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The v-*rel* oncogene product is complexed to a 40-kDa phosphoprotein in transformed lymphoid cells

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ABSTRACT **The transforming protein encoded by the v-*rel* oncogene of avian reticuloendotheliosis virus (REV-T) is a very low copy number molecule in the cytosol of transformed cells. Analysis of cytosolic extracts from a REV-T-transformed lymphoid cell line by gel filtration on Sephacryl S-300 indicated that most of the v-*rel* oncogene product, pp59$^{v\text{-}rel}$, eluted with an apparent molecular mass of 400 kDa. The size of this complex was confirmed by analysis on a fast-protein liquid chromatography gel filtration column. A 40-kDa cellular protein copurified with pp59$^{v\text{-}rel}$ on sequential gel filtration on Sephacryl S-200 and immunoaffinity chromatography with a monoclonal antibody directed against pp59$^{v\text{-}rel}$. The 40-kDa cellular protein could also be immunoprecipitated together with pp59$^{v\text{-}rel}$ from cell extracts of [^{35}S]methionine-labeled cells, suggesting that pp59$^{v\text{-}rel}$ is complexed with the 40-kDa protein in transformed lymphoid cells. Both the 59- and 40-kDa proteins were phosphorylated when the highly purified preparation containing pp59$^{v\text{-}rel}$ was incubated with [γ-^{32}P]ATP and 10 mM $MgCl_2$ *in vitro*. The identity of the kinase in the highly purified preparation containing pp59$^{v\text{-}rel}$, however, is unknown. Immune complexes recovered from extracts of REV-T-transformed lymphoid cells labeled with [^{32}P]orthophosphate also contained the 59- and 40-kDa phosphoproteins. These observations suggest that pp59$^{v\text{-}rel}$ is complexed with a 40-kDa cellular phosphoprotein to form a 400-kDa heteropolymer in the cytoplasm of transformed lymphoid cells.**

Avian reticuloendotheliosis virus (REV-T) is a replication-defective retrovirus that induces a fatal lymphomatosis within 7–10 days after infection (1). REV-T transforms very immature lymphoid cells *in vivo* and *in vitro* (2, 3). REV-T contains deletions in portions of its *gag*, *pol*, and *env* genes (4, 5) and, therefore, coreplicates with a genetically related helper virus designated reticuloendotheliosis-associated virus (6). A helper virus unrelated sequence, v-*rel*, is located near the 3′ end of the REV-T genome within envelope sequences (4, 5). The expression of v-*rel* is sufficient for transformation by REV-T. Uninfected vertebrate cells contain a sequence related to the v-*rel* oncogene (5, 7, 8). The v-*rel* oncogene, and its predicted protein, is distinct from other known transforming sequences (9–11). The v-*rel* oncogene is transcribed at low levels into a 3.0-kilobase (kb) subgenomic mRNA in transformed cells (12) and its translation product has recently been identified as a phosphoprotein of apparent molecular mass of 55–59 kDa (13–16). The predicted molecular mass based on the nucleotide sequence of the v-*rel* oncogene is 56 kDa (9). In an effort to standardize the nomenclature, the v-*rel* oncogene product will be designated pp59$^{v\text{-}rel}$. Immune complexes recovered from extracts of REV-T transformed cells with antisera directed against v-*rel* bacterial fusion proteins or with antisera against v-*rel*-related synthetic peptides display protein kinase activity in *in vitro* kinase assays (15, 17). The identity of the protein kinase measured in these assays is, however, still obscure. In this report, we describe the isolation of the v-rel protein by sequential chromatography on Sephacryl S-200 and immunoaffinity chromatography using a monoclonal antibody (mAb) prepared against the v-rel protein. Copurifying with the v-*rel* oncogene product was a 40-kDa cellular phosphoprotein. The 40-kDa cellular protein was also immunoprecipitated from cytosolic extracts of REV-T-transformed lymphoid cells with antisera directed against pp59$^{v\text{-}rel}$.

