less extreme temperature on reproductive success and larval viability. Data should be gathered for species from different habitats and should include pelagic species which may be involved to a greater extent in canal transit.

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Adenosine 3′,5′-Monophosphate–Dependent Protein Kinase of Cultured Mammalian Cells

Abstract. *Protein kinase was partially purified from Chang's liver cells, 3T6 mouse embryo fibroblasts, and HeLa cells. The rate of histone phosphorylation catalyzed by the kinase from each of these cell lines was stimulated two- to threefold by 1×10^{-6} molar adenosine 3′,5′-monophosphate. The same concentration of guanosine 3′,5′-monophosphate failed to stimulate these kinases.*

Hormone-sensitive adenyl cyclase has been demonstrated to be present in three cultured mammalian cell lines—Chang's liver cells, 3T6 mouse embryo fibroblasts, and HeLa cells (*1*). Since adenosine 3′,5′-monophosphate (cyclic AMP)–dependent protein kinase has been partially purified from a wide variety of mammalian tissues (*2*), it would be of interest to ascertain whether a cyclic AMP–dependent protein kinase is also present in the above hormone-sensitive, cultured cell lines. The data obtained in our study demonstrate the presence of a cyclic AMP–dependent protein kinase in Chang's liver cells, in 3T6 mouse fibroblasts, and in HeLa cells.

Chang's liver and HeLa cells were grown in suspension culture, and 3T6 fibroblasts were grown in stationary culture as reported (*1*). Cyclic AMP–dependent protein kinase was obtained from sonicated cells and fractionated through the ammonium sulfate step as described in the purification procedure of Kuo and Greengard (*2*). Unfractionated calf thymus histone (Schwarz/Mann) was used as substrate, and enzymatic activity was determined according to the method of Kuo and Greengard (*3*). Values for phosphorylation of histone were corrected by subtracting the small amount of radioactivity present at zero incubation time. Protein was determined by the method of Lowry *et al.* (*4*).

Each of the cultured cell lines examined contained a protein kinase that was stimulated two- to threefold by $1 \times 10^{-6}M$ cyclic AMP (Table 1). The protein kinase from 3T6 fibroblasts had the highest control activity and was stimulated to a greater extent by cyclic AMP than that from the other cell lines. Neither the HeLa cell enzyme nor that of 3T6 fibroblasts was stimulated by $1 \times 10^{-6}M$ guanosine 3′,5′-monophosphate (cyclic GMP).

Table 1. Protein kinase activity of cultured mammalian cells. Enzyme activity is expressed as picomoles of ^{32}P transferred per 100 μg of enzyme protein during a 5-minute incubation period. The numbers in parentheses are the number of separate enzymatic assays. The cyclic nucleotides, when present, were at a concentration of $1 \times 10^{-6}M$.

Cell line	Protein kinase activity		
	Control	+ Cyclic AMP	+ Cyclic GMP
HeLa	6.6 (4)	12.4 (4)	6.7 (3)
Chang's liver	17.2 (3)	40.0 (3)	
3T6 fibroblast	26.3 (3)	84.7 (3)	24.3 (2)

Kuo and Greengard (*2*) have shown that protein kinase from bovine liver is stimulated tenfold by cyclic AMP, whereas that from rat liver is stimulated only 2.5-fold. Since protein kinase has not been purified from normal human liver, it is not possible at present to determine whether the enzyme from the cultured human liver (Chang's) cells (*5*) has similar properties to that of whole human liver. In contrast to the enzyme from normal liver, adenyl cyclase from Chang's liver cells is markedly stimulated by epinephrine but is not influenced by glucagon (*1*).

Both the extent of stimulation of adenyl cyclase by catecholamines and the total adenyl cyclase activity measured in the presence of sodium fluoride are greater in 3T6 fibroblasts than in Chang's liver cells, with still lower values obtained for HeLa cells (*1*). Thus a parallel appears to exist between hormone-sensitive as well as total adenyl cyclase activity and the extent of protein kinase stimulation by cyclic AMP in these cultured mammalian cell lines.

The cyclic AMP–dependent protein kinase exists in an inactive form that is split into regulatory and catalytic subunits as a consequence of the binding of cyclic AMP to a regulatory site (*6*). The catalytic subunit is then fully active and therefore insensitive to further stimulation by cyclic AMP. The ratio of the rate of phosphorylation in the absence of cyclic AMP to that in its presence reflects the fraction of protein kinase originally in the active form. The data from HeLa cells, Chang's liver cells, and 3T6 fibroblasts suggest that approximately 50 percent of the protein kinase prepared from each one of these cell lines is in the active form.

Ours is the first report of a cyclic AMP–dependent protein kinase from cultured cells other than neuroblastoma (*7*). As was found in these studies, the kinase from neuroblastoma is also stimulated two- to threefold by cyclic AMP and is insensitive to cyclic GMP. Although neuroblastoma cells have adenyl cyclase activity, the basal activity is low and insensitive to hormonal stimulation (*8*).

