Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 812–816, February 1986
Microbiology

Expression of the oncogene of avian reticuloendotheliosis virus in *Escherichia coli* and identification of the transforming protein in reticuloendotheliosis virus T-transformed cells

(reticuloendotheliosis virus T transformation/v-*rel* oncogene protein)

NORBERT K. HERZOG AND HENRY R. BOSE, JR.

Department of Microbiology, The University of Texas, Austin, TX 78712

Communicated by Esmond E. Snell, September 16, 1985

ABSTRACT **The genome of reticuloendotheliosis virus T (REV-T) includes a unique oncogene v-*rel*, which is transcribed in low amounts into a 3.0-kilobase subgenomic mRNA in REV-T-transformed lymphoid cells. To identify the v-rel protein, REV-T DNA sequences were cloned into bacterial plasmid vectors designed to achieve expression of foreign DNA sequences in *Escherichia coli*. Portions of the v-*rel* gene were joined to the 5′ segment of the *trpE* gene. Upon induction of *trpE* with indoleacrylic acid, large amounts of trpE–v-rel fusion proteins were produced by the bacteria carrying these recombinant plasmids. Two trpE–v-rel fusion proteins were synthesized in *E. coli*, which collectively represent three-quarters of the predicted v-rel protein. Polyclonal antisera were generated to trpE–v-rel fusion proteins. These antisera were used in immunoblotting experiments to identify a 57-kDa v-rel protein in REV-T-transformed lymphoid cell lines and REV-T-infected chicken embryo fibroblast cultures. The v-*rel* gene expressed in *E. coli* under *lac* control was found to produce a 56-kDa protein. Although REV-T-transformed and Marek disease virus-transformed lymphoid cells contain c-rel mRNA transcripts, a c-rel protein could not be detected with antisera directed against v-rel fusion proteins.**

Reticuloendotheliosis virus T (REV-T) is a replication-defective retrovirus that transforms very immature lymphocytes of the B-cell series (1–3) and fibroblasts *in vitro* (4–7). REV-T-transformed non-virus-producing lymphoid cells are tumorigenic (2, 8, 9). Like other avian acute leukemia viruses, the genome of REV-T contains deletions in portions of *gag*, *pol*, and *env* genes, and it has acquired a helper virus unrelated sequence (1, 10–12). REV-A apparently acquired a turkey proto-oncogene (c-*rel*), which, by further alterations, became REV-T with the unique 1.4-kilobase (kb) oncogene v-*rel* (13–15). The v-*rel* oncogene is distinct from other known oncogenes (11, 14–16). REV-T-transformed cells contain a 3.0-kb subgenomic v-rel mRNA (17). The c-*rel* proto-oncogene encodes a 4.0-kb mRNA in cells of hematopoietic origin (17, 18). The c-*rel* proto-oncogene is transcribed in all hematopoietic tissues, but it is not transcribed at significant levels in brain or muscle (17). Furthermore, REV-T transformation of lymphoid cells does not appear to involve the activation of other known proto-oncogenes (17).

The nucleotide sequence of the entire v-*rel* gene has been determined (14, 16). A single long open reading frame within the v-*rel* nucleotide sequence has been identified, which would encode a protein of 503 amino acid residues for a total molecular mass of ≈56 kDa. The start codon for this open reading frame is within REV-A sequences, resulting in 12 amino acids derived from REV-A envelope sequences at the amino end of the v-rel protein. The protein terminates with 19 amino acids derived from envelope p20E coding sequences used in the −1 reading frame (14, 16). Efforts to immunoprecipitate the transforming protein from REV-T-transformed cells with antiserum directed against helper virus structural proteins, however, have been unsuccessful.

In this study, we have cloned v-*rel* sequences into bacterial plasmid vectors designed to achieve the expression of foreign DNA sequences in *Escherichia coli*. Two trpE–v-rel fusion proteins were synthesized in *E. coli*, which collectively represent three quarters of the predicted v-rel protein. These fusion proteins were used to produce polyclonal antisera to the v-*rel*-encoded protein. These antisera were then used to identify the transforming protein of the *rel* oncogene of REV-T.

MATERIALS AND METHODS

Bacteria and Plasmid Vectors. The plasmids pATH-1, -2, and *E. coli* RR-1 were generously provided by A. Tzagoloff and T. J. Koerner. The plasmid pUC18 and *E. coli* JM105 were provided by J. R. Walker. The minicell strain *E. coli* P678-53 was kindly provided by S. M. Payne.

