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# Autogenous Growth Factor Production by Reticuloendotheliosis Virus-Transformed Hematopoietic Cells

**Robert F. Garry and Henry R. Bose, Jr.**

*Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, Louisiana 70112 (R.F.G.); Department of Microbiology, The University of Texas at Austin, Austin, Texas 78712-1095 (H.R.B.)*

Reticuloendotheliosis virus strain T (REV-T)-transformed cells gave rise spontaneously to variants which secrete a factor that forms a distinct visible ring of precipitation (halo) surrounding colonies grown in soft agar. An $M_r$ 15,000 protein was produced at higher levels by halo variants than by nonhalo-producing cells. An assay designed to detect the formation of precipitates enabled purification of an $M_r$ 15,000 protein, p15, from serum-free medium conditioned by the growth of REV-T-transformed hematopoietic cells. Fractions enriched in p15 permitted the growth of REV-T-transformed cells under conditions where they normally failed to proliferate.

**Key words: retrovirus transformation, *v-rel*, lymphoid cell variants**

Reticuloendotheliosis virus strain T (REV-T), an avian acute leukemia virus, induces an invasive lymphoid leukosis which results in nearly 100% mortality in experimental animals within 2 wk [1]. Like other acutely transforming retroviruses, REV-T appears to have arisen by recombination of a replication-competent retrovirus with cellular genetic information [2–5]. A transduced sequence, *v-rel*, is presumed to encode the transforming function of REV-T. The product of this gene has been identified as a $M_r$ 57,000–59,000 phosphoprotein, $pp57^{v\text{-}rel}$, which contains short env-related sequences at both ends [6–12]. In transformed lymphoid cells, $pp57^{v\text{-}rel}$ is cytoplasmically located and associated with serine/threonine protein kinase activity [11–14] (Tung et al., in preparation). The phenotype of both in vivo- and in vitro-derived REV-T-transformed hematopoietic cells appeared to be that of an immature lymphoid cell [6,15–18]. Transformed nonvirus-producing cells (NP cells) have been selected after in vitro infection with REV-T in the absence of a helper virus [16]. In contrast to NP cells transformed by other avian acute leukemia viruses [19,20], REV-T-transformed NP cells induced a fatal neoplastic disease when injected into histocompatible chickens [6,21]. These results suggested that REV-T may provide the minimum required genetic information for formation of an invasive leukemic cell.

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Previously, we described spontaneously arising variants of REV-T-transformed cells which were characterized by secretion of a factor which formed a distinctive visible halo surrounding cell colonies grown in soft agar [22]. Results of experiments in which the REV-T genome was rescued from halo-producing NP-cells suggested that the halo factor was regulated by the genetic information of REV-T. Herein, we describe the identification and purification of a protein which is found in increased amounts in medium conditioned by the growth of halo-producing variant cells compared to nonhalo-producing cells or halo revertants. A possibly related protein, p15, was purified from serum-free medium conditioned by the growth of REV-T-transformed cells. Fractions containing p15 permitted the growth of REV-T-transformed cells under conditions where they normally failed to proliferate.

## MATERIALS AND METHODS

### REV-T-Transformed Hematopoietic Cells, Halo-Producing Variants, and Selection of Nonhalo-Producing Revertants

Derivation and biological characterization of REV-T-transformed hematopoietic cells and halo-producing cell variants has been described previously [16,22]. Halo-producing variants are designated with a subscript h. Unless otherwise stated, cells were propagated by seeding stationary culture flasks with 1–3 $\times$ $10^5$ cells/ml at 2–3-day intervals in RPMI 1640 medium supplemented with 10% fetal bovine serum. Selection of nonhalo-producing revertant cells was accomplished by plating halo-producing cells in 100-mm culture dishes in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% chicken serum, and 0.3% Nobel agar. After 14 days incubation, plates were inspected for formation of nonhalo-producing revertant colonies.

