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Regulation of Dome Formation in Differentiated Epithelial Cell Cultures

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Rat mammary (Rama 25) and dog kidney (MDCK) epithelial cell cultures formed 'domes' of cells due to fluid accumulation in focal regions between the culture dish and the cell monolayer. Addition of ouabain caused collapse of domes, suggesting that transport functions were required for maintenance of domes.

Dome formation in both epithelial cell lines was stimulated by a broad spectrum of known inducers of erythroid differentiation in Friend erythroleukemia cells. Among these inducers were: 1) polar solvents such as dimethylsulfoxide, dimethylformamide, and hexamethylene bisacetamide; 2) purines such as hypoxanthine, inosine, and adenosine; 3) low-molecular-weight fatty acids such as n-butyrate; and 4) conditions expected to elevate levels of cyclic AMP. In the latter group were activators of adenylate cyclase such as cholera toxin and prostaglandin E_1; cyclic AMP phosphodiesterase inhibitors such as theophylline and 1-methyl-3-isobutylxanthine; and analogs of cyclic AMP.

Induction of domes occurred 15–30 h after addition of inducer to the culture medium. Induction by chemicals was serum-dependent and required protein synthesis but not DNA synthesis. Induced dome formation was reversible after removal of inducer, requiring the continuous presence of inducer. Reversal was also observed after either removal of serum or addition of inhibitors of protein synthesis.

These results suggest the hypothesis that domes arise in these epithelial cultures by a process that is similar to cell differentiation and is influenced by cyclic AMP.

Key words: epithelial transport, differentiation, cyclic AMP, cryoprotective solvents

Recently, several tissue culture model systems have been made available for the study of morphogenesis and physiology of transporting epithelia [1–6]. Unique properties of transporting epithelia are expressed in culture by these cell cultures, notably the ability to form cell layers which act as permeability barriers between aqueous compart-

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ments. This property derives from the maintenance of specialized junctional complexes and functional polarization of the plasma membrane after transition to culture and neoplastic transformation [7, 8].

Hemispheres (0.1–1 mm in diameter) of cells, known as domes, blisters, or hemicysts, form spontaneously in densely confluent cuboidal epithelial cell cultures derived from a variety of transporting epithelia [1–9]. Domes rise and fall apparently at random over the culture dish. Domes appear to result from fluid accumulation between the cell monolayer and the plastic dish owing to the manifestation in cell culture of specialized, undirectional epithelial transport and secretory properties. Figure 1 is a schematic representation of a dome.

The focal occurrence of fluid accumulation in domes rather than uniformly under the entire monolayer suggests that dome cells may differ functionally and biochemically from cells in the surrounding monolayer. However, no morphologic features have been found which distinguish dome cells from those in the surrounding monolayer. Pickett et al [10] have demonstrated that the surface of primary mammary epithelial cell cultures in contact with the medium (equivalent to the apical or luminal surface) contained microvilli and well-developed occluding junctions. Gap junctions and desmosomes were also found in the cultures, but the luminal surface membrane of both dome cells and monolayer cells exhibited similar cell and junctional structure.

Active fluid transport is required for the maintenance of domes as shown by observations that domes are collapsed after fluid transport is inhibited by ouabain or when fluid leakage is permitted after a dome is pierced by mechanical means. However, it is important to emphasize that dome formation may represent functional changes unrelated to changes in transport systems in the plasma membrane. Changes in selective adhesion to the culture substratum or in specialized junctional contacts may play a causative role in dome formation.

A remarkable analogy between the development of domes in epithelial cell cultures and processes of cell differentiation was suggested by the following observations. A broad spectrum of compounds known as inducers of differentiation in cell culture systems, such

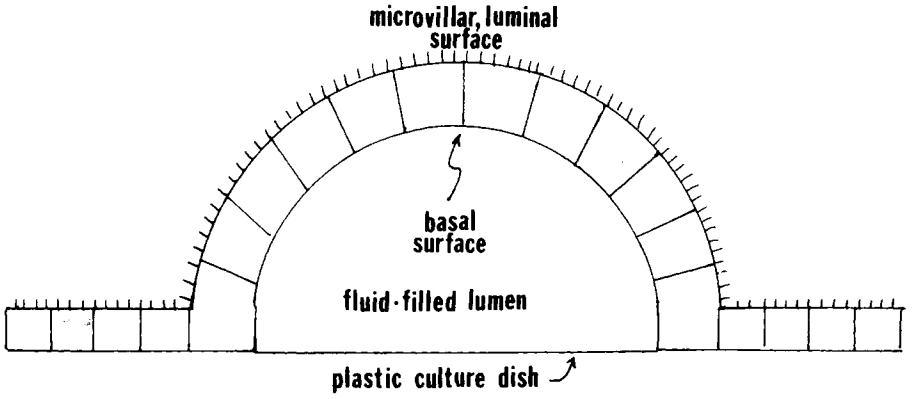

Fig. 1. Schematic representation of a dome. Microvilli are observed on the upper, apical plasma membrane surface of the dome cells and surrounding monolayer, in contact with the culture medium. Domes arise owing to transepithelial fluid transport from the culture medium through the apical membrane to the space between the cell monolayer and the culture dish via the basolateral membrane.

as Friend erythroleukemia cells [11, 12] and neuroblastoma [13], also produced a dramatic increase in both the size and frequency of dome formation in epithelial cultures within 15–30 h after addition [14, 15]. This observation suggested that induced dome formation may result from differentiation of cell types in the culture. Such a possible analogy between dome formation and cell differentiation in vitro in other cell culture model systems is pursued in this report in terms of a parallel study of agonists and antagonists of dome formation in two different dome-forming epithelial cell cultures – the Rama 25 line of rat mammary epithelial cells [6] and the MDCK kidney epithelial cell line [14].

