skeleton of these birds could probably be assigned to various modern orders. The near impossibility of diagnosing taxa of Paleogene birds on the basis of single ends of limb bones has already been emphasized (*9*) and is even more conclusively demonstrated by the fossils discussed here.

The occurrence of these birds early in the Tertiary, their reptilian-like splenial bone, the possession of an articulating frontoparietal joint, the overall lack of fusion of cranial elements, and the very generalized nature of the postcranial skeleton are sufficient to suggest that these birds, and the paleognathous palate as well, are primitive. This is supported by the existence of at least some of the features of the paleognathous palate in the early ontogeny of some neognathous birds (*10, 11*).

The palatine and pterygoid of neognathous birds have been hypothesized to be homologous with the anterior and posterior portions of the reptilian pterygoid, with the "intrapterygoid joint" being a derived character of neognathous birds (*12*). If so, this would provide further evidence that the paleognathous palate is primitive, as the intrapterygoid joint is lacking and the configuration is thus like that of the reptilian pterygoid.

If the paleognathous palate is primitive, then it cannot be used as evidence for monophyly of the ratites and tinamous. The argument that the paleognathous palate evolved from the neognathous palate (*4*) was predicated largely on the unrelated fact that ratites evolved from volant ancestors (*10*), for which reason the ratites, and consequently their palate, were considered to be "derived." The volant Tertiary paleognaths suggest the opposite evolutionary sequence. The assumption of a monophyletic origin of the ratites and tinamous from a neognathous ancestor requires a pre-Cenozoic radiation of these birds in Gondwanaland, as postulated by Cracraft (*13*). However, the occurrence of paleognathous birds in the Paleocene and Eocene of North America does not agree well with the tectonic and temporal constraints of this zoogeographical hypothesis.

The new fossil birds reported here are probably remnants of what may have been a diverse radiation of paleognathous carinates that preceded, and were possibly ancestral to, the later radiation of neognathous birds. Tinamous and ratites may have descended independently from various families or orders within this radiation of paleognaths, or some of the ratites may have evolved secondarily from neognathous birds through neoteny. A monophyletic origin of the ratites and tinamous is far from being an established fact, and the evidence suggesting that they are paraphyletic or polyphyletic (*14*) now deserves serious consideration and evaluation. A conclusive resolution of the problem will have to depend largely on new fossil evidence and more original anatomical and embryological studies of living taxa, rather than additional reinterpretations of the same data that have been brought to bear on the question in the past.

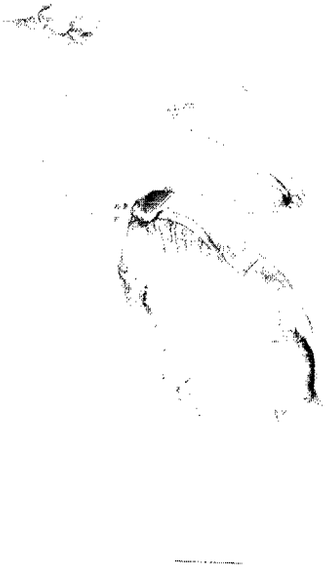

Fig. 2. Reconstructed skeleton of a volant, early Tertiary paleognathous bird based mainly on fossils from the Paleocene of Montana. Scale, 4 cm.

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23 December 1980; revised 23 April 1981

Adenosine 3′,5′-Monophosphate Modulates Thyrotropin Receptor Clustering and Thyrotropin Activity in Culture

Abstract. *A biologically active rhodamine conjugate of thyrotropin binds at 4°C to diffusely distributed membrane thyrotropin receptors which patch and become endocytosed into thyroid cells in a temperature-sensitive process. When the cells are first incubated with 8-bromo-cyclic adenosine monophosphate at 37°C, the conjugate also binds to clustered receptors at 4°C. Furthermore, 8-bromo-cyclic adenosine monophosphate reduces the amount of adenosine 3′,5′-monophosphate (cyclic AMP) induced by thyrotropin. Hence, increased intracellular cyclic AMP induces receptor patching and reduces the concentration of cyclic AMP normally induced by thyrotropin. This suggests that cyclic AMP acts both as the second messenger of thyrotropin and also as the regulator of the level of thyrotropin receptors.*

