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Glyceraldehyde 3-phosphate dehydrogenase protein and mRNA are both differentially expressed in adult chickens but not chick embryos

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ABSTRACT

We have determined the 679 nucleotide sequence of a cDNA clone which, by hybridization-translation experiments, corresponds to a 36K chick brain protein. Our studies provide a partial amino acid sequence for this protein, identifying it as chicken glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Antisera raised against purified chicken GAPDH reacted with a 36K protein present in chick brain extracts and estimated to be the fourth most prevalent protein, as determined by either Coomassie Blue staining or by in vitro translation of chick brain mRNA. The amounts of GAPDH mRNA in chick brain, liver and muscle and adult chicken brain are similar, whereas the relative amount of adult chicken muscle GAPDH mRNA is greatly elevated and that of adult liver lowered. The GAPDH protein levels showed a similar variation between tissues, suggesting that the levels of GAPDH protein are largely regulated by the amount of available GAPDH mRNA. The chicken GAPDH clone does not hybridize to rat mRNA, even though GAPDH is one of the most evolutionarily conserved proteins, indicating that selection pressures are heavier at the primary protein sequence level than at the nucleic acid sequence level for this gene, a situation contrasting to that of the tubulins.

INTRODUCTION

The developing chick brain expressed a number of abundant messenger RNA species, including mRNAs coding for tubulin and actin. We and our colleagues have generated cDNA clones corresponding to the mRNAs for actin and α and β tubulin and have shown these to be the three most abundant mRNA species in the developing chick brain (1-3). In the course of these studies, other abundant mRNAs have been cloned. We initially intended to use one of these, clone p13, corresponding to a prevalent chick brain mRNA to monitor our cloning of cDNA copies of rat brain specific mRNA. However, p13 did not hybridize in Northern blots to any rat mRNA species. We were curious as to the identity of a major avian brain mRNA species which had no homologue in the mRNA of a mammal, and so determined the nucleotide sequence of the cloned cDNA. The nucleotide sequence contained a long open triplet reading frame which, when translated, was found to encode the N-terminal two-thirds of the

glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Further studies with the cDNA clone and an antibody to purified chicken GAPDH show that both the mRNA and protein are abundantly expressed in the brain, liver and muscle of 17 day embryos. In the adult chicken, however, in the same three tissues there is a substantial regulation of the expression of GAPDH mRNA and protein.

EXPERIMENTAL

p13 represents chicken GAPDH mRNA

The cDNA clone p13 was constructed (2) by oligo dT priming the reverse transcription of polyA$^+$ RNA from chick embryo brain, followed by treatment with DNA polymerase I and S1 nuclease. The resulting double stranded cDNA was ligated to HindIII linkers, cleaved with the restriction endonuclease HindIII, and cloned in the HindIII site of pBR322.

We characterized the protein encoded by the cloned mRNA using a hybridization-translation assay (2). Chick embryo brain polyA$^+$ RNA was hybridized to p13 DNA bound to nitrocellulose filters. After extensive washing, the specifically bound RNA was released and used to program an *in vitro* translation system and the ^{35}S-methionine labeled products were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1). Unfractionated chick brain polyA$^+$ RNA programs the synthesis of many proteins (in addition to the translation products endogenous to the system): major species at 55, 53 and 46K daltons have previously been identified as α-tubulin, β-tubulin and actin respectively (1). Two other major species with SDS polyacrylamide gel mobilities of 36 and 20K daltons were also observed, as well as a large number of less prevalent species. The RNA which hybridizes to p13 programs the synthesis of a 36K dalton protein. Further experiments, described below, confirm that this 36K protein is identical to the major 36K species in the translation products of unfractionated brain polyA$^+$ RNA.

We determined the 679 nucleotide sequence of the p13 insert (Fig. 2) using the Maxam-Gilbert partial chemical degradation technique (4). The p13 sequence shows a reconstructed HindIII site proximal to the pBR322 EcoRI site, but the distal insert-vector junction is aberrant. The sequence contains only one substantial open reading frame in either orientation: the entire insert sequence is open in one frame in the opposite orientation to the pBR322 map (5). The sequence of that open frame was translated using the genetic code into a 227 residue protein which was compared by computer analysis to the Dayhoff protein sequence atlas (6). Considerable identities