### Analysis of Proteins Released Into Media Conditioned by the Growth of REV-T-Transformed Hematopoietic Cells and Halo-Producing Cell Variants

Proteins synthesized by REV-T-transformed hematopoietic cells and halo-producing cell variants were labelled by incubating the cultures in medium which lacked unlabelled methionine and was supplemented with 25 μCi/ml [$^{35}$S]methionine. After the labelling period, cell-free supernatants were prepared by centrifugation at 2,000g and by filtration through a 0.2-μm filter. Proteins released into growth-conditioned culture medium were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE, 7–15% gradient gel) and visualized by autoradiography as previously described [23].

### Development of an Assay for Precipitating Factors Produced by REV-T-Transformed Cells and Purification of a Precipitating Factor Associated With the Halo Phenotype

Serum-free medium conditioned by the growth of either halo-producing or nonhalo-producing cells was added to 10 $\times$ 75 mm polystyrene culture tubes (Falcon) containing a plug of RPMI 1640 medium supplemented with 10% fetal bovine serum and 0.3% Nobel agar. Under these conditions, a precipitate, best visualized at the interface of the liquid medium and the agar plug, formed in tubes after overnight incubation at 4°C. The amount of precipitate formed in the tube assay was greatest for those cells which formed halo-producing colonies. The precipitation assay was standardized and made semiquantitative by comparing serial twofold dilutions of culture supernatants or column fractions to dilutions of a standard consisting of serum-free medium conditioned by the

growth of RECC-UT310$_{h3}$. The amount of precipitate was then scored on a ± to + + + + + scale. One unit was defined as the amount required to produce precipitate equivalent to 1 ml of undiluted supernatant from RECC-UT310$_{h3}$ cultures. Details of a purification scheme for precipitating factors in serum-free medium conditioned by the growth of REV-T-transformed cells which was based on this assay are described in the text.

### Assay for Factors Which Permitted Growth of REV-T-Transformed Hematopoietic Cells

Cells from halo-producing and nonhalo-producing lines were seeded in RPMI 1640 medium supplemented with 10% fetal bovine serum at 2.5 × $10^4$ cells/0.25 ml/well in 24-well cluster dishes (CoStar); 0.25 ml of various dilutions of fractions purified from serum-free medium conditioned by the growth of REV-T-transformed cells was added to each well. The final cell concentration in each well was 5 × $10^4$ cells/ml. Under these conditions, REV-T-transformed hematopoietic cells which were not treated failed to proliferate. After 4 days the cells in each well were counted on a hemocytometer.

## RESULTS

### Selection of Revertants and Identification of a Protein Which Is Produced in Increased Levels by Halo-Producing Variant Cells

We have previously described the isolation and characterization of variants of REV-T-transformed hematopoietic cells which, after plating in soft agar-containing medium at low cell densities, produced a ring of precipitation (halo) around cell colonies [22]. Different lines of REV-T-transformed hematopoietic cells gave rise to these variants at different frequencies (0.5–0.01%). The halo phenotype was a stable property of the cell variants; however, we have noted that after prolonged passage in liquid medium, the culture would revert to the nonhalo phenotype. We have determined that reversion of the halo-phenotype is rare in that less than one per 10,000 colonies isolated from several halo-producing REV-T-transformed hematopoietic cell lines grown in soft agar failed to produce a halo. A photograph of one such revertant is shown in Figure 1. These results suggested that the variation to halo production was reversible and that the revertants may possess a growth advantage over halo-producing cells in liquid medium.

Transformation has been reported to result in the release of various specific proteins into the culture medium [24–27,29–35]. Because production of the halo factor appeared to be regulated by REV-T [22], it was of interest to determine the nature of this precipitating factor released in high levels from the transformed cell variants. Halo-producing REV-T-transformed cell lines, revertants, and parental cell lines were labelled in medium supplemented with [$^{35}$S]methionine which contained no unlabelled methionine. Cells were removed from the medium by low-speed centrifugation and by filtration through a 0.2-μm filter, and [$^{35}$S]methionine-labelled proteins present in the supernatants were analyzed by SDS-PAGE and autoradiography. While several labelled proteins present in medium conditioned by the growth of REV-T-transformed cells were detectable by this procedure, only one [$^{35}$S]methionine-labelled protein of $M_r$ 15,000 was specifically produced at higher levels by all halo-producing variants examined. Selected examples of [$^{35}$S]methionine-labelled proteins found in the medium of REV-T-transformed cells are shown in Figure 2. The halo-producing variant RECC-310$_{h3}$ (lane 2) produced significantly more of the $M_r$ 15,000 protein (>) than cells of a nonhalo-producing

