The GATA-6 transcription factor is expressed in cardiogenic cells and during subsequent stages of heart development in diverse vertebrate species. To gain insights into the molecular events that govern this heart-restricted expression, we isolated the chicken GATA-6 gene and used several approaches to screen for associated control regions. Our analysis of two chicken GATA-6/lacZ constructs in transgenic mouse embryos was particularly revealing. One GATA-6/lacZ construct, which has 1.5 kilobase pairs of upstream sequences along with the promoter and first intron, was expressed exclusively in the atrioventricular canal region of the heart. This expression pattern is novel and appears to mark specialized myocardial cells that induce underlying endocardial cells to initiate valve formation. The other GATA-6/lacZ construct, which has an additional 7.7 kilobase pairs of upstream sequences, was expressed in the ventricle and outflow tract in addition to the atrioventricular canal. The failure of these GATA-6 control regions to function as enhancers in transfected cardiac myocyte cultures underscores the importance of using transgenic approaches to elucidate transcriptional controls that function in the developing heart. Although the endogenous GATA-6 gene is expressed throughout the heart, our results indicate that this is effected in a heart region-specific manner.

Although commitment to the cardiogenic lineage and the events that precede cardiac myocyte differentiation are still poorly understood at the transcriptional level, major advances have recently been made in understanding the roles that various growth factors serve in this context. In particular, members of the transforming growth factor-β family of growth factors have been shown to regulate the sub-sequent proliferation and differentiation of these committed cells (1, 2). The genes that are regulated in direct response to these extracellular signals and the transcription factors that mediate these programming events within the cardiogenic lineage have not yet been elucidated.

The transcriptional controls that function within terminally differentiated cardiac myocytes have been analyzed in much greater detail owing to the relative ease of obtaining material for study. Transient transfection and direct muscle injection assays have been used to map control regions for many cardiac genes (for review, see Refs. 3 and 4), and some of these control regions have also been shown to direct heart-restricted expression in transgenic mice. Several of these control regions derive from genes that are expressed in a heart region-specific manner, and in general, the respective control regions have been found to function in a similar manner in transgenic animals. Examples include the ventricular control region from the mouse ventricular MLC-2v gene (5) and the atrial control region from the quail atrial slow MyHC3 gene (6).

Since many cardiac genes are also expressed in skeletal muscle, it is perhaps not surprising that several of the aforementioned cardiac control regions were found to contain motifs similar to those found within skeletal muscle control regions (e.g. CARG, MCAT, MEF2, and E-box). These control regions do not necessarily direct dual heart and skeletal muscle expression, however, and the factors that bind to these motifs are not necessarily the same in both types of striated muscle. For example, four basic helix-loop-helix factors (i.e. MyoD, MRF4, Myf-5, and myogenin) are expressed in skeletal muscle, but not in cardiac muscle (7). Conversely, GATA motifs are commonly found in cardiac control regions, and three GATA factors (i.e. GATA-4/5/6) that bind to these sites are expressed in the heart, but not in skeletal muscle (8–14).

Several additional heart-restricted transcription factors have been identified by other means. For example, degenerate polymerase chain reaction and low stringency screens were used to isolate clones for vertebrate Nkx2.5 factors (15–18) related to the Drosophila tinman homeodomain factor (19). Similar screens were used to isolate clones for two related basic helix-loop-helix factors, dHAND and eHAND (20–23), that are expressed in the developing heart. A factor (CMF1) has been reported to be transiently expressed in the developing heart (24, 25), and a member of the MEF2 family, MEF2C, has also been shown to be expressed early in the cardiogenic lineage (26).

It remains to be determined how the genes for these heart-restricted transcription factors are themselves regulated over the course of heart development. While members of the transforming growth factor-β family of growth factors have been shown to induce Nkx2.5 and GATA-4 gene expression in some regions of non-precardiogenic mesoderm, it is not known if these responses are direct or indirect (2). Similarly, although it is known from gene knockout mice that Nkx2.5 is required to induce eHAND gene expression, this regulation is not understood in molecular terms (15).

