the subsequent appearance of most primary tumors in the grafts depended on the presence of lymphoid cells from UV-irradiated mice in the recipient animals.

In the second approach, C3H/HeN(MTV$^-$) mice were given intravenous injections of 5×10^7 T lymphocytes that had been partially purified by separation on nylon wool columns (*4*). We had shown earlier that this procedure enriches the proportion of T lymphocytes in the population to about 85 percent and enhances the suppressive activity of the preparation (*3*). The T lymphocytes were obtained from normal or UV-irradiated C3H/HeN(MTV$^-$) mice and were injected at weeks 0, 3, 6, and 9. From weeks 0 to 12, these animals and mice that had not received injections were exposed to UV radiation for 60 minutes three times per week. The rate of primary skin tumor development in these three groups is illustrated in Fig. 2. Mice that received T lymphocytes from UV-irradiated donors developed more tumors than uninjected mice or mice given normal T lymphocytes; all tumors produced in this experiment were fibrosarcomas. Furthermore, skin cancers began to appear at around week 20 in the mice injected with the cell population containing suppressor T lymphocytes induced by UV radiation, but only after week 40 in the other two treatment groups. This result implies that nascent transformed cells are eliminated or held in abeyance by immunological means during the long latent period of UV carcinogenesis and that these transformed cells can develop into visible neoplasms only after the accumulation of an effective number of suppressor T lymphocytes.

Thomas and Burnet (*5*) proposed that the immune system may function as a defense mechanism against newly arising transformed cells. Support for this theory, which is called the theory of immunological surveillance, has been provided by studies of tumor systems involving oncogenic viruses (*6*), but its applicability to nonviral carcinogenesis remains controversial. Our demonstration that the latent period for photocarcinogenesis can be reduced by adding to the hosts T lymphocytes that specifically suppress the immune response against tumors induced by UV radiation suggests that immunological surveillance occurs during ultraviolet carcinogenesis and is partly responsible for the long latent period that accompanies the induction of these skin cancers. Immunological surveillance is important in this particular tumor system because these skin cancers are extremely antigenic (*7*) and thus are capable of being recognized and eliminated by the immune system. The fact that the skin is a particularly reactive tissue with special immunological capabilities (*8*) may contribute to the successful functioning of immunological surveillance in skin carcinogenesis.

We conclude that the suppressor T lymphocytes induced in mice by UV radiation not only inhibit the rejection of tumor transplants (*2*), but also play a decisive role in carcinogenesis. The demonstration that suppressor lymphocytes can affect the development of primary tumors in situ illustrates the importance of immunological regulatory mechanisms in the control of cancer growth in the primary host.

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Biplexiform Cells: Ganglion Cells of the Primate Retina That Contact Photoreceptors

Abstract. *Golgi-impregnated biplexiform cells of the macaque retina are neurons with cell bodies in the ganglion cell layer, an axon in the nerve fiber layer, and dendrites in the inner plexiform layer that are postsynaptic to amacrine cell processes and bipolar cell axon terminals. In these features they resemble conventional ganglion cells, but they also have processes that arise from the main dendritic arborization, extend to the outer plexiform layer, and are postsynaptic to rod photoreceptor terminals as central elements at the ribbon synaptic complex. However, ordinary retinal ganglion cell dendrites ramify in the inner plexiform layer and do not contact photoreceptors. Thus, biplexiform cells represent a previously undescribed class of neuron, part of whose synaptic input could bypass the commonly described interneuron circuitry of the vertebrate retina.*

The vertebrate retina is generally considered to consist of five major classes of neuron, each of which may contain many different structural and functional types (*1–3*). Light is transduced by photoreceptors to electrical signals. These signals are transmitted by bipolar cell interneurons to ganglion cells, whose axons convey visual information to the brain. Horizontal cells and amacrine cells (*4*) interact in this chain at the levels of the photoreceptor-bipolar cell synapse (outer plexiform layer, OPL) and the bipolar-ganglion cell synapse (inner plexiform layer, IPL), respectively. Ganglion cells have never been observed to contact photoreceptors directly. Here, I report a new class of retinal neuron found in Golgi preparations of the rhesus monkey retina which has the characteristic features of a ganglion cell, but which also directly contacts photoreceptors. These newly identified neurons are termed biplexiform cells since their processes arborize and are postsynaptic in both the OPL and IPL.

