

# GATA-4: An Integrator of Hormonal and Growth Factor Signaling in the Heart

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## ABSTRACT

Congenital heart defects in children and cardiac disease in adults are the leading cause of mortality in industrialized countries. In recent years, identification of a number of transcription factors involved in cardiogenesis has greatly enhanced our understanding of the mechanisms underlying heart formation and function. The present review will focus on the zinc finger transcription factor GATA-4 that has emerged as a key regulator of cardiac gene expression, and an important survival factor for cardiomyocytes. GATA-4 is also a nuclear effector of several signalling pathways, which modulate its function through post-translational modification of the GATA-4 protein, or regulation of its co-factors.

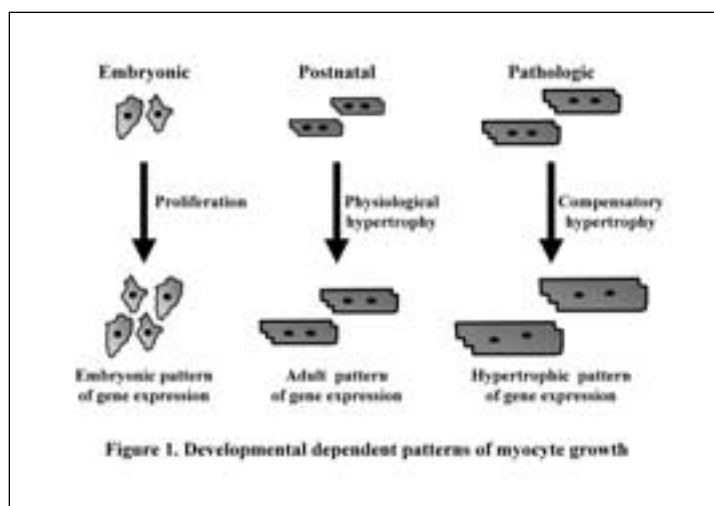
## RÉSUMÉ

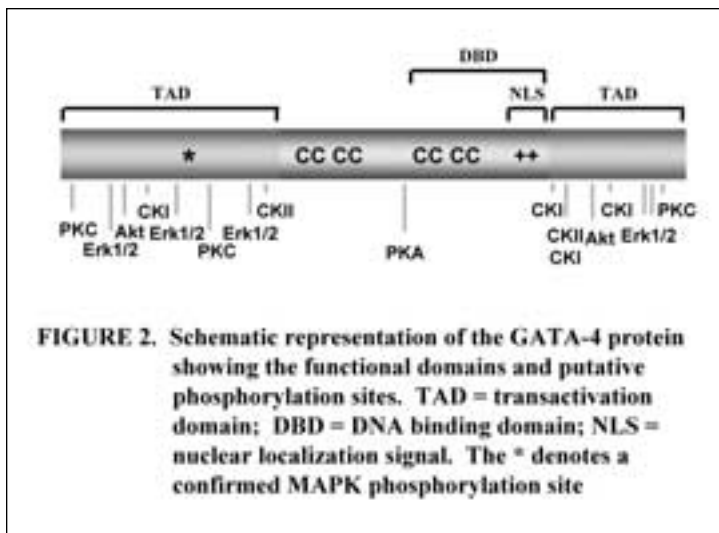
Les maladies cardiaques sont les principales causes de mortalité dans les pays industrialisés. Au cours des dernières années, l'identification de plusieurs facteurs de transcription impliqués dans la cardiogenèse, ont contribué à améliorer notre compréhension des mécanismes moléculaires qui régissent la formation et le fonctionnement du cœur. Cette synthèse portera plus particulièrement sur le facteur de transcription GATA-4, un régulateur-clé de l'expression génique et de la survie des cardiomyocytes. De plus, GATA-4

s'avère être un indispensable effecteur nucléaire de plusieurs voies de signalisation qui convergent sur GATA-4 et modulent sa fonction soit en affectant la protéine GATA-4 directement ou par le biais de leurs effets sur ses co-facteurs.

