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Down Regulation by p60$^{v\text{-}src}$ of Genes Specifically Expressed and Developmentally Regulated in Postmitotic Quail Neuroretina Cells

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The avian neuroretina (NR) is composed of photoreceptors and different neurons that are derived from proliferating precursor cells. Neuronal differentiation takes place after terminal mitosis. We have previously shown that differentiating NR cells can be induced to proliferate by infection with Rous sarcoma virus (RSV) and that cell multiplication requires expression of a functional v-*src* gene. We speculated that the quiescence of NR cells could be determined by specific genes. Cell proliferation could then result from the negative regulation of these genes by the v-*src* protein. By differential hybridization of a cDNA library, we isolated eight clones corresponding to genes expressed in postmitotic NR cells from 13-day-old quail embryos, transcriptional levels of which are significantly reduced in NR cells induced to proliferate by *ts*NY68, an RSV mutant with temperature-sensitive mitogenic activity. Partial sequencing analysis indicated that one RNA encoded the calmodulin gene, whereas the other seven showed no similarity to known sequences. By using v-*src* mutants that induce NR cell proliferation in the absence of transformation, we showed that transcription of six genes was negatively regulated by the v-*src* protein and that of four genes was correlated with NR cell quiescence. We also report that a subset of genes are specifically transcribed in neural cells and developmentally regulated in the NR. These results indicate that the v-*src* protein regulates expression of genes likely to play a role in the control of neural cell growth or differentiation.

The avian neuroretina (NR) is composed of photoreceptors and different neuronal cells that are derived from proliferating neuroectodermal cells. Differentiation of these precursor cells takes place after terminal mitosis (20). NR cells obtained from 6- to 8-day-old embryos, that is, at the stage of immature cell division, rapidly cease to divide in culture and express various markers of neuronal differentiation (18–20). The mechanisms which determine terminal mitosis of NR cells, in ovo and in vitro, are presently unknown, nor do we understand the relationship between the loss of growth capacity and establishment of differentiation programs in these cells.

We have shown that in vitro infection of embryonic NR cells with Rous sarcoma virus (RSV) results in major phenotypic alterations. Infected NR cells acquire sustained growth capacity and can be established into permanent lines which include cells with neuronal properties (41, 42). Proliferating NR cells are transformed and become tumorigenic in immunodeficient hosts (9, 10, 44).

The v-*src* gene of RSV contains the genetic information responsible for the transforming and tumorigenic properties of the virus (30). The product of v-*src* is a 60,000-molecular-weight phosphoprotein, p60$^{v\text{-}src}$, which is a membrane-bound tyrosine kinase (7, 15, 16). The mitogenic activity of RSV also depends on the expression of a functional v-*src* gene. This was demonstrated by isolating mutants of RSV that are temperature sensitive (*ts*) for their ability to induce NR cell proliferation (10, 43). Furthermore, we have described several mutants of RSV, carrying point mutations or partial deletions in the v-*src* gene, that induce NR cell division in the absence of transformation and tumorigenicity (8, 10, 11, 44). Results of these genetic analyses are consistent with the possibility that the v-*src* protein interacts with multiple substrates in NR cells. Interaction with specific substrates could induce changes in gene expression leading to NR cell division. Therefore, NR cells infected with such conditional and nonconditional mutants of RSV represent a useful model for studying the mechanisms regulating the growth of these cells.

We considered the possibility that the quiescence of differentiated NR cells depends on the expression of specific genes. Down regulation of these genes by p60$^{v\text{-}src}$ could mediate some of the phenotypic alterations leading to NR cell proliferation. In this study, we describe the isolation and preliminary characterization of cDNA clones corresponding to genes expressed in postmitotic NR cells from 13-day-old quail embryos, the transcription of which is significantly decreased in quail NR (QNR) cells expressing a functional v-*src* gene. By using v-*src* mutants inducing various phenotypes in NR cells, we show that expression of these genes is negatively regulated by p60$^{v\text{-}src}$ activity and is correlated with the quiescence of QNR cells. We also report that a subset of these genes is specifically transcribed in neural tissues and is developmentally regulated in NR cells.