Chang's liver cells, HeLa cells, and 3T6 mouse embryo fibroblasts each

contain both a cyclic AMP–dependent protein kinase and a hormone-sensitive adenyl cyclase. All the effects of cyclic AMP have been postulated to be mediated via stimulation of protein kinase (*9*). If this hypothesis is correct, then substances that increase the cyclic AMP levels should increase phosphorylation of endogenous proteins, provided that the protein kinase is not already maximally stimulated. Cyclic AMP–dependent phosphorylation of proteins has been shown to include not only histones but a number of other proteins, including phosphorylase kinase (*10*) and ribosomal protein (*11*). The presence of both adenyl cyclase and protein kinase activities in these established cultured cell lines may provide a useful system for studying the interactions of hormones, adenyl cyclase, and protein kinase as well as the regulatory role of cyclic AMP.

Note added in proof: After this manuscript was accepted, Perkins *et al.* (*12*) reported the presence of cyclic AMP–dependent protein kinase in cultured glial cells.

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Fatty Acid Ethyl Esters of Rhizopus arrhizus

Abstract. *Gas chromatographic and mass spectrometric analyses on selected lipid fractions revealed for the first time the presence of ethyl esters of long-chain fatty acids as biological products. Ethyl esters of oleic, palmitic, and stearic acids were detected in relative concentrations of 21.2, 2.4, and 1.5 percent, respectively, of the total methyl and ethyl ester fraction. Both saturated and unsaturated ethyl esters contain pronounced mass spectral fragments at a mass-to-charge ratio of 88.*

The presence of naturally occurring esters of short- and long-chain fatty acids has been documented for several plant and animal species. The complex epicuticular waxy coating of higher plant surfaces (*1*, *2*), insects (*3*, *4*), oils of certain aquatic animals (*5*), as well as the mandibular canal of the porpoise (*6*) are reported to contain waxy esters. Recently, methyl esters of long-chain fatty acids have been reported for corn pollen (*7*), chlamydospores of *Ustilago maydis* (*8*), and the sporangiospores and mycelia of *Rhizopus arrhizus* (*9*). The data in this report demonstrate for the first time the presence of novel esters of certain long-chain fatty acids containing ethyl groups.

Mycelial fragments (suspended in 0.5 ml of sterile distilled water) were inoculated onto Fothergill and Yeoman's solid medium (*10*) and grown in the dark at 25°C. After a 3-day incubation period the cultures were harvested. The mycelial mats were immediately frozen and lyophilized, and 0.434 g of the lyophilized material was extracted with a mixture of chloroform and methanol (3 : 1) followed by *n*-hexane as previously described (*9*). The freely extractable lipids were fractionated by silica gel column chromatography, and the fatty acid ester components were obtained in the neutral lipid fraction by elution with benzene. The individual methyl and ethyl esters were separated by gas-liquid chromatography, and structure confirmations were made by comparison with authentic standards by use of mass spectrometry and co-chromatographic techniques.

As previously reported (*9*), methyl esters ranging in carbon chain length from C_{14} to C_{24} were found in the neutral lipid fraction of total lipid extracts from *R. arrhizus*. The distribution of methyl esters detected under the conditions presented here is given in Fig. 1. No branched chain isomers were detected. After a 3-day growth period the significant methyl ester components were palmitate and oleate. Fluctuations in relative concentrations of these esters did occur with time but methyl palmitate and oleate remained the predominant saturated and unsaturated methyl esters. The esters of long-chain acids containing ethyl groups were also significant components (Fig. 1). The predominant ethyl esters correspond to the distribution of major free fatty acids typical for this organism (*9*). Ethyl oleate was the predominant ethyl component (21.2 percent) in the 3-day-old cultures, whereas ethyl palmitate (2.4 percent) and ethyl stearate (1.5 percent) were in lower concentrations. All values are expressed as the percentage of the total methyl and ethyl ester fraction. Relative concentration values changed significantly with age of the culture.

Standards for mass spectrometric comparison were prepared by treating the corresponding fatty acids with BF_3-ethanol or BF_3-methanol followed by extraction and gas chromatographic resolution as described previously for the preparation of esters (*9*). Approximately 85 percent of the gas chromatographic effluent was allowed to enter a Hitachi Perkin-Elmer RMU-6 by means of a glass separator operated at 250°C. The ion source was 270°C and scans were from 14.0 to 400 mass-to-charge ratio (m/e) in 10 seconds. Scans of the natural ethyl esters and the authentic standards produced equivalent spectra. For ethyl palmitate the rearrangement peak ($C_4H_8O_2^+$) at an m/e of 88 is the base peak, which is analogous to the pronounced m/e of 74 of straight-chain methyl esters. The fact that the base peak shifts from an m/e of 74 to an m/e of 88 in ethyl esters is due to the ethyl side chain being incorporated in the rearrangement ion formed by 2,3-cleavage. A methoxycarbonyl peak ($m/e = 101$) is also present. The molecular ion minus 45 ($M - 45$) in this spectrum and the $M - 43$ are both pre-