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Developmental regulation of glutamine synthetase and carbonic anhydrase II in neural retina

(glutamine synthetase gene clone/mRNA accumulation/glia neurons/cortisol induction)

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ABSTRACT **Glutamine synthetase (GS) is expressed in the neural retina only in Muller glia cells and is inducible with cortisol. A chicken genomic clone that contains at least part of the coding region for the GS enzyme was used to investigate developmental changes in the level of GS mRNA in embryonic chicken retina. A major GS transcript (≈3 kilobases) detected by the probe begins to accumulate sharply on day 15 of embryonic development. When cortisol is prematurely supplied to early embryonic retina, it induces precocious accumulation of GS mRNA and of the GS enzyme. At later ages, these effects of cortisol are significantly greater, which suggests that competence to transcribe or stabilize GS mRNA in response to stimulation with cortisol increases with development. Carbonic anhydrase II (CA-II) is expressed in early retina in all the cells, but it becomes later restricted to Muller glia. Using cloned CA-II cDNA, we detected a high level of CA-II mRNA in early retina, followed by a decline due to arrest of CA-II mRNA accumulation in differentiated neurons. As glia cells mature, CA-II mRNA and the enzyme increase to a new high level. Therefore, changes in CA-II gene expression during retina development reflect differentiation-dependent cell-type-specific control of CA-II mRNA accumulation.**

The enzymes glutamine synthetase (GS) and carbonic anhydrase II (CA-II) are differentiation markers in embryonic neural retina (1). In mature retina, both are expressed in Muller glia cells. However, their developmental patterns in embryonic retina differ markedly, raising fundamental questions concerning regulation of gene expression during cell differentiation.

In embryonic and mature retina, GS is restricted to Muller glia cells (1). In chicken embryo retina (the most thoroughly studied system), GS level is very low until day 15–16 of development. It then begins to increase sharply in response to systemic elevation of adrenal corticosteroid hormones, and it increases in 6 days >100-fold. However, GS can be induced also precociously, already in 8-day retina, by prematurely adding a corticosteroid inducer such as cortisol to embryos or to organ cultures of isolated retina tissue (2, 3). Previous indirect evidence suggested that cortisol induced GS in the retina by eliciting GS mRNA accumulation (4, 5). Here, we used a chicken GS clone to investigate changes in the level of GS mRNA in the retina during normal development and when GS is prematurely induced.

CA-II is present at high levels already in early embryonic retina (by day 5 of development) when it is found in all retina cells (6). As differentiation progresses, neurons stop expressing CA-II; by day 13, this enzyme is found only in Muller glia cells. During retina maturation, CA-II accumulates to a new high level that persists in the adult (6). It is important to note that cell proliferation in the neural retina of chicken embryo begins to decline after day 8 and ceases by day 12 of development (3). Using a CA-II cDNA clone, we investigated the accumulation and localization of CA-II mRNA during retina development in relation to changes in CA-II enzyme level.

MATERIALS AND METHODS

Neural Retina. Neural retina tissue was isolated under sterile conditions from eyes of chicken embryos (White Leghorn) at different stages of development and was stored at −70°C or directly used for RNA preparation.

RNA Preparation and Analysis. Retina tissue was disrupted by Dounce homogenization in an ice-cold solution of 0.15 M NaCl/0.01 M Tris·HCl, pH 7.5/2 mM $MgCl_2$/0.75% Nonidet P-40. Nuclei were removed by centrifugation (8 min at 1500 × *g*). The supernatant was adjusted to 2.5 mM EDTA/0.5% $NaDodSO_4$ and cytoplasmic RNA was isolated by phenol/chloroform extraction and ethanol precipitation. Poly(A)$^+$ RNA was selected by using an oligo(dT)-cellulose column (Collaborative Research, Waltham, MA, type III). Total RNA was prepared by the guanidinium isothiocyanate/CsCl method (7). Total RNA (10 μg) or poly(A)$^+$ RNA (5 μg) was denatured by heating at 60°C in 2.2 M formaldehyde/50% formamide, and it was fractionated by electrophoresis in 0.8% agarose gels containing 2.2 M formaldehyde and Mops buffer. The fractionated RNA was transferred to a nitrocellulose filter and hybridized with nick-translated DNA probes (8).

