Specification of Cell Morphology by Endogenous Determinants

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ABSTRACT We are studying the mechanisms by which cells elaborate their differentiated morphologies. Here we discuss one aspect of this issue: the specification of the detailed shape of individual cells. We describe an experimental system in which endogenous determinants of morphology are expressed. These determinants originally were detected in the morphological relationships between sister neuroblastoma cells. Approaches to analyzing these relationships are presented. The properties and behavior of the endogenous determinants have been partially characterized by further experiments, which are also described. The significance and the prospects for further analysis of our findings are discussed.

The modulation of cell shape is central to several major issues in cell biology. During differentiation, for example, animal cells elaborate an asymmetric morphology characteristic of their phenotype and appropriate for their function. Conversely, malignantly transformed cells typically have lost their asymmetry; the round shape of the newly formed daughter cells tends to persist throughout the cell cycle. In motile cells, locomotion is accomplished by a series of linked deformations of morphology. A major effort has been made in the last thirty years to understand the mechanisms by which these events proceed.

Several of the structural and molecular components of the cell that are involved in elaboration of shape have been identified. This information is largely derived from experiments performed with a variety of cells in culture, because expression of their morphology can be manipulated. For example, cells of neuronal origin, which have a spherical shape under normal culture conditions, can be induced to extend axonlike neurites. Neurite extension is both inhibited and reversed by drugs such as colchicine, which depolymerize microtubules and disrupt the normal distribution of intermediate filaments (1–3). Cytochalasin, a drug which interferes with microfilament function, also inhibits neurite extension (2). Recently, we have shown that cytochalasin blocks the secondary effect of colchicine, neurite retraction, without blocking its primary effect, microtubule depolymerization (4). Thus, microscopy and pharmacological experiments suggest that each of these three elements of the cytoskeleton—microtubules, microfilaments, and intermediate filaments—may participate in both the assumption and loss of asymmetric shape. Several groups are now involved in a molecular analysis of the detailed structure of the cytoskeleton. Our aim is to establish the precise sequence of interactions which are involved in modulation of shape.

Even when this sequence is in hand, a complementary question will remain: how are these cellular elements organized to produce the particular shape that is correct for a particular cell? We do not know the nature of the information, presumably residing in the genome, which specifies the generic features of, say, neuronal morphology as opposed to fibroblastic morphology. Nor do we know how the morphological features of the individual cell are determined. With respect to neurons, what dictates how many neurites a particular cell will extend? From where on the cell will they originate? And what will be their precise shape and branching pattern?

Formally, there are two answers to the latter question. Both were proposed at the beginning of this century by Ramon y Cajal (5). One possibility is that the detailed shape of the individual cell is controlled by extracellular cues: contacts with other cells, heterogeneities in the matrix surrounding the cell, or diffusible signals which elicit or inhibit motility. The alternative is that a particular cell, arising from a particular cell lineage, contains within it information which specifies its detailed morphology. Results consistent with both hypotheses have been obtained from observations of developing embryos. But a rigorous test of either model in vivo would require the difficult if not impossible task of isolating a cell in an animal either from its history or from its environment. Consequently, experiments with cells in culture have again provided us with our information. There are now several examples of the extracellular guidance of cell shape, notably the ability of neurites to follow heterogeneities introduced into the culture dish (6) or

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of fibroblasts to take on the shape of highly adherent patches of substratum (7).

Demonstration of intracellular information specifying shape and movement came first from the experiments of Albrecht-Buehler (8). A sense of developmental "cell lineage" and context is largely lost in cell culture, but the simplest of these lineage relationships is present among mitotic sisters. Exploiting this fact, Albrecht-Buehler showed that sister 3T3 cells have similar shapes at early times after mitosis and then walk away from one another along paths which are related to one another even though those paths take hours to generate (8). These results have formed the basis for an extensive study of the behavior and strategies of moving animal cells that has recently been reviewed (9).

We have used this approach to ask whether endogenous determinants of detailed differentiated morphology can be detected. Using the ability of neuroblastoma cells to assume typical neuronal morphologies, we have been able to demonstrate expression of those determinants. This paper's purpose is to summarize our observations and their analysis and to suggest promising approaches toward understanding the nature of these determinants.

