Journal of Cellular Biochemistry 58:237–247 (1995)

Effects of c-Jun and a Negative Dominant Mutation of c-Jun on Differentiation and Gene Expression in Lens Epithelial Cells

Jo Ann Rinaudo, Emanuel Vacchiano, and Peggy S. Zelenka

Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892-2730

Abstract We have used a retroviral vector (RCAS) to overexpress wild-type chicken c-Jun or a deletion mutant of chicken c-Jun (JunΔ7) lacking the DNA binding region to investigate the possible role of c-Jun in lens epithelial cell proliferation and differentiation. Both constructs were efficiently expressed in primary cultures of embryonic chicken lens epithelial cells. Overexpression of c-Jun increased the rate of cell proliferation and greatly delayed the appearance of "lentoid bodies," structures which contain differentiated cells expressing fiber cell markers. Excess c-Jun expression also significantly decreased the level of $\beta_{A3/A1}$-crystallin mRNA, without affecting αA-crystallin mRNA. In contrast, the mutated protein, JunΔ7, had no effect on proliferation or differentiation but markedly increased the level of αA-crystallin mRNA in proliferating cell cultures. These results suggest that c-Jun or Jun-related proteins may be negative regulators of αA- and $\beta_{A3/A1}$-crystallin genes in proliferating lens cells. © 1995 Wiley-Liss, Inc.*

Key words: protooncogenes, proliferation, crystallins, AP-1, retrovirus vectors

The nuclear protooncogene, c-Jun, plays a key role in the regulation of cell proliferation and differentiation [Vogt and Bos, 1990]. Overexpression of wild-type c-Jun generally promotes cell proliferation and has a moderate transforming effect [Bos et al., 1990; Castellazzi et al., 1991]. Conversely, differentiation is often associated with downregulation of c-Jun [Su et al., 1991; Vogt and Bos, 1990], although this is not always the case. For example, c-Jun promotes differentiation of embryocarcinoma cells [Yamaguchi-iwai et al., 1990; De Groot et al., 1990] and is transiently elevated in some differentiating neuronal and epithelial cell types [Greenberg and Ziff, 1984; Fisher et al., 1991; Rinaudo and Zelenka, 1992]. To exert its effects on cell growth and differentiation, c-Jun regulates transcription of genes containing the consensus AP1 site (TGA[C/G]TCA) or related sequences [Vogt and Bos, 1990; Hai and Curran, 1991; Ryseck and Bravo, 1991]. c-Jun binds to these DNA sites either as a homodimer or as a heterodimer with members of the Fos, Jun, or CREB/ATF family [Hai and Curran, 1991]. In addition, c-Jun forms functional complexes with nuclear hormone receptors [Diamond et al., 1990; Yang-Yen et al., 1990; Pfahl, 1993] and with NF-κB [Stein et al., 1993], further increasing the complexity and flexibility of transcriptional regulation.

The embryonic chicken lens is among the tissues in which c-Jun expression does not appear to be downregulated with differentiation [Rinaudo and Zelenka, 1992]. c-Jun mRNA is not only present as lens fiber cells differentiate, but the amount per cell appears to increase during the early stages of differentiation. This is not true of c-Fos mRNA, which rapidly disappears from the differentiating cells. Since c-Fos appears to be required in conjunction with c-Jun for stimulation of growth-promoting genes [Vogt and Bos, 1990; Holt et al., 1986], the selective loss of c-Fos during lens fiber differentiation suggests that c-Jun may complex with some other protein in differentiating lens fibers to regulate expression of differentiation-specific genes. Interestingly, many crystallin genes pos-

Received October 19, 1994; accepted October 20, 1994.

Address reprint requests to Peggy S. Zelenka, Building 6/Room 214, National Institutes of Health, Bethesda, MD 20892-2730.

Jo Ann Rinaudo's present address is National Cancer Institute, Bldg. 37/Room 3B07, NIH, Bethesda, MD 20892.

Emanuel Vacchiano's present address is Abbott Diagnostics Division, Dept. 9J4/Bldg. AP31, Abbott Park, IL 60064-3500.

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sess AP1 or AP1-like sites as potential binding sites for such complexes [Piatigorsky and Zelenka, 1992]. Among these are the chicken αA- and $\beta_{A3/A1}$-crystallin genes, both of which are upregulated following fiber cell differentiation [Hejtmancik et al., 1985].

To explore the relationship of c-Jun to cell growth and differentiation in the developing lens, we have used a replication-competent retroviral vector bearing either the full coding sequence of wild-type c-Jun or a mutation lacking the DNA binding region to overexpress the corresponding *wild-type* or mutant proteins in cultured primary lens epithelial (PLE) cells [Borras et al., 1988]. Since the mutant form of c-Jun retains the dimerization domains of the protein, it has the potential to act as a negative dominant mutation, interfering with the function of any protein capable of complexing with c-Jun. The high efficiency of this method of gene transfer has permitted us to monitor the effect of c-Jun and Jun-related proteins on lens epithelial cell proliferation and differentiation and to determine the effect of c-Jun and the altered form of c-Jun on expression of the endogenous chicken $\beta_{A3/A1}$- and αA-crystallin genes.