In this study, we undertook to screen for control regions associated with the chicken GATA-6 gene, which is expressed in early cardiogenic mesoderm as well as during subsequent stages of heart development (10, 12, 13). GATA-6 is a zinc

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fingertip transcription factor that, along with the related GATA-4 (27, 28) and GATA-5 factors, has been implicated in effecting cardiac myocyte differentiation and heart morphogenesis. Although the endogenous GATA-6 gene is expressed throughout the developing heart, our results unexpectedly reveal that this expression is attributable to several distinct control regions that function in different regions of the developing heart. Moreover, one of these control regions directs a unique pattern of expression in transgenic mouse embryos: in particular, to myocardial cells of the atrioventricular (AV) canal. Whereas this and a more distal GATA-6 control region function in transgenic mouse embryos, neither functions as an enhancer in transfected cardiac myocyte cultures. These GATA-6 control regions are thus distinct from previously described cardiac control regions in several respects.

**EXPERIMENTAL PROCEDURES**

*Isolation and Characterization of Chicken GATA-6 Genomic Clones—* A previously described chicken GATA-6 cDNA probe (12) was used to isolate three chicken GATA-6 genomic clones from a chicken genomic phage library. Standard protocols were used to purify and map these clones (29). Restriction fragments that hybridized to this GATA-6 cDNA probe were cloned into the plBluescript II KS+ plasmid (Stratagene, and the exonic regions were sequenced using primers directed against various portions of the GATA-6 cDNA sequence. These sequences, as well as the others described below, were determined on both strands using Sequenase reagents (U. S. Biochemical Corp.).

**Primer Extension and 5′-RACE**—Poly(A)+ mRNA was isolated using a FastTrack mRNA isolation kit (Invitrogen). Primer extension reactions were carried out according to a standard protocol (30) using an antisense primer directed against the 33–57-nucleotide segment of the published chicken GATA-6 cDNA sequence (12). This primer, which is marked with an arrow in Fig. 2 (A and C), was annealed at 55 °C to 5-μg aliquots of poly(A)+ mRNA.

5′-RACE reactions were performed using a 5′-AmpliFINDER kit (CLONTECH) and the primer described in the previous paragraph. The resultant polymerase chain reaction products were cloned directly into the pCR1 vector with a TA cloning kit (Invitrogen) and sequenced using SP6 and T7 primers.

*Reporter Constructs*—The promoterless pCAT plasmid was made by deleting the BglII-HindIII (simple TATA box) fragment from the pTATA/CAT plasmid (31) and replacing this with an XhoI linker site. Four restriction fragments that include the GATA-6 promoter were cloned (with XhoI linkers) into the newly engineered XhoI site of pCAT to yield the pGATA-6/CAT plasmids shown in lines 1–4 of Fig. 3A. An antisense primer to the GATA-6 gene was used as a sequencing primer to confirm that these fragments were oriented correctly so as to allow them to function as promoters for the downstream CAT gene. A NotI site was inserted into the distal end of the polylinker of the promoterless lacZ vector pDP46.21 (32) by replacing the HindIII-PstI fragment with a synthetic HindIII-PstI fragment that has a NotI site introduced between the SphI and PstI sites. (This modified vector pDP46.21 NotI) also has a NotI site downstream of the lacZ gene; thus, derivative test fragments (for transgenic assays; see below) were released by simply digesting with NotI. The derivative GATA-6/lacZ plasmids shown in Fig. 5 were made using stepwise approaches. First, the +19/+80 BamHI-SmaI fragment that spans the first intron and includes parts of the first and second exons of the GATA-6 gene was cloned into the polylinker of pDP46.21 NotI. Second, the −1467/+19 SacI-BamHI fragment was inserted (as a BamHI fragment with linkers) into the BamHI site to yield p(−1.5/+0.8)GATA-6/lacZ (see line 1 of Fig. 5). The plasmids depicted in lines 5–8 of Fig. 5 were made by inserting distal fragments into the PstI polylinker site in p(−1.5/+0.8)GATA-6/lacZ. Similarly, the plasmids shown on lines 4 and 9–12 of Fig. 5 were made by cloning fragments into the XbaI polylinker site of p(−1.5/+0.8)GATA-6/lacZ (using XbaI linkers as needed). The plasmid p(−3.0/+0.8)GATA-6/lacZ (see line 2 of Fig. 5) was made by cloning the −3.0 kb/+19 bp BamHI fragment into the BamHI site of the pGATA-6/lacZ plasmid that contains the +19/+80 BamHI-SmaI fragment (described above). Last, the plasmid p(−4.1/+1.0)GATA-6/lacZ (see line 3 of Fig. 5) was made by cloning the −4.1/+1.0 NcoI-NotI fragment (with XhoI linkers) into the XbaI site of the pDP46.21 NotI vector.