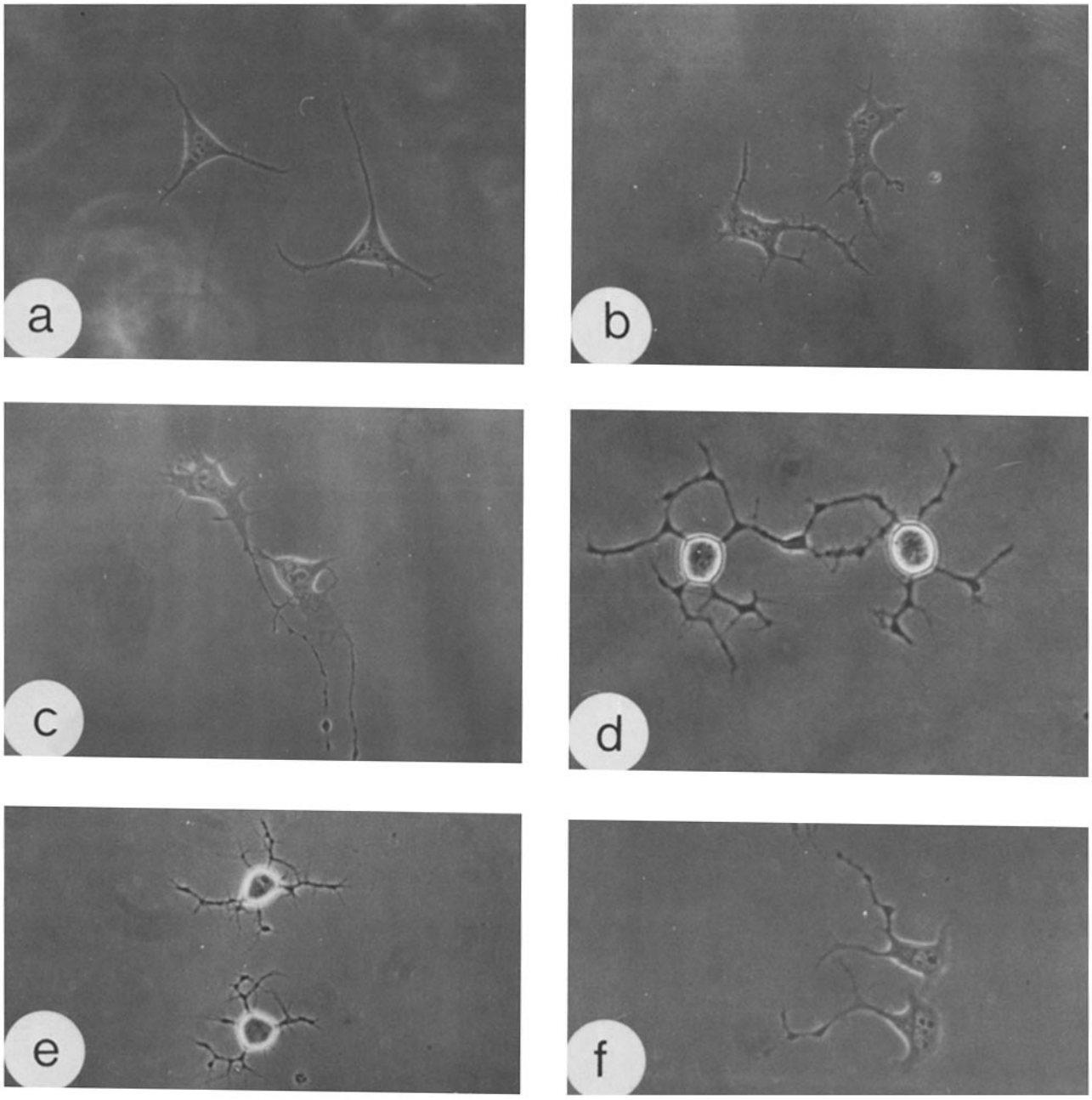


FIGURE 1 Neurite morphologies of mitotic sister neuroblastoma cells. Neuroblastoma cells were plated at low density and allowed to divide once. Neurite extension was induced by incubation with 0.1% fetal calf serum. After 8 h in low serum, pairs were photographed in live-cell chambers as described (10). The micrographs in *a–d* show pairs scored as morphologically related sister cells. The micrographs in e and *f* show sister cells which are only slightly different. The bottom cell in e displays one less neurite than the top cell, perhaps because of contact with the debris at the bottom of the field. The two cells in *f* are similar, but the top cell has two neurites and the bottom cell has one neurite that branches.

