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Cloning, Partial Sequencing, and Expression of Glyceraldehyde-3-phosphate Dehydrogenase Gene in Chick Embryonic Heart Muscle Cells*

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Two recombinant plasmids containing structural gene sequences of chick embryonic heart glyceraldehyde-3-phosphate dehydrogenase (GAP dehydrogenase) were constructed and characterized. The plasmids pGAP 30 and pGAP 36 have inserts of 1200 and 950 base pairs, respectively. The identity of the clones was established by hybrid-arrested and hybrid-selection translation assays, and by immunoprecipitation of hybrid-selected translation product with GAP dehydrogenase antiserum. Hybridization of labeled pGAP 30 DNA to size-fractionated chick heart poly(A) RNA occurred at the region on the gel corresponding to the mobility of GAP dehydrogenase mRNA. Base sequence analysis of plasmid pGAP 30 and the comparison of the amino acid sequence derived from it with that of pig muscle GAP dehydrogenase revealed that the amino acid sequence of GAP dehydrogenase is strictly conserved between the chick and pig muscle tissues. Expression of GAP dehydrogenase mRNA in developing chick heart cells in cultures was monitored by *in situ* hybridization. The GAP dehydrogenase mRNA was present in 5-h-old dividing myoblasts, in contrast to mRNAs specific for contractile proteins, which appear late in myoblast development paralleling morphogenetic differentiation of myoblasts into myocytes (Jakowlew, S. B., Khandekar, P., Datta, K., Narula, S. K., Arnold, H. H., and Siddiqui, M. A. Q. (1982) *J. Mol. Biol.* 156, 673-682).

The regulation of the glycolytic pathway by modulating enzyme activities is well established (1). One of the enzymes known to be present in several cell types in varying concentrations, depending upon the physiological state of the cell, is GAP dehydrogenase.[1] Although extensive data on enzymology and structural properties, including the amino acid sequence, of GAP dehydrogenase from various sources is available (2-7), little is known of the molecular events controlling its synthesis and regulation. The limited knowledge of the structure of GAP dehydrogenase gene and its characterization is restricted to the lower eukaryote, yeast, which contains a high concentration of the enzyme (5). Multiple polymorphic forms of GAP dehydrogenase have been identified in yeast (7-10). Recent evidence suggests that as many as three structural genes code for yeast GAP dehydrogenase, two of which are nontandemly repeated (8).

In muscle cells, where the enzyme plays a crucial role for energy supply, the activity and amount of GAP dehydrogenase is modulated with the developmental and physiological state of the cell (1, 11). Whether these variations in the enzymatic activity are transcriptionally and/or translationally controlled is not known. We have utilized chick embryonic cardiac muscle cell differentiation as a model system to investigate the molecular aspects of early embryonic gene functions and their regulation, since it offers a unique advantage in the availability of a population of cells which are predetermined and undifferentiated. These cells can be grown successfully in the laboratory to differentiate into well defined cardiac muscle tissue. In order to relate the synthesis of muscle-specific proteins during muscle cell differentiation to changes in gene activity, we have isolated, purified, and characterized the mRNAs for muscle contractile proteins, myosin light and heavy chain polypeptides. The DNA complementary to these mRNAs were subsequently cloned in *Escherichia coli* in our laboratory (12-14). In this report, we describe the construction and characterization of recombinant plasmids, pGAP 30 and pGAP 36, containing GAP dehydrogenase-specific gene sequences. Using the cloned DNA as a probe, we examined the appearance of GAP dehydrogenase mRNA in primary heart cell cultures by *in situ* hybridization.

EXPERIMENTAL PROCEDURES

White Leghorn chick eggs and embryos were purchased from the local animal farms at Hamburg, Germany, and in New Jersey. Heart tissue was excised from the embryos of desired stages, frozen immediately in liquid N_2, and stored at −80 °C until used. Oligo(dT)-cellulose (type 3) was purchased from Collaborative Research. Restriction endonucleases, terminal deoxynucleotidyl transferase, and phage DNAs were purchased from Bethesda Research Laboratories. S1 nuclease and nonlabeled deoxynucleotide triphosphates were from Boehringer Mannheim. Labeled nucleotide triphosphates and methionine were from Amersham Corp. Avian myeloblastosis viral reverse transcriptase was kindly supplied by Dr. J. W. Beard of Life Sciences, Inc., St. Petersburg, FL. Anti-rabbit GAP dehydrogenase serum raised in sheep was kindly provided by Dr. Pette, University of Konstanz, Germany.

