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Induced Expression of the Endogenous Beta Interferon Gene in Adenovirus Type 5-Transformed Rat Fibroblasts

ULRICH NIELSCH,[1] RICHARD PINE,[1] STEPHEN G. ZIMMER,[1] AND LEE E. BABISS[1]†*

Laboratory of Molecular Cell Biology, The Rockefeller University, New York, New York 10021,[1] and Department of Microbiology and Immunology, University of Kentucky Medical Center, Lexington, Kentucky 40536[2]

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Tumorigenesis is a multistep process involving both genetic and epigenetic changes resulting in altered cellular gene expression. While many phenotypic attributes of transformed cells have been described, the cellular genes responsible for these phenotypes are largely unknown. In this study, we show that the interferon-stimulated gene (ISG) ISG15 is expressed in all adenovirus type 5 (Ad5)-transformed rodent cells tested, in an E1A-dependent manner. We find that the level of ISG15 mRNA correlates with the level of the transcription factor ISGF3, which has been postulated to be the transcriptional activator of ISGs. Consistent with the activation of the interferon transduction pathway in Ad5-transformed cells, beta interferon mRNA is expressed in all but the parental untransformed cell line. The level of ISG15 mRNA in Ad5-transformed cells correlated inversely with the ability of these cells to proliferate in soft agar. This appears to have functional significance, since the phenotype of poor growth in agar could be conferred upon a cell line that grows efficiently in soft agar by using conditioned media from cells that grow poorly in soft agar. The same effect could be mimicked by applying rat interferon. We conclude that the degree of activation of the interferon signal transduction pathway explains differences in the transformation phenotypes among Ad5-transformed cell lines.

Human adenoviruses have been widely used as models for studying the process of cellular transformation. Following virus infection of rodent fibroblasts, transcription from the E1A gene results in the production of two differentially spliced mRNAs of 13S and 12S in size, which encode proteins of 289 and 243 amino acids (289R and 243R proteins), respectively (6, 8, 29). Three functionally conserved regions (CR) of the E1A proteins have been described previously (25). CR1 and CR2 are present in both E1A proteins, and CR3 is unique to the 289R protein. The E1A proteins have been shown to affect a wide variety of biological functions in virus-infected rodent cell types, including modulation of host cellular transcription. Cellular genes induced in adenovirus type 5 (Ad5)-transformed cells include those encoding brain creatine kinase (21) and hsp27 (41), while genes that are repressed include those encoding fibronectin (35) and collagenase (28). For the latter gene, the mechanisms of regulation and the functional importance have been elucidated. Additional functions associated with E1A expression include the induction of cellular DNA synthesis in quiescent cells (by CR1 and CR2), induction of a growth factor that stimulates epithelial cell proliferation (C-terminal region of the 243R protein), the physical interaction with cellular proteins (by the N terminus, CR1, and CR2), the immortalization of primary rodent cells (by CR1 and CR2), and complete morphological transformation of primary or continuous cells when coexpressed with the viral E1B gene or certain activated viral oncogenes, such as *ras* (by CR1, CR2, and CR3) (reviewed in references 7 and 13).

To understand the phenotypic traits of transformed cells in terms of changes in cellular gene expression, we have previously identified a set of genes that are differentially regulated in Ad5-transformed cells by positive/negative screening of cDNA (27b). For this screen, we used untransformed cloned rat embryo fibroblast (CREF) (12) cells and a previously characterized Ad5-transformed cell line (the O1 cell line) (4). One of the differentially regulated genes that we isolated was the interferon (IFN)-stimulated gene (ISG) ISG15, which had been previously cloned in a different context (32). We found that this gene was highly expressed by the Ad5-transformed cell line but was not expressed by untransformed CREF cells.

In this report, we demonstrate that Ad5-transformed cells constitutively express IFN-β, which results in the induction of the IFN signal transduction pathway, and IFN-stimulated gene expression. Additional studies suggest that variations in the amount of IFN gene expression among independently cloned Ad5-transformed cell lines correlates with the degree of expression of the transformed cell phenotype (as determined by anchorage-independent growth). Cells which express high levels of IFN grow poorly in soft agar, while low-IFN-expressing transformed cells display greater agar cloning efficiencies. On the basis of these observations, we propose that induction of the IFN signal transduction pathway occurs during the process of cellular transformation by Ad5, which may be relevant to the acquisition or expression of the transformed cell phenotypes.