Library Screening and Hybrid Selection. The chicken GS gene was isolated from a chicken genomic library, prepared in λ Charon 4A (9) and kindly provided by J. B. Dodgson and J. D. Engel. The library was screened with the cloned hamster GS gene, pGS113 (10), kindly provided by R. H. Wilson. Screening and purification of positive clones was according to published protocols (8). Three positive phage recombinants were isolated and one, λ-GS223, was further analyzed. λ DNA was denatured in 0.2 M NaOH for 10 min, diluted in 0.3 M Na citrate/1.5 M NaCl, and spotted (10 μg) onto nitrocellulose filters. The filters were baked at 80°C and hybridized to retina poly(A)$^+$ RNA (50 μg/ml) at 42°C for 18 hr in 50% formamide/10 mM piperazine-*N,N*′-bis(2-ethanesulfonic acid)/0.1% $NaDodSO_4$/0.4 M NaCl/4 mM EDTA/0.5 mg of tRNA per ml. The filters were washed in 10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.1% $NaDodSO_4$ at 52°C, and the RNA was eluted in 0.1% $NaDodSO_4$ at 100°C for 2 min. The eluted RNA was ethanol-precipitated and analyzed by *in vitro* translation.

***In Vitro* Translation.** Poly(A)$^+$ RNA was translated by using a rabbit reticulocyte lysate system (Amersham) in the presence of 1 μCi (1 Ci = 37 GBq) of [^{35}S]methionine per μl. Translation products were either directly analyzed by gel electrophoresis, or were first immunoprecipitated: the prod-

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Abbreviations: GS, glutamine synthetase; CA-II, carbonic anhydrase II; kb, kilobase(s).

ucts underwent reaction with preimmune rabbit serum; the precipitate was removed with *Staphylococcus aureus*; the supernatant was precipitated with antiserum directed against CA-II (6), or against GS (11), or with normal serum. Immunocomplexes were collected on *S. aureus*, washed in buffer (0.5% Nonidet P-40/0.05 M Tris·HCl, pH 7.4/0.15 M NaCl/5 mM EDTA), eluted by boiling in gel buffer containing 5% 2-mercaptoethanol, and separated by $NaDodSO_4$/polyacrylamide gel electrophoresis.

GS Induction with Cortisol. Retinas from chicken embryos at different developmental stages were organ-cultured for 24 hr in Erlenmeyer flasks in medium 199 with 10% fetal bovine serum on a gyratory shaker at 37°C (4). GS was induced with cortisol (0.33 μg/ml) (Sigma) added at the beginning of culture (4, 11). Tissue samples were assayed for GS specific activity as described (11).

Depletion of Glia Cells from Retina Tissue. Retina tissue from 13-day chicken embryos was cut into small pieces and placed in 100-ml Erlenmeyer flasks (10 retinas per flask) each containing 10 ml of medium 199 and 10% fetal bovine serum. To selectively destroy Muller glia cells (12), 1.2 mM DL-α-aminoadipic acid (Sigma) was added to each flask. The flasks were incubated on a gyratory shaker (72 rpm) at 37°C. Media were changed daily. After 48 hr, the tissue was rinsed in cold Tyrode's solution and used for RNA preparation.

