Novartis Foundation Symposium 274

HEART FAILURE: MOLECULES, MECHANISMS AND THERAPEUTIC TARGETS

2006

John Wiley & Sons, Ltd

HEART FAILURE: MOLECULES, MECHANISMS AND THERAPEUTIC TARGETS

The Novartis Foundation is an international scientific and educational charity (UK Registered Charity No. 313574). Known until September 1997 as the Ciba Foundation, it was established in 1947 by the CIBA company of Basle, which merged with Sandoz in 1996, to form Novartis. The Foundation operates independently in London under English trust law. It was formally opened on 22 June 1949.

The Foundation promotes the study and general knowledge of science and in particular encourages international co-operation in scientific research. To this end, it organizes internationally acclaimed meetings (typically eight symposia and allied open meetings and 15–20 discussion meetings each year) and publishes eight books per year featuring the presented papers and discussions from the symposia. Although primarily an operational rather than a grant-making foundation, it awards bursaries to young scientists to attend the symposia and afterwards work with one of the other participants.

The Foundation's headquarters at 41 Portland Place, London W1B 1BN, provide library facilities, open to graduates in science and allied disciplines. Media relations are fostered by regular press conferences and by articles prepared by the Foundation's Science Writer in Residence. The Foundation offers accommodation and meeting facilities to visiting scientists and their societies.

Information on all Foundation activities can be found at
http://www.novartisfound.org.uk

Novartis Foundation Symposium 274

HEART FAILURE: MOLECULES, MECHANISMS AND THERAPEUTIC TARGETS

2006

John Wiley & Sons, Ltd

Published in 2006 by John Wiley & Sons Ltd,
The Atrium, Southern Gate,
Chichester PO19 8SQ, UK

National 01243 779777
International (+44) 1243 779777
e-mail (for orders and customer service enquiries): cs-books@wiley.co.uk
Visit our Home Page on http://www.wileyeurope.com
or http://www.wiley.com

Other Wiley Editorial Offices

John Wiley & Sons Inc., 111 River Street, Hoboken, NJ 07030, USA

Jossey-Bass, 989 Market Street, San Francisco, CA 94103-1741, USA

Wiley-VCH Verlag GmbH, Boschstr. 12, D-69469 Weinheim, Germany

John Wiley & Sons Australia Ltd, 33 Park Road, Milton, Queensland 4064, Australia

John Wiley & Sons (Asia) Pte Ltd, 2 Clementi Loop #02-01, Jin Xing Distripark, Singapore 129809

John Wiley & Sons Canada Ltd, 6045 Freemont Blvd, Mississauga, Ontario, Canada L5R 4J3

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Novartis Foundation Symposium 274
x + 291 pages, 57 figures, 11 tables

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN-13 978-0-470-01597-1 (HB)
ISBN-10 0-470-01597-7 (HB)

Typeset in 10½ on 12½pt Garamond by SNP Best-set Typesetter Ltd., Hong Kong
Printed and bound in Great Britain by T. J. International Ltd, Padstow, Cornwall.
This book is printed on acid-free paper responsibly manufactured from sustainable forestry, in which at least two trees are planted for each one used for paper production.

Contents

Participants

Michael Bristow University of Colorado Health Sciences Center, Department of Cardiology, Biomedical Research Building, Room 125, Campus Box B-139, 4200 East Ninth Ave, Denver, CO 80262, USA

Kenneth R. Chien MGH Cardiovascular Research Center, Harvard Medical School, and the Harvard Stem Cell Institute, Boston, MA, USA

Stefanie Dimmeler Molecular Cardiology, Department of Internal Medicine IV, University of Frankfurt, Theodor Stern-Kai 7, D-60590 Frankfurt, Germany

Pieter A. Doevendans Department of Cardiology, Heart Lung Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

Gerald Dorn Department of Medicine, University of Cincinnati, Cincinnati, OH 45267-0592, USA

Loren J. Field Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, 1044 West Walnut Street, R4 Building, Room W376, Indianapolis, IN 46202-5225, USA

Gabor Földes *(Novartis Foundation Bursar)* Department of Internal Medicine, 1083 Budapest VIII, Koryaáni Saándor u. 2/A, Budapest, Hungary

Seigo Izumo *(Chair)* Cardiovascular Research, Novartis Institutes for BioMedical Research, 100 Technology Square, Suite 8402, Cambridge, MA 02139, USA

Arnold M. Katz (University of Connecticut School of Medicine and Dartmouth Medical School), 1592 New Boston Road, PO Box 1048, Norwich, VT 05055-1048, USA

Issei Komuro Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan

Litsa Kranias Department of Pharmacology and Cell Biophysics, University of Cincinnati, College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267-0575, USA

Leslie Leinwand Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309, USA

Andrew R. Marks Department of Physiology and Cellular Biophysics, Columbia University College of Physicians & Surgeons, 630 West 168th Street, P&S 11 511, New York, NY 10032, USA

Timothy McKinsey Myogen, Inc., 7575 West 103rd Avenue, Westminster, CO 80021, USA

Anthony Muslin Center for Cardiovascular Research – Box 8086, Washington University School of Medicine, 660 South Euclid Ave, St Louis, MO 63110, USA

Mona Nemer Institut de Recherches Cliniques de Montréal, Laboratory of Cardiac Growth and Differentiation, 110 Ave des Pins Ouest, Montréal, Quebec H2W 1R7, Canada

Eric N. Olson Department of Molecular Biology, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, TX 75390-9148, USA

Jeyaseelan Raju Novartis Institute for BioMedical Research, Cardiovascular, 100 Technology Square, Cambridge, MA 02139, USA

Nadia Rosenthal Mouse Biology Unit, EMBL Monterotondo Outstation, Via Ramarini 32, 00016 Monterotondo, Rome, Italy

Jun-ichi Sadoshima Cardiovascular Research Institute and, Department of Cell Biology and Molecular Medicine, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, 185 South Orange Ave, MSB G609, Newark NJ 07103, USA

Michael D. Schneider Department of Medicine, Baylor College of Medicine, One Baylor Plaza 506D, Houston, TX 77030, USA

Jonathan Seidman Department of Genetics, Harvard Medical School, NRB, Room 256, 77 Ave Louis Pasteur, Boston, MA 02115, USA

Suraj S. Shetty Novartis Institute for BioMedical Research, Cardiovascular, 100 Technology Square, Cambridge, MA 02139, USA

Paul C. Simpson UCSF Department of Medicine, VA Medical Center (111C8), 4150 Clement Street, San Francisco, CA 94121, USA

Peter Sugden National Heart and Lung Institute Division, Faculty of Medicine, Imperial College London, Flowers Building (4th Floor), Armstrong Road, London SW7 2AZ, UK

Introduction

Eric N. Olson

Department of Molecular Biology, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75390-9148, USA

Over the next few days we will be looking at the pathological transitions that the adult heart can undergo. In particular, we will be discussing the events of pathological cardiac hypertrophy as opposed to physiological hypertrophy. What are the mechanisms that cause the pathologically enlarged heart to progress to heart failure and dilated cardiomyopathy? There are also stimuli that can lead from a normal heart to a dilated myopathic heart without hypertrophic intermediates, and we would like to know how these transitions are regulated.