MATERIALS AND METHODS

Cells and viruses. NRs were dissected from 7- to 13-day-old quail embryos as described previously (42). QNR cultures were maintained and passaged in Eagle basal medium supplemented with 8% fetal calf serum.

The Schmidt-Ruppin strain of RSV, subgroup A (SRA), was used as the wild-type virus. The isolation and characterization of RSV mutants *ts*NY68 (32), PA101 (11), and NY315 (22) have been reported previously. Viral infection of QNR cells and measurement of NR cell growth and anchorage independence were done as previously described (10).

Construction and differential screening of a cDNA library. Polyadenylated [poly(A)$^+$] RNA (5 μg) prepared from 13-

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day-old embryonic QNR (QNR13) was selected by oligo(dT)-cellulose chromatography (4) and used to construct a cDNA library as described by Gubler and Hoffman (25). After addition of *Eco*RI linkers and subsequent digestion with *Eco*RI, the cDNAs were inserted in the *Eco*RI site of the λgt10 vector (28). The library was packaged in vitro by using a Gigapack Plus extract (Stratagene) and amplified in *Escherichia coli* C600 Hfl.

The amplified library was differentially screened with ^{32}P-labeled cDNA probes prepared from poly(A)$^+$ RNAs from QNR13 cells and QNR cells transformed with *ts*NY68 and grown at 37°C. cDNA probes were synthesized from 1 μg of poly(A)$^+$ RNA by using as primers 2.5 μg of oligo(dT) (Pharmacia) and 50 μg of random hexanucleotides (Pharmacia) under the conditions described by Maniatis et al. (38).

Duplicate replicas taken from petri dishes (12 by 12 cm) seeded with 2×10^3 PFU were hybridized with both probes for 72 h. Hybridization was performed at 65°C in 1 M NaCl–50 mM sodium phosphate (pH 6.5)–2 mM EDTA–5× Denhardt solution (1× is 0.02% each Ficoll, polyvinylpyrrolidone, and bovine serum albumin)–0.2% sodium dodecyl sulfate (SDS)–20 μg of salmon sperm DNA per ml. Filters were washed at 65°C at a final stringency of 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Plaques giving higher signals with the probe prepared from QNR13 cells than with the probe prepared from *ts*NY68-infected QNR cells grown at 37°C were picked. These clones were rescreened by the method described by Lau and Nathans (35). Each recombinant was replated at low density (100 to 300 PFU per 9-cm petri dish), and a single plaque was chosen at random to inoculate in a grid array on an agar dish (12 by 12 cm) seeded with host bacteria. Duplicate replicas were then differentially probed as described above. One additional screening was sufficient to purify differentially hybridizing recombinants.

Analytical amounts of recombinant DNAs were prepared from bacteria lysed in 6-cm dishes by the fast minipreparation procedure described by Davis et al. (23). Bacteriophage stocks were obtained from five dishes (15-cm diameter) and purified in two successive CsCl gradients. Phage DNA was extracted as described by Maniatis et al. (38).

To control inserts for cross-hybridization, all purified recombinant DNAs were digested with *Eco*RI, fractionated by electrophoresis in 1% agarose gels, and transferred to nitrocellulose filters in 6× SSC by the method of Southern (49). Filters were hybridized with each recombinant DNA radioactively labeled by nick translation under conditions used for the screening of the library. Independent inserts were subcloned in the *Eco*RI site of pGEM-1 vector (Promega Biotec).

RNA isolation and Northern (RNA) blot analysis. Total cellular RNA was isolated by the guanidium thiocyanate-cesium chloride method (14). RNAs were denatured at 60°C in a formamide-formaldehyde mixture (38), fractionated by electrophoresis in 1% agarose–2.2 M formaldehyde gels (36), and transferred to nitrocellulose filters in 20× SSC (52). Filters were hybridized with cDNA inserts radioactively labeled by nick translation under the conditions described by Wahl et al. (53). cDNA inserts were obtained by *Eco*RI digestion of pGEM-1 clones, fractionation on a 1% agarose gel, and purification by the Gene Clean method (Bio 101).

