or 13q33 seemed most likely. Turleau *et al.* (*13*) found heterozygous expression of esterase D in a patient with deletion of 13q33 and 13q34, which excludes the locus from this region. The results of these three earlier studies are not inconsistent with our assignment of the esterase D locus to band 13q14.

Retinoblastoma has served as the prototype model for hereditary human tumors. Thus, our finding that the esterase D locus maps in the same band (q14) that is responsible for predisposition to retinoblastoma (*7*, *8*) is important. Because esterase D is a polymorphic genetic marker, we now have the opportunity to determine if there is a single retinoblastoma mutation in the familial form and if the point mutations differ from the chromosome deletion form of retinoblastoma in the locus involved. If close linkage is demonstrated in the dominantly inherited cases, esterase D determination in appropriate families could be used for genetic counseling, probably including prenatal diagnosis, as well as for identification of persons at risk of developing retinoblastoma. This could allow an early diagnosis to be made and permit early institution of treatment.

The close synteny of the esterase D locus and retinoblastoma may also permit the presumptive identification of a chromosome deletion too small to be detected by cytogenetic techniques in some patients with retinoblastoma. This would be an interesting application of human gene mapping as an aid to chromosome analysis.

ROBERT S. SPARKES
MARYELLEN C. SPARKES
Division of Medical Genetics, Departments of Medicine, Pediatrics, and Psychiatry, Center for the Health Sciences, University of California, Los Angeles 90024
MIRIAM G. WILSON
JOSEPH W. TOWNER
Genetics Division, Department of Pediatrics, University of Southern California, Los Angeles County Medical Center, Los Angeles 90033
WILLIAM BENEDICT
Division of Hematology and Oncology, Childrens Hospital of Los Angeles, Los Angeles 90054
A. LINN MURPHREE
Division of Ophthalmology, Childrens Hospital of Los Angeles
JORGE J. YUNIS
Medical Genetics Division, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis 55455

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Pro-Adrenocorticotropin/Endorphin-Derived Peptides: Coordinate Action on Adrenal Steroidogenesis

Abstract. *A synthetic peptide, representing a portion of the 16K (16,000 dalton)-fragment sequence within the pro-adrenocorticotropin/endorphin precursor molecule, potentiates the steroidogenic action of the 1 to 24 portion of adrenocorticotropin [ACTH(1-24)] on the rat adrenal cortex. The peptide has 27 amino acid residues and consists of γ-melanotropin with a carboxyl terminal extension. It affects both the inner and outer adrenocortical zones of hypophysectomized animals, as evidenced by a synergistic augmentation of corticosterone and aldosterone production, respectively. The peptide can be distinguished from adrenocorticotropin by its activation of cholesterol ester hydrolase and its failure to stimulate cholesterol side-chain cleavage.*

Evidence suggests that the hormonal products of the pituitary corticotroph cell are initially synthesized as parts of a common precursor prohormone—pro-adrenocorticotropin (ACTH)/endorphin (*1*). The amino terminal region of this precursor has been designated the 16K fragment (apparent molecular weight, 16,000) (*2*, *3*) and it includes a melanotropin (MSH)-like sequence (γ-MSH) which is partially homologous with the α- and β-MSH segments of the same prohormone (*4*). No physiological activity for either the 16K fragment itself or any derivative of it has been described.

A peptide consisting of γ-MSH with a carboxyl terminal extension of 15 additional residues has been synthesized (*5*) based on the amino acid sequence of pro-ACTH/endorphin from the intermediate lobe of bovine pituitary (*4*). We report that this peptide, denoted γ_3-MSH, stimulates the activity of adrenocortical cholesterol ester hydrolase and is capable of synergistically potentiating ACTH-stimulated corticosterone and aldosterone biosynthesis in the hypophysectomized rat.

