cells

Article

MSK-Mediated Phosphorylation of Histone H3 Ser28 Couples MAPK Signalling with Early Gene Induction and Cardiac Hypertrophy

Emma L. Robinson [1,2,*,†,‡], Faye M. Drawnel [3,4,†], Saher Mehdi [1,†,§], Caroline R. Archer [3,k], Wei Liu [5], Hanneke Okkenhaug [3,¶], Kanar Alkass [6], Jan Magnus Aronsen [7,8], Chandan K. Nagaraju [1], Ivar Sjaastad [7,9], Karin R. Sipido [1], Olaf Bergmann [10], J. Simon C. Arthur [11], Xin Wang [5] and H. Llewelyn Roderick [1,9,*]

[1] Laboratory of Experimental Cardiology, Department of Cardiovascular Sciences, KU Leuven, B-3000 Leuven, Belgium; saher@wellowise.com (S.M. u@ss-253f 89o C. Aro

Citation: Robinson, E.L.; Drawnel, F.M.; Mehdi, S.; Archer, C.R.; Liu, W.; Okkenhaug, H.; Alkass, K.; Aronsen, J.M.; Nagaraju, C.K.; Sjaastad, I.; et al. MSK-Mediated Phosphorylation of Histone H3 Ser28 Couples MAPK Signalling with Early Gene Induction and Cardiac Hypertrophy. *Cells* **2022**, *11*, 604. https://doi.org/10.3390/cells11040604

Academic Editors: Paula A. Da Costa-Martins and Frank Lezoualc'h

Received: 21 December 2021
Accepted: 4 February 2022
Published: 9 February 2022

Keywords: cardiomyocyte; MSK; phosphorylated histone 3 serine 28; immediate early genes; hypertrophy

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality and morbidity worldwide [1]. While cardiac hypertrophy is initially an adaptive response to increased workload or stress, when induced by pathological cues, the response can decompensate, resulting in a decline in cardiac function and progression to heart failure. As cardiomyocytes (CM) are terminally differentiated, hypertrophy of the cardiac muscle is mediated by growth of CM and not through their proliferation [2].

Signalling pathways downstream of G-protein-coupled receptors (GPCR), such as endothelin-1 (ET-1), angiotensin II (AngII) and adrenergic receptors, play a fundamental role in the induction of pathological hypertrophic remodelling [3–6]. Mitogen-activated protein kinases (MAPK) are of particular importance in mediating the pro-hypertrophic actions, GPCR signalling contributing to regulation of protein synthesis, cell survival, metabolism and gene transcription [6,7]. MAPKs fall into four major families—the extracellular regulated kinases 1 and 2 (ERK1/2), p38 MAPK, c-Jun N-terminal kinases 1 and two (JNK1/2) and ERK5 [6,7]. All limbs of this kinase family are involved in regulating hypertrophic remodelling [6,8,9]. During cardiac hypertrophy, MAPK pathways regulate transcription via phosphorylation-dependent modulation of transcription factors such as NFAT, Elk, SRF and GATA4 [10]. Preceding the induction of expression of genes associated with the hypertrophic state, hypertrophic cues such as pressure overload and exposure to neurohormonal agonists stimulate a MAPK-dependent rapid activation of an immediate early gene (IEG) response [11–14]. This IEG response is initiated through phosphorylation-dependent activation of the activator protein 1 (AP-1) transcription factor [14–17]. The AP-1 transcription factor is a dimeric complex formed by members of the FOS (c-FOS, FOSB, FRA-1 and FRA-2), Jun (JUNB, JUND and c-JUN), activating transcription factor (ATF; ATFa, ATF2, LRF1/ATF3, ATF4 and B-ATF) and MAF families of basic-leucine zipper transcription factors, which are themselves induced as part of the IEG response [17–19]. AP-1 transcription factors also act though forming heteromeric interactions with other transcription factors such as NF B and NFAT, with known roles in CM [20,21]. In addition to showing hypertrophy-related alterations in their activation and expression, a functional role for AP-1 factors in cardiac hypertrophic remodelling is described. While AP-1 factors are generally required for hypertrophic responses in vitro [22,23], in vivo roles of different AP-1 factors are more complex. For example, in vivo deletion of *JunD* or of *c-Jun* results in a loss of the initial adaptive response to hypertrophic stimuli and an exacerbation of the deleterious remodelling to pressure overload [24–26]. Contributing to this phenotype is a reduced upregulation of sarcomeric proteins, enhanced CM apoptosis and fibrosis. *c-Fos* deletion is without effect, however [26]. Contrastingly, JunD overexpression results in ventricular dilation and reduced contractility [25], although in vitro studies suggest that JunD suppresses hypertrophic responses by inhibiting the action of c-Fos and c-Jun [22]. Taken together, these reports demonstrate the important and complex functions of AP-1 transcription factors in hypertrophic remodelling, particularly in the early adaptive responses to pathological cues.