Nuclear run-on transcription assay. Nuclei were isolated as described by Marzluff and Huang (39). To 210-μl portions of 2×10^7 isolated nuclei were added 25 μl of [^{32}P]GTP (250 μCi, 3,000 Ci/mmol) and 60 μl of 5× run-on buffer (1× is 5 mM Tris hydrochloride [Tris-HCl, pH 8], 2.5 mM magnesium acetate, 120 mM KCl, and 0.25 mM each ATP, CTP, and UTP). The reaction mixtures were incubated at 30°C for 30 min. RNA synthesis was arrested by addition of 46 U of RNase-free DNase (Boehringer Mannheim Biochemicals) in the presence of 6.6 mM $MgCl_2$ and 3.3 mM $CaCl_2$ and incubation for 45 min at 37°C. After treatment with 200 μg of proteinase K per ml in 1% SDS–5 mM EDTA–10 mM Tris-HCl (pH 7.4) at 37°C for 45 min, nuclear RNAs were obtained by phenol-chloroform extraction and ethanol precipitation. Precipitates were collected by centrifugation and suspended in 200 μl of 0.3 M NaCl–0.1% SDS–1 mM EDTA–10 mM Tris-HCl (pH 7.5). Unincorporated nucleotides were removed by chromatography on a Sephadex G50 fine column. Then 6×10^6 cpm of labeled RNA was hybridized with 2 μg of each recombinant DNA immobilized on nitrocellulose membranes in 10 mM Tris-HCl (pH 7.5)–0.2% SDS–10 mM EDTA–300 mM NaCl–1× Denhardt solution–200 μg of tRNA per ml–200 μg of poly(A) (Boehringer) per ml for 48 h at 65°C. Filters were washed twice in 2× SSC–0.1% SDS at 65°C for 15 min and then once in 0.1× SSC–0.1% SDS for 30 min.

TABLE 1. Mitogenic and transforming properties of RSV mutants in QNR cells

Virus	Proliferation		Transformation	
	37°C	41°C	37°C	41°C
None	–	–	–	–
SRA	+	+	+	+
*ts*NY68	+	–	+	–
PA101	+	–	–	–
NY315	+	+	–	–

DNA sequencing. cDNA inserts cloned in pGEM-1 were partially sequenced at both ends by the dideoxy-chain termination method of Sanger et al. (46) as adapted to double-stranded DNA by Chen and Seeburg (13).

RESULTS

Mitogenic and transforming properties of RSV mutants in QNR cells. Cultures of QNR from 8- to 13-day-old embryos are composed exclusively of neurons and glial cells that rapidly cease to divide and cannot be propagated in our culture conditions (20). Upon infection with wild-type SRA, these cells become transformed and acquire sustained proliferative capacity at 37 and 41°C. The mitogenic and transforming properties of the three RSV mutants used in this study are described in Table 1. QNR cells infected with *ts*NY68 are morphologically transformed and form large colonies in soft agar at 37°C but not at 41°C. The ability of this virus to induce QNR cell multiplication is also temperature sensitive. PA101 is a conditional mutant of RSV that carries multiple point mutations in the amino and carboxy coding portions of the v-*src* gene (40). Like *ts*NY68, this virus induces QNR cell division at 37°C but not at 41°C. However, proliferating QNR cells infected with PA101 are not morphologically transformed and form only small colonies in semisolid medium at 37°C. The v-*src* protein encoded by PA101 exhibits extremely low kinase activity at the permissive temperature (29, 43). NY315 is a nonconditional deletion mutant obtained in vitro. The v-*src* protein of NY315 lacks amino acids 2 to 15. This deletion prevents myristylation and membrane attachment of the *src* protein but does not impair its tyrosine kinase activity (21). NY315 retains full capacity to induce QNR cell proliferation but is

defective in all aspects of cell transformation (8). Therefore, the mitogenic property of v-*src* is not directly correlated with the levels of tyrosine kinase activity nor does it require association of $p60^{v\text{-}src}$ with the plasma membrane.