RESULTS

Cloning of the Chicken GS Gene. A chicken genomic library, prepared in λ Charon 4A (9) was screened using as a probe the cloned hamster *GS* gene pGS113 (10). Three positive clones were identified. The clone λ-GS223, which contains an insert of ≈7000 base pairs, was further investigated and shown to contain GS sequences by hybrid selection. Specifically, poly(A)$^+$ RNA for the hybrid selection assay was prepared from 13-day embryonic retinas in which GS was induced with cortisol. The poly(A)$^+$ RNA was hybridized to a filter to which λ-GS223 DNA had previously been bound; it was then eluted and translated *in vitro*. The major translation product, absent from the control sample, was a 42-kDa band (Fig. 1, lane 3), which is the molecular mass of GS enzyme subunits (13).

Immunoprecipitation of the translation products with GS-specific antiserum (11) precipitated the 42-kDa product (Fig. 1, lane 5). By contrast, when λ vector DNA was bound to the filter and subjected to the same processing, no specific band was detected (lanes 2 and 4). Therefore, clone λ-GS223

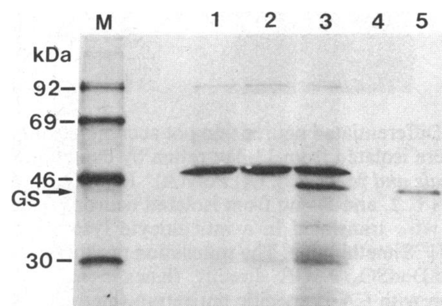

FIG. 1. λ-GS223 clone hybridizes specifically with mRNA that directs *in vitro* translation of GS. Poly(A)$^+$ RNA isolated from 13-day retina tissue in which GS was induced with cortisol was hybridized to immobilized λ DNA. The selected mRNA was washed, eluted, and *in vitro* translated in a reticulocyte lysate system. Lanes: 1, no added selected mRNA; 2 and 4, mRNA selected by λ vector DNA; 3 and 5, mRNA selected by clone λ-GS223 DNA. The translation products in lanes 4 and 5 were immunoprecipitated with GS-specific antiserum; those in lanes 1, 2, and 3 were directly resolved by 10% $NaDodSO_4$/PAGE. Lane M, size markers.

specifically hybridizes to mRNA that directs translation of GS protein and contains, at least, part of the coding region for GS.

Accumulation of GS mRNA in the Developing Retina. Using the GS gene clone, we investigated changes in the level of GS mRNA in the retina during development. RNA blot of poly(A)$^+$ RNA from retinas of different embryonic ages was hybridized with ^{32}P-labeled clone of GS gene, λ-GS223 (Fig. 2*A*). The same filter was then hybridized with ^{32}P-labeled clone of histone H3 gene pCH3dR1 (14) to visualize the histone H3 mRNA. The histone clone detects the transcripts of two histone H3 genes: the replication variant H3.2 and the replacement variant H3.3 (Fig. 2*B*). Expression of replication histone variants is correlated with DNA replication (15, 16), whereas expression of replacement histone variants is at a constitutive basal level during development and cellular differentiation (16, 17). The replication histone H3.2 mRNA, which is ≈0.5 kilobase (kb) (14), is expressed in early stages of retina development (ref. 18; see Figs. 2 and 4). The replacement histone H3.3 mRNA, ≈1.3 kb (19), maintains an apparently steady level throughout the period of cell proliferation and differentiation in the retina (see Figs. 2 and 4) and therefore was used as an internal standard for monitoring changes in expression of GS mRNA.

The GS clone detected a major transcript of ≈3 kb. At late developmental stages, this clone also detected a small amount of possibly an additional minor transcript of ≈1.4 kb (Fig. 2*C*). The major GS transcript was quantitated by scanning densitometry (see Fig. 6). Its level is low during early embryonic ages, and it begins to increase sharply on day 15 to a high level in late embryonic (see Figs. 2 and 6) and mature retina. This developmental profile of GS mRNA closely correlates with that of GS protein (1).