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MATERIALS AND METHODS

Preparation of mAb Against pp59$^{v\text{-}rel}$. A portion of the v-*rel* oncogene corresponding to the 3′ end of the transforming gene was expressed in *Escherichia coli* as a trpE/v-rel fusion protein (13). BALB/c mice were injected intraperitoneally and into foot pads with ≈60 μg of the fusion protein in complete Freund's adjuvant. Beginning 4 weeks later, the mice were repeatedly injected intravenously (60 μg of solubilized trpE/v-rel protein) at biweekly intervals. Four days after the last injection, cell fusions were performed.

Cell fusion and selection of lymphocyte myeloma hybrids were carried out by minor modifications of techniques described by Kohler and Milstein (18). The splenocytes were fused with p3-NS-1 mouse myeloma cells. A hybridoma secreting IgG that reacted with the v-rel component of the trpE/v-rel fusion protein was identified.

Preparation of mAb Sepharose-4B Affinity Chromatography Medium. Immunoglobulins were purified on a protein A-agarose column from 40 ml of ascities fluids containing mAb directed against pp59$^{v\text{-}rel}$. This mAb preparation was then attached to CNBr-activated Sepharose-4B according to the manufacturer's instructions (Pharmacia).

Immunoblotting. Immunoblotting was done as described (17). In place of ^{125}I-labeled *Staphylococcus aureus* protein A, goat anti-mouse IgG conjugated to alkaline phosphatase (Bio-Rad) was used, and the blot was stained with *p*-nitroblue tetrazolium chloride (0.3 mg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (0.15 mg/ml) in 50 mM sodium bicarbonate, pH 9.8/100 mM NaCl/5 mM $MgCl_2$.

Radiolabeling and Immunoprecipitation. The radiolabeling and immunoprecipitation of pp59$^{v\text{-}rel}$ from extracts of [^{35}S]methionine-labeled cells was done as described (17). To radioactively label the phosphoproteins in REV-T-transformed cells, exponentially growing cells were pelleted and suspended in phosphate-free RPMI 1640 medium. After 4 hr the cells (1×10^6 cells per ml) were incubated in medium containing 1 mCi of [^{32}P]orthophosphate per ml (1 Ci = 37 GBq; ICN) for 2 hr at 37°C. The cells (1×10^7) were then washed with cold Tris/saline and lysed with a Dounce homogenizer in 20 mM Tris·HCl, pH 7.5/2 mM EDTA/150 mM NaCl/0.5% (vol/vol) Triton X-100/0.05% (wt/vol) $NaDodSO_4$/50 mM β-glycerophosphate/1 mM sodium or-

Abbreviations: REV-T, reticuloendotheliosis virus strain T; mAb, monoclonal antibody.

thovanadate/1 mM benzamidine/0.2 mM phenylmethylsulfonyl fluoride after 15 min of swelling at 4°C. The cell lysate was clarified by centrifugation and 2–5 μl of affinity-purified mAb was added per ml (200 μg of total protein) of cell lysate. Immunoprecipitation was performed according to the procedure described (17).

