CYCLIC AMP AND CELL MORPHOLOGY
IN CULTURED FIBROBLASTS

Effects on Cell Shape, Microfilament and Microtubule Distribution, and Orientation to Substratum

MARK C. WILLINGHAM and IRA PASTAN

From the Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20014

ABSTRACT

The change in shape of 3T3 and L929 cells due to Bt2cAMP treatment is accompanied by altered intracellular distribution of microfilaments and microtubules. Bt2cAMP added to cells in low density culture causes (a) microfilaments to accumulate in bundles near the plasma membrane, mainly at the cell periphery, and (b) microtubules to accumulate beneath these microfilament bundles. In narrow cell processes that form characteristically in Bt2cAMP-treated L cells, microtubules accumulate in parallel arrays near the center of these processes. A new simple method for evaluating the relative distance of the cell from its underlying substratum is described. In normal medium, 3T3 cells attach to their substratum near the nucleus and at the tips of cell processes, bridging irregularities in the plastic surface. With Bt2cAMP treatment, attachment occurs at the cell edge and at many isolated points under the cytoplasm, and the cells conform more closely to irregularities of the underlying substratum. A model of the mechanism by which cAMP modulates cell shape is presented.

Cyclic 3',5' adenosine monophosphate (cAMP) regulates the shape (4, 5, 6, 9), motility (7), adhesiveness (8), growth rate (5, 9, 14, 19), and agglutination by plant lectins (4, 10, 18, 22) of fibroblastic cultured cells. The control of cell shape by cAMP has been extensively investigated at the light microscope level (5, 6, 9, 19). Recently, other studies have appeared which describe changes at the ultrastructural level that were due to treatment with Nβ,Oβ-dibutylryl cyclic adenosine 3',5' monophosphate (Bt2cAMP) or to events associated with changes in cAMP levels. Evans et al. (3) described the appearance of surface microvilli and aggregation of polysomes after insulin treatment, which, among other effects, probably causes a fall in intracellular cAMP levels. McNutt et al. (11) showed that normal Balb 3T3 or SV40-revertant Balb 3T3 cells had numerous cytoplasmic microfilaments which were diminished in an SV40-transformed derivative. cAMP levels have been shown to be high in these normal or revertant lines and low after transformation (14, 19). Porter et al. (15) described changes in surface microstructure during the cell cycle in Chinese hamster ovary cells, showing relatively few surface microvilli during S phase and more of these structures in other phases of the cell cycle. Changes in cAMP have been described in other cell lines during the cell cycle (2), the highest level generally occurring in S phase and the lowest in mitosis. More recently, Porter et al. (16) have detailed the effects of Bt2cAMP treatment on Chinese hamster ovary cells, and have shown the organization of microtubules into parallel alignment in processes of
these cells after the addition of Br2cAMP. From these studies it was suggested that cAMP influences the organization of intracellular microfilamentous and microtubular systems, and the microstructure of the cell surface.

The previous literature, however, has failed to show a central regulatory function for cAMP on both microfilamentous and microtubular systems in the same cell. Furthermore, cAMP produces different shape changes in different cell lines; some cells become spindly after cAMP treatment, while other cells flatten on their underlying substratum.

We have investigated the morphologic effects of cAMP in two cell lines. 3T3-4 cells are typical of cells which show flattened morphology after Br2cAMP treatment. L929 cells, on the other hand, typify the spindly response to elevating cAMP levels. In this paper we describe the ultrastructural changes induced by Br2cAMP in both of these cell lines. We also describe additional effects that Br2cAMP exerts on the orientation of 3T3 cells to their substratum. Using these and other previously demonstrated results, we discuss possible mechanisms by which cAMP controls the shape and motility of cells.

