the implant and the close positional relationship between this cell coating and the outgrowing axons (Fig. 2, B and C). Although, in the animal shown in Fig. 2, A and B, a few fibers grew medially for relatively long distances and seemed to terminate within the cellular coat, others grew laterally but eventually abandoned this surface to grow ventrally toward the hippocampal commissure (Fig. 2A) (*9*).

Our ultrastructural analyses suggest that the cells that encase the cellulose bridges are a form of glia, probably astrocytes. They have the ability, characteristic of young astrocytes, to actively enwrap outgrowing fibers (*10*). In addition, they are rich in glycogen granules and intermediate filaments (*11*) (Fig. 2C). We do not yet know, however, if these glia are remnants of the original sling glia population that normally occupies the subcallosal region.

It has not yet been determined whether the newly formed callosal axons have grown to appropriate or nonappropriate targets and have formed functional synapses. In addition, we do not yet know if a critical period exists postnatally that, if allowed to pass, renders the callosal axons refractory to further growth (*12*). If so, it may be possible, in older animals, to stimulate Probst's fibers by inserting bridges impregnated with freshly "harvested" embryonic glial tissues. Such procedures are a modification of those described recently by Kromer *et al.* (*13*), who used embryonic hippocampal implants as bridges to promote the regrowth of adult septal axons.

Our data suggest that substantial numbers of potentially misplaced callosal axons in acallosal mice are capable at prenatal and early postnatal stages, not only of further growth but of correctly oriented growth as well. The callosal axons display their most vigorous, contralaterally directed growth response when they are confronted with a structure that resembles (in both cell type and orientation) the environment through which the axons would have grown in the normally developing embryo.

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7. In this regard, the number of normally late arriving (after P4) callosal axons cannot solely, but perhaps partially, account for all those present.
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9. We have also used a variety of plastics that glia will not coat, and, in addition, we have quickly inserted then extracted cellulose filters as control implants. We have failed totally (0 in more than 100 animals) in our attempt to redirect axons with these substances or procedures and, thus, neither the stab wound itself nor the mere introduction of just any foreign material are sufficient stimuli to promote further callosal growth.
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Identity of HeLa Cell Determinants Acquired by Vesicular Stomatitis Virus with a Tumor Antigen

Abstract. *Growth of vesicular stomatitis virus (VSV) in HeLa cells results in progeny containing non-VSV antigens with a molecular weight around 75,000. The non-VSV antigens were detected by antiserums to HeLa cell determinants. These antiserums precipitate whole virions but do not neutralize them. Because one of the antiserums is directed to a tumor-specific surface antigen of HeLa cells, it appears that VSV specifically acquires such antigens during its passage through human tumor cells.*

When different enveloped viruses infect the same cells, they often acquire each other's surface antigens during the maturational process (*1, 2*). Vesicular stomatitis virus (VSV) is especially effective for demonstrating such phenotypic mixing because it grows in almost every cell type and acquires antigens from enveloped RNA or DNA viruses (*2, 3*). When VSV is grown in cell lines not known to harbor other enveloped viruses, the progeny will acquire a subset of the antigens normally found at the cell surface (*4–6*). With murine L cells, the "cellular" antigens acquired by VSV progeny have been identified as glycoproteins coded for by endogenous murine leukemia viruses (*7*). Therefore, with human tumor cell lines, which are not known to contain endogenous retroviruses, the "cellular" antigens acquired by VSV may, by analogy, be indicative of the expression of retrovirus-like glycoproteins.

Vesicular stomatitis virus grown in HeLa cells were precipitated, but not neutralized, by antiserums to HeLa cell determinants. Isolation of these determinants by immunoprecipitation and polyacrylamide gel electrophoresis showed two or three specific polypeptides with molecular weights between 75,000 and 100,000 (*5, 6*). Because the HeLa cells used in these experiments do not shed retrovirus-like virions (*8*), antiserum to a virus-specific glycoprotein of HeLa cells is not available. Therefore, the identity of the HeLa cell determinants acquired by VSV can be checked only with antiserum that differentiates a tumorigenic in-

