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The roles of GATA-4, -5 and -6 in vertebrate heart development

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Abstract

The transcription factors *GATA-4*, *-5* and *-6* are expressed very early in heart tissue. Essential GATA sites have been detected in several cardiac genes and the cardiac GATA factors interact with a wide variety of cofactors which synergistically increase gene expression. These multi-protein transcriptional complexes confer promoter-specificity on the GATA factors and also on the more broadly expressed cofactors. Here we summarise the data on these interactions and represent the conclusions as a GATA factor-based genetic regulatory network for the heart. Of the three cardiac GATAs, GATA-4 is by far the most extensively studied, however, loss-of-function data question its presumed dominance during heart development as opposed to hypertrophy.

Keywords: Myocardium; Endocardium; Development; Protein–protein interactions; Transcription factors

1. Introduction

The incidence of congenital heart disease is around one percent of live births, with cardiac abnormalities being the most prevalent defects reported at birth [1]. This high rate of cardiac defects is likely to stem from the complexity of heart development and therefore, to understand these cardiac pathologies, we need to define the molecular pathways involved in normal heart development. Members of the GATA family of transcription factors are amongst the first to be expressed in the developing heart. The family comprises six members, which can be subdivided into two subfamilies based on sequence similarity and expression profiles. *GATA-1*, *-2* and *-3* are involved in haematopoeisis and ectodermal patterning, whilst *GATA-4*, *-5* and *-6* are expressed in cardiac tissue and endodermal derivatives [2,3]. They all contain two zinc fingers required for binding to the sequence (A/T)GATA(A/G) and for protein–protein interactions [3]. *GATA-4*, *-5* and *-6* are expressed in overlapping but distinct spatial and temporal patterns during development, and therefore defining their individual roles presents a challenge, especially since redundancy has been posited in some instances.

2. Functional analysis

2.1. GATA-4

GATA-4 has been shown to be essential for cardiac differentiation of the embryonal carcinoma cell line, P19: a cell line induced to form beating cardiomyocytes by the addition of DMSO [4,5]. Depletion of *GATA-4* (by an antisense strategy) prevented terminal differentiation and apoptosis of the precardiac cells, whilst gain-of-function studies induced ectopic beating cardiomyocytes, even in the absence of DMSO. Thus, it has been suggested that *GATA-4* is a mediator of cardiomyocyte differentiation, proliferation and survival. However, the 60–70% of *GATA-4* null mice that survive gastrulation form differentiated myocardium apparently quite normally, although they have a deficiency in ventral morphogenesis resulting in cardia bifida [6,7]. More recent experiments performed in chick using small interfering RNAs targeted to *GATA-4* implicate loss of *N-cadherin* as a potential cause of this cardia bifida [8]. Thus, the phenotype of *GATA-4* null

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mice was mild in comparison with predictions from the P19 data. It has been suggested that this may be due to increased levels of *GATA-6* expression seen in the *GATA-4* null mice, implying a certain degree of redundancy within the GATA family [6,7,9]. This suggestion, however, was not supported by antisense experiments in chick embryos, where depletion of a single or two GATA (4, 5 or 6) transcripts resulted in normal heart formation, and even partial deletion of all three caused a failure of ventral morphogenesis (cardia bifida) with no obvious defects in cardiomyocyte maturation, reminiscent of the *GATA-4*−/− mice [10]. Thus, it seems likely that the differences in the severity of phenotypes seen reflect differences between embryos and cell lines. Consistent with this suggestion, *GATA-6* was not induced in P19 cells when *GATA-4* was ablated, unlike in *GATA-4* null mice [4].

The cardiac defects seen when *GATA-4* was inactivated are likely to be non-cell autonomous as *GATA-4*−/− ES cells contributed to an apparently normal heart in chimeric mice [6,9]. *GATA-4*−/− cells were detected in the myocardium and endocardium, in atria and ventricles [6]. Chimera studies also suggest that ventral morphogenesis defects are due to lack of *GATA-4* in the endoderm rather than the mesoderm [11]. However, gain-of-function experiments in *Xenopus* embryos caused premature expression of cardiac differentiation markers, and in presumptive ectoderm induced heart marker expression and in some cases spontaneous cardiomyocyte beating [12,13]. When endoderm induction was blocked, cardiac marker expression increased, suggesting a role for this GATA factor within the induced myocardial cells themselves. However, *GATA-5* was similarly active in this assay leaving open the possibility that *GATA-4* was fulfilling a more general requirement for GATA activity in this assay. Thus, the precise role played by *GATA-4* in cardiac mesoderm is still unclear.

GATA-4 is expressed throughout development and also in the adult heart. Clinical studies have indicated a role in human congenital heart defects (CHD) [14,15]. FISH analysis of CHD patients with a deletion of the terminal end of chromosome 8p, which contains many genes, revealed a deficiency in *GATA-4* [14]. More recent work has been able to establish a role for *GATA-4* in patients without the complications of such a dramatic deletion. A heterozygous missense mutation adjacent to the C terminal zinc finger, which is required for DNA binding and interaction with cofactors, was found in family members with CHD, specifically atrial septal defects (ASD), although additional septal and valve defects were also seen [15]. The resultant protein had diminished DNA binding, which was the likely cause of the reduced transactivational capacity observed when compared with wild type GATA-4. A second family was also identified with ASD which segregated with *GATA-4* mutations. This turned out to be a frame shift mutation, predicted to cause a premature stop codon resulting in a truncated protein or decay of the transcript. The discovery of two unrelated families in which transmission of ASD is associated with *GATA-4* mutations, strongly implicates this GATA family member in CHD.