MATERIALS AND METHODS

Cells

3T3-4 and L929 cells were propagated as previously described (6, 20). The cells were grown at 37°C in 20-cm² Falcon plastic dishes in Dulbecco-Vogt's modified Eagle's medium supplemented with 10% calf serum (Colorado Serum Co., Denver, Colo.) and penicillin-streptomycin (Flow Laboratories, Inc., Rockville, Md.) (50 U/ml each). L929 cells were subcultured either by removal by spraying with medium, or by trypsinization with 0.25% trypsin (Microbiological Associates, Bethesda, Md.). 3T3-4 cells were subcultured by treatment with 0.25% trypsin. For all morphologic studies, the cells were used only when they had been growing for at least 48 h after their last exposure to trypsin. Br2cAMP was prepared and included in the growth medium as previously described (6). Phase-contrast micrographs of these cells were taken with a Nikon or Zeiss inverted phase-contrast microscope using Polaroid (type 57 or 107) film. Shape changes of the type reported were present in over 90% of the cells examined in a minimum of 10 experiments at the light microscope level.

Processing of Cells for Transmission Electron Microscopy

Cells still attached to the culture dish were fixed at 15°C, after pouring off the medium, with 6% (0.1 M Na cacodylate-buffered, pH 7.4) glutaraldehyde (Fisher Scientific Co., Fairlawn, N. J.). They were postfixed in 2% (0.2 M collidine-buffered) OsO₄ and serially dehydrated in ethanol. In situ embedding in Epon 812 was performed by using graded additions of Epon-ethanol mixtures; the final polymerization was carried out at 55°C to prevent distortion of the plastic dish. The Epon was then separated from the plastic by torsional bending of a dish warmed to 45°C. The resulting circular block of Epon with cells on the bottom surface was cut into small blocks with a razor saw, each being mounted in the vise-chuck of a Sorvall MT2-B ultramicrotome. Side (perpendicular) sectioning required coating the cell side of the block with more liquid Epon mix and polymerizing at 55°C to provide edge support for these sections on 200-mesh copper grids. This in situ embedding method allowed us to section all cells in a controlled manner parallel or perpendicular to the substratum. Thin sections (~1000 Å, silver-gold) were cut with a Dupont diamond knife (E. I. Dupont de Nemours & Co., Wilmington, Del.) and were poststained with uranyl acetate and lead citrate. These sections were examined with a Hitachi HU-12A electron microscope. Electron microscope studies were performed in four to five separate experiments for each cell type. The morphologic changes reported occurred in over 90% of the cells examined. A minimum of 25 cells in each experiment were examined and photographed.

Method for Cell Orientation to the Substratum

Blocks of cells embedded in situ in Epon could be stained for light microscopy by diffusion of toluidine blue (0.5% in 1% sodium borate, pH 11) into the block at 60-80°C. Blocks were overlaid with this staining solution and allowed to incubate in an oven for various lengths of time. 2-3 h were required for this stain to diffuse completely through the single layer of cells adjacent to the surface of the block. At various times these blocks were removed and washed in H₂O, and the cell components that had been stained up to that time were observed with light microscopy and photographed. In this way, those components of the cell closest to the block surface, and hence the former substratum, were stained first, followed later by staining of components farther away. A minimum of 200 cells were examined with this method in each of three experiments. Even though the plastic substratum was far from smooth, the accuracy of this method in showing the actual spatial relationship of cell components to the substratum was confirmed by perpendicular sectioning of cells embedded in situ. The toluidine blue method is a simple way of establishing which parts of the cell are touching or closest to the underlying substratum.

Since the surface of different parts of the dish varied considerably in irregularity, some being quite smooth, the degree of staining varied from cell to cell. Most early staining occurred after 30-60 min, and later staining after 3 h.
RESULTS

Overall Shape Changes Due to Addition of Bt$_2$cAMP

In normal medium containing 10% serum, L929 cells are very motile (7) and appear round or ovoid in shape (Fig. 1 a). Occasionally, these cells protrude short processes and establish new attachment points to continue their migration. 3T3-4 cells also form processes, but these are generally flatter than in L cells (Fig. 2 a). The effect of Bt$_2$cAMP treatment on the morphology of these two cell lines has been reported before (6), but here we show the cells at low density to prevent cell-to-cell contact phenomena from interfering with the shape changes due solely to cAMP. L929 cells become much more spindly in shape after 24 h in medium containing 1 mM Bt$_2$cAMP (Fig. 1 b). 3T3-4 cells, on the other hand, become extremely flat and spread out over the substratum (Fig. 2 b). These morphologic changes have been shown to be accompanied by increased adhesiveness to the plastic substratum (L cells [8]; 3T3 cells, unpublished observations). Since these two cell lines are typical of the spindly (L929) and flat (3T3) morphologic responses to Bt$_2$cAMP, we used them to evaluate the accompanying ultrastructural changes produced by Bt$_2$cAMP.

