may be transported in axons at rates up to centimeters per hour (*6*). Since many neurons in the mouse cerebrum have short axons, rapid transport of protein from the perikaryon to nerve endings could be accomplished easily at such rates (*7*). McEwen and Grafstein (*8*) showed that particulate protein is transported more rapidly to nerve endings than soluble protein. The different behavior of soluble and particulate protein implied by these and previous studies (*1–3, 7*) might be due to such differential rates of transport.

An alternative explanation of these findings is that there is substantial local synthesis of protein at nerve endings. Although the nerve ending is devoid of detectable ribosomes, there are abundant mitochondria, and evidence consistent with local synthesis of mitochondrial protein at nerve endings has been presented (*2, 3*). However, in the present study, grains overlying nerve endings were not usually associated with mitochondria. Therefore, the present findings cannot be explained in this way. Austin and Morgan (*9*) and Autillio *et al.* (*10*) have shown that nerve ending fractions can incorporate amino acids into protein in vitro. These studies are consistent with incorporation of amino acids into protein in nerve endings, but the possibility that the observed incorporation is attributable to contaminants has not been completely excluded. The potentiality for local protein synthesis at nerve endings is supported by radioautographic studies of in vitro incorporation of tritiated amino acids into protein of nerve endings of the ciliary ganglion of the chick (*11*), although extensive perikaryal synthesis of protein which migrated to these nerve endings was shown in vivo (*11*).

The present study does not unambiguously demonstrate local nonmitochondrial protein synthesis at nerve endings. However, it clearly shows that newly synthesized protein appears very rapidly at nerve endings of mouse brain. Therefore, new proteins may regulate nerve ending function within minutes of their synthesis.

BERNARD DROZ
Département de Biologie, Commissariat à l'Energie Atomique, 91 Saclay, France
SAMUEL H. BARONDES
Department of Psychiatry, Albert Einstein College of Medicine, Bronx, New York 10461

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Adenosine-3′,5′-Phosphate: Identification as Acrasin in a Species of Cellular Slime Mold

Abstract. *Acrasin, the chemotactic agent responsible for aggregation in the cellular slime molds, has been identified for one species,* Dictyostelium discoideum, *as adenosine-3′,5′-phosphate.*

The cellular slime mold *Dictyostelium discoideum* has been used by a number of investigators to study the processes of cellular differentiation and morphogenesis. As long as adequate food is present the amoebas of this organism feed and reproduce by binary fission. When starved, the individual cells stream into central collecting points to form multicellular masses which become migrating pseudoplasmodia. These pseudoplasmodia later differentiate into fruiting structures consisting of both stalk and spore cells. The aggregation phase of the life cycle is mediated by chemotaxis (*1*).

The amoebas are also attracted to bacteria (*2*), and we showed that at least one of the attractants in *Escherichia coli* is adenosine-3′,5′-phosphate (3′,5′-AMP) (*3*). Subsequently, we found that the related species of slime mold *Polysphondylium pallidum* secretes 3′,5′-AMP, but is not attracted to it (*4, 5*). Our first attempts to obtain sufficient acrasin from *D. discoideum* failed, presumably because of an acrasin-inactivating enzyme first discovered by Shaffer (*6*). Chang has isolated an extracellular enzyme capable of converting 3′,5′-AMP to 5′-AMP (*7*). This discovery suggested that if the activity of this enzyme could be curtailed, and if acrasin were a substrate for this enzyme, sufficient acrasin could be accumulated to effect a chemical identification. Two different approaches to this problem were initiated: the one reported here and another reported by Konijn *et al.* (*8*). Both approaches have led to the conclusion that 3′,5′-AMP is a naturally occurring acrasin of *D. discoideum*.

Amoebas of *D. discoideum* NC-4(H) were grown in liquid culture with the use of Hohl and Raper's (*9*) modification of Gerisch's (*10*) technique for growth on dead, autoclaved bacteria. When growth was completed, the cells were centrifuged at 2500*g* for 10 minutes and suspended in 1 percent Bonner's salt solution (*11*). These preparations could be treated in one of two ways.

In the first procedure, 40 to 50 ml (2×10^6 cell/ml) were poured onto a dialysis membrane placed over 1 percent salt solution and supported by a plastic frame (*12*). The cells settled and attached themselves to the membrane. After ½ hour, the excess liquid was siphoned off. Subsequently these cells aggregated normally, formed migrating

Table 1. Results of paper chromatography of acrasin. Chromatograms were developed for 16 hours at room temperature. Acrasin activity was determined on the Konijn bioassay after eluting 1-cm strips with water. Markers were detected with ultraviolet light. The solvents used were: A, 1*M* ammonium acetate and ethanol (3 : 7), ascending; B, isopropanol, ammonia, and water (7 : 1 : 2), ascending; and C, saturated ammonium sulfate, 1*N* sodium acetate, and isopropanol (40 : 9 : 1), descending.