Construction of Recombinant Plasmids. The construction and structure of plasmids designed to express v-*rel* sequences in *E. coli* are presented in Figs. 1 and 4. Enzymes were obtained from International Biotechnologies (New Haven, CT) or New England Biolabs and were used according to manufacturers' recommendations. Plasmids were prepared according to published methods (19). Restriction fragments and linearized vectors were purified twice by agarose gel electrophoresis (19). Ligations were carried out at 14°C overnight. Transformations of *E. coli* RR-1, JM105, or P678-53 were all carried out as described by Lederberg and Cohen (20).

Induction of TrpE Expression Plasmids. Preparations and analysis of the proteins produced in *E. coli* RR-1 harboring both the pATH vectors and pATH/v-*rel* recombinant plasmids in response to *trpE* induction by indoleacrylic acid were carried out essentially as described (21, 22). Both whole cell lysates and preparations of insoluble proteins were analyzed by $NaDodSO_4$/PAGE (23). Large quantities of fusion proteins required for immunization were prepared from 1.5-liter bacterial cultures.

Gel Electrophoresis. Protein preparations were denatured by boiling for 3 min in the presence of 1 M urea/2% 2-mercaptoenthanol/2% $NaDodSO_4$. Protein samples were separated by electrophoresis in 7–15% linear gradient $NaDodSO_4$/polyacrylamide gels using 5% stacking gels (23). Electrophoresis was allowed to proceed at 3–15 mA (constant current) and was monitored by the migration of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: REV-T and REV-A, reticuloendotheliosis viruses T and A; kb, kilobase(s); bp, base pair(s); CEF, chicken embryo fibroblasts; IPTG, isopropyl β-D-thiogalactoside.

bromophenol blue. Proteins were visualized by staining with Coomassie brilliant blue. Alternatively, radiolabeled proteins in dried gels were visualized by exposure to Kodak XAR-5 x-ray film with intensifying screens. Molecular size estimations were based on the relative migration of marker proteins obtained from Bio-Rad or Pharmacia ranging in size from 14 to 200 kDa.

Minicell Isolation and Screening of Plasmid-Encoded Protein. Cultures of *E. coli* P678-53 (24) and P678-53 containing various plasmids were grown overnight at 37°C in L broth [tryptone (10 g/liter)/yeast extract (5 g/liter)/NaCl (5 g/liter)] with carbenicillin (250 μg/ml). Preparations of minicells (25) were suspended in [^{35}S]methionine assay medium with 0.5 mM isopropyl β-D-thiogalactoside (IPTG) and incubated at 37°C for 45 min. Then [^{35}S]methionine (50 μCi/ml; 1 Ci = 37 GBq) (Amersham) was added and incubation was continued for 1 hr. The minicells were washed and the amount of [^{35}S]methionine incorporated into acid precipitable material was determined by liquid scintillation counting. Equal amounts of [^{35}S]methionine-labeled proteins from each sample were analyzed by $NaDodSO_4$/PAGE and autoradiography of dried gels (26).

Immunization. The insoluble protein pellet of bacterial cell lysates was electrophoresed on 10% $NaDodSO_4$/polyacrylamide preparative gels (3 × 9 × 16 mm) at 15–20 mA (at constant current) and proteins were detected by staining with Coomassie brilliant blue. The fusion protein band from the gel was excised and emulsified in complete Freund's adjuvant (22). The initial immunization of two female New Zealand rabbits used ≈500 μg of protein. The rabbits were given booster injections of ≈250 μg of protein emulsified in incomplete Freund's adjuvant 3 weeks later. Serum was collected beginning 7 days after immunization.

Avian Cells and Virus. REV-T-transformed cells were grown as suspension cultures in RPMI 1640 medium (KC Biological, Lenexa, KS) supplemented with 5% calf serum as described (7). MSB-1 cells were maintained under similar conditions. REV-T/REV-A was obtained from the culture fluid of a REV-T-transformed bone marrow cell line (RECC-UT-1) originally described by Franklin *et al.* (4). Virus-containing supernatant fluids were harvested 48 hr after the addition of fresh medium. Chicken embryo fibroblast (CEF) cultures were established by standard methods from 10-day-old embryos of SPAFAS chickens (SPAFAS, Roanoke, IL). Secondary CEF cultures were infected with REV-T/REV-A or REV-A as described by Franklin *et al.* (5).