To put this in context, pathological hypertrophy is a major predictor of heart failure and cardiac sudden death. Heart failure affects a staggering number of individuals worldwide (5 million people, with 400 000 new cases each year in the USA). Currently, half of these individuals with late-stage heart failure die within five years, with a corresponding huge burden on the healthcare system.

Many of the people in this room have identified a range of signalling molecules from the cell membrane to the nucleus that comprise a web of pathological signalling that can drive many aspects of cardiac remodelling, leading to hypertrophy and heart failure. They can lead to alterations in contractility, and changes in gene expression, translation, Ca^{2+} handling and bioenergetics. One of the goals of this meeting is to try to sort through this complexity, to identify some of the key components of this complex disease process.

These are some of the challenges in terms of developing new heart failure therapies.

- Heart failure is complex.
- Many disease mechanisms implicated in heart failure or pathological hypertrophy are not necessarily druggable, even though we know about the mechanism.
- Many drug targets that are druggable aren't cardiac specific. Systemic delivery of small molecules that perturb a signalling pathway may have global consequences throughout the organism.
- Clinical trials in heart failure are large, expensive and lengthy. They often have survival as the endpoint.

- Many patients with heart failure are already on combinations of therapies, which complicates the analysis of new drugs that are being administered on top of the existing therapies.

The following list specifies some of the questions that I think we should consider during our discussions.

- Is heart failure a curable disease entity? Should we be thinking more about prevention or reversal, and to what extent is the disease process reversible?
- Is pathological cardiac hypertrophy a reasonable therapeutic target?
- Do we have an adequate understanding of the disease process to enable rational drug development?
- What are the opportunities and pitfalls for new drug development in this arena?
- Are pathological and physiological hypertrophies mechanistically distinct, or does the former result from over-stimulation of normal pathways? This is an important problem: if one is developing small molecules to inhibit disease processes one doesn't want to be inhibiting the normal physiological process.
- Are there common final pathways and nodal points in cardiac disease signalling, or do multiple parallel pathways lead to disease? This is an important issue in thinking about how the heart can undergo remodelling.
- What is the relative importance of cellular hypertrophy, fetal gene activation, Ca^{2+} cycling, energy metabolism, fibrosis and apoptosis? All of these are known to accompany pathological remodelling of the heart, but which are therapeutic targets?
- Later on in this meeting we will be talking about the opportunities and pitfalls for manipulating stem cells and the cardiac cell cycle: does this represent a more effective strategy than small molecule approaches?

These are questions that we will revisit during our discussions over the next few days.

Control of cardiac hypertrophy and heart failure by histone acetylation/deacetylation

Eric N. Olson, Johannes Backs and Timothy A. McKinsey*

*Myogen, Inc., 7575 W. 103rd Ave., Westminster, CO 80021 and *Department of Molecular Biology, The University of Texas Southwestern Medical Center at Dallas, 6000 Harry Hines Boulevard, Dallas, TX 75390-9148, USA*

Abstract. The adult heart responds to acute and chronic stresses by a remodelling process that is accompanied by myocyte hypertrophy, impaired contractility, and pump failure, often culminating in sudden death. Pathological growth and remodelling of the adult heart is often associated with the reactivation of a fetal cardiac gene program that further weakens cardiac performance. Recent studies have revealed key roles for histone deacetylases (HDACs) in the control of pathological cardiac growth. Class II HDACs associate with the MEF2 transcription factor, and other factors, to maintain normal cardiac size and function. Stress signals lead to the phosphorylation of class II HDACs and their export from the nucleus to the cytoplasm, with consequent activation of genes involved in cardiac growth. HDAC knockout mice are hypersensitive to stress signalling and develop massively enlarged hearts in response to various pathological stress stimuli due to an inability to counteract pathological signalling to MEF2. Strategies for normalizing gene expression in the failing heart by regulating HDAC phosphorylation and function represent potentially powerful therapeutic approaches.

2006 Heart failure: molecules, mechanisms and therapeutic targets. Wiley, Chichester (Novartis Foundation Symposium 274) p 3–19

Heart failure, the leading cause of morbidity and mortality in the Western world, is a complex disorder in which cardiac contractility is insufficient to meet the metabolic demands of the body. Diverse pathological insults can cause heart failure, including myocardial infarction, hypertension, valve abnormalities and inherited mutations in cardiac contractile and structural proteins (Frey et al 2003). Heart failure is frequently preceded by pathological cardiac hypertrophy in which cardiomyocytes increase in size, but not in number. Pathological hypertrophy is accompanied by the activation of 'fetal' cardiac genes, which encode proteins involved in contraction, calcium handling and metabolism (Fig. 1). Such transcriptional reprogramming correlates with a decline in cardiac function. Conversely, normalization of cardiac gene expression in the failing heart correlates with the

