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# GATA factors and transcriptional regulation of cardiac natriuretic peptide genes

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**Abstract**

The A- and B-natriuretic peptides (ANP and BNP) are the heart major secretory products. ANF and BNP expression is a marker of cardiomyocyte differentiation, and is regulated spatially, developmentally and hormonally. Analysis of the ANP and BNP promoters has contributed in a major way to our present understanding of the key regulators of cardiac development. It has also started to unravel the complex combinatorial interactions required for proper regulation of the cardiac genetic program. The GATA family of transcription factors initially identified as essential regulators of the two natriuretic peptide genes appears to be at the heart of the molecular circuits governing cardiac growth and differentiation. In particular, GATA-4 has emerged as the nuclear effector of several signaling pathways which modulate its function through post-translational modifications and protein–protein interactions. This review will cover our current knowledge of cardiac transcription and the role of GATA factors in embryonic and postnatal heart development.


*Keywords:* ANP/BNP; GATA factors; Transcription; Heart development; Cardiac hypertrophy

## 1. Introduction

Cardiomyocytes are terminally differentiated cells that lose their ability to proliferate shortly after birth. When challenged with increased demand of cardiac workload they grow in size as an adaptive mechanism. Nevertheless, when prolonged this compensatory mechanism becomes decompensatory resulting in congestive heart failure [1]. At the cellular level, cardiac hypertrophy is accompanied by an increase in cell surface area, increased total protein synthesis, upregulation of early response genes and the reactivation of several fetal genes such as skeletal (SK) α-actin, β-myosin heavy chain (MHC), atrial natriuretic peptide (ANP) and brain-type natriuretic peptide (BNP) [2,3]. As it is clear from the continuous research in this area, more questions have been raised concerning the underlying mechanisms implicated in inducing congenital and hypertrophic heart phenotypes. The impediment in the characterization of the mechanisms responsible for the initial stages of genetic reprogramming in hypertrophy was relieved by the discovery that ANP [4] and BNP upregulation is an exquisitely sensitive indicator of cardiac stress and in fact, it is presently used clinically for diagnostic and prognostic purposes [5]. It is therefore not surprising that the study of the mechanisms and signaling pathways involved in transcriptional regulation of these hormones has been the focus of several studies. In this respect, one of the most significant findings was the characterization of cardiac members of the GATA family of transcription factors, GATA-4, -5 and -6 which were shown to be important regulators of natriuretic peptide genes and essential for normal heart development [6,7]. Studies from our laboratory have started to elucidate the mechanisms by which GATA factors are involved in the regulation of ANP and BNP genes during embryonic, postnatal and pathologic cardiac growth. This review will be focused on the current knowledge regarding transcriptional

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regulation of the ANP and BNP genes by GATA factors, and will showcase how knowledge of cardiac gene regulation has impacted on understanding of normal and pathologic heart development.

## 2. The GATA family of transcription factors

The GATA family of transcription factors are zinc finger proteins that bind to the consensus sequence (A/T)GATA(A/G) via a DNA-binding domain [6] containing two zinc fingers to activate target genes. GATA proteins play important roles in cell differentiation and homeostasis in all eukaryotes. In mammals, six members of this family have been identified; based on sequence homology and expression pattern, they have been divided into two subgroups. The first subgroup of proteins, GATA-1, -2 and -3, is present mainly in hematopoietic cells [8]. The second subgroup includes GATA-4, -5 and -6 that are predominant in the heart, digestive system and the extra-embryonic endoderm [9–12]. These proteins show very high homology in their DNA binding domain but diverge considerably in the N- and C-regions which contain transcription activation modules (Fig. 1). For the purpose of this paper we will restrict our review to the second subgroup of GATA members.