Immunoblotting. Proteins separated by polyacrylamide gel electrophoresis were transferred onto 0.1 or 0.45 μm nitrocellulose (27). After the transfer was complete, the portion of the nitrocellulose corresponding to lanes of molecular size markers was stained with India ink (28). The remaining portion of the nitrocellulose was treated to block the remaining protein binding sites (29) and exposed to antisera (27) followed by reaction with ^{125}I-labeled *Staphylococcus aureus* protein A (30). Filters were washed, blotted dry, wrapped in plastic, and exposed to Kodak XAR-5 film using intensifying screens at −70°C.

RESULTS

Construction of Plasmids with Inducible trpE–v-rel Fusion Proteins. Expression vectors pATH-1 and -2 include the *E. coli trp* promoter, operator, leader, attenuator sequences, and the coding sequence of the *trpE* gene in a pBR322 derivative. These vectors include a polylinker region varied to allow the insertion of DNA sequences in a variety of reading frames using a number of restriction enzymes (A. Tzagoloff and T. J. Koerner, personal communication). By using the known v-*rel* nucleotide sequence (14, 16), plasmids were constructed that express a v-rel fusion protein linked to the amino terminus of *E. coli trpE* (Fig. 1). To insert v-*rel* sequences in the same reading frame as the trpE protein, the v-*rel Eco*RI [base-pair (bp) 340 of v-*rel*] to *Bam*HI (bp 876 of v-*rel*) fragment was inserted into pATH-1 within the polylinker region. This plasmid (pNH1/9B) contained 536 bp of the v-*rel* coding sequence. The amino terminus of the trpE protein (37 kDa) was fused to ≈20 kDa of the v-rel protein. A second plasmid (pNH2/17ΔB) was constructed by inserting a *Bam*HI fragment (bp 876 of v-*rel* to bp 2285 of the REV-T long terminal repeat) into the pATH-2 vector. This plasmid fused the 37 kDa of trpE to 23 kDa of the carboxyl half of the v-rel protein to generate a 60-kDa fusion protein.

TrpE–v-rel Fusion Protein Induction and Purification. The plasmids pNH1/9B and pNH2/17ΔB and the parental vectors pATH-1 and -2 were examined for the presence of fusion proteins as a result of *trpE* induction by indoleacrylic acid. Large amounts of *trpE*-regulated fusion proteins were produced that were insoluble and accumulated within *E. coli* (22, 31). Fig. 2 illustrates the results of $NaDodSO_4$/PAGE analysis of proteins from whole cell lysates and insoluble proteins present in bacteria harboring various plasmids. In Fig. 2 (lanes 5 and 6), a unique insoluble 57-kDa protein was present in bacteria carrying the plasmid pNH1/9B as predicted during planned construction. A similar protein was not seen among the insoluble proteins present in *E. coli* RR-1 (lanes 1 and 2) or in *E. coli* with the vector pATH-1 (lanes 3 and 4). To verify that the unique 57-kDa protein was encoded in part by v-*rel* sequences, a fragment of v-*rel* from *Hin*dIII in v-*rel* (bp 482) through a *Hin*dIII restriction site in the distal portion of the polylinker was removed. The resulting plasmid

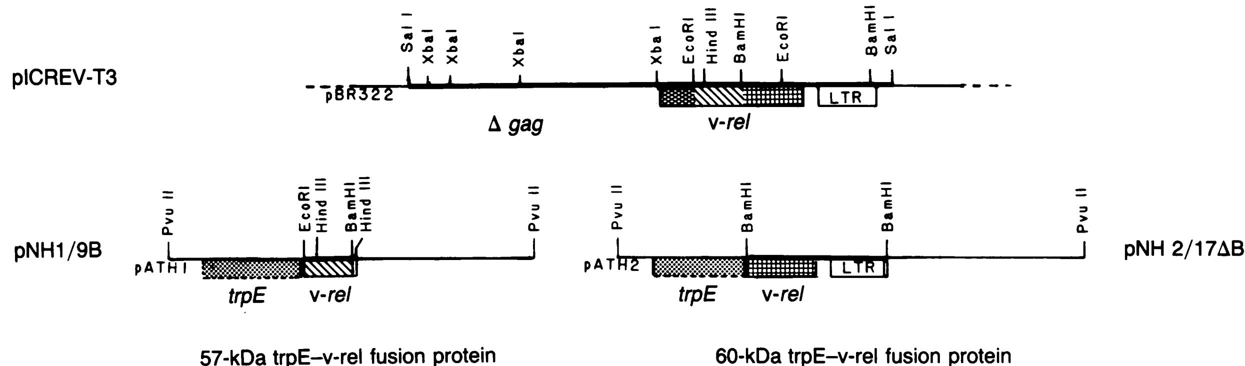

FIG. 1. Construction of pATH/v-*rel* expression vectors to achieve production of trpE–v-rel fusion proteins in *E. coli*. The plasmid pICREV-T3 was used as the source of the two *rel* fragments. The gel-purified 536-bp *Eco*RI/*Bam*HI fragment (bp 340 of v-*rel* to bp 876) was ligated into pATH-1 in frame with *trpE* to create the plasmid pNH1/9B. The plasmid pNH2/17ΔB was constructed by inserting a 1409-bp *Bam*HI fragment [bp 876 of v-*rel* to bp 2285 in the REV-T long terminal repeat (LTR)] into the pATH-2 plasmid in frame with *trpE*.

