same mobility in 3 percent, 7*M* urea polyacrylamide gels (Fig. 2C). Finally, two-dimensional oligonucleotide analysis showed that no detectable rearrangement of the viroid primary structure accompanied in vitro ligation (Fig. 2, D and E).

Our data show that an RNA ligase activity from wheat germ circularizes viroid RNA to the same extent as it ligates tRNA half-molecules. The wheat germ RNA ligase used in our experiments may well function in vivo in RNA splicing reactions, since it is capable of replacing the yeast tRNA splicing ligase in an in vitro reaction. By several criteria, the viroid circles produced in vitro could not be distinguished from natural progeny viroid RNA circles purified from infected plants.

Several features of the viroid life cycle have been described in detail. Host RNA metabolic enzymes appear to be required throughout. The mechanism of viroid replication appears to involve complementary RNA strands (*27–32*) without DNA intermediates (*33, 34*). Minus strand RNA, opposite in polarity to that of the mature viroid, arises in infected cells, with prevalent size classes on fully denaturing gels which are between two and five times the length of mature viroid RNA (*27*). Branch *et al.* have proposed a rolling circle mechanism to account for these greater than unit length minus strands (*27*).

In addition to multimeric minus strand replication intermediates, recent experiments have revealed several species of greater than unit length plus strands in PSTV-infected tomato plants (*19, 35, 36*). One straightforward way for these in vivo multimeric plus strands to participate in replication would be through cleavage by an endonuclease generating unit length linear RNA molecules with cyclic 2′,3′-phosphate termini. The viroid circularization studied here indicates one mechanism through which such cleavage products could have their ends joined and suggests an approach for determining whether the linear molecules acted on by the wheat germ ligase have specific, nonrandom terminal sequences. Alternatively, the long plus strands may be capable of self-cleavage and ligation similar to that reported for tetrahymena ribosomal RNA species (*37*).

While the mechanism of viroid pathogenesis remains a mystery, it has been proposed that disruption of host cell RNA processing, and in particular of RNA ligation, is involved (*19, 38–41*). Future studies will determine whether PSTV strains differing in the severity of their disease symptoms also differ significantly in their interactions with plant RNA ligase activities.

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12 March 1982; revised 25 June 1982.

Induction of Melanized Cells from a Goldfish Erythrophoroma: Isolation of Pigment Translocation Variants

Abstract. *Melanization was induced in some cells of a goldfish tumor cell line (GEM-81) by cultivating the cells in autologous serum. The melanized cells continued to proliferate in vitro and several clones were isolated that differed with respect to cell morphology and intracellular distribution of pigment. Some of the clones consisted of cells able to translocate their melanosomes in response to epinephrine, melatonin, or adenosine 3′,5′-monophosphate.*

Although the pigment cells of lower vertebrates have been useful for studies of differentiation and organelle translocation (*1*), their scattered distribution in the skin has hampered biochemical studies. We report here the induction, isolation, and partial characterization of several melanogenic sublines, obtained from a goldfish erythrophoroma line (GEM-81) that may be useful for such studies.

Erythrophoromas arise from xanthophores (erythrophores), which are pigment cells containing pteridines and carotenoids. Despite the retention of small amounts of pteridines, the GEM-81 cells are essentially colorless and have been maintained for over 100 passages without yielding melanogenic sublines (*2*). Previously reported instances of melanization in these cells resulted in terminal differentiation, and pigment translocation was not observed (*3*). However, the melanogenic sublines reported here continue to grow and differentiate in vitro to stages exhibiting a variety of pigment translocation characteristics. These new

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lines should prove useful in biochemical studies of the hormone-regulated translocation of pigment organelles in normal chromatophores (*4*).

Melanization was induced in uncloned GEM-81 cells maintained in a standard tissue culture medium (*5*) in which fetal calf serum had been replaced with 10 to 20 percent serum from the gold carp *Cyprinus carpio* (collected at Bay Port Fishery, Bay Port, Michigan). After 4 days a number of melanized cells were observed scattered singly or in pairs among the colorless cells. After 2 to 3 weeks these melanized cells had grown into colonies visible to the naked eye (Fig. 1A). The frequency of formation of these dark colonies was one per 3534 ± 407 cells (mean ± standard deviation) in cultures plated at a density of 100 cells per square millimeter (*6*).

Electron microscopic examination of several colonies showed that the cells contained typical melanosomes but not the pterinosomes often seen in the parent erythrophoroma cells (Fig. 1B). The absence of pterinosomes and the low frequency of melanization suggest that these colonies arose from a minor population in the parent erythrophoroma line. Consistent with this suggestion are the failure of two of the GEM-81 subclones to melanize and the fact that melanized foci occur infrequently in erythrophoromas (*7*).

The clonal nature of these colonies was suggested by their distribution in the original cultures, the continued mitosis of melanized cells, the uniform morphologies of cells in single colonies, and morphological differences between the colonies. These characteristics appeared reasonably stable when individual colonies were subcultured or cloned (*8*). However, the phenotypic stability of the cells in long-term cultures remains to be demonstrated.