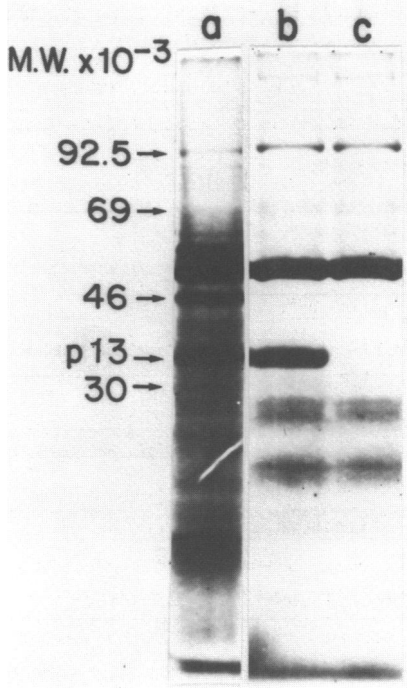

Figure 1. Hybridization-translation assay of clone p13. Autoradiogram of a 10% SDS-polyacrylamide gel (21) of ^{35}S-methionine labeled in vitro translation products. A reticulocyte lysate translation system (NEN, Boston, MA) was programmed with RNA as follows: lane a, 0.6 µg cytoplasmic polyA$^+$ RNA from 14 day chick embryo brain; lane b, chick brain RNA purified by hybridization to clone p13 bound to nitrocellulose as described by Cleveland et. al. (2); lane c, no added RNA. The positions of molecular weight markers and the position of the translation product of clone p13 are indicated at the left. The intense band of approximately 50K daltons seen in all three lanes is an endogenous product of the translation system. The predominant protein species with molecular weights 55K, 53K, and 46K have been previously identified as α-tubulin, β-tubulin and actin, respectively (1).

with the glycolytic enzyme GAPDH of other species were found (for example, 204/277 matches with the sequence of pig GAPDH, underlined in Fig. 2)--enough to show that the p13 clone represents the N-terminal two thirds of the chicken GAPDH mRNA coding region. GAPDH in other species is 36K daltons, consistent with the size of the product obtained with the hybridization-translation assay of p13.

Cloning of the mRNA for GAPDH from chick embryonic heart muscle has been recently reported by Arnold and coworkers (7). They present a nucleotide sequence which, when translated, corresponds to the carboxy terminal 136

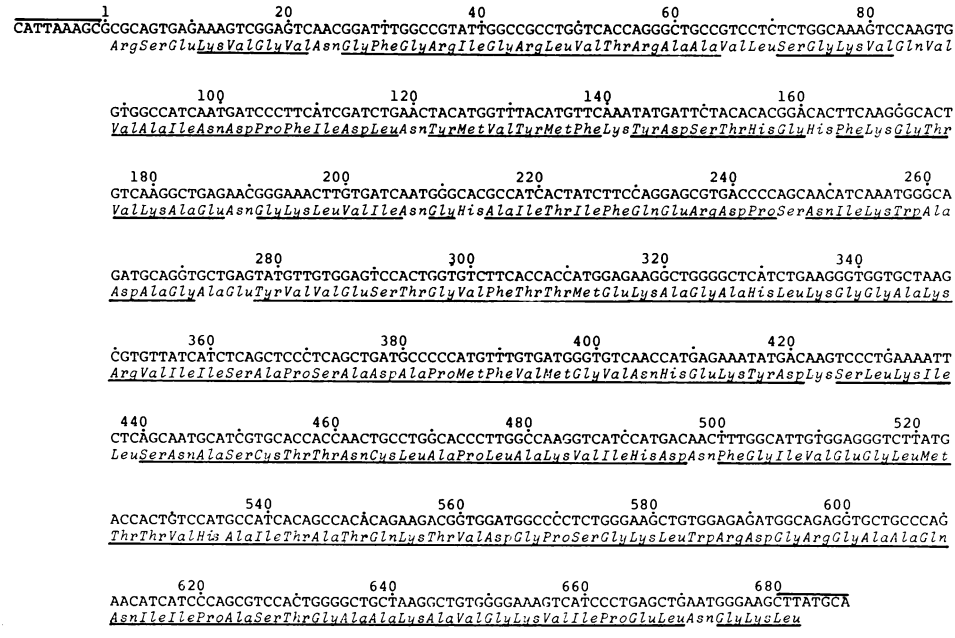

Figure 2. Nucleotide sequence of clone p13. The sequence of the sense strand of the insert is shown oriented in the HindIII site of pBR322 such that the right hand end is proximal to the EcoRI site of the vector. Residues contained in pBR322 (5) are overlined. The corresponding amino acid sequence is also shown, residues which are identical in the sequence of pig GAPDH (6) are underlined.

amino acids of GAPDH. Their sequence overlaps our sequence by 90 nucleotides and the overlapping regions of the two nucleotide sequences are identical. In both cases the derived amino sequences of the overlapping region include one residue which differs from the sequence of the pig enzyme. Together these two sequences provide the complete primary structure of chicken GAPDH. The nucleotide sequence corresponding to the carboxy terminal region of GAPDH (7) shows a site for the restriction enzyme HindIII in the position of the HindIII site which terminates our sequence indicating that the cDNA which gave rise to clone p13 was probably cleaved at this position by the HindIII treatment used in construction of the clone.

