Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8237–8241, December 1985
Medical Sciences

The putative transforming protein of S13 avian erythroblastosis virus is a transmembrane glycoprotein with an associated protein kinase activity

(acute leukemia/*env* protein/proteolytic cleavage/pulse–chase labeling/S13 *onc* protein)

M. J. HAYMAN*, G. KITCHENER*, P. K. VOGT†, AND H. BEUG‡

*Imperial Cancer Research Fund Laboratories, St. Bartholomew's Hospital, Dominion House, Bartholomew Close, London EC1A 7BE, United Kingdom; †Department of Microbiology, University of Southern California, 2025 Zonal Avenue, Los Angeles, CA 90033; and ‡European Molecular Biology Laboratories, Postfach 10,2209, Meyerhofstrasse 1, 6900 Heidelberg 1, Federal Republic of Germany

Contributed by P. K. Vogt, August 2, 1985

ABSTRACT **S13 is an avian retrovirus that transforms both fibroblasts and erythroblasts. The gene product responsible for the oncogenic effects of S13 is the env-related glycoprotein gp155. In this report we show that gp155 is a transmembrane protein with a 55-kDa cytoplasmic domain. Pulse–chase analysis shows that gp155 was cleaved posttranslationally into two glycosylated proteins, gp85 and gp70. In addition, we show that a tyrosine protein kinase activity is associated only with the gp70 protein in microsomes and in immune complexes.**

The avian retrovirus S13 was originally isolated by Stubbs and Furth (1) in the course of an investigation of strain 1 avian erythroleukemia virus. Strain 1 erythroleukemia virus exclusively caused acute leukemia, whereas S13 induced sarcomas in addition to acute erythroleukemia. Experiments have confirmed these early observations and shown that S13 represents another type of erythroblastosis virus (2). S13 is oncogenic in chickens causing small fibrosarcomas and expansion of the erythroid compartment of the bone marrow cells that results in a severe fatal anemia (2). S13 transforms both fibroblasts and erythroblasts *in vitro*, although special growth conditions are required to propagate the transformed erythroblasts (2). In this regard the cells are similar to erythroblasts transformed by the *src*, *fps*, and *erbB* oncogenes and are distinct from those transformed by avian erythroblastosis virus (AEV)-ES4 that possess the *erbA* oncogene in addition to *erbB* (3).

Analysis of the genome and gene products of S13 revealed that it was defective for replication and had a genome size of 8.5 kilobases. Nonproducer cells transformed by S13 contained a 3.5-kilobase subgenomic mRNA in addition to the genomic length RNA (4). Protein analysis of S13-transformed cells showed that they contained a transformation-specific 155-kDa glycoprotein, gp155, in addition to the *gag* gene product, Pr76 (2). This protein was immunologically related to the *env* gene product and was presumably encoded by the subgenomic mRNA. Immunological analysis of this protein and molecular hybridization analysis of the S13 genome demonstrated that the putative oncogene of S13 was not related to any of the oncogenes that are known to affect erythroid cells namely *src*, *erbA*, *erbB*, *fps*, Ha-*ras*, *abl*, *ets*, as well as the *myc*, *myb*, *fms*, *B-lym*, and *rel* oncogenes (4).

Therefore, the putative transforming protein of S13 is not only unique because it is an env-onc fusion protein but also because it may be encoded by an as yet undescribed oncogene. To understand more clearly how this protein may be responsible for erythroid and fibroblast transformation, a detailed analysis of gp155 has been carried out. In this communication we have determined the topography of gp155 and show that gp155 is posttranslationally cleaved into a gp85 and a gp70 molecule. The gp70 is a membrane protein that has an associated protein kinase.

MATERIALS AND METHODS

Cells and Cell Culture. The origin of S13 virus and the generation of transformed fibroblasts has been described earlier (2). Infection of bone marrow cells with S13 virus and generation of S13-transformed producer and nonproducer erythroblast clones was done as described (2). The origin of AEV-transformed erythroblasts (cell line HD2) has been described (5). S13-transformed fibroblasts and AEV-transformed erythroblasts were grown in standard growth medium (Dulbecco's modified Eagle's medium) supplemented with 8% (vol/vol) fetal calf serum, 2% (vol/vol) chicken serum, and 10 mM Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], pH 7.3. S13-transformed erythroblasts were grown in CFU-E medium (6) supplemented with insulin (1 μg/ml) (2).