Construction of Recombinant Plasmid—Total RNA was isolated from the frozen chick heart tissue, and poly(A) RNA was prepared by two to three passages through oligo(dT)-cellulose columns as described earlier (12). The poly(A) RNA was then fractionated by sedimentation through isokinetic sucrose gradient (15–35.9%) after denaturation in dimethyl sulfoxide (15). The RNA recovered from

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[1] The abbreviations used are: GAP dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase; DBM, diazobenzyl-methyl.

each fraction was tested by translation in nuclease-treated rabbit reticulocyte lysate (16) as described earlier (12). Fractions sedimenting between 14–18 S on the gradient that were enriched in actin and tropomyosin mRNAs also contained GAP dehydrogenase mRNA activity. RNA was recovered from these fractions and used for synthesis of cDNA as described earlier (13). The ds-cDNA was used to transform *E. coli*, strain X1776, by dA-dT tailing method using plasmid pBR322 (13). Thirty seven transformants, which were tetracycline-sensitive, were obtained with 50 ng of chimeric plasmid DNA.

Hybridization of mRNA to Cloned DNA Covalently Linked to DBM Paper—Twenty micrograms of plasmid DNA linearized with restriction endonuclease *Eco* RI was coupled to DBM filters according to the procedure of Goldberg *et al.* (17) and Paterson *et al.* (18). Poly(A) RNA (10–20 μg) from chick embryonic heart muscle was hybridized to the filter-bound DNA in 50% formamide, 0.1 M phosphate buffer (pH 7.0), 1 mM EDTA, 0.6 M NaCl, 0.06 M sodium citrate, and 0.1% sodium dodecyl sulfate. Hybridization was done for 5 h at 52 °C with shaking. The paper was then washed five times with 50% formamide in 1 mM EDTA, 10 mM Tris-HCl (pH 7.4), and 0.5% sodium dodecyl sulfate at 37 °C. The bound RNA was eluted by heating to 70 °C for 2 min in 0.5 ml of the buffer above containing 98% formamide.

Hybrid-arrested Translation—One microgram of purified plasmid DNA was digested with *Eco* RI and then hybridized with 1.0 μg of poly(A) RNA from the 16-day-old chick embryonic heart tissue according to Paterson *et al.* (18) and as described earlier (13).

Restriction Enzyme Analysis—DNA was digested with restriction endonucleases according to the procedures provided by the suppliers. The completion of the digestion was monitored using λDNA as standard. Digested and undigested plasmid DNAs were examined by electrophoresis on 0.8% and 1.0% agarose horizontal slab gels or on 5% polyacrylamide gels using *Hin*dIII fragments of λDNA and *Hae* III fragments of ØX174 DNA as markers. DNA bands were visualized by UV light after staining with ethidium bromide.

In Situ Hybridization—For *in situ* hybridization, chick heart cells from 12-day-old embryos were grown in primary culture (19) in Lab-Tek (Miles Laboratories Inc.) tissue culture slide chambers. Hybridization was done with labeled DNA probes as described earlier (14).

Hybridization to Size-fractionated Poly(A) RNA—Poly(A) RNA from 16-day-old chick embryonic heart tissue or total RNA from cultured human lymphocytes was fractionated on 1.5% agarose slab gels containing 10 mM methylmercury hydroxide (20). The RNA was then transferred to DBM paper according to Alwine *et al.* (21), except that the buffer was sodium acetate, pH 4.0. After transfer, the paper-bound RNA was hybridized to nick-translated DNA probes (21, 22).

DNA Sequencing—pGAP 30 plasmid DNA was digested with *Hin*dIII, and the resulting fragments were 5′-end labeled with [γ-^{32}P] ATP, after treatment with calf intestine alkaline phosphatase. Subsequent digestion with *Eco* RI and *Hin*dIII yielded two terminally labeled fragments which were isolated and sequenced according to Maxam and Gilbert (23). The additional 260-base pair internal *Hin*dIII fragment was strand-separated, and both strands were sequenced.

Heteroduplex Formation—Heteroduplexes were prepared and mounted for electron microscopy by the formamide procedure according to Davis *et al.* (24). The DNA was examined using a Philips EM 201 electron microscope. A numonic digitizer mapping unit was used for size analysis.