GAPDH mRNA Is the Fourth Most Abundant Message in Chick Brain

Since p13 represents the mRNA for GAPDH, a 36K protein, and since the fourth major translation product of chick brain mRNA has a molecular weight of 36,000, we suspected the two were identical. We purified GAPDH from an homogenate of adult chicken muscle by ammonium sulfate precipitation followed

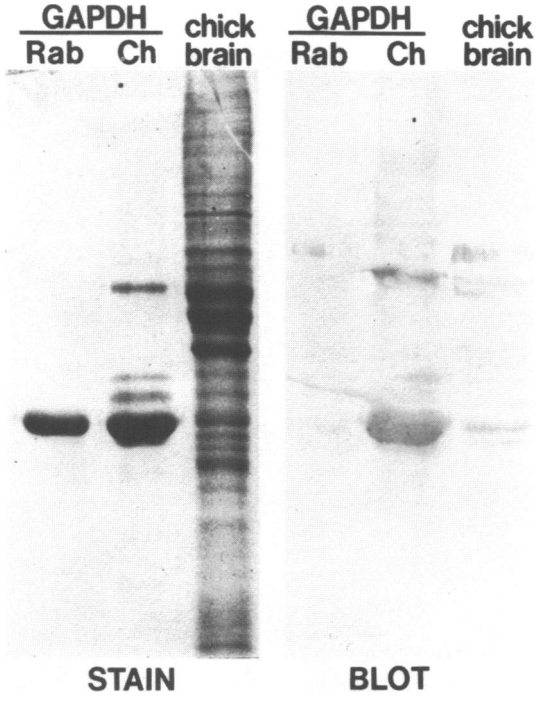

Figure 3. Gel and Western blot analysis of purified GAPDH and chick brain proteins. Rabbit GAPDH (Rab) (Sigma, St. Louis, MO), chicken GAPDH (Ch) and an SDS extract of 17 day chick embryo brain were electrophoresed on a 10% SDS-polyacrylamide gel (21). Left, Coomassie Blue staining pattern. Right, Western blot using anti-GAPDH antibody, carried out as described (10): material from a duplicate SDS gel was blotted onto nitrocellulose and incubated with rabbit anti-chicken GAPDH at a final dilution of 1:2000. After washing, bound rabbit antibodies were visualized with goat anti-rabbit IgG conjugated to horseradish peroxidase and diamino benzidine.

by several rounds of recrystallization from ammonium sulfate (8) until we judged the preparation to be at least 50% pure, as estimated by SDS-polyacrylamide gel electrophoresis (Fig. 3). The specific activity of the partially purified enzyme was determined by the method of Krebs (9) to be 65 U/mg; 100 U/mg is the activity of pure GAPDH. We used this preparation after heat denaturation (65°C, 10 minutes) as an antigen for immunizing rabbits (1 mg at day 0, 14, 21 in Freund's Adjuvant, sera collected at day 35). We incubated the antiserum with purified GAPDH and found that the antibody reduced GAPDH activity by 75%, while normal rabbit serum had no effect, suggesting that the antiserum binds GAPDH. Furthermore, when the

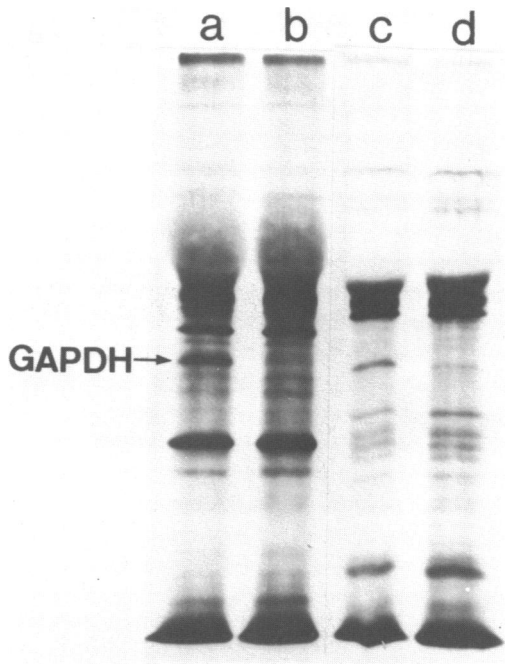

Figure 4. GAPDH is the major 36K translation product of chick brain mRNA. ^{35}S-methionine labeled translation products from 17 day chick embryo brain or muscle polyA$^+$ RNA were incubated with rabbit anti-GAPDH or normal rabbit serum. After precipitation of antibody with S. aureus bacteria, aliquots of each supernatant were subjected to electrophoresis on 10% SDS polyacrylamide gels (21). Radioactively labeled products were detected by autoradiography. (a) muscle + normal rabbit serum; (b) muscle + rabbit anti-GAPDH; (c) brain + normal rabbit serum; (d) brain + rabbit anti-GAPDH. The position of GAPDH is indicated. The distortions in the upper regions of each gel lane are probably due to overloading with unlabelled rabbit serum protein. The anti-GAPDH antibody did not completely remove all 36K material from the brain extract (lane d) but there is a clear decrease in the amount of GAPDH compared with the normal rabbit serum control (lane c).

antiserum was used to probe a Western Blot (10) (Fig. 3) on which the purified chicken GAPDH and chick brain protein extract had been separated, the antibody reacted with the chicken GAPDH, as expected. Additionally, it reacted with a 36K species in the chick brain which comigrated with the fourth major protein species as visualized by Coomassie Blue stain. Both of these have the same mobility as commercial rabbit GAPDH, which, of course, does not react in the Western because of immunological self-tolerance--the antiserum was raised in rabbit.