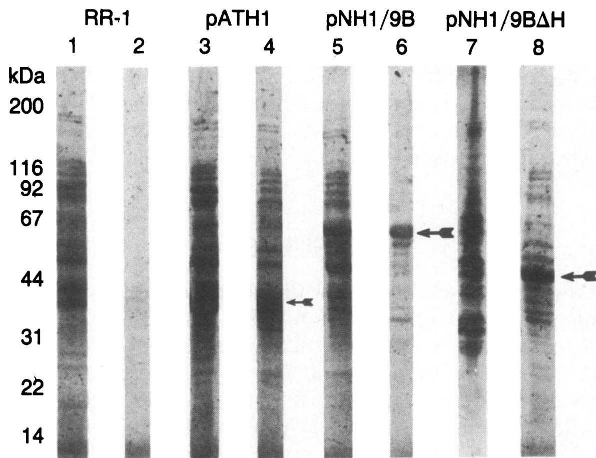

FIG. 2. NaDodSO$_4$/PAGE analysis of proteins from *E. coli* RR1 carrying no plasmids (RR1) and those transformed with plasmids pATH-1, pNH1/9B, and pNH1/9BΔH. Samples (1 ml) of each culture either were used to prepare whole cell lysates (odd-numbered lanes) in 50 μl of Laemmli NaDodSO$_4$/polyacrylamide gel sample buffer or were used to prepare insoluble protein (even-numbered lanes). The insoluble proteins were denatured in 50 μl of Laemmli buffer, and 10 μl of both whole cell lysates and insoluble proteins were analyzed on 7–15% linear gradient NaDodSO$_4$/polyacrylamide gels. Arrows indicate unique fusion protein products produced from plasmids pNH1/9B and pNH1/9BΔH.

(pNH1/9BΔH) would encode a truncated version (42 kDa) of the pNH1/9B-encoded fusion protein. In Fig. 2 (lanes 7 and 8) such a protein was seen among the insoluble proteins of *E. coli* carrying the plasmid pNH1/9BΔH. Similar NaDodSO$_4$/PAGE analysis revealed the presence of a 60-kDa insoluble fusion protein in bacteria with the plasmid pNH2/17ΔB. We have, therefore, created two fusion proteins that utilize approximately three-quarters of the coding sequence of the v-rel protein.

The Identification of v-rel-Related Proteins in Avian Cells. The trpE–v-rel fusion proteins from bacteria with plasmid pNH1/9B were separated by preparative NaDodSO$_4$/PAGE. The 57-kDa fusion protein was excised from the gel and used to immunize rabbits. Serum taken 3 weeks after immunization was used in immunoblotting experiments. Proteins from bacterial and cellular extracts of REV-T-transformed cells, MSB-1 cells (a herpesvirus-transformed lymphoid cell line), and CEF cultures were separated on polyacrylamide gels. The proteins were transferred to nitrocellulose and then probed with the serum against the fusion proteins (27). Sera taken from two rabbits, both immunized with the 57-kDa fusion protein, were found to recognize a 57-kDa protein in extracts of bacteria carrying pNH1/9B (Fig. 3*A*). A 42-kDa fusion protein was detected in bacteria with pNH1/9BΔH, which encodes for the truncated fusion protein. Low levels of the 37-kDa trpE protein were detected in bacteria carrying pATH-1. REV-T-transformed lymphoid cell lines (UTC41, UT370, UT371, UT-1, UTBB5, UT377, UTES01, UT024) contained a single 57-kDa protein that reacted with the antisera against the v-rel fusion proteins. This is consistent with the size of the v-rel protein (55,915 Da) as predicted by the v-*rel* nucleotide sequence (14, 16). A 57-kDa v-rel protein was also detected in REV-T-infected CEF cultures (Fig. 3*B*). The antisera directed against the fusion protein encoded by the plasmid pNH2/17ΔB also recognized a 57-kDa v-rel protein in REV-T-transformed cells. Duplicate filters of those shown in Fig. 3 were incubated with sera taken from the rabbits prior to immunization with the fusion proteins. Preimmune serum did not react with any avian or insoluble bacterial proteins. The addition of the bacterial fusion proteins was found to compete with the detection of the 57-kDa protein in immunoblots of proteins from REV-T-transformed cells. The 37-kDa trpE protein did not affect the detection of the 57-kDa v-rel protein.