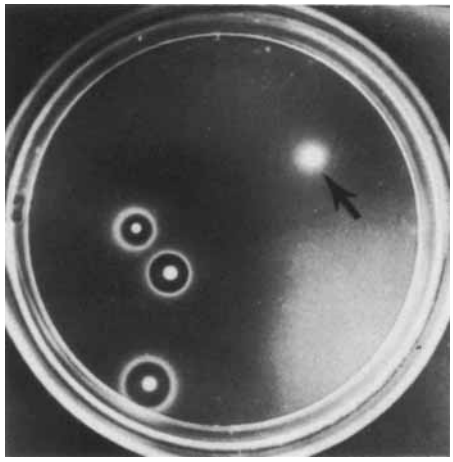

Fig. 1. Selection of nonhalo-producing revertant cells. Halo-producing cells from a line of REV-T-transformed cells (RECC-UT310$_{h3}$) were plated at a density of 5–10 cells/ml in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% chicken serum, and 0.3% Nobel agar. After 14 days incubation, plates were inspected for formation of nonhalo-producing revertant colonies. Approximately one out of 10,000 colonies failed to form a visible halo (arrow). The plate was illuminated from underneath with a circular fluorescent lamp.

revertant (lane 5) or the parental line (lanes 3 and 4). If the gel was overloaded with protein (lane 3, <), p15 was detectable in the supernatant from the parental cell line. These results indicate that the halo variation is due to increased or constituitive release of a factor normally produced by REV-T-transformed hematopoietic cells, rather than production of a novel factor. Because our labelling procedure detects only those proteins which contain methionine, we cannot exclude the possibility that halo variants overproduce other proteins which do not contain methionine.

The halo phenotype was not correlated with the production of immunoglobulins. One transformed cell line derived after in vitro infection with REV-T (in the presence of helper virus REV-A) has been shown to transiently secrete proteins which precipitate with polyvalent rabbit antiserum to the heavy and light chains of chicken IgM [6]. As previously described, labelled proteins of the appropriate molecular weight for the heavy and light chains of chicken IgM were secreted by cells of line RECC-UT317 (Fig. 2, lane 1, open arrowheads). Increased production of the $M_r$ 15,000 protein was not observed in this cell line, which suggested that halo production does not require secretion of immunoglobulins. Cells from a Marek's disease virus (MDV)-transformed line, another example of an immortalized chicken lymphoid cell, also release numerous proteins into culture supernatants. However, the MDV-transformed cells failed to release a protein which comigrated with the $M_r$ 15,000 protein in amounts detectable by this methodology (Fig. 2, lane 6).

## Development of an Assay for Precipitating Factors Produced by REV-T-Transformed Cells

Initial attempts to demonstrate the presence of precipitating factors in liquid medium conditioned by the growth of the halo-producing variants were complicated by the presence of a particulate fraction in bovine serum. This particulate material was present