To confirm the identity of GAPDH and the 36K chick brain translation

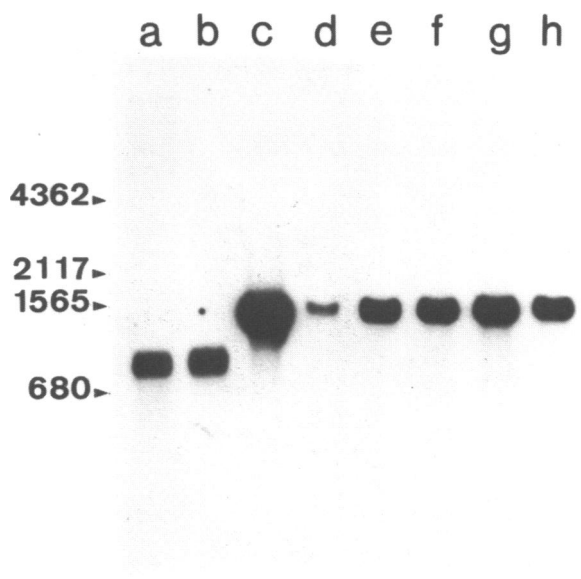

Figure 5. Northern blot analysis of chicken GAPDH mRNA. RNA samples from tissues of adult and embryonic chickens, and from adult rats were electrophoresed on denaturing formaldehyde, 1.5% agarose gels, transferred to nitrocellulose, and hybridized with a mixture of clone p13 and rat brain cDNA clone p1B15, each labeled to a specific activity of 2-3 x 10^8 cpm/μg by nick translation (22). Blots were washed in 0.2X SSC at 68°C. Chicken samples were of total cellular RNA extracted as described (23); 20 μg were loaded per lane. Rat samples were cytoplasmic polyA$^+$ RNA preparations (24); 2 μg per lane. Lanes: a, rat kidney; b, rat brain; c, adult chicken muscle; d, adult chicken liver; e, adult chicken brain; f, chick embryo muscle; g, chick embryo liver; h, chick embryo brain. The positions of size markers are indicated in bases. The melting temperature of hybrids formed with the p13 insert (53% G+C) in 0.2X SSC is 78°C (28,29) and since each 1% mismatch lowers the T_m by 1°C, our 68°C wash reflects a 90% stringency.

product, we translated unfractionated chick brain polyA$^+$ RNA in vitro, immunoprecipitated the products with rabbit anti-GAPDH or normal rabbit serum, and analyzed the supernatants by gel electrophoresis (Fig. 4). Rabbit anti-GAPDH but not normal rabbit serum removed the majority of the 36K product from the gel profile, indicating that the major species is indeed GAPDH. Therefore, the fourth major chick brain mRNA codes for GAPDH.

GAPDH mRNA Is Regulated During Chicken Development

We used p13 DNA as a hybridization probe for Northern blot analysis (11) of the total cytoplasmic RNA of various chicken and rat tissues, including developing chick brain from 17 day eggs. p13 hybridizes to a chick brain RNA species with gel mobility of about 1400 nucleotides, a length quite suffi-

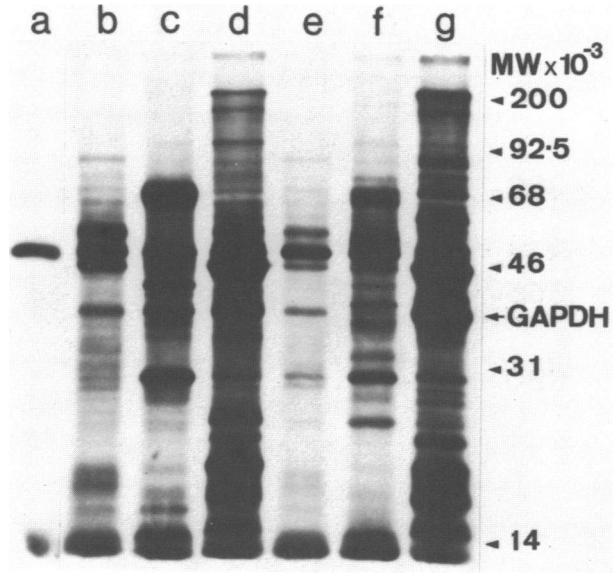

Figure 6. Translation products of chicken mRNA from different tissues. RNA was extracted from chicken tissues (23) and the polyadenylated fraction purified by oligo dT cellulose chromatography (25). One μg of each preparation was translated in vitro and the ^{35}S-methionine labeled products were electrophoresed on a 10% SDS-polyacrylamide gel. Lanes: a, no added RNA; b, chick brain; c, chick liver; d, chick muscle; e, adult brain; f, adult liver; g, adult muscle. The positions of molecular weight markers and of GAPDH are indicated.