Heart disease can often be attributed to the death of cardiac muscle cells. Strong evidence supports a role for *GATA-4* (and *GATA-6*) in apoptosis and adult cardiomyocyte survival during hypertrophy [16], which may provide insights into their roles during embryogenesis. Survival genes such as *HGF* and *Endothelin-1* activated MEK/ERK-dependent signalling pathways which increased the DNA binding activity of GATA-4 via phosphorylation of serine 105 [16]. A subsequent increase in anti-apoptotic gene expression was seen thereby regulating cell survival. In contrast, when cardiomyocyte apoptosis was stimulated, a decrease in *GATA-4* expression was seen [17]. It has been demonstrated that apoptosis can be rescued by the restoration of *GATA-4* (or *GATA-6)*, clearly showing that *GATA-4* can regulate cell survival in the adult heart. During hypertrophy, inhibition of GATA-4 activity can also be achieved by regulation of its nuclear accumulation [18]. GSK3β decreased nuclear GATA-4 by stimulating nuclear export, probably by direct phosphorylation of GATA-4 and export via Crm1, a nuclear exportin. Taken together, it is apparent that *GATA-4* is important for later aspects of heart development, such as valve and septum formation, which is affected in patients with CHD, and is also likely to be important in hypertrophy.

2.2. GATA-5

Null mutations in *GATA-5* caused no obvious cardiac defects in the mouse, whereas in zebrafish a similar phenotype to *GATA-4* null mice was seen (cardia bifida), raising the possibility that functions may have been differentially assigned to these two GATA factors during evolution [3,19]. However, these mice may express a truncated form of GATA-5, containing both zinc fingers and the C-terminal activation domain, which would still be transcriptionally active, thus a role for *GATA-5* during murine cardiogenesis cannot yet be excluded [20]. In fact, chicken *GATA-5* is differentially regulated by two alternative non-coding exons [21]. This is a common feature in members of the GATA family, mouse *GATA-1* [22], human and mouse *GATA-2* [23,24], human and mouse *GATA-3* [25] and human and mouse *GATA-6* [26] genes all possess two distinct promoter-first exon combinations. In the case of *GATA-5*, two distinct isoforms were produced [21]. Interestingly, one isoform encoded a truncated protein which only contains one zinc finger. This isoform excluded exon 2 which contains the translation start site (which was targeted in the *GATA-5* null mouse), yet a functionally active protein was still produced.

In contrast to the *GATA-4* null mice, which express wild type levels of many of the terminal differentiation markers [6,7], *faust* (GATA-5) mutant zebrafish exhibit a reduction in the expression of many of these markers [19]. In addition, the expression of the early regulator *Nkx2.5* was also down regulated, thus both early and later cardiac events were affected in these mutants. Over expression of *GATA-5* in zebrafish embryos was not only able to increase the expression

of *Nkx2.5*, *GATA-4*, *-6* and later myocardial genes in cardiac tissue but also induced ectopic expression of some of these genes in non-cardiac tissue [19]. Occasionally such regions could spontaneously contract. More recently, a requirement for *GATA-5* has been demonstrated for the endocardial differentiation of a mouse cell line derived from cardiac mesoderm [27]. Inhibition of GATA-5 in these cell lines, induced towards an endocardial fate by retinoic acid, blocked terminal differentiation at a pre-endocardial stage. This observation is consistent with the expression pattern of *GATA-5* which becomes restricted to the endocardium during development [28]. Furthermore, *faust* embryos lack endocardial cells [19]. These data suggest that the role of GATA-5 during cardiogenesis may be conserved across species.

2.3. GATA-6

Little information on the role of *GATA-6* during cardiogenesis has emerged from *GATA-6* null mice, which die prior to heart induction, at E5.5–7.5, due to defects in extraembryonic endoderm [29,30]. Thus, of the mammalian GATA factors it appears that *GATA-6* is required at the earliest stage of development. *GATA-6*−/− ES cells were able to contribute to the heart in chimeric embryos and to differentiate into myocardium in vitro, indicating that the loss of GATA-6 protein does not cause cell autonomous cardiac defects. Gain-of-function experiments in *Xenopus* embryos, however, suggested that *GATA-6* does play a role in cardiogenesis [31]. *GATA-6* expression in heart precursors decreases as cardiac machinery gene expression commences. Elevating *GATA-6* beyond this time delayed the onset of terminal differentiation. Once degradation of the exogenous mRNA had occurred, maturation proceeded, resulting in an increased thickness in the myocardium. Therefore, these data indicated that *GATA-6* may hold the cardiac precursors in a progenitor and proliferative state, needing to be downregulated for heart cells to mature. More recent loss-of-function experiments in *Xenopus* and zebrafish, using antisense morpholino oligonucleotides, have shown for the first time that GATA-6 is required for differentiation of the cardiac lineage during embryogenesis [32]. The requirement appears to be in the maturation of the cardiac progenitors rather than in their initial induction. It has also been demonstrated with large dorsal marginal zone explant conjugates from *Xenopus* embryos that GATA-6 is required within the cardiac mesoderm itself but also in the adjacent deep anterior endoderm. Finally, the similarity between the phenotypes of BMP inhibition and GATA-6 depletion suggested a role for *GATA-6* in this signalling pathway, and it was shown to regulate *BMP-4* expression, presumably via the functional GATA sites in the promoter [32,33]. This function for GATA-6 in the developing myocardium explains the lack of phenotype in chimeras and embryoid bodies, where BMP-4 would be available from surrounding wild type cells or serum.

