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REVIEW

# Cellular mechanisms of cardiac hypertrophy

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Hypertrophy is the principal response of the heart to overload from any cause, including hypertension, myocardial infarction, valvar heart disease, and dilated cardiomyopathy. In the Framingham study, left ventricular hypertrophy was found on echocardiography in 15–20% of adults.[1] This is an important observation because hypertrophy is a strong, independent predictor of cardiovascular death[2] and is associated with diastolic dysfunction.[3] Adult cardiac myocytes are highly specialised, terminally differentiated cells which have lost the ability to divide. The increase in heart muscle mass seen in cardiac hypertrophy occurs predominantly through an increase in myocyte size rather than number,[4] though some claim evidence of mitotic division when the left ventricular mass exceeds 350 g.[5]

At a cellular level the events leading to cardiac hypertrophy can be broadly divided into three stages: extracellular hypertrophic stimulus; intracellular signal transduction; and activation of nuclear events which allow development of the hypertrophic phenotype.

## Extracellular hypertrophic stimulus: haemodynamic vs non-haemodynamic factors

Haemodynamic factors, typified by pressure and/or volume overload, have long been known to cause hypertrophy in humans. Many animal models of hypertrophy have been developed. These involve increasing pressure load (for example, by aortic or renal artery banding) or volume load (for example, by anaemia). Recent work has demonstrated that haemodynamic overload is only part of a complex interaction between mechanical, neural, hormonal, and genetic factors that culminates in cardiac hypertrophy. The clinical importance of non-haemodynamic factors is shown by the observation that therapeutic normalisation of blood pressure in hypertensive patients only produces partial regression of left ventricular hypertrophy.[6] Regression is more appreciable with some antihypertensive drugs than others despite equivalent reductions in blood pressure.[6]

Experiments in vivo are unable to distinguish clearly between the relative contributions of the different factors leading to hypertrophy. As well as having direct effects, many neurohumoral stimuli can produce hypertrophy indirectly as a consequence of haemodynamic changes or activation of other neurohumoral mechanisms. Longer term responses cannot be studied because isolated heart preparations are not viable for more than a few hours. The neonatal rat ventricular cardiomyocyte culture, first described by Simpson and Savion[7] in 1982, provides an opportunity to study the effects of single factors in isolation. This well established model shows many of the features of hypertrophy seen in adult ventricular cardiac myocytes in vivo (fig 1). Within 30 minutes of exposure to a hypertrophic stimulus early response genes are activated.[8] At 6–12 hours there is induction (recapitulation) of embryonic genes such as atrial natriuretic factor (ANF),[9] $\beta$ myosin heavy chain ($\beta$ MHC),[10] and skeletal muscle $\alpha$ actin.[11] Downregulation of $\alpha$ MHC has also been observed.[10] Between 12 and 24 hours there is upregulation of constitutively expressed contractile protein genes such as myosin light chain-2 (MLC-2)[12] and cardiac $\alpha$ actin.[11] These changes culminate in increased cell size without cell division, increased cell protein and RNA content, and increased production and assembly of individual contractile proteins into sarcomeric units.[12] In human cardiac muscle features of hypertrophy are similar, except that skeletal $\alpha$ actin and $\beta$ MHC are already the predominant isoforms in adults.[13] Neurohumoral hypertrophic factors identified or confirmed using the cultured cardiac myocyte system include $\alpha$ adrenergic agonists,[14] endothelin 1,[15,16] angiotensin II,[17] and various polypeptide growth factors.[18,19] The hypertrophic effects of pressure/volume overload in vivo may involve mechanical stretch, and indeed stretching of cardiac myocytes cultured on deformable surfaces has been shown to reproduce the cellular features of hypertrophy[20] as well as activate multiple signal transduction pathways.[21]

