unique among the cellular slime molds. Instead of small vesicles pinching off from the ends of the individual cisternae, the latter swell up completely so that they resemble the sections of a citrus fruit (Fig. 1H). All the cells within the young sorocarp have many of these stacks of swollen vesicles. A thread-like material embedded in a mucous matrix develops abundantly within the vesicles (Fig. 1I). Substances very similar to that contained within the vesicles can be seen between the individual cells and in the walls of the stalk surrounding them (Fig. 1, J and K). The vesicles appear to migrate to the plasma membrane of the cell, fuse with it, and release their contents to the outside (Fig. 1J). While much of the mucous material containing some thread-like matter forms the matrix around the cells, most of the thread-like material accumulates in the walls of the neck (Fig. 1K) and especially in the thickened base of the stalk. This is believed to give support to the stalk and its sorus.

While the sorocarp is developing and the cells are moving upward during stalk formation, all the cells have a fairly amoeboid appearance. However, after the sorocarp has achieved its full height, the cells within begin to show a division of labor. Those cells nearest the base are amoeboid and continue to produce dictyosomes that accumulate and deposit stalk material. Those farther up are much more rounded, have very few dictyosomes, and are beginning to develop into spores. The cells in the upper neck are elliptical, lack dictyosomes, and are forming thick walls as they mature into spores.

At the culmination of sorogenesis, the encysted cells emerge from the apex of the stalk into a mucous matrix and become the sorus. At this point, the neck region of the sorocarp is almost devoid of cells. However, a group of amoeboid cells remains in the base and continues to manufacture stalk material even after the spores emerge. It seems likely that the mucous material surrounding these cells may absorb moisture from outside the sorocarp and thereby exert enough pressure within the stalk to cause it to elongate and the spores to rise and be expelled. Evidence of this was obtained by removing immature sorocarps from the bacterial culture and placing their bases in contact with a layer of water on a slide. The developing spores and mucous material rapidly emerged from the stalk apex. A similar mode of cell movement, in which the sorogenic cells eject hydrophilic material that swells and causes the cells to rise in the formation of an aerial fruiting body, was recently reported in an unrelated organism, the ciliate *Sorogena stoianovitchae* (*4*).

The origination of dictyosomes from the outer membrane of the nuclear envelope is an uncommon but not unique phenomenon. Although the Golgi apparatus originates from the endoplasmic reticulum in most organisms investigated, its formation from the nuclear envelope has been demonstrated in several systems, including sorogenesis in apparently unrelated cellular slime molds (*5*, *6*) and the embryonic stages of some higher plants and animals (*7*). The aggregating amoebas of the acrasids *Guttulinopsis nivea* (*5*) and *Copromyxa protea* (*C. arborescens*) (*6*) form structures from the nuclear envelope resembling rudimentary dictyosomes. Also, in *Endemosarca hypsalyxis*, an organism possibly related to plasmodiophorids, small vesicles termed "amplexi" migrate from the nuclear envelope to form small dictyosome-like structures (*8*). However, in each case these dictyosomes remain very small and do not bear any resemblances to the large vesicles seen in sorogenic cells of *F. alba*.

Perhaps the more significant observation is that the sorogenic cells of *F. alba* are stimulated to form conspicuous, unusually shaped dictyosomes at the beginning of aggregation, that these organelles function in the accumulation of stalk material, and that they disappear before the cells encyst. This series of events occurs only during one specific phase of the life cycle. The stimulus that elicits such an intriguing response remains to be identified.

MARY C. DEASEY
LINDSAY S. OLIVE
Department of Botany, University of North Carolina, Chapel Hill 27514

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Mesenchymal Cells from the Human Embryonic Palate Are Highly Responsive to Epidermal Growth Factor

Abstract. *An established line of mesenchymal cells from the human embryonic palate is highly sensitive to the stimulatory effect of epidermal growth factor on growth, labeled thymidine incorporation, and ornithine decarboxylase activity. The results suggest that epidermal growth factor may play a key role in development of various human embryonic and fetal tissues.*

Formation of the secondary palate occurs early in human development (weeks 6 to 8 of gestation) and involves a complex series of steps involving both the growth and differentiation of epithelial and mesenchymal cells (*1*). These events are presumably under the control of a number of hormones and growth factors (*2–4*). In the study described here we investigated the effects of epidermal growth factor (EGF) on growth and other parameters of an established line of human embryonic palatal mesenchymal (HEPM) cells cultured in a serum-free hormone-supplemented medium and on the binding of EGF to cell surface receptors.