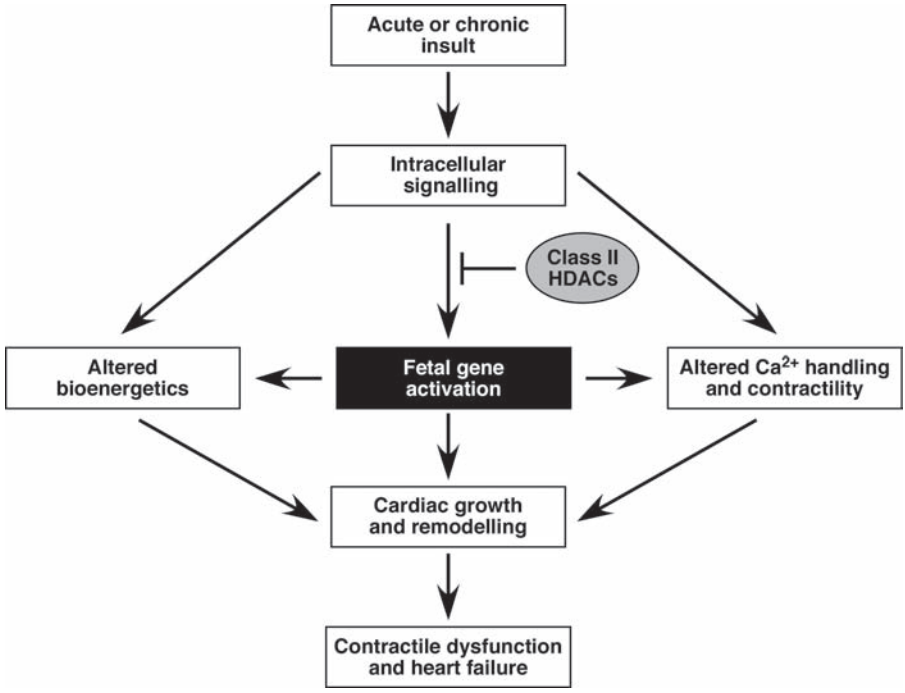

FIG. 1. A central role of histone acetylation/deacetylation in cardiac remodelling during pathological hypertrophy and heart failure.

restoration of cardiac function (Abraham et al 2002, Lowes et al 2002, Blaxall et al 2003). There is a major need for the development of novel therapeutics, preferably new drugs, that will prevent progression of pathological hypertrophy to heart failure and will improve long-term function of the failing heart. Thus strategies to control cardiac gene expression represent attractive, yet untested, therapeutic approaches.

Transcription factors are generally considered to be poor drug targets due to their lack of enzymatic activity and inaccessibility in the nucleus. However, we and others have recently found that cardiac stress response pathways control cardiac gene expression by modulating the activities of chromatin-remodelling enzymes, which act as global regulators of the cardiac genome (McKinsey & Olson 2004). Here we discuss approaches for manipulation of chromatin-remodelling enzymes and the signalling pathways that modulate them as a means of normalizing abnormalities in cardiac gene expression during heart disease.

Signalling pathways involved in cardiac hypertrophy and heart failure

A wide variety of neurohumoral and mechanical stimuli act through a web of signalling pathways to drive pathological cardiac hypertrophy and heart failure. Many hypertrophic agonists stimulate cell surface receptors that couple with Gαq to mobilize intracellular calcium, with consequent activation of downstream kinases and the calcineurin phosphatase (Chien 1999, Molkentin & Dorn 2001, Frey & Olson 2003, Olson & Schneider 2003). An important question in the field is how these upstream signalling events are linked to the transcriptional machinery that drives cardiac remodelling. Are there nodal points in these pathways that can be therapeutically targeted, or do different upstream signalling pathways act through parallel, independent pathways to control the cardiac growth response? As discussed below, class II histone deacetylases (HDACs) have emerged as integrators of diverse stress response pathways and signal transducers to the cardiac genome.

Transcriptional remodelling during cardiac hypertrophy and heart failure

A hallmark of maladaptive cardiac growth and remodelling is the up-regulation of fetal cardiac and stress response genes. The differential regulation of the two myosin heavy chain (MHC) isoforms, α and β, in the stressed myocardium has a profound effect on cardiac contractility (Braunwald & Bristow 2000). αMHC, which is up-regulated in the heart after birth, has high ATPase activity, whereas βMHC has low ATPase activity. Pathological remodelling of the heart in rodent models is accompanied by up-regulation of βMHC expression and down-regulation of αMHC, with consequent reduction in myofibrillar ATPase activity and reduced shortening velocity of cardiac myofibres, leading to eventual cardiac dysfunction. Remarkably, minor changes in αMHC content of the heart can have a profound influence on cardiac performance (Herron & McDonald 2002). Because the human heart contains only a small percentage of αMHC, there has been controversy regarding the potential significance of MHC isoform switching in humans. Nonetheless, there is compelling evidence supporting a role for changes in MHC isoform switching in the pathogenesis of heart failure in humans. Other changes in cardiac gene expression during hypertrophy and failure are also likely to contribute to cardiac demise.

Control of gene transcription by histone acetylation and deacetylation

Changes in histone acetylation and deacetylation represent a central mechanism for the control of gene expression in response to extracellular stimuli (Fischle et al 2003). Acetylation of histones by histone acetyltransferases (HATs) promotes transcription by relaxing chromatin structure, whereas histone deacetylation by HDACs reverses this process, resulting in transcriptional repression.

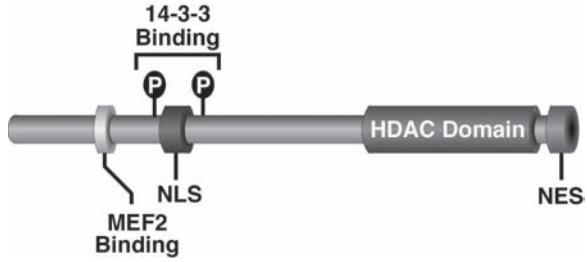

FIG. 2. Schematic of class II HDACs. The structure of class II HDACs is shown. Two phosphorylation sites flanking the NLS serve as binding sites for 14-3-3 proteins, which promote nuclear export in response to extracellular signals. NES, nuclear export sequence; NLS, nuclear localization sequence.

There are two classes of HDACs that can be distinguished by their structures and expression patterns (Verdin et al 2003). Class I HDACs (HDAC1, HDAC2 and HDAC3), which are expressed in all tissues, are comprised simply of a catalytic domain. In contrast, class II HDACs (HDAC4, HDAC5, HDAC7 and HDAC9) are most abundant in striated muscle tissue and brain and contain a distinct structure with an N-terminal regulatory domain followed by a C-terminal catalytic domain (Fig. 2).

Class II HDACs interact avidly with the MEF2 transcription factor, which regulates fetal cardiac and stress-responsive genes (McKinsey et al 2002). Notably, the transcriptional coactivators p300 and GRIP, which possess histone acetyltransferase activity, bind the same region of MEF2 as class II HDACs (Youn et al 2000, Chen et al 2002). Thus, MEF2 can function either as a transcriptional activator or repressor, dependent on the type of chromatin-modifying enzymes to which it is bound.

Control of cardiac growth by signal-dependent regulation of class II HDACs

The N-terminal regulatory regions of class II HDACs interact with a variety of positive and negative cofactors. This domain also contains conserved phosphorylation sites for calcium/calmodulin-dependent (CaM) kinase, protein kinase D (PKD) and other kinases involved in hypertrophic signalling (McKinsey et al 2000a, 2000b, 2001, Grozinger & Schrieber 2000, Wang & Yang 2001). Phosphorylation of these sites creates binding sites for the 14-3-3 family of chaperone proteins, which mediate nuclear export of class II HDACs and consequent derepression of HDAC target genes (Fig. 3).