Although ERK activation is highly correlated with induction of IEG expression during CM hypertrophic responses [11,27], the mechanism linking these events is not resolved. In other tissues however, IEG expression is induced following MAPK pathway activation via a mechanism involving phosphorylation of serines 10 and 28 (H3S10 and H3S28) in the histone H3 NH_2-terminal tail at IEG loci, termed the nucleosomal response [28,29]. Phosphorylated histone H3 creates a permissive environment for induction of transcription through recruitment of scaffn17(C)55(57(pr)18(oteinf)-255(such)-263(as)-25(14-3-3s)-25((family)-562

during the nucleosomal response, histone H3 is phosphorylated by the mitogen and stress-activated kinases (MSK1/2) [30–33]. MSKs are nuclear-localised kinases that are activated by an initial phosphorylation by upstream MAPK including ERK1/2 and subsequent autophosphorylation [34,35]. MSK1 and the highly homologous kinase MSK2 are both expressed in the heart and are activated in response to hypertrophic stimuli [36–38]. While

2.4. Preparation of Neonatal Rat Ventricular Cardiomyocytes (NRVMs)

Primary neonatal rat ventricular CM (NRVMs) were isolated from 3–4-day-old male and female Wistar pups and cultured as described previously [44]. Cultures were >95% pure. Adenoviral infections were as previously described [44]. Agonist treatments diluted in serum-free medium were applied 24 h post-infection with adenovirus. Endothelin-1 (ET-1), Iso and PD184352 (PD) were used at final concentrations of 100 nM, 10 nM and 1 M, respectively. PD was applied for 30 min prior to hypertrophic agonist application (ET-1 / Iso). Control cellular experiments (no treatment) were treated with the same volume of vehicle only (DMSO for ET-1 and PD).

2.5. Isolation and Culture of Adult Rat Ventricular Cardiomyocytes (ARVMs)

Male Wistar rats (Harlan; ~200 g) were anaesthetised by CO_2 inhalation and sacrificed by cervical dislocation. ARVMs isolation by Langendorff and collagenase digestion, culture

2.15. Chromatin-Immunoprecipitation (ChIP)

heart was immediately removed for dissection. Whole hearts were removed and placed in ice-cold PBS briefly to remove excess blood, dissected using a sterile surgical scalpel in PBS on ice and weighed on a microbalance before snap-freezing in liquid nitrogen and being stored at 80 C.

2.17. Adenoviral Methods

Adenoviruses were produced and amplified in HEK293 cells and purified as previously described [44]. Adenoviruses to express the WT and catalytically dead D565A mutant (DN) of MSK1 were generated using the AdEasy method by sub-cloning the cDNA for MSK1 or its mutant from a pCMV5 backbone (kindly provided by Prof D Alessi, University of Dundee) into pShuttle CMV [39]. PacI-digested recombinant plasmids were transfected into HEK293 cells and crude adenovirus was harvested after 10–14 days. Adenoviruses for dominant negative (DN)-Jun and AP-1 luciferase were purchased from Vector Biolabs (Malvern, PA, USA). All viruses were amplified in HEK293 cells, purified using the Vivapure Adenopack 100 (Sartorius, Gottingen, Germany) and titrated by end-point dilution in HEK293 cells.

2.18. Analysis of Luciferase Reporter Activity

The AP-1 luciferase reporter was expressed using an adenoviral vector and luciferase activity was determined using a luciferase assay kit from Promega (Madison, WI, USA) as previously described [42].