Female Sprague-Dawley rats (Holtzman) weighing 160 to 180 g were individ-

Table 1. Response of rat adrenocortical glands to γ_3-MSH injected intravenously 7 minutes before the animals were killed. The experiments and serum steroid determinations were performed as described in the text and the legend to Fig. 1. The adrenal glands were rapidly enucleated in situ to eliminate zona glomerulosa and tissue, and the glands from animals in each group were then pooled. The mitochondrial and 105,000*g* supernatant fractions were prepared by differential centrifugation (*16*) for determination of cholesterol side-chain cleavage (*16*) and cholesterol ester hydrolase (*8*) activities, respectively. Protein was measured by the method of Bradford (*17*). Comparison of serum steroid results between groups was performed by analysis of variance; $N = 5$ for all groups. Enzyme activities were measured in triplicate or quadruplicate.

Group	Peptide given (ng)		Concentration in serum of		Enzyme activity (nmole/min-mg protein)†	
	ACTH(1-24)	γ_3-MSH	Corticosterone (μg/dl)*	Aldosterone (ng/dl)*	Cholesterol ester hydrolase	Cholesterol side-chain cleavage
A			2.2 ± 0.3	0.7 ± 0.2	1.54 ± 0.06	0.34 ± 0.24
B	10 (1 mU)		26.9 ± 2.8	10.5 ± 2.2	1.62 ± 0.11	1.58 ± 0.32
C		10	3.7 ± 1.0	2.8 ± 0.4	2.01 ± 0.15	0.41 ± 0.14
D	10	10	63.0 ± 1.7‡	66.6 ± 4.8‡	2.19 ± 0.07	3.05 ± 0.34

*Mean ± standard error. †Mean ± 2 standard deviations. ‡$P < .01$ compared with groups B and C.

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ually caged in rooms illuminated from 0600 to 1800 hours. They were given free access to rat chow and water. Transaural hypophysectomies were performed 24 hours before we began the experiments. The animals were lightly anesthetized with ether, and then the peptide (or peptides) in 0.20 ml of neutralized peptide diluent (*6*) was injected into the jugular vein 7 minutes before the animals were killed by decapitation. Controls received diluent only. Adrenal tissue from the inner zones and trunk blood were rapidly collected for enzyme and serum steroid assays.

As shown in Fig. 1, the apparent median effective dose (ED_{50}) for ACTH(1-24) (Cortrosyn; Organon) stimulation of corticosterone was decreased from 12 ng (1.2 mU) to 3 ng (0.3 mU), and for aldosterone from 22 ng (2.2 mU) to 2 ng (0.2 mU), when 100 ng of γ_3-MSH was also present. We achieved dose-response shifts of nearly comparable magnitude with as little as 1 ng of γ_3-MSH.

Table 1 shows that 10 ng of γ_3-MSH, given alone, elicited only slight increases in the concentrations of corticosterone and aldosterone in the serum (group C). Moreover, γ_3-MSH had no effect on cholesterol side-chain cleavage activity in tissue derived from the inner adrenocortical zones. However, this peptide stimulated cholesterol ester hydrolase activity to 131 percent of that in controls. Conversely, an increase in hydrolase activity was not observed with ACTH(1-24), but ACTH did stimulate cholesterol side-chain cleavage activity and increased serum adrenal steroid concentrations as expected (group B). The effects of each peptide could therefore be qualitatively distinguished from one another. When both agents were administered together (group D), all variables were increased relative to controls (group A).

The hydrolysis of cholesterol esters in the adrenal cortex provides substrate from one of several available cholesterol pools for steroid biosynthesis by the gland (*7*). We previously reported, however, that the increase in activity of adrenocortical cholesterol ester hydrolase observed in rats subjected to needle-injection stress is not reproduced by the administration of ACTH to hypophysectomized animals (*8*). Stress-induced activation requires the pituitary and is abolished by prior dexamethasone suppression. Furthermore, a non-ACTH peptide in rat pituitary homogenate is capable of stimulating the enzyme (*8*). Other groups have previously suggested that steroidogenesis in the inner (*9*) and outer (*10*) adrenocortical zones of dog, rat, and man may be under the influence of some additional, unidentified pituitary factor.