MATERIALS AND METHODS

Cells and viruses. Monolayer cultures of CREF cells and Ad5-transformed cell lines were maintained in Dulbecco modified Eagle's medium (DME) supplemented with 10% defined bovine calf serum (HyClone). In340 virus (phenotypically wild type) (18)-transformed CREF cell lines 1 through 6 and the 2-6 cell line were independently cloned. Cell lines of the O1, A2, and E series have been previously described (4, 5). The F4 cell line was derived from a focus of Ad5-transformed rat embryo brain cells. Conditioned medium was obtained by growing CREF and O1 cells to confluence in DME containing 10% defined bovine calf serum. The cells

* Corresponding author.
† Present address: Department of Cell Biology, 3,3194, Glaxo Inc., 5 Moore Drive, Research Triangle Park, NC 27709.

were refed, and the conditioned medium was harvested 24 h later. Rat IFN was obtained from Lee Biochemicals. Cell doubling times were measured by plating 4×10^4 of the appropriate cells per 35-mm plate in DME containing 10% defined calf serum. Triplicate plates were used for each 24-h time point to count the total number of cells per plate over a 4-day assay period. Doubling times were calculated by determining the slope of the growth curve.

Anchorage-independent growth. Soft-agar cloning assays were performed as previously described (3). Briefly, approximately 5×10^3 or 5×10^4 cells from each cell line assayed were suspended in DME containing 4% Noble agar and seeded on 0.8% agar base layers prepared in the same medium. Plates (60 mm) were fed 3 days, 1 week, and 2 weeks postplating with 3 ml of 0.4% agar in DME medium, and microscopic colonies were counted after 2 to 3 weeks at 37°C. Conditioned medium was briefly centrifuged prior to mixing with agar to remove detached cells which might seed the plates. When mixed with agar, the final concentration of conditioned medium per plate was 0.75×.

RNA analysis. Cytoplasmic RNA was isolated from untransformed CREF cells and virus-transformed CREF cells as previously described (2). The production of stable mRNAs from the viral E1A gene was scored by using a ^{32}P-labelled SP6-generated antisense E1A probe. Following hybridization and T_2 RNase digestion, the products were sized on denaturing acrylamide gels. The T_2 RNase protection assay for the viral E1A mRNAs has been previously described (2). Rat ISG15 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA levels were compared among the adenovirus-transformed cell lines by Northern (RNA) hybridization analysis. Twenty micrograms of total cytoplasmic RNA was loaded into each lane of a 1.0% agarose formaldehyde gel, and after electrophoresis the RNA was blotted on a Hybond-N filter (Amersham). The filters were prehybridized and hybridized with the use of ^{32}P-labelled randomly primed cDNA probes in a mixture of 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 sodium citrate), 10× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 2 mM EDTA, 50 mM sodium phosphate (pH 7.2), and 100 μg of salmon sperm DNA per ml. After hybridization, the filters were washed to a final stringency of 0.5× SSC–0.2% SDS at 65°C and exposed for autoradiography.

IFN mRNA was detected in Ad5-transformed cells or rat IFN-treated CREF cells by polymerase chain reaction (PCR) amplification and Southern blot analysis (34). One microgram of cytoplasmic RNA from CREF cells, Ad5-infected CREF cells, or O1 cells was DNase treated. The cDNA was synthesized by using reverse transcriptase and oligo(dT) primers at 42°C. RNA not treated with reverse transcriptase served as a negative control, and rat genomic DNA (1 μg) served as a positive control. For amplification, the reaction was carried out with 0.25 U of *Taq* polymerase in a final reaction volume of 80 μl containing 10 mM Tris (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.01% gelatin, 200 μM each deoxynucleotide triphosphate, and 140 ng each of primers 1 and 2 (primer 1, AGCACTGGGTGGAATGAGACTA; primer 2, GACCTTTCATAATGCAGTAGATT). Amplification was carried out for 30 cycles (1 min, 94°C; 2 min, 52°C; 2 min, 72°C; 3-s extension of the 72°C period in each cycle; and a final period of 10 min at 72°C).

Preparation of nuclear extracts and gel retardation assays. Nuclear extracts were prepared as previously described (19), using a modification of the protocol of Dignam et al. (11). Gel retardation analysis was performed as previously described (19). All probes were 3′ end labeled with ^{32}P and incubated with 2.5 μg of nuclear protein extract in 20 μl of binding reaction mixture containing 4 μg of poly(dI-dC) · poly(dI-dC) for 30 min at room temperature. After incubation, 4 μl of this mixture was subjected to electrophoresis on a 8.5% acrylamide gel in the absence or presence of unlabelled PRDI+II DNA or homologous DNA (50-fold molar excess).