2.19. Small Interfering RNA (siRNA) Knockdown

Stealth™ siRNAs were purchased from Invitrogen. To achieve sufficient knockdown of *Msk1* or *Brg1*, two siRNAs targeti [(].)-15(o)-15(gt)-15(d [(].)-15(f)-15(i)-15(r)3(e)-326(

3. Results

3.1. Endothelin-1 Stimulates ERK-Dependent Phosphorylation of Histone H3 Serine 28

Figure 1. Neurohumoral signalling-induced ERK1/2 activation results in histone H3S28 phosphorylation at IEG promoters. (**A**). Immunoblot analysis of pH3S10 and pH3S28. Left: Representative immunoblots from 1 NRVM preparation probing for pH3S10 and pH3S28 in acid-extracted histones

3.2. MSK1/2 Is Activated following ET-1 Stimulation in an ERK1/2 Dependent Manner

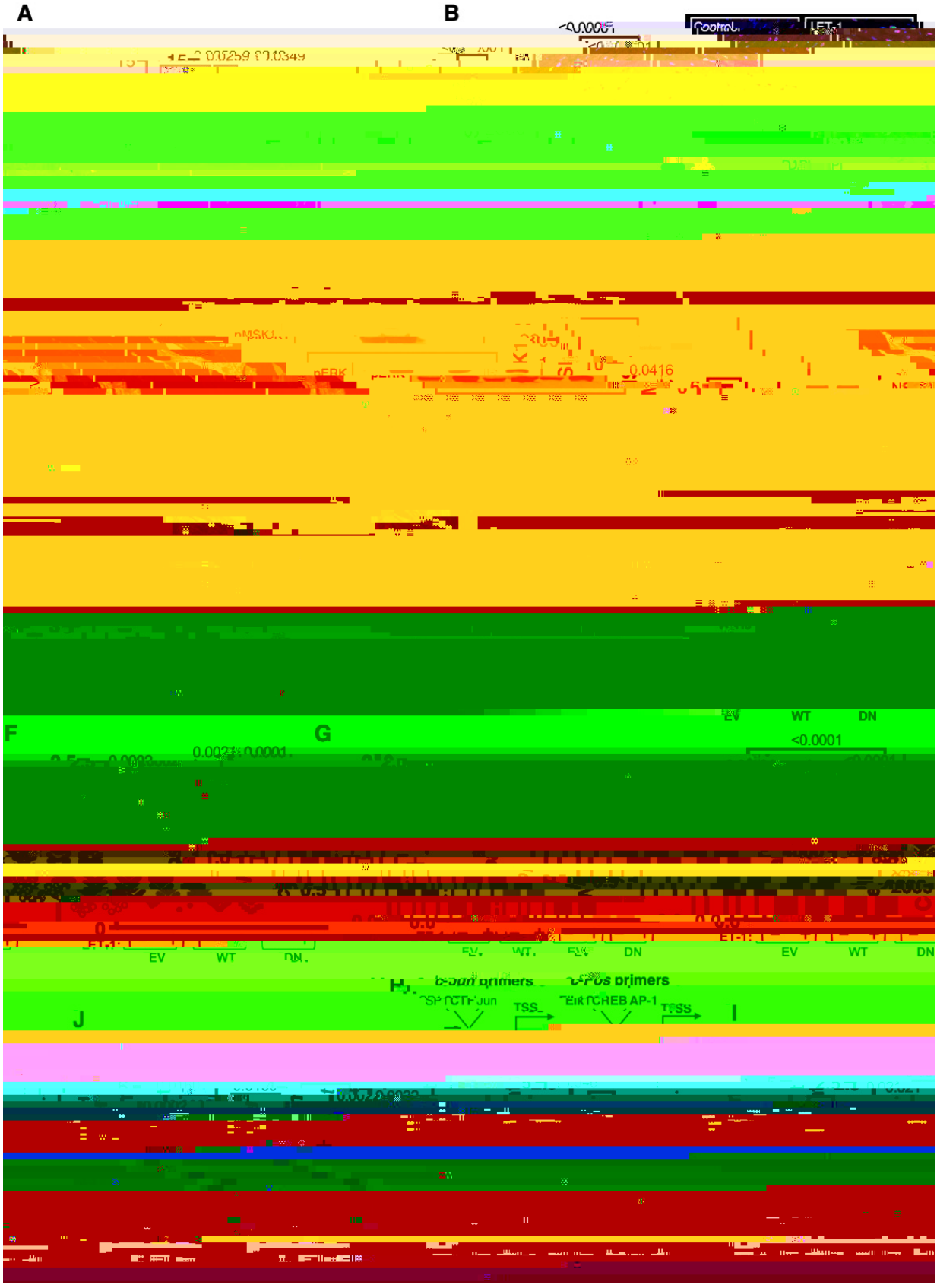

Figure 2. Activated MSK is required for histone H3S28 phosphorylation, recruitment of BRG1 to chromatin and IEG induction in CM. (**A**). Immunoblot showing levels of phosphorylated (activated)