*Cell Culture and Transient Transfection—* Primary cultures of cardiac fibroblasts and cardiac myocytes were prepared using a slightly modified protocol (33, 34). Chick embryo hearts were dissected from embryonic day 7 chick hearts and serially digested at 37 °C with fresh aliquots of 0.05% trypsin in calcium- and magnesium-free saline. After each 5-min digestion cycle, the sample was vortexed gently, hearts were allowed to settle, and the supernatant was collected. The supernatants from the first two cycles contained mostly cardiac fibroblasts; these were collected into growth medium (M199 medium, 10% fetal bovine serum (FBS), 10% tryptose phosphate, and 1% penicillin/streptomycin/kanamycin (PSK) antibiotics) and then passed through a 75-μm nylon cell strainer, centrifuged, and resuspended in the same medium. These cells were passaged three to four times (in T75 flasks; splitting 1:3 each time) in the same medium before being used for transfection assays (see below). The supernatants from the next five cycles contained mostly cardiac myocytes; after inactivating the trypsin (with M199 medium, 5% heat-inactivated FBS, and 1% PSK), the cells were passed through a 75-mm nylon cell strainer, centrifuged, and resuspended in M199 medium and 1% PSK for 12–18 h before being used for transfection assays (see below). These cardiac myocytes displayed spontaneous contractile activity in culture.

Cos-7 cells, which were obtained from American Type Culture Collection, were grown in Dulbecco’s modified Eagle’s medium, 10% FBS, and 1% PSK. Chicken LHm hepatoma cells (35) were grown inWaymouth’s medium, 10% FBS, and 1% PSK as originally described.

Transient transfections were performed using LipofectAMINE reagent (Life Technologies, Inc.). Experiments were processed according to the manufacturer’s instructions. The following volumes of LipofectAMINE per number of cells were found to be optimal for transfection assays and hence were used for the experiments described in this report: cardiac myocytes, 4.5 μl/1 × 10⁶ cells; cardiac fibroblasts, 4.0 μl/5 × 10⁶ cells; LMH cells, 5.5 μl/4 × 10⁶ cells; and COS-7 cells, 4.0 μl/10⁶ cells. The molar amounts of reporter plasmids were held constant for each experiment; differences in the weight amounts of DNA were adjusted (to a total of 2.0 μg) with a generic plasmid (pBluescript II KS+). When CAT test plasmids were used, 0.3 μg of pSV-β-galactosidase (Promega) was used as an internal control. When lacZ test plasmids were used, 0.2 μg of pGL3-control (Promega) served as the internal control. Cells were exposed to the transfection mixture for 6 h.

For cardiac fibroblasts, LMH cells, and COS-7 cells, the respective growth media (described above) were switched at this time. Cardiac myocytes were initially switched to M199 medium plus 2% heat-inactivated FBS and 1% PSK and were subsequently (12 h later) switched to M199 medium plus 1% PSK.

Transfected cells were harvested and lysed with reporter lysis buffer (Promega) after 48 h. Aliquots (2–10 μl) of cell extracts were assayed for luciferase activity in a Monolight 1000 luminometer (Analytical Luminescence Laboratory) using a Promega luciferase assay system kit. Aliquots (20–50 μl) of cell extracts were assayed for β-galactosidase activity in 96-well plates using a β-galactosidase enzyme assay system kit (Promega); data were quantified at 420 nm with a Rainbow Reader (SLT Lab Instruments). For CAT test constructs, after β-galactosidase assay, the rest of each extract was heated for 10 min at 65 °C. CAT activities for these samples were assayed with 14C using a standard chromatographic method. The CAT activity was measured within the linear range in each case.

*Hypersensitive Site Analysis—* DNase I hypersensitive (HS) sites were identified over the GATA-6 gene region using an established protocol (36). Chick heart and brain nuclei were prepared from day 12 embryos and digested with 0.1–1.0 μg/ml DNase 1 for 10 min at 37 °C. The DNA samples were isolated, digested with KpnI or HindIII and EcoRI, Southern-blotted, and probed with a random primer-labeled 3.0–2.7 HindIII-KpnI fragment.