Shape Changes Due to a Fall in Cyclic AMP Levels

The morphologic response to treatment with Bt$_2$cAMP is slow. Extensive process formation and flattening is evident only after treatment for 24 h or longer. In contrast, retraction of cell processes occurs more rapidly. Johnson et al. (7) have reported that 20 min after replacement of Bt$_2$cAMP-containing medium with normal medium, L929 cells have retracted almost all their extended processes. Willingham et al. (20) have isolated a mutant of 3T3 cells (3T3cAMP$^{m}$) in which cyclic AMP levels were observed to fall from 42 to 27 pmol/mg nucleic acid 2 min after a dish of cells was shifted from a 39°C incubator to 23°C. They found that adhesiveness was unchanged until 5 min after the temperature shift, when it diminished. Cell processes remained extended until 15 min after temperature shift and then retracted. We have now further investigated the relationship of a fall in cyclic AMP levels to process retraction, employing these 3T3cAMP$^{m}$ cells. In one set of experiments, we changed the temperature of these cells and immediately sprayed them with overlying medium to mechanically detach cell processes. Upon detachment, the cell processes retracted slightly, but were still in the extended form. Later, 10–15 min after the initial temperature change, full retraction occurred. The retraction of any one process required 10–30 s. In another set of experiments we treated these cells with EDTA immediately after temperature change to decrease their adhesiveness to the substratum (22). Decreased adhesiveness was confirmed since the cells could be readily removed from the substrate by gently shaking 5 min after EDTA treatment. The cell processes did not begin to retract until 10–15 min after the temperature was changed and cyclic AMP levels had fallen, whether the cells were still loosely attached to or had been shaken free of the substratum.

Preincubation of these cells for 15 min with L

![Figure 1](https://example.com/figure1.png)  
**Figure 1** Cell shape of L929 cells with and without Bt$_2$cAMP treatment. L929 cells were grown in 10% calf serum medium alone (a) or with 1 mM Bt$_2$cAMP for 24 h (b). Phase contrast; $\times$ 260.
mM Bt2cAMP or a phosphodiesterase inhibitor (methylisobutylxanthine, 0.5 mM) prevented the retraction of cell processes (20). This treatment also prevented the retraction of cell processes after disattachment with mechanical agitation or EDTA treatment. All these experiments suggest that full retraction of cell processes requires some active metabolic process and that a fall in cyclic AMP levels does not induce this process in a manner analogous to release of a "coiled spring" from the substratum.

Effects of Prior Trypsin Treatment

We studied the effect of Bt2cAMP treatment on morphology only after cells were cultured for longer than 48 h after their last exposure to trypsin, because the shape of both L929 and 3T3 cells is affected by exposure to trypsin. During the first 24 h after cells are subcultured with 0.25% trypsin, both L and 3T3 cells are very flat and appear similar to Bt2cAMP-treated 3T3 cells (results not shown). Their shape does not return to "normal" until after the next mitotic event; after trypsin treatment some cells may not divide for up to 48 h. The morphology of the cells is then stable as long as cell-to-cell contact does not occur. The fact that trypsinized cells display an altered morphology for up to 48 h raised the possibility that membrane-bound enzymes, such as adenylyl cyclase and cyclic AMP phosphodiesterase (17) (and hence cyclic AMP levels), may also be affected for a prolonged period. Indeed, it is possible that the flattened morphology produced by trypsin treatment may be due to high cAMP levels.