REV-T-transformed lymphoid cells, which contain approximately equivalent amounts of c-rel and v-rel mRNA transcripts (17), contained only a 57-kDa v-rel protein. MSB-1 cells, which also contain c-rel transcripts, did not contain a c-rel protein that could be detected by either of these antisera. In uninfected avian fibroblast cultures, a protein of ≈190 kDa was detected by immunoblotting with antisera directed against the v-rel fusion proteins (Fig. 3*B*). The detection of this large rel-related protein in immunoblots of CEF proteins was substantially reduced by the addition of competing trpE–v-rel fusion proteins, but not by the trpE

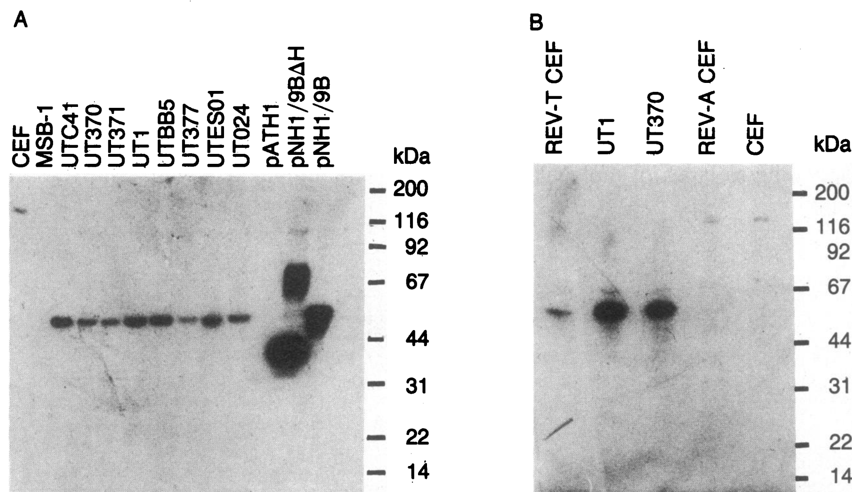

FIG. 3. Immunoblotting analysis of avian cells. The samples (25 μg of protein) were separated on 7–15% linear gradient NaDodSO$_4$/polyacrylamide gels. Proteins were electrophoretically transferred to nitrocellulose filters (0.1 μm or 0.45 μm), which were incubated with anti-trpE–v-rel fusion sera. (*A*) CEF, REV-T-transformed cell lines derived by *in vitro* transformation of chicken spleen cells (UTC41, UT370, UT371, UTBB5, UT377, UT024), embryonic spleen cells (UTES01), bone marrow cells from a moribund REV-T/REV-A-infected chicken (UT-1), and MSB-1 cells that are a herpesvirus-transformed avian lymphoid cell line. (*B*) Proteins from uninfected CEF cultures (CEF), REV-T/REV-A-infected CEF cultures (REV-T/REV-A CEF), and REV-A-infected CEF cultures (REV-A CEF). REV-T-transformed lymphoid cells (UT-1, C4-1) are also included. Incubation of duplicate filters with preimmune serum yielded no detectable activity to any protein present in these cells.

37-kDa protein. This protein, however, has not been consistently detected.