Several independent lines of evidence point to important roles of class II HDACs in the control of cardiac growth in response to stress signalling. (1) Hypertrophic

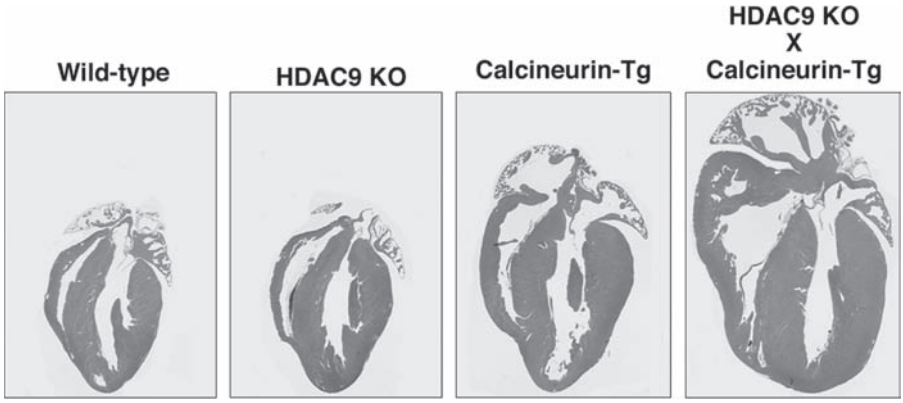

FIG. 3. Hypersensitivity of HDAC9 knockout mice to calcineurin signalling. Histological sections of adult mouse hearts of the indicated genotypes are shown. HDAC9 knockout (KO) mice have hearts of normal size at early age. Transgenic mice harboring a cardiac-specific calcineurin transgene (Calcineurin-Tg) develop cardiac hypertrophy, which is exacerbated in an HDAC9 KO background.

signals induce the nuclear export of class II HDACs and stimulate MEF2 activity (Zhang et al 2002, Bush et al 2004, Vega et al 2004). (2) Forced overexpression of signal-resistant HDAC5 or HDAC9 mutant proteins prevents hypertrophy of cardiomyocytes in response to diverse agonists (Zhang et al 2002). (3) Knockout mice lacking HDAC5 or HDAC9 are hypersensitive to cardiac stress and develop cardiomegaly and eventual cardiac failure in response to stresses such as pressure overload or constitutive calcineurin activation (Zhang et al 2002, Chang et al 2004). (4) Abnormal cardiac growth of HDAC knockout mice correlates with superactivation of the MEF2 transcription factor (Zhang et al 2002), which suggests a causal relationship between MEF2 activity and the development of cardiac hypertrophy.

Consistent with a repressive role of class II HDACs in cardiac growth, several studies have implicated HATs in the stimulation of cardiac growth. For example, the HAT p300 associates with and enhances the transcriptional activity of the MEF2 and the GATA4 transcription factors, which regulate fetal cardiac genes (Yanazume et al 2003). In addition, overexpression of p300 induces hypertrophy of primary cardiomyocytes.

Given the apparent role of class II HDACs as nuclear integrators of hypertrophic signals, there has been intense interest in identifying the signalling pathways that impinge on these transcriptional repressors. Therapeutic strategies to sustain the repressive function of class II HDACs by blocking their signal-dependent nuclear export could provide clinical benefit in the treatment of pathologic cardiac remodelling.

Multiple kinases leading to class II HDACs

Many hypertrophic agonists activate protein kinase C (PKC). Recently, we showed that PKC signalling leads to the phosphorylation of the same sites in HDAC5 that are phosphorylated by CaMK (Vega et al 2004). The PKC family includes at least 12 different isoforms, many, but not all, of which are expressed at appreciable levels in the myocardium. PKC signalling drives HDAC5 nuclear export via a downstream kinase, PKD (Fig. 4). Based on studies with protein kinase inhibitors,

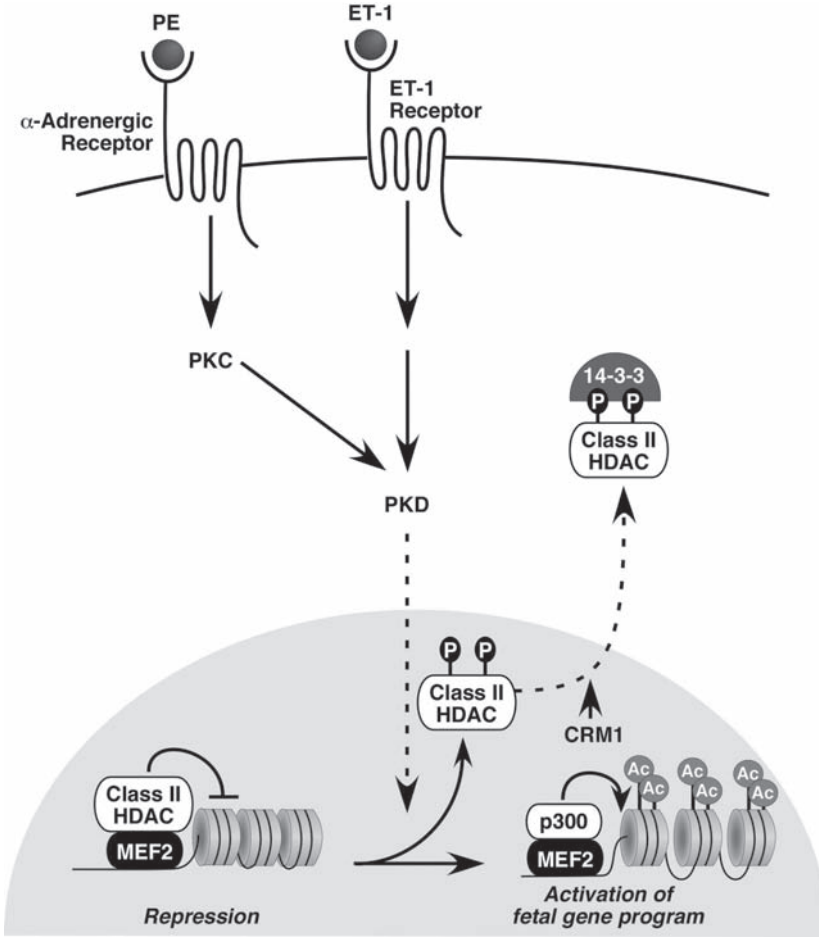

FIG. 4. Signal-dependent regulation of cardiac gene expression by class II HDACs. PE and ET1 both induce cardiomyocyte hypertrophy and fetal gene activation via PKD. PE stimulates PKC, which activates PKD, whereas ET1 signalling bypasses PKC. PKD phosphorylates class II HDACs, promoting their export from the nucleus to the cytoplasm, with consequent association of MEF2 with p300, histone acetylation and chromatin remodelling.

phenylephrine (PE) appears to induce HDAC5 nuclear export by a pathway involving an atypical PKC isoform that phosphorylates PKD, which delivers the signal directly to the two critical serines in HDAC5. In contrast, endothelin acts through a PKC-independent pathway to activate PKD and induce HDAC5 nuclear export.