3. Combinatorial interactions

Forced expression of *GATA-4* in P19 cells showed that it was not sufficient to initiate cardiomyocyte differentiation unless the cells were aggregated, suggesting that additional factors are required for GATA-4 dependent transcriptional activity [4]. There are several other families of transcription factors that play roles in cardiogenesis [34]. The temporal and tissue specific expression of cardiac genes during cardiogenesis is likely to be controlled by combinatorial interactions between members of these transcription factor families. Furthermore, the specific roles for GATA-4, -5 and -6 in cardiogenesis are likely to be determined by these interactions. The interaction of ubiquitously expressed factors such as p300 and YY1 with the tissue restricted GATA factors suggests that in cardiomyocytes GATA factors are acting as tissue specific accessory factors for the participation of these ubiquitous factors in cardiogenesis [35–37].

In order to integrate the rapidly expanding number of interactions, we have begun to build genetic regulatory networks (Fig. 1) [38]. Regulatory connections between two genes must satisfy three criteria: (1) the expression of the putative target is affected by perturbation of its activator or repressor; (2) the expression patterns of the target and its upstream activator/repressor must be consistent with their proposed relationship; (3) the promoter/enhancers of the target gene must contain binding sites for the activating/repressing transcription factor or the target must be shown to respond directly to the upstream factor. A connection that satisfies all three criteria is illustrated using a solid line. Connections that satisfy two criteria are shown with a dotted line. In several cases, multiple factors have been shown to complex at one binding site. Here, the line representing this connection is solid, but composed of colours representing each factor that interacts at that DNA binding site. The network is accessible on the worldwide web in interactive form [90], where the inputs or outputs from individual genes can be highlighted (see for example Fig. 2). Clicking on individual connections takes you to the evidence for that connection.

To date many of the combinatorial interactions between the GATA factors and other cardiac transcription factors have been defined for GATA-4 alone. In addition to interactions with other families, studies in postnatal cardiomyocytes demonstrated that GATA-4 and -6 can functionally interact with each other, at a single GATA element, to synergistically activate the *ANF* and *BNP* promoters (Fig. 3A) [39]. Functional interaction and co-operativity required both zinc fingers and the C-terminal activation domain of GATA-4, but not its DNA binding activity.

3.1. Nkx2.5

Several studies have shown that GATA-4 and Nkx2.5 directly interact at the protein level to regulate the expression of the *ANF*, *α-cardiac actin* and *cardiac restricted ankyrin repeat protein* (CARP) promoters [40]. At the *ANF* promoter,