RESULTS

ISG15 mRNA expression is constitutive in all Ad5- and Ad2-transformed rodent fibroblast cells. To determine whether the induction of ISG15 is a general phenomenon, several independently cloned Ad5-transformed CREF cell lines were assayed for ISG15 mRNA expression (Fig. 1A). All CREF cells transformed by wild-type Ad5 (clones 1 to 6 and clone 2-6) expressed ISG15 mRNA, while the parental CREF cell line did not. The E1A protein has three conserved regions; two (CR1 and CR2) are essential for transformation, while the third (CR3) is unnecessary (16). Cell lines (O1 and A2) expressing the E1A 243R protein (containing CR1 and CR2) and a truncated E1A 289R protein that lacks a functional CR3 also expressed ISG15 mRNA. Transformed CREF cell lines established by using viruses which were completely defective in E1A 13S or 12S mRNA expression provided additional information (Fig. 1A). Both *dl*1500 virus-transformed cell lines (no E1A 289R protein) expressed ISG15 mRNA but at greatly reduced (15-fold) levels compared with the pm975 virus-transformed (no E1A 243R protein) cell line (Fig. 1A and data not shown). Therefore, the induction of ISG15 mRNA expression can be increased by the transactivating function of CR3 but does not absolutely require CR3.

When the levels of E1A, E1B, E4, and ISG15 mRNAs were compared, no clear correlation was observed (Fig. 1B and data not shown) (1, 5). However, E1A expression was absolutely required for ISG15 expression (Fig. 1C). The A2 cell line has been previously shown to display a cold-sensitive transformation phenotype (2, 3). This cell line was established at the permissive temperature of 37°C, as a focus of transformed cells. When these cells are cultured at the nonpermissive temperature of 32°C, the cells fail to pile up and revert to characteristics resembling those of the untransformed parental CREF cell line. At this nonpermissive temperature, we found that ISG15 mRNA expression was extinguished (Fig. 1C). This was not the sole result of the incubation conditions, since clone 2-6 cells continued to express ISG15 mRNAs at 32°C. Since the loss of the transformed cell phenotype by the A2 cell line is not the result of altered viral early gene transcription (2, 3), these findings demonstrate an absolute minimal requirement for E1A expression for ISG15 gene induction. We cannot rule out the possibility that E1B proteins are participating in the induction of ISG15 expression, since we have not yet established a CREF cell line that expresses only the viral E1B gene product(s). However, the observations of Shiroki et al. (39) suggest a role for the E1B 19-kDa protein in IFN gene regulation, which could suggest a dual requirement for both E1A and E1B gene expression.

ISG15 gene expression was not restricted to transformation of immortal rodent cells, since its expression could be detected following transformation of primary or secondary rodent cells by Ad2 and Ad5 (Fig. 1D). F4 cells (primary rat brain cells transformed by Ad2) and E11 cells (Ad5-transformed secondary rat embryo fibroblasts) (5) clearly express ISG15 mRNA. The phenotypically progressed E11nmt cell line (a cell line established from a nude mouse tumor of E11)