Isolation of the v-*rel* Oncogene Product from a REV-T-Transformed Cell. A nonvirus-producing REV-T-transformed cell line (RECC4 1) was used as a source of the v-rel protein. Exponentially growing cells in RPMI 1640 medium (7 liters) supplemented with 5% fetal bovine serum were pelleted, washed once with phosphate-buffered saline, and homogenized with a Polytron unit in 100 ml of 25 mM Tris·HCl, pH 7.5/2 mM EDTA/10 mM EGTA/0.2 mM phenylmethylsulfonyl fluoride/1 mM benzamidine/0.1% (vol/vol) 2-mercaptoethanol. The mixture was centrifuged at 45,000 rpm (Beckman Ti 50.2 rotor) for 30 min. The supernatant fluids were collected, concentrated to 5 ml by vacuum dialysis, and subjected to gel filtration on a Sephacryl S-200 (Pharmacia) column (2.5 × 90 cm) equilibrated and developed with 50 mM Tris·HCl, pH 7.5/0.1 mM EGTA/0.2 M NaCl/0.2 mM phenylmethylsulfonyl fluoride/1 mM benzamidine/0.1% (vol/vol) 2-mercaptoethanol. The eluted fractions were analyzed by immunoblotting using the mAb (see above) and polyclonal antibodies directed against two nonoverlapping regions comprising the middle and amino-terminal sections of the v-rel protein, which were expressed in *E. coli* as trpE/v-rel fusion proteins (13, 17). Fractions containing the v-*rel* oncogene product were pooled and loaded onto the mAb affinity column (1 × 2.5 cm). The column was equilibrated in 25 mM Tris·HCl, pH 7.5/0.2 mM phenylmethylsulfonyl fluoride/1 mM benzamidine/0.05% (vol/vol) 2-mercaptoethanol. The column was washed extensively with 25 mM Tris·HCl, pH 7.5/150 mM NaCl/0.2 mM phenylmethylsulfonyl fluoride/1 mM benzamidine/0.05 (vol/vol) 2-mercaptoethanol, followed by 10 mM sodium phosphate (pH 6.0) containing 0.05% (vol/vol) 2-mercaptoethanol, until no protein was detected in the fractions. The v-*rel* oncogene product was then eluted with 10 mM sodium phosphate, pH 6.0/0.05% (vol/vol) 2-mercaptoethanol/1 M $MgCl_2$, and dialyzed against 50 mM Tris·HCl, pH 7.5/0.2 mM phenylmethylsulfonyl fluoride/1 mM benzamidine/0.1% (vol/vol) 2-mercaptoethanol.

RESULTS

Preparation of a mAb Against pp59$^{v\text{-}rel}$. A mAb directed against the protein product of the 3′-terminal region of the v-*rel* oncogene synthesized in *E. coli* as a trpE/v-rel fusion protein has been produced (12). This mAb immunoprecipitated a protein of 59 kDa in extracts of [^{35}S]methionine-labeled REV-T-transformed cells (Fig. 1*A*, lane 2). The mAb prepared against pp59$^{v\text{-}rel}$ also recognized a 59-kDa protein by immunoblot analysis of extracts obtained from unlabeled transformed cells (Fig. 1*B*, lane 2). This mAb failed to react with proteins in uninfected avian lymphoid cells (data not shown).

The v-*rel* Oncogene Product Is Complexed with a 40-kDa Cellular Protein in Transformed Lymphoid Cells. Exponentially growing REV-T-transformed nonvirus-producing lymphoid cells were physically disrupted and the cytosolic extract was concentrated. Initially, the cytosolic extract was subjected to gel filtration on Sephacryl S-200, which separates molecules from 10 to 150 kDa. However, on Sephacryl S-200 all of the pp59$^{v\text{-}rel}$ was detected in the void volume by immunoblotting, indicating that pp59$^{v\text{-}rel}$ does not exist as a monomer in transformed lymphoid cells. Cytosolic extracts of REV-T-transformed cells were, therefore, subjected to gel filtration on Sephacryl S-300, which effectively separates molecules from 10 to 1500 kDa. Each fraction was analyzed by immunoblotting to detect pp59$^{v\text{-}rel}$ (Fig. 2*A*). The majority of the v-*rel* oncogene product eluted with an apparent molecular mass between 350 and 400 kDa (Fig. 2*B*) suggesting that the v-*rel* oncogene product is either a homopolymer or that it is complexed to cellular proteins in REV-T-transformed lymphoid cells.