MATERIALS AND METHODS

Construction of Retroviral Vectors

The replication-competent avian retroviral vector, RCAS [Hughes and Kosik, 1984], was used in conjunction with the adaptor plasmid, Cla12Nco [Hughes et al., 1987], for all constructions. Sequence overlap extension [Ho et al., 1989] and polymerase chain reaction (PCR) [Mullis and Faloona, 1987; Saiki et al., 1988] were used to eliminate an internal NcoI site (738/743 bp) in the chicken *c-jun* coding sequence [Nishimura and Vogt, 1988] and to amplify a DNA fragment consisting of the *c-jun* coding sequence with NcoI and BamH1 sites at the 5′ and 3′ ends, respectively. This fragment was inserted into NcoI and BamH1 sites of the adaptor plasmid and cloned into the ClaI site of the RCAS vector. Clones having the proper orientation were identified by SalI digestion. Sequencing of the resulting clones confirmed that the plasmid contained the chicken *c-jun* coding sequence with a conservative C to A substitution at nucleotide 739. This retroviral vector is referred to as RCAS/jun.

To construct a plasmid lacking the c-Jun DNA binding domain [Nishimura and Vogt, 1988; Vogt and Bos, 1990], sequence overlap extension [Ho et al., 1989] was used to delete 21 bp (1,019–1,039 bp) from the chicken *c-jun* coding sequence (Fig. 1a). The resulting fragment was amplified by PCR, inserted into the NcoI and BamH1 sites of the adaptor plasmid, and cloned into the ClaI site of the RCAS vector, as described above. This vector is denoted RCAS/junΔ7. Sequencing of the cloned product confirmed that the region 1,019/1,039 bp had been deleted (Fig. 1b).

The luciferase (luc) gene was obtained as a pGL2 vector from Promega (Madison, WI). An internal ClaI site (1,365/1,370 bp) was eliminated by a conservative G to A conversion at 1,368 bp using sequence overlap extension [Ho et al., 1989], and the resulting product was cloned into the ClaI site of the RCAS vector by means of the adaptor plasmid, using NcoI and BamH1 sites added to the luc gene during PCR. The resulting vector is called RCAS/luc.

Transfection and Production of Retroviral Stocks

Chicken embryo fibroblasts (CEF) from 12-day-old chicken embryos of the EV-O strain (kindly provided by M. Federspiel) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum, 5% calf serum, 3% (wt/vol) tryptose phosphate broth, penicillin (100 U/ml), and streptomycin (100 μg/ml). After reaching confluency, cells were plated at a density of 1×10^6 cells/60 mm culture dish and incubated at 39°C, 5% CO_2. Using a previously described transfection protocol [Chepelinsky et al., 1985], 10 μg of $CsCl_2$ banded retroviral DNA (RCAS, RCAS/jun, RCAS/junΔ7, or RCAS/luc) was incorporated into the fibroblast cultures by $CaPO_4$-mediated transfection. CEFs were subcultured every second day for 8–10 days. At that time, the culture medium containing the infectious retrovirus was collected, passed through a 0.45 μm filter, and frozen at −86°C. Medium was also collected from mock-transfected CEFs cultured the same length of time for use in control experiments.

Lens Cell Culture and Viral Transduction

Primary lens epithelial cultures (PLE) were prepared from 14-day-old chicken embryos using SPAFAS research quality eggs (SPAFAS, Norwich, CT) as previously described [Borras et al., 1988]. After 3 h in culture to allow cell attachment, 100 μl of medium from the retrovi-

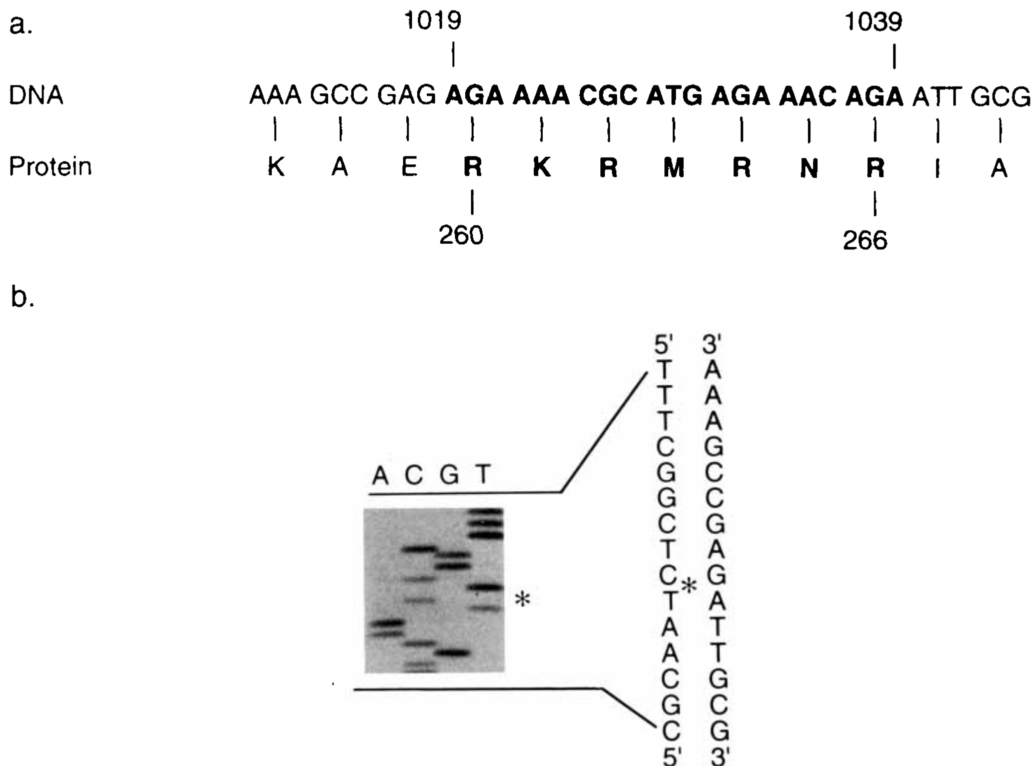

Fig. 1. **a:** Sequence of the chicken c-jun coding region (1,010/1,045 bp) showing the nucleotides and amino acids (boldface) deleted from the junΔ7 construct. **b:** Sequencing gel of the antisense strand of the junΔ7 construct corresponding to 1,010/1,045 bp of the chicken c-jun cDNA, showing successful deletion of the DNA binding region (1,019/1,039) at the position marked *.