## Signal transduction

Growth factors (for example, fibroblast growth factors, insulin-like growth factor I) and phorbol esters, which are mitogenic for most cell types, induce hypertrophy in cardiac myocytes.[18,22–24] There are notable analogies between events early in mitosis and early in

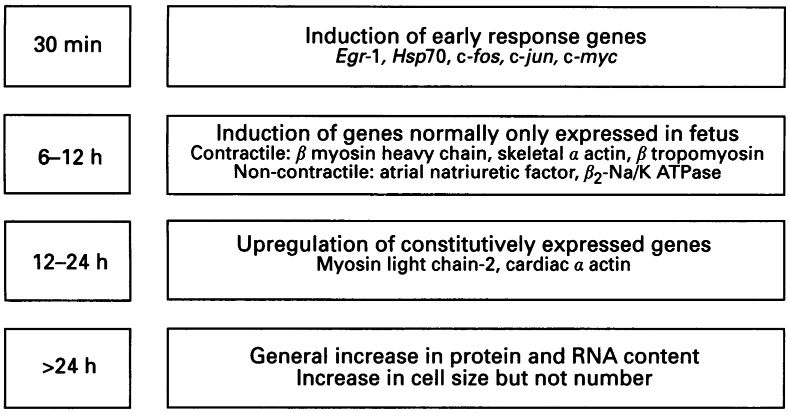
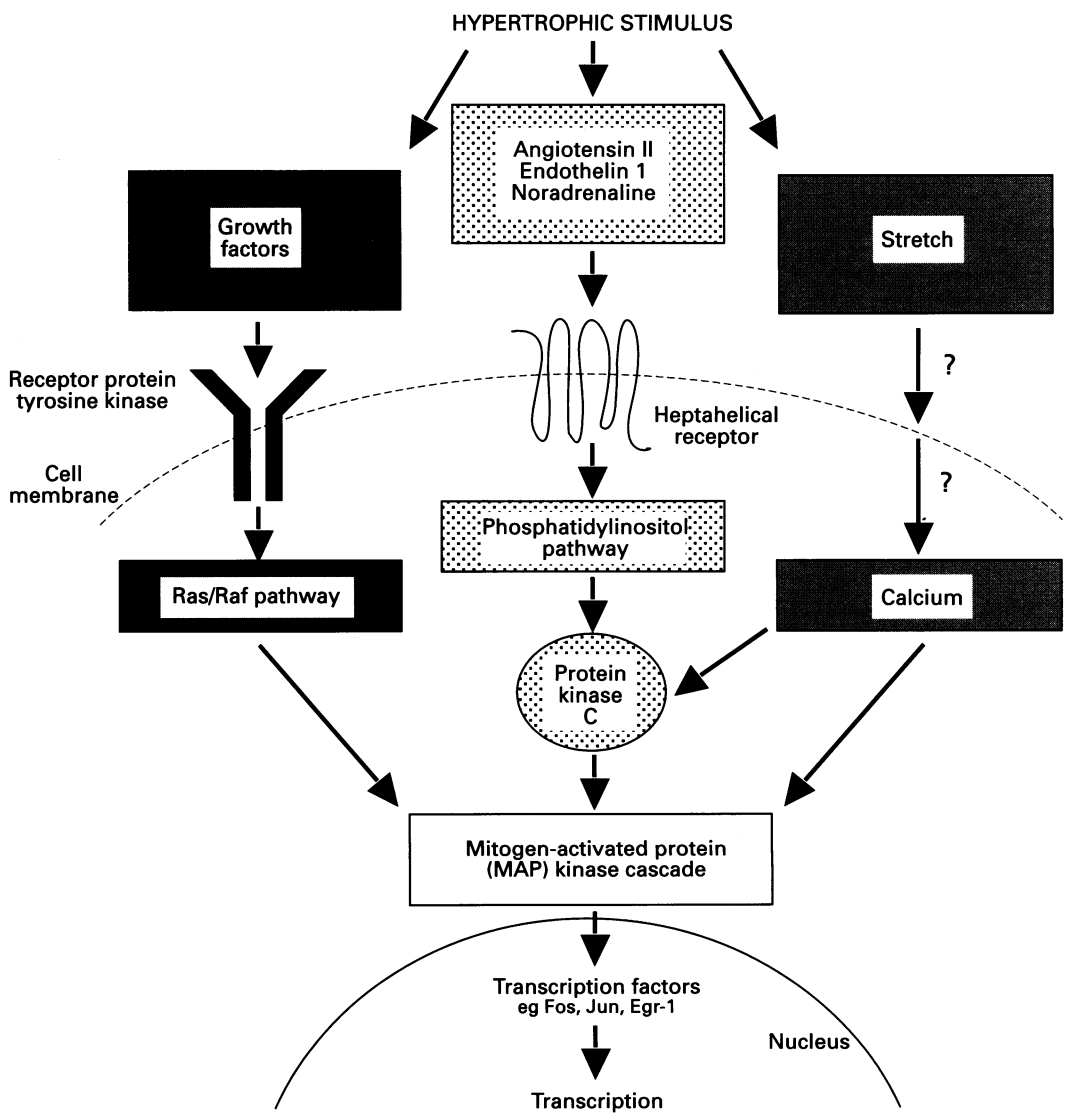