Mitotically Related Neuroblastoma Cells Are Morphologically Related

The repertoire of detailed neurite morphologies displayed by neuroblastoma cells is extremely large, as judged by easily visualized parameters. The number of neurites extended by a single cell varies from one to at least nine, at which point they become difficult to count. Even among cells with the same number of neurites, other variables expand the range of morphologies: the relative position of origin of the neurites about the cell perimeter, the length of the neurites, their thickness, and their branching pattern. As a result, viewing a population of neuroblastoma cells that have been induced to extend processes gives the impression that the shape of each cell is arrived at randomly.

However, when the culture conditions are adjusted so that mitotic sister cell pairs are readily distinguished, a pattern of morphology emerges. At least 60% of cells which are mitotically related are also morphologically related in striking detail (10). The morphological relationships are expressed in each of the parameters of morphology listed above: neurite number, position, length, thickness, and branching pattern. Some examples of morphologically related sister cells are shown in Figs. 1*a–d*. The related neurite patterns of daughter cells are not dependent upon cues left behind on the substratum by their progenitors, because the same proportion of relatedness is found when the pairs are generated by mitosis in suspension and then allowed to attach and extend neurites. It is likely that the true proportion of related sister cells is even higher. For example, an extending neurite frequently retracts when it touches another cell or a piece of debris, thus perturbing the detailed shape. Also, the time-course of neurite extension in sister cells is not synchronous, so that, at the time of scoring, one cell of a pair may not have completely elaborated its shape.

Several questions arise in assessing the significance of these findings. The crucial one is: with what probability could such morphological relationships have arisen by chance? Underlying this question is a methodological problem inherent in an analysis of data of this sort. The relationships we see are detected by our own visual system, and it is possible that the eye stresses elements of symmetry and eschews unrelated features. In the absence of an objective measuring device for analyzing cell morphology, we have used our data to approach this problem in other ways. In Fig. 2, we have scrambled the individual cells in a set of twelve sister pairs and thus generated new pairs related by chance rather than by mitosis. Tracings of the cells were used, sacrificing some details of morphology, to avoid the influences of the photographic background. These chance pairs represent the results we would obtain by random growth. In no case do we generate morphologically related pairs if the cells are not mitotically related. This is true even if the two cells in a chance pair display the same number of