Generation of Tumor-Bearing Rat Anti-S13 Protein Antisera. S13-transformed rat (FR) cells were obtained by cocultivation of S13-producing chicken fibroblasts and normal rat FR cells. Before cocultivation the chicken cells were treated with mitomycin-C (10 μg/ml) for 2 hr. Foci of transformed FR cells appeared in the mixed cultures after 2 weeks of cocultivation. They were picked with a capillary pipette and grown into mass cultures. The S13-transformed FR cells used in the present study came from a focus of polygonal and round transformed cells. S13 virus could be rescued from these cells by cocultivation with chicken fibroblasts if the chicken cells were also infected with avian leukosis helper virus. Without helper virus no S13 could be rescued from the S13-transformed FR clone, suggesting that no helper viruses were integrated in the rat cell genome. These cells were injected intraperitoneally into 14-day-old (Fisher × Wistar)F_1 rats and serum collected 6–8 weeks later by which time small tumors were present. This tumor-bearing rat antiserum will be referred to hereafter as TBR antiserum.

Antisera. The rabbit anti-gag (anti-p27), anti-env, and anti-src sera have been described (7, 8).

Immunoprecipitation. Cells were labeled with [^{35}S]methionine (Amersham) or [^{3}H]glucosamine (Amersham) as described (9–11). Lysates prepared as described (10) were immunoprecipitated, analyzed on $NaDodSO_4$/polyacryl-

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: AEV, avian erythroblastosis virus; TBR, tumor-bearing rat.

amide gels, and fluorographed as described (9). To increase efficiency of immunoprecipitation by rat anti-S13 antisera the *Staphylococcus aureus* bacteria used for collecting the immune complexes were sometimes preincubated with 5 μg of affinity-purified rabbit anti-rat IgG per 10 μl of *S. aureus* suspension. Immunoprecipitation of gag- and env-related proteins by TBR anti-S13 antisera was blocked by using disrupted virus as described (12). The natural helper virus of S13 virus, S13 associated virus (2), was harvested from mass cultures of S13 associated virus-infected fibroblasts (2), purified, and extracted as described (12).

Proteinase K Digestion of Microsomes. Microsomes were prepared from S13-transformed erythroblasts as described (7). The microsomes from 10^7 cells were then digested with varying concentrations of proteinase K for 90 min on ice as described (13, 14).

Tunicamycin Treatment. S13-transformed erythroblasts were incubated with tunicamycin (5 μg/ml, Sigma) in growth medium for 2 hr. This medium was removed and replaced with methionine-free growth medium containing 200 μCi (1 Ci = 37 GBq) of [^{35}S]methionine and tunicamycin (5 μg/ml), and the cells were incubated for a further 60 min. Cell lysates were then immunoprecipitated as described above.

Protein Kinase Assays. Protein kinase assays were performed either on microsomes as described by Gilmore *et al.* (15) or on immunoprecipitated complexes (16).

RESULTS

Topography of the S13 gp155 Molecule. Analysis of the gp155 molecule has shown that it is glycosylated and is immunologically related to the env gene product (2). If gp155 is a transmembrane protein, it should be asymmetrically inserted into membranes. Therefore, proteinase protection experiments were performed to determine whether gp155 is indeed a transmembrane protein and to examine its position in the membrane. Since microsomes should have the same sidedness as the endoplasmic reticulum, the carboxyl-terminal domains of membrane proteins should be sensitive to proteinase K, whereas the amino-terminal domain should be protected by the membrane. This method has been used to assess the asymmetry of a wide variety of transmembrane proteins (13, 14).

S13-transformed nonproducer erythroblasts were labeled for 20 min with [^{35}S]methionine, and microsomes were prepared from these cells. To test for stability in the absence of proteinase, microsomes were incubated on ice for 90 min, lysed, and immunoprecipitated with anti-env antiserum. The gp155 is found in the microsomes (Fig. 1*A*, lane 5), and essentially all of the gp155 could be recovered in the microsomal fractions (data not shown). For proteinase digestion, amounts of microsomes equivalent to those analyzed in Fig. 1*A* (lane 5) were incubated with various amounts of proteinase K for 90 min on ice. Fig. 1*A* (lanes 1–4) shows that as the amount of proteinase is increased a 100-kDa molecule is immunoprecipitated concomitant with loss of gp155 (Fig. 1*A*, lane 1). The loss of radioactivity in this protein relative to that in gp155 is due either to deterioration of the microsome structure during the incubation and subsequent loss of material or to the possibility that the digested carboxyl-terminal domain is relatively rich in methionine. These data show that gp155 consists of a 55-kDa cytoplasmic domain and a 100-kDa amino-terminal domain.