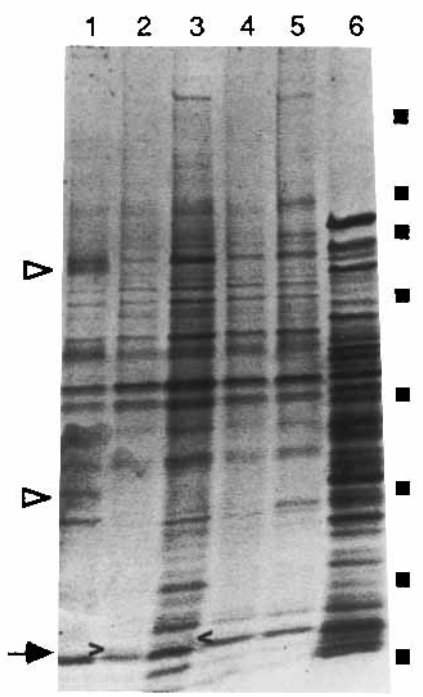


Fig. 2. Identification of a protein produced in elevated levels by halo-producing cells. Halo-producing REV-T-transformed cell lines, revertants, and parental cell lines were labeled for 4 hr in medium supplemented with [$^{35}$S]methionine which contained no unlabelled methionine. Cells were removed from the medium by low-speed centrifugation and by filtration though a 0.2-μm filter, and labelled proteins present in the supernatants were analyzed by SDS-PAGE and autoradiography. One protein of $M_r$ 15,000 (p15) was specifically produced in higher levels by this halo-producing variant and all variants examined (closed arrow and sideways "V" signs). Open arrowheads to the left of lane 1 represent IgM-related peptides. **Lane 1**: Nonhalo-producing, IgM-secreting variant RECC-UT317. **Lane 2**: Halo-producing variant $310_{h3}$. **Lanes 3** and **4**: Nonhalo-producing parental cell line RECC-UT310. **Lane 5**: Nonhalo-producing revertant RECC-UT$310_{h3}$R1. **Lane 6**: Marek's disease virus-transformed chicken lymphoid cell, MDCC-MSB1. Lane 3 and 6 were loaded with five times the amount of total protein as other lanes. $M_r$ markers (■ to the right of lane 6) are from top to bottom myosin (200k), β-galactosidase (116k), phosphorylase B (94k), bovine serum albumin (67k), ovalbumin (43k), carbonic anhydrase (30k), soybean trypsin inhibitor (20k), and lysozyme (14k).

in delipidized serum (fetal or newborn) and in serum which had been clarified by centrifugation at 10,000g. Therefore, attempts were undertaken to isolate precipitating factors from serum-free medium conditioned by the growth of the REV-T-transformed cells. Both halo-producing variants and parental cell lines were able to maintain growth under serum-free conditions for several weeks; however, serum-free medium conditioned by the growth of either halo-producing or nonvariant cells failed to form precipitates upon prolonged incubation. We concluded that serum factors or conditions imposed by growth in soft agar were required for the formation of either the precipitating factor or the precipitate. To test these possibilities cell-free, serum-free medium conditioned by the growth of REV-T-transformed cells was added to tubes containing a plug consisting of RPMI 1640 medium supplemented with 10% fetal bovine serum and 0.3% Nobel agar. Under these conditions, a precipitate best visualized at the interface of the liquid

medium and the agar plug formed in tubes after overnight incubation (Fig. 3). Analysis of the composition of the precipitates by SDS-PAGE revealed proteins of $M_r$ 150,000 and 67,000 and lower amounts of an $M_r$ 15,000 protein (data not shown). If serum was excluded from the agar plug, a precipitate failed to form. This suggested that the formation of the precipitate in the tube assay was the result of interaction between a factor present in medium conditioned by the growth of REV-T-transformed cells and serum proteins which diffused from the agar-containing plug. The amount of precipitate in the tube assay correlated directly with the formation of halos by colonies grown in soft agar (Table I). While all nonhalo-forming REV-T-transformed cell lines examined, including revertants, produced low levels of the precipitating factor in a 24-hr period, this amount was eight to 32 times less than that produced by the halo-forming variants. These results provide further evidence that the halo phenotype is associated with overproduction or constituitive production of a factor normally produced by REV-T-transformed cells. MDV-transformed cells, chicken spleen, bursa, or thymus cells, and REV-T-transformed chick embryo cells failed to release precipitating factors detectable in this assay.

## Purification of a Precipitating Factor Associated With the Halo Phenotype

The development of the rapid simple assay for precipitating factors produced by REV-T-transformed hematopoietic cells permitted purification of the responsible factor. Halo-producing variant cells were washed extensively and grown in serum-free medium for 24–48 hr. Cells were removed by centrifugation at 2,000g and the supernatants were subjected to clarification by a further centrifugation step at 10,000g. Clarified cell-free supernatants were fractionated by ammonium sulfate, precipitated, and dialyzed against a balanced salt solution. The bulk of the precipitating activity was present in the 40–

