"YOU CAN OBSERVE A LOT BY JUST WATCHING."
(Yogi Berra)

Some General Comments on My Approach to Studying Problems of Embryonic Development

The development of multicellular organisms has three fundamental processes. First, apparently similar cells differentiate into distinctive cell types (cytodifferentiation). Second, groups of cells conspire to form distinctive tissues (histogenesis) and then organs (organogenesis). Third, there is growth, caused by increase in cell number, cell volume, and amount of extracellular matrix, leading to increases in mass and volume of the embryonic and then adult organism. Morphogenesis is a generic term that applies to developmental processes resulting in the generation of new form and, by definition, typically involves all three of the aforementioned processes. Imagine this! Each person reading this book (and indeed all multicellular organisms on earth), arose from a single cell, the zygote, resulting from the fusion of a paternal gamete, the spermatozoon, and a maternal gamete, the egg or ovum. Two important thoughts occur to me here. The first is that we still lack much of the understanding of the fundamental details of precisely how this incredible transformation of a zygote into a person (or a fly, frog, or mouse for that matter) occurs. The second is that I am surprised, dumbfounded even, about how much we have learned about these processes in my lifetime.

The study of cytodifferentiation requires research primarily at the subcellular level beginning with the genes, that is, DNA, and how the genetic information encoded in the DNA is transcribed into RNA and then translated into proteins. The essence of cytodifferentiation is the expression of selected genes in the complete genome, leading to the accumulation of a cell-specific ensemble of functionally active proteins. Ultimately, we need to know how these proteins interact and somehow produce the cytoplasmic proteins and assemblages of organelles that characterize each differentiated cell type. The molecular composition of genes, their location on the chromosomes, and the nature of their molecular and cellular expression is a dominant and fashionable area of current
embryological investigation. And now, with all the hullabaloo over the sensational and truly exciting essential completion of the massive Human Genome Project, many embryologists have come to believe that we are now on the brink of solving the secret of development. Far from it. Important though this project and other research on the genes is and how engrossing our pursuit, we have so far largely ignored the complicated pathways in the cytoplasm starting from the genes. These pathways lead to the differentiation of the many different, characteristic tissue cells—neurons or intestinal epithelia or red blood cells, to name just a few, or even the cytoplasmic organelles of which they are composed.

To be sure, the genes contain the genetic information for development and provide the starting point for cytodifferentiation, but how the proteins they produce interact and synthesize other chemical agents, and, finally morphological structures, is yet the big mystery in the study of development. For example, a gene on the mammalian Y (male) chromosome, when somehow appropriately activated at the right time and in the right gonadal precursor cells, produces a "testis determining protein," leading to differentiation of an indifferent gonad into a testis instead of an ovary. Other cells in the testes then produce the enzymes that catalyze the synthesis of the male sex hormone, testosterone, which then acts on the sexually indifferent genitalia, ultimately causing formation of a penis and scrotum rather than a clitoris and labia majora. Testosterone also alters brain development and ultimately some behaviors. I have oversimplified the actual cascade of events that leads to sexual differentiation, but the point is that there are long, complicated pathways of interactions that, in the end, are far from the initial genetic determinants. These interactions are called epigenesis and they operate normally when the reactant tissues are in the right place at the right time and competent to react.

Besides simply fertilization, cell divisions and growth, embryonic development of animals results from some rather strange combinations of two key processes, one of which is cell differentiation, and the other of which is active cell movements. These key processes are often closely related. For example, cell movement first begins in earnest as a result of some primitive cell differentiation. Cascades of cell differentiation are triggered when one set of cells move in the embryo to a new location, contacting new neighbors, and inducing new rounds of cytodifferentiation. Most researchers who work on developmental problems have focused on some aspect of differentiation, and the mechanisms that control it.

My own research, in contrast, has been mostly concerned with the other main set of phenomena that form anatomy, namely cell locomotion and cell rearrangements in general. Cells do a remarkable amount of active moving around in embryos, more than any sensible person would ever have expected, and also more than anybody has yet been able to explain. Maybe it is because all the really serious researchers have focused their attention on differentiation.
 Somehow it got left for me and my graduate students to discover a rather surprising fraction of what anyone has been able to find out about the cell motility aspects of how embryos work. People used to assume that it was growth, in the sense of cells being pushed around by one another’s enlargement, or maybe by cell divisions. In plant development, that is partly true; but much less true in animals.