## INTRODUCTION

Cardiomyocytes respond to growth stimulation via two distinct pathways depending on their developmental stage (Fig. 1). During embryonic life, heart growth involves cell proliferation but the post-natal heart grows essentially by increasing the size but not the number of its cardiomyocytes, a phenomenon known as hypertrophic growth. In the ventricles, post-natal growth (i.e. physiologic hypertrophy) is accompanied by a genetic switch that involves down-regulation of embryonic genes -like the atrial natriuretic peptide (ANP) - and upregulation of the adult pattern of gene expression. Another type of hypertrophic growth often referred to as compensatory or pathologic hypertrophy, occurs in response to work-overload of the post-natal heart; work-overload





may be due to a variety of physical or hormonal stimuli such as increased pressure, mechanical stretch, vasoactive hormones and myocardial infarction. Although in these cases, myocyte hypertrophy is initially a compensatory response, it often leads to decompensatory, cardiac dysfunction and ultimately, heart failure. Understanding the molecular mechanisms required for proper myocyte function is therefore of great scientific, medical and economic relevance. Because each stage of heart development is characterized by a distinct pattern of gene expression, defining the mechanisms that regulate gene transcription constitutes an important step towards understanding the molecular basis of myocyte function. Interestingly, many embryonic genes are reexpressed during pathologic hypertrophy, a finding that has led to the hypothesis that the mechanisms of gene transcription in embryonic and hypertrophic (pathologic) growth are similar.

### The ANP gene, an exquisite marker of cardiac growth

In order to define transcriptional control of cardiac growth, we have used as marker the gene encoding atrial natriuretic peptide (ANP), the major secretory product of the heart. ANP is a hypotensive hormone with natriuretic and diuretic properties that acts on target organs via membrane receptors that have guanylate cyclase

activities. The physiologic importance of the ANP system was evidenced by the phenotype of mice in which the ANP receptor or the ANP genes were inactivated leading in both cases to hypertension [reviewed in (13)]. The ANP gene is expressed predominantly in the heart, where its transcription is dynamically regulated in a spatial and temporal manner. In particular, ventricular expression of ANP characterizes the embryonic but not postnatal heart as ANP transcription is rapidly downregulated after birth but is again upregulated in conditions of pathologic hypertrophy, be it in animal models or human subjects [reviewed in (16)]. In fact, increased ventricular ANP level is a widely accepted hallmark of the genetic switch that accompanies pathologic hypertrophy, and measurement of the resulting increased plasma ANP is routinely used in clinical settings for the diagnosis of cardiac dysfunction (13). A genomic fragment containing the first 700 bp of ANP upstream sequences is sufficient to recapitulate the cardiac and temporal expression of the ANP gene. Work carried out in our laboratory over the past 10 years identified, within this region, numerous cis-regulatory elements required for proper cardiac transcription. The transcription factors that bind these DNA sequences were also characterized, including GATA-4 (Fig. 2), a cardiac-enriched member of the GATA family of zinc finger proteins, which were shown to play crucial roles in hematopoiesis (6).

### GATA-4 and cardiac transcription factor

Members of the GATA family of transcription factors are zinc finger proteins that bind specifically to (A/T)GATA(A/G) DNA sequences (1). The founding member of this family, GATA-1, as well as GATA-2 and GATA-3, is largely restricted to the hematopoietic lineage, and targeted disruption of their genes have revealed an essential non-redundant function for each of these factors in hematopoiesis. Analysis of cardiac-specific promoters led to the cloning of an additional member of the GATA family, GATA-4, whose expression is mainly restricted to the heart and gonads

(6). GATA-4 can be detected in the bilateral cardiac primordia and, together with Nkx2-5, constitutes the earliest markers of heart field induction. Later, GATA-4 transcripts and proteins are detected throughout the myocardium and endocardium and persist at all stages of heart development. Transfection studies in noncardiac cells established that GATA-4 is a potent transactivator of numerous cardiac promoters.