Several studies have shown that GATA-4, -5 and -6 bind to similar consensus sequences in cardiac [13–15] and some gastric promoters [16–18] enhancing their activity. This raises the possibility for some functional redundancy among these proteins, in particular with the overlapping of GATA-4 and GATA-6 expression in cardiomyocytes [13,19]. It was widely speculated that the two proteins may be able to compensate for each other in heart development. This notion was supported by the fact that both proteins show similar potency in activating several cardiac genes including troponin C, ANP and BNP [12,13]. Moreover, the down-regulation of either GATA-4 or GATA-6 proteins in primary cardiac myocytes resulted in reduced expression of several cardiac genes [20]. Finally, gain of function studies in xenopus embryos implicated both GATA-4 and GATA-6 in cardiogenesis [21]. On the other hand, other studies have argued for functional specificity of these proteins. First, the GATA-4–Nkx2.5 transcriptional synergy was not observed when GATA-4 was substituted by GATA-6 [22]. Second, although GATA-4 and GATA-6 regulate ANP and BNP genes with the same potency, GATA-4 is more powerful in modulating α- and β-MHC [6,20]. Third, phenylephrine stimulation as well as other hypertrophic agonists upregulate GATA-4 expression, DNA-binding and transcriptional activity (discussed below) without any detectable effect on GATA-6. Importantly, loss of function studies either through protein knock down in xenopus or chick embryos [23,24] or in vitro models of cardiogenesis [25] as well as gene inactivation in mouse [26–28] or zebrafish [29] have clearly established the essential role of each GATA factor in embryogenesis and in heart development. In this respect, we have recently reported differential expression of GATA-4, -5 and -6 proteins at the single cell level using specific antibodies against the C-terminal which is the least conserved area between these proteins [19]. In this study the expression pattern of GATA-4 and -6 suggested strong involvement of both proteins in early vasculogenesis and mesoderm–endoderm signaling. However, expression of both proteins showed divergent patterns in several organs and cell types such as the embryonic ectoderm, neural tube and neural crest-derived cells where GATA-6 but not GATA-4 was present [19].

## 3. GATA factors and cardiac development

The significance of GATA factors in the heart was first suggested by the finding of conserved GATA sites in the

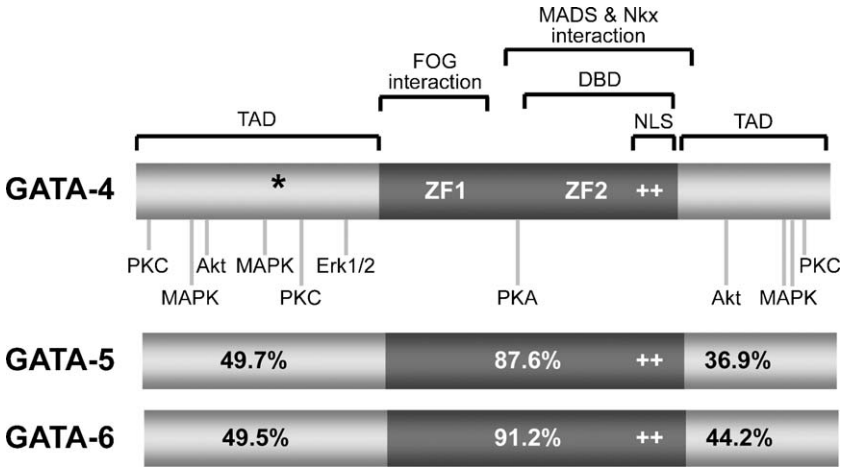


Fig. 1. Schematic representation of the GATA-4 protein showing the functional domains and putative phosphorylation sites. ZF=Zinc finger, TAD=transactivation domain, DBD=DNA binding domain; NLS=nuclear localization signal. The * denotes a confirmed MAPK phosphorylation site. The percentage shown represents the degree of homology for each protein vs GATA-4.

regulatory region of many cardiac promoters and the activity of GATA-4 to potently activate their promoters [30]. Subsequently, several lines of evidence were provided by different in vivo models confirming the role of GATA-4 in cardiogenesis. GATA-4 null mice were not viable and died as early as 7–8 days post-coitum and lacked a primitive heart tube [26,27]. Targeted disruption of GATA-4 resulted in *cardia bifida*, an embryonic heart abnormality that is characterized by the looping, rather than fusion, of the bilateral myocardial rudiments. This phenomenon was also observed when GATA-4 expression was interrupted in chicken embryo using the siRNA strategy [24] which was attributed to the absence of N-cadherin, cell adhesion molecule, expression at the midline. Moreover, targeted mutation of a region in the first zinc finger of GATA-4, which is involved in protein–protein interaction, resulted in a hypoplastic myocardium as well as septation defects [28]. GATA-6 gene knock out was embryonic lethal at gastrulation, with apparent differentiation defects in the ectoderm and part of the visceral endoderm [31,32]. This early lethality did not allow assessment of GATA-6 role in the heart. However, recent studies using antisense strategies have demonstrated an essential role for GATA-6 in xenopus heart development [23,28]. Finally, GATA-5 mutation also resulted in *cardia bifida* and absence of endocardial cells in zebrafish [28,29]. In mice, targeted mutation of the GATA-5 locus which removed the N-terminal region of the protein resulted in defects in the female genitourinary tract but had no effect on cardiac development [33]. Although definitive proof for GATA-5 and -6 role in the heart will have to await development of heart specific models of gene inactivation, the body of evidence available clearly supports an essential non-redundant role for each GATA-4, -5 and -6 in heart development. For GATA-4, this is underscored by the finding that mutations in the human GATA-4 gene are linked to congenital heart defects [34].