In order to form tissues and organs, you need to get the correct differentiated cell types to be spatially arranged into their proper geometrical patterns. The sensible way to accomplish this would be to have molecular signals control gene activation in the correct spatial patterns, so that cells differentiate at the locations where they are needed, and then just stay there. In other words, the early embryonic cells that are located where the liver needs to be would simply differentiate into liver cells. Those cells that are in the various places where muscles are needed would differentiate into muscle cells, and so on for the thousand or so different cell types in our bodies. That would be the sensible way, and much of development really is approximately that way: in higher plants, virtually all of it is, but animal development also depends strongly on active cell movements. For example, in your body, all the voluntary muscle cells, the ones you use to bend your arms and legs by volition, begin their development along the middle of the back. We still have many muscles in our backs; so their cells didn’t move very far. On the other hand (literally!), all the muscles in your hands and feet, and everywhere else but in the middle of your back, were formed by actively migrating precursors of muscle cells. There are lots of other examples, including the pigment cells about which I did some of my early research in Goodrich’s and Willier’s labs now these many years ago. Melanoblasts originate in the middle of the back, as part of the primitive nervous system, and then migrate to many different locations in the body, where they then differentiate into pigment cells.

When people write, as just about all of the embryology textbooks do nowadays, that embryos form anatomy by each cell somehow being told what to do, or “knowing where it is” relative to other cells, and then activating the appropriate genes corresponding to their location, then that is something of an over-simplification. If humans had designed embryos, then that’s how we would have designed them to work: each cell would just be told how to differentiate, according to position, and then it would stay there. To a large part, that really is what happens in embryos; and since that’s how we would have designed it, then it is comprehensible to us when embryos do that. It makes sense to us, and we can recognize those aspects of what is going on. If only there weren’t so much of this strange cell rearrangement going on, with lots of kinds of cells starting differentiation in one location, and then crawling off somewhere else, then embryonic development would make a lot more sense. We would then be able to explain it all much better than we do, and would probably be able to control it
medically much more effectively. There wouldn’t be gastrulation, neurulation, neural crest cell migration, and cells would be able to sort out by type when randomly mixed together.

Cell sorting, which interested me for a time, and to which I made some significant contributions (see Chapter 7), is a good example of the incomprehensibility of the motility aspects of development. Although H.V. Wilson discovered this phenomenon, in primitive marine invertebrates, he resisted the idea that the cells could be rearranged by cell type. For many years, he thought they must be switching from one differentiated cell type to another; and after that he tried to prove that the phenomenon was entirely a matter of differentiation of previously undifferentiated archecocytes; and that was more than 30 years after he had discovered sorting. He just didn’t think of the anatomical arrangements of cells as being produced by properties of individual cells, neither adhesive properties, motility, nor any other intrinsic properties. The bricks in a wall got their locations by someone putting them in place; nobody knows how to make bricks that can crawl up forming walls and climb into place, under their own power. If the bricks and other components of a house did have intrinsic, structure-creating properties, then if a bulldozer knocked down a house, its bricks, etc. would be able to sort out to form a new house (or a row of cottages) where the old house had been. This is one way to think about cell sorting and tissue morphogenesis, but it leaves open many questions about exactly what cell properties are related to the formation of which parts of our anatomy.