MSK in NRVMs PD and stimulated ET-1 for 10 min. pMSK is normalised to -Actinin (-Act) as a loading control. Left: Representative immunoblot. Right: Quantification of pMSK relative to control-vehicle-treated cells. N = 5. (**B**). Confocal immunofluorescence analysis of pMSK in CM in ventricular cardiac sections prepared from rats infused with ET-1 or Iso for 15 min. CM nuclei were demarcated by pericentriolar material 1 (PCM-1; in magenta) perinuclear staining. Nuclei are stained with DAPI (blue) and pMSK in green. Left: Quantification of nuclear pMSK in PCM-1-positive nuclei. N = 4, 200–400 CM nuclei per sample. Right: Confocal images of heart sections from animals treated as indicated. Scale bar = 20 m. (**C**). Representative confocal images of immunostained NRVMs showing expression of FLAG-tagged WT-MSK and DN-MSK adenoviruses (AdV). Nuclei are stained with DAPI (blue), Beta-Actin in green and FLAG-tagged MSK in red. (**D**). Immunoblotting for pMSK, pERK and FLAG-tagged MSK AdV in NRVMs infected with either empty vector (EV), WT-MSK1 AdV or DN-MSK1 AdV and treated 15 min with ET-1, normalised to GAPDH as a loading control. **Left**: Representative immunoblot. **Right**: Quantification of immunoblot, relative to EV. N = 5. (**E**). Immunoblotting for phosphorylated histone H3S28 in NRVMs infected with either empty vector (EV), WT-MSK1 AdV or DN-MSK1 AdV treated 15 min with ET-1, normalised to total histone H3 (T-H3) as a loading control. **Left**: Representative immunoblot. **Right**: Quantification of immunoblot data. N = 6. (**F**). Effect of DN-MSK expression on *c-Fos* expression in NRVMs treated with ET-1 for 10 min. *c-Fos* expression was determined by RT-qPCR. Data are presented relative to empty vector. For WT-MSK data (**left**), EV ctrl and WT-MSK ctrl, N = 10, EV ET-1 and WT-MSK ET-1, N = 6. For DN-MSK data (**right**), N = 6. (**G**). Analysis of hypertrophic responses in NRVMs infected with EV or DN-MSK1 AdV treated ET-1 for 24 h. **Left**: RT-qPCR expression analysis of *Nppa* / *Anf* mRNA in NRVMs. Data are presented relative to EV untreated cells. For EV ctrl, EV ET-1, WT-MSK ctrl and WT-MSK ET-1, N = 8. For DN-MSK ctrl and DN-MSK ET-1, N = 6. **Right**: Cell area (m^2) as a measure of hypertrophy in NRVMs. N = 4, 50–80 cells per condition. * indicates significantly different from EV transduced NRVM not treated with ET-1; $p < 0.0001$ (**H**

3.4. MSK1-Mediated Phosphorylation of H3S28 Recruits BRG1, a Component of the BAF60 Chromatin Remodelling Complex to IEG Loci

IEG induction and pathological cardiac remodelling involves the action of Brahma-related gene-1(BRG1; encoded by gene *SMARCA4*), a component of the BAF (BRG1 / Brahma

To determine the wider relevance of the role of MSK in hypertrophic responses, we examined whether increased expression of *Msk1/2*