RESULTS

Construction and Identification of Recombinant Plasmids Containing GAP Dehydrogenase-specific DNA Sequences—The RNA fractions sedimenting between 15–18 S markers on isokinetic sucrose gradient (see under "Experimental Procedures") were enriched in mRNA directing the synthesis of proteins of 35,000–45,000 daltons. The mRNA from these fractions were pooled and used for the synthesis of ds-cDNA and cloning in *E. coli*, strain X1776, using the plasmid pBR322. Although the enrichment for GAP dehydrogenase mRNA activity was only 3- to 5-fold, it was possible to select the potential GAP dehydrogenase-specific transformants by eliminating most of the transformants for abundant mRNAs, *e.g.* myosin light chains and actin. Initial screening was performed using ^{32}P-labeled 15–18 S poly(A) RNA, and myosin light chains and actin clones were then eliminated by screening with the respective cloned DNA probes. Based on hybrid-selection translation (see below) of the remaining clones, two transformants, pGAP 30 and pGAP 36, were chosen as candidates for GAP dehydrogenase-specific clones for further characterization.

The identity of the DNA inserts in pGAP 30 and pGAP 36 was documented by translation of mRNA selected by annealing to DNA isolated from the plasmids. The DNA linearized

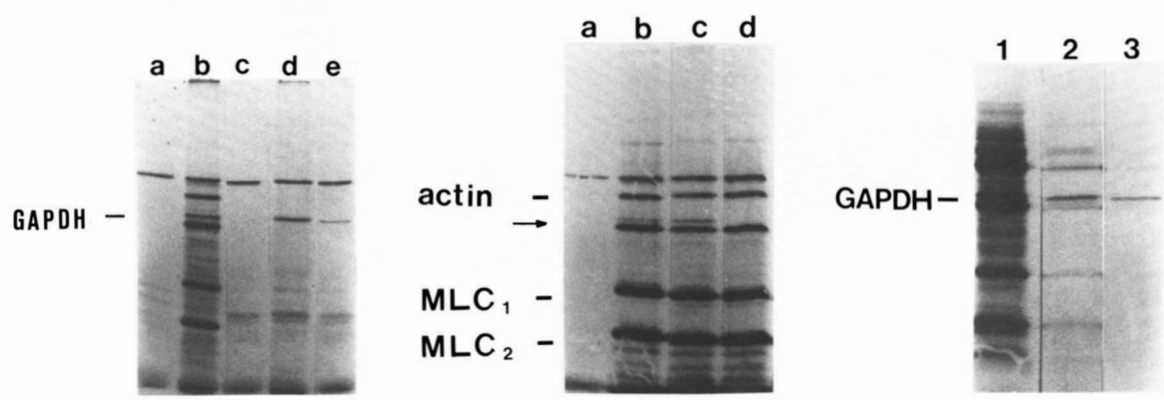

FIG. 1. **Hybrid-selection translation and identification of translation products.** *Left*, hybrid-selection translation. Plasmid DNA (10 μg) which was linearized, denatured, and coupled to DBM paper (20) was hybridized to 5 μg of total chick heart poly(A) RNA. The paper-bound RNA was eluted and translated in mRNA-dependent rabbit reticulocyte lysate as described under "Experimental Procedures." *a*, nuclease-treated lysate; *b*, cytoplasmic poly(A)$^+$ chick heart RNA without prior hybridization; *c*, RNA hybridized to plasmid pBR322 DNA; *d*, RNA hybridized to plasmid pGAP 30 DNA; *e*, RNA hybridized to plasmid pGAP 36 DNA. *Center*, immunoprecipitation of translation products of RNA hybridized to pGAP 30 DNA. The RNA eluted from the DBM-bound pGAP DNA was translated in rabbit reticulocyte lysate as described under "Experimental Procedures." The product was immunoprecipitated with antiserum made against rabbit GAP dehydrogenase. *1*, cell-free products directed by total chick heart mRNA; *2*, immunoprecipitate from total chick heart mRNA-directed translation products with rabbit GAP dehydrogenase antiserum; *3*, immunoprecipitate from hybrid-selected mRNA-directed translation products with rabbit GAP dehydrogenase antiserum.