gp155 has been shown to contain carbohydrate units by incorporation of radioactive mannose (4). By analogy with all other transmembrane glycoproteins, these carbohydrate units should be located on this exposed amino-terminal region. To determine the extent of this carbohydrate structure, cells were incubated in the presence of an inhibitor of *N*-linked carbohydrate addition, tunicamycin, and immunoprecipitated with anti-env antiserum. Fig. 1*B* shows that this treatment generates a nonglycosylated 95-kDa protein. Therefore, gp155 is composed of a 95-kDa polypeptide of which the above-mentioned 55-kDa segment is oriented toward the cytoplasm, and 40 kDa is on the outside of the cell and carries 60 kDa of carbohydrate.

Posttranslational Cleavage of gp155. The data above show that the anti-env antiserum can react with the amino-terminal domain of gp155. This domain should be exposed on the outside of the cell; however, cell surface immunofluorescence with this same antiserum gave a surprisingly weak reaction on nonproducer S13-transformed erythroblasts (data not shown). Therefore, either gp155 remains intracellular or the amino-terminal env domain is removed from the molecule and is not exposed on the cell surface. Pulse–chase analysis of gp155 has shown it to be turned over with a half-life of ≈2 hr but no products were detected (4). How-

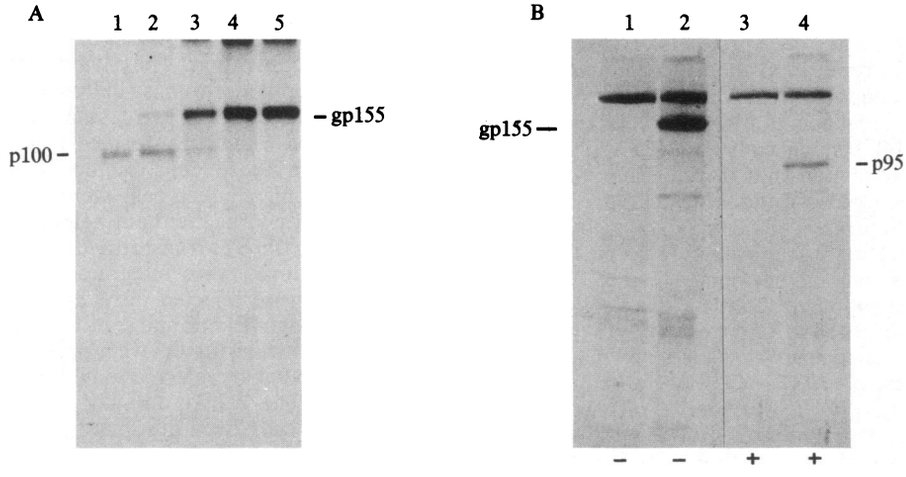

FIG. 1. Transmembrane orientation and extent of glycosylation of gp155. (*A*) Microsomes purified from S13-transformed nonproducer erythroblasts were treated with the following concentrations of proteinase K. Lanes: 1, 20 μg; 2, 4 μg; 3, 0.8 μg; 4, 0.16 μg; 5, 0 μg. The microsomes were then solubilized and immunoprecipitated with anti-env antiserum, and the immune complexes were electrophoresed on $NaDodSO_4$/polyacrylamide gels. (*B*) S13 nonproducer erythroblasts were labeled with [^{35}S]methionine either in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of tunicamycin (5 μg/ml), then were lysed and immunoprecipitated with normal rabbit serum (lanes 1 and 3) or anti-env antiserum (lanes 2 and 4).

ever, the anti-env antisera used for these experiments were not of high titer. Therefore, the pulse–chase analysis was repeated using a high-titer anti-env antiserum which reacts with gp85 and gp37 (7). Fig. 2*A* shows that by using this antiserum two proteins that appear during the chase period can be immunoprecipitated, a faint diffuse 85-kDa band and a more discrete 70-kDa band. The 85-kDa protein is difficult to visualize due to its diffuse nature and appears to contain relatively few methionine residues. It can be seen more clearly by using radioactive sugar (see Fig. 4). Thus, it appears that gp155 is cleaved posttranslationally during its biosynthesis.