Retinas of rhesus monkeys (*Macaca mulatta*) were Golgi-impregnated and prepared as whole flat-mounts (*5*, *6*). Eight of 14 retinas contained Golgi-impregnated biplexiform cells; the most found in any one retina was six and the total number was 19. Biplexiform cells, as viewed in flat preparations (Fig. 1) and radial sections (Fig. 2A), have relatively small 9-μm round, smooth cell bodies which are located in the ganglion cell layer. Three or four main dendritic processes, 1.5 to 2 μm in diameter, arise from the cell body and subsequently give rise to secondary and tertiary branches forming, overall, a loose wavy, radiate dendritic pattern, about 100 μm in span, confined to the inner one-half of the IPL. Four axons of somatic origin were impregnated up to 10 μm, whereas three

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axons of dendritic origin were impregnated up to 300 μm (Fig. 1) and were 1 μm or less in diameter except for irregularly spaced, slight, elliptical varicosities. Fine, 0.5-μm processes arise from the main dendritic arborization, ascend radially, sometimes branching, through the IPL and inner nuclear layer (Fig. 2A) to the OPL where they terminate as small knoblike swellings, or follow a long, 100-μm, tortuous, horizontal course, bearing occasional short terminals (Fig. 1). These terminals appear to be randomly distributed, without the tight clustering characteristic of horizontal and bipolar cell dendritic terminals, and separated by 10 to 100 μm.

Electron microscopy of Golgi-impregnated biplexiform cells (*7*) shows that in the IPL their dendrites are postsynaptic to amacrine cells at conventional synapses and are in contact with large bipolar cell axon terminals identified as those of rod bipolar cells, in the innermost stratum of the IPL. These bipolar cell contacts, examined in serial sections, resemble conventional (nonribbon) synapses even though the other synapses made by the rod bipolar cell terminals onto unimpregnated processes were of the ribbon type (*8*). No synapses onto the biplexiform cell processes in the outer one-half of the IPL on their way to the OPL were seen. In the OPL, the biplexiform cell processes entered rod synaptic spherules and terminated as central elements of the triad at the ribbon synaptic complex (Fig. 2, B and C), similar to the endings of rod bipolar cell dendrites. As central elements at the ribbon synaptic complex, the biplexiform cell terminations lie opposite presynaptic structures in the rod spherule, the synaptic ribbon, and synaptic vesicles, and are presumed to be postsynaptic. No contacts with cones were seen. The one cell that was most extensively sectioned (480 sections) and studied made contacts with nine different rod photoreceptor terminals. Eight of these contacts were formed by the terminal swellings which projected on short stalks from the arborizations in the OPL. One contact was formed by a varicosity similar to that in Fig. 1 (open arrow) located along one of the processes running parallel to the receptor bases in the OPL. Since the area spanned by the biplexiform cell processes in the OPL is much greater than that which was actually sampled by electron microscopy, a more reasonable estimate of the number of contacts with rod spherules can be made by counting the number of terminals and varicosities by light microscopy, with the knowledge that these structures form central elements at rod triads. The average number of such terminals per biplexiform cell (N = 15 cells) was 25, with a range of 10 to 36. The density of biplexiform cells per unit area was estimated from a preparation in which three stained cells were separated about 100 μm. If this represents an upper limit on intercellular distance, there are about 100 biplexiform cells per square millimeter of retinal surface at this location (5 mm from foveal center). Although no cells have been found closer than 4 mm from the center of fovea, this does not exclude biplexiform cells from central retina, since they contact rods, and rods are found on the foveal slope (*2*).

Thus, based on both their morphology and synaptic connections, biplexiform cells are a unique class of retinal neuron. Some features of this neuronal class are shared with other main classes of retinal cells, and a comparison of these features can provide some clues as to their function. The most obvious comparison to be made is with ganglion cells. Like ganglion cells, biplexiform cells have cell bodies in the ganglion cell layer, postsynaptic dendrites in the IPL, and an axon in the nerve fiber layer. The major difference is that biplexiform cells have, in addition to the other features, processes which ascend to the OPL and contact rods. Another difference, which perhaps is minor, from ordinary ganglion cells lies in the synaptic contacts with bipolar cells. The biplexiform cells are postsynaptic at nonribbon synapses and, unlike other ganglion cells studied in the mammalian retina, are postsynaptic to rod bipolar cell axon terminals. This latter synaptic arrangement is a feature of the rod-amacrine system in cat retina where only amacrine cells have been found postsynaptic to rod bipolar cells (*9*).