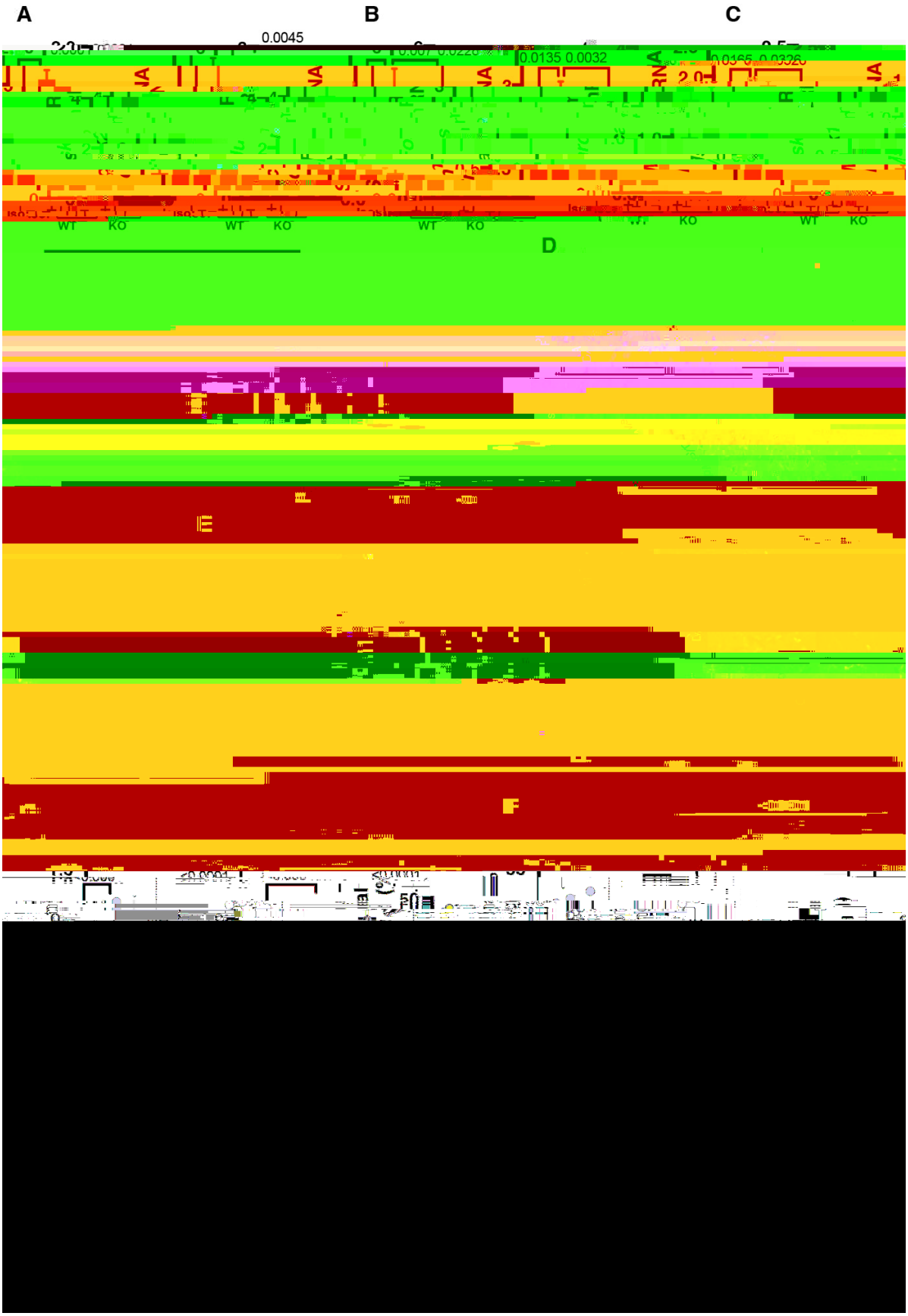

Figure 3. IEG activation and cardiomyocyte hypertrophy is suppressed in vivo in MSK1/2 KO mice. (**A**). RT-qPCR analysis of *Msk1* (**Left**) and *Msk2* (**Right**) mRNA expression in left ventricle from *Msk1/2*

Together, these data show conservation of the MSK pathway to IEG induction in human hearts and support our hypothesis that the MSK/pH3S28/IEG axis is necessary to bring about the initial stages of the CM pathological hypertrophic response (Figure 5).

Figure 5. Graphical abstract of main findings of this study indicating pathway by which MSK couples GPCR activation with IEG induction during the cardiac hypertrophic response.

4. Discussion

MAPK pathway regulation of the expression and activity of IEGs is the key to stress-mediated induction of cardiac hypertrophic responses. Here we identified MSK1/2, a kinase-activated downstream of ERK1/2, as being necessary for the initiation of IEG expression in response to pathological hypertrophic cues. MSK1/2 elicited this response through phosphorylation of histone H3S28 allowing recruitment of the ATP-dependent chromatin remodeller, BRG1. In the absence of this response, gene expression changes and tissue remodelling associated with cardiac hypertrophy was attenuated. Notably, live-cell functional assays and analysis of post-mortem human hypertrophic hearts revealed conservation of this mechanism in humans. These data are summarised in the cartoon in Figure 5.