FIG. 2 (*right*). **Hybrid-arrested translation of chick heart poly(A) RNA.** One microgram of chick heart total poly(A) RNA was hybridized with pGAP 30 DNA (1.0 μg) and subjected to translation in nuclease-treated rabbit reticulocyte lysate as described under "Experimental Procedures." The products were analyzed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and examined by fluorography (13). *a*, endogenous background of the lysate; *b*, total poly(A)$^+$ chick heart RNA; *c*, total poly(A)$^+$ chick heart RNA hybridized to DNA from plasmid pBR322; *d*, total poly(A)$^+$ RNA hybridized to DNA from plasmid pGAP 30. The *arrow* indicates the position of rabbit muscle GAP dehydrogenase. *MLC*, myosin light chain.

with restriction enzyme *Eco* RI and covalently bound to DBM paper was hybridized with total poly(A) RNA from the 16-day-old chick embryonic heart (21). The RNA hybridized to DNA bound to paper was eluted and translated as before. Fig. 1 (*left*) (*lanes d* and *e*) shows that the RNAs eluted from plasmid pGAP 30 and pGAP 36 DNA yielded a polypeptide that migrates identically with nonlabeled GAP dehydrogenase from rabbit muscle. The fast moving minor bands are apparently nonspecifically bound RNA products, since they were present in pBR322 DNA-bound filter papers (*lane c*) and in control samples (*lane a*). We have also examined the translation product of the hybrid-selected mRNA by two-dimensional electrophoretic separation (12). The migration pattern of the hybrid-selected peptide was identical with that of nonlabeled GAP dehydrogenase from rabbit muscle (data not shown). When the *in vitro* translation product was incubated with sheep antiserum against rabbit GAP dehydrogenase, the immunoprecipitated product migrated as 36,000-dalton protein on sodium dodecyl sulfate-polyacrylamide gel (Fig. 1 (*center*)). High conservation of the amino acid sequence was reported for GAP dehydrogenase enzymes from organisms as diverse as lobster and pig (3); hence the rabbit GAP dehydrogenase antiserum cross-reaction with chicken GAP dehydrogenase was not surprising. Additional bands of lower intensity when total poly(A) RNA was used (Fig. 1 (*center*), *lane 2*) were due to nonspecific binding.

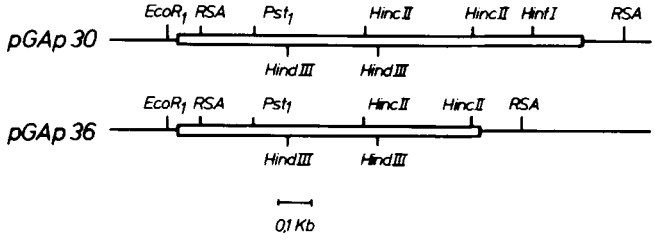

FIG. 3. **Partial restriction map for plasmids pGAP 30 and pGAP 36.** Plasmid DNAs were digested by restriction enzymes indicated, and the resulting fragments were analyzed on 0.8% agarose gel as described in text. *Eco* RI and *Bam* HI did not cleave the plasmid DNA. *kb*, kilobase pairs.

The ability of plasmid DNAs to arrest the translation of GAP dehydrogenase mRNA in total poly(A) RNA was tested next. As shown in Fig. 2 (*right*), the DNA from the clone pGAP 30 specifically abolished the translation of a 36,000-dalton polypeptide (*lane d*) which migrates identically with the authentic GAP dehydrogenase on 10% sodium dodecyl sulfate-polyacrylamide gel. Prehybridization with control plasmid pBR322 DNA (*lane c*) and mock hybridization, *i.e.*