Preparation of TBR Antiserum Against S13-Transformed Cells. For two reasons it seemed desirable to prepare antiserum specific for the S13 onc-specific domain of the gp155 molecule and its cleavage products. First, such antisera would allow us to characterize more precisely the products during pulse–chase analysis, something that was not possible with even our best anti-env antiserum. Also, these antisera would allow us to define the product of the *onc* gene sequences of S13 virus immunologically. Previous analysis has shown that rats carrying fibrosarcomas induced by AEV and by the avian myelocytomatosis virus MC29 produce antibodies against the oncogene products of these two viruses (10, 17). Therefore, we transformed rat fibroblasts with S13 and were able to isolate several transformed clones (the isolation of the clones and their properties will be described in detail elsewhere). Analysis of the proviruses contained in these cells led to the identification of a rat fibroblast clone from which S13 virus could be rescued. This clone of S13-transformed nonproducer cells contained a single integrated provirus and synthesized gp155 (data not shown). Rats were injected with these cells, and serum samples were taken ≈6 weeks later when the animals showed signs of small tumors. Fig. 2*B* shows a pulse–chase analysis of immunoprecipitated lysates of S13-transformed cells using one of these antisera. The antiserum contains antibodies against gp155 as well as to Pr76, as expected, since S13 carries a complete *gag* gene (Fig. 2*B*, lane 4). In addition, the antiserum very efficiently immunoprecipitates the 70-kDa cleavage product of gp155 formed during the chase period (Fig. 2*B*).

To test the TBR antiserum for antibodies directed against the putative *onc* gene product of S13 virus, immunoprecipitation was done by using TBR antiserum that has been adsorbed with virion proteins, including those from the natural helper virus of S13 virus, S13 associated virus (2). This treatment was sufficient to block the precipitation of gp155 and the 70-kDa protein by the anti-env antiserum (Fig. 3*A*). However, when TBR antiserum was used, this treatment blocked the immunoprecipitation of Pr76 and its products completely, but the precipitation of gp155 and the 70-kDa protein remained unchanged (Fig. 3 *B* and *C*). This observation indicates that the TBR antisera indeed react with the onc-specific domains on the gp155 and 70-kDa molecules. The diffuse gp85 protein was not detected efficiently during these experiments and, therefore, it is unknown whether the TBR antiserum still precipitated this protein.

The 85-kDa and 70-kDa Proteins Are Glycosylated. gp155 contains a very large amount of carbohydrate (Fig. 1*B*). To study whether these carbohydrate units were present on one or both of the cleavage products, cells were labeled overnight with [^{3}H]glucosamine, and lysates were immunoprecipitated with the TBR antiserum. Fig. 4 shows that all three proteins were labeled with [^{3}H]glucosamine. Therefore we shall hereafter refer to the cleavage products of gp155 as gp85 and gp70, respectively. Unfortunately, we were not able to use tunicamycin to determine the carbohydrate content of these two proteins, since this inhibitor also blocked the proteolytic cleavage of the precursor molecule (data not shown).

gp70 Has an Associated Protein Kinase Activity. To date, two viral oncogenes have been described that code for integral plasma membrane proteins, *erbB* and *fms* (11, 14, 18, 19). The products from both of these oncogenes have been demonstrated to possess an associated tyrosine kinase activity (14–16). Therefore, although previous analysis has shown that gp155 does not have any detectable kinase activity, we decided to reexamine this question using both anti-env and TBR antisera under the specific conditions that have been used to detect kinase activity in the v-*erbB* gene product (15, 16).

First, an assay for membrane-associated kinase was done. Microsomes were prepared, were permeabilized with