MSK1/2-mediated phosphorylation of histone H3S10 and H3S28 transduces MAPK activation in response to mitogenic stimulation activation to induction of IEG expression in a wide range of tissues [32,33,55,57]. Until now, a mechanism involving MSKs acting as histone kinases contributing to the induction of IEG and of hypertrophic gene expression via a nucleosomal response has not been demonstrated in CM. MSKs have been reported

ERK, results in cardiomyopathy, however [62]. Notably, loss of ERK2, which represents 50–70% of ERK activity in the heart, attenuates the initial compensatory phase of the hypertrophic response and causes a direct progression to a cardiomyopathic phenotype associated with substantial CM death [60]. Surprisingly, conditional deletion of both ERK alleles does not prevent pathological hypertrophic growth [63]. ERK1/2 likely makes different contributions to the different forms of hypertrophy—while ERK1/2 mediates concentric growth responses to stimulus, it prevents eccentric growth [7,63,64]. As we suggest for MSKs, ERK2 is not required for physiological cardiac remodelling in response to 4 weeks of swim training, indicating independent pathways for adaptive hypertrophy in response to pathological or physiological stimuli [60]. A similar requirement for the acute adaptive hypertrophic growth and repression of maladaptive growth is reported for c-Jun NH_2 terminal kinase (JNK1) [65]. Through deletion of this kinase, mice exhibit a loss of adaptive hypertrophic responses and subsequent direct progression to cardiac dilation [65]. Moreover, MKK4, which lies upstream of JNK and p38MAPK, is also required for this hypertrophic response [5]. Consistent with these actions of JNK, c-Jun acts in a protective manner, preventing maladaptive responses to stress [26]. While JunD also protects CM from maladaptive remodelling, its activity is decreased in pathology, leading to a reduction in AP-1 complexes in which it participates, thereby resulting in a greater influence of other AP-1 factors on downstream signalling [24,25]. As a consequence, pathological cardiac remodelling develops. Further contributing to any differences between the effects of loss of MSK activity on IEG signalling compared to knockout of individual AP-1 factors or

Notably, CaMKII contributed to a delayed but sustained elevation of global pH3S28 but not to the early peak in pH3S28 observed in response to catecholaminergic stimulation [70]. Our description of a role for MSK in the phosphorylation of histone H3S28 at IEG loci in the minutes following agonist stimulation may suggest a model whereby MSKs mediate the induction of IEGs during hypertrophy whereas CaMKII-dependent phosphorylation of histone H3S28 is involved in the control of the expression of genes involved in later stages of the hypertrophic response. While CaMKII may indeed play a role at certain gene loci, the substantial decrease in histone H3S28 phosphorylation in *Msk1/2* KO animals observed in this study would however suggest that MSKs make a substantial contribution

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cells11040604/s1, Figure S1, Supplementary Figure S1: IEG

Abbreviations

Ascending aortic banding, AB; cytosine b-D-arabinofuranoside, ara-C; Histone 3, H3; Histone H3 Serine 10, H3S10; phosphorylated histone H3S10, p-H3S10; Histone H3 Serine 28, H3S28; phosphorylated histone H3S28, pH3S28; immediate early gene, IEG; isoproterenol, Iso; PD184352, PD.

References

1. Timmis, A.; Townsend, N.; Gale, C.P.; Torbica, A.; Lettino, M.; Petersen, S.E.; Mossialos, E.A.; Maggioni, A.P.; Kazakiewicz, D.; May, H.T.; et al. European Society of Cardiology: Cardiovascular Disease Statistics 2019. *Eur. Heart J.* **2020**, *41*, 12–85. [CrossRef] [PubMed]
2. Alkass, K.; Panula, J.; Westman, M.; Wu, T.D.; Guerquin-Kern, J.L.; Bergmann, O. No Evidence for Cardiomyocyte Number Expansion in Preadolescent Mice. *Cell* **2015**, *163*, 1026–1036. [CrossRef] [PubMed]
3. Drawnel, F.M.; Archer, C.R.; Roderick, H.L. The role of the paracrine/autocrine mediator endothelin-1 in regulation of cardiac contractility and growth. *Br. J. Pharmacol.* **2013**, *168*, 296–317. [CrossRef] [PubMed]
4. Wang, J.; Gareri, C.; Rockman, H.A. G-Protein-Coupled Receptors in Heart Disease. *Circ. Res.* **2018**, *123*, 716–735. [CrossRef] [PubMed]
5. Liu, W.; Zi, M.; Jin, J.; Prehar, S.; Oceandy, D.; Kimura, T.E.; Lei, M.; Neyses, L.; Weston, A.H.; Cartwright, E.J.; et al. Cardiac-specific deletion of mkk4 reveals its role in pathological hypertrophic remodeling but not in physiological cardiac growth. *Circ. Res.* **2009**, *104*, 905–914. [CrossRef]
6. Rose, B.A.; Force, T.; Wang, Y. Mitogen-activated protein kinase signaling in the heart: Angels versus demons in a heart-breaking tale. *Physiol. Rev.* **2010**, *90*, 1507–1546. [CrossRef]
7. Bueno, O.F.; De Windt, L.J.; Tymitz, K.M.; Witt, S.A.; Kimball, T.R.; Klevitsky, R.; Hewett, T.E.; Jones, S.P.; Lefer, D.J.; Peng, C.F.; et al. The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *EMBO J.* **2000**, *19*, 6341–6350. [CrossRef]
8. Garrington, T.P.; Johnson, G.L. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr. Opin. Cell. Biol.* **1999**, *11*, 211–218. [CrossRef]
9. Heineke, J.; Molkentin, J.D. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat. Rev. Mol. Cell. Biol.* **2006**, *7*, 589–600. [CrossRef]
10.