There also appears to be specificity among class II HDACs with respect to their responsiveness to upstream signals. For example, HDAC5 and HDAC9 are not responsive to CaMKII signalling, whereas HDAC4 is efficiently exported from the nucleus to the cytoplasm by activated CaMKII. We have pinpointed the specific residues in HDAC4 that confer CaMKII responsiveness and have shown that HDAC4 contains a specific docking site for CaMKII that is not present in other class II HDACs. Intriguingly, although HDACs 5 and 9 cannot respond directly to CaMKII signalling, they can be exported from the nucleus in the presence of HDAC4 and activated CaMKII. We have shown that HDAC4 dimerizes with these class II HDACs and thereby confers CaMKII responsiveness to them.

Paradoxical effects of HDAC inhibitors on cardiac growth

The enhanced cardiac growth response of knockout mice lacking HDAC5 and HDAC9 predicts that HDAC inhibitors, which are currently in use as anti-cancer drugs, would also promote cardiac growth. Paradoxically, HDAC inhibitors have the opposite effect—that is, they inhibit cardiac hypertrophy (Antos et al 2003, Kook et al 2003).

The surprising ability of HDAC inhibitors to prevent cardiac hypertrophy raises interesting questions about the enzymatic target of these inhibitors and their mechanism of action. One interpretation of these findings is that one or more HDACs play a pro-hypertrophic role, such that their inhibition prevents cardiac growth (Fig. 5). The HDAC inhibitors shown to block hypertrophy inhibit both class I and II HDACs. However, based on the well-documented role of class II HDACs as repressors of cardiac growth and fetal gene expression, we postulate that HDAC inhibitors are most likely to act on class I HDACs to prevent hypertrophy. Perhaps the target genes of class I HDACs are dominant over those of class II HDACs.

What might be the gene targets of pro-hypertrophic HDACs? We speculate that such HDACs are required for repression of genes whose products repress hypertrophy. Accordingly, inhibition of these HDACs could result in derepression of such anti-hypertrophic genes and a consequent block to hypertrophy. Expression of the cyclin-dependent protein kinase inhibitor p21 has been shown to be upregulated by HDAC inhibitors in cancer cells, resulting in inhibition of cell growth. It is interesting in this regard that p21 has been implicated in the inhibition of cardiomyocyte hypertrophy (Hassig et al 1997, Nozato et al 2000).

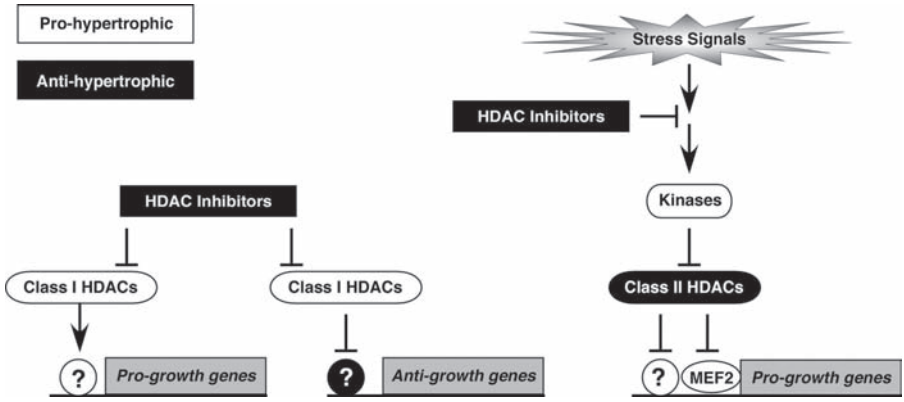

FIG. 5. A model to account for the roles of HDACs in cardiac growth. Stress signals activate prohypertrophic kinases that inactivate class II HDACs, leading to activation of MEF2 and pro-hypertrophic genes. Other transcription factors may also be regulated by class II HDACs. Class I HDACs may repress expression of anti-growth genes or potentially may activate pro-growth genes. HDAC inhibitors may act on class I HDACs or potentially may perturb stress signalling. Lighter grey denotes pro-hypertrophic effectors. Black denotes anti-hypertrophic effectors.

While most studies to date have focused on the roles of HDACs in the deacetylation of histones and consequent effects on gene expression, these enzymes can deacetylate a variety of cellular proteins. Thus, it is not unreasonable to anticipate that changes in the acetylation of other types of proteins, such as components of signalling pathways or the cytoskeleton, might also be affected by HDAC inhibitors and might thereby disrupt hypertrophic signalling.

Regardless of the precise mechanism, the fortuitous discovery that HDAC inhibitors prevent cardiac hypertrophy and normalize cardiac gene expression in the face of stress points to intriguing possibilities for the use of such inhibitors in the treatment of hypertrophy and heart failure in humans. Importantly, HDAC inhibition results in downregulation of βMHC expression with a concomitant increase in levels of αMHC. HDAC inhibitors therefore have the potential to not only antagonize deleterious cardiac growth, but also to increase myofibrillar ATPase activity and improve contractility in the failing heart.

Future prospects

The signal-dependent control of cardiac growth by differential association of HDACs and HATs with MEF2 is illustrative of the mechanism of action of transcriptional coactivators and corepressors. Indeed, we have recently identified

several other coactivators that stimulate cardiac gene expression during development and disease. These include myocardin, a cofactor of SRF, CAMTA, a cofactor of Nkx2.5, and TAZ, a cofactor of Tbx5. In the future, it will be important to determine whether these different transcriptional partnerships act redundantly or uniquely in the heart and how they may respond to the signalling inputs that control cardiac growth and function.