Isolation of differentially regulated cDNAs. A cDNA library was prepared in the λgt10 insertion vector by using mRNA from postmitotic NR cells dissected from 13-day-old quail embryos (QNR13). At this stage, all neuronal types have completed terminal mitosis and are undergoing differentiation. The library contained about 5×10^6 independent clones per μg of cDNA. The average size of inserts was about 1.4 kilobase pairs (kbp), ranging up to 5.5 kbp. About 20,000 plaques were screened by differential hybridization with radioactively labeled probes complementary to mRNAs from either QNR13 cells or proliferating QNR cells infected with *ts*NY68 and maintained at 37°C. After rescreening, eight recombinant phages were found to reproducibly give stronger hybridization signals with the cDNA probe prepared from QNR13 cells than with that obtained from *ts*NY68-infected cells. Cross-hybridization of the cDNAs indicated that each recombinant clone contained a different insert. These inserts were subsequently subcloned into the pGEM-1 plasmid vector for further characterization and sequencing.

Northern blot analysis of total RNAs from QNR13 cells showed that each clone hybridized to a single mRNA species. Transcript sizes varied from 6.5 to 2.2 kilobases (kb) (Fig. 1, lanes a). The levels of these RNAs in QNR13 cells were in the range observed for abundant and relatively rare RNA species. The most abundant (clone 5) represented about 0.15% and the less abundant (clone 9) represented about 0.002% of the entire library. The steady-state levels of these RNAs were markedly reduced in proliferating QNR cells infected with *ts*NY68 at 37°C. Densitometrical analysis of the Northern blots showed that the intensities of hybridization signals in *ts*NY68-infected cells were between 3-fold (clone 1) and over 200-fold (clone 12) lower than those detected in QNR13 cells (Fig. 1, lanes b).

To obtain preliminary information on the genes corresponding to the cDNA clones we isolated, we sequenced about 200 nucleotides from either end of each clone and searched for possible homologies in a sequence data base (GenBank). The 5′ portion of clone 5 was found to be strongly homologous (95%) to a chicken calmodulin gene (45). The seven other clones appeared to be distinct from sequences present in the data base. The sequenced portions at the 5′ end of clones 1, 4, 7, 9, and 12 included an open reading frame, whereas partial sequencing of both ends of clones 3 and 14 did not reveal the presence of an open reading frame, suggesting that these two inserts contained only 3′ noncoding sequences.

Nuclear run-on experiments. We examined the transcription rates of the genes corresponding to the eight selected clones in isolated nuclei from QNR13 cells and from *ts*NY68-infected cells at 37°C (Fig. 2). The results of the nuclear run-on assays showed that the transcription rates of seven genes (corresponding to clones 3, 4, 5, 7, 9, 12, and 14) were markedly reduced in *ts*NY68-infected cells compared with normal QNR13 cells. Hence, expression of these genes in QNR cells containing a functional v-*src* gene appeared to be regulated at the transcriptional level. In contrast, the rate of clone 1 transcription was comparable in normal and in *ts*NY68-infected cells, suggesting that expression of this gene was down regulated at a posttranscriptional level.

Identification of six mRNAs regulated by v-*src*. Both proliferation and transformation of QNR cells infected with RSV depend on the continuous expression of a functional v-*src* protein. To determine whether transcription of mRNAs corresponding to the selected clones was regulated by v-*src* protein activity, we examined the effects of $p60^{v\text{-}src}$ inactivation on the expression of these genes. We analyzed by Northern blotting RNAs extracted from *ts*NY68-infected QNR cells maintained at 37°C and from duplicate cultures shifted to 41°C for 48 h (Fig. 3). The cDNA clones could be classified into two categories. Levels of mRNAs corresponding to clones 1, 3, 4, 7, 12, and 14 increased in cells transferred to 41°C and were comparable to those detected in QNR13 cells. In contrast, shifting the cultures to 41°C had no effect on the levels of mRNAs corresponding to clones 5 and 9, which remained lower than those detected in QNR13 cells.