23. Omura, T.; Yoshiyama, M.; Yoshida, K.; Nakamura, Y.; Kim, S.; Iwao, H.; Takeuchi, K.; Yoshikawa, J. Dominant negative mutant of c-Jun inhibits cardiomyocyte hypertrophy induced by endothelin 1 and phenylephrine. *Hypertension* **2002**, *39*, 81–86. [CrossRef] [PubMed]
24. Hilfiker-Kleiner, D.; Hilfiker, A.; Kaminski, K.; Schaefer, A.; Park, J.K.; Michel, K.; Quint, A.; Yaniv, M.; Weitzman, J.B.; Drexler, H. Lack of JunD promotes pressure overload-induced apoptosis, hypertrophic growth, and angiogenesis in the heart. *Circulation* **2005**, *112*, 1470–1477. [CrossRef]
25. Ricci, R.; Eriksson, U.; Oudit, G.Y.; Eferl, R.; Akhmedov, A.; Sumara, I.; Sumara, G.; Kassiri, Z.; David, J.P.; Bakiri, L.; et al. Distinct functions of junD in cardiac hypertrophy and heart failure. *Genes Dev.* **2005**, *19*, 208–213. [CrossRef] [PubMed]
26. Windak, R.; Muller, J.; Felley, A.; Akhmedov, A.; Wagner, E.F.; Pedrazzini, T.; Sumara, G.; Ricci, R. The AP-1 transcription factor c-Jun prevents stress-imposed maladaptive remodeling of the heart. *PLoS ONE* **2013**, *8*, e73294. [CrossRef] [PubMed]
27. Amirak, E.; Fuller, S.J.; Sugden, P.H.; Clerk, A. p90 ribosomal S6 kinases play a significant role in early gene regulation in the cardiomyocyte response to G(q)-protein-coupled receptor stimuli, endothelin-1 and alpha(1)-adrenergic receptor agonists. *Biochem. J.* **2013**, *450*, 351–363. [CrossRef] [PubMed]
28. Clayton, A.L.; Rose, S.; Barratt, M.J.; Mahadevan, L.C. Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation. *EMBO J.* **2000**, *19*, 3714–3726. [CrossRef] [PubMed]
29. Dyson, M.H.; Thomson, S.; Inagaki, M.; Goto, H.; Arthur, S.J.; Nightingale, K.; Iborra, F.J.; Mahadevan, L.C. MAP kinase-mediated phosphorylation of distinct pools of histone H3 at S10 or S28 via mitogen- and stress-activated kinase 1/2. *J Cell. Sci.* **2005**, *118*, 2247–2259. [CrossRef]
30. Drobic, B.; Perez-Cadahia, B.; Yu, J.; Kung, S.K.; Davie, J.R. Promoter chromatin remodeling of immediate-early genes is mediated through H3 phosphorylation at either serine 28 or 10 by the MSK1 multi-protein complex. *Nucleic Acids Res.* **2010**, *38*, 3196–3208. [CrossRef]
31. Duncan, E.A.; Anest, V.; Cogswell, P.; Baldwin, A.S. The kinases MSK1 and MSK2 are required for epidermal growth factor-induced, but not tumor necrosis factor-induced, histone H3 Ser10 phosphorylation. *J. Biol. Chem.* **2006**, *281*, 12521–12525. [CrossRef] [PubMed]
32. Josefowicz, S.Z.; Shimada, M.; Armache, A.; Li, C.H.; Miller, R.M.; Lin, S.; Yang, A.; Dill, B.D.; Molina, H.; Park, H.S.; et al. Chromatin Kinases Act on Transcription Factors and Histone Tails in Regulation of Inducible Transcription. *Mol. Cell.* **2016**, *64*, 347–361. [CrossRef] [PubMed]
33. Soloaga, A.; Thomson, S.; Wiggin, G.R.; Rampersaud, N.; Dyson, M.H.; Hazzalin, C.A.; Mahadevan, L.C.; Arthur, J.S. MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. *EMBO J.* **2003**, *22*, 2788–2797. [CrossRef]