ral-producing CEFs was added. After 2 days the medium was changed and the PLEs maintained in culture for another 2 days, after which RNA was prepared using RNAzol extraction (TelTest, Friendswood, TX).

For certain experiments, secondary cultures of infected PLEs were prepared after 7 days of culture by trypsinization for 5 min with trypsin DeLarco (Gibco/BRL, Grand Island, NY). Dissociated cells were replated at constant density in collagen-coated 35 mm dishes or 24-well plates. Cell numbers were determined by counting dissociated cells in a hemacytometer.

Assays for Expression of Transferred Genes

PLEs infected with either the pGL-2 vector or RCAS/luc were maintained in culture for 4 days. To assess the distribution of cells expressing the luciferase gene, culture dishes containing infected cells were placed directly on Polaroid Type 57 film in the dark, and 250 μl of luciferin substrate was added. After 60 min exposure the film was developed. The cell cultures were then fixed, stained with eosin, and photographed.

Expression of mRNA from the RCAS retrovirus and the constructs RCAS/jun and RCAS/junΔ7 as well as expression of endogenous mRNAs for αA- and $\beta_{A3/A1}$-crystallins was monitored by RT/PCR as previously described [Rinaudo and Zelenka, 1992]. PCR oligos were as follows:

1. For RCAS/jun and RCAS/junΔ7, spanning the vector/insert boundary: upstream—ATCGATTCTAGACCACTGTGGCCA (1/24 bp in RCAS vector); downstream—AACCCGTTGCTGGACTGGATGATG (537/561 bp in *c-jun*).
2. For chicken αA-crystallin [Thompson et al., 1987]: upstream—TTCGACCAGTTTTTGGAGAG (66/86 bp); downstream—CAGGCTCAGATCTTCAGGAGA (241/261 bp).
3. For chicken $\beta_{A3/A1}$-crystallin [McDermott et al., 1992]: upstream—ATGGGCGAAGCAGCTGTACCG (6/26 bp); downstream—GGGAGCGAATGTTGTCGAAAC (181/201 bp).

All PCR products were radiolabeled with α[^{32}P]dCTP and separated on 8% polyacrylamide/urea gels in parallel with ^{32}P-labeled DNA molecular weight markers (Gel Marker I; Research Genetics, Huntsville, AL). Radioactivity was quantitated by autoradiography and densitometry.

Expression of c-Jun and JunΔ7 proteins was assayed by immunoblotting following a standard protocol [Towbin et al., 1979]. Cultured cells were lysed with 2× SDS polyacrylamide gel electrophoresis sample buffer [Laemmili, 1970]. Proteins were separated by electrophoresis on 12% SDS polyacrylamide gels and electroeluted onto 0.2 μm nitrocellulose filters, and immunoblots were probed with a 1:200 dilution of anti(c-jun) antibody (anti-PEP2; Oncogene Science, Manhasset, NY).

RESULTS

Efficiency of RCAS-Mediated Gene Transfer

To assess the efficiency of RCAS-mediated gene transfer into primary cultures of lens cells, PLEs were transduced with the RCAS/luc retroviral vector. After 4 days, the luciferin substrate was added to the cultures, and the dishes were placed on Polaroid film to obtain a direct photograph of their luminescence (Fig. 2a). The same culture dishes were then fixed and stained to identify the regions of the dish containing cells (Fig. 2b). The areas of luminescence closely corresponded to the regions occupied by stained cells, indicating that efficient transfer of the luciferase gene had occurred and most, if not all, cells expressed the luciferase enzyme.

Expression of RCAS/jun and RCAS/junΔ7

Expression of RNA corresponding to the RCAS/jun and RCAS/junΔ7 retroviral vectors was assessed by RT/PCR using PCR primers that spanned the junction between the RCAS vector and the *c-jun* or *junΔ7* insert (Fig. 3A). A PCR product of the expected size (302 bp) was observed in cultures infected with RCAS/jun or RCAS/jun(Δ7) but not in RCAS-infected or uninfected controls. A less intense PCR product was also observed in the RCAS/jun- and RCAS/junΔ7-infected cells in the absence of reverse transcriptase. This is not unexpected, since the RCAS vector is replication competent and the cells, therefore, contain endogenous cDNA. The absence of this product in uninfected or RCAS-infected cultures indicates that it is not derived from genomic DNA. Infection of cultures with the parent RCAS vector was confirmed by RT/PCR, using oligonucleotides specific for the retroviral *env* gene (not shown).