As shown in Figure 3, GATA-4 binds the proximal ANP promoter where it physically and functionally interacts with several other transcription factors to regulate compartment-specific gene expression as well as hormonal response (4,19,20). Thus, a multimeric protein complex coordinated by GATA-4 is targeted by various extracellular stimuli and controls transcriptional changes of ANP and other cardiac genes. How cell signalling modulates this multimeric complex has not been fully elucidated. Bioinformatic analysis mapped several putative phosphorylation sites on GATA-4 (Fig. 2) and work in our laboratory and elsewhere confirmed that GATA-4 can be regulated by several kinases, including the ERK and p38 MAP kinases (3,14). In fact, GATA-4 appears to be an essential nuclear mediator of the Rho family of small GTPases that coordinate Rho effects on gene expression and cytoskeletal remodeling (3). Although recent work in our lab indicates that other kinases also directly regulate GATA-4 (Wang et al, unpublished data), it is important to note that GATA-4 activity may be indirectly regulated by numerous intracellular pathways that target GATA-4 collaborators (Fig. 4). These include the calcium-calciurin pathway which targets transcription factor NFAT which was shown to play a role in cardiac hypertrophy (18) as well as the protein kinase C and JNK pathways which target the AP1 proteins jun/fos, and the CAM kinase and p38 MAPK which target the Mef2 proteins (11,21). In the next years, it will be interesting to determine, through proteomics and mass spectroscopy, the exact composition of the GATA complex in cardiomyocytes at different developmental stages and in response to various agonists, and how different post-translational modifications influence the ability of GATA-4 to assemble distinct, transcriptionally active complexes.

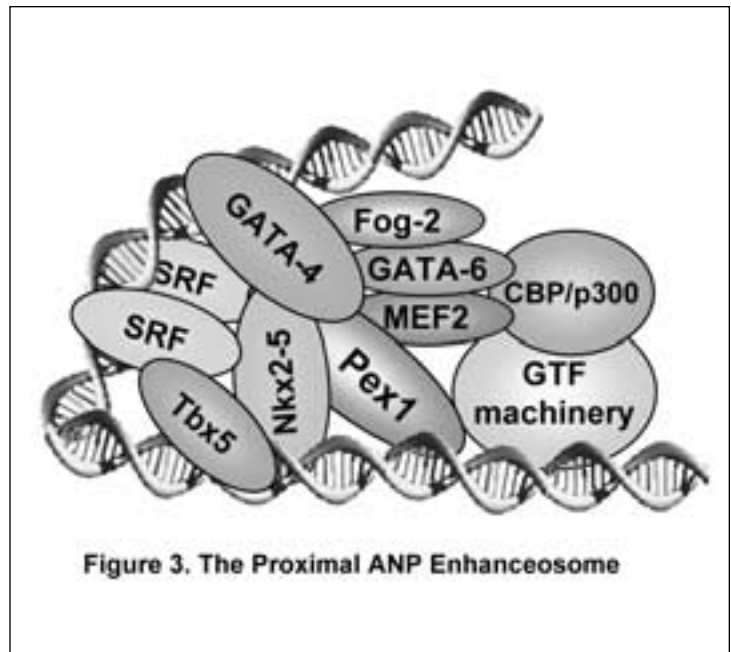


Figure 3. The Proximal ANP Enhanceosome

### GATA-4, a survival factor for cardiomyocytes

In addition to its function as a potent activator of cardiac genes, gain- and loss-of-function studies in various experimental models indicated that GATA-4 is essential for cardiomyocyte survival, proliferation and differentiation. For example, in *drosophila*, the GATA-4 ortholog pannier, is required for proliferation of cardioblasts (5), a result consistent with our finding that embryonic stem cells in which GATA-4 protein was downregulated undergo apoptosis at a cardioblast stage (7). Mice lacking both GATA-4 alleles die *in utero* due to a migration defect of precardiac cells which fail to form a primitive heart tube (17). In human, GATA-4 haplo-insufficiency is associated with congenital heart defects (22). These results indicate that GATA-4 is an essential component of cardiogenesis and suggest that GATA-4 may be required for the action of one or more growth factors required for cardiomyocyte survival/ proliferation/differentiation. Indeed, GATA-4 is one of the earliest targets of the TGF/BMP family of cardiac inducers (23). GATA-4 is also a target of retinoic acid and may mediate its effects during cardiac development (12). In support of the hypothesis that

GATA-4 is a target of cardioregulators, we found that overexpression of GATA-4 enhances *in vitro* cardiogenesis of embryonic stem cells (7).