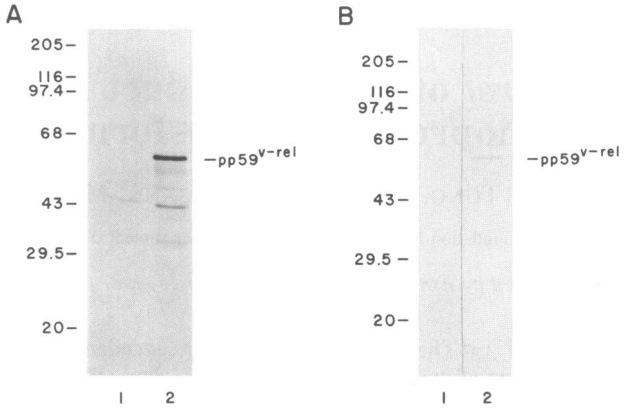

FIG. 1. Identification of the v-*rel* oncogene product in a REV-T-transformed lymphoid cell line by mAb directed against pp59$^{v\text{-}rel}$. (*A*) Immunoprecipitation of [^{35}S]methionine-labeled v-rel protein from extracts of metabolically labeled REV-T-transformed lymphoid cells. Lanes: 1, immune complex recovered from cell extracts with rabbit preimmune serum; 2, immune complex recovered with mAb directed against pp59$^{v\text{-}rel}$. (*B*) Immunoblot analysis of REV-T-transformed cell extracts at 25 μg of protein per lane. Lanes: 1, immunoblot with normal mouse serum; 2, immunoblot with mAb directed against pp59$^{v\text{-}rel}$. Positions of marker proteins from top to bottom (in kDa) are as follows: myosin, β-galactosidase, phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor.

Isolation of pp59$^{v\text{-}rel}$ from Transformed Lymphoid Cells. The polypeptides present in cytosolic extracts obtained from a REV-T-transformed cell line were separated by NaDodSO$_4$/PAGE and detected by silver staining (Fig. 3*A*, lane 2). The proteins in the cytosolic extract were then separated by gel filtration on Sephacryl S-200. The v-*rel* gene product eluted from Sephacryl S-200 in the void volume together with numerous other proteins (Fig. 3*A*, lane 3). The eluate containing pp59$^{v\text{-}rel}$ was then subjected to affinity chromatography with a mAb directed against pp59$^{v\text{-}rel}$. This highly purified preparation contained two major proteins as well as minor polypeptide species (Fig. 3*A*, lane 4). The major proteins had an apparent molecular mass of 59 and 40 kDa. When the preparation from Sephacryl S-200 was subjected to immunoaffinity chromatography with a column constructed with immunoglobulins from unimmunized mice, pp59$^{v\text{-}rel}$ was not detected by immunoblotting. The 40-kDa protein also failed to bind to this column. Immunoblot analysis revealed that only the 59-kDa protein was recognized by the mAb directed against v-*rel* (Fig. 3*B*). Similar results were obtained with two polyclonal antisera that were directed against the middle and amino-terminal region of the v-rel protein (Fig. 3*C*). Likewise, a polyclonal antisera generated against the carboxyl-terminal region recognized a single polypeptide (59 kDa) in transformed lymphoid cells and failed to react with the 40-kDa protein. It is unlikely, therefore, that the 40-kDa protein represents a proteolytic fragment of pp59$^{v\text{-}rel}$, since antisera generated against all the sequences present in pp59$^{v\text{-}rel}$ failed to react with the 40-kDa protein in the purified preparation as well as extracts of REV-T-transformed cells. These results indicate that the 40-kDa protein is not a protein that shares antigenic determinants with pp59$^{v\text{-}rel}$ or a proteolytic fragment of the v-*rel* oncogene product.