				200									210
GGC	AGA	GGU	GCU	GCC	CAG	AAC	AUC	AUC	CCA	GCG	UCC	ACU	GGG	GCU
Gly	Arg	Gly	Ala	Ala	Gln	Asn	Ile	Ile	Pro	Ala	Ser	Thr	Gly	Ala
									220					
GCU	AAG	GCU	GUG	GGG	AAA	GUC	AUC	CCU	GAG	CUG	AAU	GGG	AAG	CUU
Ala	Lys	Ala	Val	Gly	Lys	Val	Ile	Pro	Glu	Leu	Asn	Gly	Lys	Leu
				230									240
ACU	GGA	AUG	GCU	UUC	CGU	GUG	CCA	ACC	CCC	AAU	GUC	UCU	GUU	GUU
Thr	Gly	Met	Ala	Phe	Arg	Val	Pro	Thr	Pro	Asn	Val	Ser	Val	Val
									250					
GAC	CUG	ACC	UGC	CGU	CUG	GAG	AAA	CCA	GCC	AAG	UAU	GAU	GAU	AUC
Asp	Leu	Thr	Cys	Arg	Leu	Glu	Lys	Pro	Ala	Lys	Tyr	Asp	Asp	Ile
				260									270	
AAG	AGG	GUA	GUG	AAG	GCU	GCU	GCU	GAU	GGG	CCC	CUG	AAG	GGC	AUC
Lys	Arg	Val	Val	Lys	Ala	Ala	Ala	Asn	Gly	Pro	Leu	Lys	Gly	Ile
									280					
CUA	GGA	UAC	ACA	GAG	GAG	CAG	GUU	GUC	UCC	UGU	GAC	UUC	AAU	GGU
Leu	Gly	Tyr	Thr	Glu	Asp	Gln	Val	Val	Ser	Cys	Asp	Phe	Asn	Gly
				290									300	
GAC	AGC	CAU	UCC	UCC	ACC	UUU	GAU	GCG	GGU	GCU	GGC	AUU	GCA	CUG
Asp	Ser	His	Ser	Ser	Thr	Phe	Asp	Ala	Gly	Ala	Gly	Ile	Ala	Leu
									310					
AAU	GAC	CAU	UUC	GUC	AAG	CUU	GUU	UCA	UGG	UAU	GAC	AAU	GAG	UUU
AsN	Asp	His	Phe	VAL	Lys	Leu	Val	Ser	Trp	Tyr	Asp	AsN	Glu	Phe
				320									330	
GGA	UAC	AGC	AAC	CGU	GUU	GUG	GAC	UUG	AUG	GUC	CAC	ACG	GCA	UCC
Gly	Tyr	Ser	AsN	Arg	Val	Val	Asp	Leu	Met	Val	His	Thr	Ala	Ser
AAG	GAG	UGA												
Lys	Glu													

FIG. 4. **Partial mRNA sequence of GAP dehydrogenase derived from plasmid pGAP 30.** The internal *Hin*dIII fragment of 260 base pairs together with the flanking sequences to the left and to the right were sequences as described under "Experimental Procedures." The corresponding amino acid sequence is given using the only possible open reading frame. Amino acids different from their counterparts in the pig enzyme are underlined by *solid bars*. The Asp to Asn variation is indicated by a *broken line*.

without DNA (*lane b*), resulted in translation of all polypeptides expected. Identical results were obtained with pGAP 36 DNA (data not shown).

DNA Base Sequence Analysis—Final proof that the cloned plasmids contain inserts coding for chicken GAP dehydrogenase mRNA came from the base sequence analysis of plasmid pGAP 30. Prior to DNA sequencing, the restriction sites for various endonucleases were determined for plasmids pGAP 30 and pGAP 36 according to Smith and Birnstiel (26). The insert sizes for these clones were estimated to be 1200 and 950 base pairs, respectively. The restriction maps were identical for the two plasmids with the exception of one *Hin*fI site which was missing from pGAP 36 (Fig. 3). The internal 260-base pair *Hin*dIII fragment and its surrounding sequences were analyzed according to Maxam and Gilbert (23). The partial mRNA sequence derived from the pGAP 30 DNA and the 136 amino acids deduced from the mRNA (Fig. 4) showed extensive homology to the GAP dehydrogenase enzymes from pig and lobster (3). Out of 136 amino acids, 126 were identical with the pig enzyme (92% conservation); a slightly lower extent of homology (75%) exists between the yeast and chicken enzyme in the same region (25). The sequence analysis also indicates the orientation of the mRNA within the cloned sequence. The 3′-end of GAP dehydrogenase mRNA is located on the left side of the restriction map, near the *Eco* RI site of pBR322, whereas the 5′-end of the clone is located toward the right, close to the RSA site of pBR322 (see Fig. 3).

Heteroduplex Analysis—Heteroduplex of pGAP 30 and pGAP 36 were made after linearizing the plasmid DNA with restriction enzyme Sal I. The representative micrographs are shown in Fig. 5. The sizes of pGAP 30 and pGAP 36 inserts represented by the deletion loops were 1.2 ± 0.08 and 0.95 ± 0.08 kilobase pairs, respectively, which agrees with the restriction data. The positions of the loops map at the same distance from the Sal I site as expected.