There is no doubt that the P19 embryonal carcinoma cell line has significantly contributed to our understanding of cardiac differentiation (reviewed in [35]), particularly in identifying the signaling pathways involved at various stages of cardiomyocyte development. When treated with DMSO, P19 cells differentiate into beating cardiomyocytes. The inhibition of GATA-4 expression using the antisense strategy blocked cardiomyocyte differentiation [36] and the cells underwent apoptosis at a cardioblast stage [25]. This was the first evidence that GATA-4 was dispensable for commitment of cells to the cardiogenic lineage, but essential for survival of cardiogenic precursors. This in turn placed GATA-4 at the top of the transcription factor cascade regulating cardiomyocyte differentiation. Several studies have since confirmed that GATA-4 is a crucial activator of the cardiac differentiation program. On one hand, ectopic expression of GATA-4 was shown to enhance cardiogenesis [25] and on the other, GATA-4 was found to be an upstream activator of other cardiac transcription factors, most notably Nkx2.5 and MEF2 (myocyte enhancer factor), a function conserved in drosophila and mammals [37,38]. The results also suggested the ultimate contribution of other factors in the very early stages of cardiac cell commitment possibly through a compensatory effect of GATA-6 [26,27] which was shown to play a role in the proliferation of pre-cardiac cells [39]. How do GATA factors acquire specificity? The data so far suggests that this may result from differential regulation of the proteins themselves which can occur at several levels, including transcriptional and post-transcriptional [40–43]. Additionally, accumulating evidence suggests that differential interaction between GATA proteins and other transcription factors contributes to functional specificity, likely a result of the differential activity of GATA complexes on target genes.

In this review we will restrict our discussion to the GATA co-factors involved in the regulation of the ANP and BNP promoters. The role of the combinatorial interaction between GATA factors and its collaborators in cardiomyocyte growth and hormonal response will also be discussed.

## 4. The GATA-4 pathway of transcription regulation: lessons learnt from the NP promoters

ANP and BNP are the heart's major secretory products and as such, they represent markers of cardiomyocyte differentiation. In addition, their expression is modulated by spatial, developmental and hormonal cues and this occurs primarily at the transcriptional level. Consequently, the ANP and BNP promoters have been used as models for the study of cardiac-specific transcription. Structural and functional analysis of the ANP and BNP promoters revealed the presence of conserved *cis*-acting elements containing GATA motifs within their proximal promoter regions; these sequences were essential for promoter activity in both atrial and ventricular myocytes (reviewed in [30]). ANP contains two GATA sites at −120 bp and −280 bp as well as a low affinity site around −101 bp, whereas BNP has three GATA binding sites one at −30 bp and two adjacent sites at −90 bp (Fig. 2). GATA-4 was found to have a stimulatory effect on both genes, and, decreased GATA-4 levels downregulated ANP and BNP transcripts in cardiac cells indicating that both genes are direct GATA targets [13].

In mammals, ANP is strictly expressed in the developing heart initially in both atria and ventricles but at later developmental stages, its expression becomes limited to the atria and the ventricular trabeculae [44,45]. Structural analysis of the rat ANP promoter showed that the first 700 bp of flanking sequences were sufficient for cardiac- and stage-specific expression in both atrial and ventricular myocytes [46]. In a recent study, the normal spatial and temporal pattern of ANP was also achieved with 600 bp of upstream sequences from the xenopus ANP gene [47]. In the case of BNP, the proximal 114 bp of the rat promoter are sufficient for maximal expression in cardiomyocytes but additional upstream sequences are required to restrict