MATERIALS AND METHODS

Cell Culture

Rama 25 (cuboidal), Rama 29 (elongated), and Rama 30 (elongated) clonal epithelial cell lines derived from a dimethylbenzanthracene-induced rat mammary tumor [6] were obtained from Dr. D. Bennett, Imperial Cancer Research Fund Laboratories, London. These cell lines were propagated in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, 50 ng/ml bovine insulin and 50 ng/ml hydrocortisone. It was necessary to replace cultures of Rama 25 from frozen low-passage stocks when they had reached passage numbers above 30 owing to appreciable infiltration by elongated epithelial cell types which arise spontaneously in these cultures [6].

The MDCK epithelial cell line derived from dog kidney [16] was obtained from Dr. R. Holley, Salk Institute. The MDCK cell line, and MDCK clone 4, a subline with a high frequency of spontaneous dome formation, were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, nonessential amino acids, hypoxanthine, putrescine, biotin, lipoic acid, vitamin B-12, ascorbic acid, glutathione, p-aminobenzoic acid, trace metals, and linoleic acid [17].

All cell growth was at 37°C in an atmosphere of 10% CO_2 in air.

Quantitation of Domes in Epithelial Cell Cultures

Cultures were fixed with 5% glutaraldehyde in phosphate-buffered saline for 15 min at room temperature. This solution was aspirated, and cells were stained with Giemsa, washed three times with water, and dried in air. This procedure permitted the identification of dome foci on dried monolayers. The number of domes was counted on duplicate 35-mm dishes using 8 to 20 fields of either 0.13 cm^2 (Bausch & Lomb stereozoom microscope) or 0.07 cm^2 (Zeiss stereo microscope). Larger numbers of fields were examined for cultures with an average of fewer than 10 domes per field.

Determination of Intracellular Levels of Cyclic AMP

Medium was removed from epithelial cell monolayers on 5-cm dishes and 1 ml of 50 mM Na acetate, pH 4.75, was added. Harvesting was carried out rapidly at 4°C. Cells were removed from the dish by scraping with a rubber spatula, the dish was rinsed with an additional 1 ml of buffer, and a tube containing the combined 2 ml of extract was immediately placed in a boiling-water bath for 5 min. Then the extract was cooled and acetylated as described previously [18]. Samples were stored frozen at −15°C. Acetylated samples were analyzed for cyclic AMP content by a radioimmunoassay method (New England Nuclear kit NEX-132).

Two-Dimensional Electrophoresis of Labeled Proteins

Epithelial cell cultures on 5-cm dishes were labeled either 2 h or 15 h in the presence of 1 ml of medium containing 0.5 mCi ^{35}S-methionine (Amersham) at 37°. Cultures were washed three times with ice-cold phosphate-buffered saline and then rapidly solubilized by scraping at 4° in the presence of staphylococcal nuclease, 0.3% sodium dodecylsulfate (SDS), 1% mercaptoethanol, deoxyribonuclease I, and ribonuclease A according to the procedure described by Garrels [19]. Samples were lyophilized, dissolved in a sample buffer composed of urea, NP-40, ampholytes (pH 6–8), and dithiothreitol, and then analyzed by the improved high-resolution two-dimensional polyacrylamide gel electrophoresis (PAGE) technique recently described by Garrels [19]. Gels were fixed and stained [20], then processed for fluorography as described by Bonner and Laskey [21]. Dried gels were exposed to flash-presensitized X-ray film (Kodak, XR-5).

Materials

Hexamethylene bisacetamide (HMBA) was generously donated by Dr. R. Reuben, Columbia University, and prostaglandin E_1 was obtained through the courtesy of Dr. J. Pike, Upjohn. Cholera toxin was purchased from Schwartz-Mann. Dimethylsulfoxide (DMSO) was from Mallinckrodt, 1-methyl-2-pyrrolidinone (MPR) was from Eastman, and N-methylacetamide was from Aldrich. Cytochalasin B was obtained from Aldrich. Sodium butyrate was prepared by neutralizing butyric acid (Fisher). Other chemicals were purchased from Sigma.

RESULTS

Cryoprotective Agents as Inducers of Dome Formation

Confluent dense cell cultures of either Rama 25 mammary cells or MDCK kidney cells exhibited a low spontaneous frequency of dome formation – less than 10 domes per 1 cm^2 [14, 15]. Spontaneously occurring domes were dependent on the presence of serum and occurred in patches on the cell monolayer, rather than at random all over the dish.

Addition of certain categories of compounds to dense cultures increased dome formation above the spontaneous level, beginning at 15–30 h (Fig. 2). Table I compares the relative dome-forming response of each cell line at the optimal concentration of each inducer. Polar solvents known as cryoprotective agents [22] and inducers of erythroid differentiation in Friend erythroleukemia cells [11, 12] were among the most effective inducers of dome formation in both the kidney and mammary cell lines. Polar solvent inducers listed in Table I differed in relative potency, both in terms of optimal concentration required for maximal induction, and the magnitude of commitment to dome formation. Thus, dimethylformamide (DMF), hexamethylene bisacetamide, and 1-methyl-2-pyrrolidinone were among the most effective, both on a concentration basis and in terms of response. Dimethylsulfoxide was moderately inductive, and acetamide and diethylene glycol (DEG) were least effective.