Hybridization to Size-fractionated Poly(A) RNA—To ascertain whether GAP dehydrogenase-specific mRNA sequences can be identified in a mixed population of chick heart muscle mRNA, nick-translated pGAP 30 [^{32}P]DNA was hybridized to increasing concentrations of total chick heart poly(A) RNA which was fractionated on 1.5% methylmercury hydroxide gel and transferred to DBM paper. As shown in Fig. 6, hybridization was observed predominantly to an RNA of about 12 S size (*lane b, panel B*) expected of GAP dehydrogenase mRNA. The fast moving hybridizable material was due to degradation of RNA. The intensity of hybridization was clearly dependent on the concentration of RNA. The ^{32}P-labeled pGAP 30 DNA did not hybridize to RNA from human lymphocytes (*lane f, panel B*).

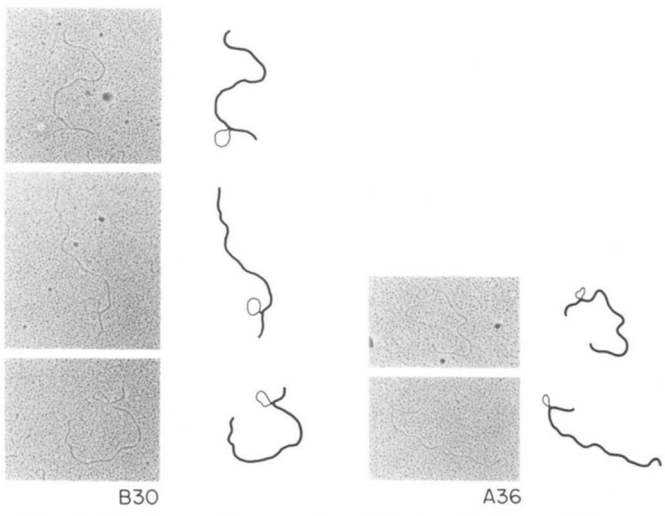

FIG. 5. **Electron micrographs of heteroduplexes between pGDH30, pGDH36, and pBR322 DNAs.** Heteroduplexes were formed between pBR322 and pGAP30 DNA (*B30*) and pGAP36 DNA (*A36*) after linearizing the DNAs with Sal I as described under "Experimental Procedures." The diagrammatic representations are shown on the right. *Left*, pGAP30; *right*, pGAP36.

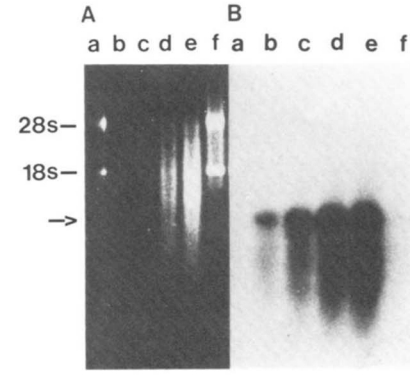

FIG. 6. **Hybridization of labeled pGAP 30 DNA to size-fractionated RNA.** ^{32}P-labeled pGAP 30 DNA (2×10^6 cpm) was hybridized to various RNAs fractionated on a methylmercury hydroxide gel as described under "Experimental Procedures." *A*, the gel, which was stained with ethidium bromide; *B*, the autoradiograph of the RNA-bound paper after hybridization with ^{32}P-labeled pGAP 30 DNA. *a*, 2 μg of 28 S and 18 S RNA; *b–e*, 1 μg, 2 μg, 4 μg, 6 μg of poly(A)$^+$ RNA from embryonic chick heart; *f*, 10 μg of total RNA from human lymphocytes.

In Situ Hybridization to RNA in Chick Heart Cells in Culture—To examine the appearance of GAP dehydrogenase mRNA in these cells, nick-translated pGAP 30 [^{3}H]DNA was hybridized *in situ* to chick embryonic heart cells in culture at different stages of development (Fig. 7 (*left*)). The specificity of hybridization was demonstrated by the clear differences in intensity of silver grains between the labeled pBR322 and pGAP 30 DNA probes. Prior treatment of cells with pancreatic RNAse also caused a significant loss of silver grains (Fig. 7 (*right*)). These results indicate that pGAP 30-specific mRNA can be detected in as early as 5-h-old single myoblast cells in culture, suggesting that GAP dehydrogenase synthesis is an early event during development of cardiac muscle cells in culture. In contrast, myosin heavy chain mRNA appears only after 48 h of incubation, paralleling the morphogenetic differentiation and appearance of myofibrillar structure in cardiac myoblasts (14).