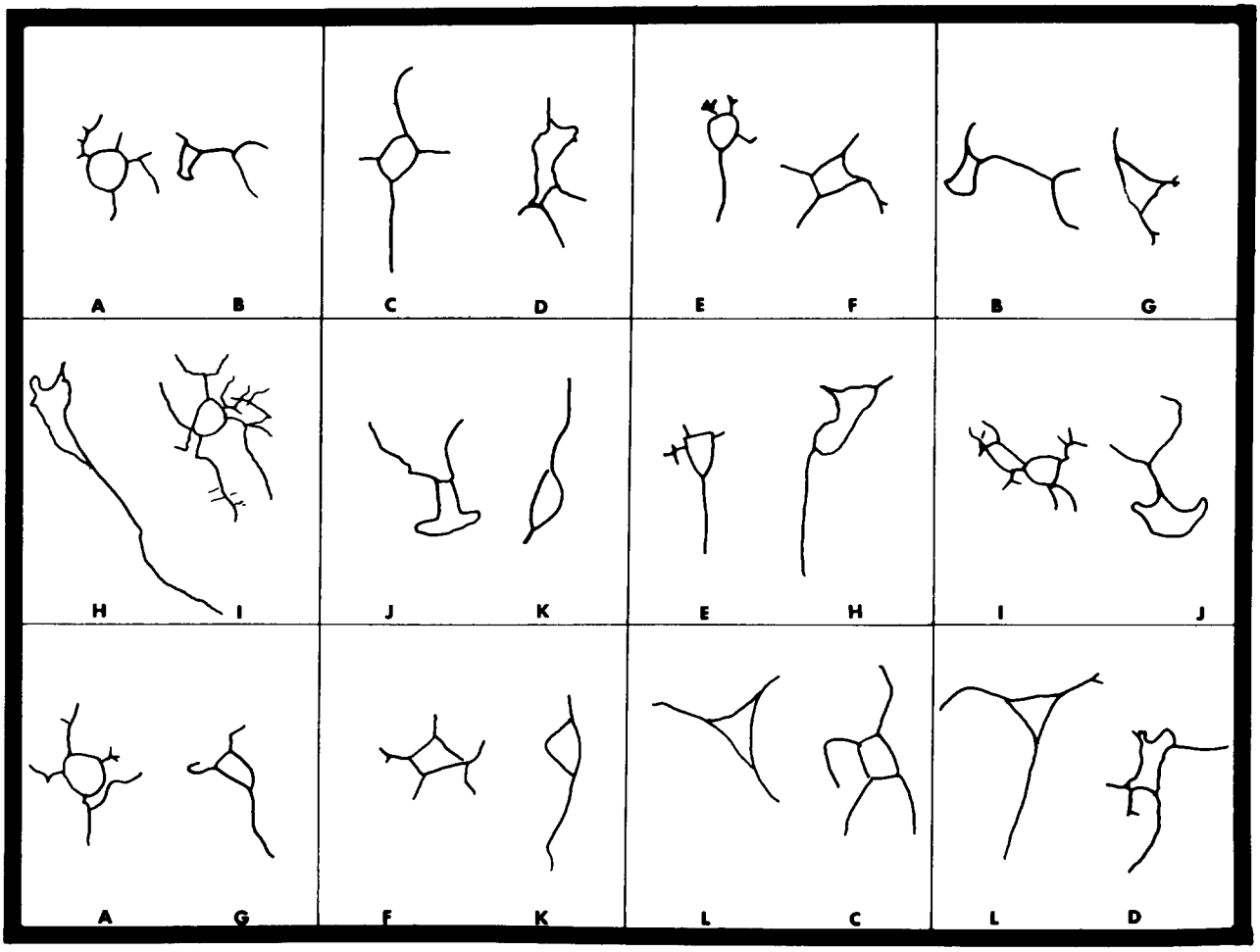


FIGURE 2 Random generation of pairs. Tracings of each of the 24 cells in 12 pairs were made from micrographs. The tracings were then rearranged into new pairs at random. No morphologically related pairs are generated by this procedure. The letters beneath each cell identify the mitotic pair from which it was taken.

neurites (for example, when one cell each from pairs E and F are matched, in the top row of Fig. 2).

The same conclusion is reached by a statistical analysis of our data. To permit such a treatment, we reduced the morphology of a cell to just the one parameter most easily and rigorously quantified—that is, the number of neurites. We counted the neurites on each of the cells in 81 mitotic pairs. These results are displayed in the histogram in Fig. 3. The graph demonstrates the tendency of sister cells to have the same number of neurites or to differ by only one neurite (for example, see Fig. 1*e* and *f*) The probability that such relationships could have arisen by random pairing of the cells in the same population is extremely small (Chi-square test, $P < 0.001$). This outcome supports the notion that only cells which are mitotically related are also morphologically related, even when only one element of morphology is used as a criterion.

Another question, more difficult to resolve, is the possibility that the large repertoire of morphologies seen can in fact be broken down into a limited range of repertoires. That possibility arises when cells are followed through successive rounds of mitosis. We have previously shown that clonal populations of four cells can be morphologically related (10, 11). More frequently, the cells of these groups are very much like one another but differ slightly in detail (Figs. 4 and 5). This sort of relationship can persist through subsequent rounds of division, to clonal groups of eight and sixteen cells. That precise details may vary slightly among the cells of such a group could be due to artifacts. For example, the cells are crowded and, as noted above, the resulting contacts can perturb neurite patterns. In addition, the cells in these groups are no longer synchronous, with respect to either neurite extension or division. When clonal populations are allowed to expand and are subcultured, the complete repertoire of neurite morphologies present in the parental line is observed. Some features of morphology can be cloned—for example, round vs. polygonal cell bodies (Figs. 6 and 7). But among 119 subclones examined, even the range in the number of neurites per cell shows no clonal restriction (11). The exact pathway by which the detailed relationships between daughter cells is eventually randomized is now under study.

Recapitulation of Detailed Morphology after Loss of Asymmetry

We detected endogenous determinants of shape by the relatedness of sister cells. The symmetrical antecedent of daughter cells is the mitotic cell from which they arose. In that mitotic cell, the elements of the cytoskeleton are distributed between the daughters as they form. That cytoskeleton is then rearranged and underlies the morphology we see. Therefore, we have asked whether perturbations of the cytoskeleton can disrupt the endogenous determinants of shape (12).