Expression of v-*rel* Sequences Using *lacZ*. To confirm that the 57-kDa protein detected in REV-T-transformed cells is the v-*rel*-encoded polypeptide, a plasmid was constructed to express the native v-rel protein. The *Xba* I (bp 27 of v-*rel*) to *Sal* I (bp 86 distal to the REV-T long terminal repeat) fragment of the REV-T genome was inserted into the polylinker region of pUC18 (32, 33) (Fig. 4). Mixtures of ligated fragment and plasmid DNA were transformed into *E. coli* JM105, plated on L agar containing 500 μg of carbenicillin per ml with 2.5-ml agar overlays containing 0.5 mM IPTG and 0.5 mg of XGal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside) per ml. In the plasmid pNHX/SW10, the *Xba* I site places v-*rel* sequences into the same reading frame as *lacZ*. However, protein synthesis initiated at the *lacZ* start codon would encounter a TGA triplet at the 12th codon downstream of the *Xba* I site. The v-*rel* ATG is another 5 codons further downstream. Translation beginning at the v-*rel* ATG would terminate at the v-*rel* stop codon, resulting in the synthesis of the v-rel protein that is not a lacZ fusion protein. Therefore, plasmid pNHX/SW10 confers the ampicillin-resistance Lac^- phenotype on *E. coli* JM105. The plasmid pNHX/SW10 was transformed into the *E. coli* strain P678-53. Minicells were purified from cultures of *E. coli* P678-53 and from transformants carrying pUC18 and pNHX/SW10. The polypeptides synthesized upon *lacZ* induction with IPTG were labeled with [^{35}S]methionine and separated by $NaDodSO_4$/PAGE. The majority of the proteins synthesized in minicells correspond to those encoded by plasmids (25, 34). The autoradiogram of the protein gel in Fig. 5*A* revealed that pNHX/SW10 encodes a 56-kDa protein not detected in minicells that lack plasmids or minicells containing pUC18 without the v-*rel* insert. Utilization of the v-*rel* coding sequence in the production of the 56-kDa protein seen in minicells with pNHX/SW10 was confirmed by the deletion of a fragment from *Hin*dIII (bp 482) in v-*rel* to a *Hin*dIII site in the distal polylinker region in pNHX/SW10. As illustrated in Fig. 5*B*, minicells carrying the plasmid pNHX/SΔH did not synthesize the 56-kDa protein. Therefore, the 56-kDa protein produced in minicells with pNHX/SW10 utilizes v-*rel* coding sequences in the proper reading frame. The size of the v-rel protein produced in the *lacZ* expression vector corresponded to the predicted size based on the nucleotide sequence and was somewhat smaller than the v-rel protein detected in REV-T-transformed cells. The 56-kDa protein produced in minicells carrying pNHX/SW10 was recognized by antisera generated to both fusion proteins.

DISCUSSION

The genome of REV-T includes a unique oncogene v-*rel*, which is located in the envelope region (11, 14–16). The insertion of the v-*rel* oncogene within envelope sequences closely resembles the arrangement of v-*mos* into the *env* gene within the genome of Moloney murine leukemia virus (35). To identify the product of the v-*rel* oncogene, we have used trpE–v-rel fusion proteins produced in bacteria to generate specific antibodies against the v-rel protein. Two plasmids that encode trpE–v-rel proteins were constructed, which include approximately three-quarters of the v-rel protein. Antisera to the fusion proteins were used in immunoblotting experiments to identify a 57-kDa protein in REV-T-transformed lymphoid cell lines and REV-T/REV-A-infected CEF cultures. A 57-kDa protein was not found in REV-A-infected CEF cultures or in MSB-1 cells. Minicells carrying the plasmid constructed to express the native v-rel protein (pNHX/SW10) were found to synthesize an IPTG-inducible 56-kDa v-rel protein. This protein was also recognized by the antisera directed toward the fusion proteins. This confirms that the v-rel protein is 56 kDa, as was predicted by the nucleotide sequence. Possible differences in the covalent modifications of the v-rel protein made in *E. coli* may account for its slightly smaller size when compared with the v-rel protein detected in avian cells.

The oncogenes transduced by various retroviruses are involved in the process of intracellular or intercellular transmission of signals regulating growth and cell division. The v-*rel* oncogene is distinct from other transforming genes, and how it functions to interfere with proper lymphocyte differentiation is unknown. The v-rel protein is not glycosylated and, like the v-*mos* gene product, is found predominantly in the cytoplasm.

The identification of the gene product encoded by c-*rel* is problematic. The complete nucleotide sequence of the c-*rel* gene has not been defined, so an estimate of the size of the c-*rel* gene product is not available. Hematopoietic cells contain a single c-*rel* transcript of ≈4.0 kb (11, 17). In lymphocytes transformed by REV-T or Marek disease virus, which contain c-*rel* transcripts, a c-rel-specified protein could not be detected by using antisera generated against the v-rel fusion proteins. Avian lymphoid cells transformed by avian leucosis virus and chicken syncitial virus also failed to express a detectable protein related to v-rel. Uninfected avian fibroblasts, however, contained a single protein species of ≈190 kDa, which could be detected by immunoblotting using antisera directed against the two different trpE–v-rel fusion proteins. Fibroblasts, infected but not morphologically transformed by REV-T, contained the 57-kDa v-rel protein