In summary, a common feature of cardiac remodelling regardless of aetiology is fetal cardiac gene induction, which is likely to contribute to cardiac demise through dysregulation of genes encoding proteins involved in cardiac contractility. Histone deacetylation plays a key role in the control of cardiac growth in response to stress signalling. The regulation of class II HDAC function by stress signalling pathways opens opportunities for therapeutically manipulating cardiac gene expression through modulation of protein kinase pathways. In addition, the finding that HDAC inhibitors prevent hypertrophy has the potential to allow for the rapid advancement of compounds into human patients for the treatment of pathological hypertrophy and heart failure.

Acknowledgements

Work in the lab of Eric Olson was supported by grants from the National Institutes of Health, the Donald W. Reynolds Cardiovascular Clinical Research Center, The Muscular Dystrophy Association, The Robert A. Welch Foundation, and the Texas Advanced Technology Program.

References

Abraham WT, Gilbert EM, Lowes BD et al 2002 Coordinate changes in Myosin heavy chain isoform gene expression are selectively associated with alterations in dilated cardiomyopathy phenotype. Mol Med 8:750–760

Antos CL, McKinsey TA, Dreitz M et al 2003 Dose-dependent blockade to cardiomyocyte hypertrophy by histone deacetylase inhibitors. J Biol Chem 278:28930–28937

Blaxall BC, Tschannen-Moran BM, Milano CA, Koch WJ 2003 Differential gene expression and genomic patient stratification following left ventricular assist device support. J Am Coll Cardiol 41:1096–1106

Braunwald E, Bristow MR 2000 Congestive heart failure: fifty years of progress. Circulation 102:IV14–23

Bush E, Fielitz J, Melvin L et al 2004 A small molecular activator of cardiac hypertrophy uncovered in a chemical screen for modifiers of the calcineurin signaling pathway. Proc Natl Acad Sci USA 101:2870–2875

Chang S, McKinsey TA, Zhang CL, Richardson JA, Hill JA, Olson EN 2004 Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. Mol Cell Biol 24:8467–8476

Chen SL, Loffler KA, Chen D, Stallcup MR, Muscat GE 2002 The coactivator-associated arginine methyltransferase is necessary for muscle differentiation: CARM1 coactivates myocyte enhancer factor-2. J Biol Chem 277:4324–4333

Chien KR 1999 Stress pathways and heart failure. Cell 98:555–558
Fischle W, Wang Y, Allis CD 2003 Histone and chromatin cross-talk. Curr Opin Cell Biol 15:172–183
Frey N, Olson EN 2003 Cardiac hypertrophy: the good, the bad, and the ugly. Annu Rev Physiol 65:45–79
Grozinger CM, Schreiber SL 2000 Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization. Proc Natl Acad Sci USA 97: 7835–7840
Hassig CA, Tong JK, Schreiber SL 1997 Fiber-derived butyrate and the prevention of colon cancer. Chem Biol 4:783–789
Herron TJ, McDonald KS 2002 Small amounts of alpha-myosin heavy chain isoform expression significantly increase power output of rat cardiac myocyte fragments. Circ Res 90: 1150–1152
Kook H, Lepore JJ, Gitler AD et al 2003 Cardiac hypertrophy and histone deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop. J Clin Invest 112:863–871
Lowes BD, Gilbert EM, Abraham WT et al 2002 Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. N Engl J Med 346:1357–1365
McKinsey TA, Olson EN 2004 Cardiac histone acetylation—therapeutic opportunities abound. Trends Genet 20:206–213
McKinsey TA, Zhang CL, Lu J, Olson EN 2000a Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. Nature 408:106–111
McKinsey TA, Zhang CL, Olson EN 2000b Activation of the myocyte enhancer factor-2 transcription factor by calcium/calmodulin-dependent protein kinase-stimulated binding of 14-3-3 to histone deacetylase 5. Proc Natl Acad Sci USA 97:14400–14405
McKinsey TA, Zhang CL, Olson EN 2001 Identification of a signal-responsive nuclear export sequence in class II histone deacetylases. Mol Cell Biol 21:6312–6321
McKinsey TA, Zhang CL, Olson EN 2002 MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends Biochem Sci 27:40–47
Molkentin JD, Dorn II GW 2nd 2001 Cytoplasmic signaling pathways that regulate cardiac hypertrophy. Annu Rev Physiol 63:391–426
Nozato T, Ito H, Tamamori M et al 2000 G1 cyclins are involved in the mechanism of cardiac myocyte hypertrophy induced by angiotensin II. Jpn Circ J 64:595–601
Olson EN, Schneider MD 2003 Sizing up the heart: development redux in disease. Genes Dev 17:1937–1956
Vega RB, Harrison BC, Meadows E et al 2004 Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. Mol Cell Biol 24:8374–8385
Verdin E, Dequiedt F, Kasler HG 2003 Class II histone deacetylases: versatile regulators. Trends Genet 19:286–293
Wang AH, Yang XJ 2001 Histone deacetylase 4 possesses intrinsic nuclear import and export signals. Mol Cell Biol 21:5992–6005
Yanazume T, Hasegawa K, Morimoto T et al 2003 Cardiac p300 is involved in myocyte growth with decompensated heart failure. Mol Cell Biol 23:3593–3606
Youn HD, Grozinger CM, Liu JO 2000 Calcium regulates transcriptional repression of myocyte enhancer factor 2 by histone deacetylase 4. J Biol Chem 275:22563–22567
Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN 2002 Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. Cell 110: 479–488

DISCUSSION

Leinwand: Have you looked at CAMTA in your constitutively active *Gsk3* mouse hearts?

Olson: You are referring to experiments in which we have expressed a mutant form of *Gsk3* in the heart, which can render hearts resistant to hypertrophy. We haven't looked at how CAMTA is regulated in these hearts, but this would be an interesting thing to do. We are now beginning to look at CAMTA in many different mouse models of cardiac disease.

Rosenthal: It looks to me as if cardiomyocytes have a decision to make. If PKC is activated, it can either activate PKD and give cardiac hypertrophy, or it can activate CAMTA and give cardiac hyperplasia. What do you think is making the difference?

Olson: That's a good question. The data on CAMTA in hyperplasia are all based on gross overexpression. I don't know whether this is its *in vivo* function yet. Recent data also indicate that CAMTA can induce cardiomyocyte hypertrophy. So, it remains to be determined how it regulates one response versus the other. Perhaps hypertrophy occurs in myocytes that are unable to re-enter the cell cycle.

Muslin: The docking site you showed on HDAC4 had an arginine residue and there were a lot of leucines. In some ways this seems similar to the D-box that Roger Davis described for Jnk. Have you compared this with the D-box?

Olson: That is an interesting point. What does the D-box look like?