To determine whether the RNA carried by the retroviral vectors was translated into protein, cell extracts from infected and uninfected cells were immunoblotted using an antibody specific for c-Jun. High levels of c-Jun and the mutated form, JunΔ7, were found in PLEs transduced with the corresponding RCAS vectors (Fig. 3B). Wild-type c-Jun migrated with an apparent molecular weight of about 40 kDa, as has been observed in other systems [Berko-Flint et al., 1994; Okuno et al., 1991]. The JunΔ7 product migrated slightly faster, consistent with its lower molecular weight. Endogenous c-Jun expression

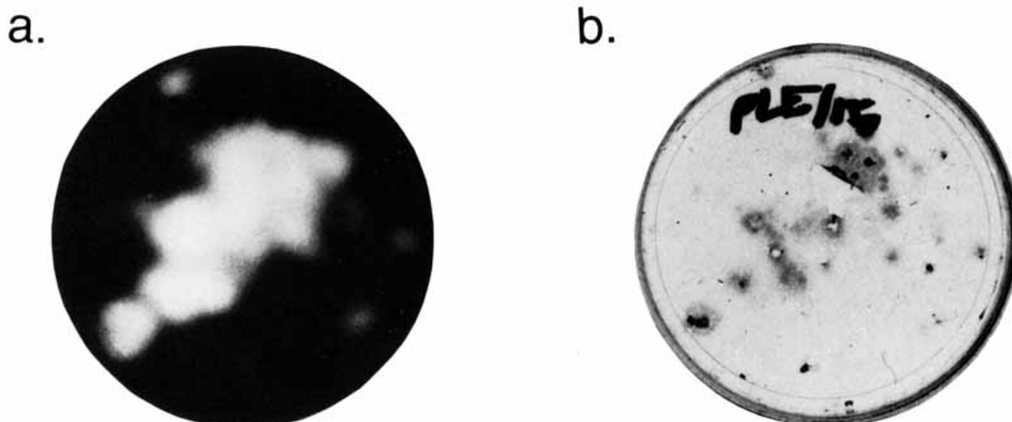

Fig. 2. Efficiency of transduction with the RCAS retroviral vector. **a:** PLEs transduced with RCAS/luc were cultured 4 days. After adding 250 μl of luciferin substrate, luminescence was recorded by placing the culture dish on Polaroid Type 57 film for 1 h. **b:** Eosin staining of the same culture.

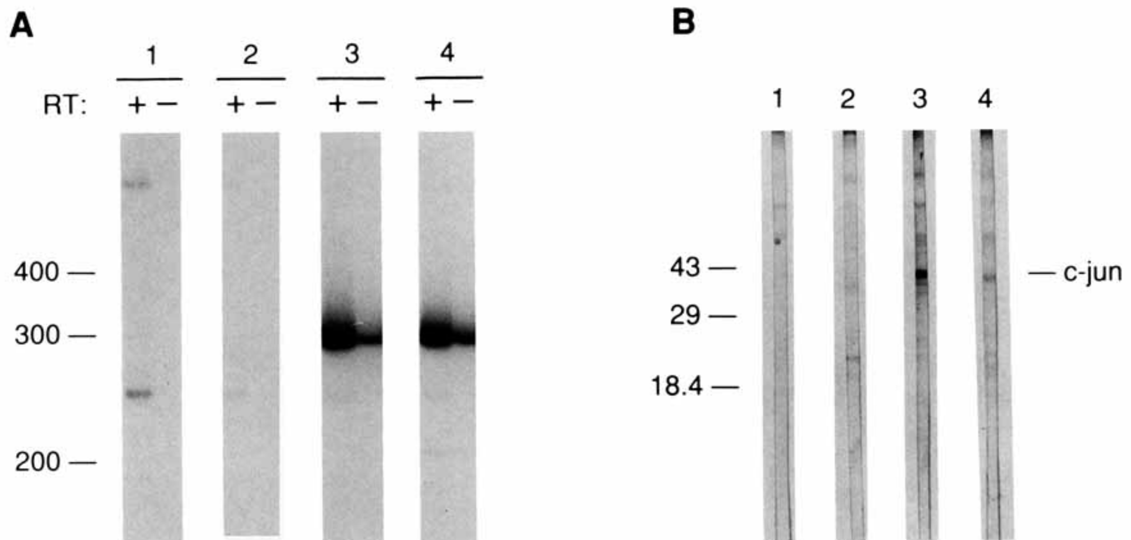

Fig. 3. Expression of RCAS/jun and RCAS/jun(Δ7) **A:** PCR was performed in the presence (+) or absence (−) of reverse transcriptase (RT) using oligonucleotides spanning the junction between the *env* gene of the RCAS vector and the coding sequence of c-jun or jun(Δ7). **Lane 1:** Control, mock-infected with supernatant from uninfected CEFs. **Lane 2:** Infected with the parent vector, RCAS. **Lane 3:** Infected with RCAS/jun. **Lane 4:** Infected with RCAS/jun(Δ7). The position of DNA size markers is indicated at left. **B:** Immunoblots of cellular proteins using an antibody specific for c-Jun. Lanes 1–4 are as in A. The position of protein molecular weight markers is indicated at left.

was very low in the uninfected control cultures and was not affected by infection with the parent vector, RCAS.