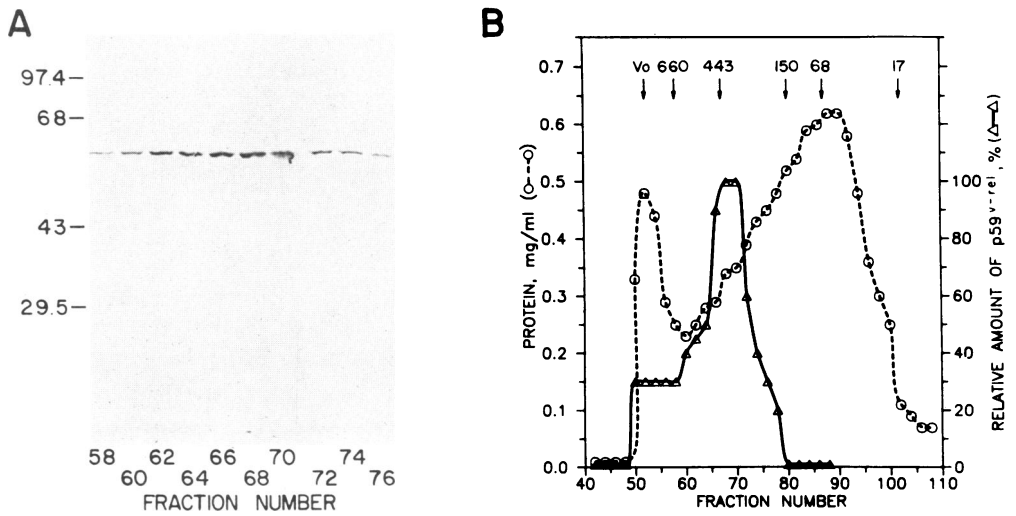

FIG. 2. Analysis of the v-*rel* oncogene product from a REV-T-transformed cell extract by chromatography on Sephacryl S-300. The column (2.5 × 90 cm) was equilibrated in 50 mM Tris·HCl, pH 7.5/0.1 mM EGTA/0.2 M NaCl/1 mM benzamidine/0.2 mM phenylmethylsulfonyl fluoride/0.1% (vol/vol) 2-mercaptoethanol. (*A*) Cell extracts were prepared and the eluted fractions were assayed for pp59$^{v\text{-}rel}$ by immunoblotting analysis with mAb directed against pp59$^{v\text{-}rel}$. (*B*) The relative amount of the v-*rel* oncogene product in each fraction was quantitated by densitometry and expressed as percentage of the peak fraction. Arrows indicate the void volume V_o and the elution positions of the standard marker proteins, thyroglobulin (660 kDa), apoferritin (433 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (68 kDa), and myoglobin (17 kDa).

The observation that pp59$^{v\text{-}rel}$ exists as a larger complex and copurifies with a 40-kDa cellular protein suggests that pp59$^{v\text{-}rel}$ may form a heteropolymer with the 40-kDa protein in the cytoplasm of REV-T-transformed lymphoid cells. If pp59$^{v\text{-}rel}$ and the 40-kDa protein are complexed in transformed cells, the 40-kDa protein should immunoprecipitate with pp59$^{v\text{-}rel}$. Logarithmically growing REV-T-transformed cells were labeled with [^{35}S]methionine and extracts were prepared and immunoprecipitated with mAb against pp59$^{v\text{-}rel}$. As indicated in Fig. 4, mAb against pp59$^{v\text{-}rel}$ precipitated both pp59$^{v\text{-}rel}$ and a 40-kDa cellular protein. The complex, however, was detergent sensitive, since the 40-kDa protein could only be coprecipitated with pp59$^{v\text{-}rel}$ in the absence of $NaDodSO_4$ and sodium deoxycholate and when the level of Nonidet P-40 in the immunoprecipitation reaction was 0.5%. Immunoglobulins (IgG) fractionated from polyclonal antisera generated against the amino-terminal, middle, and carboxyl-terminal regions of pp59$^{v\text{-}rel}$ also precipitated pp59$^{v\text{-}rel}$ and the 40-kDa cellular protein (data not shown). The 40-kDa protein also coprecipitated together with pp59$^{v\text{-}rel}$ from other independently derived REV-T-transformed lymphoid cell lines.

To more accurately define the size of the pp59$^{v\text{-}rel}$ 40-kDa complex, the complex eluted from the immunoaffinity col-