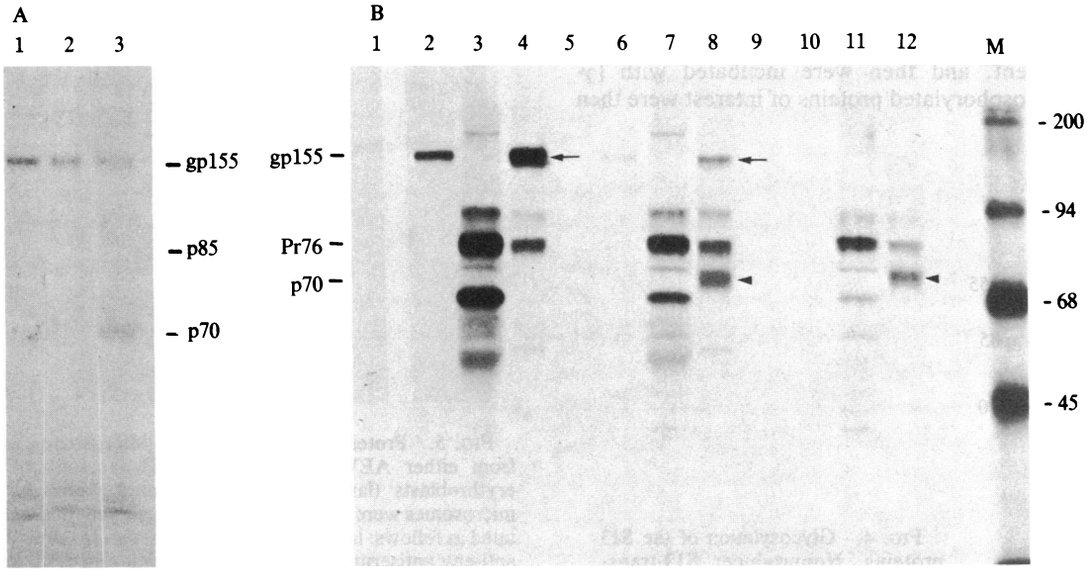

FIG. 2. Pulse–chase analysis. S13-transformed nonproducer erythroblasts were labeled for 20 min with [^{35}S]methionine and then chased for various time periods. Cells were harvested and analyzed by immunoprecipitation. (*A*) Immunoprecipitates collected from extracts with the anti-env antiserum were electrophoresed. Lanes: 1, the pulse; 2, 30-min chase; and 3, 90-min chase. (*B*) Extracts were immunoprecipitated with the following antisera: lanes 1, 5, and 9, normal rabbit serum; lanes 2, 6, and 10, anti-env antiserum; lanes 3, 7, and 11, anti-p27 antiserum; lanes 4, 8, and 12, TBR antiserum. Lanes: 1–4, pulse; 5–8, 1-hr chase; 9–12, 2-hr chase. Lane M, molecular size standards in kDa.

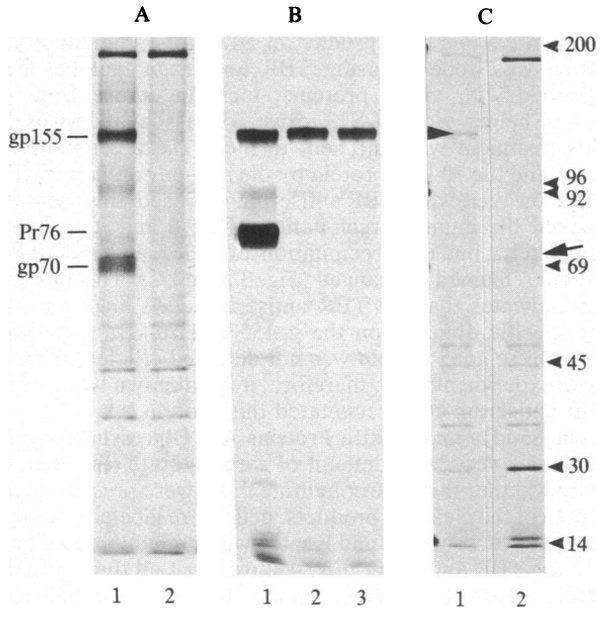

FIG. 3. Detection of S13-specific domains on gp155 and gp70 by immunoprecipitation with TBR antiserum. (*A*) Extracts from S13-transformed nonproducer erythroblasts labeled for 2 hr with [^{35}S]methionine were immunoprecipitated with rabbit anti-env antiserum in absence (lane 1) or presence (lane 2) of 100 μg of competing viral protein (15). Note the complete block of gp155 and gp70 immunoprecipitation by adsorption with viral protein. (*B*) S13-transformed nonproducer erythroblasts were labeled with [^{35}S]methionine for 30 min and immunoprecipitated with TBR antiserum in absence (lane 1) or presence of 100 μg of competing virus (ref. 15; lane 2) or in the presence of 100 μg of competing virus and 75 μg of S13 associated virus extract (lane 3). Efficiency of immunoprecipitation was increased by preincubation of *S. aureus* with rabbit anti-rat IgG. (*C*) S13-transformed nonproducer erythroblasts were pulse-labeled with [^{35}S]methionine for 20 min (lane 1) and then chased in cold CFU-E medium for additional 90 min (lane 2). Immunoprecipitation of cell extracts by TBR antiserum in presence of 100 μg competing viral protein reveals gp155 (arrowhead, lane 1) and gp70 (arrow, lane 2). Molecular size standards in lane 2 from the top are as follows: myosin heavy chain, 200 kDa; phosphorylase *b*, 96 kDa; phosphorylase *a*, 92 kDa; bovine serum albumin, 69 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 30 kDa; lysozyme, 14 kDa.