Normal function of the thyroid gland is regulated by the pituitary through the hormone thyrotropin (TSH). This hormone binds to specific receptors on the membrane of thyroid cells and induces a variety of biological responses (*1–3*). Furthermore, TSH activates a thyroid adenylate cyclase and many of its responses are mimicked by analogs of adenosine 3′,5′-monophosphate (cyclic AMP). Therefore, it is generally accepted that TSH response is regulated by the concentration of cyclic AMP (*4*, *5*). Since increased intracellular cyclic AMP, mediated by either TSH (*6*) or by other hormones (*7*, *8*), desensitizes the thyroid adenylate cyclase (E.C. 4.6.1.1), this could serve as a feedback mecha-

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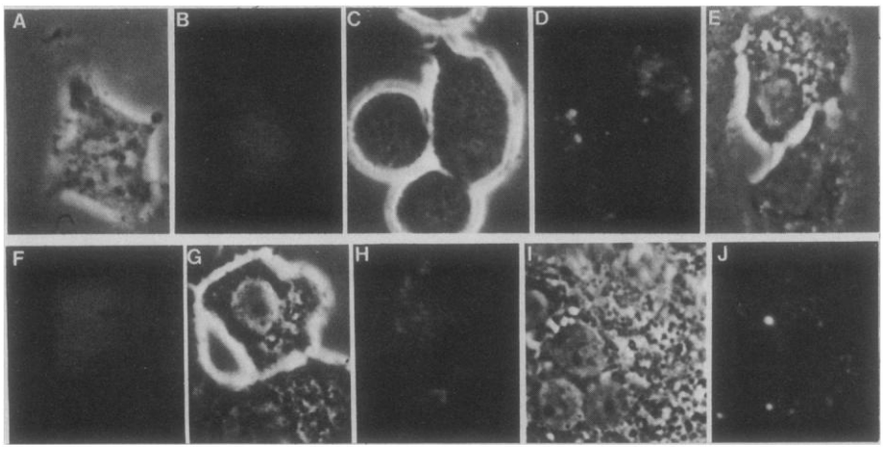

Fig. 1. The distribution of R-TSH on $FRTL_5$ cells in the absence or presence of 8-Br–cyclic AMP. The $FRTL_5$ cells were grown for 18 hours on glass cover slips in the absence of TSH. Diffuse labeling of R-TSH was detected after 1 hour of incubation at 4°C with 500 ng of R-TSH. (A) Phase and (B) fluorescence micrographs of the same field. Fluorescent patches of R-TSH were observed when these cells were incubated for an additional 15 minutes at 37°C. (C) Phase and (D) fluorescence micrographs of the same field. Diffuse labeling of R-TSH was observed when the cells were incubated for 30 minutes at 37°C with 1 m*M* adenosine monophosphate and then incubated for an additional hour with R-TSH (500 ng/ml) at 4°C. (E) Phase and (F) fluorescence micrographs of the same field. Fluorescent patches were visualized when the cells were directly labeled at 37°C for 15 minutes with R-TSH (500 ng/ml). (G) Phase and (H) fluorescence micrographs of the same field. When the cells were first incubated with 1 m*M* 8-Br–cyclic AMP for 30 minutes at 37°C and then washed with ice-cold medium and incubated for an additional hour with R-TSH (500 ng/ml) at 4°C, R-TSH bound to preclustered TSH receptors. (I) Phase and (J) fluorescence micrographs of the same field (×1360).

nism for the regulation of the TSH-sensitive adenylate cyclase in thyroid cells (*5*).

We discovered (*9*) that the concentration of TSH receptors on the surface of thyroid cells can be regulated by receptor-mediated endocytosis. In that study we reported the preparation of a biologically active fluorescent conjugate of bovine TSH labeled at the free amino groups with tetramethylrhodamine isothiocyanate (R-TSH). This conjugate retains 25 percent of the binding affinity of native TSH toward TSH receptors of membranes from bovine thyroid cells and 25 percent of the capacity of the native hormone in stimulating the production of cyclic AMP in cultures of rat thyroid cells (*9*). This conjugate also binds specifically to diffusely distributed membrane receptors on thyroid cells. At 37°C the fluorescent hormone forms visible patches that become internalized and subsequently degraded (*9*). Receptor clustering induced by TSH seems to play a role in the activation of the thyroid adenylate cyclase. It was reported (*10*) that bivalent antibodies against TSH receptor isolated from the serum of patients with Graves' disease mimic TSH and activate the thyroid adenylate cyclase (*10*). Since maximal stimulation of cyclic AMP production induced by TSH was observed prior to the formation of visible patches of the fluorescent hormone, we concluded that TSH receptor microclusters, which are too small to be detected by fluorescence microscopy, rather than visible patches, play a role in the activation of the thyroid adenylate cyclase (*9*, *11*).