34. Malakhova, M.; Kurinov, I.; Liu, K.; Zheng, D.; D'Angelo, I.; Shim, J.H.; Steinman, V.; Bode, A.M.; Dong, Z. Structural diversity of the active N-terminal kinase domain of p90 ribosomal S6 kinase 2. *PLoS ONE* **2009**, *4*, e8044. [CrossRef]
35. McCoy, C.E.; Campbell, D.G.; Deak, M.; Bloomberg, G.B.; Arthur, J.S. MSK1 activity is controlled by multiple phosphorylation sites. *Biochem. J.* **2005**, *387*, 507–517. [CrossRef]
36. Deak, M.; Clifton, A.D.; Lucocq, L.M.; Alessi, D.R. Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. *EMBO J.* **1998**, *17*, 4426–4441. [CrossRef]
37. Markou, T.; Cieslak, D.; Gaitanaki, C.; Lazou, A. Differential roles of MAPKs and MSK1 signalling pathways in the regulation of c-Jun during phenylephrine-induced cardiac myocyte hypertrophy. *Mol. Cell. Biochem.* **2009**, *322*, 103–112. [CrossRef]
38. Markou, T.; Hadzopoulou-Cladaras, M.; Lazou, A. Phenylephrine induces activation of CREB in adult rat cardiac myocytes through MSK1 and PKA signaling pathways. *J. Mol. Cell. Cardiol.* **2004**, *37*, 1001–1011. [CrossRef]
39. Alessi, D.R. The protein kinase C inhibitors Ro 318220 and GF 109203X are equally potent inhibitors of MAPKAP kinase-1beta (Rsk-2) and p70 S6 kinase. *FEBS Lett.* **1997**, *402*, 121–123. [CrossRef]
40. Markou, T.; Lazou, A. Phosphorylation and activation of mitogen- and stress-activated protein kinase-1 in adult rat cardiac myocytes by G-protein-coupled receptor agonists requires both extracellular-signal-regulated kinase and p38 mitogen-activated protein kinase. *Biochem. J.* **2002**, *365*, 757–763. [CrossRef]
41. Naqvi, S.; Macdonald, A.; McCoy, C.E.; Darragh, J.; Reith, A.D.; Arthur, J.S. Characterization of the cellular action of the MSK inhibitor SB-747651A. *Biochem. J.* **2012**, *441*, 347–357. [CrossRef] [PubMed]
42. Arthur, J.S.; Cohen, P. MSK1 is required for CREB phosphorylation in response to mitogens in mouse embryonic stem cells. *FEBS Lett.* **2000**, *482*, 44–48. [CrossRef]
43. Wiggin, G.R.; Soloaga, A.; Foster, J.M.; Murray-Tait, V.; Cohen, P.; Arthur, J.S. MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts. *Mol. Cell. Biol.* **2002**, *22*, 2871–2881. [CrossRef] [PubMed]
44. Higazi, D.R.; Fearnley, C.J.; Drawnel, F.M.; Talasila, A.; Corps, E.M.; Ritter, O.; McDonald, F.; Mikoshiba, K.; Bootman, M.D.; Roderick, H.L. Endothelin-1-stimulated InsP3-induced Ca2+ release is a nexus for hypertrophic signaling in cardiac myocytes. *Mol. Cell.* **2009**, *33*, 472–482. [CrossRef]
45. Drawnel, F.M.; Wachten, D.; Molkentin, J.D.; Maillet, M.; Aronsen, J.M.; Swift, F.; Sjaastad, I.; Liu, N.; Catalucci, D.; Mikoshiba, K.; et al. Mutual antagonism between IP(3)RII and miRNA-133a regulates calcium signals and cardiac hypertrophy. *J. Cell. Biol.* **2012**, *199*, 783–798. [CrossRef]

46.

, 604