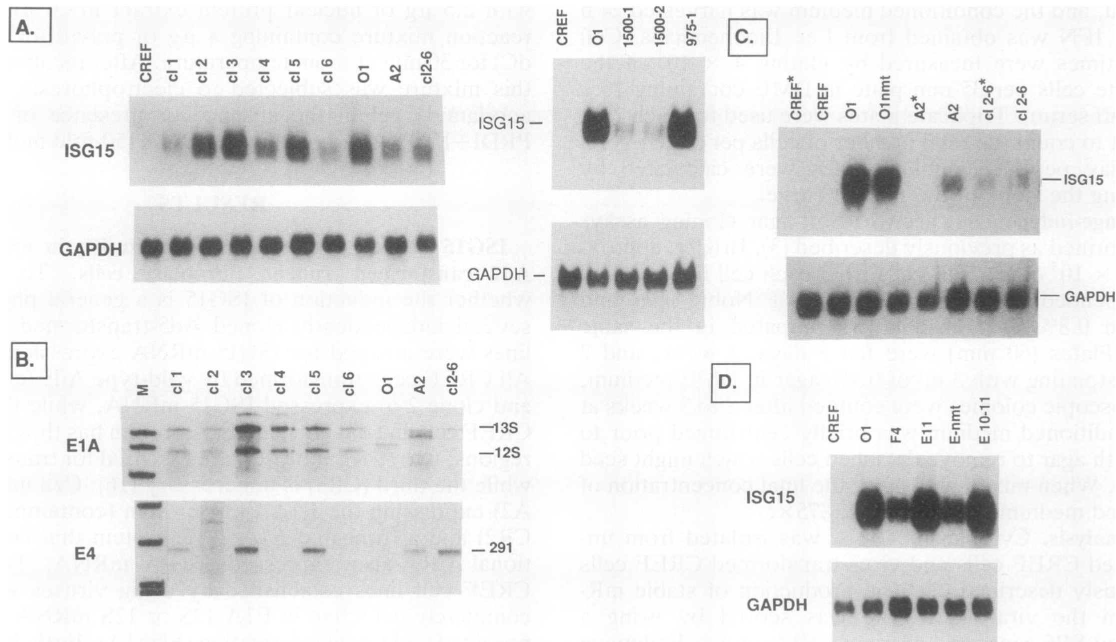

FIG. 1. Induction of ISG-15 gene expression among different adenovirus-transformed rodent cell types. Cytoplasmic RNA was isolated from the cell lines named above each panel and was analyzed by Northern hybridization or T_2 RNase protection. Individual clones of wild-type Ad5-transformed CREF cell lines (clones [cl] 1 through 6 and clone 2-6), the O1 and A2 cell lines (which express only CR1 and CR2 of the E1A protein), pm975-transformed (express only the E1A 289R protein) and *dl*1500-transformed (express only the E1A 243R protein) CREF cell lines (A and C), and Ad5-transformed primary or secondary rodent cells (D) were analyzed for ISG15 and GAPDH mRNAs by Northern hybridization using ^{32}P-labelled rat cDNA probes. (B) Relative levels of E1A and E4 mRNAs were measured by T_2 RNase protection. The antisense labelled probes, generated by transcription from the bacterial SP6 promoter, and the sizes of the RNase-resistant products indicated to the right of the panels have been described previously (2, 19). The E1A mRNAs expressed by the O1 and A2 cell lines are not wild type in size since they contain mutations which affect the coding potential of the 13S mRNA and CR3 of the larger E1A protein (4). Specifically, the O1 cell line was established by transfecting CREF cells with a plasmid containing the E1A and E1B genes derived from the H5*dl*101 virus (3). This E1A gene contains a deletion of nucleotides 1008 to 1012, relative to the 5′ end of the viral genome (nucleotide position 1). The T_2 RNase protection assay using RNA obtained from this cell line results in protected RNAs of 467 nucleotides (12S, but underrepresented because of the inefficient use of the 12S donor splice site [unpublished observation] and a truncated 13S-specific RNA of 509 nucleotides. The A2 cell line was established from a focus of transformed cells following Ad5*hr*1 virus infection of CREF cells (5). This virus contains a point mutation in the E1A gene at nucleotide 1055. As a result, protected E1A-specific RNAs of 467 nucleotides (12S) and 556 nucleotides are observed. For panel C, the cell lines indicated with an asterisk were maintained for 6 weeks at 32°C prior to analysis of ISG15 mRNA levels.

showed a reduction (15-fold) in ISG15 mRNA levels compared with the E11 cell line. An azacytidine-induced revertant of the E11nmt cell line (E10-11, phenotypically similar to the E11 cell line) (5) regained a high level of ISG15 gene expression (11-fold increase compared with E11nmt), similar to the E11 cell line. The selective down-regulation of ISG15 expression in E11nmt cells may suggest that ISG15 mRNA levels are inversely correlated with the transformation phenotype.

The IFN signal transduction pathway is operating in Ad5-transformed cells. The expression of ISG15 mRNA has been observed exclusively following induction by type I IFN (IFN-α or -β). The interaction of type I IFN with the IFN receptor activates a signal transduction pathway that causes ISGF-3α and ISGF-3λ to associate in the cytoplasm and form the transcription factor ISGF-3 (23). This activated complex is translocated to the nucleus, where it binds to the IFN-stimulated response element (ISRE), which is necessary and sufficient for transcriptional activation of ISGs (24, 31). To determine whether ISG15 induction in Ad5-transformed cells was due to increased levels of nuclear ISGF-3, gel retardation assays were carried out, using nuclear extracts prepared from the cell lines shown in Fig. 2 and using the ISRE of the ISG15 gene as the probe. CREF cells show constitutive levels of ISGF-1 and ISGF-2/IRF-1 (24, 25), while ISGF-3 is present only following treatment with rat IFN.

All of the Ad5-transformed cell lines, as well as nude mouse tumor cells derived from the transformed cell lines, show constitutive but variable relative ISGF-3 DNA-binding activity. This finding suggests that ISG15 expression in Ad5-transformed cells is due to the presence of activated ISGF-3. While for all cell lines tested there was a direct correlation between ISG15 mRNA levels and ISGF3 DNA-binding activities, we cannot exclude the possibility that additional regulatory proteins are contributing to the rate of ISG15 mRNA expression. It should be noted that the slight variation in ISGF3 DNA-binding activity for both the O1 and O1nmt cells was not reproducible. As was the case for ISG15 mRNA expression, there was no apparent correlation between high ISGF-3 DNA-binding levels and the particular E1A protein species expressed.