*Production and Analysis of Transgenic Mouse Embryos—* The p(−9.2/+0.8)GATA-6/lacZ plasmid and the p(−1.5/+0.8)GATA-6/lacZ plasmid (see lines 4 and 1 of Fig. 5) were used to derive founder lines. The founder lines were crossed to the GATA-6/lacZ vector pDP46.21 NotI as described above. These GATA-6/lacZ inserts were isolated free of plasmid sequences by NotI digestion followed by preparative agarose gel electrophoresis. The inserts were purified using a QiAquick gel extraction kit (QIAGEN Inc.); serially extracted with phenol (three times), phenol/chloroform, and chloroform; and then precipitated with ethanol. The inserts were resuspended in phosphate-buffered saline.
Heart Region-specific GATA-6 Transgene Expression

**RESULTS**

**Isolation and Characterization of the Chicken GATA-6 Gene**—We previously isolated a chicken GATA-6 cDNA clone (12), and in the present study, we used this probe to isolate three overlapping chicken GATA-6 genomic clones. An analysis of these genomic clones revealed five GATA-6 coding exons (denoted E2–E7) (Fig. 1A) that are each flanked by consensus splice sites (Fig. 1B). Based on the results of a polymerase chain reaction analysis of genomic DNA (data not shown), we infer that the remainder of the GATA-6 open reading frame (which was not included in any of our genomic clones) is encoded by a single exon (denoted E7) (Fig. 1A).

The 5’-end of the chicken GATA-6 gene was mapped using primer extension and 5’-RACE assays. The GATA-6-specific antisense primer for these assays was directed against a unique sequence from what proved to be the second exon (E2) of this gene (Fig. 2, A and C). This primer yielded a 118-nucleotide cDNA product from stomach and liver (but not brain) mRNA samples. Note that the ladder does not correspond to the sequence of the first exon. C, the sequence of the largest 5’-RACE product (obtained using embryonic (day 6) mRNA as template) is in accord with the results of the primer extension analysis shown B. Note that the sequences above and below the dashed line represent sequences from the first and second exons (E1 and E2), respectively. The transcriptional start site (at position +1) and the BamHI site (at position +19) are in boldface (see also Fig. 1C).

There were several features that are typical of a promoter. For example, 1) the transcriptional start site (position +1) maps to a purine embedded within a pyrimidine-rich tract; 2) an AT-rich motif (albeit not a consensus TATA box) maps to the purine embedded within a pyrimidine-rich tract; 2) an AT-rich motif (albeit not a consensus TATA box) maps to the motif (albeit not a consensus TATA box) maps to the motif; 2) an AT-rich motif (albeit not a consensus TATA box) maps to the motif. In COS-7 cells, the promoter functioned in cardiac myocytes, and although less active in cardiac fibroblasts and LMH cells, these signals were clearly above the background levels of the promoterless pCAT plasmid (compare lines 4 and 5). In COS-7 cells, the GATA-6...
promoter plasmid was only 3-fold more active than the promoterless p/CAT plasmid.

The addition of progressively more distal sequences as well as intronic sequences to the core GATA-6 promoter plasmid increased transient expression to various extents in the four cell types assayed (Fig. 3B, compare lines 1–4). These results suggest that the core GATA-6 promoter is flanked by several distinct positive control regions. However, since the effect of any given nonpromotor fragment was rather modest (particularly in cardiac myocytes), we did not attempt to resolve the respective control regions further using this approach.

The GATA-6 Gene Is Flanked by Proximal and Distal HS Sites—The results of the preceding transient expression assays suggest that 1) the core GATA-6 promoter preferentially functions in cardiac myocytes; 2) a positive control region(s) maps within the first intron; and 3) at least two positive control regions map upstream of the minimal promoter. To begin to evaluate whether these (or more distal) control regions might regulate the endogenous GATA-6 gene in the heart, we exploited the fact that functional control regions are usually marked at the chromatin level by DNase I HS sites (40).

The probing strategy that we used to survey the GATA-6 gene region for HS sites is outlined in Fig. 4A. As indicated by the results shown in Fig. 4C, it is clear that HS sites mark the promoter and first intron (HS-2 and HS-3, respectively) in the heart, where the GATA-6 gene is expressed, but not in the brain, where the GATA-6 gene is silent. These results provide further support for a transcriptional control region(s) within the first intron of the GATA-6 gene; however, we cannot rule out that the activity of this region in transfection assays (Fig. 3B) is effected at a post-transcriptional level. The results of the HS site analysis presented in Fig. 4B also suggest that another control region(s) may reside ~6 kb upstream of the gene since this region is marked by a HS site (HS-1) in the heart, but not in the brain.