At a Friday Evening Lecture in Woods Hole just two years ago I had the pleasure of attending a clear, refreshingly unpretentious lecture describing beautiful, elegant work. The lecture was about how a research group had identified the genes and the proteins necessary for the continued mitosis of the primitive precursors of gametogenic cells and the migration of these primitive sex cells in the worm Caenorhabditis elegans. The lecture then ended, as if that is all that needs to be said or done. There was no mention of the big unsolved problems before us, i.e., how do the genes, and the proteins they produce, lead to continued mitosis and the migration of whole cells? There was no mention of the myriad possible connections between the chemical beginning and the cellular end. Unfortunately this is typical of the presentations of such results by many of those working at the level of DNA. They lay the chemical genetic basis, often brilliantly, but do not pursue the subsequent molecular and cellular work or even mention the need for it. This gives the audience and in particular young students the idea that laying the initial DNA-RNA-protein basis for an eventual cellular trait suffices. Moreover, although this field of contemporary biological research is certainly of outstanding importance and even glamorous, unfortunately in the rush to explain everything on the initial molecular (genic) level, many equally important aspects of development are nowadays getting less attention than they
deserve. These include the differentiation of cytoplasmic organelles and cytoskeleton, the cell surface, the cells themselves as motile interacting units, cell movement, rearrangement and contractility in the shaping of organs, (not to even mention evolutionary aspects of development).

Additionally, there is also an unfortunate, childish arrogance displayed by some molecular biologists that implies with scorn that if biological research is not molecular it is not really scientific or at least less so. Insinuations of this sort abound in conversations and lectures, and are well-documented by the distinguished entomologist, E.O. Wilson, in his autobiography, but I will mention only one of a number that I have personally experienced. It occurred during the question period after my lecture at the University of Virginia on some of our research about 20 years ago. After a few questions, a man at the back of the room, whom I recognized as the current head of the Department of Biology and whose field of research was protein structure, arose to offer the following comment. “This is interesting work you have presented, but it is all descriptive.” He was being critical, meaning that the work was not molecular. If I were younger, I might have been floored by this gentle but direct affront. But I was no longer young. I responded that, “Yes, much of what I presented is descriptive, but not all. Some was experimental. Tell me, what is wrong with description? All science begins with careful description, as, for example, using x-ray diffraction to study protein structure.” Accompanied by a few smiles and some tittering in the audience, Trinkaus had yet again emerged triumphant!

What is totally ignored in much of the research on gene action is precisely what is studied in an area of research known as “developmental genetics.” Here, as I define it, the emphasis is more on the embryological consequences of gene activity than on the gene itself (for many “das Ding an sich” of biology). The investigators create mutants and observe them, not merely the end result in the damaged embryo or adult, but work to trace step by step the deviant developmental directions taken in the genesis of the abnormality. Much of the developmental genetics of Drosophila, the mouse, the chick, the zebra fish, and the worm Caenorhabditis has used this approach with great profit and has in this way revealed some of the hitherto unknown intricacies of normal development.

Yet another approach to the study of development is to ignore the genes, fundamental as they are in providing genetic information, and to study the development of the normal phenotype on a cellular and cytoplasmic (and molecular) level. This was the old-fashioned, traditional way of embryological research in days of yore, before we knew about the chemical nature of genes or much about gene expression during development. It has taught us most of what we know about development. This approach, for example, is how we first learned about stem cells, a subject of intense current biomedical research—by research a long time ago on the neural crest, the most spectacular of embryonic stem cells.
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Also, transplantation of nuclei many years ago led directly to the contemporary cloning of mammals. One of the central objectives of this approach to the study of development is to understand cell motile behavior during morphogenesis. We need to know how cells change shape and move, how they generate motility, and how cells’ collective behavior forms groups or clusters of cells, sheets and other cell masses that ultimately form tissues and organs. Now, with the incredible advances in cell and molecular biology and powerful new techniques for studying the cell surface and the cytoplasm, there is more and more emphasis on the mechanisms of the movements of cells and their contact behavior. A pressing human need for this approach to development is posed by the problem of cancer. Most malignant cancers spread by cell movement, the invasion of neighboring tissues and organs, and by metastasis, movement of cancer cells to different regions of the body after invasion of the blood stream and the lymphatic system. By continuing this invasiveness, cells move out of these vessels to invade other parts of the body. For these reasons and out of plain old dumb curiosity I have dedicated most of my research life to the study of this grand problem of cell movement within the organism.