Substance	R_F	Distance from origin (cm)
	Solvent A	
Acrasin	0.34 to 0.41	
3′,5′-AMP	.34 to .41	
5′-AMP	.07 to .10	
	Solvent B	
Acrasin	0.30 to 0.42	
3′,5′-AMP	.30 to .38	
5′-AMP	.08 to .11	
	Solvent C	
Acrasin		9 to 11
3′,5′-AMP		9 to 12
5′-AMP		22 to 27

pseudoplasmodia, and then fruited. The salt solution beneath the dialysis membrane accumulated quantities of stable acrasin [as measured by the Konijn bioassay (*13*)] because of the inability of the inactivating enzyme to pass through the membrane to reach it (*14*).

The other procedure consisted of adding 2 g of dry Dowex 1 chloride (Bio-Rad AG 1-X8, 200 to 400 mesh) to a 500-ml cell suspension (2×10^6 cell/ml). The *p*H of the mixture was adjusted to 7.8 with NaOH, and the mixture was placed on a magnetic stirrer for several hours. These preparations bound acrasin to the resin beads and made it less susceptible to attack by the enzyme. With the Konijn bioassay for acrasin, it was possible to demonstrate an accumulation of elutable acrasin on the resin with time (*14*). The resin was washed free of amoebas with distilled water, and then eluted with 10 ml of 0.1*N* HCl. The eluate was neutralized with NaOH and placed in a boiling water bath for 5 minutes to eliminate any remaining phosphodiesterase. The eluate was then treated with 100 mg of Norit A, which was washed three times with water, and the acrasin was eluted twice with 10 ml of a solution of ethanol, ammonia, and water (50 : 2 : 48). The final product was stable and was concentrated by vacuum evaporation at 60°C and stored at 4°C.

Both of these procedures accumulated amounts of biological activity equivalent to 10^{-10} mole of commercial 3′,5′-AMP (Calbiochem). Acrasin collected by both of these methods was identified as 3′,5′-AMP by the following criteria. Because acrasin adsorbs on Dowex 1 chloride, it must be negatively charged. It is of sufficiently low molecular weight to be dialyzable. It can withstand 20 to 30 minutes of boiling at neutral *p*H without appreciable loss of activity. It can be adsorbed onto Norit. Paper chromatography on three different solvent systems shows that acrasin behaves identically to 3′,5′-AMP (Table 1). Activity completely disappeared after incubation for 45 minutes at 37°C with a cyclic phosphodiesterase purified from bovine heart (*15*). This enzyme preparation appears to be specific for 3′,5′-cyclic nucleotides—no other nucleotide or nucleotide phosphodiesterase activity has ever been detected (*15*).

Even with the protection afforded by the procedures described, only very low quantities have been collected. For this reason, other methods of characterizing the molecule (such as its predicted ultraviolet absorption spectrum) have not been possible. Nevertheless, the data presented here, together with the fact that 3′,5′-cyclic nucleotides are the only nucleotides of a large number tested which show acrasin activity (*16*), strongly suggests that 3′,5′-AMP is an acrasin of *D. discoideum*.

It is clear from previous work that the amoebas of *D. discoideum* respond chemotactically to 3′,5′-AMP (*3*). We have shown that it is also secreted by this species. From this it is apparent that 3′,5′-AMP is a natural acrasin of *D. discoideum*. It must be added, however, that the possibility remains that other acrasins exist—we have studied only those extracellular molecules which are either dialyzable or negatively charged.

DAVID S. BARKLEY*
Department of Biology,
Princeton University,
Princeton, New Jersey

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* Present address: Department of Neuropathology, Harvard Medical School, Boston, Massachusetts.

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Tumor Induction in Developing Frog Kidneys by a Zonal Centrifuge Purified Fraction of the Frog Herpes-Type Virus

Abstract. *Injection of frog embryos* (Rana pipiens) *with a zonal centrifuge purified fraction of herpes-type virus (prepared from virus-containing Lucké tumors) resulted in a high incidence of kidney tumors. This partially purified oncogenic fraction contained a high concentration of an enveloped form of the frog herpes-type virus (adjacent fractions lacked this particle and were not oncogenic), which suggests that this form of the virus plays a role in the genesis of the Lucké tumor.*

The association of herpes-type viruses (HTV) with malignancies of a variety of vertebrates has recently attracted a great deal of attention. In man, interest has focused upon the EB virus found in tissue culture cells derived from patients with Burkitt lymphoma (*1*). Evidence has also been accumulating to indicate that a herpes-type virus is involved in the etiology of Marek's disease, a lymphoproliferative disease of chickens (*2*).

The EB virus can be cultured and harvested in amounts sufficient for the preparation of cell-free, relatively clean virus inoculum; but, although this virus has been tested for tumorigenicity in a variety of experimental animals, tumors have never been produced, and an adequate assay system is unavailable. On the other hand, an assay system is available for Marek's disease; tissue culture cells containing herpes-type virus have produced typical Marek's disease lesions when injected into newly hatched chicks. Nevertheless, attempts to produce Marek's disease with *cell-free* virus preparations have been unsuccessful. Thus, although suspect as oncogenic agents, herpesviruses have failed to produce tumors in either of these two systems when prepared as cell-free extracts; and some investigators have interpreted this inability as a reflection of the unstable nature of herpes virions.