Since biplexiform cells are involved in the rod system of the retina in at least

Fig. 1. Camera lucida drawing of a Golgi-impregnated (Colonnier-rapid) biplexiform cell in a whole flat preparation of the rhesus monkey retina. Dendrites originate from the cell body and a long axon arises from a dendrite. Processes arising from the main dendritic arborization ascend through the inner nuclear layer (indicated by broken lines) where they terminate (arrowheads) or follow a long course with few branches before terminating as small knoblike swellings (solid arrows). Varicosities are often located along the axon in the nerve fiber layer and are sometimes seen on the processes which ramify in the outer plexiform layer (open arrow).

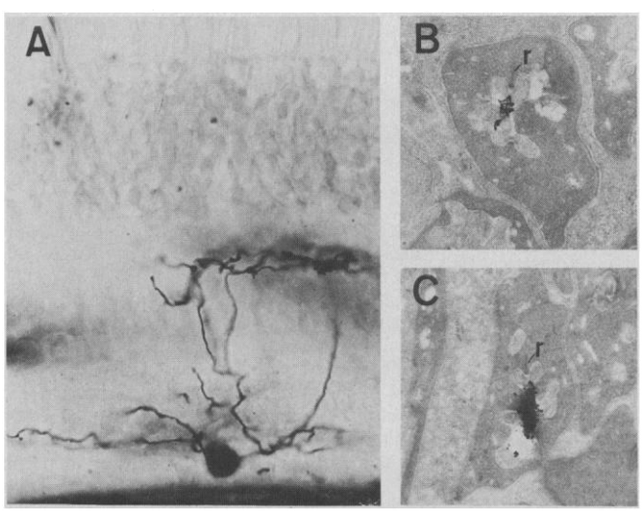

Fig. 2. Light and electron micrographs of Golgi-impregnated biplexiform cells of rhesus monkey retina. (A) A radial section of a Golgi-impregnated biplexiform cell, with a cell body in the ganglion cell layer, dendrites confined in the inner one-half of the inner plexiform layer, and processes which arise from the main dendritic arborization and ascend through the inner plexiform and inner nuclear layers to the outer plexiform layer (×637). (B and C) Electron micrograph of Golgi-impregnated biplexiform cell terminals in the outer plexiform layer. The impregnated, electron-opaque processes lie opposite synaptic ribbons (*r*) in the rod synaptic endings (×9450).

two different points, the rods themselves and the rod bipolars, and possibly a third (amacrine synapses), it seems likely that they engage in a temporal sequencing of the rod signals, and furthermore could transmit scotopic signals to the brain more rapidly than ordinary types of retinal ganglion cells since they could possibly bypass the usual interneuron circuitry of the retina (*10*).

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5. Retinas of adult rhesus monkeys were isolated from the choroid and pigment epithelium in oxygenated Eagle's tissue culture medium, placed on wax sheets, and covered with a fixative of 2.5 percent glutaraldehyde in sodium cacodylate for 30 minutes to 1 hour. The fixative was replaced with an aqueous solution of 5 percent glutaraldehyde and 4 percent $K_2Cr_2O_7$ [M. Colonnier, *J. Anat.* **98**, 327 (1964)] or 0.2 percent OsO_4 and 2.4 percent $K_2Cr_2O_7$ [F. Valverde, in *Contemporary Research Methods in Neuroanatomy*, W. J. Nauta and S. O. F. Ebbesson, Eds. (Springer-Verlag, New York, 1970), pp. 12–31] or a mixture of both of these solutions. The retinas were placed between sheets of Whatman No. 50 filter paper, covered by Whatman No. 4 filter paper, sandwiched between glass microscope slides, and lightly bound with rubber bands. This package was immersed in the dichromate solution for 2 to 3 days, then placed in 1 percent $AgNo_3$ for 2 to 3 days, and subsequently processed by routine techniques.
6. After biplexiform cells were found and studied in rhesus retinas, a number of them were also impregnated in another macaque species, *M. fascicularis*; biplexiform cells identical in size and form to those of *M. mulatta* were found in this other species.
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8. Bipolar cell axon terminals in the IPL usually contain a presynaptic ribbon and there are two postsynaptic elements. This arrangement is termed a dyad [J. E. Dowling and B. B. Boycott, *Proc. R. Soc. London Ser. B* **166**, 80 (1966)], but R. A. Allen [in *The Retina: Morphology, Function and Clinical Characteristics*, B. R. Straatsma, M. O. Hall, R. A. Allen, F. Crescitelli, Eds. (Univ. of California Press, Los Angeles, 1969), pp. 101–143] and M. T. T. Wong-Riley [*J. Neurocytol.* **3**, 1 (1974)] report that bipolar cell axon terminals also form nonribbon synapses.
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Isolation of Human Oncogene Sequences (v-*fes* Homolog) from a Cosmid Library