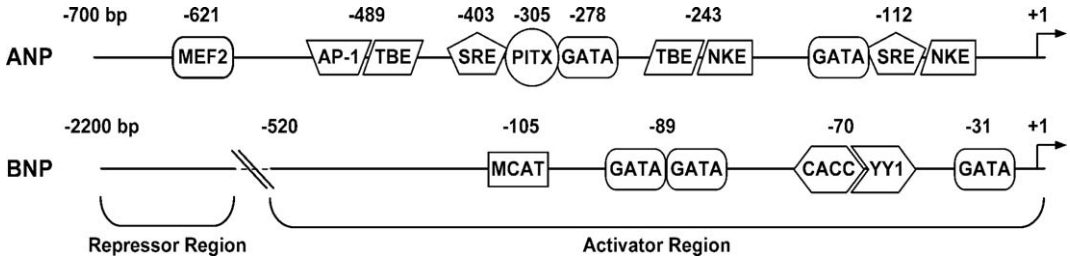


Fig. 2. Structural organization of the promoter of the two cardiac natriuretic peptide genes. Shown are the confirmed *cis*-acting elements for which the cognate *trans*-acting factor has been identified.

expression to the heart [10]. In addition to the GATA sites, the cardiac regulatory regions of both ANP and BNP contain several other *cis*-regulatory elements that are binding sites for cardiac restricted or muscle enriched transcription factors. Interestingly, several of these factors were shown to cooperate with GATA proteins in ANP and BNP regulation.

For example it was found that the ANP gene was a direct downstream target for Nkx2.5, a member of the NK family of homeobox protein and a key regulator of heart development. Nkx2.5 DNA binding sites (NKx2.5 response Element, NKE) were found to be essential for chamber- and developmental stage-specific activity of the ANP promoter which was highly inducible by the addition of Nkx2.5 in heterologous cells [48]. ANP transcripts were absent in Nkx2.5 null mice [49] and mutation of the NKE site of the ANP promoter resulted in significant reduction in promoter activity in atrial cells [47,50]. Structural examination of the ANP promoter shows evolutionary conservation of a GATA element near the NKE, suggesting the existence of functional interaction between the two transcription factors. Consistent with this, GATA-4 and Nkx2.5 synergistically activated the ANP promoter in several cell types including cardiomyocytes [50–52]. This synergy was attributed to direct physical interaction between GATA-4 and Nkx2.5 by which GATA-4 induces a conformational change in Nkx2.5 that unmasks its activation domain. The Nkx2.5–GATA-4 synergy is not restricted to ANP and is observed on several other cardiac promoters [51,53]. Analysis of the regulatory region of early cardioregulators like MEF2 and Nkx2.5 also indicate that their transcription is likely governed by combinatorial interaction between GATA and NK proteins [54]. Although the GATA-4–Nkx2.5 cooperativity was initially characterized as a possible mechanism contributing to atrial specificity (discussed below), it appears to be the target and nuclear effector of bone morphogenetic proteins (BMPs) during early stages of cardiomyocyte differentiation [55] and of stretch mediated hypertrophy (discussed later).

Interestingly, in xenopus embryos, mutation of the proximal GATA element modulated the spatial activity of the ANP promoter, a phenotype that was also seen with the NKE mutation. Under these conditions ANP transcription was no longer restricted to the atria and persisted in the ventricle and outflow track during heart development [47]. These results further support the role of Nkx2.5 and GATA factors in spatial control of ANP transcription. Moreover, the observation that GATA sites are essential for ANP promoter activity in this assay further confirm the in vivo relevance of the GATA pathway for cardiac transcription.

## 5. How could GATA-4 and NKx2.5 specify atrial transcription?

Since both proteins are present in all myocardial cells during development and in the adult heart [56,57], it is possible that the restricted expression of ANP in the atria might be attributed to the displacement of a Nkx2.5 repressor. This hypothesis could be supported by the finding that ANP expression persisted in the ventricle of mice overexpressing Nkx2.5 throughout the myocardium [58] and the upregulation of ANP promoter activity in ventricular cardiomyocytes when the NKE site was mutated [48]. Several studies have suggested putative repressors of the Nkx2.5 activity such as the NK2 and 3 family of homeodomain proteins [59–61]. While these proteins are able to bind to the ANP NKE site, no in vivo evidence is available yet in support of this mechanism.