*Figure 1 Sequence of changes in the cardiac myocyte after exposure to a sustained hypertrophic stimulus.*

hypertrophy. The pattern of early response gene expression is similar,[8 25] and the re-expression of fetal genes which is a characteristic of cardiac hypertrophy (fig 1) also occurs in mitotically active, differentiated non-cardiac cells. For example, re-induction of α fetoprotein is seen in regenerating hepatocytes.[26] These observations have led to the hypothesis that the signal transduction mechanisms may also be broadly conserved between cell types, although the end response may differ. This concept is supported by the ubiquity of many intracellular signalling proteins. Hypertrophy may thus be the only form of growth response available to terminally differentiated adult cardiac myocytes. Many of the hypotheses concerning signal transduction in hypertrophy are based on the similarities between the early stages of hypertrophy and mitosis.

Growth related intracellular signalling pathways are activated and inactivated within minutes of a stimulus reaching the cell surface, an altered phosphorylation state of the participant proteins is often a feature of this response. In vivo studies may therefore be inappropriate as the relevant proteins cannot be isolated and stabilised quickly enough for accurate assays. As mentioned earlier, cardiac myocyte cultures are ideally suited to the study of signal transduction.[27 28] A simplified representation of the putative intracellular signalling pathways leading to hypertrophy is shown in fig 2. This diagram illustrates the current general hypotheses of signal transduction but is by no means complete. Three broad categories of hypertrophic stimulus act

*Figure 2 Putative signal transduction pathways for cardiac myocyte hypertrophy.*



on the cell. Firstly, growth factors bind to receptors which have a tyrosine kinase activity, initiating a signalling cascade which includes the oncoproteins Ras and Raf-1.[29 30] Secondly, angiotensin II, endothelin 1 and $\alpha$ adrenergic agonists all bind to specific G protein-linked receptors (characterised by seven membrane-spanning helices) resulting in activation of the phosphatidylinositol pathway and protein kinase C.[15 31 32] Thirdly, stretching of myocytes stimulates as yet unidentified mechanoreceptors which may lead to increased intracellular calcium.[21] These three major pathways seem to converge on the mitogen-activated protein (MAP) kinase cascade[21 27] which indirectly modulates transcriptional activity. The MAP kinase cascade may thus be viewed as amplifying integrating signals from a diverse variety of receptors. Formal proof of the importance of many of these pathways in cardiac myocyte hypertrophy is still lacking. For example, there is considerable evidence for the involvement of the more proximal elements (Ras[29] and protein kinase C[33]) in triggering hypertrophy, but evidence for the role of the MAP kinase cascade is still circumstantial.[21 27 28] The extent of "cross-talk" between pathways is largely unknown but is likely to be considerable. Stretching of myocytes may exert indirect hypertrophic effects via paracrine mechanisms.[21] Calcium from both extracellular and intracellular sources seems to be important for the hypertrophic response[21 34] although its exact role is still to be defined.