cient to contain the coding region for a 36K dalton protein (approximately 1000 nucleotides). It hybridizes to approximately the same extent with chick liver and muscle RNA and adult brain RNA (Fig. 5). There was substantially less (~10 fold) hybridization to adult liver RNA and significantly more (~5 fold) hybridization to adult muscle RNA. The discrete nature of the hybridization bands suggests that there was little, if any, degradation of these RNA preparations, and that the differences in band intensity reflect real differences in cytoplasmic mRNA concentration. No RNA in rat brain or kidney extracts hybridized with p13. However, a rat cDNA clone, p1B15 (24), hybridized to the two rat RNA preparations (but not to chicken), demonstrating that the rat mRNA was not degraded. Thus, the GAPDH mRNA is equally present in various chick tissues, but dramatically regulated in adult tissues, and is not homologous to the rodent GAPDH mRNA which is presumably present in the rat tissues.

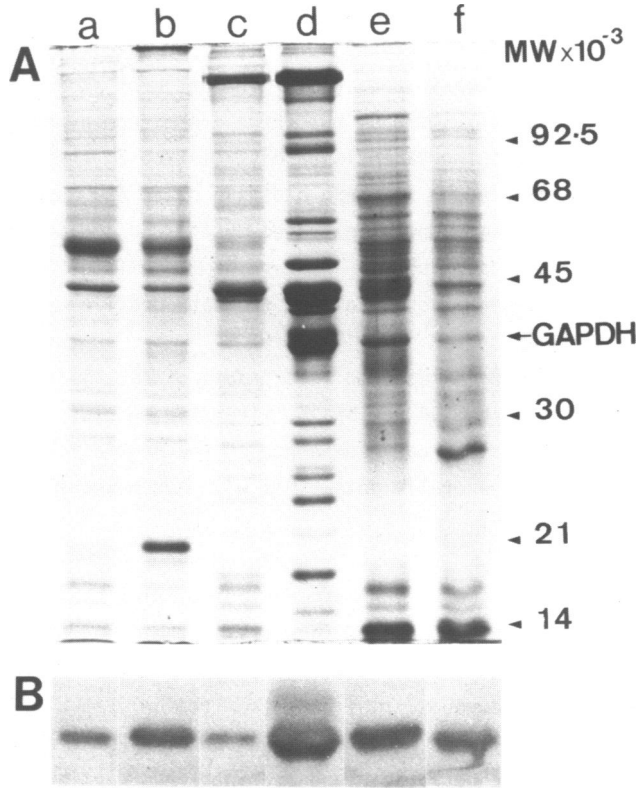

Figure 7. A) Electrophoretic patterns of proteins in adult and embryonic chick tissues. Frozen tissues were disrupted (Polytron, Brinkmann Instruments, Westbury, N.Y.) in 5 volumes phosphate buffered saline (pH 7.4), added to an equal volume of 4% SDS, 10% β-mercaptoethanol and boiled for 5 min. After centrifugation at 10,000 xg for 10 min., 60 μg of the supernatants were electrophoresed on a 10% SDS-polyacrylamide gel. The gel was stained with Commassie Blue and photographed. Lanes: a, chick brain; b, adult brain; c, chick muscle; d, adult muscle; e, chick liver; f, adult liver. The positions of molecular weight markers and of GAPDH are indicated. B) Western blot of extracts as in A prepared as in Figure 3. The photograph was cut to show the 36K region.

GAPDH Protein Concentration Reflects GAPDH mRNA Concentration

Since GAPDH mRNA appears to be dramatically regulated in adult chickens, we investigated the levels of GAPDH protein in those tissues and in the translation products of the polyA$^+$ RNA from each tissue. Figure 6 shows the *in vitro* translation patterns of the RNA from each tissue. The levels of the major 36K protein species seem to mimic the levels of GAPDH mRNA in these tissues (Fig. 5). The overall gel profile of each tissue is quite

different, and each changes with age. Figure 7 shows the Coomassie Blue stained SDS-polyacrylamide gel protein profile of brain, liver and muscle extracts from both chick embryo and adult. The patterns show some similarity to the protein profiles of Figure 6, but there are also many differences. The major 36K species has the same tissue distribution in the dissected tissues as GAPDH mRNA analyzed on Northern blots in brain and muscle. Both adult and chick liver seem somewhat elevated in the 36K species compared to the mRNA levels in those tissues. Western blotting (Fig. 7B) of the same extracts with the anti-GAPDH serum follows the same profile as the major 36K species.