The increase in the levels of mRNAs corresponding to clones of the first group, following cell transfer to 41°C, either could be correlated with $p60^{v\text{-}src}$ inactivation or could result merely from the elevation of temperature. Therefore, we examined these mRNAs in QNR cells infected with wild-type SRA, which induces cell transformation and proliferation at both temperatures. We detected comparably low levels of the six mRNAs in SRA-infected cells grown at either temperature. We conclude that the transcription of these mRNAs in QNR cells infected with *ts*NY68 is down

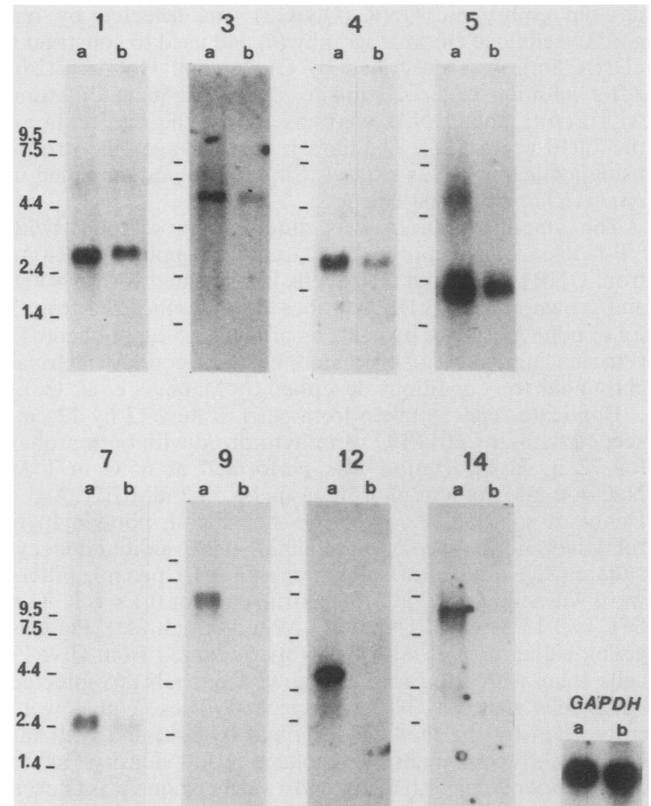

FIG. 1. Northern blot analysis of cDNA clones expressed in postmitotic QNR13 cells and down regulated in proliferating QNR cells. Total RNA (10 μg) from QNR13 cells (lanes a) and from *ts*NY68-infected proliferating QNR cells (lanes b) was denatured by formamide-formaldehyde treatment, fractionated on 1% agarose–2.2 M formaldehyde gels, blotted, and hybridized with ^{32}P-labeled cDNA inserts. Northern blots were also hybridized with a chicken glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe (37) to assess the total amount of RNA in each lane. The sizes of RNAs are expressed in kilobases.

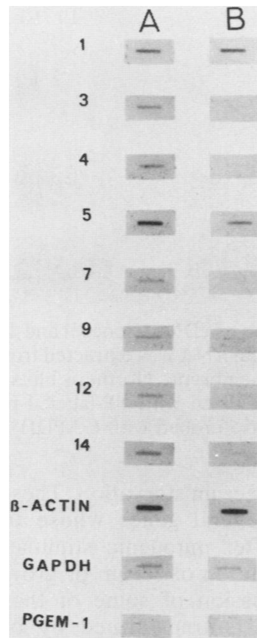

FIG. 2. Nuclear run-on analysis. Linearized recombinant plasmid DNAs (2 μg) from the indicated clones, blotted on nitrocellulose filters, were hybridized with 6×10^6 cpm of nuclear run-on transcripts prepared from QNR13 cells (A) and from *ts*NY68-infected QNR cells at 37°C (B). Plasmid DNAs containing mouse β-actin (3), chicken GAPDH, and pGEM alone were used as controls.

regulated as a consequence of $p60^{v\text{-}src}$ activity at 37°C and correlates with the inactivation of $p60^{v\text{-}src}$ at 41°C.