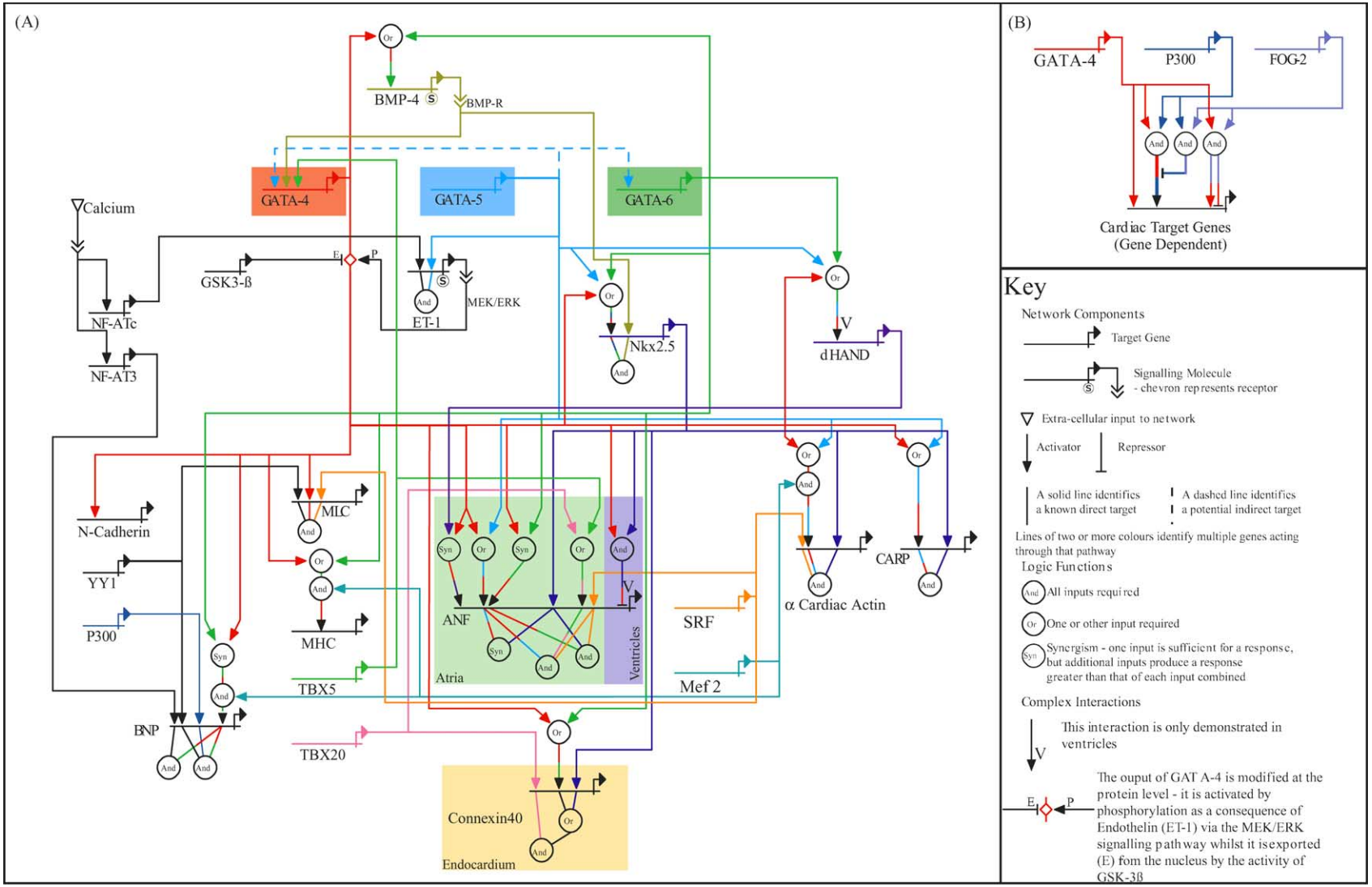

Fig. 1. GATA based genetic regulatory network for heart development. Panel A shows the genetic regulatory interactions identified for the GATA factors during heart development (see also web reference [90]). The output of each gene is shown as activating or repressing target genes. At each target gene any regulatory interactions that have been described are represented as logical interactions (‘and’, ‘or’ or ‘syn’). The various elements of the network can be identified using the key. Panel B illustrates the complex interactions of FOG-2 and p300 with GATA-4. p300 increases GATA-4 activity in the absence of FOG-2. FOG-2 and GATA-4 form a complex in the absence of p300 and can either repress or activate target genes. When both FOG-2 and p300 are present, FOG-2 binds p300, so reducing the activity of GATA-4 by competition.

GATA-4 and Nkx2.5 were shown to be mutual co-factors in its synergistic activation (Fig. 3B) [41]. Structural and functional studies indicated that the carboxy-terminal zinc-finger and a C-terminal extension of GATA-4 binds to the C-terminal auto-repressive domain of Nkx2.5, causing a conformational change exposing the Nkx2.5 activation domains. GATA-5 can substitute for GATA-4 in this interaction but GATA-6 cannot, suggesting that the interaction with Nkx2.5 can convey functional specificity to GATA factors during development (Fig. 3C) [41]. Binding of both the Nkx and GATA proteins to their respective DNA elements within the *ANF* promoter was required for maximal synergy, but synergy was also detected when the GATA binding sites were absent [42,43]. This suggested that Nkx2.5 can recruit GATA-4 or -5 into a transcriptionally active complex at the *ANF* promoter. Despite the expression of additional Nkx2 family members in the cardiac mesoderm, combinatorial interactions with other cardiac transcription factors have been defined for Nkx2.5 alone [44]. The synergistic GATA/Nkx2 interaction appears to be evolutionarily conserved between the fly, nematode and mammals [2].

The *Xenopus ANF* gene promoter has a high degree of conservation with its mammalian counterparts [45]. This indicates that the transcriptional mechanisms that regulate *ANF* expression are conserved among evolutionarily diverse organisms. Mutation of individual binding sites in embryos