To answer this question, cells which had extended neurites were incubated briefly with the microtubule-depolymerizing drug, Nocodazole. After 30 min, the neurites had retracted completely in almost all the cells. The microtubules were essentially completely depolymerized, as judged by both biochemical and microscopic criteria. In addition, the microfilaments and intermediate filaments of the cell were completely rearranged. In the cell with neurites, these structures were colinear with the cell processes, but after incubation with Nocodazole they were confined to the cell body. When the drug was removed, almost all of the cells re-extended neurites within 2 h. By live-cell microscopy we followed individual cells from their original morphology through the drug treatment and during recovery. Using the same parameters that were applied to judging relationships between sister cells, we have shown that at least 60% of the cells recapitulated their original detailed morphology. It is possible that the original neurites left behind on the substratum some cues which guided the re-extending neurites. However, a subset of about half the cells moved across the substratum during neurite retraction. The same proportion of these cells also recapitulated their original neurite morphologies. Thus, re-extension of the same relative shape from new positions on the substratum occurs with the same frequency as re-extension of the same neurites in the same absolute position on the substratum. Conversely, cells which remain in the same position are not confined to recapitulate their original shapes. Typical results are shown in Figs. 8 and 9.

This result demonstrates that endogenous determinants of morphology survive disruption of the overall pattern of the cytoskeleton and survive an interruption in their expression. In addition, these experiments separate the detection of morphological determinants from mitotic sister relationships; the determinants are expressed even by single cells.

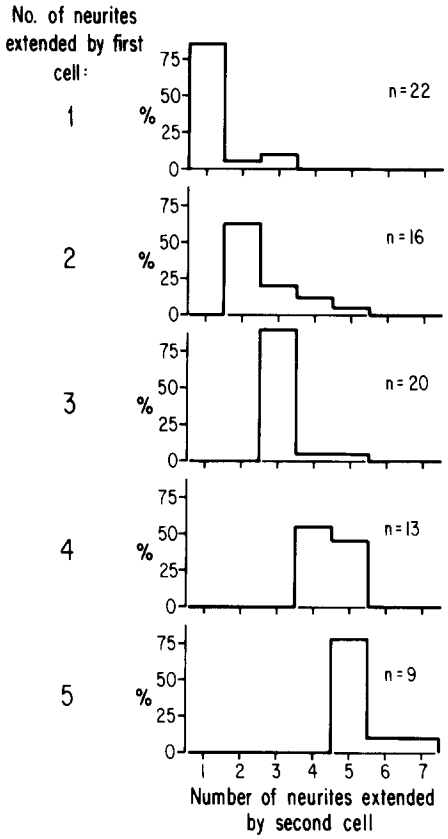


FIGURE 3 Statistical analysis of neurite number in sister pairs. The number of neurites on each cell in a set of 81 sister pairs were counted from micrographs. The pairs were divided into groups according to the lower number of neurites displayed by either cell ("No. of neurites extended by first cell", ranging from 1 to 5). The number of pairs in each group is given as "n". The distribution of the neurite number in the second cell of each pair is displayed as percentage in each histogram. Not included in the figure is one pair containing two cells with six neurites each. Chi-square analysis shows that the probability that these relationships would arise from random recombination of the cells in this population is very low ($P < 0.001$).