Transient Expression Assays Fail to Provide Evidence for a Classical GATA-6 Enhancer—To determine whether the distal and/or intronic HS sites mark classical enhancers, the respective GATA-6 genomic fragments were inserted downstream of the CAT gene in the pCAT promoter plasmid, which contains the SV40 promoter, and transfected into cardiac myocytes. Neither of these fragments displayed enhancer activity in this assay (data not shown).

We next addressed whether the GATA-6 gene might be flanked by distal control regions that function only in combination with the homologous GATA-6 promoter and/or other proximal control regions. Overlapping fragments that collectively span the ~9.2/–1.5 region were thus cloned into the parental p(−1.5/+0.8)GATA-6/lacZ plasmid, which includes several positive control regions in addition to the core GATA-6 promoter (Fig. 3B), and assayed in transfected cardiac myocyte cultures. The results of these transient expression assays are summarized in Fig. 5. Once again, we found no evidence for a classical enhancer associated with this gene. On the other hand, these results do suggest that a negative element lies within the ~3.0/–1.5 region since each of the three reporter plasmids that harbor this region displays 2-fold lower expression than the parental plasmid (compare lines 2–4 with line 1). This fragment also reduced expression 2-fold when assayed in the context of the heterologous pCAT control plasmid (data not shown).

A 10-kb Fragment That Spans the Chicken GATA-6 Promoter Directs Heart-restricted Expression in Transgenic Mice—Although transient expression assays have been used to detect a plethora of transcriptional control regions, negative results must be interpreted with caution. In particular, control regions that escape detection in transfection assays can sometimes be shown to function in transgenic assays (for example, see Ref. 41). With this in mind, we undertook to test whether a 10-kb...
fragment that spans the GATA-6 promoter and first intron as well as all three of the documented HS sites could function in transgenic mouse embryos.

Since lacZ reporter plasmids can be assayed in transgenic mice, the p(−9.2/−0.8)GATA-6/lacZ plasmid that we previously constructed (Fig. 5, line 4) was well suited for our purposes. As evidenced by the results shown in Fig. 6, this plasmid directed heart-restricted expression in both of the transgenic mouse embryos we obtained. In one embryo (panel A), the transgene was expressed only in the heart, whereas in the other embryo (panel E), the transgene was expressed at a few small sites in addition to the heart. A sagittal section of the former embryo is shown in panel B; a more detailed view of the heart region is shown in panel C. Note that intense staining is seen in the myocardial layer of the ventricle (v) and in the proximal region of the outflow tract (ot). In contrast, expression of the transgene was more sporadic in the atrium (a). A parasagittal section through the same embryo (panel D) shows that the transgene is also expressed in the myocardial layer of the atrioventricular canal (av) that lies between the primitive atrium and ventricle.

The Chicken GATA-6 Promoter-proximal Region Directs Expression Exclusively to the Atrioventricular Canal of Transgenic Mice—In light of the failure of transient expression assays to provide evidence for a distal control region associated with the GATA-6 gene, it was of interest to determine if distal sequences contributed to the heart-restricted expression of the p(−9.2/−0.8)GATA-6/lacZ transgene. The p(−1.5/−0.8)GATA-6/lacZ plasmid (Fig. 5, line 1) was suited to resolve this issue and was used accordingly. Remarkably, all three of the resultant transgenic mouse embryos expressed this truncated transgene in a similar highly restricted manner (Fig. 7). In particular, as shown in panels A–C, expression of this transgene was confined in each case to a small region of the heart (assayed around embryonic day 11). Sagittal sections of the hearts of these transgenic embryos (panels D–F) revealed that expression was restricted in each case to myocardial cells that are confined to the atrioventricular canal and thus overlie the endocardial cushion.

DISCUSSION

Since avian, amphibian, and mammalian species display similar tissue-restricted patterns of GATA-6 gene expression, we anticipated that the chicken GATA-6 gene region might contain tissue-restricted control regions that are sufficiently well conserved to function in transgenic mice. Indeed, we obtained evidence for two such control regions within a 10-kb chicken GATA-6 genomic fragment. However, these two control regions did not function in two different tissues, as might have been expected considering the rather complex pattern of endogenous GATA-6 expression, but rather in two different regions of one organ (heart) that expresses the endogenous GATA-6 gene.