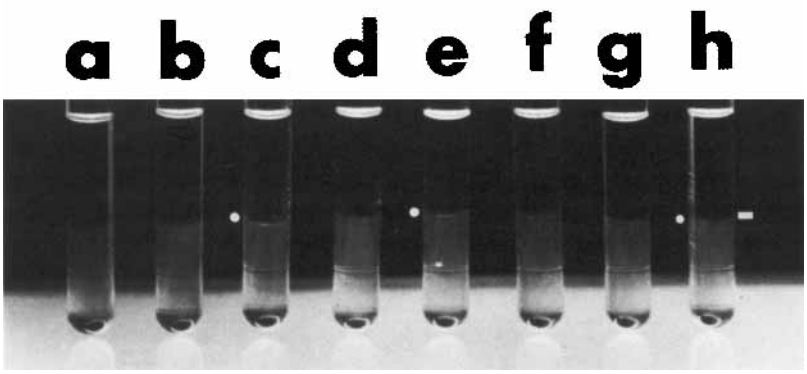


Fig. 3. Assay for precipitating factors produced by REV-T-transformed hematopoietic cells. Serum-free medium conditioned by the growth of REV-T-transformed cells was added to tubes containing a plug of agar containing medium and incubated overnight at 4°C. Under these conditions, a precipitate best visualized at the interface of the liquid medium and the agar plug (white bar) formed in tubes conditioned by the growth of REV-T-transformed cells. The amount of precipitate formed by conditioned medium from halo-producing variants (c, e, h, white symbol) was significantly higher than from nonhalo-producing parental cells or revertants (b, d, f, g). Tube **a**: Unconditioned medium. Tube **b**: Nonhalo-producing cell line RECC-UT313. Tube **c**: Halo-producing variant RECC-UT313$_{h1}$. Tube **d**: Nonhalo-producing cell line RECC-UT310. Tube **e**: Halo-producing cell line RECC-UT310$_{h3}$. Tube **f**: Nonhalo-producing revertant RECC-UT310$_{h1}$R1. Tube **g**: Nonhalo-producing cell line RECC-UT316. Tube **h**: Halo-producing variant RECC-UT316$_{h1}$.

**TABLE I. Precipitate Produced by Media Conditioned by Growth of Halo-Producing or Nonhalo-Producing REV-T-Transformed Cells***

| Cell type | Amount of precipitate |
|---|---|
| RECC-UT | ±/ − |
| RECC-UT310 | +/ + + |
| RECC-UT313 | ± |
| RECC-UT316 | ± |
| RECC-UT317 | ± |
| RECC-UT327 | ± |
| RECC-UT1$_{h1}$ | + + + |
| RECC-UT310$_{h3}$ | + + + + + |
| RECC-UT310$_{h5}$ | + + + + |
| RECC-UT313$_{h1}$ | + + + |
| RECC-UT316$_{h1}$ | + + + |
| RECC-UT327$_{h1}$ | + + + +/ + + + + + |
| RECC-UT310$_{h3}$R1 | +/ + + |
| RECC-UT313$_{h1}$R1 | + |
| MDCC-MSB1 | − |
| Spleen cells | − |
| Thymus cells | − |
| Bursal cells | − |
| REV-T-transformed CE cells | − |
| Medium control | − |

*Serum-free medium (1 ml) conditioned by the growth of either halo-producing or nonhalo-producing cells was added to 10 × 75 mm polystyrene culture tubes (Falcon) containing a plug of RPMI 1640 medium supplemented with 10% fetal bovine serum and 0.3% Nobel agar. Culture supernatants were also obtained from MDCC-MSB-1, chicken spleen, thymus, and bursal cells (Ficol-Hypaque purified) and from REV-T-transformed chick embryo (CE) cells. Unconditioned medium served as a control. After overnight incubation in the cold, the amount of precipitation was quantitated by comparing serial two-fold dilutions of culture supernatants to dilutions of a serum-free medium conditioned by the growth of RECC-UT310$_{h3}$. The amount of precipitate was scored on a ± to + + + + + scale (+ + + + + equal to 1 ml of undiluted supernatant from RECC-UT310$_{h3}$, + equal to a 16-fold dilution, ± to a 32-fold dilution). The ranges of variable results are given.