Several lines of evidence indicate that atrial specificity requires an additional transcription factor bound to the ANP promoter. For example, a heterologous promoter driven by the NKE or GATA site is similarly active in both atrial and ventricular myocytes [48] and mutation of the GATA site results in similar decreases in ANP promoter activity in these cells [13]. In contrast, mutation of the NKE in the context of the ANP promoter decreases promoter activity only in atrial cells [48]. This led to the hypothesis that atrial specificity results from combinatorial interaction of Nkx2.5 with another transcription factor bound to the ANP promoter. This factor was later identified as Tbx5, a cardiac-specific member of the T-box family, whose gene is mutated in Holt Oram Syndrome [62].

The ANP promoter contains three T-box elements (TBE) which are binding sites for Tbx transcription factors. Several studies have established that Tbx proteins are important regulators of the ANP gene. First, Tbx5 binds with high affinity to the TBE site on the ANP promoter and potently activate promoter activity in a dose dependent manner [62]. Second, Tbx5 null mice lack ANP expression whereas

heterozygous mice show reduced ANP mRNA levels, indicating that ANP transcription is unusually sensitive to Tbx5 dosage [62]. In xenopus embryos, the time of activation and the efficiency of ANP promoter expression are also dependent on a functional TBE demonstrating the conservation of Tbx regulation of ANP [47]. This work also confirmed the presence of a functional interaction between NKx2.5 and Tbx5 that may well explain why mutations in either protein lead to similar cardiac defects in human [63,64]. Another T-box factor, Tbx2 which, in contrast to Tbx5 is a transcriptional repressor, was also shown to regulate ANP transcription. In this case, Tbx2, which is expressed in the atrioventricular canal, could act to restrict spatial expression of ANP within the heart [45]. In addition to Nkx2.5, Tbx5 also interacts with GATA-4 to synergistically activate the ANP promoter; interestingly a mutation in GATA-4 that abrogates Tbx5 interaction was found in patients with cardiac septal defects [34]. These results suggest the presence of a multiprotein complex (enhanceosome) required for proper cardiac transcription and heart morphogenesis. Disruption of any component of this complex might cause similar cardiac abnormalities. The molecular analysis of such complex might therefore identify novel candidate genes linked to congenital heart disease.

In addition to these factors, ANP transcription is also regulated by the MADS box proteins, MEF2 and serum response factor (SRF) which play important roles in muscle cells including cardiomyocytes. Interestingly, MEF2 activation of ANP is independent of its DNA binding to a low affinity site present around −500 bp (Fig. 2); in fact, MEF2 acts as a cofactor of GATA-4 and modulates promoter activity via the GATA binding site [65]. SRF, which is essential for mesoderm formation [66] and a key regulator of skeletal and smooth muscle differentiation, is also an important regulator of cardiac genes, including ANP. Two SRF binding sites (SREs) are present on the ANP promoter, a distal high affinity site and a proximal low affinity site flanked by two GATA elements (Fig. 2). In vitro and in vivo analysis of promoter activity clearly established the importance of both sites for ANP promoter activity although their relative importance depends on developmental stages and hormonal status [46,47,67]. Importantly, SRF and GATA-4 form a ternary complex on the proximal GATA-SRE element which likely serves to recruit other cofactors to the promoter [67]. Several lines of evidence indicate that this ternary complex is targeted by positive and negative cardioregulators ([67] and our unpublished work). It is important to note that, while the interaction between GATA factors and MADS proteins was first established on the ANP promoter, this functional cooperation extends to other genes [65,67].

Recently it has been shown that ANP is also a downstream target of the bicoid/paired-like homeobox transcription factor family PITX2 [68] which plays an important role in left–right asymmetry of the heart. The PITX protein binds to the DNA sequence 5′-TAATCC-3′ [69] to differentially activate the ANP promoter via its different isoforms, A, B and C. Importantly, only PITX2C, which is the major isoform expressed in the heart, was capable of synergistically activating the ANP promoter in the presence of Nkx2.5. This interaction might contribute to the high level of ANP found in left ventricle. Since Nkx2.5 interacts with GATA-4 it is intriguing to test the possible interaction between PITX and GATA-4 or the formation of a complex between NKX2.5–GATA-4 and PITX.

In addition to ANP, the BNP promoter has also served to elucidate novel pathways for transcriptional regulation in the heart. The structure of the proximal BNP promoter is evolutionarily conserved and contains two GATA motifs, one YY1, one CACC box-binding protein [10] and an MCAT motif at −100 bp [70] (Fig. 2). The MCAT element has been linked to BNP promoter activation in response to interleukin-1β, an inflammatory cytokine, through a p38 kinase-dependent pathway [71]. GATA-4 was found to recruit YY1 protein to enhance BNP transcription by forming a transcriptional complex with the c-AMP response element-binding protein [72]. This binding required the GATA-4 but not the YY1 DNA-binding site.