Muslin: It has three lysine or arginine residues followed by two leucines or isoleucines. The motif is similar to what you showed. Obviously, Jnk is a proline-directed kinase, and here you have an arginine-directed kinase, but the comparison might be interesting.

Olson: Tim McKinsey, you have worked with Jnk. Have you ever looked at whether it might regulate HDAC4?

McKinsey: No. We know that Jnks don't regulate HDAC5 though.

Schneider: Along the lines of Nadia Rosenthal's question and the potential role of CAMTA2 in cardiac cell cycle control, activation of PKC *in vivo* doesn't result in a markedly hyperplastic phenotype in myocardium. This suggests that what you have unmasked by forced expression of CAMTA2 is a way to inhibit cardiac cell cycle regulation rather than an effect of the endogenous protein and its pathophysiological state of activation. I am curious as to whether you have looked in cultured cells to see whether forced expression of CAMTA2 can override cell cycle constraints. If we are titrating out pocket proteins, this would be an expected phenotype.

Olson: We have put CAMTA into a virus. When we infect neonatal myocytes with this it clearly induces their growth, both hyperplastic and hypertrophic. We

haven't yet done this in adult myocytes. Neonatal myocytes can undergo a few addition rounds of division, so I am not sure whether CAMTA2 is prolonging the proliferation or driving post-mitotic cells into the cell cycle.

Schneider: Have you looked in skeletal myocytes, where the dichotomy between proliferative growth and terminal differentiation is even clearer?

Olson: We haven't done that yet. With respect to its role in pocket protein biology, the CG1 motif binds weakly to a sequence related to the E2F binding sequence, so we are exploring whether CAMTA2 might have an effect on E2F activity.

Dorn: There are some *in vivo* expression data on PKC showing that if you activate endogenous PKCε or δ, rather than overexpress a wild-type or constitutively active PKC, you get a hyperplastic heart. This results in hypertrophy of the organ due to increased calculated number of normally proportioned cells. I believe these data support what you are suggesting: perhaps it is the timing of the growth stimulus in the early neonatal period, when the cardiac myocytes continue to proliferate, rather than the nature of the growth stimulus *per se* that determines whether growth is hyperplastic or hypertrophic.

Seidman: Your screen for atrial natriuretic factor (ANF) luciferase was in COS cells. You identified a molecule that in the end binds Nkx2.5. But I don't understand why you found this: there isn't much Nkx2.5 in COS cells.

Olson: I think there must be an endogenous NK-type protein in COS cells that it is utilizing.

Seidman: Did you look for binding to Tbx5?

Olson: It doesn't bind strongly to Tbx.

Seidman: Your PKD model involves looking at neonatal myocytes. Is there a possibility that the regulation in neonatal myocytes is different from that in adult myocytes?

McKinsey: It is always a concern. However, there is an abundance of PKD in adult myocytes and it can be readily activated by various agonists. I believe the pathways will be conserved from neonatal to adult myocytes, but this still needs to be proven formally.

Olson: Mike Bristow, don't you have data that HDACs are nuclear in human adult cardiomyocytes and they become cytoplasmic in failing hearts?

Bristow: Yes, we have data that in failing heart there is less nuclear HDAC5 compared with non-failing hearts, as if it has been exported from the nucleus. With HDAC4 there is a greater amount in the cytoplasm in failing versus non-failing hearts, again as if it has been nuclear exported. The two HDACs differ in terms of where they are primarily found, but in both cases there is evidence of nuclear export in failing heart.

Sugden: This takes me back to work on calmodulin overexpression in the hearts of transgenic mice by Gruver et al (1993). Is there any connection here with the protein that you are describing?

Olson: That paper showed that overexpression of calmodulin will drive hypertrophy

Sugden: They said that there was also significant cardiac myocyte hyperplasia.

Olson: So there may be a tie in then.

Field: It was through the ANF promoter and it turned off in the ventricle, so it was only a transient burst effect.

Sugden: You talked in general terms about PKC. There are many PKC isoforms, and I believe that each may have a different, independent mode of regulation and different sustrate preferences. When 'PKC' is used in rather general terms, I wonder what is going on and which isoform is involved.

Olson: Tim McKinsey did a lot of this work on PKCs, so he may want to elaborate on this. The atypical, Ca^{2+}-independent PKCs in our hands were the strongest inducers of HDAC5 nuclear export.

Sugden: Was this with wild-type or constitutively activated PKCs?

McKinsey: These were constitutively activated.

Sugden: Was this with the pseudosubstrate site mutation?

McKinsey: Yes. We looked at a panel of PKCs for their ability to drive PKD-dependent nuclear export of HDAC5. PKCε and δ were the strongest activators. Eric Olson, with CAMTA it is PKCα: did you look at the other isoforms?

Olson: We have only looked at the effects of PKCα on CAMTA2 activity so far.

Schneider: I have a question about the bait in the Gal4 HDAC screen. Did you take the step of mutationally inactivating the HDAC, or under the conditions of that screen does the recruitment of the VP16 activation domain override the functional activity of the bait?

Olson: That's a good question. The way we engineered this is that we deleted the whole catalytic domain, so it is just the N-terminus. For the aficionados, this is analogous to MITR, which is a naturally occurring splice variant.

Muslin: In terms of the nuclear export of CAMTA, I was wondering whether there are potential 14-3-3 binding sites or other potential binding partners that you have identified.

Olson: I don't think that CAMTA exports by a 14-3-3-dependent pathway. There is not an obvious site. We have narrowed down the region required for export and import. There is clearly a phosphorylation site there, but we still need to figure out how this works.

Sadoshima: I am interested in the differences between class I and class II HDACs. Is class I using a similar mechanism as class II to affect cardiac hypertrophy?

Olson: You have raised an important point. The genetics and biochemistry suggest strongly that class II HDACs are repressors of cardiac hypertrophy and pathological remodelling, and that this mechanism is blocked by upstream kinases. The paradox comes from work by our lab and others showing that HDAC

inhibitors (such as trichostatin A; TSA) can also block hypertrophy. This is the opposite of what we would expect. We'd expect HDAC inhibitors to function like a genetic deletion of an HDAC, and sensitize the cell to hypertrophic stimuli. On the basis of this we have proposed a model. Class II HDACs function as suppressors of growth, but class I HDACs counterbalance the function of class IIs, leading to the repression of anti-growth genes. HDAC inhibitors such as TSA lead to the expression of anti-growth genes by blocking the activity of class I HDACs. HDAC inhibitors are now deep into clinical trials for anti-cancer treatment. They are extremely well tolerated with few side-effects. It is thought that one of their mechanisms of action is to up-regulate p21, which is a negative regulator of cell growth. This may be the mechanism by which class I HDACs are functioning. HDAC inhibitors might also be acting far upstream from histone acetylation. It will likely turn out that components of hypertrophic and stress-responsive signalling pathways have acetylated components. HDAC inhibitors might be knocking out these pathways far upstream of the genome. A prediction of this model would be that the class I HDACs are pro-hypertrophic, whereas class II HDACs are anti-hypertrophic. Consistent with such a model, we have over-expressed class I HDACs in the heart and this leads to massive cardiac growth. In contrast, if you express the class IIs in this setting you will have a shrunken heart if you have one at all.