70% ammonium sulfate fraction. This cut was applied to a DEAE-cellulose column, which failed to retain the activity. The excluded fractions containing the peak of precipitating activity were pooled and then applied to a phosphocellulose column, which retained most of the activity. The precipitating activity was eluted from the phosphocellulose column by increasing the NaCl concentration of the column buffer to 0.1 M. A summary of the purification protocol and a typical enrichment and recovery are presented in Table II. In this experiment, the purification was 183-fold with approximately a 20% recovery of activity. The peak from such a purification was sharp (three to five 1-ml fractions from a 100-ml column volume) and contained predominantly a single protein, with an identical $M_r$, 15,000, to the protein previously identified in metabolic-labelling experiments as being overproduced by halo-producing cells (Fig. 4). Because the recovery of this protein, designated p15, by this purification scheme yielded five-

**TABLE II. Purification of Precipitating Activity From Serum-Free Medium Conditioned by the Growth of REV-T-Transformed Hematopoietic Cells***

| Purification step | Volume (ml) | Protein (mg) | Activity (units)[a] | Recovery (%) | Purification (fold) |
|---|---|---|---|---|---|
| Cell-free supernatant | 1200 | 28.14 | 1,200 | 100 | 1.0 |
| 40–70% ammonium sulfate | 7.5 | 12.58 | 722 | 60.2 | 1.3 |
| DEAE-cellulose chromatography | 10.5 | 1.51 | 601 | 50.1 | 9.3 |
| Phosphocellulose chromatography | 5.0 | 0.03 | 242 | 20.1 | 183.1 |

*Halo-producing variant cells from line RECC-UT310$_{h3}$ were washed extensively and grown in serum-free medium for 48 hr. Cells were removed by centrifugation at 2,000g and the supernatants were subjected to clarification by a further centrifugation step at 10,000g. The bulk of the activity detected by the assay described in the legend of Figure 3 in clarified cell-free supernatants was present in a 40–70% ammonium sulfate fraction. This cut was dialyzed and applied to a DEAE-cellulose column; excluded fractions containing the peak of precipitating activity were pooled and then applied to a phosphocellulose column. The precipitating activity was eluted from the phosphocellulose column by increasing the NaCl concentration of the column buffer to 0.1 M.

[a]One unit of activity is defined as the amount required to produce precipitate equivalent to 1 ml of undiluted cell-free supernatant.

to tenfold lower yields from medium conditioned by nonhalo-producing cells compared to medium conditioned by halo-producing cells (not shown), the $M_r$ 15,000 proteins may be identical.

## Quantitation of Growth Factor Activity in Fractions Enriched in p15

Growth factors are induced after transformation by many retroviruses, and several transforming viruses encode oncoproteins which are related either to growth factors or to their cellular receptors [24–34]. Therefore, it was of interest to determine if the purified fractions containing p15 possessed growth factor activity. REV-T-transformed hematopoietic cells grow well in serum-free medium; however, they would not proliferate when cultured at densities less than $5 \times 10^4$ cells/ml. Therefore, cells from a variety of halo-producing and nonhalo-producing lines were seeded at $5 \times 10^4$ cells/ml and exposed to various concentrations of a fraction enriched in p15. Addition of this fraction, in amounts which contained only nanogram levels of protein, permitted the growth of both halo-producing and nonhalo-producing cells lines in a concentration-dependent manner under these restrictive conditions of low cell density (Fig. 5). It is of interest that two to four times as much factor was required to promote the growth of halo-producing variant cells as compared to their parental cells or nonhalo-producing revertants. Conditioned medium from REV-T-transformed cell lines or the fractions containing p15 failed to detectably stimulate the growth of quiescent chicken embryo cells or chicken splenic lymphocytes.