Ultrastructural Changes after Bt2cAMP Treatment

After Bt2cAMP treatment, we observed consistent alterations in the ultrastructural morphology of microfilamentous and microtubular systems, reported here, and microvillin changes, reported elsewhere (23).

In both L929 cells (Fig. 3 a) and 3T3-4 cells (Fig. 4 a), a layer of submembranous microfilaments is apparent under the plasma membrane, being much more prominent in 3T3 cells (Fig. 4 a). These microfilaments were identified at higher magnification to be of the 50-70-Å type (results not shown). The width of the microfilament region at the cell edge increases after Bt2cAMP treatment (1 mM, 24 h). Submembranous microfilaments are arranged in dense bundles under the plasma membrane at the lateral cell borders in both cell types (Figs. 3 b and 4 b).

In untreated cells, occasional microtubules are evident deeper in the cytoplasm than microfilaments (Figs. 3 a and 4 a). After Bt2cAMP treatment, the number of microtubules appears to increase (Figs. 3 b and 4 b), but the difference is not dramatic in 3T3 cells (Fig. 4 b). Particularly striking is the alignment of these microtubules into parallel arrays in the center of cell processes, dramatically evident in the spindly, long cell processes of L929 cells (Fig. 5).
FIGURE 3  Ultrastructural appearance of the edge of an L929 cell process with and without Bt-cAMP treatment. L929 cells were cultured with (b) or without (a) 1 mM Bt-cAMP for 24 h, processed for sectioning parallel to the substratum, and examined with transmission electron microscopy, as described in Materials and Methods. In normal medium (a), L cells display occasional microtubules (long arrow) and a few, barely visible 70-Å microfilaments (short arrow) near the cell edge. After Bt-cAMP treatment for 24 h (b), the cell edge shows prominent areas of density discernible, under close inspection, as 70-Å microfilaments (short arrow). Microtubules (long arrow) increase dramatically in number, being arranged in longitudinal arrays deeper within the cytoplasm. \( \times 50,000; \text{bar} = 0.2 \mu m. \)

Bundles of microfilaments and microtubules are also evident in sections taken perpendicular to the substratum which show submembranous dense microfilament bundles at the edge of Bt-cAMP-treated 3T3 cells (Fig. 6). This perhaps explains the high density of the cell edges observed in flattened 3T3 cells with phase microscopy (Fig. 2 b).

In Bt-cAMP-treated 3T3 cells, these microfilament bundles were most pronounced at the edges of flattened cells. This could come about by
FIGURE 4. Ultrastructural appearance of the edge of a 3T3-4 cell with and without Bt2cAMP treatment. 3T3-4 cells were cultured with (b) or without (a) 1 mM Bt2cAMP for 24 h, and processed and examined with transmission electron microscopy as for Fig. 3. In normal medium (a), loose mats or webs of 70-Å microfilaments lie just beneath the plasma membrane both at the cell edge (as shown here, short arrows) and over the rest of the cell (not shown). Microtubules (long arrow) appear sporadically, usually oriented longitudinally and deeper in the cytoplasm aligned following extended cell processes. After treatment with Bt2cAMP for 24 h (b), the edge of the flattened cell shows a large mass of 70-Å microfilaments bundled just under the plasma membrane (short arrow), and the microtubules deeper in the cytoplasm appear more numerous and more uniformly aligned. This effect on microtubules is considerably more difficult to quantitate than the accumulation seen in L929 cells after Bt2cAMP treatment (Fig. 3 b). 100-Å microfilaments can be seen usually associated with microtubules deep in the cytoplasm (a) but their number or arrangement does not appear strikingly changed after Bt2cAMP treatment. × 50,000; bar = 0.2 μm.
redistribution of previously randomly arranged microfilament mats into dense clusters at the cell periphery or by the assembly of new microfilaments. Furthermore, microtubular elements seemed to accompany the microfilament bundles suggesting that they, too, might have undergone some intracellular redistribution. In flattened 3T3 cells, these dense microfilament bundles also occurred next to the substratum at locations away from the cell edge and closer to the nucleus, resulting in the long, straight, fibrous bundles previously described as “stress fibers” (Fig. 9 a) (1). These bundles are seen in perpendicular section in Fig. 7 b.