DISCUSSION

We have constructed and characterized two recombinant DNA plasmids, pGAP 30 and pGAP 36, that contain the structural gene for the chick heart GAP dehydrogenase. The size of the larger of the two inserts (plasmid pGAP 30) is 1200 base pairs. Based on the amino acid sequence data from various sources, the enzyme is a single molecular species of about 335 amino acids with a molecular weight of 36,000 daltons (2–4). This agrees with our estimation of the size of GAP dehydrogenase mRNA from the chick heart tissue. Since 1005 nucleotides are required to code for 335 amino acids, the plasmid pGAP 30 DNA should encompass all the 3′ noncoding and presumably the whole coding sequence. The plasmid pGAP 36 is about 250 base pairs shorter than pGAP 30. Since the orientation of the inserts is identical in both plasmids, we conclude that pGAP 36 lacks the 5′-end sequence.

The identity of the plasmids was established by several criteria. The Amp^r transformants were screened with both the cDNA made from GAP dehydrogenase-enriched mRNA frac-

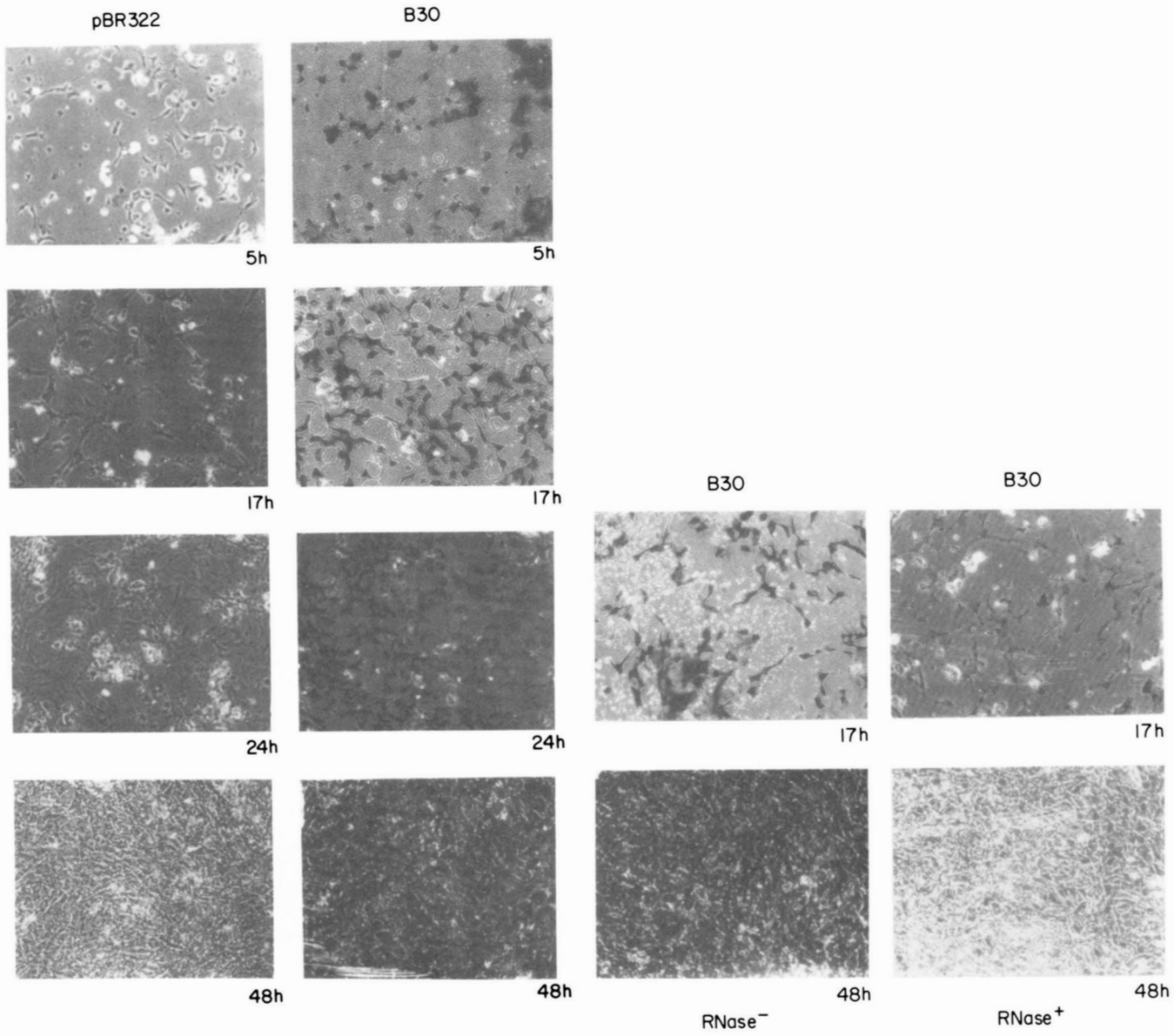

FIG. 7. ***In situ* hybridization.** Twelve-day-old chick embryonic heart cells were grown in culture, immobilized, and processed for *in situ* hybridization with nick-translated ^{3}H-labeled pGAP 30 DNA as described under "Experimental Procedures" and elsewhere (14). *Left*, pBR322 and pGAP 30 (*B30*) DNAs were hybridized to cells in culture at times indicated; *right*, cells were treated with pancreatic RNase prior to hybridization with ^{3}H-labeled pGAP 30.

tion and the cloned DNA probes specific for myosin light chains and actin, the two major constituent mRNAs of the GAP dehydrogenase-enriched poly(A) RNA fraction. The potential GAP dehydrogenase-specific clones were then examined by hybrid-arrested and hybrid-selection translation assays. The hybridization of labeled pGDH30 DNA to the size-fractionated chick heart poly(A) RNA occurred only at the region on the gel expected for GAP dehydrogenase mRNA size. These results combined with the specific immunoprecipitation of translation product with antiserum against pig GAP dehydrogenase established the authenticity of the GAP dehydrogenase-specific plasmids.