The number of dome foci increased quantitatively as a function of polar solvent inducer concentration in both kidney and epithelial cells, as shown in Figure 3 [14, 15]. A maximal number of domes per area was reached, characteristic of the particular inducer

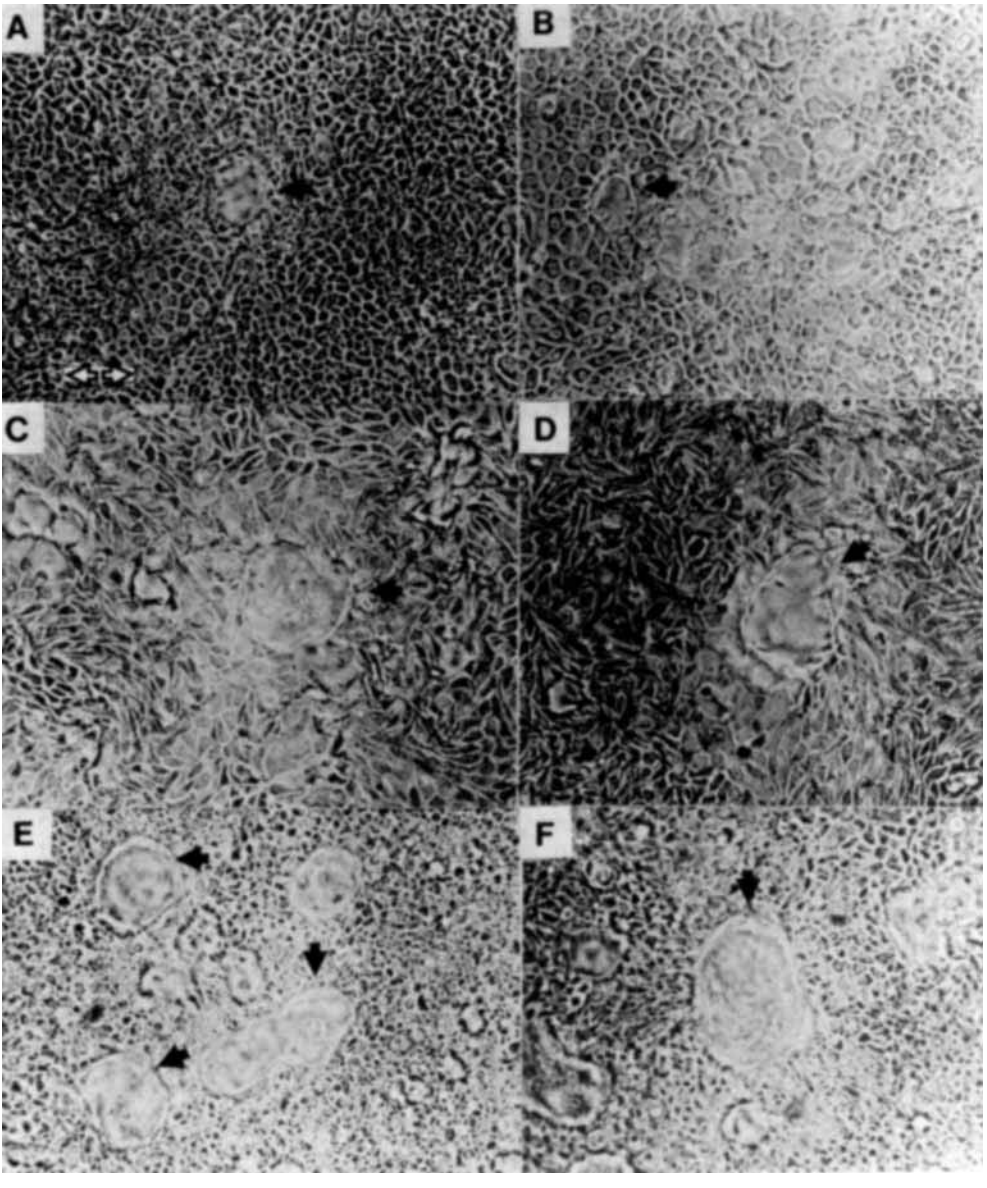

Fig. 2. Spontaneous and induced dome formation in MDCK kidney cells. A: No addition. B: Sodium n-butyrate, 2.5 mM. C: DMF, 190 mM. D: DMF, 190 mM plus cytosine arabinoside, 25 μM. E: Adenosine, 1 mM. F: Cyclic AMP, 1 mM plus theophylline, 1 mM. The indicated compounds were added with medium change to confluent cell cultures. Bar represents 100 μm. From Lever [14], with permission.