Effect of c-Jun and JunΔ7 on Proliferation

c-Jun is known to play an essential role in cell proliferation [Vogt and Bos, 1990] and can transform cells when expressed at high levels [Bos et al., 1990]. To determine the effect of c-jun overexpression on chicken lens epithelial cells, RCAS-, RCAS/jun-, and RCAS/junΔ7-infected cells and uninfected controls were trypsinized and replated at constant cell density. After 3 days, while all the cultures were still subconfluent, the cells were again trypsinized and cell numbers were determined as a measure of the proliferation rate. The number of cells in the RCAS/jun-infected cultures was significantly greater than in the RCAS- or RCAS/junΔ7-infected cultures or in the uninfected controls (Fig. 4). Interestingly, cultures infected with RCAS/junΔ7 showed no inhibition of proliferation as compared to controls, although the mutated protein was present at elevated levels (Fig. 3B).

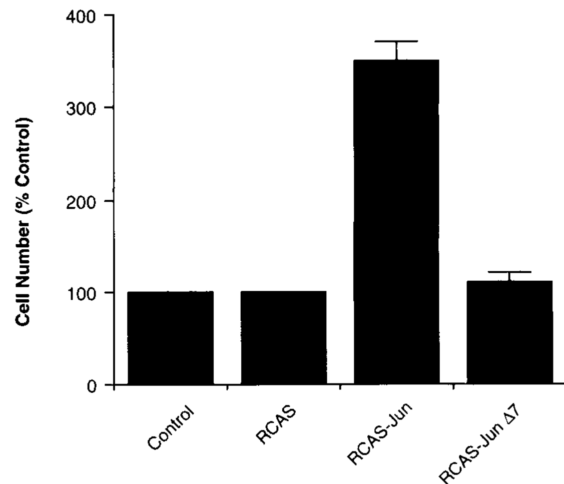

Fig. 4. Effect of RCAS/jun and RCAS/jun(Δ7) on cell proliferation. Secondary cultures of PLEs were plated at 2×10^5 cells/25 cm^2 flask. Three days after replating, cells were again trypsinized, and dissociated cells were counted in a hemacytometer. Control, uninfected PLEs.

Effect of C-jun and JunΔ7 on Differentiation

The effect of overexpression of c-Jun or expression of JunΔ7 on differentiation was assessed by the spontaneous appearance of lentoid bodies, highly refractile, morphologically distinct aggregates of nondividing cells that express high levels of crystallin proteins [Okada et al., 1971; Menko et al., 1984]. Secondary cultures of infected and uninfected cells were plated at constant cell density and allowed to reach confluency. Six days postconfluency, lentoid bodies occupied extensive areas of the dishes plated with RCAS-infected control cells or cells in-