As mentioned above, GS can be precociously induced by cortisol in Muller glia cells in early embryonic retina (1, 11). At very early ages, GS inducibility is low; at later ages, it is higher (2, 3). We investigated whether this increased inducibility reflected greater ability to accumulate GS mRNA. Retinas from embryos of different ages were cultured for 24 hr in the presence or absence of cortisol. Poly(A)$^+$ RNA was isolated and analyzed by RNA blotting and hybridization with the cloned GS gene (Fig. 3). At all ages, cortisol elicited

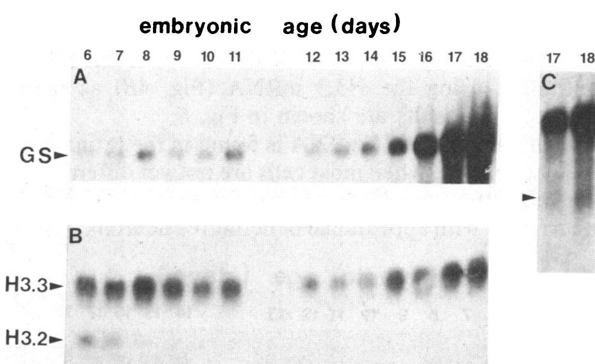

FIG. 2. Accumulation of GS mRNA in developing retina. RNA blot analysis of retina poly(A)$^+$ RNA (≈5 μg per lane) isolated at indicated days of development. The RNA was size-fractionated by electrophoresis and blotted to nitrocellulose filter. (*A*) The filter was hybridized with ^{32}P-labeled clone of GS gene, λ-GS223. A major transcript of ≈3 kb, which accumulates to a high level at late stages of development, was detected. (*B*) The same filter was hybridized with the ^{32}P-labeled clone of histone H3 gene, pCH3dR1. Replacement histone H3.3 mRNA (≈1.3 kb) and replication histone H3.3 mRNA (≈0.5 kb) were detected. Histone H3.3 mRNA was used as reference for quantitating GS mRNA by scanning densitometry (see Fig. 6). (*C*) Hybridization with the cloned GS gene detected at late stages a second minor transcript (arrow) of ≈1.4 kb, which can be seen in a short-time exposure of the blot.

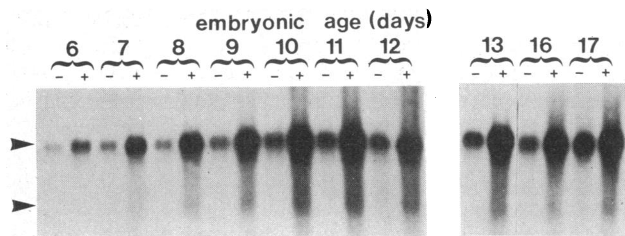

FIG. 3. Induction by cortisol of GS mRNA accumulation. Retina tissue isolated at indicated days of development was organ-cultured for 24 hr in the presence (+) or absence (−) of cortisol. Poly(A)$^+$ RNA was isolated, size-fractionated by electrophoresis, and blotted to nitrocellulose filters. The filters were hybridized with ^{32}P-labeled clone of the GS gene λ-GS223. Two bands were detected: a major one at ≈3 kb (upper arrowhead) and a minor one at ≈1.4 kb (lower arrowhead). An age-dependent increase in accumulation of both transcripts is observed in retina treated with cortisol. A small increase in GS mRNA level in cultures without cortisol is probably due to corticosteroid hormones in the serum supplementing the culture medium.

increases in levels of the major (≈3 kb) and the putative minor (≈1.4 kb) GS transcripts (as well as of GS enzyme). However, the increases were significantly greater at later than at earlier ages (Fig. 3). A small increase in GS mRNA level was also observed in the absence of cortisol; this was possibly due to the presence of a low amount of corticosteroid hormones in the serum supplement. The high levels of GS mRNA at days 16 and 17 were due to the preceding *in vivo* induction of GS in the retina, as explained in the Introduction (see Fig. 2).

