic data for these models suggest a greater degree of preload and afterload in the AOB model. Our data show that inhibition of NO production can rescue the stretch-induced down-regulation of LPP, suggesting that the stretch-induced down-regulation of LPP is NO dependent. NO has been shown to modify focal adhesions where a significant amount of the protein resides [22]. NO can have a direct effect on Zn^{2+} containing transcription factors by ejecting the cation from zinc fingers [23]. Mechanical stretch also increases NO production [17] which could in turn lead to the degradation of LPP through the mechanisms mentioned previously. This is consistent with our finding that inhibition of NO production up-regulates the protein expression. NO can facilitate post-translational regulation of gene expression by directly affecting mRNA stability and translation [24]. Finally, NO could have an indirect effect on LPP protein expression through its interaction with numerous signaling pathways [25].

In conclusion, we have shown that LPP is highly expressed and regulated in the myocardium but its highest expression is in cardiac fibroblasts. The protein is down regulated in the adult heart but re-expressed following cardiac hypertrophy induced by hemodynamic overload. These findings suggest that the protein may play a role in mechano-dependent growth and this link is regulated through nitric oxide signaling. To determine additional roles of LPP, it is likely that double or even triple knockouts would have to be produced because of its functional overlap with zxyin and TRIP6.

Acknowledgments

We would like to thank Dr. David Geenen for his help in providing the cardiac tissue of the animal models of heart failure. This research was supported by American Heart Association 0630307N, British Heart Foundation, The European Commission and the Royal Society.

References

[1] Tsyba, L., Nikolaienko, O., Dergai, O., Dergai, M., Novokhatska, O., Skrypkina, I. and RYnditch, A. (2011) Intersectin mutlidomain adaptor proteins: regulation of functional diversity. Gene 473, 67–75.

[2] Wang, Y. and Gilmore, T.D. (2001) LIM domain protein Trip6 has a conserved nuclear export signal, nuclear targeting sequences, and multiple transactivation domains. Biochim. Biophys. Acta 1538, 260–272, http://dx.doi.org/10.1016/S0167-4889(01)00077-5.

[3] Vervenne, H.B., Crombez, K.R., Delvaux, E.L., Janssens, V., Van de Ven, W.J. and Petit, M.M. (2009) Targeted disruption of the mouse Lipoma Preferred Partner gene. Biochem. Biophys. Res. Commun. 379, 368–373, http://dx.doi.org/10.1016/j.bbrc.2008.12.074.

[4] Petit, M.M., Fradelizi, J., Golsteyn, R.M., Ayoubi, T.A.Y., Menichi, B., Louvard, D., Van de Ven, W.J.M. and Friederich, E. (2000) LPP, an actin cytoskeleton protein

related to zyxin harbors a nuclear export signal and transcriptional activation capacity. Mol. Biol. Cell 11, 117–129.

[5] Li, B., Zhuang, L., Reinhard, M. and Trueb, B. (2003) The lipoma preferred partner LPP interacts with alpha-actinin. J. Cell Sci. 116, 1359–1366.

[6] Hansen, M.D.H. and Beckerle, M.C. (2005) Opposing roles of zyxin/LPP ACTA repeats and the LIM domain region in cell–cell adhesion. J. Biol. Chem. 281, 16178–16188.

[7] Calvet, C.M., Oliviera Jr., F.O.R., Araujo-Jorge, T.C. and Pereiera, M.C.S. (2009) Regualtion of extracellular matrix expression and distribution in *Trypanosoma cruzi*-infected cardiomyocytes. Int. J. Med. Microbiol. 299, 301–312.

[8] Petit, M.M., Swarts, S., Bridge, J.A. and Van de Ven, W.J.M. (1998) Expression of reciprocal fusion transcripts of the HMGIC and LPP genes in parosteal lipoma. Cancer Genet. Cytogenet. 106, 18–23.

[9] Rogalla, P., Lemke, I., Kazmierczak, B. and Bullerdiek, J. (2000) An identical HMGIC-LPP fusion transcript is consistently expressed in pulmonary chondroid hamartomas with t(3;120(q27–28;q14–15). Genes Chromosomes Cancer 29, 363–366.

[10] Gorenne, I., Nakamoto, R.K., Phelps, C.P., Beckerle, M.C., Somlyo, A.V. and Somlyo, A.P. (2003) LPP, a LIM protein highly expressed in smooth muscle. Am. J. Physiol. Cell Physiol. 285, C674–C685, http://dx.doi.org/10.1152/ajpcell.00608.200200608.2002.

[11] Jin, L., Hastings, N.E., Blackman, B.R. and Somlyo, A.V. (2009) Mechanical properties of the extracellular matrix alter expressin of smooth muscle protein LPP and its partner palladin; relationship to early atherosclerosis and vascular injury. J. Muscle Res. Cell Motil. 30, 41–55.