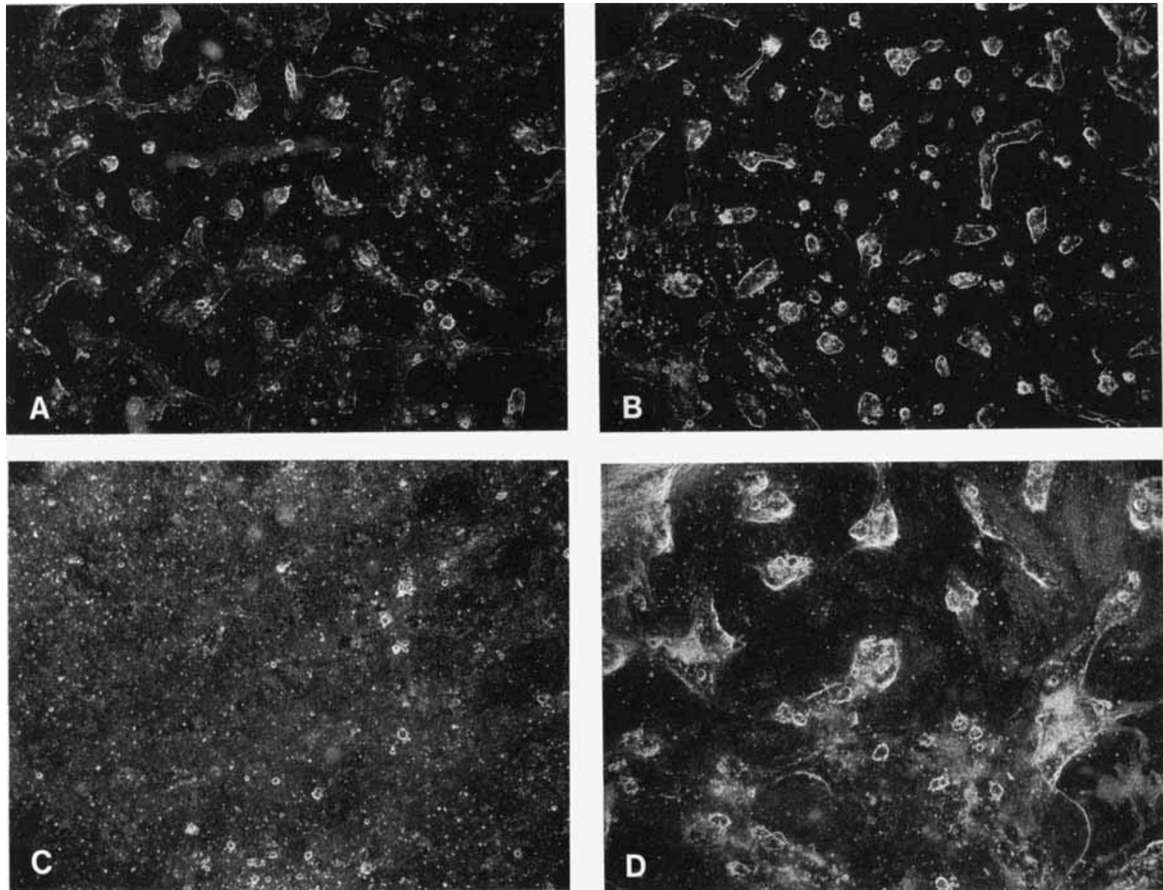

Fig. 5. Effect of RCAS/jun and RCAS/jun(Δ7) on lentoid body formation. Confluent secondary cultures of PLEs were maintained in culture until lentoid body formation was observed. **A:** Control cultures infected with parent vector, RCAS, 6 days after confluency. **B:** Cultures infected with RCAS/jun(Δ7), 6 days after confluency. **C:** Cultures infected with RCAS/jun, 6 days after confluency. **D:** Cultures infected with RCAS/jun, 14 days after confluency.

fected with the RCAS/junΔ7 vector (Fig. 5A,B). Cells infected with RCAS/jun, however, were still vigorously proliferating, as determined by labeling with ^{3}H-thymidine (not shown) and contained only a few, small lentoids (Fig. 5C). While overexpression of c-Jun clearly delayed lentoid formation, it did not entirely prevent it. By 14 days postconfluency, RCAS/jun-infected cultures also showed extensive lentoid body formation (Fig. 5D).

To test whether the lentoid bodies formed in cultures infected with RCAS, RCAS/jun, and RCAS/junΔ7 expressed proteins characteristic of differentiated lens fiber cells, lentoid bodies were detached from the cultures and isolated for protein analysis. The protein profiles obtained from the lentoid bodies of RCAS-, RCAS/jun-, and RCAS/junΔ7-infected cultures were markedly different from those of the corresponding epithelial monolayers but closely resembled the protein profile of embryonic chicken lens fiber cells (Fig. 6A). In particular, lentoid bodies contained a high concentration of a 50 kDa protein that comigrated with lens fiber cell δ-crystallin. Immunoblotting of proteins derived from lentoids and epithelial monolayers with an antibody raised against the lens fiber cell membrane protein, MIP26 [Menko et al., 1987], demonstrated that this protein was expressed in the lentoids but not in the monolayer epithelial cells of RCAS-, RCAS/jun-, and RCAS/junΔ7-infected cultures (Fig. 6B). The identity of the cross-reacting, high molecular weight protein seen only in the lentoids is not known. These findings demonstrate that the lentoids seen in these cultures display biochemical, as well as morphological, evidence of differentiation.

Since c-Jun overexpression promoted proliferation and cells must cease proliferating to differentiate, the appearance of differentiated

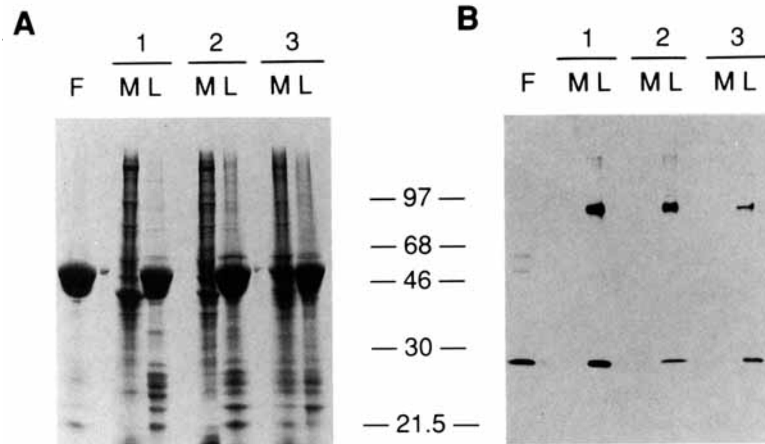

Fig. 6. Differentiation markers in lentoid bodies from RCAS/jun-infected PLEs. Equal amounts of protein extracted from lens fibers of 14-day-old chicken embryos (F) or from lentoid bodies (L) or the surrounding monolayers (M) of lens epithelial cell cultures were electrophoresed on SDS polyacrylamide gels and transferred to nitrocellulose for immunoblotting. **A:** Coomassie blue stained gel, showing prominent δ-crystallin band at 50 kD in lens fibers (F). Lane 1M,L: Monolayer cells and lentoids of RCAS-infected control cultures. **Lane 2M,L:** Monolayer and lentoids of RCAS/jun-infected cultures. Lane 3M,L: Monolayer and lentoids of RCAS/junΔ7-infected cultures. **B:** Immunoblot using anti(MIP26) antibody. Lanes are labeled as in A. The chicken MIP26 protein migrates as a 28kDa protein on SDS polyacrylamide gels [Menko et al., 1987]. The position of molecular weight markers is indicated between panels A and B.

cells in cultures infected with RCAS/jun was unexpected. To test whether the cells that formed lentoid bodies in the RCAS/jun-infected cultures continued to overexpress c-Jun, lentoid bodies were selectively detached and c-Jun expression was examined by immunoblotting. As shown in Figure 7, levels of c-Jun in the lentoid bodies appeared to be as high as in the monolayer of cells that did not form lentoids.