Abstract. *To define the human homolog (or homologs) of transforming sequences (v-*fes *gene) common to Gardner (GA) and Snyder Theilen (ST) isolates of feline sarcoma virus (FeSV), a representative library of human lung carcinoma DNA in a cosmid vector system was constructed. Three cosmid clones were isolated containing GA/ST FeSV v-*fes *homologous cellular sequences, within 32- to 42-kilobase cellular inserts representing 56 kilobases of contiguous human cellular DNA. Sequences both homologous to, and colinear with, GA or ST FeSV v-*fes *are distributed discontinuously over a region of up to 9.5 kilobases and contain a minimum of three regions of nonhomology representing probable introns. A thymidine kinase selection system was used to show that, upon transfection to RAT-2 cells, the human c-*fes *sequence lacked detectable transforming activity.*

That RNA transforming viruses contain acquired cellular genes accounting for their capacity to transform cells in culture and induce tumors of various histological classes in vivo is well established (*1*). Although such cellular derived "oncogenes" have been described only in animal model systems (*1*), their existence makes possible the isolation of related human genomic sequences. Extensive noncoding sequences within the cellular homologs of many such viral transforming genes (*2, 3*) represent a major difficulty for their cloning in conventional phage and plasmid systems. Because of this problem and the desirability of obtaining such genes with sufficiently extensive flanking sequences for studies of cellular regulatory controls influencing their expression, we used a cosmid cloning vector (*4–6*) in our study. This system involves the use of plasmids containing lambda cos sequences (cohesive ends) permitting insertion of large DNA fragments, in vitro packaging, and transduction to *Escherichia coli*.

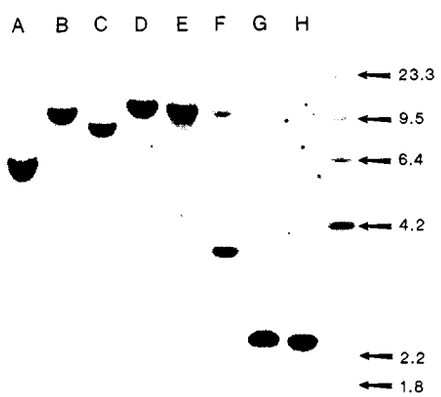

Fig. 1. Restriction patterns of cellular homologs of transformation-specfic DNA sequences common to GA and ST FeSV. (A) High molecular weight DNA's were prepared from kitten lung embryo cells; (B) CCL64 mink lung cells; (C) NIH/3T3 mouse embryo cells; (D and G) normal human lung tissue; and (E, F, and H) human lung carcinoma tissue. DNA samples (20 μg) were digested with Eco RI (A to E), Bam HI (F), or Kpn I (G and H), separated by electrophoresis on 0.75 percent agarose gels, transferred to nitrocellulose, and analyzed by hybridization according to previously described procedures (*19*). ^{32}P-Labeled DNA digested with Hind III is included as a molecular weight standard.

To define the cellular homolog of the GA/ST transforming (v-*fes*) gene, we used a molecular probe corresponding to a 0.5-kb Pst I restriction fragment within the ST FeSV (feline sarcoma virus) acquired sequence (v-*fes* S_L) (*2, 7*). By Southern blot analysis (*8*), single bands of hybridization were observed at molecular weights of between 7.0 and 12.5 kb in cat, mink, mouse, and human Eco RI restricted DNA's (Fig. 1, A to E). Restriction of DNA's from both normal human lung and human lung carcinoma with Bam HI or Kpn I resulted in generation of single bands hybridizing at 3.8 kb (Fig. 1F) and 2.4 kb (Fig. 1, G and H), respectively.

A cosmid library was constructed and screened for v-*fes* homologous sequences (Fig. 2). Three clones were initially selected on the basis of hybridization to v-*fes* S_L and were propagated for restriction endonuclease analysis. Two of the clones contained a 12.0-kb Eco RI restriction fragment with homology to both v-*fes* S_L and v-*fes* S_R while only the 9.8-kb 5′ region of this fragment was represented in the third clone. Further restriction enzyme analysis indicated that sequences within these clones were overlapping and represented a 58-kb contiguous region of the human genome. The orientation and positioning of the cellular inserts within these clones, both relative to each other and to the cosmid vector, are summarized in Fig. 3. For purposes of fine structure mapping, the above described 12.0-kb Eco RI v-*fes* homologous restriction fragment was subcloned in plasmid pBR328.

As a prerequisite to further analysis of the above described clones it was first necessary to isolate and prepare molecular probes corresponding to the complete

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