[12] Petit, M.M., Mols, R., Schoenmakers, E.F., Mandahl, N. and Van de Ven, W.J. (1996) LPP, the preferred fusion partner gene of HMGIC in lipomas, is a novel member of the LIM protein gene family. Genomics 36, 118–129, http://dx.doi.org/10.1006/geno.1996.0432.

[13] Boateng, S.Y., Belin, R.J., Geenen, D.L., Margulies, K.B., Martin, J.L., Hoshijima, M., de Tombe, P.P. and Russell, B. (2007) Cardiac dysfunction and heart failure are associated with abnormalities in the subcellular distribution and amounts of oligomeric muscle LIM protein. Am. J. Physiol. Heart Circ. Physiol. 292, H259–H269, http://dx.doi.org/10.1152/ajpheart.00766.2006.

[14] Boateng, S.Y., Senyo, S.E., Qi, L., Goldspink, P.H. and Russell, B. (2009) Myocyte remodeling in response to hypertrophic stimuli requires nucleocytoplasmic shuttling of muscle LIM protein. J. Mol. Cell Cardiol. 47, 426–435, http://dx.doi.org/10.1016/j.yjmcc.2009.04.006.

[15] Boateng, S.Y., Seymour, A.M.L., Bhutta, N.S., Dunn, M.J., Yacoub, M.H. and Boheler, K.R. (1998) Sub-antihypertensive doses of ramipril normalize sarcoplasmic reticulum calcium ATPase expression and function following cardiac hypertrophy in rats. J. Mol. Cell Cardiol. 30, 2683–2694.

[16] Boateng, S.Y., Lateef, S.S., Mosley, W., Hartman, T.J., Hanley, L. and Russell, B. (2005) RGD and YIGSR synthetic peptides facilitate cellular adhesion identical to that of laminin and fibronectin but alter the physiology of neonatal cardiac myocytes. Am. J. Physiol. Cell Physiol. 288, C30–C38, http://dx.doi.org/10.1152/ajpcell.00199.2004.

[17] Cheng, T.H., Chen, J.J., Shih, N.L., Lin, J.W., Liu, J.C., Chen, Y.L. and Chen, C.H. (2009) Mechanical stretch induces endothelial nitric oxide synthase gene expression in neonatal rat cardiomyocytes. Clin. Exp. Pharmacol. Physiol. 36, 559–566, http://dx.doi.org/10.1111/j.1440-1681.2008.05100.x.

[18] Colas, E., Muinelo-Romay, L., Alonso-Alconada, L., Llaurado, M., Monge, M., Barbazan, J., Gonzalez, M., Schoumacher, M., Pedrola, N., Ertekin, T., et al. (2012) ETV5 cooperates with LPP as a sensor of extracellular signals and promotes EMT in endometrial carcinomas. Oncogene, http://dx.doi.org/10.1038/onc.2011.632.

[19] Grunewald, T.G.P., Kammerer, U., Kapp, M., Eck, M., Dietl, J., Butt, E. and Honig, A. (2007) Nuclear localisation and cytosolic overexpression of LASP-1 correlates with tumor size and nodal-positivity of human breast carcinoma. BMC Cancer 7, 198.

[20] Guo, B., Sallis, R., Greenall, A., Petit, M., Jansen, E., Young, L., Van de Ven, W. and Sharrocks, A. (2006) The LIM domain protein LPP is a coactivator for the ETS domain transcription factor PEA3. Mol. Cell. Biol. 26, 4529–4538.

[21] Kassel, O., Schneider, S., Heilbock, C., Litfin, M., Göttlicher, M. and Herrlich, P. (2004) A nuclear isoform of the focal adhesion LIM-domain protein Trip6 integrates activating and repressing signals at AP-1 and NF-κB-regulated promoters. Genes Dev. 18, 2518–2528.

[22] Goligorsky, M.S., Abedi, H., Noiri, E., Takhtajan, A., Lense, S., Romanov, V. and Zachary, I. (1999) Nitric oxide modulation of focal adhesions in endothelial cells. Am. J. Physiol. Cell Physiol. 276, C1271–C1281.

[23] Kröncke, K.D. and Carlberg, C. (2000) Inactivation of zinc finger transcription factors provides a mechanism for a gene regulatory role of nitric oxide. FASEB 13, 166–173.

[24] Bogdan, C. (2001) Nitric oxide and the regulation of gene expression. Trends Cell Biol. 11, 66–75.

[25] Kim, H., Shim, J., Han, P.L. and Choi, E.J. (1997) Nitric oxide modulates the c-Jun N-terminal kinase/stress-activated protein kinase activity through activating c-Jun N-terminal kinase kinase. Biochemistry 36, 13677–13681.