GAP dehydrogenase from different tissues of the same organism are structurally very similar (27), and the enzyme from a variety of sources share extensive homology. As such, the immunological cross-reaction between the chick embryonic heart tissue and the rabbit GAP dehydrogenase is not surprising. We have determined the nucleic acid sequence of a large part of plasmid pGAP 30 DNA. Using the only possible open reading frame, we derived the amino acid sequence of 136 amino acids from the COOH terminus of chicken GAP dehydrogenase. The correspondence between the amino acids coded for by plasmid pGAP 30 and the amino acid sequence of pig GAP dehydrogenase reflects the conserved nature of GAP dehydrogenase enzymes. The 3′ noncoding sequence of chicken GAP dehydrogenase mRNA is about 230 nucleotides in length (unpublished results). A comparison of the coding and noncoding regions by cross-hybridization analysis of the DNA sequences of the respective isolated genes would establish the extent of homology between different tissues and organisms.

Chick embryonic heart cells in culture, which undergo a distinct morphogenetic differentiation, could, in principle, provide a useful experimental system to investigate the molecular events associated with cardiac muscle differentiation. Recent observations on appearance of mRNAs for the cardiac muscle structural proteins suggest that these proteins are synthesized coordinately by the burst of corresponding mRNAs at the

time of myoblast differentiation (28, 29). Using *in situ* hybridization assay with a probe specific for myosin heavy chains, we observed a striking difference in appearance of hybridizable RNA between dividing myoblasts and the differentiated 4-day-old myocytes (14). This suggested that myosin heavy chain mRNA is transcribed after 48 h of incubation paralleling morphogenetic differentiation of myoblasts into myocytes. In contrast to myosin heavy chains, however, the GAP dehydrogenase-specific transcripts were apparently present in cells as early as 5-h-old myoblasts, suggesting that expression of GAP dehydrogenase genes precedes that of myosin. Using *in vitro* translation assay and hybridization of poly(A) RNA with a cloned DNA probe, it was recently shown that the mRNAs coding for GAP dehydrogenase in different embryonic tissues are structurally similar (27). Little is known, however, of the control of these developmentally regulated proteins. The availability of specific DNA probes for GAP dehydrogenase and myosin polypeptides will thus facilitate the analysis of the mechanism underlying the activation of gene transcription during early embryonic development.

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