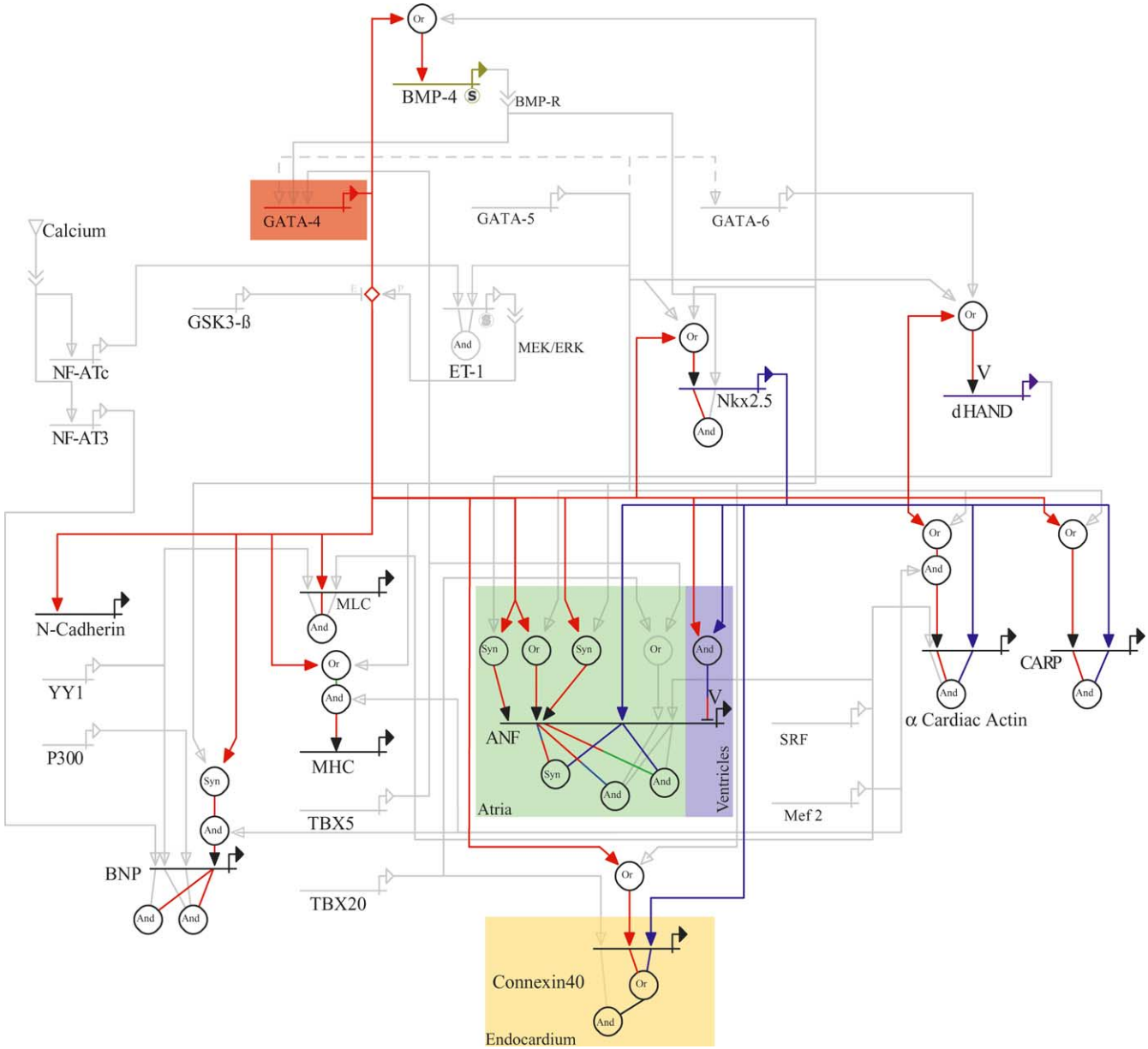

Fig. 2. Genetic regulatory network highlighting the outputs from GATA-4 and Nkx2.5. Downstream interactions involving GATA-4 and Nkx2.5 are highlighted to facilitate clarity. This is achievable for all genes in the network, both upstream and downstream, at web reference [90].

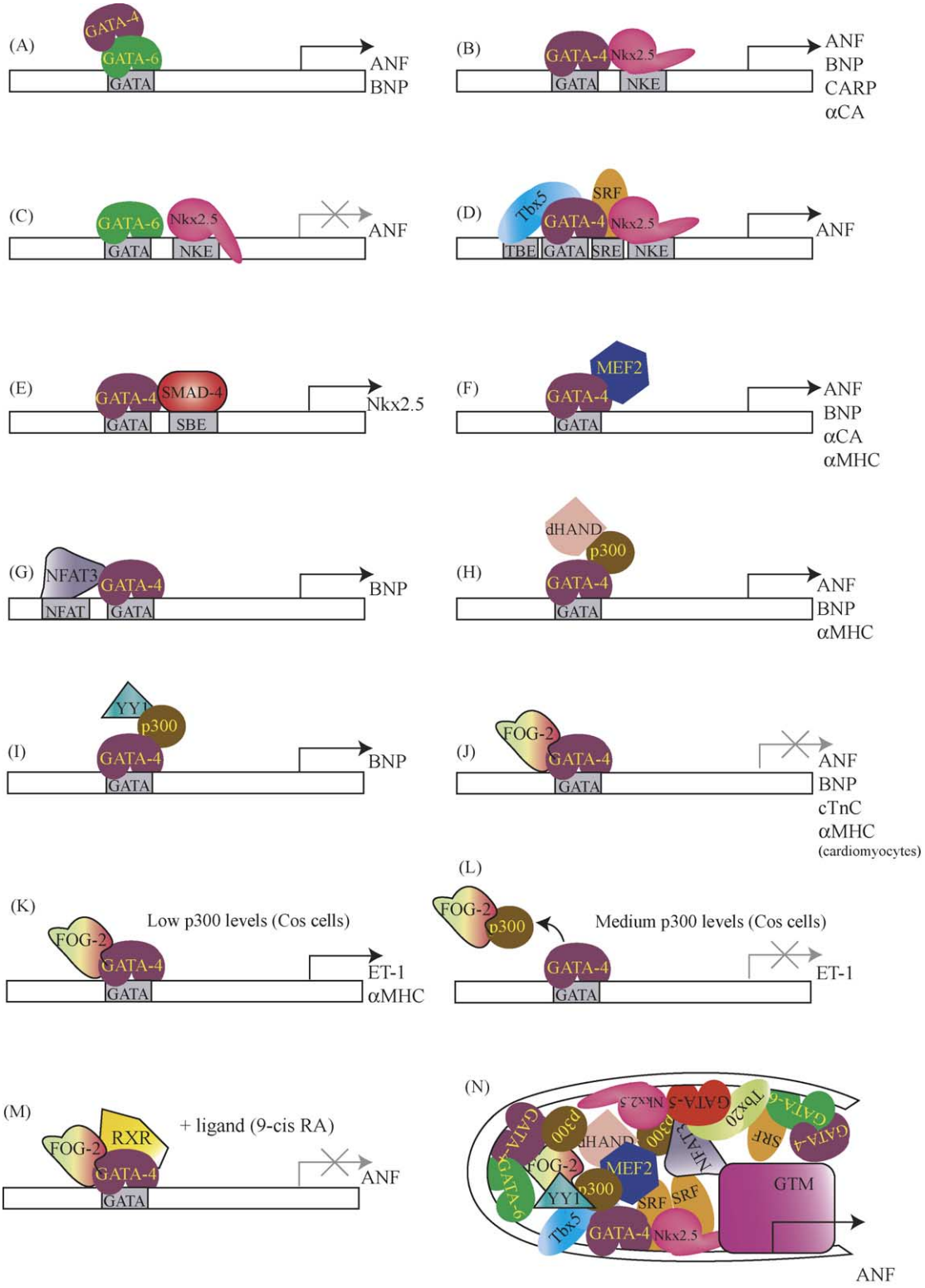

Fig. 3. Combinatorial interactions of GATA factors. GATA-4, -5 and -6 are able to interact with cofactors to activate or repress gene expression. Parts (A)–(M) represents protein complexes shown to interact on various promoters. Part (N) depicts a hypothetical multi subunit complex on the *ANF* promoter. All the proteins shown in the complex, with the exception of NFAT3 and YY1 which interact on the BNP promoter (a related family member), have been identified to synergistically co-operate with GATA factors on *ANF*. The spatial binding of transcription factors on the DNA does not necessarily represent an in vivo situation but highlights the complexity of this promoter. GTM = general transcriptional machinery.

expressing an *ANF* transgene indicated that the T-box element (TBE), the distal GATA site and the serum response elements (SREs) are essential for *ANF* transcription. This is in agreement with the combinatorial interactions described and highlights the importance of the distal GATA site in these interactions (Fig. 3D). The proximal GATA site and the Nkx element (NKE), however, had little effect on transgene expression in the myocardium but were important in the restriction of expression to the atrium. This indicates that the DNA binding activity of Nkx2.5 is dispensable in the activation of *ANF* expression and that binding sites for GATA, serum response factor (SRF) and Tbx5 are sufficient for recruitment to, and synergistic activation of, the promoter [46]. Thus, the proximal GATA and NKE binding sites may be important for the assembly of a repressor complex required for atrial specific expression of the *ANF* gene. In support of this, mutation of the NKE site caused an up-regulation of *ANF* promoter activity in ventricular tissue, consistent with an inhibitory role for this site [41]. Thus, GATA sites within a promoter may have distinct functions.