Down regulation of four mRNAs is correlated with the mitogenic activity of $p60^{v\text{-}src}$. To determine whether the down regulation of these six mRNAs was correlated with cell transformation or proliferation, we investigated their expression in QNR cells infected with the conditional mutant PA101, which induces cell proliferation in the absence of transformation. Northern blots of RNA extracted from mutant virus-infected cells, maintained at 37°C or transferred to 41°C, were probed with the cDNA clones. The steady-state levels of mRNAs corresponding to clones 3, 4, 12, and 14 significantly increased when PA101-infected cells were transferred to 41°C for 48 h (Fig. 4A). Conversely, transcription of these mRNAs was down regulated within 24 h, when cell proliferation resumed after the cultures were returned to 37°C (data not shown). This indicated that expression of these mRNAs was regulated similarly in QNR cells infected with either temperature-sensitive mutant. In contrast, we detected comparable levels of mRNAs corresponding to clones 1 and 7 in PA101-infected cells maintained at either temperature (data not shown). Therefore, expression of these two genes was not regulated by the v-*src* protein activity encoded by PA101.

We also examined expression of the mRNAs detected by clones 3, 4, 12, and 14 in QNR cells infected with the nonconditional mutant NY315 (Fig. 4B). This virus, like PA101, induces QNR cell proliferation in the absence of transformation. We observed a marked decrease in the levels of these RNAs in NY315-infected cells compared with those detected in QNR13 cells and in *ts*NY68- or PA101-infected cells maintained at 41°C. mRNA levels were as low as those induced by wild-type virus and by either conditional mutant at 37°C. Therefore, accumulation of these four

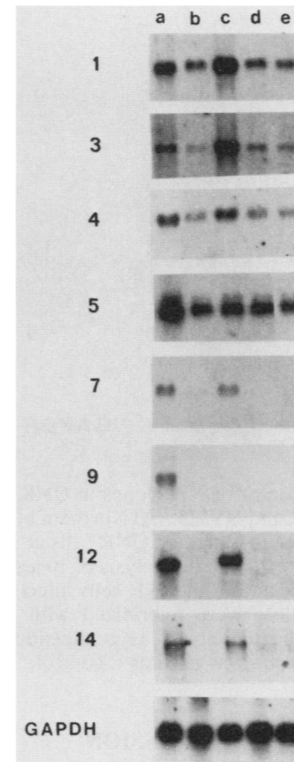

FIG. 3. Northern blot analysis of mRNAs regulated by v-*src*. Total RNA was extracted from QNR13 cells (lane a), from *ts*NY68-infected QNR cells maintained at 37°C (lane b) and at 41°C (lane c), and from SRA-infected QNR cells maintained at 37°C (lane d) and at 41°C (lane e). Northern blots containing 10 μg of each RNA were hybridized with ^{32}P-labeled cDNA probes from the indicated clones. Hybridization of Northern blots with the GAPDH probe was used as a control.

mRNAs appeared to be correlated with the quiescence of QNR cells.

Expression of cDNA clones in quail tissues and during NR development. As a preliminary step toward understanding the functions of the genes corresponding to the cDNA clones, we investigated their expression in various tissues from 13-day-old quail embryos and in cultured quail fibroblasts (Fig. 5). The clones were distributed into three groups. Sequences from clones in the first group (1, 4, and 14) corresponded to mRNAs detected in all tissues examined. Those from clones in the second group (3 and 7) were detected only in the NR and the brain. Finally, expression of the sequences in clones 9 and 12 exhibited tightly restricted tissue specificity, since the corresponding mRNAs were detected in QNR13 cells but not in other tissues.