The HEPM cell line (CRL 1486) was established for us by the American Type Culture Collection (Rockville, Maryland). These mesenchymal cells, pre-

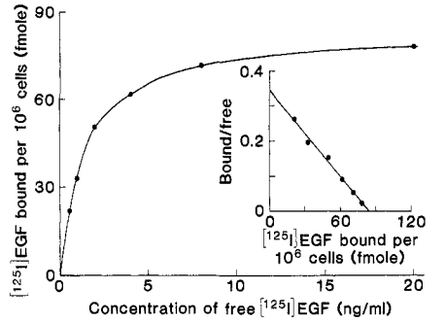

Fig. 1. Specific [^{125}I]EGF binding to HEPM cells. The HEPM cells were plated at a density of 2×10^5 cells in 3 ml of complete DMEM in 35-mm dishes and grown for 72 hours as described. Labeled EGF binding experiments were carried out according to the methods reported previously (*24*).

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sumably representing the undifferentiated fibroblast-like cells from the palatal shelves, were established from a single human abortus (normal) at the time of palatal shelf elevation, but prior to epithelial contact. These cells were maintained in culture in Dulbecco's modified Eagle's minimum essential medium (DMEM) containing 10 percent fetal or newborn calf serum (heat-inactivated), penicillin, and streptomycin (complete DMEM) in 75-cm^2 tissue culture flasks (Falcon) under 95 percent air and 5 percent CO_2 in a humidified atmosphere at 37°C. The medium was changed every 3 days, and when the cells became confluent they were harvested with 0.05 percent trypsin and 0.02 percent EDTA solution (Gibco) and subcultured.

Growth of HEPM cells was superior in a serum-free improved minimum essential medium (IMEM) supplemented with EGF, fibroblast growth factor (FGF), insulin, fetuin, transferrin, glutamine, penicillin, streptomycin, and Hepes (complete IMEM) (*5*) compared to that obtained in serum-supplemented complete DMEM (Table 1, experiment 1). When EGF, FGF, or insulin was removed from complete IMEM separately, the most dramatic decrease in cell growth resulted from removal of EGF. Addition of EGF by itself to IMEM supplemented only with fetuin and transferrin (basal IMEM) resulted in as much growth as that observed in complete IMEM. Stimulation of HEPM cell growth by EGF was dose-dependent and was observed at concentrations as low as 0.5 ng/ml (data not shown). Epidermal growth factor stimulated the incorporation of [^{3}H]thymidine into HEPM cells more than twofold above controls, which was much greater than the stimulation observed in the presence of FGF or insulin (Table 1, experiment 2). Epidermal growth factor was also a potent inducer of ornithine decarboxylase (ODC) activity, which is normally increased in cells undergoing rapid proliferation (*6, 7*). It also is of interest that under these conditions, EGF produced a tenfold stimulation in the levels of prostaglandin E_2.

Since these results suggested that HEPM cells were highly sensitive to EGF, we next examined the binding of labeled EGF to cell surface receptors. As shown in Fig. 1, ^{125}I-labeled mouse EGF was bound with high affinity to a class of saturable binding sites. Scatchard plot analysis (*8*) revealed the existence of a single population of high-affinity binding sites (5.2×10^4 receptors per cell) with a dissociation constant (K_d) of $2.7 \times 10^{-10} M$. The binding of labeled EGF was highly specific, since FGF, insulin, or a variety of other growth factors were not able to compete with labeled EGF in the binding assay (data not shown). However, when HEPM cells were first incubated in basal IMEM containing EGF for 3 to 72 hours and the binding assay was performed in the absence of EGF, 98 percent of the EGF binding sites were lost, presumably due to down regulation of EGF receptors (*9–12*).