CA-II mRNA in Embryonic Retina. The developmental pattern of CA-II in chicken embryo retina differs markedly from that of GS. The level of CA-II is already high on the 5th day of development, and then it declines and increases again during retina maturation (1, 6). We investigated how these changes are related to levels of CA-II mRNA.

Poly(A)$^+$ RNA from retinas of 5- to 18-day embryos was analyzed by RNA blotting using the cloned CA-II cDNA as a probe. Retina replacement histone H3.3 mRNA served as reference. The blot was hybridized, first with ^{32}P-labeled clone of the histone H3 gene pCH3dR1 (14), and then with ^{32}P-labeled clone of CA-II cDNA pPE5-0.3 (20). At all ages, a single band of CA-II mRNA (≈2 kb) was detected by the CA-II cDNA (Fig. 4*A*). The level of CA-II mRNA was quantitated, using the H3.3 mRNA (Fig. 4*B*) as internal standard. The results are shown in Fig. 6.

A high level of CA-II mRNA is found in the retina on day 5 of development, when most cells are not yet differentiated. Soon thereafter, the level of this mRNA declines sharply concurrently with appearance of definitive neurons. It begins to increase again on about day 14 to a new high level that persists in adult retina.

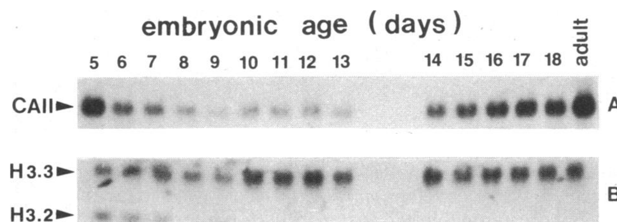

FIG. 4. Accumulation of CA-II mRNA in developing neural retina. RNA blot analysis of poly(A)$^+$ RNA (≈5 μg per lane) isolated at indicated days of development. The RNA was size-fractionated by electrophoresis and blotted to nitrocellulose filter. (*A*) Hybridization with ^{32}P-labeled clone of CA-II cDNA pPE5-0.3 detected a single transcript of ≈2 kb, which accumulates to a high level at early and late developmental stages. (*B*) Hybridization of the same filter with the ^{32}P-labeled clone of histone H3 gene pCH3dRI, detected histone H3.3 mRNA and H3.2 mRNA.

Restriction of CA-II Gene Expression to Glia Cells. Immunohistochemical studies (4) have revealed striking changes in localization of CA-II enzyme during retina development. On the 5th day, this enzyme is present in virtually all the cells; as differentiation progresses, neurons no longer contain CA-II, whereas in Muller glia its amount increases. To investigate the basis for these changes, we determined whether differentiated neurons contain CA-II mRNA. Neurons were isolated from 13-day retina by depleting Muller glia cells with α-aminoadipic acid, which selectively destroys ≈90% of these cells (12). In the first tests, poly(A)$^+$ RNA isolated from glia-depleted and from whole retina tissue was *in vitro* translated. The translation products were immunoprecipitated with CA-II-specific antiserum (6) and analyzed by gel electrophoresis (Fig. 5*A*). Whereas the translation products of poly(A)$^+$ RNA from whole retina tissue contained abundant CA-II protein, those of poly(A)$^+$ RNA from isolated neurons contained only trace amounts of CA-II protein, most likely reflecting some contamination of the neurons with residual glia cells. Next, poly(A)$^+$ RNA from isolated neurons and from whole retina was analyzed by RNA blotting and hybridization with CA-II cDNA (Fig. 5*B*). In contrast to the abundance of CA-II mRNA in whole retina, isolated neurons yielded only a faint band of this mRNA, most likely derived from residual glia cells. The possibility that differentiated neurons accumulate unprocessed CA-II transcripts was examined by RNA blotting of total neuronal RNA. Hybridization with CA-II cDNA did not detect CA-II-specific transcripts (data not shown). Therefore, differentiated retina neurons do not accumulate CA-II transcripts.