GATA-4 was also shown to recruit another class of transcription factors to its target promoters. In a recent study both BNP and ANP promoters were synergistically activated by GATA-4 and the basic helix–loop–helix (bHLH) protein dHand [73]. Structure function analysis indicated that the zinc finger domain of GATA-4 physically interacts with the bHLH domain of dHand. This domain was also found to bind the p300 coactivator, forming a higher order complex over the GATA binding site. dHand and its closely related family member eHand are expressed in a complementary manner in the left and right ventricle and have been shown to be essential for proper heart development [74,75]. At present the ANP and BNP promoters are the only known cardiac promoters targeted by the hand proteins and, as such, they will allow dissection of the mechanisms of action of these important cardiac regulators.

## 6. Genetic reprogramming in cardiac hypertrophy: GATA-4 at the heart of the program

In the adult heart, cardiac hypertrophy is an adaptive mechanism by which the heart responds to the increase in workload due to humoral, hemodynamic or pathologic conditions (reviewed [1]). It is well established that under these circumstances, ANP and BNP transcription is significantly increased (reviewed in [5]). The nature of the transcription factors and the exact signaling cascades that are involved in the induction of these genes and other cardiac genes in response to hypertrophic stimuli remain undefined. Recently, GATA-4 emerged as a key regulator of the adaptive response of the heart. First, it was found that an intact GATA binding site in the promoter of the angiotensin II type 1a (AT1) [76] and the β-MHC genes [77] was

important for the induction of both genes in response to pressure overload. The second line of evidence was provided by the finding that the calcineurin stimulated nuclear factor-activated T lymphocytes (NF-AT3) was a GATA-4 collaborator, and its overexpression was sufficient to induce cardiac hypertrophy [78]. This finding suggested that GATA-4 may be an effector of the calcium–calcineurin pathway that underlies at least a subclass of hypertrophy. The role of GATA-4 as a nuclear target and effector of other signaling pathways activated by hypertrophic stimuli was further emphasized by the finding that GATA binding sites on the ANP promoter were essential for its response to the hypertrophic hormone endothelin (ET-1) [67]. The GATA binding site on the ET-1 promoter was also essential for activation of the promoter in response to $\alpha_1$-adrenergic agonists [65]. The essential role of GATA-4 in cardiomyocyte hypertrophy was underscored by the finding that antisense GATA-4 vectors specifically interfered with cardiomyocyte response to ET-1 and $\alpha_1$-adrenergic agonists [40]. Conversely, overexpression of GATA-4 in cardiomyocytes mimicked the hypertrophic action of these stimuli [40]. These findings were confirmed in whole animals, whereby transgenic mice overexpressing GATA-4 exhibited progressive cardiac hypertrophy and enhanced ANP and BNP expression despite the low increase of GATA-4 levels [79]. Numerous other studies have since confirmed the critical role of GATA-4 in the adaptive response of the heart.

In in vivo studies, pressure overload induced by either arginine–vasopressin infusion [80] or aortic coarctation [76] enhanced GATA binding activity over the BNP promoter. This binding was specific for GATA-4 and was inhibited by ET-1 receptor antagonists, indicating that pressure overload leads to the activation of ET-1–GATA-4 signaling pathway [80].

Similarly, in whole organ experiments, increased wall-stress induced by inflating a balloon in the left ventricle enhanced GATA binding activity [81]. Under these conditions BNP–GATA-4 binding activity, but not GATA-5 or -6, was transiently increased as early as 30 min of wall stretching. Again, the infusion of ET-1 as well as AT-1 receptor antagonists blocked the increased GATA binding activity, induced by wall-stretching. The exact mechanisms underlying the essential role of GATA-4 as an effector of various cardioregulators are presently the subject of intense investigations. They will likely be numerous and will depend upon the nature, duration and strength of the stimulus. At this point, the body of evidence suggests that the GATA-4 protein is able to integrate different signaling cascades by two main processes (Fig. 3). On the one hand, the GATA-4 protein appears to be itself a direct downstream target for several kinases, including the MAP Kinases ERK [41] and p38 [40]. Studies from our laboratory (Wang and Nemer, unpublished data) also indicate that GATA-4 is a direct target of PKCs. On the other hand, GATA-4 associates with other transcription factors, such as NFAT, SRF, MEF2 and FOS/JUN [82], which are themselves targets of several signaling pathways activated in cardiac hypertrophy.