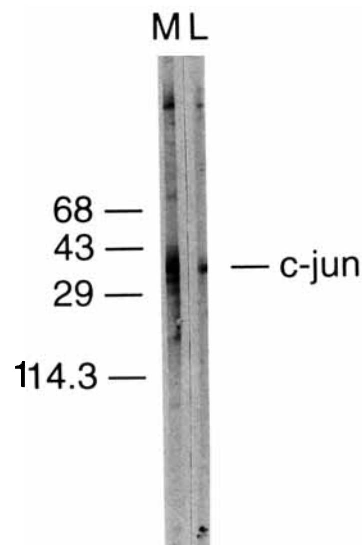

Fig. 7. Immunoblotting with anti(c-Jun) antibody of proteins present in monolayer cells (**M**) remaining after removal of lentoids and in lentoids (**L**) of RCAS/jun-infected PLEs. Lentoids were isolated by gentle agitation of PLE cultures 14 days after cells reached confluency. The position of molecular weight markers is indicated at left.

Effects of RCAS/jun and RCAS/junΔ7 on Crystallin Gene Expression

Many crystallin genes contain potential AP-1 or CREB sites in their 5′-flanking regions [Piatigorsky and Zelenka, 1992]. In the chicken, the $\beta_{A3/A1}$-crystallin gene contains an AP-1 site (-264/-258) [McDermott et al., 1992], while the αA-crystallin gene contains both AP-1 (at -571/-565) and CREB-like sites (at -138/-130 and -108/-100) [Thompson et al., 1987]. To determine whether overexpression of the wild-type c-Jun protein or expression of the mutated form, JunΔ7, affects endogenous expression of these crystallin genes, PLEs were transduced with RCAS/jun or RCAS/junΔ7, and total cellular RNA was isolated after 4 days, a time when all cultures were subconfluent and no lentoid bodies had formed. RT/PCR was then performed to determine the relative levels of αA- and $\beta_{A3/A1}$-crystallin mRNAs (Fig. 8A,B). Overexpression of c-Jun via the RCAS/jun vector had no effect on levels of αA-crystallin mRNA relative to controls (Fig. 8A) but significantly depressed expression of $\beta_{A3/A1}$-crystallin mRNA (Fig. 8B). In contrast, RCAS/junΔ7, which carries the deletion mutation of *c-jun,* greatly increased levels of αA-crystallin mRNA (Fig. 8A) but had no effect

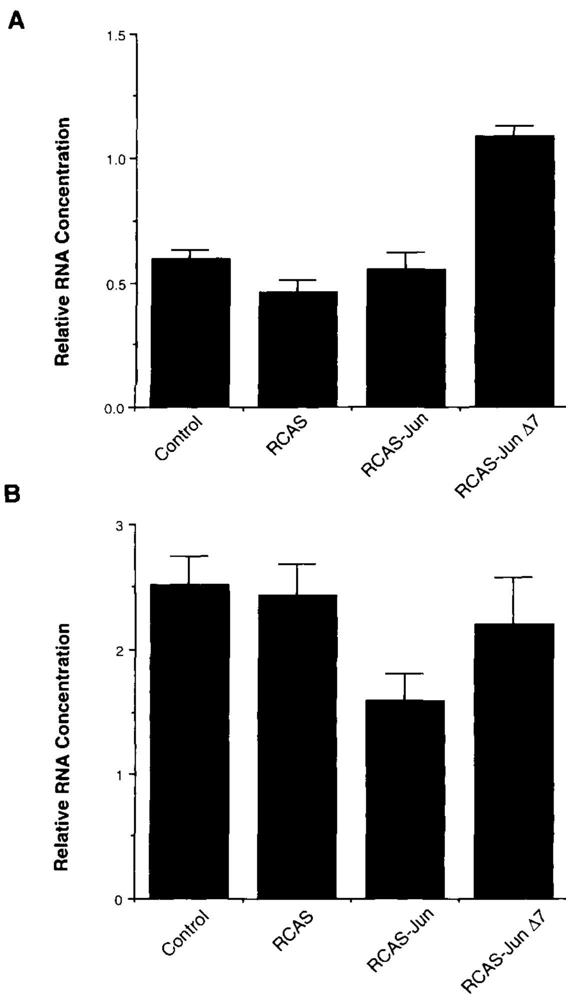

Fig. 8. Effect of RCAS/jun and RCAS/junΔ7 on crystallin gene expression in PLEs. Levels of (**A**) αA-crystallin mRNA or (**B**) $\beta_{A3/A1}$-crystallin mRNA were assayed by competitive RT/PCR with a DNA internal standard. PLE cultures were either mock-infected (control) or infected with RCAS parent vector, RCAS/jun, or RCAS/junΔ7. Bars represent the mean of three experiments ± standard error.

on expression of $\beta_{A3/A1}$-crystallin mRNA (Fig. 8B).

DISCUSSION

Infection of embryonic chicken lens epithelial cells with the RCAS/jun retrovirus vector led to a marked stimulation of cell proliferation, as has previously been observed with chicken embryo fibroblasts and myoblasts [Bos et al., 1990; Su et al., 1991]. This observation not only demonstrates that some of the excess c-Jun expressed in the PLEs is biologically active, but also indicates that the response of cultured lens epithelial cells to elevated levels of c-Jun is similar to that of other cell types.