TABLE I. Categories of Inducers of Dome Formation in Mammary and Kidney Epithelial Cell Mass Cultures

Compound	Concentration (mM)	Response: Number of domes	
		Mammary cell line (RAMA 25)	Kidney cell line (MDCK)
A. Polar compounds			
Dimethylsulfoxide	200	++	+
N,N′-dimethylformamide	140	++++	++++
N,N-dimethylacetamide	20	++++	0
1,3-Dimethylurea	500	++++	++
Acetamide	1,000	+	0
1-Methyl-2-pyrrolidinone	25	++++	++
N-methylacetamide	100	++	++
Pyridine-1-oxide	50	+++	0
Diethylene glycol	90	+	0
1,1,3,3-Tetramethylurea	10	++++	0
Hexamethylene bisacetamide	5	++++	++++
B. Purines			
Hypoxanthine	1	++	++
Inosine	1	+++	++
Adenosine	1	+++	+++
C. Butyric acid	3	++	++++
D. Cyclic nucleotides			
Cholera toxin, 5 μg/ml		+	+
Prostaglandin E_1, 5 μg/ml		+	+
Dibutyryl cyclic AMP, 1 mM, plus theophylline, 1 mM		++++	++++
Dibutyryl cyclic GMP	1	0	0
8-Bromo-cyclic AMP	1	+++	+++
Theophylline	1	+++	+++
1-Isobutyl-3-methylxanthine	1	+++	+++

Symbols: ++++, highest response in terms of numbers and size of domes; +++, good response; ++, moderate response; +, weak but significant response; 0, no detectable induction of domes above spontaneous level.
From Lever [14], with permission.

Induction of domes was reversible, requiring continuous presence of inducer. After removal of inducer, the majority of domes contracted then disappeared over a period of 15–30 h, reaching an incidence corresponding to that observed without addition of in- and the cell line. Above these optimal inducer concentrations decreased dome formation and cell death was observed. The average size of domes showed much less variation with inducer concentration than the number of domes per unit area.
ducer. By contrast, these inducers triggered an irreversible differentiation of Friend erythroleukemia cells [11, 12].

Dome Formation Represents a Specific Cellular Response

Several observations indicated that domes are the result of specific cellular functions, rather than artifacts of toxicity resulting from exposure to these nonphysiologic compounds. First, induction of domes by these chemicals is a property unique to certain epithelial cell lines with the morphologic polarization, electrical resistance, and permea-

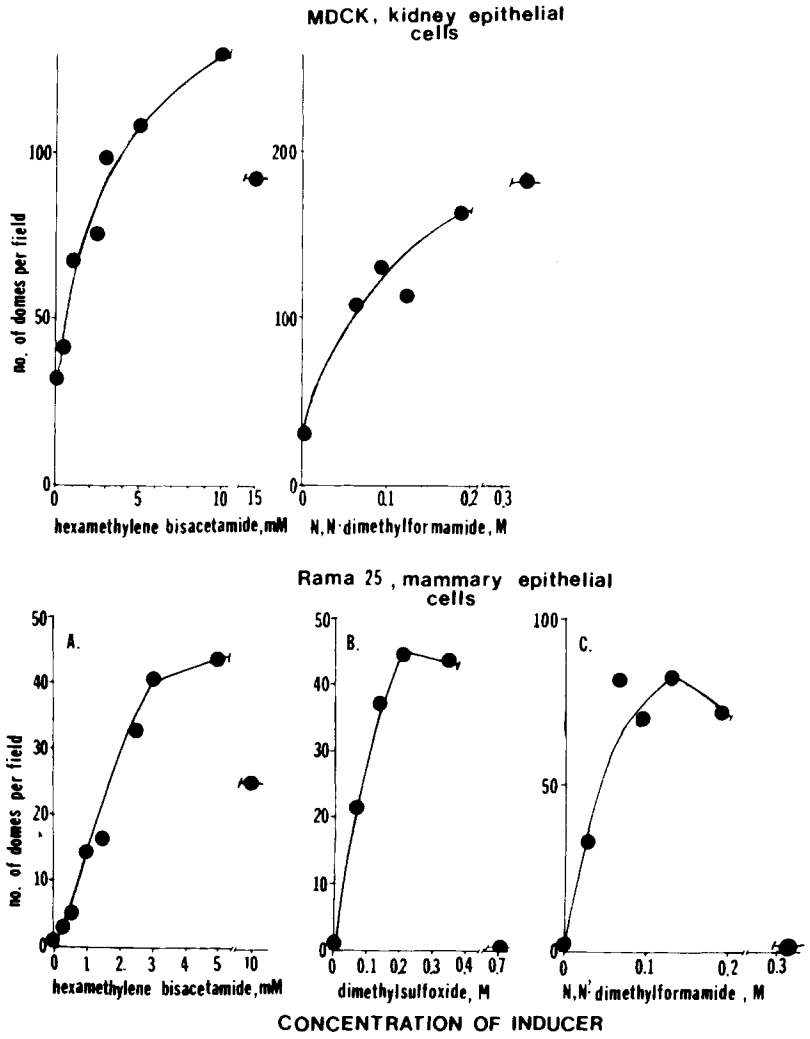

Fig. 3. Induction of domes as a function of inducer concentration. Domes were counted 3 days after addition of the indicated concentrations of inducer to confluent cultures of either Rama 25 mammary epithelial cells or MDCK kidney epithelial cells.

bility properties of transporting epithelia. None of the compounds listed in Table I caused dome formation in either 3T3 fibroblasts, BSC-1 epithelial cells, or certain non-dome-forming cell sublines (Rama 29, Rama 30) which could be isolated from Rama 25 cultures [14, 15].