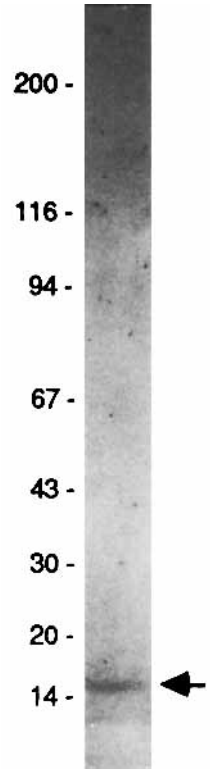


Fig. 4. SDS-PAGE analysis of a protein fraction enriched in precipitating activity and purified from medium conditioned by the growth of REV-T-transformed hematopoietic cells. Precipitating activity was purified from serum-free medium conditioned by the growth of RECC-UT310$_{h3}$ by the procedure outlined in Table I. Three fractions (3 ml from a 100-ml column volume) containing the peak of activity were pooled and 25 μl (approximately 0.5 μg protein) was analyzed on a 7–15% SDS-polyacrylamide gel. Proteins were detected by staining with Commassie Brilliant Blue dye. These fractions contained predominantly a $M_r$ 15,000 protein (**arrow**). Markers run in a parallel lane and indicated by their $M_r$ ($\times 10^{-3}$) are, from top to bottom, myosin, β-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme.

## DISCUSSION

Hematopoietic cells transformed by REV-T possess an extremely rapid generation time in vitro and a high cloning efficiency in soft agar-containing medium. The REV-T system may, therefore, provide an appropriate system with which to study by cytogenetic approaches those events which result in formation of a transformed lymphoid cell with invasive potential. Previous studies had revealed that REV-T-transformed cells give rise spontaneously to variants which secrete a factor that forms a distinct visible ring of precipitation (halo) surrounding colonies grown in soft agar [20]. A $M_r$ 15,000 protein was overproduced by halo variants and a $M_r$ 15,000 protein, p15, has been purified from serum-free medium conditioned by the growth of REV-T-transformed hematopoietic cells. Fractions enriched in p15 permitted the growth of REV-T-transformed cells under conditions in which they normally failed to proliferate; however, halo-producing cells required more of the growth-promoting activity than nonhalo-pro-

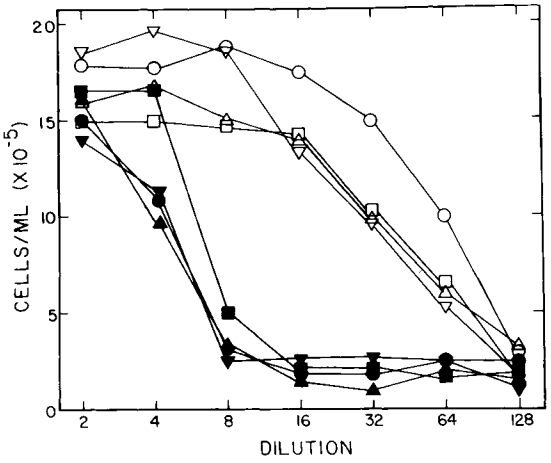


Fig. 5. Growth factor activity associated with fractions enriched in p15. Cells from halo-producing and nonhalo-producing lines were exposed to various concentrations of p15-enriched fractions purified as described in Table I ($5 \times 10^4$ cells/ml was final cell density in a volume of 0.5 ml). The initial dilution tested (2-fold) contained approximately 0.5 μg protein from the column fractions/ml of culture medium. After 4 days the cells in each well were counted on a hemocytometer. Values represent the average of triplicate determinations. Symbols: (○) nonhalo-producing cell line RECC-UT313; (●) halo-producing variant RECC-UT313$_{h1}$; (△) nonhalo-producing cell line RECC-UT310; (▼) halo-producing cell line RECC-UT310$_{h3}$; (▽) nonhalo-producing revertant RECC-UT310$_{h3}$R1; (▲) halo-producing cell line RECC-UT310$_{h5}$; (□) nonhalo-producing cell line RECC-UT316; (■) halo-producing variant RECC-UT316$_{h1}$.

ducing cells. Collectively, these results suggest that p15 is responsible for both halo formation and growth-promoting activity. If this is the case, the ability of p15 to precipitate various proteins may be related to its ability to stimulate growth of REV-T-transformed cells. Indeed, several multivalent proteins, including antibodies and lectins, may initiate division of hematopoietic cells by cross-linking cell surface proteins. Our results do not, however, exclude the possibility that an undetected protein in nanogram amounts in our preparation of p15 could be responsible for the precipitating activity or for the growth-promoting activity we have detected.