Ad5-transformed cells constitutively express the IFN-β gene. The activation of ISGF-3 and the expression of ISG15 suggested that Ad5-transformed cell lines may be expressing type I IFNs or some other factor capable of activating the IFN signal transduction pathway. To test this, conditioned medium from O1 cells was applied to CREF cells, and

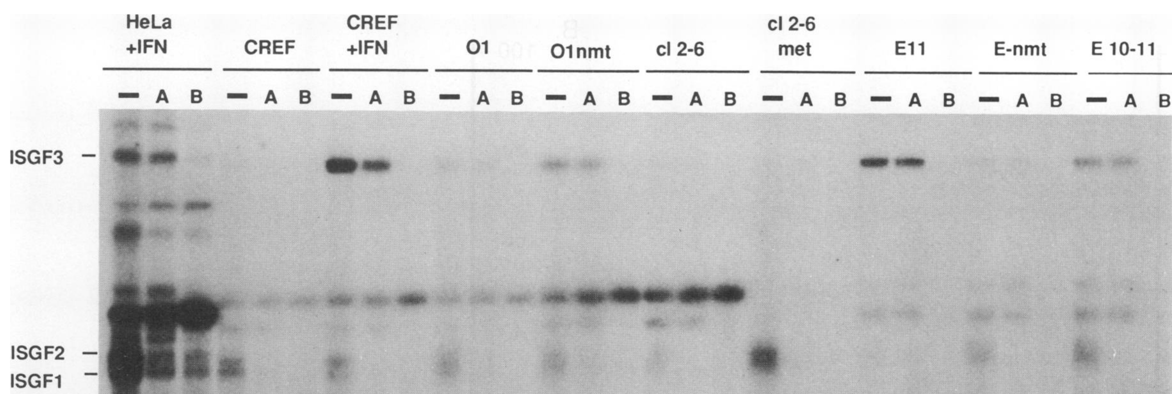

FIG. 2. Constitutive ISGF3 expression in Ad5-transformed rodent cells. Nuclear extracts were prepared from each cell line shown as previously reported (19). The clone 2-6 (cl 2-6) met cell line was established from a metastatic lung tumor nodule following the injection of clone 2-6 cells into a nude mouse. Human HeLa S3 cells were treated with 5 ng of λ-IFN per ml for 12 h and 500 U of human IFN-α per ml for 2 h, and CREF cells were treated with 500 U of rat IFN per ml for 2 h. A ^{32}P-labelled oligonucleotide (1 ng) containing the ISG15 ISRE sequence (capable of binding ISGF1, ISGF2/IRF1, and ISGF3 factors) was used in the gel mobility shift binding reactions with 2.5 μg of protein extract. Shifted complexes were competed for specifically by the addition of a 100-fold molar excess of unlabelled (A) PRDI+II oligonucleotide (from the human IFN-β promoter, which has no ISGF3-binding ability) (lanes A) or a homologous probe (lanes B).

induction of ISG15 mRNA was observed (Fig. 3A). ISG15 expression was also induced in CREF cells treated with rat IFN. Further analysis revealed that IFN-β mRNA was expressed by both Ad5 virus-infected CREF cells and the O1 cell line but not by the untransformed parental CREF cell line (Fig. 3B). We used a PCR-based approach to detect IFN-β mRNA, since the levels of IFN mRNA are often relatively low (14, 33). These results, taken together with the observation that O1 conditioned medium had antiviral activity equivalent to 100 to 300 U of rat IFN per ml whereas CREF cell conditioned medium had no clear antiviral effects (less than 10 U/ml), suggest that ISG15 expression is due to autocrine expression of IFN-β in Ad5-transformed cells. Further proof of this suggestion might be provided by experiments using anti-rat IFN antibodies, which are not currently available.