We investigated the transcription of these two genes during earlier stages of NR development. We probed mRNAs extracted from NR dissected from 7- and 11-day-old quail embryos (QNR7 and QNR11, respectively) with cDNA clones 9 and 12 (Fig. 6). QNR7 cells contain essentially immature proliferating cells. At this stage, only ganglion cells are differentiated, whereas most cell types of QNR11 are no longer able to divide and undergo differentiation. mRNAs hybridizing to each cDNA clone were detected in QNR11 but not in QNR7 cells. In addition, the levels of these mRNAs increased between days 11 and 13 of development. These results showed that expression of these two genes was developmentally regulated in the NR.

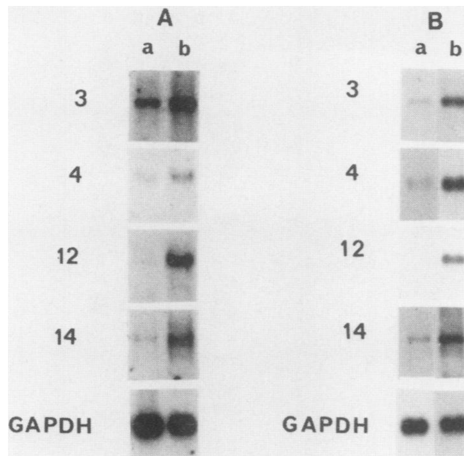

FIG. 4. Expression of cDNA clones in QNR cells infected with RSV mutants PA101 and NY315. (A) Northern blot analysis of 10 μg of total RNA from PA101-infected QNR cells at 37°C (lane a) and at 41°C (lane b). (B) Northern blot analysis of 10 μg of total RNA from QNR13 cells (lane b) and from QNR cells infected with the NY315 mutant (lane a). Blots were hybridized with ^{32}P-labeled cDNA probes from the indicated clones. Hybridization of Northern blots with the GAPDH probe was used as a control.

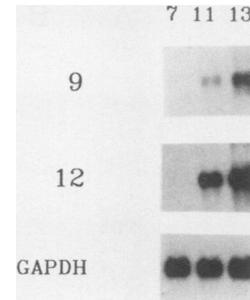

FIG. 6. Expression of cDNA clones 9 and 12 is regulated during NR development. Total RNA was extracted from NR cells of 7-, 11-, and 13-day-old quail embryos. Northern blots containing 10 μg of each RNA were hybridized with ^{32}P-labeled probes from clones 9 and 12. Blots were also probed with GAPDH.

DISCUSSION

Changes in gene expression which accompany induction of cell division have been studied mostly in cultured fibroblasts, which divide spontaneously. Growth of these cells can be arrested by serum starvation. These studies led to the identification of several genes whose transcription is increased shortly after mitogenic stimulation of growth-arrested cells by addition of serum or growth factors (2, 35). Constitutive expression of some of these genes was observed in fibroblasts transformed by oncogenes (5, 51). Recent reports described the isolation of genes that are negatively regulated by mitogenic stimulation of fibroblasts (6, 47). One such gene was also shown to be down regulated by p60$^{v\text{-}src}$ (6). Together, these results indicate that the cell cycle is regulated by a balance between positive and negative stimuli (17).