The stimulation of proliferation in RCAS/jun-infected PLEs was coupled with an inhibition of differentiation as judged by the delay in formation of lentoid bodies in infected cultures. However, differentiation was not entirely blocked by c-Jun expression. Ultimately, large numbers of cells were able to form lentoid bodies and accumulate δ-crystallin and MIP26, an indication that they had undergone differentiation [Menko et al., 1984, 1987]. Moreover, the lentoid bodies formed in these cultures still expressed high levels of c-Jun protein as determined by immunoblotting. Studies by other investigators have shown that myoblasts overexpressing c-Jun behave similarly: differentiation of myoblasts to myotubes is only partially inhibited, and myotubes containing elevated levels of c-Jun protein are observed [Su et al., 1991]. The ability of both lens epithelial cells and myoblasts to differentiate in the presence of excess c-Jun suggests that these cell types may ultimately inactivate c-Jun or sequester it in complexes that lack growth promoting activity.

The RCAS/jun vector used in the present study contains the full coding sequence of chicken *c-jun* but lacks the 3′ untranslated region, including several AUUUA repeats that confer instability on the mRNA [Shaw and Kamen, 1986]. Deletion of this region thus stabilizes the mRNA, permitting higher levels of both mRNA and protein to accumulate [Bos et al., 1990]. The protein, however, still retains a regulatory region in the amino terminus that makes it susceptible to inactivation [Auwerx and Sassone-Corsi, 1991; Baichwal et al., 1991; Adler et al., 1992]. Thus, although infection with this vector caused marked overexpression of c-Jun protein in cultured PLEs, it is not known what fraction of this protein was active. Indeed, previous studies were unable to detect an increase in AP-1 binding activity in nuclear extracts of chicken embryo fibroblasts transduced with a similar retroviral construct bearing chicken *c-jun* [Hadman et al., 1993], suggesting that fibroblasts are able to inactivate the bulk of the excess c-Jun. Nevertheless, chicken embryo fibroblasts infected with this retroviral construct showed a moderate level of transformation [Bos et al., 1990], suggesting that even a small excess of active c-Jun may have important biological consequences.

The present data indicate that c-Jun overexpression has an inhibitory effect on expression of the $\beta_{A3/A1}$-crystallin gene in proliferating PLE cultures. Although these experiments are unable to determine whether this effect is mediated by direct binding of c-Jun to the $\beta_{A3/A1}$-crystallin promoter, this gene does possess a consensus AP1 site at -264/-258, which is part of a larger region shown to have strong enhancer activity [McDermott et al., 1992; McDermott, 1994]. A recent study of the chicken $\beta_{A3/A1}$-crystallin promoter [McDermott, 1994] suggests that the c-Jun homodimer may be one of several proteins in chicken lens nuclear extracts able to bind to the AP-1 site. Since overexpression of c-Jun would be expected to lead to increased levels of c-Jun homodimers [Kovary and Bravo, 1991], the present results are consistent with the possibility that binding of the c-Jun homodimer to the AP1 site at -264/-258 may have a negative effect on transcription of the $\beta_{A3/A1}$-crystallin gene in proliferating lens epithelial cells.

Expression of the mutated form of c-Jun (JunΔ7) in cultured lens epithelial cells had no measureable effect on the rate of cell proliferation or differentiation, although the mutated protein was efficiently expressed in the cultured cells. A similar result has been reported using a negative dominant mutation of c-Fos that lacks a functional DNA binding domain [Okuno et al., 1991]. Nonetheless, expression of the mutated c-Jun protein (RCAS/junΔ7) in proliferating cultures of lens epithelial cells had a marked effect on expression of the endogenous αA-crystallin gene. The increase in αA-crystallin expression observed in RCAS/junΔ7-infected cells was specific, since this construct did not affect $\beta_{A3/A1}$-crystallin expression. Moreover, this effect cannot be attributed to differentiation, since it was observed 4 days after infection in rapidly proliferating cultures that showed no morphological signs of differentiation. This finding suggests that JunΔ7 is able to relieve repression of the chicken αA-crystallin gene in proliferating lens cells, presumably by dimerization with a negative regulatory protein [Hai and Curran, 1991; Vogt and Bos, 1990].

Analysis of the chicken αA-crystallin promoter has identified several cis acting elements responsible for positive and negative regulation of transcription [Klement et al., 1989, 1993; Matsuo et al., 1991; Matsuo and Yasuda, 1992]. Although these elements contain no consensus AP1 or CRE sites, a divergent CRE has recently been identified at -138/-130 [Cvekl et al., 1994]. The available evidence suggests that CREB binds this region in lens cells, exerting a positive effect on αA-crystallin expression, while AP1 family proteins bind the same site in fibroblasts and may be responsible for the low level of expression in these cells [Cvekl et al., 1994]. The present study indicates that negative regulation of αA-crystallin transcription also occurs in lens cells under conditions that favor cell proliferation. Moreover, the data support the possibility that AP1 family proteins are involved in this negative regulation, as in fibroblasts, since it was relieved by the negative dominant construct, JunΔ7. Following differentiation and the loss of c-Fos [Rinaudo and Zelenka, 1992], the spectrum of AP1/CREB proteins presumably shifts to favor positive-acting proteins, such as CREB, leading to increased expression of αA-crystallin. Further study of the AP1/CREB/ATF family of transcription factors in proliferating and differentiating lens cells is needed to clarify their respective roles.

ACKNOWLEDGMENTS

We thank Dr. Peter Vogt for the chicken *c-jun* cDNA clone, Dr. Steven Hughes for the RCAS retroviral vector and Cla12Nco adaptor plasmid, Dr. Mark Federspiel for virus-free chicken embryo fibroblasts as well as much helpful advice, Dr. Sue Menko for antibody to MIP26, and Dr. Chris Sax and Dr. Graeme Wistow for critically reading the text.

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