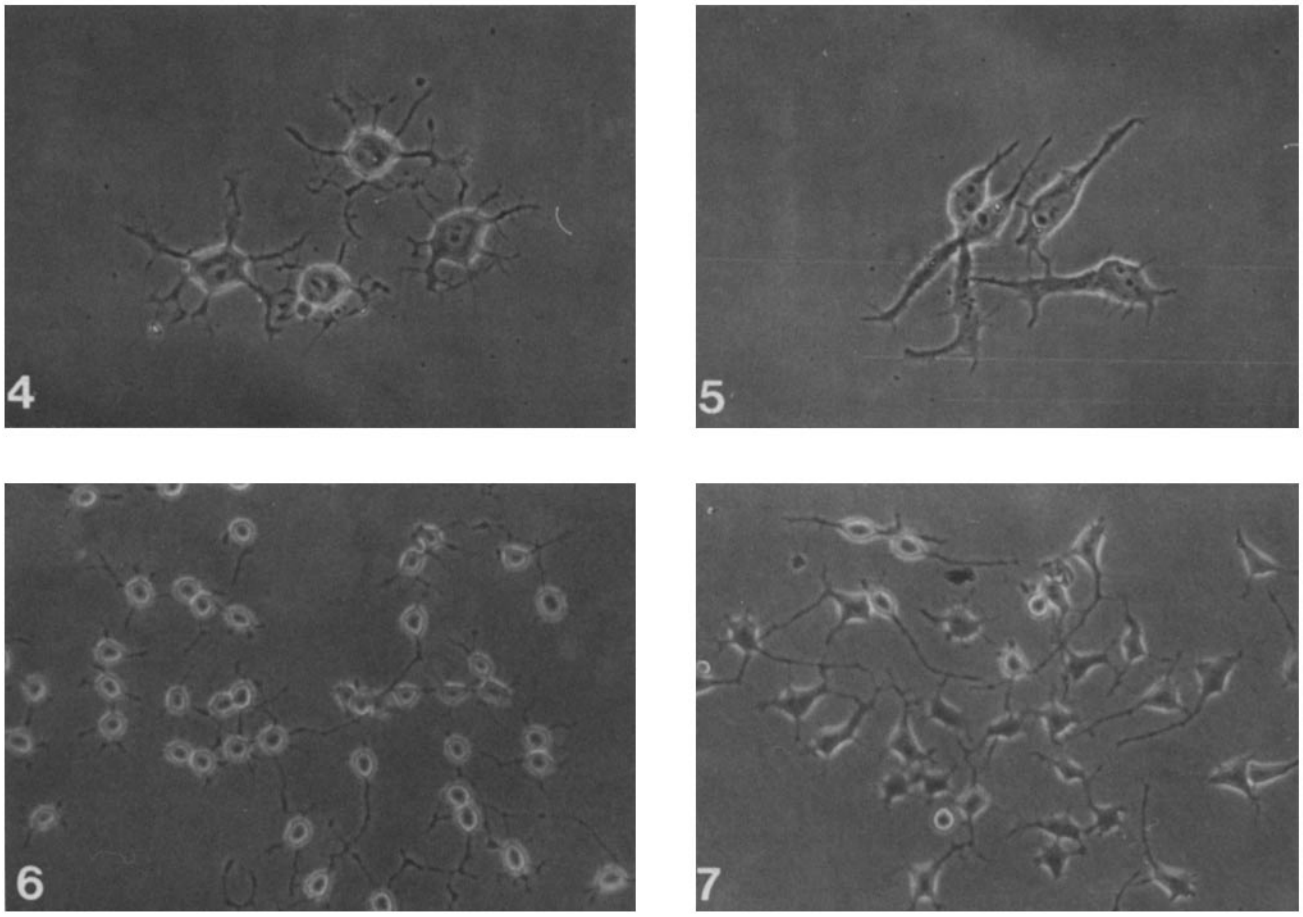


FIGURE 4–7 Clonal populations of neuroblastoma cells. Although clonal groups of four cells can be related in detail, the individual cells frequently differ slightly. The cells in Fig. 4 display between 6 and 8 neurites. Two of the cells in Fig. 5 have two neurites; the other two cells have extended only one. Possible explanations for these differences are discussed in the text. Fully expanded clones, after subculturing, can show some common features such as shape of the cell body (Figs. 6 and 7), but there is no evidence that detailed morphology can be cloned.