The essential role of GATA-4 during development prompted us to analyze its role in the terminally differentiated postnatal heart. For this, we engineered adenoviral vectors that express sense or antisense GATA-4 transcripts and used them to infect post-natal cardiomyocytes in primary cultures. Decreased GATA-4 protein levels led to decreased expression of several cardiac genes including ANP, BNP, and amylosin heavy chain (2). Remarkably, the cellular response of myocytes to hypertrophic stimuli like endothelin-1 (ET1) and  $\alpha$ 1-adrenergic agonists was also blunted as evidenced by the inability of cells to reorganize their cytoskeletal or increase their size (3). Ectopic expression of GATA-4 mimicked the hypertrophic changes elicited by ET-1 and  $\alpha$ 1 adrenergic agonists suggesting that GATA-4 is essential, and its activation, sufficient for the adaptive response of post-natal cardiomyocytes. This conclusion is supported by several studies showing that GATA elements are required for activation of cardiac genes in response to *in vivo* pressure or volume overload (8,10,15), and that GATA-4 levels and/or activity are upregulated in *in vivo* models of cardiac hypertrophy (9) and our unpublished data.

### Conclusions and perspectives

The discovery and characterization of GATA-4 represent a major advance in our understanding of the mechanisms underlying cardiac function. GATA-4 is central to embryonic cardiomyocyte growth, to maintenance of the differentiated state of postnatal cardiomyocytes and their adaptive response to work overload. That a single transcription factor can exert such pleiotropic effects highlights the efficiency of the cell but also raises important questions as to how a given protein can mediate distinct function. Obviously, protein-protein interactions as well as post-translational modifications must play essential roles in this process. In 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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### REFERENCES

1. Charron, F. and M. Nemer. 1999. GATA transcription factors and cardiac development. *Sem.Cell Dev.Biol.* 10:85-91.
2. Charron, F., P. Paradis, O. Bronchain, G. Nemer, and M. Nemer. 1999. Cooperative interaction between GATA-4 and GATA-6 regulates myocardial gene expression. *Mol.Cell.Biol.* 19:4355-4365.
3. Charron, F., G. Tsimiklis, M. Arcand, L. Robitaille, Q. Liang, J. D. Molkentin, S. Meloche, and M. Nemer. 2001. Tissue-specific GATA factors are transcriptional effectors of the small GTPase RhoA. *Genes Dev.* 15:2702-2719.
4. Durocher, D., F. Charron, R. Warren, R. J. Schwartz, and M. Nemer. 1997. The cardiac transcription factors Nkx2-5 and GATA-4 are mutual cofactors. *EMBO J.* 16:5687-5696.
5. Gajewski, K., N. Fossett, J. D. Molkentin, and R. A. Schulz. 1999. The zinc finger proteins Pannier and GATA4 function as cardiogenic factors in *Drosophila*. *Development* 126:5679-5688.
6. Grepin, C., L. Dagnino, L. Robitaille, L. Haberstroh, T. Antakly, and M. Nemer. 1994. A hormone-encoding gene identifies a pathway for cardiac but not skeletal muscle gene transcription. *Mol.Cell.Biol.* 14:3115-3129.