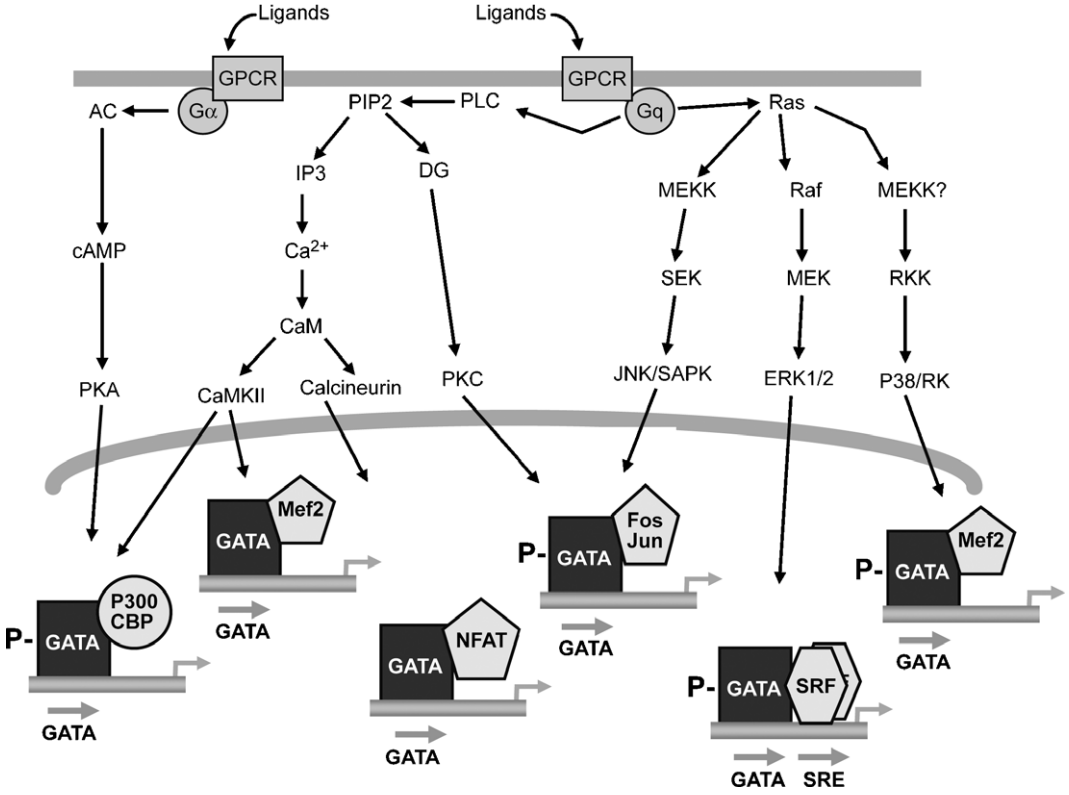


Fig. 3. Convergence of cell signaling on GATA-4. Please note how GATA-4 is targeted for phosphorylation by different kinase cascades.

## 7. Conclusions and prospective

Analysis of cardiac natriuretic peptide gene transcription has uncovered fundamental regulatory pathways for cardiomyocyte growth and differentiation. There has been a remarkable impact of this knowledge at the clinical level, be it in terms of the development of rapid and reliable biochemical tools for the diagnosis of cardiac dysfunction or in terms of molecular and genetic understanding of congenital heart defects. In this respect, the discovery and characterization of GATA-4 represent a major advance in our understanding of the mechanisms of heart function. GATA-4 is central to embryonic cardiomyocyte survival, to maintenance of the differentiated state of the postnatal heart and to its adaptive response. That a single transcription factor can exert such pleitropic effects highlights the efficiency of the cell but also raises important questions as to how a given protein can mediate distinct functions. Obviously, protein–protein interactions as well as post-translational modifications must play essential roles in this process. In the coming years, the identification of GATA-4 target genes as well as GATA-4 collaborators at different stages of cardiomyocyte growth will further enhance our understanding of the role of GATA-4 in the heart and also of the molecular basis of myocyte growth and function. Finally, knowledge of GATA-4 upstream regulators might offer new avenues for pharmacologic regulation of GATA-4 for purposes of cardioprotection.

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