So I think the real issue may be whether my work can yet be connected to any or all of the new discoveries in molecular biology. And, in that respect, I share my critics’ regret. Unfortunately, neither I nor they have been very successful in connecting the mechanical aspects of embryonic development to their molecular aspects. Maybe they think, if they ignore the mechanical aspects, then they will go away; and that we can just pretend that turning genes on is all there is to it. Or maybe they think nobody should study subjects that are not immediately “connectable” to what is already known. At least that criticism would be more logical than complaining that my work is more descriptive than theirs. If I knew how to connect my discoveries to theirs, then I would have done so with great delight. Indeed, my students and I have tried as hard as we could to make such connections, admittedly without much success. But we often felt as if we were trying to dig a tunnel from one side of a mountain, or build a bridge from one side of a river, and people on the other side were not helping, by digging from their side, toward where we have managed to reach.

Until this point in my book, I have placed emphasis on our work on the movements of masses of cells, particularly of a certain cell sheet and a certain syncytial sheet, setting somewhat aside for reasons of clarity our often accompanying studies of the contacts and movements of cells as individuals and in small groups. This was an oversimplification. Cells move and rearrange within cell sheets. Individual cells congregate to form cell masses of various contours and degrees of contact. Now in this chapter the emphasis will be on some of our primitive understanding of mechanisms of movement of discrete cells. Their motility begins after the cessation of cleavage and continues throughout much of early embryogenesis and, indeed, continues in some cases
normally in the adult, as in the migrations of white blood cells, during wound closure, during regeneration, and abnormally, as in cancer.

**Cells into Organs**

My heavier involvement in the motility of individual cells was commensurate with the composition of a little book on cell movements during embryogenesis. This book, which finally appeared in 1969, began as part of a multiple textbook series for undergraduates edited by my old friend Clem Markert. He asked me to write a volume on embryonic induction, organizers, and the like. As I began assembling my thoughts in preparation I soon came to realize that I didn’t want to write on the assigned topic at all but instead on morphogenetic cell movements, a subject that passionately interested me and that I had already recently reviewed. Moreover, I believed that there should be a volume on this subject in the series. I discussed my change of heart with Clem and he agreed, showing remarkable flexibility.

I wrote my book mainly during our summers in the South of France, in Le Castellet, where distracted only by the sun, the sea and petanque, I was free to read, reflect and write on problems close to my heart. It was a perfect way to do it. Being away from Yale and Woods Hole, I had virtually no distractions. I wrote partly from memory and partly with the aid of a large collection of reprints I brought with me, stuffed in my briefcase and one of the suitcases. I would take care of corrections and illustrations back in New Haven. Since I never learned to type, my good wife took time off from the beach and from our charming life in Provence to transform my scrawlings into a legible typescript using an old typewriter we had rented in Marseilles. As I was in the midst of my writing, we had a visit in Le Castellet from my brother, Charles, the Renaissance historian, on his way home from a summer of teaching in Florence, Italy. He asked me about the book and the title. I mumbled something like *Morphogenetic Movements* and he said, “Terrible. Boring. What’s it about?”

“It’s about how cells move around in the embryo and get together to form tissues and organs.”

“Ah,” said he, “*Cells into Organs,*” and I instantly knew that I had a suitable title.

To help make the book seem important, I later added a rather pretentious subtitle: *The Forces that Shape the Embryo.* In addition to being a distinguished scholar, Charles was good at titles. One of his books on intellectual life in the Renaissance was titled *Adversity’s Noblemen* and his latest and most ambitious work is titled *In His Image and Likeness.* For my book we designed an elegant cover, using real images of contacting fibroblasts from one of my papers (Figure 11.1).
As I got going on my book I pretty soon abandoned pitching it as an undergraduate textbook. Instead, I wrote it more as an extended essay on the movements of a subset of all cells, namely those bereft of cilia and flagella, during the early development of a number of carefully chosen, well-studied organisms. The book would be intended not just for advanced undergraduates but also for graduate students and for biologists outside the field of motility who might thereby become interested in cell movements. I made a concerted effort to point out what was known but also what needed to be investigated. From comments and reviews and by numerous citations in professional journals, I was soon pleased to find that the book served not only these purposes but also as a monograph for embryologists and cell biologists already working in the field. Several of my former students said they got good new ideas for their own research from the book. Great! To my immense gratification, my book entertained and enlightened not only neophytes but also helped experienced scientists. Although I hadn’t expected this outcome when I wrote the book, I was happy to learn that my work was useful to a broad spectrum of readers.