Second, addition with inducer of inhibitors of protein synthesis (such as cycloheximide or puromycin), or the Na^+ pump inhibitor ouabain, or any one of the cytoskeletal disruptive agents cytochalasin B, colcemid, and vinblastine, blocked the chemical induction of domes [14, 15]. If any of these inhibitors were added to cultures after the appearance of domes induced by DMF, domes largely disappeared from the culture. Thus, protein synthesis,

cytoskeletal organization, and Na^+,K^+ ATPase activities were required for induction and maintenance of domes. Finally, domes resulting after chemical induction resembled morphologically those which occurred spontaneously (Fig. 2).

By contrast, DNA synthesis was not a requirement for chemical induction of domes. Thus, DMF converted over 50% of the cells in the culture to participation in dome formation. Addition with DMF of concentrations of hydroxyurea or cytosine arabinoside that inhibited ^{3}H-thymidine incorporation by 99% did not affect the final number of domes in the culture [14, 15].

Dome Formation by Cell Subpopulations

Induction by these chemicals did not occur as the result of selective proliferation of cells which could make domes spontaneously. Rather, additional cell subpopulations were induced to make domes. This conclusion was based on the experiment shown in Table II. Individual colonies were first isolated, then grown without inducer for 11 days. Then colonies were tested for inducibility by these chemicals. Table II shows that 13.5% of colonies tested formed domes in the total absence of inducer in the case of the mammary cell line and 16.6% in the case of the kidney cell line. This indication that only a certain subpopulation of cells can form domes spontaneously may account for the patch-like occurrence of spontaneous dome formation in the parental culture. Addition of either 140 mM DMSO or 190 mM DMF to colonies grown nonselectively greatly increased the incidence of dome-forming colonies in both the mammary epithelial cells and the kidney epithelial cells. Since in this protocol all colonies tested were isolated before exposure to inducer, the inducer is not acting by selecting the survival of colonies. Therefore, the finding that increased numbers of colonies form domes in the presence of inducer compared with spontaneous incidence suggests that these inducers recruit an increased number of preexisting cells in the population to form domes compared with the number capable of forming domes spontaneously. The conclusion that inducers are not acting

TABLE II. Frequency of Colonies Which Form Domes Spontaneously or in Response to Various Inducers

Addition	Number of colonies tested	Average % colonies with domes
A. RAMA 25 (mammary)		
No addition	2,143	13.5
Dibutyryl cyclic AMP, 0.2 mM, plus theophylline, 1 mM	1,091	44.0
Butyrate, 2.5 mM	640	76.5
DMSO, 140 mM	1,097	58.0
DMF, 190 mM	1,099	88.0
B. MDCK (kidney)		
No addition	380	16.6
DMSO, 140 mM	297	44.8
DMF, 190 mM	400	58.3

Isolated colonies were obtained by plating 200 cells per 90-mm dish in the absence of inducer. The cloning efficiency of MDCK cells varied from 60 to 100% and that of RAMA 25 cells varied from 32 to 37%. At 11 days after plating, when colonies were 0.5–1 cm in diameter, the medium was changed and the indicated additions were made. Cultures were fixed and stained after 3 days. Data from Lever [14].

by allowing selective proliferation of cell populations that form domes spontaneously is reinforced also by the observation that inhibition of DNA synthesis does not block the chemical induction of domes.

As a corollary, these data show that certain subpopulations of cells do not make domes in the presence of inducers. For example, Table II shows that 50–60% of MDCK colonies do not form any domes in the presence of either DMSO or DMF.

Another point concerning these data should be noted. Different inducers, including categories discussed below, stimulated different numbers of Rama 25 colonies to form domes. Since the Rama 25 cell line, a putative stem cell line of the mammary gland which undergoes a reproducible differentiation in culture to other cell types [6] was derived from a single cell by two rigorous cloning procedures [6], this observation suggests the possibility that subpopulations of the culture responding to different inducers may arise in vitro with high frequency.

Induction of Domes by Purines

Certain purines and their derivatives caused increased dome formation (Table I). Adenosine was one of the most effective inducers in this category.

Cyclic AMP as a Positive Regulator of Dome Formation

A possible clue to physiologic mechanisms involved in dome formation was provided by the observation that various conditions expected to elevate intracellular levels of cyclic AMP also caused increased incidence of domes in both the mammary and kidney cell culture systems. Thus, compounds known to activate adenylate cyclase activity, such as cholera toxin [23] and prostaglandin E_1 [24] also produced a small but reproducible increase in dome formation. Inhibitors of cyclic AMP phosphodiesterase activity [25], such as theophylline and 1-methyl-3-isobutylxanthine, caused a much more dramatic increase in dome formation. Furthermore, analogs of cyclic AMP, such as $N^6O^{2'}$-dibutyryl cyclic AMP or 8-bromo-cyclic AMP, caused an increased dome formation which was potentiated after the further addition of phosphodiesterase inhibitors. It seems unlikely that the inductive effects of dibutyryl cyclic AMP are due to breakdown to butyric acid, itself an inducer, since the same concentration of dibutyryl cyclic GMP was ineffective (Table I). Furthermore, this concentration of dibutyryl derivative would yield 1 mM butyric acid upon complete hydrolysis, a concentration of butyrate below the level required for induction.

Interestingly, several, but not all, of the polar solvent category of inducers, as well as n-butyrate, caused an elevation of cyclic AMP levels (Table III). While this unexpected observation may partially explain their inductive effects according to the hypothesis that cyclic AMP levels regulate dome formation, it does not explain the much greater dome-forming response observed with these chemicals compared with that observed to accompany similar levels of cyclic AMP triggered by other agents.