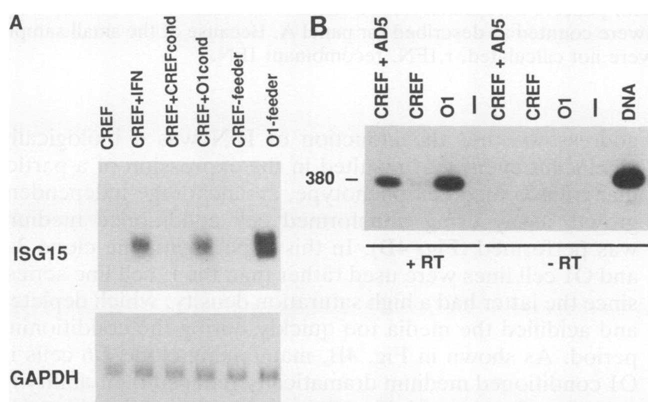

FIG. 3. Induction of ISG15 mRNA in Ad5-transformed cells as a result of autocrine IFN-β release. (A) CREF cells (CREF-feeder) and O1 cells (O1-feeder) were grown to confluence in DME containing 10% defined bovine calf serum (HyClone). The cells were refed, and the conditioned media were harvested 24 h later. Conditioned medium or rat IFN (500 U/ml) was added to CREF cells at the mid-log stage. Following this treatment for 6 h, cytoplasmic RNA was prepared. ISG15 and GAPDH mRNA levels were determined by Northern hybridization analysis. (B) Cytoplasmic RNA isolated from the cell lines shown above each lane was converted to DNA by using oligo(dT) primers and reverse transcriptase (+RT), or incubated in the absence of enzyme (−RT), and amplified by PCR. The IFN-β PCR product was visualized by Southern hybridization using a ^{32}P-labelled rat IFN-β cDNA probe cloned by us.

High expression of IFN correlates with reduced cloning efficiency in soft agar. Since IFN expression in cells is tightly controlled and usually observed only for a brief period during viral infections (20, 40), it was unexpected to find constitutive IFN expression in Ad5-transformed cell lines, particularly since they do not shed virus. Furthermore, since IFNs have clear antitumor activity (10) and may even represent a class of tumor suppressor genes (30), induction of IFN-β gene expression during Ad5 transformation of rodent fibroblast cells may appear paradoxical. However, there is precedent for autocrine expression of IFN in human cancer cells (9). Taking into account the pronounced effects of IFNs on transformed cells, it seemed likely that the extent of activation of the IFN pathway could influence the transformed cell phenotype. Therefore, we compared ISG15 expression with growth in soft agar (Fig. 4A), which has been used as a measure of the transformed cell phenotype and usually correlates with tumorigenic potential (4, 5). All of the virus-transformed cell lines exhibited an inverse relationship between the level of expression of ISG15 mRNA and the efficiency of growth in soft agar (Fig. 4A). An analysis of the tumor-derived cell lines established from the O1, clone 2-6, and E11 transformed cells revealed that only the E11nmt cells exhibited a progressed phenotype, as scored by both growth in soft agar (Fig. 4A) and tumorigenicity (5). To determine whether this was a reflection of differences in the rate of proliferation among these cell lines, cell doubling times were determined (Table 1). All of the cells, including the parental CREF cell line, had comparable doubling times. Furthermore, treating CREF cells (Table 1) or the Ad5-transformed cells (data not shown) with exogenous rat IFN failed to produce an antiproliferative state. Therefore, we can distinguish between the antiproliferative and anti-anchorage-independent growth effects. Since there is a correlation between the levels of IFN-β production and ISG15 mRNA levels (based on an antiviral assay using conditioned media; data not shown), we used ISG15 mRNA levels as a measure of the degree of induction of the IFN