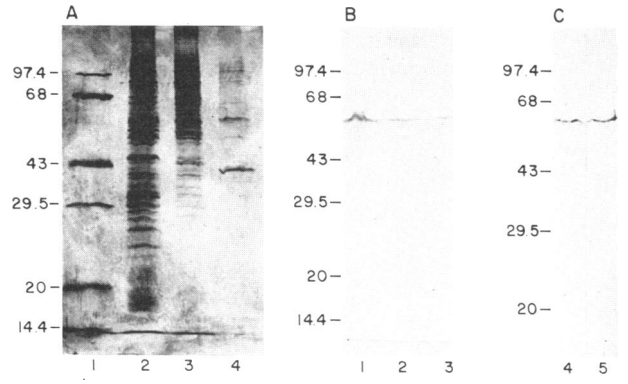

FIG. 3. Purification of the v-*rel* oncogene product from cell extract by chromatography on Sephacryl S-200 and immunoaffinity chromatography. (*A*) Proteins detected by silver staining in cytosolic extracts of REV-T-transformed lymphoid cells. Lanes: 1, molecular mass markers described in Fig. 1; 2, unfractionated cytosolic extract; 3, fraction containing pp59$^{v\text{-}rel}$ from chromatography on Sephacryl S-200; 4, proteins from sequential Sephacryl S-200 and immunoaffinity chromatography. (*B*) Detection of pp59$^{v\text{-}rel}$ by immunoblotting using mAb directed against the carboxyl-terminal region of pp59$^{v\text{-}rel}$. Lanes: 1, unfractionated cell extract; 2, fraction containing pp59$^{v\text{-}rel}$ from Sephacryl S-200 chromatography; 3, fraction containing pp59$^{v\text{-}rel}$ from sequential Sephacryl S-200 and immunoaffinity chromatography. (*C*) Detection of pp59$^{v\text{-}rel}$ using polyclonal antibodies directed against the amino-terminal and middle regions of pp59$^{v\text{-}rel}$. Fraction containing pp59$^{v\text{-}rel}$ from sequential Sephacryl S-200 and immunoaffinity chromatography was treated with antisera against the amino-terminal (lane 4) and middle (lane 5) regions of pp59$^{v\text{-}rel}$.

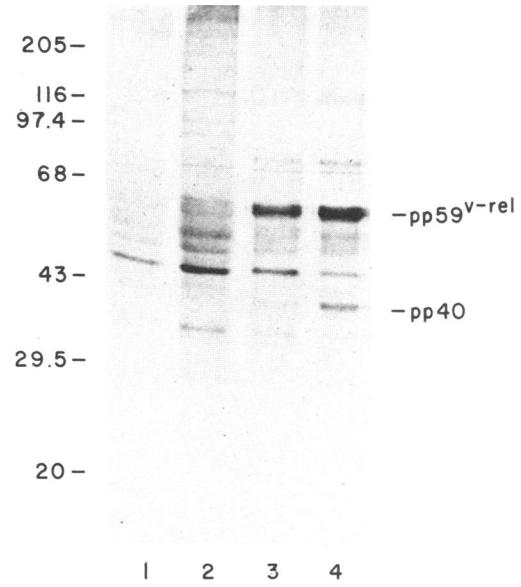

FIG. 4. Immunoprecipitation of ^{35}S-labeled proteins from REV-T-transformed cells with mAb directed against pp59$^{v\text{-}rel}$. Transformed lymphoid cells were grown in the presence of [^{35}S]methionine and extracts were precipitated with mAbs in high detergent buffer (lanes 1 and 3) or in buffer containing only 0.5% Nonidet P-40 (lanes 2 and 4). Proteins precipitated with mouse preimmune serum are shown in lanes 1 and 2, while proteins precipitated with mAb against pp59$^{v\text{-}rel}$ are shown in lanes 3 and 4.