GAPDH activity has been measured in extracts of rat tissues (12) and is found in the ratio 81:63:294 for brain, liver and muscle. Given the species difference and the clear age dependence of the regulation in chicken, these values seem to be generally compatible with our measurements.

DISCUSSION

We have identified a major mRNA of developing chick brain as coding for the glycolytic enzyme GAPDH. The identity of the mRNA corresponding to clone p13 was determined unambiguously from the clone's nucleotide sequence: an amino acid sequence translated from the only open triplet reading frame showed 90% homology with the known sequence of GAPDH from the pig. Further experiments using both the clone and anti-GAPDH antibodies demonstrate that GAPDH and its mRNA are abundant species in the brain of the chick embryo.

Our sequence does not provide an N-terminus for the primary translation product of GAPDH mRNA or for mature chicken GAPDH (which, in fact, DNA sequencing cannot provide). Two observations seem appropriate. Because the HindIII site at the N-terminal end of the sequence was not perfectly formed, as would have been expected had cloning gone exactly as designed, the 5' sequence must be regarded as potentially unfaithful until it becomes synchronous with other GAPDH sequences (9 nucleotides). Second, there is a precedent for variable N-terminal extensions of GAPDH as evidenced by the recently determined human GAPDH sequence (13). Our sequence is best characterized as providing suggestive, but not necessarily unequivocal, evidence that the chicken GAPDH primary translation product is N-terminally extended compared to the mature pig GAPDH sequence.

Our studies provide a large portion of the amino acid sequence of chicken GAPDH. This sequence shows extensive homologies with the known se-

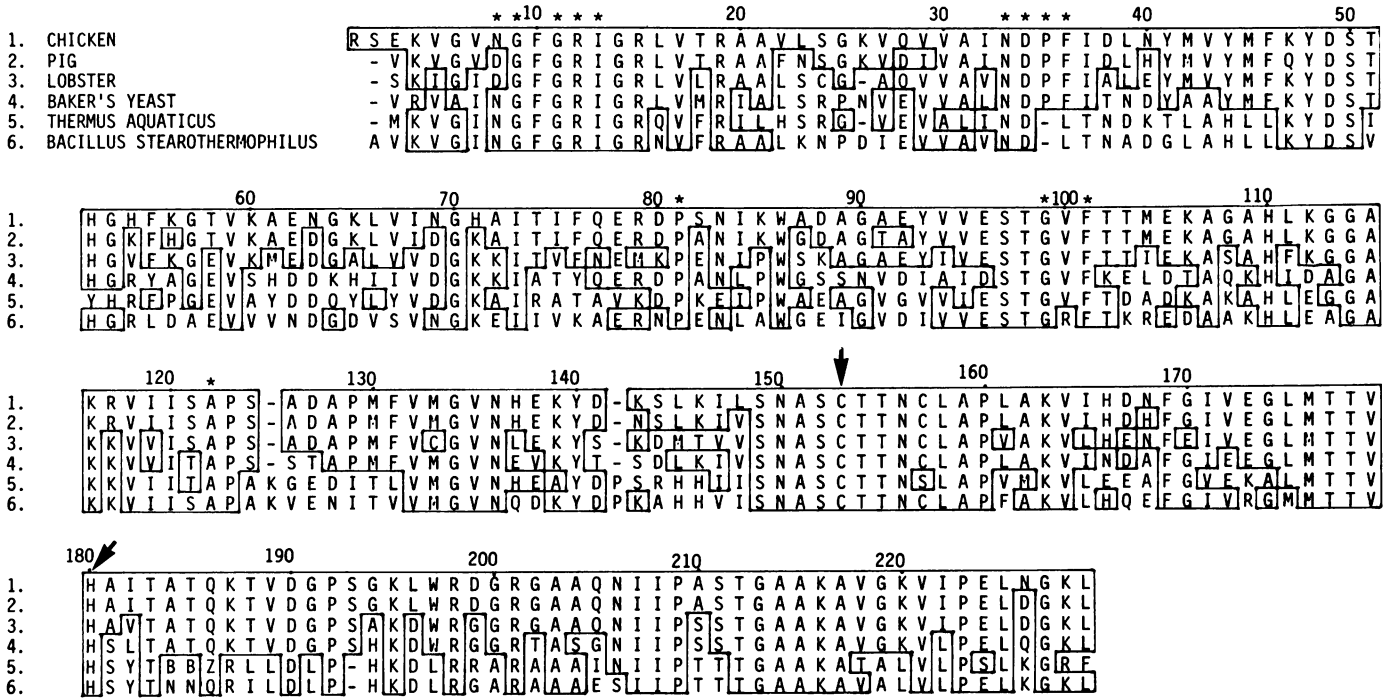

Figure 8. Comparisons of amino acid sequences of GAPDH from chicken and other species. The data for all sequences except chicken were taken from ref. 6, except that ambiguities in yeast GAPDH were corrected by reference to the sequence of yeast GAPDH clone pgap 491 (26). Amino acid residues homologous to the chicken GAPDH sequence are boxed. Residues important for catalysis are indicated by arrows; residues involved in NAD binding by * (27).