The melanized colonies could be distinguished morphologically by cell shape, state of pigment dispersion (or aggregation), and responses to epinephrine, melatonin, and adenosine 3′,5′-monophosphate (cyclic AMP). Individual colonies or clones were composed of flat and amorphous cells (Fig. 1C) or of fusiform to dendritic cells (Fig. 1D). A few colonies of dendritic cells contained dense, perinuclear aggregates of melanosomes when unstimulated by hormones or cyclic nucleotides (Fig. 1E). In all the other colonies the melanosomes were uniformly dispersed throughout the cells.

Most of the colonies consisting of dendritic cells with aggregated melanosomes responded to cyclic AMP by melanosome dispersion (Fig. 1F). When cyclic AMP was withdrawn the melanosomes reaggregated, a process that was greatly accelerated by epinephrine or melatonin (Fig. 1G). Since the translocation was indistinguishable from that seen in normal melanophores, these were designated melanophoroma cells (*9*). Among the colonies whose cells contained dispersed melanosomes, some reversibly aggregated their pigment in response to epinephrine or melatonin and were also termed melanophoromas. Others did not translocate their melanosomes in response to any of these agents and were designated melanocytomas (*9*).

In their response to autologous serum and such agents as 4β-phorbol-12-myristate-13-acetate and dimethyl sulfoxide, the parent GEM-81 line is one of a few tumor cell types that can undergo differentiation in vitro (*3*). The stable phenotype of the uncloned GEM-81 line under standard culture conditions and the induction by carp serum of melanocytoma and melanophoroma cells suggest that this line may contain minor populations of "stem" cells determined to differentiate by melanization. The basis for this difference in response and the nature of the inductive substance are unknown.

The melanized sublines described here are fundamentally different from other melanomas in that some develop the capacity for pigment organelle translocation. The only other melanized tumor cells able to translocate melanosomes are the melanomas that arise in swordtail/platyfish hybrids in vivo (*10*), but these cells do not proliferate in culture. The clones described here thus represent examples of tumor cells that retain the capacity for both hormone-regulated melanosome translocation and growth.

An interesting prospect is the use of these clones to study the role of the cytoskeleton in intracellular motility (*11*). The variants described here, along with a number of normal goldfish chromatophores (*4*), provide a family of closely related cells that differ in the resting distribution of pigment organelles and in the ability to respond to hormones and cyclic AMP by reversible pigment

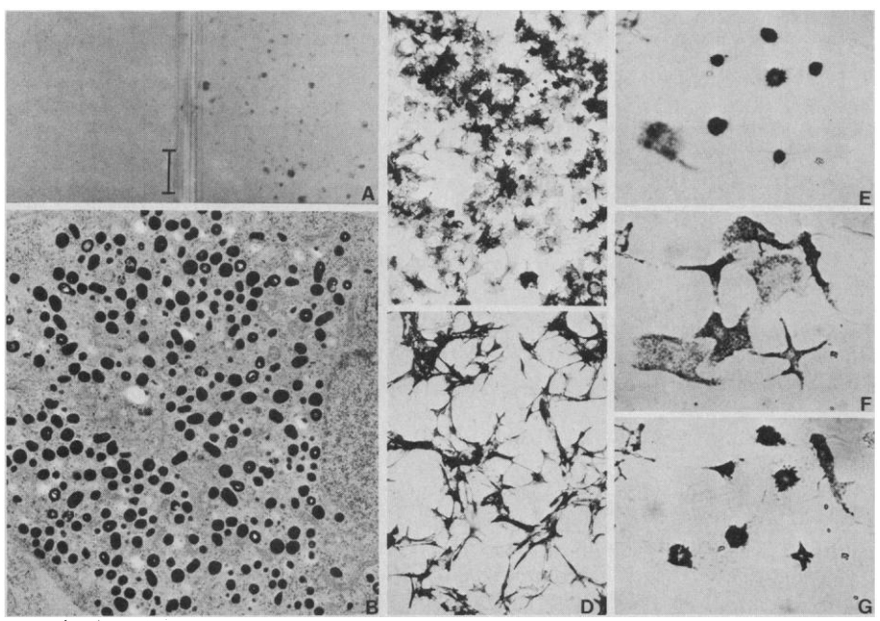

Fig. 1. (A) Two cultures of uncloned GEM-81 cells. The culture on the left was maintained in standard medium (*5*) for 6 weeks. The culture on the right was kept in medium supplemented with 20 percent carp serum and shows many melanized colonies. Mitosis was observed in the colonies; the generation time averaged 3 to 4 days but varied between colonies. Differences in mitotic rates and perhaps cell migration probably account for the variation in colony size. Scale bar, 2 cm. (B) Transmission electron micrograph of a GEM-81 cell induced by carp serum, containing typical melanosomes but no pterinosomes. The section is of a dendritic melanophoroma cell with aggregated melanosomes similar to those shown in (E) (×5200). (C and D) Cultures of melanized GEM-81 cells 6 weeks after subcultivation, prior to cloning (*8*). The melanized cells in each case were derived from a single colony and therefore display homogeneous amorphous (C) or dendritic (D) morphology. The melanosomes in both are constitutively dispersed (×60). (E) Cloned melanophoroma cells with constitutively aggregated melanosomes. Treatment with 1 m*M* cyclic AMP causes the melanosomes in most cells to disperse within 5 minutes (F). Exchanging the medium with 0.1 m*M* epinephrine causes reaggregation of the melanosomes within the same period (G). One cell responds to cyclic AMP but not to epinephrine; it is unclear whether such differences are due to intraclonal heterogeneity or temporal variations in sensitivity, as reported for melanocyte-stimulating hormone in other systems (*14*) (×165).