DISCUSSION

The expression program of a gene during embryonic development depends on regulatory signals derived, in part, from the gene sequence and, in part, from the cell's microenvironment. Determination of the developmental programs of several genes in a particular cell type might help to identify

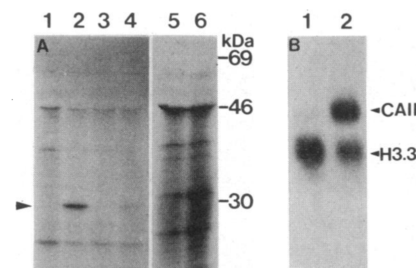

FIG. 5. Differentiated neurons do not accumulate CA-II mRNA. Neurons were isolated from 13-day retina by depletion of glia cells (see *Materials and Methods*). (*A*) Poly(A)$^+$ RNA (2 μg) from whole retina (lanes 1, 2, and 5) and from isolated neurons (lanes 3, 4, and 6), was *in vitro* translated in a reticulocyte lysate system in the presence of [^{35}S]methionine. The translation products were resolved by 13% $NaDodSO_4$/PAGE directly (lanes 5 and 6) and, after precipitation with CA-II specific antiserum (lanes 2 and 4) or with normal rabbit serum (lanes 1 and 3), were visualized by autoradiography. Lanes 5 and 6 were exposed for a shorter time. CA-II protein is 30 kDa. Trace amounts of CA-II protein in lane 4 are probably derived from residual glia cells. (*B*) RNA blot analysis of poly(A)$^+$ RNA (5 μg) from isolated neurons (lane 1) and from whole retina (lane 2). The filter was hybridized with ^{32}P-labeled clone of CA-II cDNA (pPE5-0.3) autoradiographed, and hybridized again with ^{32}P-labeled clone of histone H3 gene (pCH3dRI). The autoradiograms of the first and the second hybridization were superimposed. CA-II mRNA is absent in isolated neurons (lane 1); H3.3 mRNA is detected in isolated neuron and in retina tissue.

these signals and to define their roles in regulation of gene expression.

In adult retina, CA-II and GS are coexpressed in Muller glia cells. However, their developmental patterns in embryonic retina are very different. Fig. 6 describes the changes in the levels of GS mRNA and CA-II mRNA during retina development and includes, for comparison, the accumulation patterns of c-src mRNA and replication histone mRNA, which we have previously studied (18). The developmental programs of these four transcripts differ markedly and characteristically.

Accumulation of GS mRNA increases sharply on day 15 of embryonic development subsequent to systemic elevation of adrenal corticosteroid hormones. This increase is closely correlated with a rapid many-fold increase in the amount of GS protein (1), suggesting that regulation of GS gene expression in retina Muller glia cells involves changes in GS mRNA transcription or stabilization due to hormone action. In fact, premature addition of cortisol to retina tissue at early developmental stages elicits precocious increases in GS mRNA accumulation and in GS enzyme level. These cortisol-induced increases are significantly greater at later than at very early embryonic stages (Fig. 3)—i.e., the ability to transcribe or stabilize GS mRNA in response to stimulation with cortisol increases as the retina develops. The progressively greater GS inducibility could be due to (*i*) differentiation-related changes in the Muller glia cells; (*ii*) increase in number of inducible Muller cells; (*iii*) onset of glia–neuron cell interactions that are prerequisite for GS induction (11, 21). It is not due to changes in the level of cortisol-binding receptors (22). The possibility also exists that, in addition to the increase in GS mRNA accumulation, the translational rate of GS mRNA increases with development resulting in greater induction of GS enzyme by cortisol.