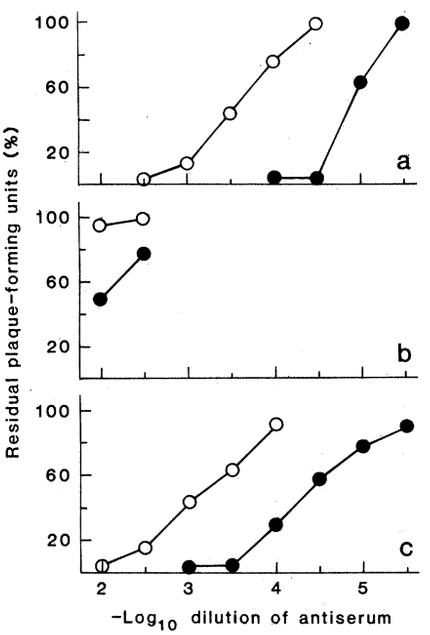

Fig. 1. Immunoprecipitation of VSV made in HeLa (●) or in HMB2 melanoma cells (○) with antibodies to HeLa cell surface antigens and *S. aureus* cells. Details concerning viruses, cells, and antiserums have been reported (*5, 6, 9*). The antiserums used were (a) sheep antiserum to VSV (tsO45) made in HeLa cells absorbed with VSV-infected Chinese hamster ovary cells, (b) rabbit antiserum to tumorigenic HeLa–human diploid cell hybrids absorbed with corresponding nontumorigenic hybrids, and (c) rabbit antiserum to HeLa cells unabsorbed. One hundred plaque-forming units of VSV in 50 μl were mixed with 50 μl of diluted antiserum, incubated for 30 minutes at room temperature, and then reacted with 1 ml of 0.2 percent *S. aureus*. The mixtures were incubated for an additional 60 minutes at room temperature, then centrifuged for 7 minutes in a microcentrifuge (Eppendorf). Supernatants were plaque-assayed on Chinese hamster ovary cells for residual VSV infectivity.

traspecies hybrid of a HeLa cell and a diploid cell from its nontumorigenic counterpart (*9, 10*). The rationale behind this assay, again by analogy to some of the animal systems, is the possibility of an association between the expression of retrovirus *env* gene products and tumorigenicity. Moreover, growth of VSV in several other human tumor cell lines indicates that VSV acquires specific determinants that do not readily undergo serologic competition from extracts of normal cells (*5*).

In the initial assay, three different antiserums were compared for their ability to precipitate VSV grown in HeLa cells. These antiserums were (i) sheep antiserum to VSV (tsO45) grown at nonpermissive temperature in HeLa cells and absorbed with wild-type VSV-infected Chinese hamster ovary cells (*5*), (ii) rabbit antiserum to tumorigenic HeLa cell–diploid cell hybrids absorbed with nontumorigenic HeLa cell–diploid cell hybrids (*9*), and (iii) rabbit antiserum to HeLa cells (*6*). *Staphylococcus aureus* (Cowan strain I) was added to detect immunoprecipitation, and the immune complexes were precipitated by centrifugation, followed by a determination of the infectivity of residual virus in the supernatant (*11*).

All three serums specifically precipitated whole VSV grown in HeLa cells, but did not react to the same degree with VSV grown in human melanoma cells (Fig. 1). The latter served as a control for nonspecific human antigens (*5*). The antiserum to hybrid cells (*9*) was much less reactive than the other two serums, but the relative specificity for VSV grown in HeLa cells was apparent (Fig. 1b).

To determine which polypeptides acquired by VSV from HeLa cells were detected by these three antiserums, highly purified, radioiodinated VSV grown in HeLa cells was first precipitated with antiserum to VSV and *S. aureus*; the pellet was then washed and solubilized in detergent (*6*). After the bacterial complexes were removed by centrifugation the supernatant was mixed with each of the three HeLa cell–specific antiserums, and the precipitated antigens were examined by gel electrophoresis (Fig. 2). As markers a preparation of VSV labeled in its structural proteins G, N, NS, and M was subjected to electrophoresis. Because of the different strengths of the antiserums used, only qualitative comparisons could be made. Three polypeptides, migrating slower than the VSV glycoprotein G, were immunoprecipitated. The two faster migrating bands were precipitated by both the sheep antiserum to VSV and the antiserum to HeLa cells (lanes a and c in Fig. 2, respectively), whereas the tumor-specific antiserum (lane b in Fig. 2) precipitated the two slower migrating bands. The antiserum to HeLa cells precipitated all three bands.

Thus, one band was in common to all three antiserums. The relationship between the three polypeptides can only be surmised at this time. Preliminary evidence suggests that the two slower migrating bands may be related to each other as precursor to product (*12*). The major tumor-specific antigen of HeLa cells is glycosylated and phosphorylated and is also at the cell surface (*9*); similar determinations on the antigens acquired by VSV will be necessary. The fastest migrating band may represent a human-specific antigen that is found in small amounts on diploid cells but is amplified in rapidly growing human tumor cells. Current efforts are aimed at establishing monoclonal antibodies that would further establish the identity and determine the relationship, if any, between the precipitated HeLa cell determinants.