Nkx2.5 and *GATA-4* have been identified as downstream targets of BMP signalling [47–50]. Studies in *Xenopus* indicated that BMP signalling was required for cardiomyocyte maturation but not initial induction [51,52]. Identification of GATA sites in the *BMP* promoter suggest that a positive feedback loop may exist between GATA and BMP-4, and in vivo a role for GATA-6 in the maintenance of *BMP-4* and *Nkx2.5* expression has been defined [32,33]. A recent study demonstrated that GATA-4 can physically interact, through its C-terminal zinc finger and adjacent basic domain, with the N-terminal domain of SMAD-1 and -4, the intracellular mediators of BMP signalling, to synergistically activate the *Nkx2.5* promoter in P19 cells (Fig. 3E) [53]. GATA-5 and -6 can also physically interact with SMAD, and GATA-6 has been shown to functionally substitute for GATA-4 in this co-operation. Therefore, GATA factors are able to regulate BMP signalling by two mechanisms: by affecting the expression levels of BMP and by interacting with downstream effectors of BMP signalling.

3.2. MADS proteins

Targeted disruption of the *SRF* gene in mice indicated that this member of the MADS domain family of proteins is critical for cardiac mesoderm development [54]. SRF can physically interact with GATA-4 or Nkx2.5 to synergistically activate the *ANF* and *α-actin* genes in cardiomyocytes [40]. GATA-5 and -6 can also synergise with SRF on the *ANF* and *α-actin* promoters. More recent studies demonstrated that GATA-4 can form part of a triad complex with Nkx2.5 and SRF to more efficiently activate cardiac genes [46,55]. SRF was shown to be responsible for recruiting Nkx2.5 and GATA-4 to the *α-cardiac actin* promoter in promyocardial and embryonic stem cells, and formation of the complex appeared to enhance the SRF DNA binding affinity [46] (as illustrated in Fig. 3D). Whether it was SRF or Nkx2.5 that was responsible for recruiting GATA-4 to the complex has not been determined but it is thought that the unmasking of the Nkx2.5 C-terminal repressive domain by GATA-4, in combination with the increased DNA binding affinity of SRF by the complex, was responsible for the robust synergistic activation seen with all three factors.

Another subfamily of the MADS-box family of transcriptional regulators, myocyte enhancer factor-2 (MEF-2) proteins, have been implicated in cardiomyocyte maturation and differentiation [56,57]. GATA-4 recruited MEF2 to the *ANF* promoter, which does not contain MEF-2 binding sites, and the physical interaction between these two proteins resulted in its synergistic activation (Fig. 3F) [58]. GATA-6, but not GATA-5, can substitute for GATA-4 in this synergistic interaction demonstrating further functional differences between GATA-4, -5 and -6. *α-Cardiac actin*, *α-myosin heavy chain* and *BNP* were also identified as targets for GATA-4 and MEF2C co-operation.

3.3. Tbx family

Co-expression of Tbx5 and GATA-4 in HeLa cells and primary cardiomyocytes resulted in co-operative activation of the *ANF* promoter [15] similar to that observed for Tbx5 and Nkx2.5 [59]. The similar effect of the Tbx5 missense mutations, isolated from families with Holt-Oram syndrome, on interaction with GATA-4 and Nkx2.5, as demonstrated by co-immunoprecipitation assays, suggested that a complex consisting of Tbx5, Nkx2.5 and GATA-4 may function in the regulation of a subset of cardiac genes [15]. Another cardiac expressed T-box factor, Tbx20, has been shown to physically interact with Nkx2.5, GATA-4 and -5, -6 was not analysed [60]. Nkx2.5, GATA-4 or -5 and Tbx20 can interact in combinations on the promoter region of the *ANF* gene or the endocardial *Gja5* (connexin40) gene to synergistically activate cardiac gene expression in fibroblasts, with the greatest effects occurring with a combination of the three, further supporting the proposition that an Nkx2.5, Tbx, GATA complex operates in the regulation of specific cardiac genes. Tbx20 was also shown to preferentially collaborate with GATA-5, over GATA-4 and -6, and Nkx2.5 to synergistically activate the *Gja5* promoter, demonstrating functional differences between the subfamily members in regulating cardiac gene transcription. In the mouse, GATA-5 is the predominant factor expressed with Tbx20 in the endocardium and it has been shown to regulate endocardial differentiation in vitro [27]. Hence, the regulatory relationship between Tbx20 and GATA-5 may be important in endocardial development [60].

3.4. NF-AT transcription factors

The multigene family of transcription factors, NF-AT (nuclear factor of activated T-cells), alter gene expression in response to calcium signaling. A co-operative role for GATA-5 and NF-ATc in endocardial transcription has been identified in in vitro studies using the GATA-dependent promoter of the

endothelial gene *ET-1*, which was preferentially activated by GATA-5 [27]. Physical interaction of GATA-5 and NF-ATc was not demonstrated in this study and functional interaction of NF-ATc with GATA-4 or -6 was not discounted. A second member of this gene family, NF-AT3, has been shown to interact via its C-terminal DNA binding Rel homology domain with the C terminal zinc finger of GATA-4, as well as GATA-5 and -6 (Fig. 3G) [61]. The interaction with GATA-4 led to a synergistic activation of cardiac gene expression, during hypertrophy. Taken together, it is likely that co-factors help to restrict GATA factor activity to myocardium and endocardium.