The cloned GS gene detected in RNA blots a major and a minor transcript (Figs. 2 and 3). It is not known whether the minor one represents an intact GS transcript and, if it does, how it is related to the major transcript. It is of interest that a Chinese hamster cell line selected to overproduce GS (10) also expresses a major and a minor GS transcript; both, reportedly, containing the entire nucleotide sequence for the GS enzyme (23). The major GS transcript in the retina and in the hamster cell line (≈3 kb and ≈2.8 kb, respectively) is much longer than required to code for the 42-kDa GS subunit. The minor transcript (≈1.4 kb) is long enough to code for the GS subunit. It remains to be determined whether, in the retina, both the major transcript and the putative minor transcript are translated into GS enzyme, and if both are derived from the same gene.

Unlike GS, the level of CA-II mRNA is high already on day 5 of development; then it declines and increases again during retina maturation. Whereas GS is restricted to glia cells throughout retina development (1), CA-II is expressed at early developmental stages in both glia and neuron precursors and only later becomes confined to glia cells (6). Our finding that CA-II mRNA is absent in differentiated neurons suggests the following rationale for the biphasic profile of CA-II mRNA in developing retina. In very early retina, CA-II mRNA level is high since all the cells express CA-II. Later, its level declines because differentiated neurons (≈80% of retina cells) cease to accumulate it and CA-II expression becomes restricted to glia cells. Although cell proliferation in the retina stops by day 12, CA-II mRNA accumulates during late embryonic development to a high level that persists in mature retina (Fig. 6). These changes in CA-II mRNA accumulation and cellular localization closely correspond to changes in the amount of CA-II enzyme (6), which suggests that regulation of CA-II gene expression in the retina involves cell-type-specific changes in mRNA transcription or stabilization: arrest of CA-II gene expression characterizes neuronal differentiation; in developing glia cells its expression increases.

The level of the replacement histone H3.3 mRNA (Figs. 2 and 4) does not change markedly throughout the period of cell proliferation and retina development. The replication histone H3.2 mRNA is detected only at early stages of retina development and its accumulation profile correlates with the program of cell multiplication in the developing retina (18).

Accumulation of the cellular protooncogene c-src mRNA also shown in Fig. 6 (18) peaks at a time when cell multiplication in the retina declines and ceases; its increase closely coincides with that of c-src protein product (24), suggesting that also the regulation of c-*src* gene involves changes in mRNA transcription or stabilization, and that its expression is associated with cell differentiation rather than with cell proliferation (18). Unlike GS and CA-II, c-src is expressed, at least predominantly, in retina neurons (18).

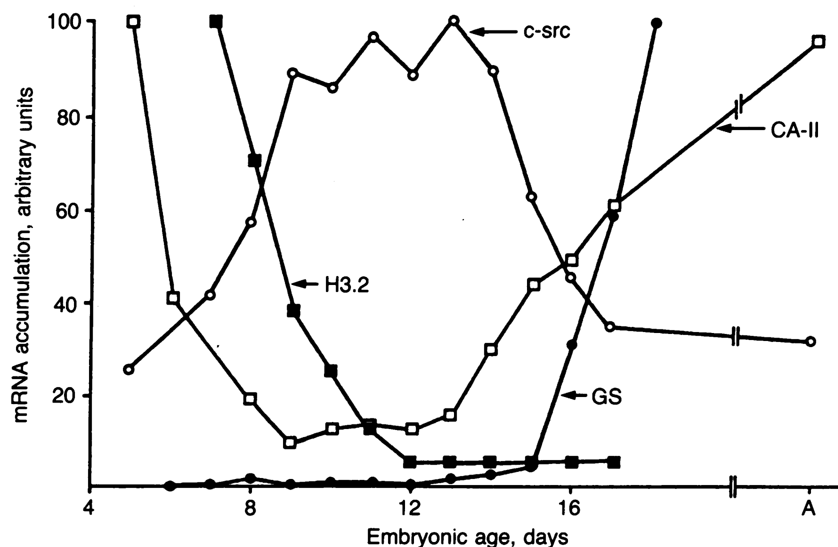

FIG. 6. Relative changes in accumulation of GS, CA-II, c-*src*, and H3.2 gene transcripts during retina development. Autoradiograms of RNA blots were densitometrically scanned and the levels of GS (see Fig. 2), CA-II (see Fig. 4), c-*src* (18), and H3.2 (18) gene transcripts were quantitated. The replacement histone H3.3 mRNA, which was used as an internal reference, represents the base line in this graph. The highest level of each transcript was given the arbitrary value of 100.