translocation. Hence organelle motility is controlled exogenously and the translocation-defective variants may be useful in characterizing the cellular components involved in this process (*12*). Preliminary studies have shown that the inability of erythrophoroma cells to carry out cyclic AMP–induced carotenoid droplet dispersion may be due to a defective protein kinase (*13*). Similar studies with the melanized variants should provide additional information.

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30 April 1982; revised 16 July 1982

Quantitative Autoradiographic Mapping of Herpes Simplex Virus Encephalitis with a Radiolabeled Antiviral Drug

Abstract. *2′-Fluoro-5-methyl-1-β-D-arabinosyluracil (FMAU) labeled with carbon-14 was used to image herpes simplex virus type 1–infected regions of rat brain by quantitative autoradiography. FMAU is a potent antiviral pyrimidine nucleoside which is selectively phosphorylated by virus-coded thymidine kinase. When the labeled FMAU was administered 6 hours before the rats were killed, the selective uptake and concentration of the drug and its metabolites by infected cells (defined by immunoperoxidase staining of viral antigens) allowed quantitative definition and mapping of HSV-1–infected structures in autoradiograms of brain sections. These results show that quantitative autoradiography can be used to characterize the local metabolism of antiviral drugs by infected cells in vivo. They also suggest that the selective uptake of drugs that exploit viral thymidine kinase for their antiviral effect can, by appropriate labeling, be used in conjunction with clinical neuroimaging techniques to define infected regions of human brain, thereby providing a new approach to the diagnosis of herpes encephalitis in man.*

Herpes simplex virus type 1 (HSV-1) causes severe sporadic encephalitis in man with high mortality and morbidity (*1*). The course of this illness can be altered by treatment with the antiviral drug vidarabine (*2*), and newer drugs with more selective antiviral action may further improve the therapeutic outcome (*3, 4*). However, a critical factor in successful management of herpes encephalitis is early diagnosis. Brain biopsy is at present the only definitive means of early diagnosis, leaving the clinician with a difficult decision that may result in delayed or inappropriate therapy (*5*).

In an effort to find a noninvasive, accurate diagnostic test for HSV-1 encephalitis, we have examined the possibility of exploiting the selective uptake by infected cells of an antiviral drug that serves as substrate for virus-coded thymidine kinase. By using radioactively labeled 2′-fluoro-5-methyl-1-β-D-arabinosyluracil (FMAU), a potent new antiviral agent (*6*), and quantitative autoradiography (*7*), we have now demonstrated the selective uptake of FMAU by HSV-1–infected brain regions in the rat. We predicted that, after penetration into the brain, labeled FMAU would be selectively phosphorylated by viral thymidine kinase expressed in infected cells, and that the metabolic products (nucleotides and perhaps DNA) "trapped" within these cells could be assessed quantitatively in autoradiograms of brain sections in a manner analogous to that of [2-^{14}C]deoxyglucose phosphate (*7*). We here present data showing that infected regions do indeed selectively sequester and concentrate labeled FMAU and that these regions can be mapped autoradiographically.

Focal encephalitis was produced in female CD rats (Charles River Laboratories; 150 to 200 g) by intraocular inoculation of the right eye with 2.6×10^6 plaque-forming units of the F-strain of HSV-1 suspended in a volume of 20 μl (*8*). The intraocular route was chosen because it avoids the need for direct intracerebral injection with attendant trauma and because a reproducible, anatomically stereotyped focal encephalitis ensues (*9*). In some experiments, the rats were immunosuppressed by intraperitoneal injection of cyclophosphamide (250 mg/kg) administered 1 day after viral challenge in order to produce more severe brain infection. The rats were killed on day 5 or 6 after inoculation of the virus. Radioactive FMAU (labeled with ^{14}C at the number 2 position of the pyrimidine ring) with a specific activity of 47.5 μCi/mg (12.3 nCi/nmole) was injected intravenously (5 mg/kg) (*10*). The [^{14}C]FMAU was injected into three rats 24 hours before they were killed, and into the remaining rats 6 hours before they were killed. Samples of arterial blood were obtained at frequent intervals and analyzed for ^{14}C activity by liquid scintillation counting. Arterial blood pressure was monitored continuously and arterial blood gases were sampled intermittently by way of femoral cannulae. Previous studies have shown that in the rat most FMAU is excreted unchanged in the urine and only a minor fraction undergoes metabolic alteration (*11*). The animals were killed by injection of a lethal dose of pentobarbital (42 mg). Their brains were removed rapidly, frozen in Freon (cooled to −40°C in dry ice)

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