The chicken GATA-6 distal control region(s) was found to be required for expression in the ventricle and outflow tract segments of the heart in transgenic mouse embryos. The expression pattern of the GATA-6/lacZ transgene that includes this distal control region is similar to the transgenic pattern dictated by the HF-1 control region from the mouse MLC-2v gene (5). Since the latter control region contains a critical MEF2 site, one might also expect to find an MEF2 site within the GATA-6 distal control region. These control regions are distinct in other respects, however, and may use different means to effect similar expression patterns. For example, the inactivity of the GATA-6 control region in transfection assays stands in contrast to the MLC-2v control region. Also, the GATA-6 control region is located far upstream of the gene, whereas the HF-1 control region lies within the MLC-2v promoter.

The chicken GATA-6 proximal control region(s) was found to direct a unique expression pattern in transgenic mouse embryos. In particular, the truncated GATA-6/lacZ transgene was only expressed in myocardial cells that reside within the AV canal. These cells are known to play a special role in heart development; namely, they induce the underlying endocardial cells to undergo an epithelial to mesenchymal transition (42, 43). This transition serves to restructure the endocardial cushion and to initiate valve formation. Although several endogenous genes are known to be preferentially expressed in AV myocardial cells (44–46), it is not known how any of these genes are regulated at the molecular level. We presume that the GATA-6/lacZ transgene is regulated in response to the same signal(s) that regulates these endogenous genes. However, since two of the latter genes (BMP-2 and BMP-4) encode growth factors that can induce the expression of another GATA gene (GATA-4) earlier in the general cardiogenic program (2), it is possible that these growth factors function in an autocrine manner to regulate the GATA-6/lacZ transgene in these specialized AV myocardial cells. In any event, it should be possible to resolve how this precise heart region-specific control is achieved at the molecular level since the relevant control region(s) must lie within a 2.3-kb GATA-6 genomic fragment. This is a topic of great biomedical relevance since valve abnor-
malities are a major cause of congenital birth defects.

Whereas the larger GATA-6 genomic fragment directed robust expression in the primitive ventricle, this fragment functioned poorly in the primitive atrium. The ability of the endogenous GATA-6 gene to be expressed throughout the primitive heart may thus require other control regions that reside outside the 10-kb segment assayed in this study. Alternatively, the poor expression of this transgene in the atrium may be related to the use of a heterologous (chick/mouse) transgenic assay. The basis for the lack of transgene expression in other tissues (such as gut) that express the endogenous GATA-6 gene is similarly ambiguous. Whereas homologous (mouse/mouse) transgenic assays may resolve some of these uncertainties, there is obviously no guarantee that the relevant control regions will lie within a fragment of comparable (10 kb) size. Indeed, a much larger (20 kb) fragment that spans the promoter for the heart-restricted chicken GATA-5 gene failed to direct heart-restricted expression in transgenic mouse embryos.2 Thus, despite the fact that the GATA-4/5/6 genes display similar expression patterns, the spatial arrangements of the respective control regions may be quite distinct.

In contrast to numerous heart-restricted control regions that have been reported in the literature (5, 6, 47, 48), the GATA-6 distal control region(s) does not appear to function in transfaction assays. Several possible explanations can be advanced to account for why this control region might function in ventricular myocytes of transgenic mice, but not in transfected ventricular myocytes. For example, the control region may effect a change in methylation (49) or alter the organization of a chromatin domain (50). Alternatively, the control region may only function during a particular stage of development (51). Future studies will be directed at evaluating these possibilities. The present results also underscore the question of whether transgenic assays will be required to find evidence for control regions associated with other heart-restricted transcription factor genes.

We do not know if the GATA-6 proximal control region, which directs AV canal-specific expression in transgenic mice, can function in transfaction assays. This is a difficult issue to address since AV myocardial cells would have to be isolated in reasonable quantities, and the transcriptional milieu that is unique to these cells would have to remain stable in culture. Whereas efforts to analyze these specialized myocardial cells have been hampered by the lack of a unique molecular marker, it may be possible to circumvent this technical limitation by creating lines of transgenic mice that express markers of inter-

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2 C. MacNeill and J. B. E. Burch, unpublished result.
est under the control of the GATA-6 proximal control region. This line of investigation is currently in progress.

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