We report here that cyclic AMP modulates both TSH receptor clustering and the potency of the hormone to stimulate the adenylate cyclase of thyroid cells. At 4°C, R-TSH binds to diffusely distributed membrane receptors on thyroid cells which patch and become internalized when the temperature is subsequently raised to 37°C. However, if the thyroid cells are first incubated with 8-bromocyclic AMP at 37°C and then labeled with R-TSH at 4°C the fluorescent hormone binds to aggregated receptors. Under similar conditions 8-Br–cyclic AMP reduces the cyclic AMP concentration induced by TSH. These experiments show that cyclic AMP modulates TSH receptor clustering and TSH activity and suggests that the feedback response induced by TSH occurs at the level of receptor clustering.

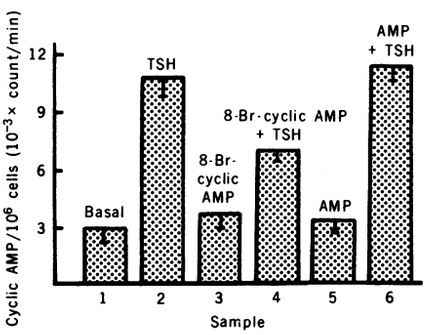

Fig. 2. The effect of 8-Br–cyclic AMP on TSH-induced stimulation of cyclic AMP production in $FRTL_5$ cells. The $FRTL_5$ cells were labeled with [^{3}H]adenine for 2 hours at 37°C. Then the cells were treated with various reagents and their cyclic AMP content determined. 1, Basal; 2, TSH, 2.5 mU/ml; 3,1 m*M* 8-Br–cyclic AMP; 4, 1 m*M* 8-Br–cyclic AMP followed by 2.5 mU of TSH; 5, 1 m*M* AMP; and 6, 1 m*M* adenosine monophosphate followed by 2.5 mU of TSH. Each bar represents the average of four different experiments each performed in triplicate. Error bars indicate the calculated standard deviation.

For these experiments we used a rat thyroid cell line, $FRTL_5$ (Fisher rat thyroid cells grown in 5 percent serum) established by Ambesi-Impiombato *et al.* (*12*, *13*). This cell line responds to TSH, synthesizes thyroglobulin, and accumulates iodide (*12*). The fluorescent hormone bound to the cells was visualized with a sensitive, image-intensified microscopy system (*14*, *15*).

When $FRTL_5$ cells that were initially maintained at 37°C were incubated for 1 hour at 4°C in the presence of R-TSH (500 ng/ml), the fluorescent conjugate appeared diffusely distributed over the cell surface (Fig. 1, A and B). When these cells were incubated for 10 minutes at 37°C, the fluorescent TSH aggregated into visible patches (Fig. 1, C and D). Such patches were also observed when the $FRTL_5$ cells were labeled with R-TSH for 15 minutes at 37°C (Fig. 1, G and H) or when the cells were first incubated with 1 m*M* 8-Br–cyclic AMP for 30 minutes at 37°C and then washed with ice-cold medium for 10 minutes and incubated for an additional 60 minutes at 4°C with R-TSH (500 ng/ml). Here, R-TSH bound to clustered receptors on the $FRTL_5$ cells (Fig. 1, I and J) in contrast to the diffusely distributed receptors observed in the absence or presence of 8-Br–cyclic AMP at 4°C. Apparently, 8-Br–cyclic AMP induced a temperature-sensitive clustering of TSH receptors and therefore R-TSH binds at 4°C to clustered receptors. Prior incubation of $FRTL_5$ cells at 37°C with 1 m*M* adenosine monophosphate did not induce TSH receptor clustering (Fig. 1, E and F).

We also found that 8-Br–cyclic AMP also affects the ability of TSH to stimulate the thyroid adenylate cyclase (*16*). When $FRTL_5$ cells were first incubated with 1 m*M* 8-Br–cyclic AMP for 30 minutes at 37°C and then treated with TSH, the concentration of cyclic AMP induced by TSH was lower than in the control experiment where the cells were not exposed to 8-Br–cyclic AMP (see Fig. 2).