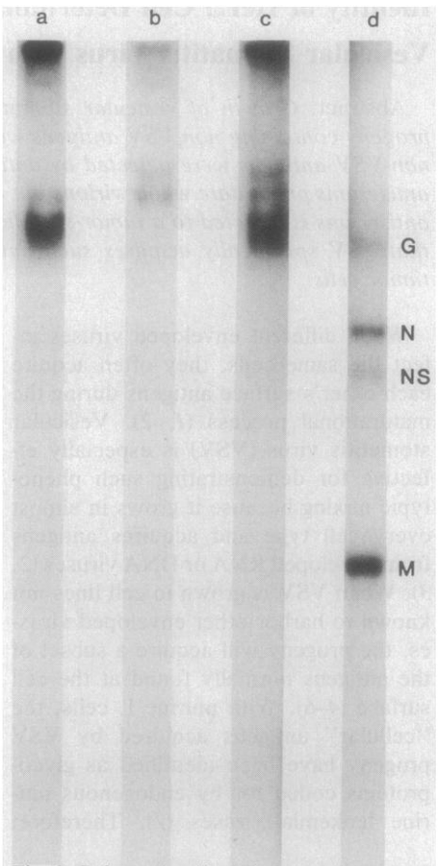

Fig. 2. Polyacrylamide gel electrophoresis of cellular determinants found on VSV grown in HeLa cells. Purified VSV grown in HeLa cells was labeled with radioactive iodine as described (*6*). A sample of VSV containing approximately 400,000 count/min was purified further by immunoprecipitation with antiserum to VSV and *S. aureus*. The sedimented virions were disrupted with RIPA buffer (1 percent Triton X-100, 1 percent deoxycholate, 0.1 percent sodium dodecyl sulfate, 0.15*M* NaCl, and 0.05*M* tris, *p*H 7.2) to release virus-associated proteins. Portions of this supernatant were incubated with *S. aureus* and the following three serums: (lane a) sheep antiserum to VSV (tsO45) grown in HeLa cells and absorbed with VSV-infected Chinese hamster ovary cells, at a dilution of 1:5; (lane b) rabbit antiserum to tumorigenic HeLa–diploid cell hybrids absorbed with corresponding nontumorigenic hybrids, used undiluted; and (lane c) rabbit antiserum to HeLa cells, unabsorbed, at a dilution of 1:10. The bacterial pellets were then subjected to electrophoresis as described (*6*). Marker VSV grown in baby hamster kidney cells and labeled with radioactive iodine is shown in lane d.

Despite our lack of knowledge concerning the mechanism by which VSV acquires such specific proteins during its budding process, VSV provides a powerful biological tool for concentrating and highlighting human cell surface antigens that appear to be not just tumor-associated but tumor-specific. Immunoprecipitation of such VSV grown through tumor cells and the sensitivity of the plaque assay on residual virus provide a rapid assay for selecting monoclonal antibodies against potential tumor-specific cell determinants. Also, such sensitive plaque assays and the monoclonal antibodies may provide a rapid screening procedure for diagnosing the presence of tumor antigens or host antibodies to these antigens. This in turn may lead to an understanding of the role such antigens may have in neoplasia.

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Adrenocorticotropic Hormone Causes Long-Lasting Potentiation of Transmitter Release from Frog Motor Nerve Terminals

Abstract. *Exposure of frog neuromuscular preparations to adrenocorticotropic hormone for several minutes increased both nerve-evoked and spontaneous transmitter release for several hours. No changes in postsynaptic sensitivity to transmitter were detected. The long-lasting potentiation shows little sensitivity to changes in extracellular calcium concentration and seems to be entirely presynaptic in origin.*

Recent studies suggest that adrenocorticotropic hormone (ACTH) has a broad distribution in the nervous system (*1*) and exerts a wide spectrum of physiological, behavioral, and biochemical actions (*2*), in addition to its well-known function in stimulating the adrenal cortex. In this report we show that ACTH and some closely related peptides produce a long-term increase in amplitudes of end-plate potentials (EPP's) and frequency of miniature end-plate potentials (mEPP's) in frog cutaneous pectoris and sartorius neuromuscular preparations. These effects appear to be entirely presynaptic in origin.

Purified porcine ACTH(1-39) (Sigma), synthetic melanocyte-stimulating hormone (α-MSH) (Sigma), synthetic ACTH(4-10) (Peninsula), and ACTH(1-24) (Organon) at concentrations of 0.2 to 2 m*M* in water were kept frozen until ready for use, then diluted into saline at 0.4 to 10 μ*M*. The normal Ringer solution contained 116 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, and 5 m*M* D-glucose, buffered at *p*H 7.2 with 5 m*M* Hepes. Nerve-evoked contractions were blocked by adding *d*-tubocurarine chloride (1 to 5 μg/ml) (Sigma) or by lowering $CaCl_2$ and adding $MgCl_2$, adjusting NaCl to maintain isosmolarity; $[Na^+]$ varied less than 5 percent, which was too little to affect release appreciably (*3*). Preparations were constantly superfused with Ringer solution at temperatures between 12° and 20°C (kept to within 1°C throughout each experiment). Conventional nerve stimulation and microelectrode techniques were used to study one or two muscle fibers at a time. The EPP's and mEPP's were recorded on tape and chart recorders and analyzed with the aid of a microcomputer. Tension was measured with a force transducer and preamplifier (Grass).