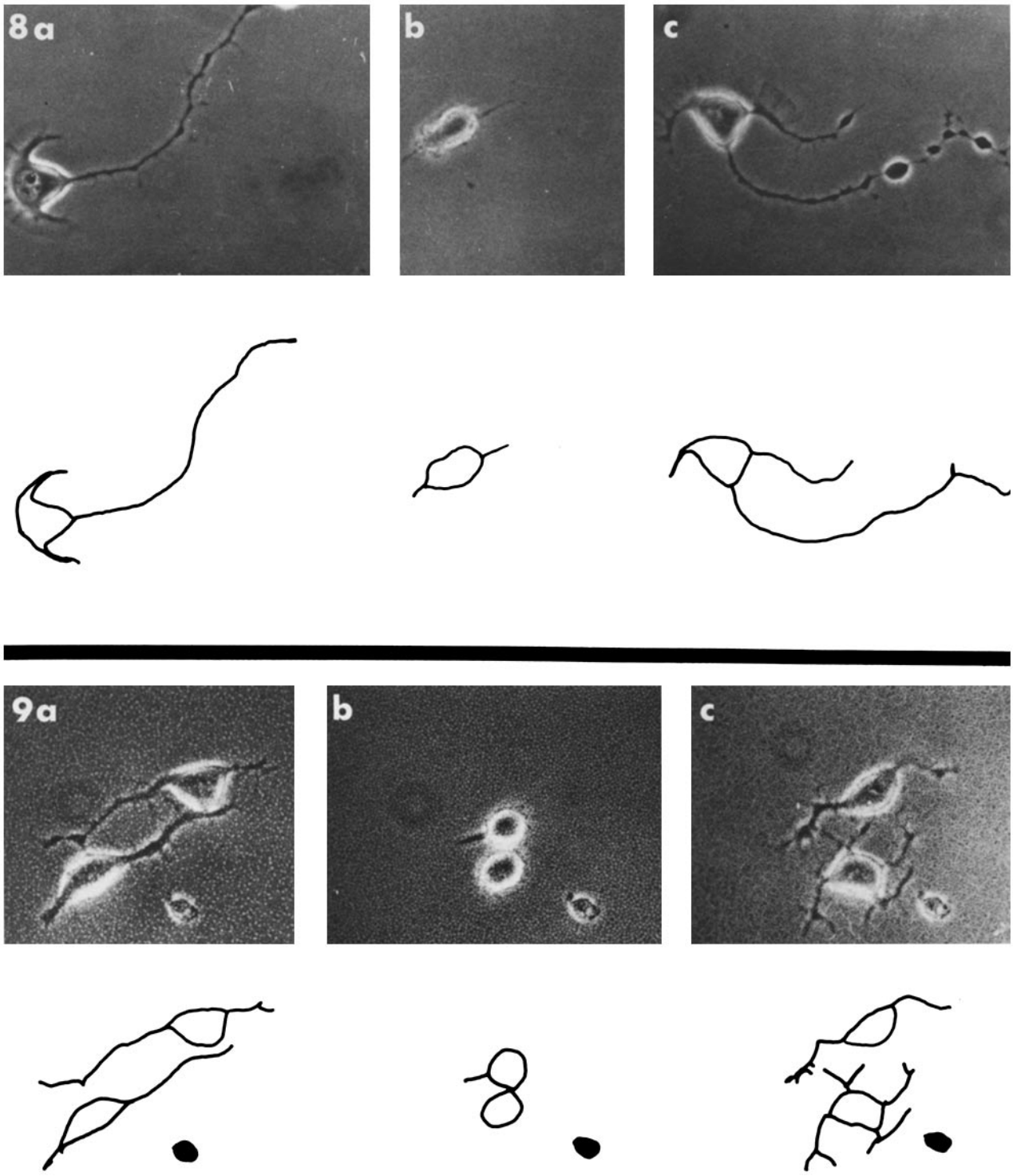
Analyzing Endogenous Determinants of Morphology: Prospects and Significance

The experiments described above demonstrate that endogenous determinants of detailed morphology are expressed in cultured cells. These determinants are mitotically heritable, at least through two rounds of division. The goals of further investigation are to determine how the determinants are transmitted to daughter cells, where they are stored in the cell, and by what mechanism they are expressed. Clearly, the answers to these questions are related to one another.

Our results to date do not identify the location of the determinants but they do suggest a limited list of candidates. It is possible that detailed cell morphology is specified by structures which nucleate the assemby of the cytoskeletal fibers that underlie that morphology. Recent evidence implies that the properties of cytoplasmic microtubule-organizing centers (MTOCs), studied by several groups (13), are consistent with such a role. In the accompanying paper, Brinkley et al. (14) reports that MTOCs from different cell types nucleate characteristic numbers of microtubules that grow to characteristic lengths. Their work also provides an approach for assaying the ability of MTOCs to specify the topography of the microtubule network. This hypothesis is also consistent with previous demonstration that the orientation of the centrioles, with which MTOCs are frequently associated, is correlated with the direction of migration in fibroblastic cells (15).