quences of GAPDH from pig, lobster, yeast and two species of bacteria (Fig. 8). The number of differences in amino acid residues between these sequences correlate well with the evolutionary relationships of the various species. That is, chicken GAPDH is most closely related to pig GAPDH and the chicken and pig enzymes show almost equal divergences from GAPDH of lower organisms. Nevertheless, this is one of the most highly conserved of proteins, retaining 50% sequence identity over long eons of evolution, equivalent to a mutation rate of 2.2 PAMs (accepted point mutations) per 100 million years (14). This preservation of structure has been previously described (15) and can be clearly seen in the large blocks of conserved residues, particularly in regions 9-14, 33-66 and 149-160, which contain amino acids believed to be involved in the active site, such as the catalytic cysteine (residue 153)or residues involved in binding the cofactor NAD. Other regions of the molecule show more variation in primary sequence; these are largely at sites which are on the surface of the protein (15). Here too, the chicken enzyme follows the same pattern as the other sequences and there are several positions which contain chicken-specific amino acid residues. Many of the differences between the various sequences are conservative (for example, substituting val for ala at position 43), suggesting that the highly evolved three dimensional structure of the protein has little tolerance for changes in its primary structure at some positions; other sites seem slightly more variable.

Even though GAPDH is a protein whose primary sequence is extensively conserved through evolution and the sequences of the chicken and pig enzymes are so closely (~90%) related, no hybridization between p13 and rat mRNA could be detected. The rat enzyme is immunoprecipitated by our antibody to chicken GAPDH (data not shown). We calculate our hybridization stringency to be about 90%, so lack of cross-hybridization might not be so surprising. Nonetheless, it appears that the nucleotide sequences of the GAPDH coding region have drifted somewhat more extensively than the primary amino acid sequence. This contrasts with what has been observed for the α and β tubulins (3) which have conserved both their nucleotide and their amino acid sequences.

That an enzyme should be encoded by such an abundant mRNA in the chick embryo brain was a considerable surprise--we had anticipated that the major 36K protein would be structural, as are actin and the tubulins. We therefore examined the expression of GAPDH mRNA and protein in other tissues of adult and embryonic chickens. To assess the protein, we produced an antibody

against purified chicken GAPDH. In 17 day chick embryos there are approximately equal amounts of both GAPDH and GAPDH mRNA in brain, liver and breast muscle. In the same tissues in adult chickens, however, there is a considerable variation in the levels of both protein and mRNA: relative to the amounts in the embryo, brain is approximately unchanged, liver is decreased and muscle is increased. The same changes are seen when GAPDH mRNA levels are quantitated directly by Northern blotting or indirectly by in vitro translation and when GAPDH protein levels are examined by staining. In brain and muscle at the two ages there is a good correlation between the concentration of GAPDH protein and its mRNA, suggestive that the molecular basis for regulating the amount of GAPDH protein in different tissues is by controlling the steady state levels of cytoplasmic GAPDH mRNA. This could operate at the level of transcription or RNA processing and degradation, or all of these. In liver, particularly in the adult, there is rather more GAPDH protein present than would be predicted from the amount of GAPDH mRNA, suggesting that there may be additional regulation at the translational level in this tissue, possible influencing rates of GAPDH protein synthesis or degradation. Of course, metabolic changes, such as variations in the NAD^+/NADH ratio (16) may alter enzyme activity acutely without any effect on the total concentration of the protein, but our results suggest that the chronic activity levels of this enzyme are set, most probably at the level of the gene.

GAPDH is a key enzyme of carbohydrate metabolism, functioning both in glycolysis and gluconeogenesis, and is therefore probably constitutively expressed in all cells. Yet different tissues may have unique demands for glucose metabolism and may contain different activities of glycolytic enzymes. Unfortunately, little is known about the metabolic physiology of either the chicken or the egg and we can only guess at the reasons for the different observed levels of GAPDH. The major energy source in the egg is fat and to some extent protein, while carbohydrates provide only a few percent of the total energy metabolism of the egg (17). The metabolism of fatty acids is not dependent on glycolysis and animals cannot convert fatty acids to carbohydrate. Why, then, do embryonic tissues contain so much GAPDH? It is possible that GAPDH could play a structural role, but its levels can be explained on purely physiological grounds. In mammals, the brain is completely dependent on glucose as an energy source under non-fasting conditions. If this is also true for birds, then we may expect a significant level of GAPDH activity in the brain at all stages of develop-

ment, enabling the brain to utilize any available glucose. Similarly, muscle tissue has a high demand for glucose and GAPDH constitutes over 10% of the soluble proteins in muscles of many species. It is not surprising, therefore, that we find an increase in GAPDH between the muscles of the relatively inactive embryo and the active adult.