In summary, the ultrastructural changes in L cells after Bt2cAMP treatment consisted of an accumulation of microtubules, particularly in the center of spindly processes (Fig. 5), with a smaller accumulation of microfilaments along the lateral cell border (Fig. 3 b). However, in 3T3 cells after this treatment there was a dramatic accumulation of microfilaments along the lateral cell border (Figs. 4 b, 6, 7 b), with a less striking effect on microtubules (Fig. 4 b).

**Orientation of 3T3 Cells to the Substratum before and after Bt2cAMP Treatment**

In observing cells in perpendicular section (Fig. 7), it became evident that the plastic surface itself was quite irregular, with “hills” and “valleys.” At low density, 3T3 cells generally were attached to the hills of the plastic surface. Attachment points were at the tips of cell processes, under the nucleus and occasionally in between, depending on the irregularities in the substratum (Fig. 7 a). Generally, the surface irregularities (valleys) varied in width from one-fifth to one-twentieth of the length of a stretched cell. By using the toluidine blue diffusion technique and light microscopy, the...
points of closest apposition of the cell to its substratum could be evaluated in many cells. Most 3T3 cells with one or two extended processes showed consistent close apposition of the cell process tip to the substratum and single or multiple central points of attachment near the nucleus (Fig. 8). Further diffusion of toluidine blue gradually stained more and more of the intervening cytoplasm, indicating that either this portion of the cell was elevated off the plastic or that the cell was bridging a valley in the plastic surface. The latter has been shown to be more likely by perpendicular sectioning and by observation at the ultrastructural level (Fig. 7a).

After Bt2cAMP treatment, the orientation of 3T3 cells to their substratum was strikingly different. The toluidine blue staining method shows that the entire peripheral cell edge is in close apposition to the substratum (Fig. 9). In addition, the cells are attached at multiple locations near the nucleus. Particularly noteworthy is the fact that the cells are often in close apposition to the substratum near bundles of microfilaments. In perpendicular section, the closeness of the cell to the substratum was confirmed. The cells were attached to the substratum down in the valleys as well as on the hills. These differences in orientation to irregularities were present in 90% of the cells examined over these valleys (a minimum of 20 cells in each experiment). The toluidine blue method allows resolution of the small differences in cell orientation to the substratum. It also demonstrates the arrangement of microfilament bundles in flattened cells. Most of the bundles over the cytoplasm are oriented pointing to either the corners of the flattened cell or to some isolated point in the interior of the cell (Fig. 9).

In contrast to 3T3 cells, the relationship of L929 cells to the irregularities in the substratum was variable. Some of the cells bridged the irregularities and some did not. Treatment with Bt2cAMP had no obvious effect on this relationship.

DISCUSSION

In this paper we have investigated the effect of Bt2cAMP treatment on the morphology of L929 and 3T3 cells by both light and electron microscopy. We have also investigated the effect of Bt2cAMP on the relationship of these cells to their underlying substratum. We find that Bt2cAMP treatment promotes the extension of existing cellular processes. Bundles of microfilaments accumulate in intimate association with the plasma membrane of these processes, and microtubules accumulate deeper in the cytoplasm. Our results indicate that cyclic AMP acts to determine cell shape.
FIGURE 7 3T3-4 cells sectioned perpendicular to the substratum with and without Bt_{cAMP} treatment.

3T3-4 cells grown in plastic dishes were fixed and embedded in situ. Blocks from these embedded preparations were prepared for perpendicular sectioning. The plastic surface (S) was quite irregular, and in normal medium (M) 3T3-4 cells often spanned long gaps in the surface, stretching between "hills" (short arrow) on the plastic (a). When treated with 1 mM Bt_{cAMP}, these cells frequently adhered down into the "valleys" (long arrow) as well (b). Note also that treated cells (b) appear thinner than cells in normal medium (a). Dense microfilament bundles (after Bt_{cAMP} treatment) are visible not only at the cell edge, but also at points in the cytoplasm nearer the nucleus, seen in parallel section as "stress fibers" (arrow *) (b). (a) = × 8,200; (b) = × 11,000; bar = 1 μm.

by altering the formation and movement of cell processes and raise some questions about the function of microfilaments and microtubules.