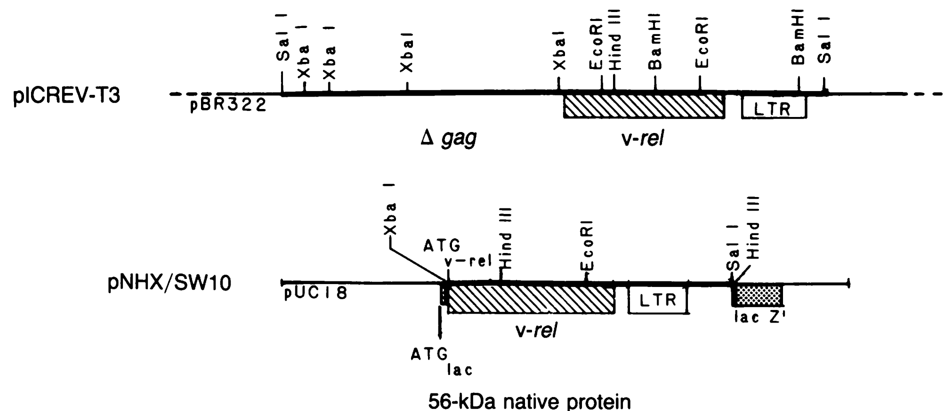

FIG. 4. A plasmid was constructed to express the v-*rel* gene in bacteria utilizing the *lac* promoter and operator. The *Xba* I (bp 27 of v-*rel*) to *Sal* I [bp 86 distal to the REV-T long terminal repeat (LTR)] fragment of pICREV-T3 was gel-purified. This fragment was ligated into the gel-purified plasmid pUC18 and selected on the basis of the disruption of the *lacZ* gene resulting in the Lac^- phenotype in *E. coli* JM105.

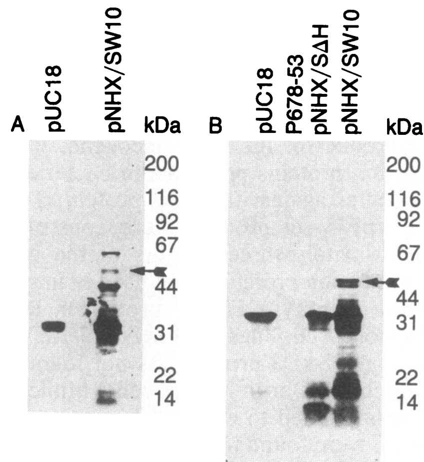

FIG. 5. NaDodSO$_4$/PAGE analysis of proteins synthesized in purified minicells exposed to IPTG as an inducer of the *lacZ* gene. Proteins labeled with [^{35}S]methionine from minicells of *E. coli* P678-53 and minicells harboring the plasmids were separated on 7–15% linear gradient NaDodSO$_4$/polyacrylamide gels. The dried gels were exposed to Kodak XAR-5 x-ray film. (*A*) Labeled proteins produced in minicells harboring pUC18 and pNHX/SW10. (*B*) Labeled minicell proteins encoded by plasmids pUC18, pNHX/SW10, and pNHX/SΔH, and protein in minicells harboring no plasmids (P678-53).

and the 190-kDa protein. The size of this protein does not correspond to the coding capacity of a 4.0-kb c-rel mRNA described in cells of hematopoietic origin. Protease mapping experiments are being performed to define the relationship, if any, between the v-rel 57-kDa protein and the large protein detected in avian fibroblasts. Immunoblotting experiments have also revealed the presence of c-rel-related proteins in *Drosophila melanogaster* and in the cytoplasm of sea urchin eggs. The expression of c-rel-related proteins among such evolutionary diverse organisms as the chicken, fruit fly, and sea urchin strongly suggests that the c-rel protein is involved in some fundamental cellular process.

We wish to express our sincere gratitude to Howard Temin for his generosity in supplying the cloned REV-T DNA (pICREV-T3). We also wish to thank Alex Tzagoloff and T. J. Koerner for the pATH expression vectors that we used. We thank Irving Weissman and Kathy Spindler for valuable discussions and suggestions. We acknowledge the excellent technical help of Will Bargmann as well as the patience and cooperation of Rosie and Josie. This work was supported by the Public Health Service Grants CA 33192 and CA 26169 from the National Cancer Institute and Grant F849 from the Robert A. Welch Foundation. N.K.H. is a predoctoral trainee supported by Grant CA 09182 from the National Cancer Institute.