nonionic detergent, and then were incubated with [γ-^{32}P]ATP. The phosphorylated proteins of interest were then isolated by immunoprecipitation. This assay has been used successfully for both v-src (20) and v-erbB (ref. 15 and unpublished results). The assay was done with S13-transformed erythroblasts and with AEV-transformed erythroblasts as control cells. As shown in Fig. 5*A*, anti-erbB antiserum precipitated the phosphorylated erbB proteins from ^{32}P-labeled microsomes from AEV transformed cells (lane 3) but anti-env antiserum did not (lane 2); in contrast, anti-env serum precipitated phosphorylated gp70 from labeled microsomes from S13-transformed cells (lane 5), but anti-erbB antiserum did not (lane 6). Therefore, gp70 is phosphorylated in microsomes, and analysis shows that the amino acid being labeled is tyrosine (data not shown).

Since it was possible that gp70 was being phosphorylated by cellular membrane-associated kinases, an immune-complex kinase assay was also done under the reaction conditions that allowed detection of the erbB kinase activity (16). In this assay the S13 proteins are purified from the other cell proteins by immunoprecipitation, then the immune complexes are incubated with [γ-^{32}P]ATP, and the labeled protein(s) are identified by NaDodSO$_4$/PAGE. Again, AEV-transformed erythroblasts were used as controls to test the specificity of our sera, whereas antibodies to c-src (contained in both S13- and AEV-transformed erythroblasts) served as a positive control for the reaction. gp70, but not gp155, immunoprecipitated with the S13-TBR antiserum was phosphorylated (Fig. 5*B*, lane 4), whereas no phosphorylated proteins were immunoprecipitated from AEV-transformed erythroblasts (lane 1). In contrast, the anti-erbB antiserum immunoprecipitated erbB proteins from AEV-transformed erythroblasts (lane 2) but not from S13-transformed cells (lane 5). The anti-src antiserum detected c-src kinase activity in both cell types. This result demonstrates that only gp70, the cleavage product of gp155, was phosphorylated presumably by an associated kinase activity whereas uncleaved gp155 and gp85 were not phosphorylated and thus have low

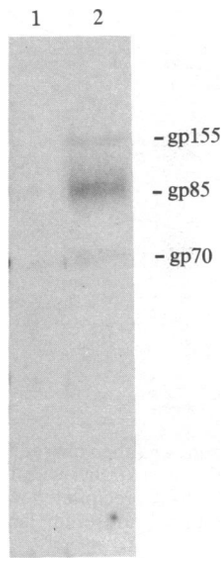

FIG. 4. Glycosylation of the S13 proteins. Nonproducer S13-transformed erythroblasts were labeled for 16 hr with [^{3}H]glucosamine (500 μCi), the cells were harvested and were analyzed by immunoprecipitation. Lane 1, normal rat serum; lane 2, TBR antiserum.