3.5. p300

A ubiquitously expressed general regulator of transcription, p300, can directly interact with GATA-4 to synergistically activate the *ANF*, *α-MHC* and *β-MHC* promoters in fibroblasts [62]. p300 is a member of the same family of proteins as cAMP-response binding protein (CBP). It possesses histone acetyltransferase activity and acts as a transcriptional co-activator for many DNA binding factors [63]. More recent in vitro studies in HeLa cells and primary cardiomyocytes indicate that GATA-4 and dHAND physically interact to form a complex, and the interaction of dHAND with p300 is necessary for the functional synergy of GATA-4 and dHAND on cardiac promoters (Fig. 3H) [37]. Functional synergism was shown to require the GATA site within the promoter and not the dHAND site [37]. In contrast to Nkx2.5, NF-AT3, MEF-2 and SRF, that interact with the C-terminal zinc finger, and FOG-2, that interacts with the N-terminal zinc finger of GATA-4, p300 can bind to either finger. Thus, p300 may act as a scaffold protein in the assembly of multisubunit transcription factor complexes for specific cardiac promoters, thereby conferring further specificity [37]. p300 can also physically interact with GATA-5 in vivo, and co-operate to activate the *ANF* promoter [35].

MEF-2 has also been shown to physically interact with p300 [64–66]. Gel mobility shift analysis of MEF-2 binding activity in a programmed reticulocyte lysate identified p300 as part of the shifted complex, indicating the presence of endogenous p300 [66]. Interestingly, the characterised interactions of GATA-4 with Nkx2.5, SRF, MEF-2 or NF-AT utilised programmed reticulolysates, hence these factors may have been interacting through p300 [37]. Thus, p300 may play a central role in the functional and physical interactions of cardiac transcription factors with the GATA factors facilitating the formation of multisubunit complexes.

3.6. YY1

Another ubiquitously expressed general regulator of transcription is the zinc-finger protein YY1 which is capable of activating and repressing transcription [67]. It can also cause bending of DNA and chromatin remodelling. In transactivating the *BNP* promoter in Hela and CV1 cells, it can synergistically co-operate with GATA-4 via GATA and not YY1 binding sites [36]. However, interaction of YY1 with GATA-4 was dependent upon the DNA binding domain of YY1 and appeared to be mediated via the CBP/p300 class of proteins (Fig. 3I). In *Xenopus* transgenics, the pan-myocardial expression of the cardiac specific marker *myosin light chain 2* (MLC2) was dependent upon the combined activities of GATA factors, SRF and YY1 [68]. GATA-4 and SRF can simultaneously bind to the *MLC2* promoter in vitro. In presumptive ectoderm, simultaneous over expression of GATA-4 and SRF resulted in the synergistic activation of the promoter. In the promoter region a YY1 binding site overlaps with the SRE and inactivation of this site led to a broadening and weakening of XMLC2 promoter activity. The ability of YY1 to act as both a repressor and an activator led the authors to suggest that the repressor activity of YY1 may be responsible for restricting expression to the cardiac muscle, while its activating ability positively regulated promoter activity. YY1 may also be involved in promoting loading of SRF to the low affinity SRE. Together these results suggest that a functional interaction between a GATA factor and SRF is important in *XMLC2* regulation and both the SRE and YY1 sites are required for heart specific expression. Thus, combinatorial interactions involving GATA proteins appear to be important in harnessing the activity of ubiquitous factors, such as p300 and YY1, for cardiac gene expression.

3.7. FOG

The founding member of the *Friend of GATA-1* (FOG) family of cofactors, *FOG-1*, is expressed predominantly in developing haematopoietic cells and interacts with GATA-1 in the mediation of erythroid and megakaryocytic maturation [69]. A recent study involving transgenic rescue of the haematopoietic defect of *FOG-1*−/− mice uncovered an unsuspected role for *FOG-1* in the development of the outflow tract and valves of the heart [70]). *FOG-1* expression was found to correspond with these structures and is proposed to function with the GATA-4, -5, -6 subfamily in their development.

The expression patterns of *GATA-4*, *-5* and *-6*, and a second FOG factor, *FOG-2*, overlap in the developing heart. FOG-2 can interact with the N-terminal zinc fingers of all GATA factors [71–73]. During cardiogenesis, FOG-2 appears to repress the transcriptional activity of GATA-4 (Fig. 1B) [72,74]. Functional analysis of the FOG-2 protein resulted in the identification of an N-terminal repressor domain, distinct from the GATA-4 binding domain, which was both necessary and sufficient for repression of GATA-4 dependent transcription [72]. Interestingly the interaction between FOG-2 and GATA-4 can act to synergistically activate or repress GATA-dependent promoters, depending on the cardiac promoter and particular cell line used (Fig. 1B, 2J and K) [73]. FOG-2 can physically interact with the transcriptional co-activator, p300, suggesting that FOG-2 may compete with GATA-4 for binding to p300 [75]. Maximal GATA-4 activation requires p300

thus when bound to FOG-2, p300 can no longer co-activate GATA-4 resulting in the repression of GATA-4 dependent transcriptional activity (Fig. 3L). Therefore it would seem that levels of p300 in different cell types determine the extent of FOG-2-dependent transcriptional repression.