Adenosine monophosphate did not affect the TSH-sensitive adenylate cyclase. Neither 8-Br–cyclic AMP by itself nor AMP by itself affected the basal level of cyclic AMP (see Fig. 2).

Hence, elevated intracellular cyclic AMP induces receptor patching and reduces the concentration of cyclic AMP normally induced by TSH.

Receptor internalization induced by insulin and epidermal growth factor (EGF) (*15*, *17*) seems to be related to the well-characterized process of insulin and EGF-induced receptor loss or down regulation (*18*, *19*). However, cyclic AMP does not affect the down regulation of insulin or EGF but it does induce down regulation of TSH receptors. This raises the interesting possibility that part of the regulation of TSH activity occurs at the level of receptor clustering when cyclic AMP production is stimulated and that cyclic AMP acts both as the second messenger of TSH and also as the regulator of the level of its membrane receptor.

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19 June 1981; revised 9 September 1981

Type III Hyperlipoproteinemia Associated with Apolipoprotein E Deficiency

Abstract. *Subjects with type III hyperlipoproteinemia develop premature atherosclerosis and have hyperlipidemia due to an increase in cholesterol-rich very low density lipoproteins (VLDL) of abnormal electrophoretic mobility. Apolipoprotein E is a major protein constituent of VLDL and appears to be important for the hepatic uptake of triglyceride-rich lipoproteins. A new kindred of patients with type III hyperlipoproteinemia is described in which no plasma apolipoprotein E could be detected, consistent with the concept that type III hyperlipoproteinemia may be due to an absence or striking deficiency of apolipoprotein E.*

Patients with type III hyperlipoproteinemia (HLP) often develop premature coronary artery and peripheral vascular disease and may have palmar, tubo-eruptive, and tendinous xanthomas (*1*). The hyperlipidemia in these subjects generally is not detectable prior to the third decade of life and is exacerbated by obesity, increased caloric intake, and hypothyroidism (*1*). Type III HLP patients have increased concentrations of plasma cholesterol and triglyceride (*1*). The concentrations of low density lipoprotein (LDL) (density 1.019 to 1.063) and high density lipoprotein (HDL) may be normal or decreased in these subjects (*2*); chylomicrons are often present in the fasting state, and very low density lipoprotein (VLDL) (density < 1.006 g/ml) and intermediate density lipoprotein (IDL) (density 1.006 to 1.019 g/ml) are increased (*1*). The VLDL isolated from type III HLP plasma has an increased ratio of cholesterol to triglyceride, and

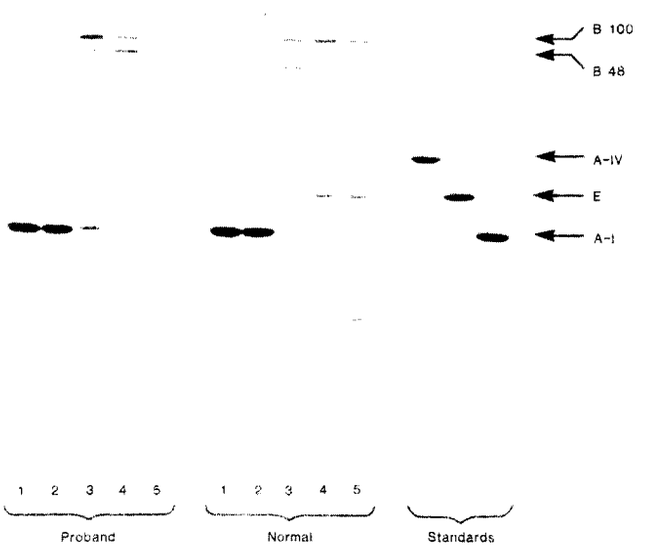

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of plasma lipoproteins from a normal subject and the proband showing no detectable apoE, but the presence of apoB-48 and apoA-IV in the proband's lipoproteins. Identical 15-μg protein loads of 1, HDL_{2a+3}; 2, HDL_{2b}; 3, LDL; 4, IDL, and 5, VLDL were run on gels. Apolipoprotein A-IV, apoE, and apoA-I standards are shown.

0036-8075/81/1211-1239$01.00/0