The comparative presentation in Fig. 6 of mRNA levels of the different genes provides an overview of their developmental programs in the retina and raises questions concerning the nature of the signals involved in the timing and coordination of these programs. One possible candidate is cell interactions. It is known, for example, that contact interactions between glia and neurons render the glia cells responsive to GS induction with cortisol (1, 21). Something similar may apply more generally: it is conceivable that, as the various cells in the developing retina progressively establish specific cell contacts, their membrane interactions generate signals that influence regulation of gene expression (1).

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1. Moscona, A. A. & Linser, P. (1983) *Curr. Top. Dev. Biol.* **18**, 155–188.
2. Piddington, R. & Moscona, A. A. (1967) *Biochim. Biophys. Acta* **141**, 429–432.
3. Moscona, M. & Moscona, A. A. (1979) *Differentiation* **13**, 165–172.
4. Moscona, A. A., Moscona, M. M. & Saenz, N. (1968) *Proc. Natl. Acad. Sci. USA* **61**, 160–167.
5. Moscona, M., Frenkel, N. & Moscona, A. A. (1972) *Dev. Biol.* **28**, 229–241.
6. Linser, P. & Moscona, A. A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7190–7194.
7. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1978) *Biochemistry* **18**, 5294–5299.
8. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
9. Dodgson, J. B., Strommer, J. & Engel, J. D. (1979) *Cell* **17**, 879–887.
10. Sanders, P. G. & Wilson, R. H. (1984) *EMBO J.* **3**, 65–71.
11. Linser, P. & Moscona, A. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6476–6480.
12. Linser, P. & Moscona, A. A. (1981) *Dev. Brain Res.* **1**, 103–119.
13. Sarkar, P. K., Fischman, D. A., Goldwasser, E. & Moscona, A. A. (1972) *J. Biol. Chem.* **247**, 7743–7749.
14. Engle, J. D., Sugarman, B. J. & Dodgson, J. B. (1985) *Nature (London)* **297**, 434–436.
15. Robbins, E. & Borum, T. W. (1967) *Proc. Natl. Acad. Sci. USA* **57**, 409–416.
16. Brown, D. T., Wellman, S. E. & Sittman, D. B. (1985) *Mol. Cell. Biol.* **5**, 2879–2886.
17. Wu, R. S. & Bonner, W. M. (1981) *Cell* **27**, 321–330.
18. Vardimon, L., Fox, L. E. & Moscona, A. A. (1986) *Mol. Cell. Biol.*, in press.
19. Brush, D., Dodgson, J. B., Choi, O.-R., Wilkins Stevens, P. & Engle, J. D. (1985) *Mol. Cell. Biol.* **5**, 1307–1317.
20. Yoshihara, C. M., Federspiel, M. & Dodgson, J. B. (1984) *Ann. N.Y. Acad. Sci.* **429**, 332–334.
21. Linser, P. & Moscona, A. A. (1983) *Dev. Biol.* **96**, 529–534.
22. Saad, A. D. & Moscona, A. A. (1985) *Cell Differ.* **16**, 241–250.
23. Hayward, B. E., Hussain, A., Wilson, R. H., Woodcock, V., McIntosh, B. & Harris, T. J. R. (1986) *Nucleic Acids Res.* **14**, 999–1008.
24. Sorge, L. K., Levy, B. T. & Maness, P. F. (1984) *Cell* **36**, 249–257.