In the liver, however, there is a considerable decrease in GAPDH content in the adult relative to the embryo. Measurements of GAPDH enzyme activity during development show a similar pattern: a rise in activity peaking at 18 embryonic days, dropping 3-4 fold thereafter to the level of the adult (18). The increase in enzyme activity correlates well with the time course of glycogen deposition in the liver (19), suggesting that in the embryonic liver GAPDH may function largely in the direction of gluconeogenesis using carbohydrate or, more likely, amino acid precursors. Other glycolytic enzymes follow similar activity time courses as GAPDH during development (18). Immediately after hatching, the glycogen stores of the embryo, mainly in the liver, are rapidly depleted, indicating a massive release of glucose. GAPDH activity shows a similar rapid decline after hatching to the lower levels of the adult. This activity is probably sensitive to diet and the low levels of GAPDH in the adult may be related to special feeds which promote egg laying. In the embryo, however, where diet is of no importance, the high levels of GAPDH may not only be a consequence of the metabolic demands of the embryo but perhaps also a preparation for the nutritional changes that occur after hatching.

For this study it was useful to produce an antibody to the protein product corresponding to the cloned mRNA. In this particular case, the preparation of the enzyme was simple and efficient because the sequence matched an already known and well characterized protein. In other cases, however, particularly when the cloned gene product is unknown, it might be necessary to adopt an alternative strategy and generate appropriate antibodies directed against short synthetic peptide fragments of the protein sequences (20).

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REFERENCES

1. Cleveland, D.W., Kirschner, M.W. and Cowan, N.J. (1978) Cell 15: 1021-1031.
2. Cleveland, D.W., Lopata, M.A., MacDonald, R.J., Cowan, N.J., Rutter, W.J. and Kirschner, M.W. (1980) Cell 20:95-105.
3. Valenzuela, P., Quiroga, M., Zaldivar, J., Rutter, W.J., Kirschner, M.W. and Cleveland, D.W. (1981) Nature 289:650-655.
4. Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74:560-564
5. Sutcliffe, J.G. (1978) Cold Spring Harbor Symp. Quant. Biol. 43:77-90.
6. Dayhoff, M.O., Hunt, L.T., Barker, W.C., Orcutt, B.C., Yeh, L.S., Chen, H.R., George, D.G., Blomquist, M.C., Fredrickson, J., Johnson, G.C., PSQ Version 4, Apr. 30, 1982, National Biomedical Research Foundation, Georgetown University Medical Center.
7. Arnold, H.H., Domday, H., Weibauer, K., Datta, I. and Siddiqui, M.A.Q. (1982) J. Biol. Chem. 257:9872-9877.
8. Amelunyen, R.E. and Carr, D.O. (1975) Methods in Enzymology 41:264-267.
9. Krebs, E.G. (1955) Methods in Enzymology 1:407-411.
10. Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76: 4350-4354.
11. Thomas, P. (1980) Proc. Natl. Acad. Sci. USA 77:5201-5205.
12. Shonk, C.E. and Boxer, G.E. (1964) Cancer Res. 24:709.
13. Nowak, K., Wolny, M. and Banas, T. (1981) FEBS Letters 134:143-146.
14. Dayhoff, M.O., In: Atlas of Protein Sequence and Structure, Vol. 5, supplement 3, p. 3.
15. Olson, K.W., Moras, D., Rossman, M.G. and Harris, J.I. (1975) J. Biol. Chem. 250:9313-9321.
16. Krebs, H.A. and Veech, R.L. (1970) In: Pyridine Nucleotide Dependent Dehydrogenases, H. Sund, Ed., p. 413-434, Springer-Verlag, Berlin.
17. Romanoff, A.L. (1967) Biochemistry of the Avian Embryo, Wiley and Sons, New York.
18. Rinaudo, M.T. (1962) Enzymologia 24:230-236.
19. Rinaudo, M.T. (1961) Experientia 17:30-31.
20. Sutcliffe, J.G., Shinnick, T.M., Green, N., Liu, F.-T., Niman, H.L. and Lerner, R.A. (1980) Nature 287:801-805.
21. Laemmli, U.K. (1970) Nature 227:680-685.
22. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113:237-251.
23. Levy, D.E., Lerner, R.A. and Wilson, M.C. (1982) Proc. Natl. Acad. Sci. USA 79:5823-5827.
24. Sutcliffe, J.G., Milner, R.J., Bloom, F.E. and Lerner, R.A. (1982) Proc. Natl. Acad. Sci. USA 79:4942-4946.
25. Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69:1408-1412.
26. Holland, J.P. and Holland, M.J. (1980) J. Biol. Chem. 255:2596-2605.
27. Harris, J.I. and Waters, M. (1976) In: The Enzymes, Boyer, P., Ed., Vol. 13, 3rd Edition pp. 1-49, Academic Press, New York.
28. Marmur, J. and Doty, P. (1962) J. Mol. Biol. 5:109-118.
29. Dove, W.F. and Davidson, N. (1962) J. Mol. Biol. 5:467-476.