1. Beug, H., Muller, H., Grieser, S., Doederlein, G. & Graf, T. (1981) *Virology* **115**, 295–309.
2. Lewis, R. B., McClure, J., Rup, B., Niesel, D. W., Garry, R. F., Hoelzer, J. D., Nazarian, K. & Bose, H. R., Jr. (1981) *Cell* **25**, 421–432.
3. Shibuya, T., Chen, I., Howatson, A. & Week, T. W. (1982) *Cancer Res.* **42**, 2722–2728.
4. Franklin, R. B., Maldonado, R. L. & Bose, H. R., Jr. (1974) *Intervirology* **3**, 342–352.
5. Franklin, R. B., Kang, C. Y., Wan, M. K. & Bose, H. R., Jr. (1977) *Virology* **83**, 313–321.
6. Hoelzer, J. D., Franklin, R. B. & Bose, H. R., Jr. (1979) *Virology* **93**, 20–30.
7. Hoelzer, J. D., Lewis, R. B., Wasmuth, C. R. & Bose, H. R., Jr. (1980) *Virology* **100**, 462–474.
8. Beug, H., Kirchbach, A. V., Doederlein, G., Conscience, J. F. & Graf, T. (1979) *Cell* **18**, 375–390.
9. Graf, T. & Beug, H. (1978) *Biochim. Biophys. Acta* **516**, 264–299.
10. Breitman, M. L., Lai, M.-C. & Vogt, P. K. (1980) *Virology* **100**, 450–461.
11. Chen, I. S. Y., Mak, T. W., O'Rear, J. J. & Temin, H. M. (1981) *J. Virol.* **40**, 800–811.
12. Gonda, M. A., Rice, N. R. & Gilden, R. V. (1980) *J. Virol.* **34**, 743–751.
13. Wilhelmsen, K. C. & Temin, H. M. (1984) *J. Virol.* **49**, 521–529.
14. Wilhelmsen, K. C., Eggleton, K. E. & Temin, H. M. (1984) *J. Virol.* **52**, 172–182.
15. Wong, T. C. & Lai, M. M. C. (1981) *Virology* **111**, 289–297.
16. Stephens, R. M., Rice, N. R., Hiebsch, R. R., Bose, H. R., Jr., & Gilden, R. V. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6229–6233.
17. Herzog, N. K., Bargmann, W. J. & Bose, H. R., Jr. (1986) *J. Virol.*, in press.
18. Chen, I. S. Y., Wilhelmsen, K. C. & Temin, H. M. (1983) *J. Virol.* **45**, 104–113.
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Lederberg, E. M. & Cohen, S. N. (1974) *J. Bacteriol.* **119**, 1072–1074.
21. Kleid, D. G., Yansura, D., Small, B., Dowbenko, D., Moore, D. M., Grubman, M. J., McKercher, P. D., Morgan, D. O., Robertson, B. H. & Bachrach, H. L. (1981) *Science* **241**, 1125–1129.
22. Spindler, K. R., Rosser, D. S. E. & Berk, A. J. (1984) *J. Virol.* **49**, 132–141.
23. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
24. Adler, H. I., Fischer, W. D., Cohen, A. & Hardigree, A. A. (1966) *Proc. Natl. Acad. Sci. USA* **57**, 321–326.
25. Roozen, K. J., Fenwick, R. G., Jr., & Curtiss, R., III (1971) *J. Bacteriol.* **107**, 21–33.
26. Meagher, R. B., Tait, R. C., Betlach, M. & Boyer, H. W. (1977) *Cell* **10**, 521–536.
27. Burnette, W. N. (1981) *Anal. Biochem.* **112**, 195–203.
28. Hancock, K. & Tsang, V. (1983) *Anal. Biochem.* **133**, 157–162.
29. Johnson, D. A., Gautsch, J. W., Sportsman, J. R. & Elder, J. H. (1984) *Gene Anal. Tech.* **1**, 3–8.
30. Markwell, M. A. K. (1982) *Anal. Biochem.* **125**, 427–432.
31. Goeddel, D. V., Kleid, D. G., Bolivar, F., Heynekar, H. L., Yanasura, D. G., Crea, R., Hirose, T., Kraszewski, A., Hakura, K. & Riggs, A. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 106–110.
32. Norrander, J., Kempe, T. & Messing, J. (1983) *Gene* **26**, 101–106.
33. Yaenisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33**, 103–119.
34. Levy, S. B. (1971) *J. Bacteriol.* **108**, 300–308.
35. Van Beveren, C., Galleshaw, J. A., Jonas, V., Berns, A. J. M., Doolittle, R. F., Donoghue, D. J. & Verma, I. M. (1981) *Nature (London)* **289**, 258–262.