During and after a 15- to 30-minute exposure to ACTH, the amplitudes of the synaptic potentials increased slowly to a level sometimes exceeding twice the control value (Fig. 1A). Figure 1B shows representative EPP's from an experiment on a sartorius nerve-muscle preparation. This increase in amplitude was generally sustained: the preparation neither recovered from the effects of ACTH nor responded to a second trial of hormone for as long as 4 hours after the first exposure. The increase in amplitude ranged from 16 to 106 percent, depending on hormone concentration (Fig. 2), in 17 cutaneous pectoris muscle fibers from 12 frogs. Sartorius preparations varied more: in three of seven fibers we saw little or no increase in amplitude in response to ACTH (*4*).

The augmentation of synaptic potentials was observed also with α-MSH and ACTH(1-24), peptides closely related to ACTH, although slightly higher concentrations were necessary than with ACTH(1-39) (Fig. 2). ACTH(4-10) produced no discernible response.

The EPP amplitudes were analyzed for quantal content by the methods of failures and of coefficient of variation (*5*).

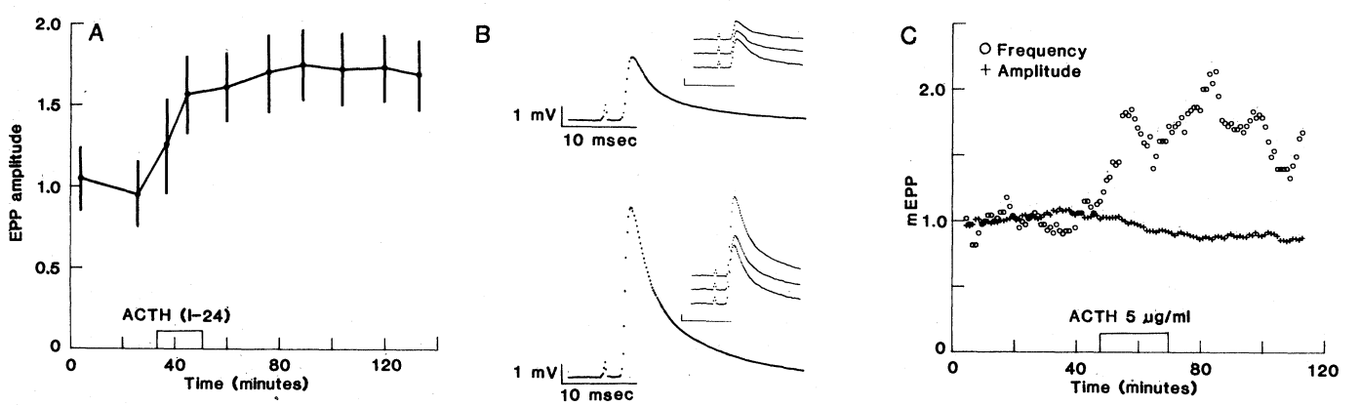

Fig. 1. (A) Effect of ACTH on EPP amplitudes. After a 30-minute control period, ACTH(1-24) was applied to a cutaneous pectoris preparation at a concentration of 1.3 μ*M* for 18 minutes ($[Ca^{2+}]$ = 0.9 m*M*, $[Mg^{2+}]$ = 6 m*M*). Each point is the average of 100 EPP's evoked by nerve stimulation at 0.33 Hz. Error bars are standard deviations; the standard error of the mean was less than 3 percent (about the size of the data marks). The ordinate is EPP amplitude normalized to the average during the control period. The ACTH-induced increase in this fiber was 69 percent and was stable until the penetration was lost. (B) Average of two groups of 100 EPP's recorded from a sartorius muscle fiber before (top) and 200 seconds after (bottom) 3.9 μ*M* ACTH(1-39) was added to the bathing medium (0.5 m*M* $[Ca^{2+}]$, 2.5 m*M* $[Mg^{2+}]$). Insets show individual EPP's from the same period. All calibration bars are 1 mV and 10 msec. (C) Effect of ACTH on mEPP frequency and amplitude. After a 45-minute control period, ACTH(1-39) was added for 20 minutes at a concentration of 1.3 μ*M* (5 μg/ml) ($[Ca^{2+}]$ = 1.8 m*M*, $[Mg^{2+}]$ = 0 m*M*). Values were normalized to averages obtained during the control period. Frequencies and amplitudes were calculated by counting and measuring the mEPP's in 1-minute time periods. Because of the very low mEPP frequency (0.1 to 0.2 Hz), frequency and amplitude data were subsequently smoothed with a moving average (bin width, 10 minutes; Δt = 1 minute).