An alternative model is that the endogenous determinants of neuroblastoma morphology may reside at the cell surface. The motility of neuroblastoma cells is largely confined to the tips of growing axons. It is their persistent motion relative to the cell body that elaborates the final neuronal morphology. There may be unique structural or molecular features at localized regions of the cell surface that specify which parts of the cell will be motile. These and other possibilities are under investigation in several laboratories.

The expression of endogenous determinants of detailed morphology can be included in the catalog of cellular abilities which biologists now compile. There is no unequivocal evidence that those determinants participate in the modulation of cell shape in vivo. It seems likely that cell shape is specified by the interaction of both internal and external information. Moreover, any dichotomy between these two modes is less relevant than the elements they share in common. Regardless of where the information comes from, the important problem is to understand how that information is transduced by the cell to result in the ordered patterns of motility and morphology which are the center of organization in complex organisms.



FIGURES 8–9 Recapitulation of neurite morphologies after reversible microtubule assembly. Both figures show the original cell morphologies (*a*), the same cells after 30 min in 0.1 mg/ml Nocodazole (*b*), and the same cells 2 h after the drug was removed (*c*). Tracings of each micrograph are provided for clarity. The single cell in Fig. 8 originally extended one long neurite and two shorter ones. After retraction and reextension, the same neurite pattern is displayed from a different position on the substratum. A positional marker—debris on the substratum—has been cropped from the micrograph. Both of the sister cells in Fig. 9 have extended two neurites. During incubation with Nocodazole, the upper cell moves with respect to a piece of debris in the lower righthand corner of the field. Upon recovery, the upper cell again extends two neurites but from new positions on the substratum. The lower cells, which did not move, now extends four neurites.

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REFERENCES

1. Seeds, N. W., A. Gilman, T. Amano, and M. W. Nirenberg. 1970. Regulation of axon formation by clonal lines of a neural tumor. *Proc. Natl. Acad. Sci. U. S. A.* 66:160–167.
2. Yamada, K. M., B. S. Spooner, and N. K. Wessells. 1970. Axon growth: roles of microfilaments and microtubules. *Proc. Natl. Acad. Sci. U. S. A.* 66:1206–1212.
3. Jorgenson, A. O., L. Subrahmangan, C. Turnbull, and V. I. Kalnins. 1976. Localization of the neurofilament protein in neuroblastoma cells by immunofluorescent staining. *Proc. Natl. Acad. Sci. U. S. A.* 73:3192–3196.
4. Solomon, F., and M. Magendantz. 1981. Cytochalasin separates microtubule disassembly from loss of asymmetric morphology. *J. Cell Biol.* 89:157–161.
5. Jacobson, M. 1978. Developmental Neurobiology. Plenum Press, Inc., New York.
6. Weiss, P. 1958. Cell contact. *Int. Rev. Cytol.* 7:1217–1221.
7. Carter, S. B. 1967. Haptotaxis and the mechanism of cell motility. *Nature (Lond.).* 213: 256–267.
8. Albrecht-Buehler, G. 1977. Daughter 3T3 cells. Are they mirror images of each other? *J. Cell Biol.* 75:595–603.
9. Albrecht-Buehler, G. 1981. The control of tissue cell movement. *J. Natl. Cancer Inst.* In press.
10. Solomon, F. 1979. Detailed neurite morphologies of sister neuroblastoma cells are related. *Cell.* 16:165–169.
11. Solomon, F., and A. Zurn. 1981. The cytoskeleton and specification of neuronal morphology. *In* Cytoskeleton and the Architecture of Nervous Systems. R. J. Lasek and M. J. Shelanski, editors. The Neuroscience Research Program, Boston. In press.
12. Solomon, F. 1980. Neuroblastoma cells recapitulate their detailed neurite morphologies after reversible microtubule disassembly. *Cell.* 21:333–338.
13. Solomon, F. 1980. Organizing microtubules in the cytoplasm. *Cell.* 22:331–332.
14. Brinkley, B. R., S. M. Cox, D. A. Pepper, L. Wible, S. L. Brenner, and R. L. Pardue. 1981. Tubulin assembly sites and the organization of cytoplasmic microtubules in cultured mammalian cells. *J. Cell Biol.* 90:554–562.
15. Albrecht-Buehler, G. 1977. Phagokinetic tracks of 3T3 cells: parallels between the orientation of track segments and of cellular structures which contain actin or tubulin. *Cell.* 12:333–339.