Binding of labeled EGF was highest in HEPM cells among a variety of normal human cells investigated (Table 2). In conjunction with this, HEPM cells responded to EGF the most dramatically by increased growth, DNA synthesis, and ODC activity, which were 95, 63, and 159 percent increased over non-

Table 1. Effects of EGF, FGF, and insulin on HEPM cells in culture. For measurement of cell growth, HEPM cells were plated at a density of 5×10^4 cells in 35-mm plastic tissue culture dishes (Costar) containing 2 ml of Dulbecco's modified Eagle's minimum essential medium (DMEM) supplemented with 10 percent fetal or newborn calf serum, glutamine (0.6 mg/ml), penicillin (50 U/ml), and streptomycin (50 μg/ml) (complete DMEM). After 24 hours, the medium was changed to 2 ml of a serum-free improved minimum essential medium (IMEM) (*5*) containing fetuin, transferrin (basal IMEM), and one of the following: EGF (20 ng/ml), FGF (200 ng/ml), or insulin (5 μg/ml). At the end of 72 hours in culture, the cells were harvested and counted. For measurement of [^{3}H]thymidine incorporation into HEPM cells, the cells were grown as described above for 5 to 7 days until confluent. The media were changed every 2 days. The cells were exposed for the last 4 to 6 hours to [^{3}H]thymidine (1 μCi/ml) (New England Nuclear), and incorporation was determined by trichloroacetic acid precipitation. For assay of ODC activity, the cells were plated at 2×10^6 cells in 5 ml of complete DMEM in 35-mm dishes and cultured for 72 hours. The medium was then changed to a serum-free basal IMEM containing hormone or growth factors as indicated for 4 hours at 37°C. The cell layers were then removed, sonicated, and centrifuged. The resulting supernatants were used for assessment of ODC activity according to the method of Janne and Williams-Ashman (*21, 22*). Protein was measured by the method of Lowry *et al.* (*23*). Values shown are means ± standard errors for six (cell number) or triplicate (labeled thymidine incorporation and ODC activity) dishes. Values in parentheses represent the percentage relative to control.

EGF	FGF	Insulin	Cell number ($\times 10^5$ per dish)	[^{3}H]Thymidine incorporation in 10^6 cells ($10^5 \times$ count/min)	ODC activity per hour (pmole CO_2/mg protein)
			Experiment 1		
+	+	+	11.3 ± 0.2 (100)		
−	+	+	7.6 ± 0.1 (68)		
+	−	+	11.8 ± 0.2 (105)		
+	+	−	9.7 ± 0.1 (86)		
DMEM + 10% FCS			7.4 ± 0.2 (66)		
			Experiment 2		
−	−	−	4.2 ± 0.1 (100)	2.5 ± 0.2 (100)	44 ± 12 (100)
+	−	−	7.9 ± 0.1 (188)	5.6 ± 0.2 (222)	107 ± 10 (243)
−	+	−	4.6 ± 0.1 (110)	4.1 ± 0.2 (161)	53 ± 1 (120)
−	−	+	5.3 ± 0.1 (126)	4.9 ± 0.1 (193)	78 ± 9 (170)
+	+	+	8.4 ± 0.2 (200)	5.8 ± 0.4 (222)	106 ± 21 (241)

Table 2. The binding of ^{125}I-labeled mouse EGF and the effects of EGF on various human cells in culture. All cell lines used in this experiment were obtained from the American Type Culture Collection. The cells were maintained in culture as described. For binding experiments, the cells were incubated with labeled EGF (20 ng/ml) in the presence or absence of nonlabeled EGF (2 μg/ml) (obtained from Collaborative Research). Values shown are means ± standard errors for triplicate dishes. For examination of EGF effects, cells were cultured either in basal IMEM (nontreated group) or in basal IMEM containing EGF (20 ng/ml).

Cells	[^{125}I]EGF bound (pmole per 10^5 cells)	Cell growth	Percentage stimulation over nontreated cells	
			[^{3}H]thymidine incorporation	ODC activity
CRL 1486 (HEPM)	78 ± 3	95	63	159
CRL 1475 (fetal skin fibroblast)	12 ± 1	54	27	−25
CRL 1502 (fetal skin fibroblast)	6 ± 1	25	25	15
CRL 1510 (fetal skin fibroblast)	18 ± 1	12	61	−67
CCL 137 (fetal lung fibroblast)	25 ± 1	23	50	41
CCL 13 (liver epithelial cell)	68 ± 2	−28	−72	21
CCL 27 (heart cell)	69 ± 3	−29	−39	62
CCL 17 (oral epidermoid carcinoma)	123 ± 2	38	46	7

treated controls, respectively. Various human fetal cells, including skin and lung fibroblasts, showed lower binding of labeled EGF compared to HEPM cells and were less responsive to EGF. Adult liver and heart cells were able to bind labeled EGF to a similar extent as HEPM cells; however, EGF suppressed their growth and DNA synthesis, and changed their cell shape to a more fibroblastic appearance (not shown). It is noteworthy that a human oral epidermoid carcinoma cell line (CCL 17) (*13*) has an extremely high number of EGF receptors, approximately 60 percent of that found in A431 cells (3×10^6 per cell) (*14*) and that its basal ODC activity is higher than in other cell lines listed in Table 2.