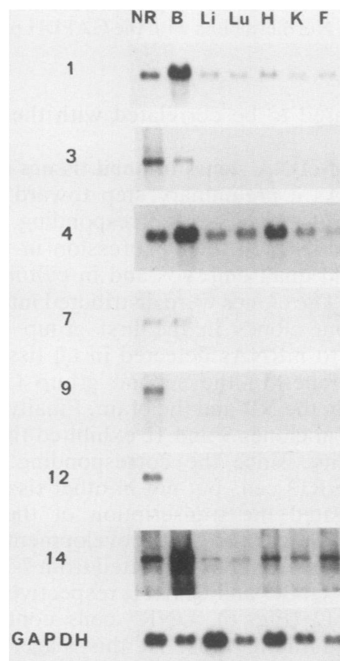

FIG. 5. Expression of cDNA clones in quail embryo tissues. Total RNA was extracted from neuroretina (NR), brain (B), liver (Li), lung (Lu), heart (H), and kidney (K) of 13-day-old quail embryos and from cultured quail embryo fibroblasts (F). Northern blots containing 10 μg of each RNA were hybridized with radioactively labeled cDNA probes from the indicated clones. Hybridization with the GAPDH probe was used as a control.

In contrast to cultured fibroblasts, differentiated NR cells are subjected to regulatory mechanisms that restrict spontaneous cell division in vivo and in tissue culture. In our model system, cell proliferation depends exclusively on v-*src* activity. This study was based on the assumption that the state of NR cell quiescence is determined by specific genes. We reported the isolation of eight cDNA clones corresponding to genes expressed in postmitotic QNR cells that are down regulated in QNR cells transformed and induced to proliferate as a result of v-*src* expression.

We found that one clone (clone 5) shares 95% homology with the chicken calmodulin gene (45). The sequenced portions of clones 1, 4, 7, 9, and 12 which contained amino acid coding information showed no similarity to known sequences, including those of genes previously shown to be down regulated in RSV-transformed fibroblasts or in fibroblasts stimulated by mitogens (1, 6, 24, 26, 47). Sequencing of the entire coding region of the clone 12 gene confirmed that this cDNA contained sequences of a previously unidentified gene (Guermah et al., unpublished data). Further sequencing of 800 bases in the 3′ coding portion of clone 4 also showed an absence of similarity to known sequences (Guermah, unpublished results). The cDNA inserts of clones 3 and 14 contained noncoding sequences, presumably derived from the 3′ end of these two mRNAs, greater than 6 kb in length. Because expression of clone 3 was restricted to neural cells, it is unlikely that this mRNA is transcribed from one of the genes previously shown to be down regulated by v-*src*. However, further sequencing is required to confirm this hypothesis and to determine whether clone 14, which is expressed in other tissues, also corresponds to a novel gene.

We showed that down regulation of six genes was continuously correlated with p60$^{v\text{-}src}$ activity in QNR cells infected with *ts*NY68. Clones 3, 4, 12, and 14 were also down regulated in cells induced to proliferate in the absence of transformation by mutant PA101 or NY315. Therefore,

negative regulation of these four genes appears to be correlated primarily with the mitogenic property of v-*src*. In addition, it does not depend on the levels of tyrosine kinase activity nor does it require myristylation and membrane association of p60^{v-src}. In contrast, expression of the clone 1 and 7 genes was not regulated by v-*src* activity in PA101-infected QNR cells. Transcription levels of these mRNAs were not influenced by cell division and appeared to be correlated with the transforming activity of p60^{v-src}.

An interesting result of this study was the isolation of cDNA clones corresponding to genes preferentially expressed in neural tissues. The clone 3 and 7 mRNAs were detected in NR and brain cells, whereas transcription of the clone 9 and 12 mRNAs was restricted to the NR and was developmentally regulated. Neither mRNA was detected in proliferating immature cells from 7-day-old embryos. Therefore, transcription of these two genes in QNR cells is correlated with terminal mitosis and acquisition of differentiated functions. Together, these results show that the v-*src* protein down regulates expression of genes likely to serve important functions in NR cells. These genes constitute potential models for studying tissue-specific and developmental regulation of gene expression.

The recent finding of tumor suppressor genes suggests that transcriptional repression of genes represents an important mechanism in cell transformation (12, 33, 34, 48, 50). Products of such genes are believed to act as negative control elements of cell proliferation. Whether down regulation of the genes described in this study is a cause or a consequence of the induction of NR cell proliferation by p60^{v-src} remains to be determined.

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