A number of acutely transforming viruses have been shown to encode oncoproteins which are related to growth factors or to the cellular receptors for growth factors [29–34]. For example, the transforming protein of simian sarcoma virus (SSV), p28$^{v\text{-}sis}$, is extensively homologous to one of the two subunits of platelet-derived growth factor (PDGF-2/B) and may stimulate autocrine growth of SSV-transformed cells through interactions with PDGF receptors on the cell surface [29–31]. Other retroviruses have been shown to influence cell-encoded growth factors, as has been extensively documented in the case of transforming growth factor α (TGFα) induction by murine sarcoma virus [25–28]. Association of p15 with growth factor activity suggests that it might have a direct role in hematopoietic cell transformation by REV-T. Autogenous production of a growth factor may permit tumor cell growth under conditions in which proliferation is limited normally. Alternatively, the virus-regulated growth factor may be involved in other aspects of REV-T tumorigenesis such as in the induction or activation of cells involved in immunosuppression [1,21,36].

The observation that more factor was required to promote the growth of halo-

producing variant cells than their parental cells or non-halo-producing revertants suggests that the nonhalo-producing cells may be more sensitive to the action of the factor. It is possible that the halo variants may have more or higher-affinity receptors for the factor. This may also provide an explanation for the observation that nonhalo revertants eventually overgrow cultures of halo-producing cells. All REV-T-transformed hematopoietic cells may require p15 or an associated factor for their growth. Occasionally variants may arise which are rather insensitive to the growth-promoting activity, and in order to survive they must compensate by producing higher levels of the factor. The halo phenotype could be a reflection of this high level of p15 production.

Transformation of chicken spleen cells with REV-T rescued from halo-producing cells always resulted in the formation of halo-producing cells, whereas virus rescued from nonhalo-producing cells [22] or from nonhalo-producing revertants (Garry, unpublished observation) did not give rise to halo-producing cells at a high frequency. This indicated that secretion of the precipitating activity, which we have now associated with p15, was regulated by the genetic material of REV-T. It is unclear whether p15 is the product of a viral or cellular gene. Nonvirus-producing (NP) cells transformed by replication-defective REV-T in the absence of a helper virus produce p15. REV-T contains extensive deletions in the pol and env regions, but a full-length gag region [8,9]. However, heterospecific antisera which react with RE virus structural proteins failed to immunoprecipitate a $M_r$ 15,000 protein from NP cells [6,16] or to interfere with the tube assay for precipitating factors (data not shown). These results suggest that p15 is not the product of a REV-T structural gene.

If p15 represents the product of a cellular gene, then several possible mechanisms exist to account for the influence of REV-T genetic information on the secretion of p15. One possibility is that $pp57^{v\text{-}rel}$ modulates expression of p15. The *v-rel* gene product shares distant sequence similarities both to oncoproteins which are protein kinases and to the epidermal growth factor receptor [8,9]. Recently, we and others have shown that $pp57^{v\text{-}rel}$ is cytoplasmically located and associated with serine/threonine protein kinase activity [11–14] (Tung et al., in preparation). The production of several growth factors is known to be regulated by a complex series of events [33–35,37]. The *v-rel*-encoded protein could interface with such a regulatory system to mediate secretion of p15.

Additional characterization of p15 will be necessary to determine the significance, if any, of p15 in REV-T transformation. Because overproducer cells provide a relatively abundant source of the factor, it should be possible in future studies to define, either through the use of monospecific antibodies or by direct sequencing of the factor, the actual relatedness of the halo-forming activity to the growth-promoting activity, and to determine whether p15 is virally or cellularly encoded. It will also be important to determine whether p15 is required for growth of REV-T-transformed cells under a variety of conditions. Further study may also reveal regulatory interactions between the growth factor we have isolated and the REV-T-encoded oncoprotein which has a cytoplasmic location.

## ACKNOWLEDGMENTS

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