Changes in Synthesis of Specific Proteins Accompanying Dome Formation

Total cellular proteins labeled biosynthetically with ^{35}S-methionine were analyzed by high-resolution two-dimensional polyacrylamide gel electrophoresis. Two narrow pH ranges, pH 5–7 (shown in Fig. 4) and 6–8, were used, each range resolving an average of 395 and 495 spots, respectively, after detection by fluorography – with a region of overlap between the two ranges. Figure 4 compares the pattern obtained after Rama 25 cells were induced to form domes with DMF (50% of the cells in the culture participating in domes) compared with uninduced controls. The arrow indicates a region where major changes in levels of specific proteins were observed in preliminary experiments.

TABLE III. Intracellular Levels of Cyclic AMP in the Presence of Inducers of Dome Formation: RAMA 25 Mammary Cells

Addition	Cyclic AMP[a] (pmoles/mg protein)
Medium change only	1.67 ± 0.15
Theophylline, 1 mM	2.07 ± 0.20
1-Methyl-3-isobutylxanthine, 1 mM	3.06 ± 0.39
Cholera toxin, 5 μg/ml	2.22 ± 0.19
n-Butyrate, 3 mM	3.03 ± 1.1
Dimethylformamide, 190 mM	3.15 ± 0.70
Dimethylsulfoxide, 140 mM	1.77 ± 0.44
Hexamethylene bisacetamide, 5 mM	3.43 ± 1.0

[a]Determinations were made using extracts from confluent RAMA 25 cells 24 h after addition of inducer, as described under Materials and Methods. Values are mean ± SD.

This approach may reveal groups of proteins that are necessary for induction, or that are necessary for maintenance and functional expression of domes, as a preliminary to their biochemical or immunologic identification.

DISCUSSION

Several eukaryotic cell culture systems have been proposed as models to study stages of cell differentiation in terms of molecular events [26]. Whereas in many studies it has not been possible to control either the direction or degree of differentiation, in certain cases several compounds have been identified which trigger a greater magnitude of differentiation of the cell population at a defined time, greatly simplifying experimental approaches.

The pattern of differentiation in these model systems depends on the nature of the cell type rather than the nature of the inducer. Various categories of inducers which promote a program of differentiation in Friend erythroleukemia cells include polar cryoprotective solvents, butyric acid, purines, ouabain, and actinomycin D [11]. Erythroid cultures induced by these compounds undergo a partially normal program of terminal erythroid differentiation leading to heme production, changes in surface glycoproteins, spectrin production, synthesis of globin mRNA and globins, increase in activities of heme synthetic enzymes, and synthesis of hemoglobins. Many of these same inducers also promote neurite extension in neuroblastoma cells [13]. The optimal concentration and relative magnitude of cellular response for each inducer of dome formation in polarized epithelial cell cultures is strikingly similar to their optimal concentration and relative effectiveness in triggering different programs of differentiation characteristic of other cell lines such as neuroblastoma or Friend cells.

Another demonstrated similarity between induction of Friend cell differentiation and induction of domes is that in both cases it has been demonstrated that each inducer triggers only a portion of the cell population to undergo differentiation [11]. These systems differ in that induced dome formation is reversible, whereas erythroid differentiation is terminal [11]. Also, ouabain, a Friend cell inducer [11], blocks dome formation, possibly by direct inhibition of transport systems necessary to maintain domes.