## Nuclear transcription factors

Induction of the early response genes (fig 1) is a marker of the early phase of hypertrophy both in vivo and in vitro.[8 35] The early response genes code for proteins that regulate transcription of other genes.[36] Many early response genes (eg c-*fos*, c-*jun* and c-*myc*) are proto-oncogenes, the cellular homologues of transforming viral oncogenes. Italics refer to the gene; for the corresponding protein product the name is capitalised (for example, Fos). Induction of the early response genes, with appearance of the corresponding messenger RNA, occurs within 30 minutes of exposure of the cell to a stimulus. Synthesis of new proteins is not required for this induction. This implies that the transcription factors for the early response genes do not need to be newly synthesised, and only require stimulus-induced modification to confer DNA-binding activity. For example, MAP kinase phosphorylates the 62 kDa ternary complex factor which then, in conjunction with serum response factor, binds to the serum response element of the c-*fos* promoter to induce transcription of c-*fos*.[25] MAP kinase also phosphorylates Jun facilitating heterodimer formation with Fos. This Jun-Fos heterodimer is known as activating protein 1 (AP1) and binds to a specific, 9 base pair motif found in the promoter regions of the ANF and skeletal $\alpha$ actin genes (fig 1).[36 37] The ANF promoter also contains a consensus sequence for another transcription factor, *Egr*-1.[38] The promoter regions responsible for the inducibility and tissue specificity of other genes involved in hypertrophy are under intensive investigation.[9 39]

Not all the early response genes have been definitively linked to cardiac myocyte hypertrophy. Induction of the proto-oncogene c-*myc* is required for mitosis in vascular smooth muscle cells.[40] Induction of cardiac c-*myc* by pressure and volume overload suggested it could also be important for hypertrophy.[41] Transgenic mice genetically engineered to overexpress c-*myc* 20-fold do indeed have cardiac enlargement, but this is due to myocyte hyperplasia and not hypertrophy.[42] The prevailing current view is that c-*myc* is involved in the regulation of cardiac myocyte proliferation during development, and does not influence the adaptive hypertrophic response of differentiated cells. In the adult heart subjected to a hypertrophic stimulus, c-*myc* induction is probably important for the proliferation of non-myocytic cells which contribute to the increase in cardiac mass.[43]

## Relevance in humans

The study of cardiac hypertrophy at the cellular level in humans is difficult. Pharmacological experiments to elucidate mechanisms in vivo are limited by the multifactorial nature of hypertrophy. Samples of ventricular muscle, obtained from explanted hearts or by percutaneous biopsy, may not be representative of the whole organ as they are almost without exception from patients in whom cardiac hypertrophy is not the predominant pathology. Fresh, normal cardiac tissue is not available for experimental controls. Thus there is little direct information about the cellular mechanisms of hypertrophy in humans. However, cell membrane receptors and intracellular signalling proteins are highly conserved between mammalian species and the triggering events for cellular hypertrophy in humans are likely to resemble closely those in the various animal models used. We have detected the presence of signalling proteins such as protein kinase C and MAP kinase in human heart (Lazou *et al*, in press). Cardiac myocyte cultures are useful for unravelling intracellular signalling events and for rational preliminary testing of new molecular or pharmacological interventions. Confirmation in vivo is being made possible by advances in the breeding of transgenic animals—homologous recombination and manipulation of embryonic stem cells make it possible to create lines of animals in which a specific gene in the signalling pathway is either overexpressed or inactivated.[42 44] This approach should allow the rigorous analysis of specific signalling mechanisms during cardiac hypertrophy in vivo.

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