Patterns of Cell Movement

L929 cells tend to be round or ovoid, often with two rather small processes, one at each end of the cell. In this discussion, we distinguish a cell process, which is a relatively large extension from the cell (> 5 μm in length), from smaller structures such as ruffles, blebs, and microvilli, which are often part of a cell process. Cell process extension involves attachment and "movement" (that is, motion of a portion of the cell) of the process mainly at its advancing edge or tip. When viewed in time-lapse cinematography, L cells show small processes that are actively extending and retracting. 3T3 cells have many broad processes, some of which may occupy the entire side of one cell. When viewed by time-lapse cinematography, these processes, too, are actively extending and retracting.
FIGURE 8 3T3-4 cells grown in 10% serum medium and stained after embedding by toluidine blue diffusion. 3T3-4 cells grown in normal medium were embedded in situ. The entire Epon block surface was then exposed to toluidine blue staining solution at 60°C for varying lengths of time and photographed by light microscopy. Early staining occurred mainly under nuclei (short arrow) and at the tips of cell processes (long arrow) (a). Further diffusion stained more of the cytoplasm (arrow *) (b, c) until the entire cell was completely stained (d). × 510.

(unpublished data). These time-lapse films also show that, after treatment with 1 mM Bt2cAMP for a few hours, processes are still present but that they fail to retract. Over the following 12 h the narrow processes of L cells extend, eventually becoming the long narrow processes characteristic of L929 cells treated with Bt2cAMP. As mentioned above, the processes of 3T3 cells are very large, often occupying an entire cell border; thus, their extension leads to a broad flat cell rather than an elongated cell.

Substratum Adhesiveness and Process Retraction

It is already known that treatment of cells with Bt2cAMP leads to an increase in overall adhesiveness to the substratum. This is based on the fact...
Figure 9 3T3-4 cells treated with 1 mM Bt2cAMP and stained, after embedding, by toluidine blue diffusion. 3T3-4 cells were grown in 10% serum medium with 1 mM Bt2cAMP for 24 h. Toluidine blue diffusion was carried out as in Fig. 8. Early staining occurred mainly at the cell edges (short arrow), along "stress fibers" (long arrow), and at intermediate points near the nucleus (arrow *) (a, b). Deeper staining reached the peripheral cytoplasm (arrow C) (b) and later the nucleus (N) itself (c), finally staining the entire cell (d). × 510.

that treatment of cells with Bt2cAMP decreases the rate at which trypsin or EGTA removes these cells from the substratum (8). From the studies of substratum orientation presented in this paper, it is evident that treatment with Bt2cAMP causes 3T3 cells to become closely apposed to the substratum, particularly near bundles of microfilaments. These bundles are especially prominent along cell borders and in cell processes at attachment points. Thus, part of the inability of cell processes to withdraw or retract after Bt2cAMP treatment could be due to their increased adhesiveness to the substratum.

However, increased adhesiveness might not be the only reason that processes fail to retract. We have investigated the retraction of processes in the absence of adhesion to substratum by removing
3T3cAMP cells (20) from their substratum either by mechanical means or with EDTA. We then observed the rate at which their processes retracted. If these cells were maintained in 1 mM Bt2cAMP, process retraction was prevented. However, if Bt2cAMP were not present, the low cAMP levels induced in these cells by temperature change caused the cells to retract their processes and round up. Thus, Bt2cAMP was probably affecting the function of structures lying within cellular processes.