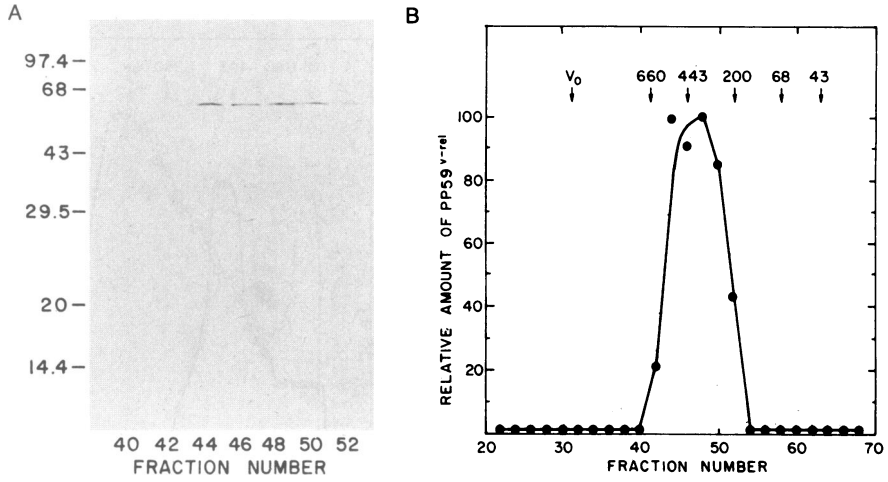

FIG. 5. Analysis of highly purified v-*rel* oncogene product preparation by chromatography in FPLC Superose-12. After chromatography in mAb affinity chromatography, the highly purified preparation containing v-*rel* oncogene product was subjected to gel filtration on FPLC Superose-12. The column (1 × 30 cm) was equilibrated as described in Fig. 2 and the flow rate was 1 ml/min. (*A*) The fractions (1 ml) were assayed for v-rel protein by immunoblotting analysis as in Fig. 2. (*B*) The relative amount of the v-*rel* oncogene product in each fraction was quantitated as in Fig. 2. The elution positions of the void volume and marker proteins are indicated in Fig. 2.

umn was analyzed on a fast-protein liquid chromatography (FPLC) gel filtration column (Superose-12). The presence of $pp59^{v\text{-}rel}$ in fractions obtained from this FPLC column were detected by immunoblotting. As shown in Fig. 5, the complex has an apparent molecular mass of ≈400 kDa.

Protein Kinase Activity Is Associated with the v-*rel* Oncogene Product. To determine whether this highly purified preparation obtained by sequential gel filtration on Sephacryl S-200 and mAb affinity chromatography had an associated kinase activity, the highly purified preparation containing $pp59^{v\text{-}rel}$ and the 40-kDa cellular protein were incubated in the presence of 10 mM Mg^{2+} and [γ-^{32}P]ATP for various time intervals. As shown in Fig. 6, both the 59-kDa protein and 40-kDa protein were phosphorylated *in vitro*. The 97-kDa protein present in the purified preparations of $pp59^{v\text{-}rel}$ that was phosphorylated *in vitro* was not consistently detected in the $pp59^{v\text{-}rel}$ 40-kDa complex. The kinase associated with the purified preparation containing $pp59^{v\text{-}rel}$ and the 40-kDa protein also phosphorylated an exogenous substrate (casein) on serine residues (data not shown). It is unclear whether the kinase activity associated with the highly purified preparation containing $pp59^{v\text{-}rel}$ is intrinsic to $pp59^{v\text{-}rel}$, the 40-kDa protein, or a kinase that copurified with the $pp59^{v\text{-}rel}$ 40-kDa complex.

The 40-kDa Protein That Is Complexed with $pp59^{v\text{-}rel}$ Is Phosphorylated in Intact Transformed Cells. Since the 40-kDa protein that copurified with $pp59^{v\text{-}rel}$ became phosphorylated in an *in vitro* kinase reaction, we determined whether the 40-kDa protein was also phosphorylated in transformed cells. REV-T-transformed lymphoid cells were labeled with [^{32}P]orthophosphate. Extracts were then immunoprecipitated with the mAb against $pp59^{v\text{-}rel}$ and the immunoprecipitate was analyzed by $NaDodSO_4$/PAGE followed by autoradiography. As shown in Fig. 7, $pp59^{v\text{-}rel}$ and the 40-kDa protein were phosphorylated *in vivo*. These results suggest that both the $pp59^{v\text{-}rel}$ and the 40-kDa protein that is associated with the v-*rel* oncogene product in REV-T-transformed lymphoid cells may undergo phosphorylation *in vivo*.

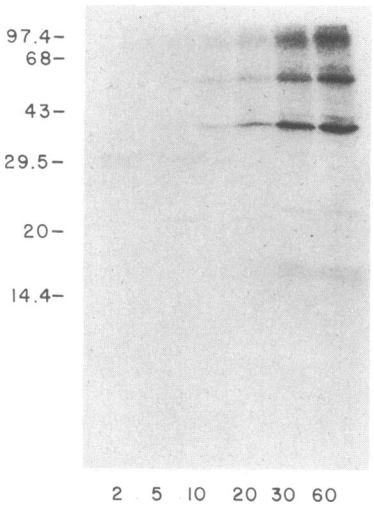

FIG. 6. Phosphorylation of the highly purified preparation containing the v-*rel* oncogene product. The purified preparation eluted from the mAb affinity column was incubated at 30°C in 50 mM Tris·HCl, pH 7.5/0.1 mM EGTA/0.1% (vol/vol) 2-mercaptoethanol/10% (vol/vol) glycerol/10 mM $MgCl_2$/0.1 mM [γ-^{32}P]ATP. Reaction was stopped at the times indicated (min) by heating the incubation mixture for 5 min at 100°C with an equal volume of denaturing sample buffer. The mixture was subjected to $NaDodSO_4$/PAGE, washed extensively, dried, and autoradiographed.