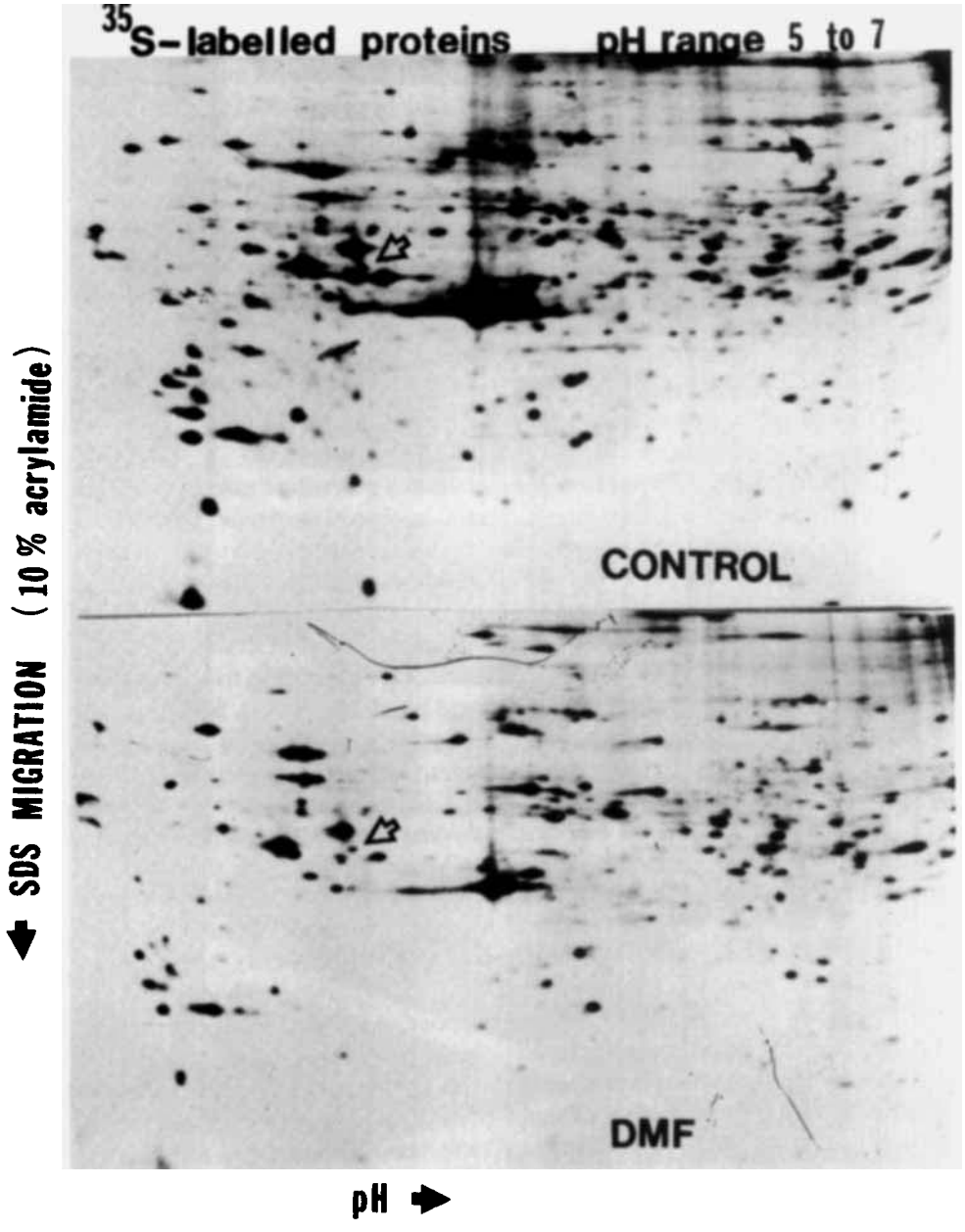

Fig. 4. Two-dimensional polyacrylamide gel electrophoresis of total proteins from Rama 25 mammary epithelial cells after induction of domes by DMF compared with uninduced controls. Cultures were labeled 15 h with ^{35}S-methionine added at 3 days after addition of 1.5% DMF with medium change. Isoelectric focusing was carried out using pH 5–7 ampholytes. Total labeled protein applied to each gel, estimated after precipitation of an aliquot with 5% trichloroacetic acid, was 1.15×10^5 cpm for the control and 8.8×10^4 cpm for the DMF-induced culture.

The degree of experimental manipulation provided by the identification of inducers of dome formation, as well as the use of clonal epithelial cell cultures, should provide a new approach to investigation of differentiation of epithelial cells into polarized fluid-transporting cells. The Rama mammary cell line has been reported to further differentiate in vitro into several morphologically discrete, nonpolarized mammary epithelial cell types [6].

It is not known whether the program of events leading to dome formation in cell culture resembles the normal pattern of secretory epithelial development. Also, it is not known whether changes in any biochemical or functional markers accompany dome formation.

A major question is whether these inducers affect the various patterns of differentiation characteristic of different cell types by similar mechanisms. Furthermore, within a single cell system it is pertinent to ask whether each category of inducer acts by a different mechanism or whether the effects of these compounds intersect within a common pathway.

The polar solvent inducers have nonspecific and generalized effects, making it difficult to identify their obligatory targets. Several mechanisms – including conformational changes in DNA or chromatin proteins [27] or plasma membrane structures [28] induced by solvent effects on water structure – have been proposed. DNA breakage has been proposed to explain their action on Friend cell differentiation [29].

Reuben et al [30] have examined the relationship between structure and activity of the polar solvent inducers of erythroid differentiation. Conclusions from this study were that effective inducers contained both planar and polar components, with optimal activity observed when polar groups were separated by a polymethylene chain of 5–6 methylene residues as in HMBA. Since HMBA can be taken up and metabolized by cells [31], a metabolic product of this molecule may be the active inducer.

Herskovits et al [32] have measured unfolding of globular proteins by monoalkyl- and dialkyl-substituted formamides and ureas. There does not appear to be a direct correlation between the effectiveness of these compounds as inducers of differentiation and their ability to unfold proteins as estimated by solvent denaturation midpoints.

In addition to its effects on erythroid induction in Friend cells [11], sodium butyrate also affects granulopoiesis of mastocytoma cells [33], and causes induction of alkaline phosphatase [34], morphologic transformations, and altered ganglioside sialyltransferase activity [35] in HeLa cells. Sodium butyrate inhibited cytochalasin B-induced multinucleation in NRK (normal rat kidney) cells [36]. Altenberg et al [37] reported that butyrate reversed the morphology of virally transformed cells to normal, accompanied by a striking elaboration of cytoplasmic microfilaments and microtubules. n-Butyrate has been reported to inhibit histone deacetylation [38, 39]. Butyrate and purines such as adenosine may act by elevating cyclic AMP levels, as reported for other cell systems [40, 41].

Observations that some, but not all, inducers caused elevated levels of cyclic AMP, taken together with the finding of sublines of cells which respond to DMF but not to DMSO, suggest that multiple mechanisms may operate. Furthermore, examples could be found where different types of inducers triggered a similar elevation of intracellular cyclic AMP levels but caused a markedly different final response in terms of numbers of domes. However, the possibility remains that different cell types in these cultures may differ in their individual cyclic AMP levels. Therefore, estimations of intracellular cyclic AMP carried out using the total cell population may not reveal a specific response of a target cell.