Microtubules and Microfilaments

Arrays of microtubules are prominent in the center of the processes of Bt2cAMP-treated L929 cells. It is easy to imagine how these arrays act as a cytoskeleton to maintain these long narrow structures. Such arrays also exist in the narrow processes of Chinese hamster ovary cells treated with Bt2cAMP (16). However, it is difficult to picture how microtubules participate in maintaining the flattened structure of 3T3 cells. Some microtubules are present under the membrane of the broad processes of these cells, but they are not very prominent. On the other hand, microfilaments are very prominent in both 3T3 and L cells after Bt2cAMP treatment. In untreated 3T3 cells, the microfilaments are arranged in thin mats near the cell surface (results not shown). After Bt2cAMP treatment, the mats of microfilaments near the cell surfaces are evident as thick bundles. Such bundles are present at lateral cell margins and also as "stress fibers" throughout the cytoplasm in intimate association with the plasma membrane near the substratum surface (Fig. 8 b). It seems very likely that these bundles of microfilaments which contain actin and can bind myosin (1) participate in contractile processes in cells. Our proposal is that cyclic AMP inhibits contractile processes and promotes the accumulation of bundles of microfilaments.

Model of Cyclic AMP Action

A scheme by which cAMP may act to control cell shape, motility, and adhesiveness is presented in Fig. 10. It is likely that microfilaments have a role in the retraction of cell processes, and that the contractile protein actin, known to be present in microfilaments (1), interacts in some way with the plasma membrane of the cell. The intimate association of microfilaments with the plasma membrane and the fact that myosin has been identified in the plasma membrane (21) support this concept. It is not known if microfilaments themselves are capable of contraction. We assume that the assembly of microtubular subunits is required for certain types of cell process extension. cAMP is pictured as a central regulator of both systems. High cAMP levels would promote assembly of microtubules and inhibit microfilament-dependent functions (i.e. retraction of processes). Low cAMP levels would result in microfilament-mediated contraction of cell processes and disassembly of microtubules. Apparently, attachment to a substratum is necessary to demonstrate changes in shape subsequent to Bt2cAMP treatment, because L cells growing in spinner culture are round and do not show any change in shape after treatment with Bt2cAMP.

Cell-to-Cell Contact

In chick heart fibroblastic cells (1) or Balb-3T3 cells (11), cell-to-cell contact produces the forma-
tion of microfilament bundles near the point of contact. Since cAMP levels rise in Balb 3T3 cells when the cells come into contact and crowd together (14), we suggest that local cell-to-cell interactions produce a local elevation of cAMP in the region of cell-to-cell contact. These local high levels of cyclic AMP would, in turn, promote the formation of microfilament bundles near contact points between cells.

**Contractile Proteins**

Treatment of cells with Bt2cAMP does not cause any change in the total content of biochemically measurable tubulin (12), actin, or myosin (13). Thus, Bt2cAMP appears to act on the assembly of microtubules from tubulin, and microfilaments from actin and possibly myosin and other components. Indeed, Porter et al. (16) have shown that Bt2cAMP treatment results in a statistical increase in microtubular elements in CHO cells. We find that there is also an increase in microtubules in L929 cells treated with Bt2cAMP. Whether the number of microfilamentous structures similarly changes, or whether the changes we observe are due to redistribution of existing structures, is not yet established.

**CONCLUSION**

The morphologic reactions to cAMP could be explained as follows: L cells emit single, thin processes which migrate over the substratum, attached at the process tip. Raising the cAMP levels allows the process to continue to form and enlarge, but prevents retraction. Cyclic AMP also stimulates microtubular assembly; the microtubules strengthen the enlarging cell process. This unrelenting forward extension without concomitant retraction results in an extended thin process, firmly attached at its tip.

3T3 cells emit broad ruffling cell borders which migrate over the substratum attached over a broad area. With rising cAMP levels, the retraction of these edges is prevented, and the firmly attached edges continue slowly to extend away from the nucleus, often in multiple directions around the cell. As a result, the final cell morphology is very flat, since these cells are attached to the substratum along their entire cell border. The extensive microfilamentous bundles that accumulate and perhaps participate in attachment to the substratum are inhibited by cyclic AMP and, therefore, the cell edges do not retract.

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