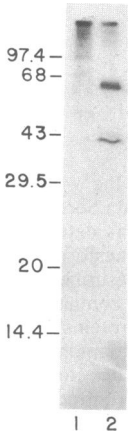

FIG. 7. Immunoprecipitation of phosphoproteins from REV-T-transformed cells with mAb directed against $pp59^{v\text{-}rel}$. Transformed lymphoid cells were grown in the presence of [^{32}P]orthophosphate, and extracts were precipitated with mAb against v-rel protein as described. Lanes: 1, proteins precipitated with mouse preimmune serum; 2, proteins precipitated with mAb against $pp59^{v\text{-}rel}$.

DISCUSSION

The v-*rel* oncogene of avian reticuloendotheliosis virus plays a critical role in the transformation of lymphoid cells of avian species. In this manuscript, we have characterized the viral oncogene product of v-*rel*, which has been purified to near homogeneity by sequential gel filtration on Sephacryl S-200 and immunoaffinity chromatography with a mAb against $pp59^{v\text{-}rel}$. When cytosolic extracts obtained from REV-T-transformed cells were analyzed by gel filtration, all of the $pp59^{v\text{-}rel}$ eluted as a 400-kDa complex. The 400-kDa complex contained $pp59^{v\text{-}rel}$ and a 40-kDa protein as well as some minor protein species. The 40-kDa protein that copurified with $pp59^{v\text{-}rel}$ on sequential Sephacryl S-200 and immunoaffinity chromatography failed to react with polyclonal antisera directed against amino-terminal, middle, and carboxyl-terminal regions of $pp59^{v\text{-}rel}$ and is, therefore, not related to $pp59^{v\text{-}rel}$. The 40-kDa protein was also immunoprecipitated from transformed lymphoid cells with mAb directed against $pp59^{v\text{-}rel}$ and is a phosphoprotein. The subunit stoichiometry of the components in the complex remains to be determined. A 40-kDa protein also coprecipitates with $pp59^{v\text{-}rel}$ in REV-T morphologically transformed avian fibroblasts (19).

A few other transforming proteins have been found to be associated with cellular proteins. The transforming protein of Rous sarcoma virus, $pp60^{v\text{-}src}$, coprecipitates with 50- and 90-kDa phosphoproteins from infected avian fibroblasts (20, 21). The transforming proteins encoded by Fujinami and Yamaguchi sarcoma viruses, which are distinct from $pp60^{v\text{-}src}$, also bind to pp50 and pp90 in transformed cells (22). The 90-kDa cellular protein is a heat shock protein (20).

The transforming protein of polyomavirus, middle T antigen, is associated with $pp60^{c\text{-}src}$, the cellular homolog of v-*src*, in immune complexes obtained from transformed cells (23, 24). Likewise, the large T antigen of simian virus 40 forms a stable complex with a cellular protein p53 (25).

The nature of protein kinase associated with preparations containing $pp59^{v\text{-}rel}$ remains unresolved. In this study, we have shown that a kinase activity is present in the highly purified preparation of $pp59^{v\text{-}rel}$ obtained by sequential gel filtration on Sephacryl S-200, followed by immunoaffinity chromatography with mAb directed against $pp59^{v\text{-}rel}$. Both $pp59^{v\text{-}rel}$ and the 40-kDa cellular protein undergo phosphorylation *in vitro* and *in vivo*. The protein kinase activity may be an intrinsic property of either the v-*rel* oncogene product or the 40-kDa protein or a kinase that copurified with the $pp59^{v\text{-}rel}$ 40-kDa complex.

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