Our data indicate that HEPM cells, compared to various human adult and fetal cells investigated in the present study, are the most sensitive to stimulation by EGF, whereas FGF and insulin at concentrations known to be stimulatory in other cell systems (*15*) do not stimulate HEPM cell growth to a significant extent. Thus, our findings, together with the proposed role of EGF in the control of growth of various target cells (*16*), suggest that HEPM cells are one of the target cells for EGF during development. These results are consistent with our previous studies showing that EGF affects terminal differentiation of the medial epithelial cells in the rodent palate as well as mesenchymal cells of the palatal shelves in organ culture (*3, 17*). We proposed (*18*) the presence of an embryonic form of EGF in the midgestation mouse embryo. It appears that this form of EGF, which may be similar to sarcoma growth factor (*19*), may be important in development of various embryonic and fetal tissues (*20*). Since HEPM cells are derived from the palatal shelves of a human embryo, these cells may be useful for studies on the physiological and biochemical role of hormones and growth factors in the regulation of embryonic and fetal development.

TOSHIYUKI YONEDA*
ROBERT M. PRATT†
Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205

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* Present address: Second Department of Oral and Maxillofacial Surgery, Osaka University Dental School, Osaka, Japan.
† Present address: Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Building 17, P.O. Box 12233, Research Triangle Park, N.C. 27709.

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Tumor-Induced Anorexia in the Wistar Rat

Abstract. *The transplantable Leydig cell tumor of Wistar rats, LTW(m), caused decreased food consumption and weight loss in the host within 2 weeks of implantation. The tumor was small, did not metastasize, and did not affect several parameters of biochemical function. When the tumors were removed, increases in food intake and body weight occurred within 72 hours and were sustained. Reimplantation of tumors caused anorexia to recur. Parabiotic pairs of rats with tumor in one partner also experienced weight loss. Those rats in parabiosis with tumor-bearing rats gained less weight than those in parabiosis with control rats. These observations suggest that the LTW(m) tumor causes anorexia and that this anorexia is mediated by a circulating substance.*

Tumor-induced anorexia is of major concern to physicians, as it often leads to the development of fatal cachexia (*1*). Its cause is unknown. Research has been hampered by the difficulty of human experimentation and the lack of well-characterized animal models. With humans there often is variability in the presentation of the syndrome, lack of tissue for study, and confounding of data by therapy. An animal model of tumor-induced anorexia should display several features. The tumors should be small and not metastasize. They must not cause structural or biochemical derangements that could produce nonspecific loss of appetite. Ideally, long-term survival of the animal should be possible, and loss of appetite should be reversible on removal of the neoplasm. Tumors used in previous animal studies of tumor-induced anorexia (*2*) did not meet these criteria. We report a rodent tumor system that appears suitable for the study of anorexia.

To evaluate tumors that might provide models of cancer anorexia, we studied the records of the tumor bank maintained by the Mason Research Institute (*3*). One tumor that appeared to arrest weight gain despite its small size was the Leydig cell tumor of Wistar rats, LTW(m). For comparison, a tumor of the Wistar rat not associated with weight loss, the breast carcinosarcoma MT/W9a-B, was also selected for study.

The first experiment was performed to study the effect of these tumors on the weight of male rats (*4*). Beginning 18 days after implantation there was an obvious arrest of weight gain in the rats with LTW(m) tumor, while the rats with breast tumor were relatively unaffected. Between days 18 and 35, seven control rats gained 26 ± 2 g (mean ± standard error); seven breast tumor–bearers gained 34 ± 4 g; and eight LTW(m) tumor–bearers lost 2 ± 2 g ($P < .001$). When the rats were killed the LTW(m) tumors weighed 1.5 ± 1.2 g and the breast tumors weighed 6.3 ± 10.1 g. In another experiment, eight control rats gained an average of 57 g while eight tumor-bearing rats gained only 9 g over 28 days ($P < .01$). Final tumor weight was 1.0 ± 0.1 g. A biochemical profile (*5*) revealed no major differences between normal and tumor-bearing rats except for significantly lower alkaline phosphatase in tumor bearers (141 ± 82 versus 241 ± 31 mU/ml). Glucose levels in both groups were slightly elevated, probably due to the ether used to anesthetize the rats. All animals were sub-

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