In this context, the results reported here with γ_3-MSH are particularly intriguing. The peptide appears to potentiate steroid output from the inner zones of the adrenal cortex by stimulating the rate of cholesterol ester hydrolysis, thereby augmenting the amount of substrate available for steroidogenesis. ACTH, in contrast, increases the interaction of this substrate with the cytochrome P-450 side-chain cleavage system (*11*). The effect of γ_3-MSH on the inner zones is therefore manifested in a way that is qualitatively distinct from that of ACTH. Administration of both peptides together produces a synergistic increase in serum corticosterone. A similar mechanism may explain the increase observed in serum aldosterone concentration (*12*). These data confirm our present results demonstrating that ACTH(1-24) and γ_3-MSH stimulate corticosterone production by collagenase-dispersed rat adrenocortical cells. They also confirm our results with ACTH(1-24) and a brief tryptic digest of the 16K fragment on adrenocortical function both in vitro and in vivo (*13*).

Neither the 16K fragment nor any derivative of it has been reported in the circulation. However, the coordinate release of other pro-ACTH/endorphin products in vivo has been demonstrated (*14*), and ACTH, β-lipotropic hormone, and the 16K fragment are secreted together from rat anterior pituitary cells in culture (*2*). Furthermore, there is evidence that at least a part of the 16K fragment pool in rat intermediate pituitary cells undergoes proteolytic processing (*15*). We believe therefore that the γ-MSH region of the 16K fragment may have a significant role in adrenocortical control.

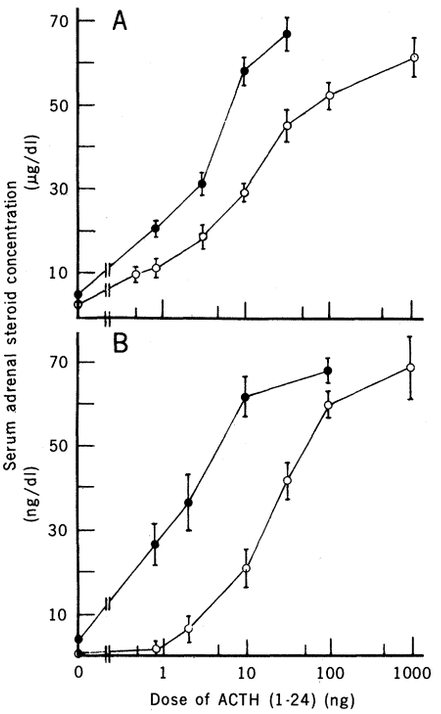

Fig. 1. Response curves for (A) serum corticosterone and (B) aldosterone in hypophysectomized female rats to increasing doses of ACTH(1-24) with (closed circles) and without (open circles) 100 ng of γ_3-MSH. Corticosterone was measured as fluorogenic steroid (*18*) with an ethanolic H_2SO_4 reagent. Serum aldosterone was determined by radioimmunoassay after extraction and immunoprecipitation (*19*). Both steroids were assayed in duplicate on individual serum samples. The values plotted represent means ± standard error (bars). Amounts of ACTH(1-24) and γ_3-MSH of equivalent mass are approximately equimolar. Ten nanograms of ACTH(1-24) = 1 mU.

ROBERT C. PEDERSEN
ALEXANDER C. BROWNIE
Department of Biochemistry, State University of New York at Buffalo, Buffalo 14214

NICHOLAS LING
Laboratories for Neuroendocrinology, Salk Institute for Biological Studies, La Jolla, California 92037

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Evolutionary Conservation of Repetitive Sequence Expression in Sea Urchin Egg RNA's

Abstract. *Cloned repetitive DNA sequences were used to determine the number of homologous RNA transcripts in the eggs of two sea urchin species,* Strongylocentrotus purpuratus *and* S. franciscanus. *The eggs of these species contain different amounts of RNA, and their genomes contain different numbers of copies of the cloned repeats. The specific pattern of repetitive sequence representation in the two egg RNA's is nonetheless quantitatively similar. The evolutionary conservation of this pattern suggests the functional importance of repeat sequence expression.*