Seidman: Is the cardiac enlargement you observe with over-expression of class I HDACs hyperplasia or hypertrophy?

Olson: This is hypertrophy.

Field: When you showed the CAMTA image in the COS cells, you said you had both cytoplasmic and nuclear expression of the wild-type protein. If I remember rightly, there are some cells which had very obvious nuclear localization, but in others it was through the entire cell. Have you tried to correlate cell cycle stage with the subcellular localization?

Olson: We haven't done this yet. You are right: if we look at the CAMTA localization in COS cells or other cell types, the majority have it distributed in the nucleus and cytoplasm, but there is a subset where it will be in one place or the other. The obvious question is whether this correlates with the phase of the cell cycle.

Simpson: Relative to the set of questions you posed in the introduction, what do you think you are looking at in this system with HDAC export? Is this a good thing or a bad thing in terms of myocardial remodelling? You are sort of using the fetal program as a symbol of pathological remodelling.

Olson: Your question really touches on one of the key issues I hope to resolve in this meeting. I think we should throw this question out to the audience.

Sugden: I worry that you may be looking at ANF expression *per se* rather than anything that is necessarily related to hypertrophy. Although ANF expression has

been used extensively as a criterion of the hypertrophic response, the correlation may not be as strictly linked as previously thought. How many of the processes that have been linked to ANF expression, such as HDAC export, do you think need to occur for the hypertrophic response (as opposed to increased ANF expression)? Is one sufficient? What are the targets of these molecules? *Ex vivo*, the system can sometimes be manipulated to give a result that you want to see, but I am not clear what is happening *in vivo* in terms of whether a single change in terms of location of HDAC is sufficient to drive hypertrophy. It could be that you need a multiplex of these factors going in and out of the nucleus at different times in order to establish the overall phenotype.

Olson: This is one of the issues we need to confront at this meeting. Are many of these things operating independently and in parallel, or are there nodal points that can be therapeutically targeted? My own bias is that the phosphorylation of class II HDACs is a nodal point, on the basis of the results of their genetic deletion. If they are deleted then the heart becomes sensitized to stress, and if one converts a serine to a non-phosphorylatable residue it blocks the hypertrophic response. There are other pathways involved, but how they cross-talk with this pathway is an open question.

Sugden: PKC seems to be an essential feature.

Katz: We talk about the hypertrophic response as if it was a single response, but heart failure is a syndrome encompassing many abnormalities, including cell elongation, cell thickening and reversion to the fetal phenotype. We now know that hypertrophy is good and bad at the same time, so that to look at organ size, cell size or cell number may over-simplify the endpoint. To relate signal transduction to the clinical reality is going to be difficult as you set out beautifully in your introductory remarks: testing any form of therapy will be very difficult until you have defined a subset of patients in whom that therapy seems rational. As an example, the blocking of the gp130 pathway was believed to be a good thing because cytokines, as we all know, are evil. It turns out that this is actually the wrong result. Until we know more about the relationship between the growth patterns and human disease, simply to know the details of these regulatory mechanisms may not be all that helpful.

Muslin: Another fundamental issue we should discuss is whether it is growth that is the primary problem with heart failure, or whether it is 'growth plus'. When growth is extreme it can be deleterious, but in general it is growth plus apoptosis or fibrosis or cell elongation that causes the problem.

Olson: One of the original questions I posed was, what is the importance of hypertrophy versus fetal gene activation, Ca^{2+} cycling, contractility, energy metabolism, fibrosis or apoptosis? Is it adequate to block any one of these or do we need to be looking for upstream effectors that are controlling all these things globally?

Nemer: We are taking it for granted that ANF is an indicator of hypertrophy. Some of the early work we did shows that ANF goes up in response to stress way before there is hypertrophy. The way we look at hypertrophy is probably the way that people looked at cancer 20 years ago. So many things can get you there. When we look at organ size in these transgenics or knockouts, it is just saying that something has happened. It doesn't mean it was pro-growth; it may have been anti-growth.

Seidman: I agree that ANF provides an indicator of at least one hypertrophic pathway. What are the other genes in this pathway? Are they regulated by the same transcriptional activators?

Nemer: To qualify this, the binding site on ANF that is 'well characterized' is actually not so well characterized. Just about everything we have tested can bind over this proximal element, which is 50 base pairs or so. It is up-regulated in response to just about any stimulus.

Rosenthal: From the point of view of a cardiologist, wouldn't it be important to know what the functional output of those big hearts is? Does a hyperplastic heart have the same ejection fraction as a hypertrophic heart? I've been told by cardiologists that unless I can show this I can't publish my paper on fixing hearts.

Dovendans: I would support that strongly as a cardiologist. The functional assay should be done before you publish.

Schneider: Nadia Rosenthal adds an important element to what the phenotyping of the models has to be. I would argue that another one illustrated by the example Arnie Katz gave by the example of the gp130 knockout is the response to stress. One could have a heart that is enlarged and appears to function relatively normally at baseline, whether it does or doesn't have the hallmarks of fetal gene activation to a high degree. Whether this heart is a normal heart or a severely diseased one can be unmasked by the response of the enlarged heart to aortic banding, ischaemic stress and mating with different genetic models that activate single cardiac signalling pathways. The issue of basal versus provokable phenotypes provides one portal to answering the question about whether large hearts are endangered.

Katz: From a clinical standpoint, what is often most important is what the heart will be like six months or six years from now. Today's haemodynamics are interesting, but what is going to happen to the heart in the future? Progression also needs to be defined. Is it cell elongation, apoptosis or necrosis? These are but three ways that the heart can deteriorate, each of which has its own set of control mechanisms. To take the complexity of signal transduction and then juxtapose this with the complexity of the clinical syndrome is going to be an incredible challenge for all of us to sort out.

Leinwand: One thing we have found interesting is that the mice that express constitutively active Gsk3 are blocked in pathological responses, but they are