Retinoids are compounds derived from dietary Vitamin A and have been shown to regulate late cardiac growth and development, and hypertrophy. Retinoids bind to ligand activated nuclear receptor transcription factors termed retinoid receptors. Retinoid X receptors (RXRs) are generally believed to be the master regulators of the retinoid signalling pathway. The failure of the ventricular myocardium to develop normally in *RXRα*-null mice is evidence of this [76,77]. A novel mechanism by which retinoids can regulate cardiac gene expression through direct interaction with GATA-4 and FOG-2 has been proposed recently (Fig. 3M) [78]. The interaction of FOG-2 with RXR in a ligand-dependent manner was believed to be responsible for recruiting FOG-2 to the GATA-4 interface for the subsequent repression of *ANF* promoter activity. The DNA binding domain of RXR was shown to interact with the C-terminal zinc finger region and site(s) within the C-terminal domain of the GATA-4 protein. The N-terminal zinc finger domain was necessary for interaction with FOG-2, hence it is possible that GATA-4 binds to both proteins simultaneously.

In summary, combinatorial interactions with other transcription factors involved in cardiogenesis are likely to bestow temporal, tissue and gene specificity on the cardiac GATA factors. Data for GATA-5 and -6 equivalent to those for GATA-4, however, are currently lacking.

4. Regulation of GATA-4, -5 and -6 expression

It is becoming apparent that cardiac genes are regulated in a modular fashion by multiple enhancers. For example, *Nkx2.5* is controlled by multiple cardiac-specific enhancers that contain essential GATA sites [79,80]. Similarly, the *dHAND* cardiac enhancer can be subdivided into two distinct subregions that drive expression in the right ventricle or outflow tract [81]. This recurring theme extends to members of the GATA family expressed in the heart. In chicken, two control regions were shown to regulate expression of *GATA-5* [21]. The distal region was activated in the embryonic endoderm, whilst the other control region functioned in the heart and late gut. Two distinct heart specific enhancers were also located upstream of chicken *GATA-6* [82,83]. Characterisation of these regulatory regions in transgenic mouse assays demonstrated that the distal enhancer activated expression in the cardiac crescent and linear heart tube [83]. Expression was subsequently downregulated in the atria and ventricles, whilst expression in the outflow tract persisted. This enhancer contained Nkx2.5 binding sites which were required for its activity. The more proximal enhancer directed expression in the atrioventricular (AV) canal at very early stages and persisted in mature cells in the AV conductive system [82,83]. Similar studies of mouse *GATA-6* also identified an Nkx-dependent enhancer [84]. However, the endogenous expression of *GATA-6* was not completely recapitulated in these experiments making it likely that other regulatory elements are still to be found. Recently a zebrafish *GATA-4* cardiac enhancer was discovered [85]. The more proximal regulatory region directed expression to the bulboventricular valve and rostral (ventricular) part of the heart tube, whilst the distal elements were required for AV valve and caudal (atrial) expression of *GATA-4*. T-box binding sites located within the distal region of the enhancer have been shown to be essential for expression, suggesting that *GATA-4* may be a direct target for T-box transcription factors, such as Tbx5, in the heart. Consistent with this, Tbx5 null mice fail to express *GATA-4* in the myocardium [86].

5. Summary

Few would argue that *GATA-4*, *-5* and *-6* are unimportant in development of the myocardium. Nevertheless, much of the data supporting their importance derives from experiments in cell culture. The data from developing embryos is currently inconclusive. For example loss-of-function data in mouse and chick embryos suggest either no role in specification and differentiation of the myocardium, or at most a redundant one. In contrast, experiments in *Xenopus* and zebrafish embryos reveal critical functions for both GATA-5 and -6. It will be important to determine if this extends to the mouse in view of the potentially active protein remaining in the *GATA-5* null. A strong role for GATA-5 in endocardium formation is starting to emerge, where it is required in both mammals and zebrafish. *GATA-6* is required for maintenance of the myocardium, but not its initial specification, in both *Xenopus* and zebrafish. An important link between BMP signalling and GATA-6 explains the ability of *GATA-6* null ES cells to contribute to heart tissue in chimeric embryos and embryoid bodies.

For *GATA-4*, the evidence is strong that it plays a role during late heart development such as heart septation and valve formation. The cardiac malformations seen in humans when *GATA-4* is disrupted are similar to those seen with Holt-Oram syndrome mutations in *Tbx5*, and when *Nkx2.5* is mutated [87–89]. However, its role during early development of the heart is unclear. The earliest appears to be in endoderm where it is required for ventral closure and morphogenesis of the cardiac primordia. Furthermore, *GATA-4* is unable to compensate for the loss of GATA-6 in the drive towards terminal differentiation [32]. Collectively, it appears that *GATA-4* is not required for cardiac myocyte differentiation in the embryo.

Transcription of the GATA genes appears to be regulated in a modular fashion, whilst complex formation is a major determinant of transactivational activity. They are able to interact with a wide variety of partners in many different tissues: both with ubiquitously expressed and tissue-restricted

transcription factors. Identification of multisubunit complexes such as GATA-4/Nkx2.5/SRF/Tbx5 allows more precise specificity both within the GATA family and for those transcription factors which are more broadly expressed. Only in cells where all the components are expressed can the complex activate gene expression. A major role for p300 in the assembly of the multisubunit complexes has been identified, potentially acting as a scaffold for other cofactors. Fig. 3N shows a schematic representation of a potential multisubunit complex. With the exception of NFATc and YY1, which have only been tested on the promoter of *BNP*, the other factors have all been shown to synergistically activate the *ANF* promoter with members of the GATA family. Further control is conferred by the interaction of GATA factors with repressors such as FOG-2.

In conclusion, the roles of *GATA-4*, *-5* and *-6* in control of heart gene expression have been extensively studied. However, studies to date mainly concentrate on *GATA-4*, especially with respect to interactions, and relationships with *GATA-5* and *-6*, which may be more relevant to early heart development, will be important to define in the future.

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