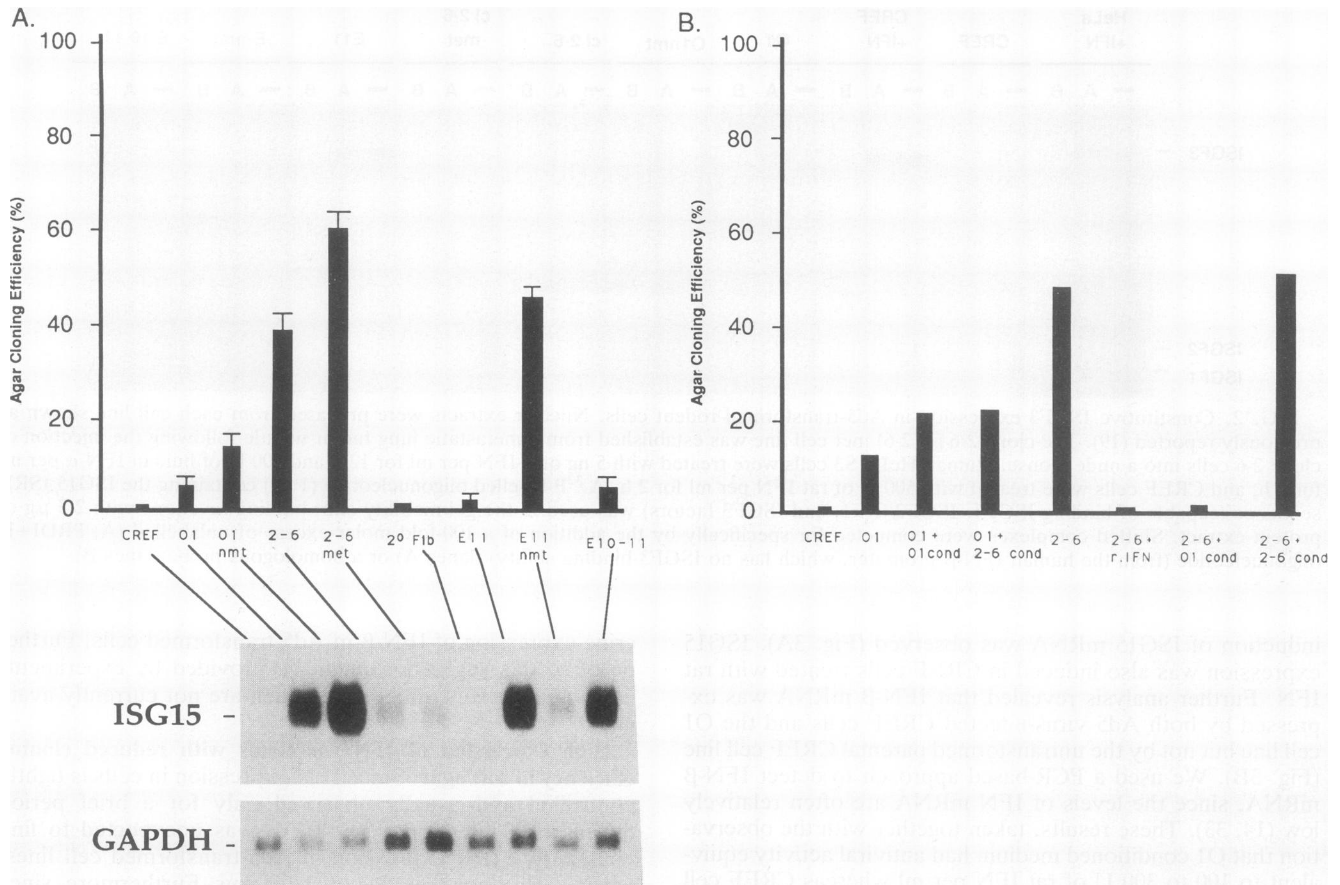

FIG. 4. Evidence that Ad5-transformed cells which express high levels of ISG15 mRNA show a reduced capacity to proliferate in soft agar. (A) Approximately 5×10^3 or 5×10^4 cells from each of the cell lines shown were suspended in DME medium containing 0.4% Noble agar and seeded on 0.8% agar base layers prepared in the same medium. Each value is the mean number of colonies counted per plate ± standard deviation from a total of six to eight plates. Secondary cultures of rat embryo fibroblasts (2° Fib) were used since they were the parental cells used to establish the Ad5-transformed E11 cell line. (B) As for panel A except that fresh DME was substituted with conditioned medium obtained from the 2-6 or O1 cell line where indicated. Colonies were counted as described for panel A. Because of the small sample size in this assay (two 60-mm plates per sample), standard deviations were not calculated. r.IFN, recombinant IFN.

signal transduction pathway. Furthermore, we have shown that all of our Ad5-transformed cell lines can be induced to express higher amounts of ISG15 mRNA by treatment with exogenous rat IFN (27a). Therefore, differences in the cloning efficiencies in soft agar among all of the cell lines tested cannot be explained simply by changes in their susceptibility to IFN.

Conditioned medium from a high-IFN-expressing transformed cell can reduce the agar cloning efficiency of a transformed cell which expresses lower levels of IFN. To begin to address whether the induction of IFN was a biologically significant event that resulted in the expression of a particular transformed cell phenotype, an anchorage-independent growth assay using transformed cell conditioned medium was performed (Fig. 4B). In this experiment, the clone 2-6 and O1 cell lines were used rather than the E cell line series, since the latter had a high saturation density, which depleted and acidified the media too quickly during the conditioning period. As shown in Fig. 4B, maintaining clone 2-6 cells in O1 conditioned medium dramatically reduced their ability to grow in soft agar, and this effect could be mimicked by using a matched concentration (300 U/ml, based on an antiviral assay) of rat IFN. We have determined that the O1 and clone 2-6 cells express between 100 to 300 and 30 to 100 U of IFN per ml, respectively. In contrast, O1 conditioned medium had no effect on the efficiency of anchorage-independent growth of the O1 cell line, which demonstrates that the effect of O1 conditioned medium on clone 2-6 cells was not due simply to the inability of the medium to support growth in agar as a result of accumulation of toxic metabolites. Thus, the growth characteristics of Ad5-transformed cells in soft agar are due to the release of a soluble factor, which is most likely IFN-β.