Although these phenomena are complex, these families of cell lines and categories of inducers will provide a useful experimental system for correlation of biochemical changes which accompany induction of domes. Specific parameters which may be investigated in this model system are 1) the synthesis and regulation of specialized junctions; 2) the synthesis of functionally and structurally polarized plasma membranes; and 3) the coupling of transport and hormone receptor-effector systems that are possibly present on opposite sides of the cell.

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REFERENCES

1. Leighton J, Brada Z, Estes LW, Justh G: Science 163:472, 1969.
2. Leighton J, Estes LW, Mansukhani S, Brada Z: Cancer 26:1022, 1970.
3. Enami J, Nandi S, Haslam S: In Vitro 8:405, 1973.
4. Visser AS, Prop FJA: J Natl Cancer Inst 52:293, 1974.
5. Owens RB, Smith HS, Hackett AJ: J Natl Cancer Inst 53:261, 1974.
6. Bennett DC, Peachey LA, Durbin H, Rudland PS: Cell 15:283, 1978.
7. Misfeldt DS, Hamamoto ST, Pitelka DR: Proc Natl Acad Sci USA 73:1212, 1976.
8. Cerijido M, Robbins ES, Dolan WJ, Rotunno CA, Sabatini DD: J Cell Biol 77:853, 1978.
9. McGrath CM: Am Zool 15:231, 1975.
10. Pickett PB, Pitelka DR, Hamamoto ST, Misfeldt DS: J Cell Biol 66:316.
11. Marks PA, Rifkind RA: Annu Rev Biochem 47:419, 1978.
12. Marks PA, Rifkind RA, Terada M, Reuben RC, Gazitt Y, Fibach E: In Golde DW, Cline MJ, Metcalf D, Fox CF (eds): "Hematopoietic Cell Differentiation." New York: Academic, 1978, p 25.
13. Palfrey C, Kimhi Y, Littauer UZ, Reuben RC, Marks PA: Biochem Biophys Res Commun 76:937, 1977.
14. Lever JE: In Ross R, Sato G (eds): "Cold Spring Harbor Conference on Cell Proliferation," vol 6. Cold Spring Harbor, New York: Cold Spring Harbor Press, 1979, p 727.
15. Lever JE: Proc Natl Acad Sci USA 76:1323, 1979.
16. Gaush CR, Hard WL, Smith TF: Proc Soc Exp Biol Med 122:931, 1966.
17. Holley RW, Kiernan JA: Proc Natl Acad Sci USA 71:2908, 1974.
18. Harper JF, Brooker GJ: J Cyclic Nucleotide Res 1:207, 1975.
19. Garrels J: J Biol Chem 254:7961, 1979.
20. Fairbanks G, Steck TL, Wallach DFH: Biochemistry 10:2606, 1971.
21. Bonner WM, Laskey RA: Eur J Biochem 46:83, 1974.
22. Nash T: J Gen Physiol 46:167, 1962.
23. Finkelstein RA: Crit Rev Microbiol 2:553, 1973.
24. Kantor HS, Tao P, Kiefer HC: Proc Natl Acad Sci USA 71:1317, 1974.
25. Chasin M, Harris DN: In Greengard P, Robison GA (eds): "Advances in Cyclic Nucleotide Research." New York: Raven, 1976, p 225.
26. Newmark P: Nature 272:756, 1978.
27. Terada M, Nudel U, Fibach E, Rifkind RA, Marks PA: Cancer Res 38:835, 1978.
28. Lyman GH, Preisler HD, Paphadjopoulos D: Nature 262:360, 1976.
29. Scher W, Friend C: Cancer Res 38:841, 1978.
30. Reuben RC, Khanna PL, Gazitt Y, Breslow R, Rifkind R, Marks PA: J Biol Chem 253:4214, 1978.

31. Reuben RC, Marks PA, Rifkind RA, Terada M, Fibach E, Nudel U, Gazitt Y, Breslow R: In Ikawa Y (ed): "Oji International Seminar on Genetic Aspects of Friend Virus and Friend Cells." New York: Academic, 1978.
32. Herskovits TT, Behrens CF, Siuta PB, Pandolfelli ER: Biochim Biophys Acta 490:192, 1977.
33. Mori Y, Akedo H, Tanaka K, Tanigaki Y, Okada M: Exp Cell Res 118:15, 1979.
34. Griffin MJ, Price GH, Bazzell KL, Cox RP, Ghosh NK: Arch Biochem Biophys 164:619, 1974.
35. Simmons JL, Fishman PH, Freese E, Brady RO: J Cell Biol 66:414, 1975.
36. Altenberg BC, Steiner S: Exp Cell Res 118:31, 1979.
37. Altenberg BC, Via DP, Steiner SH: Exp Cell Res 102:223, 1976.
38. Boffa LC, Vidali G, Mann RS, Allfrey VG: J Biol Chem 253:3364, 1978.
39. Candido EPM, Reeves R, Davie JR: Cell 14:105, 1978.
40. Clark RB, Seney MN: J Biol Chem 251:4239, 1976.
41. Storrie B, Puck T, Wenger L: J Cell Physiol 94:69, 1978.