7. Grepin, C., G. Nemer, and M. Nemer. 1997. Enhanced cardiogenesis in embryonic stem cells overexpressing the GATA-4 transcription factor. *Development* 124:2387-2395.
8. Hasegawa, K., S. J. Lee, S. M. Jobe, B. E. Markham, and R. N. Kitsis. 1997. cis-Acting sequences that mediate induction of beta-myosin heavy chain gene expression during left ventricular hypertrophy due to aortic constriction. *Circulation* 96:3943-3953.
9. Hautala, N., H. Tokola, M. Luodonpaa, J. Puhakka, H. Romppanen, O. Vuolteenaho, and H. Ruskoaho. 2001. Pressure overload increases GATA4 binding activity via endothelin-1. *Circulation* 103:730-735.
10. Herzig, T. C., S. M. Jobe, H. Aoki, J. D. Molkentin, A. W. Cowley, Jr., S. Izumo, and B. E. Markham. 1997. Angiotensin II type1a receptor gene expression in the heart: AP- 1 and GATA-4 participate in the response to pressure overload. *Proc.Natl.Acad.Sci.USA* 94:7543-7548.
11. Kolodziejczyk, S. M., L. Wang, K. Balazsi, Y. DeRepentigny, R. Kothary, and L. A. Megeney. 1999. MEF2 is upregulated during cardiac hypertrophy and is required for normal post-natal growth of the myocardium. *Curr.Biol.* 9:1203-1206.
12. Kostetskii, I., Y. Jiang, E. Kostetskaia, S. Yuan, T. Evans, and M. Zile. 1999. Retinoid signalling required for normal heart development regulates GATA-4 in a pathway distinct from cardiomyocyte differentiation. *Dev.Biol.* 206:206-218.
13. Levin, E. R., D. G. Gardner, and W. K. Samson. 1998. Natriuretic peptides. *N.Engl.J.Med.* 339:321-328.
14. Liang, Q., R. J. Wiese, O. F. Bueno, Y. S. Dai, B. E. Markham, and J. D. Molkentin. 2001. The transcription factor GATA-4 is activated by extracellular signal-regulated kinase 1- and 2-mediated phosphorylation of serine 105 in cardiomyocytes. *Mol.Cell Biol.* 21 :7460-7469.
15. Marttila, M., N. Hautala, P. Paradis, M. Toth, O. Vuolteenaho, M. Nemer, and H. Ruskoaho. 2001. GA T A4 mediates transcriptional activation of the B-type natriuretic peptide gene expression in response to hemodynamic stress. *Endocrinology* 142:4693-4700.
16. McBride, K. and M. Nemer. 2001. Regulation of the ANF and BNP promoters by GATA factors: Lessons learned for cardiac transcription. *Can.J.Physiol.Pharmacol.* 79:673-681.
17. Molkentin, J. D., Q. Lin, S. A. Duncan, and E. N. Olson. 1997. Requirement of the transcription factor GA T A4 for heart tube formation and ventral morphogenesis. *Genes Dev.* 11 :1061-1072.
18. Molkentin, J. D., J. R. Lu, C. L. Antos, B. Markham, J. Richardson, J. Robbins, S. R. Grant, and E. N. Olson. 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93:215-228.
19. Morin, S., F. Charron, L. Robitaille, and M. Nemer. 2000. GATA-dependent recruitment of MEF2 proteins to target promoters. *EMBO J.* 19:2046-2055.
20. Paradis, P., N. Dali-Youcef, F. W. Paradis, G. Thibault, and M. Nemer. 2000. Overexpression of angiotensin II type 1 receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. *Proc.Natl.Acad.Sci.USA* 97:931-936.
21. Passier, R., H. Zeng, N. Frey, F. J. Naya, R. L. Nicol, McKinsey, TA, P. Overbeek, J. A. Richardson, S. R. Grant, and E. N. Olson. 2000. CaM kinase signalling induces cardiac hypertrophy and activates the MEF2 transcription factor in vivo [comment]. *J.Clin.Invest.* 105:1395-1406.
22. Pehlivan, T., B. R. Pober, M. Brueckner, S. Garrett, R. Slaugh, R. Van Rheeden, D. B. Wilson, M. S. Watson, and A. V. Hing. 1999. GATA4 haploinsufficiency in patients with interstitial deletion of chromosome region 8p23.1 and congenital heart disease. *Am.J.Med.Genet.*83:201-206.
23. Schultheiss, T. M., J. B. Burch, and A. B. Lassar. 1997. A role for bone morphogenetic proteins in the induction of cardiac myogenesis. *Genes Dev.* 11 451-62:26.