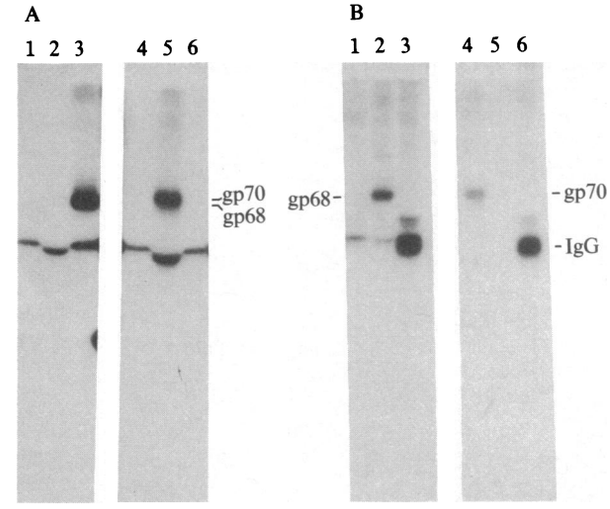

FIG. 5. Protein kinase assays. (*A*) Microsomes were prepared from either AEV (lanes 1–3) or S13-transformed nonproducer erythroblasts (lanes 4–6) and incubated with [γ-^{32}P]ATP. The microsomes were then extracted with detergent and immunoprecipitated as follows: lanes 1 and 4, normal rat serum; lanes 2 and 5, rabbit anti-env antiserum; and lanes 3 and 6, rat anti-erbB antiserum. (*B*) Immunoprecipitated complexes were prepared from AEV- (lanes 1–3) and S13-transformed nonproducer erythroblasts (lanes 4–6) with TBR antiserum against the S13 proteins (lanes 1 and 4), rat anti-erbB antiserum (lanes 2 and 5), and rabbit anti-src antiserum (lanes 3 and 6). IgG indicates the location of the immunoglobulin heavy chains labeled by the src kinase.

or undetectable kinase activity. This result is consistent with that from the membrane-associated kinase assay (Fig. 5*A*).

DISCUSSION

The avian retrovirus S13 causes fibrosarcoma and erythroid leukemia *in vivo* and is capable of transforming fibroblasts and erythroblasts *in vitro* (1, 2). The env-related protein, gp155, has been proposed to be an env-onc fusion protein that is responsible for transformation by S13 virus *in vivo* and *in vitro* (2, 4). The structural and functional characterization of gp155 reported in this communication supports this proposal.

gp155 is synthesized as an integral transmembrane protein consisting of a 100-kDa amino-terminal domain and a 55-kDa carboxyl-terminal region. The amino-terminal domain consists of a 40-kDa polypeptide with 60 kDa of carbohydrate. Pulse–chase analysis showed that gp155 was an intracellular precursor that was posttranslationally cleaved into two products, gp85 and gp70. Both of these cleavage products reacted with anti-env antiserum, albeit weakly, whereas gp85 and gp70 were specifically and efficiently recognized by TBR antiserum raised against S13-transformed rat fibroblasts. Moreover, *in vitro* protein kinase assays showed that gp70 was associated with a tyrosine kinase whereas the gp155 and gp85 molecules were not.

Our data on the structure and biosynthesis of the S13 env-related proteins allow some tentative conclusions as to where the onc sequences of S13 are located within the primary translation product, gp155. The normal *env* gene product is synthesized as a glycosylated precursor of 95 kDa of which only 2 kDa protrudes into the cytoplasm of infected cells (21). This protein is cleaved and further glycosylated to yield gp85 and gp37, which form a disulfide-linked dimer in which only gp37 is an integral membrane protein.

A comparison of this biosynthetic pathway with that of the S13 env-related protein leads to the tempting speculation that the env proteolytic cleavage site is still present in gp155. Consequently, the gp85 molecule released by cleavage of gp155 would be equivalent to the gp85 that is released from the 95-kDa *env* gene product. Consistent with this interpretation of our data is the observation that subgroup A-host range virus is sometimes released, when subgroup B-helper virus is used to rescue S13 nonproducer cells (2), implying that S13 virus encodes enough of the *env* gene to determine the subgroup specificity, which is a known property of gp85. Also, this hypothesis predicts that gp37-encoded material is located at the amino terminus of gp70, which is related to env. Our hypothesis also predicts that the *onc* sequences are located in gp70. The kinase activity associated with this protein and the presence of a 55-kDa cytoplasmic domain in gp155 is consistent with this interpretation. Ultimately, only the molecular cloning and sequencing of the S13 genome will determine whether these predictions are correct.

The demonstration of kinase activity associated with gp70 is of interest, since so far most oncogene products known to transform avian erythroid cells also have an associated kinase activity—i.e., *erbB*, *src*, and *fps*, with the ets-myb protein of E26 providing the only exception. The oncogene contained in the S13 genome has not yet been identified. However, the finding that the putative transforming protein of S13 is a glycosylated transmembrane protein with kinase activity is reminiscent of the *erbB* and *fms* oncogenes, although neither of these kinases show any detectable homology with S13 (4). Both *erbB* and *fms* have been proposed to be related to growth factor receptor genes (11, 14). The *erbB* gene has recently been shown to be homologous to the gene coding for the epidermal growth factor receptor gene (22, 23), and the *fms* gene is related to the gene coding for the receptor of the mononuclear phagocyte growth factor CSF-1 (24). Given the structure of gp70, it is tempting to speculate that this protein also is related to a growth factor receptor gene. In this case the associated kinase activity is of particular interest. *erbB* and *fms* have been truncated and are presumed to provide growth-promoting signals, possibly related to the kinase activity, in the absence of hormone growth factors (reviewed in ref. 25). Consistent with this is the observation that forms of these proteins in the endoplasmic reticulum and in the plasma membrane have associated kinase activity (14–16), whereas normally only the plasma membrane form of growth factor receptor has kinase activity that is regulated by its interaction with the growth factor. However, the S13 protein kinase is still regulated in some way, since only gp70 possesses this activity. This regulation could be at the level of posttranslational cleavage or could be due to some other posttranslational event. The identification of the oncogene contained in the S13 genome, and its subsequent molecular analysis, will provide the answers to these questions.