TABLE 1. Doubling times of CREF and Ad5-transformed CREF cell lines

Cell line	Doubling time (h)[a]
CREF	19
CREF + recombinant IFN (500 U/ml)	22
O1	14
O1nmt	15
clone 2-6	19
clone 2-6 met	17

[a] Calculated as described in Materials and Methods.

DISCUSSION

Altered expression of specific cellular genes is the driving force for the processes of carcinogenesis and tumor progression. As selective pressures are imposed upon a transformed cell, variants from within the originally clonal population can emerge as dominant. While many cellular genes have been shown to be expressed at distinct phenotypically defined stages of these events, a direct causal role for these genes has only occasionally been demonstrated.

In this report, we have characterized the mechanism by which ISG15 mRNA is constitutively expressed in Ad5-transformed rodent cells. We show that all Ad5-transformed cells constitutively express IFN-β. Consistent with IFN-β expression, we observed that Ad5-transformed cells show elevated levels of the DNA-binding protein complex ISGF3, which is the transcriptional activator of ISGs.

How IFN gene expression might play a role in the process leading to the transformed cell state remains unclear. However, it is clear from our findings that IFN expression can modulate at least one transformed cell phenotype, that is, to limit cell growth in soft agar. We used ISG15 expression as a marker for the activation of the IFN pathway. However, since a considerable number of genes are IFN inducible (we have scored the expression of several IFN-inducible genes in the Ad5-transformed cells lines [27a]), it remains to be determined which of the genes regulates the ability of these cells to proliferate in soft agar. ISG15 expression is, however, potentially interesting in itself. The gene product shares homology with ubiquitin and may be involved in targeting proteins for degradation (15), which would be a posttranslational mechanism for modifying cellular functions. In support of this type of gene regulation, the recent studies of Scheffner et al. (37) have in fact shown that the wild-type antioncoprotein p53 is a target for ubiquitin degradation.

Another important group of IFN-inducible genes are the major histocompatibility complex (MHC) class I genes. The expression of these genes has been shown to be down-regulated in Ad12-transformed rodent cells and up-regulated in Ad5-transformed cells (38). While the two viruses transform CREF cells at similar efficiencies (data not shown), this difference in MHC class I expression can in some instances explain why Ad12 virus but not Ad5 virus can induce tumors in immunocompetent syngeneic rats. Since MHC expression can be restored in Ad12-transformed cells by IFN (17), it is tempting to speculate that Ad12-transformed cells fail to constitutively express IFN-β. We have recently confirmed this hypothesis by demonstrating that both ISGF3 and NF-κβ DNA-binding activities (both positively regulate MHC class I gene expression) are reduced in Ad12- but not Ad5-transformed CREF cells (27). Thus, the failure of Ad12-transformed cells to activate the IFN signal transduction pathway explains their low levels of MHC class I expression and their greater oncogenicity.

It has been reported previously that adenovirus infection of human HeLa cells suppresses ISG15 induction by IFN (32). Specifically, it was shown that the E1A 243R protein was responsible for this effect. While this finding appears to be in conflict with our observations (particularly since our highest ISG15-expressing O1 cell line expresses only the E1A 243R protein), virus infection of human cells may not be a relevant model for this gene in transformed rodent cells. An example of such a situation is the induction of MHC class I genes during infection of rodent cells by Ad12 and suppression of these genes in transformed cells (36, 38). Therefore, the rate of E1A expression (exogenous during infection versus endogenous following stable integration of the viral DNA into the host genome; addressed in reference 1) and chromosomal context of the viral genome could determine whether the IFN signal transduction pathway is activated. This may also help to explain why human 293 cells fail to constitutively express ISG15 mRNA (data not shown). Here we must consider species variations and the rate of endogenous viral E1A and/or E1B gene expression.

The ability of Ad5-transformed cells to activate constitutively the IFN pathway allows the virus to modulate many genes through a single gene product, which ultimately confers a number of phenotypic traits on the cell, including reduced growth in soft agar and an antiviral state. It is, however, somewhat surprising that Ad5-transformed cells seem to limit their transformation phenotype by expressing IFN. This phenomenon might indicate an obligatory activation of the IFN pathway at an early stage of the transformation event by Ad2 or Ad5. Finally, it should be noted that the induction of IFN gene expression is not unique to Ad2- and Ad5-transformed cells. Simian virus 40 large T antigen has been shown to induce endogenous IFN-α and -β gene expression in both human HeLa and 293 cells (22). This finding also suggests that T antigen can complement some aspect of the IFN signal transduction pathway that either is not fully induced by the viral E1A and E1B proteins or is lacking in 293 cells.

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