The sea urchin genome contains 10^5 to 10^6 repetitive sequence elements belonging to several thousand nonhomologous families. These repeat families are represented in nuclear RNA's and in egg RNA in a manner specific to the state of differentiation (*1, 2*). The individual repetitive sequence fragments were isolated by S1 nuclease digestion of partially renatured DNA from *Strongylocentrotus purpuratus*, and cloned by the addition of chemically synthesized restriction enzyme recognition sequences (*3*). The cloned fragments were labeled and strand-separated, and were used as probes to detect RNA's homologous to individual repeat families. The results can be summarized as follows: (i) All of nine cloned repeat sequences studied are represented in nuclear RNA's and egg RNA, and at least 80 percent of the various repeat families in the genome are represented in egg RNA. This is in marked contrast to single copy sequences, of which only a minor fraction are found in nuclear or egg RNA's. (ii) Each repeat sequence family is represented to a particular extent in each RNA. The sequence concentrations of transcripts complementary to particular cloned repeats may differ more than 100-fold in a given RNA, and different families are highly represented in each RNA investigated. Thus the levels of representation are a function of the state of differentiation. (iii) Both strands of each cloned repeat are represented in all the RNA's studied. The complementary repeat transcripts in general reside on different RNA molecules, and probably derive from asymmetric transcription of separate multiple genomic copies oriented oppositely (*4*). A number of repeat elements of each sequence family are probably utilized in transcription. Recent studies (*5*) have shown that many of the single copy maternal messenger RNA transcripts stored in the egg are linked covalently with short repetitive sequence elements, and most of the repeat transcripts are associated with this interspersed RNA fraction.

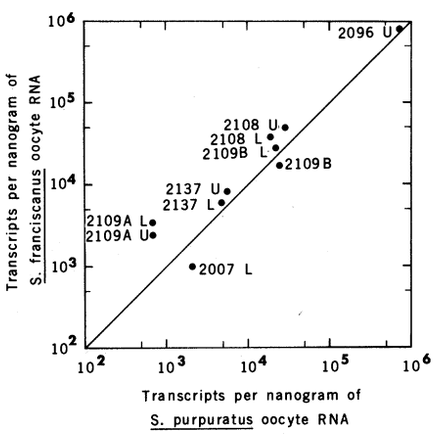

Fig. 1. Comparison of repeat sequence concentrations in the RNA of *Sf* and *Sp* eggs. Data are from *S. purpuratus* (abscissa) and *S. franciscanus* (ordinate) of Table 1. The solid line with slope 1 is the equivalence curve that would represent equal transcript concentrations in the eggs of the two species.

We now report experiments demonstrating that the specific pattern of repeat sequence representation in egg RNA has been quantitatively conserved since the divergence of two sea urchin species. The lineages leading to these species, *S. purpuratus* (*Sp*) and *S. franciscanus* (*Sf*), apparently separated 15 to 20 million years ago (*6*). They are of particular interest since their genomes contain clearly different numbers of copies of many repeat sequences (*7*). An issue addressed in the following experiments is whether the pattern of repeat sequence expression reflects these large evolutionary changes in repetitive sequence family size, or is independent of them.

In Table 1 are listed the results of measurements made with six cloned repeat fragments. The two strands of each cloned repeat were separated and reacted individually with *Sp* and *Sf* egg RNA's. The fraction of the total egg RNA complementary to each probe sequence was calculated from either kinetic or titration measurements (legend to Table 1) [see (*1*), the source of much of the *Sp* data listed]. In order to estimate the number of specific RNA transcripts, the mass of RNA per egg was multiplied by the specific fraction of the RNA hybridizing with the cloned probe. This calculation is of interest because the diameter of the *Sf* egg is about 120 μm compared to 80 μm for *Sp*, and thus the *Sf* egg is three times larger in volume. Measurements by the phloroglucinol method (*8*) show that the mass of RNA per average *Sf* egg is 8.3 ng compared to about 3 ng for the *Sp* egg (*9*). Thus the concentration of total RNA (most of which is ribosomal RNA) in the eggs of the two species is almost the same. In Table 1 the number of transcripts complementary to each cloned probe per egg is given for *Sp* and *Sf*, respectively; these values have been normalized for the egg RNA content of each species to give the number of specific transcripts per nanogram of total RNA. Measurement of transcript prevalence by kinetic or titration analysis is accurate only to within a factor of about 2.

The conclusions (*1*) for *Sp* egg RNA hold as well for *Sf* egg RNA (Table 1). Apart from clone CSp2096, which in several ways is atypical (*10*), the specific representation of the individual clones differs in *Sf* egg RNA by factors as large as 50. Both strands of all but one cloned repeat are also represented in *Sf* egg

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