The authors thank Drs. P. Enrietto and J. Wyke for their comments on the manuscript and Andrea Sterlini, Joyce Newton, and Glennis Harding for typing. This work was supported by Grants CA 13213 and CA 29777 from the National Cancer Institute.

1. Stubbs, E. L. & Furth, J. (1935) *J. Exp. Med.* **61**, 593–616.
2. Beug, H., Hayman, M. J., Graf, T., Benedict, S. H., Wallbank, A. M. & Vogt, P. K. (1985) *Virology* **145**, 141–153.
3. Kahn, P., Adkins, B., Beug, H. & Graf, T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7122–7126.
4. Benedict, S. H., Maki, Y. & Vogt, P. K. (1985) *Virology* **145**, 154–164.
5. Beug, H., von Kirchbach, A., Doederlein, G., Conscience, J. F. & Graf, T. (1979) *Cell* **18**, 375–390.
6. Radke, K., Beug, H., Komfeld, S. & Graf, T. (1982) *Cell* **31**, 643–653.
7. Hayman, M. J. (1978) *Virology* **85**, 475–486.
8. Stoker, A. W., Enrietto, P. J. & Wyke, J. A. (1984) *Mol. Cell. Biol.* **4**, 1508–1514.
9. Hayman, M. J., Royer-Pokora, B. & Graf, T. (1979) *Virology* **92**, 31–45.
10. Hayman, M. J., Ramsay, G. M., Savin, K., Kitchener, G., Graf, T. & Beug, H. (1983) *Cell* **32**, 579–588.
11. Beug, H. & Hayman, M. J. (1984) *Cell* **36**, 963–972.
12. Beug, H., Graf, T. & Hayman, M. J. (1981) *Virology* **111**, 201–210.
13. Rothman, J. E. & Lodish, H. F. (1977) *Nature (London)* **269**, 775–780.
14. Rettenmeier, C. W., Roussel, M. F., Quinn, C. O., Kitchingman, G. R., Look, T. A. & Sherr, C. J. (1985) *Cell* **40**, 971–981.
15. Gilmore, T., DeClue, J. E. & Martin, G. S. (1985) *Cell* **40**, 609–618.
16. Kris, R. M., Lax, J., Gullick, W., Waterfield, M. D., Ullrich, A., Fridkin, M. & Schlessinger, J. (1985) *Cell* **40**, 619–625.
17. Vennstrom, B., Kahn, P., Adkins, B., Enrietto, P. J., Hayman, M. J., Graf, T. & Luciw, P. (1984) *EMBO J.* **3**, 3223–3229.
18. Hayman, M. J. & Beug, H. (1984) *Nature (London)* **309**, 460–462.
19. Roussel, M. F., Rettenmeier, C. W., Look, A. T. & Sherr, C. J. (1984) *Mol. Cell. Biol.* **4**, 1999–2009.
20. Dehayza, P. & Martin, G. S. (1985) *Virology* **143**, 407–421.
21. Schwartz, D. E., Tizard, R. & Gilbert, W. (1983) *Cell* **32**, 853–858.
22. Downward, J., Yarden, Y., Mayes, E., Scrace, G., Totty, N., Stockwell, P., Ullrich, A., Schlessinger, J. & Waterfield, M. D. (1984) *Nature (London)* **307**, 521–527.
23. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle, N., Waterfield, M. D. & Seeburg, P. H. (1984) *Nature (London)* **309**, 418–425.
24. Sherr, C. J., Rettenmeier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41**, 665–676.
25. Hunter, T. (1984) *J. Natl. Cancer Inst.* **73**, 773–786.