Figure 11.1. The scuffed cover, giving testimony to the usefulness of the first edition of *Cells into Organs.* Reprinted with permission of Prentice-Hall.
Motility of Individual Cells in the Embryo

About this time two enthusiastic new graduate students arrived from little Union College, where both had received good solid training in embryology from a former Yale graduate student, Ray Rappaport. Ray is one of the most creative people trying to understand how cells divide (cytokinesis). I persuaded one of these new students, Richard Ebstein, to join me at Woods Hole to begin a study of the genesis and morphology of the movement of deep cells in the *Fundulus* blastoderm before and during gastrulation, using time-lapse cinemicrography to speed up their movements. Dick was a bright, talkative, funny guy and great fun to have in the lab. The other student, Gary Conrad, returned to Salisbury Cove, in Maine, to continue his work with Rappaport. With Dick’s able assistance and boundless energy, I had a very profitable summer. No one had previously studied the motility of individual cells moving before and during gastrulation of a living vertebrate embryo using time-lapse cinemicrography. Many of you have probably seen time-lapse films of a flower blooming or a butterfly hatching from its chrysalis. Basically, this technique works by taking individual frames of movie film at say, one minute intervals, and then projecting the film at say 16 frames per second. This collapses a slow event by a factor of 960. High-speed cinemicrography can be employed in the opposite manner. Specially designed cameras can take thousand of frames a second and then project them at 16 frames per second, slowing down a quick process. You may have seen films of a speeding bullet shattering glass or a car slowly disintegrating after a violent collision. Anyway, frog, chick and mouse embryos are too opaque to see much of what goes on inside them during morphogenesis. In contrast, in the nearly transparent *Fundulus* embryos, we could not only see the cells as individuals, but also follow their detailed changes in surface features, shape, and movement. Although time-lapse cinemicrography is an old technique, Dick and I were neophytes. We were provided with many adventures trying one interval or another, stabilizing the microscope, and deciding which cells to follow and what to focus on. We were limited to old-fashioned bright-field microscopic optics because phase-contrast microscopy is useless in a three-dimensional system. Furthermore, Nomarski differential interference contrast (DIC) optics was not available. But we learned much with the proper use of our ancient but excellent Zeiss optics, and because of our synergistic enthusiasms for the new venture. The results were gratifying, real eye-openers, both because they were new, and, more importantly, because they yielded a great deal of new information about the onset of cell movement *in vivo*, i.e., in the living embryo.

As in most organisms, the cleavage blastomeres of *Fundulus* are quiescent, except, of course, during cell division. At first, *Fundulus* blastomeres divide rapidly, say every hour or so (depending on the temperature), but soon, the rate of cell division declines. Then, soon after the near cessation of cleavage, the
mid-blastula transition begins along with the appearance of new messenger RNA and the onset of cell movement. We discovered that the onset of cell motility appears gradually. The first signs of it are gentle, barely perceptible undulations of the cell surface. As these surface undulations increase in magnitude over a period of about 30-40 seconds, one of them suddenly expands to form a large, clear, hemispheric blister that we have come to call a bleb, essentially a small mobile cortical blister. Then, seconds later another bleb appears. It takes seconds for blebs to form in real time, but when movement is speeded up by time-lapse cinemicrography, their appearance is explosive and truly spectacular. They quickly retract and are just as quickly replaced by new ones. At first, few cells form blebs, but, with time, the number increases steadily until by the late blastula stage, before the beginning of gastrulation, almost all deep cells are forming and retracting blebs and the blastoderm is converted into a bubbling, jostling cell mass. What a show! During this frantic but brief phase of development the cells do not change location; they just sit there merrily blebbing—exercising in place, as it were. In this phase each bleb is a clear, hyaline hemisphere totally lacking cytoplasmic organelles such as mitochondria, Golgi and endoplasmic reticulum. Transmission electron microscopy showed that these organelles are excluded from the bleb by a dense barrier of actin-containing microfilaments at the base of each bleb. Blebs seem to form as the plasma membrane peels away from the microfilament-rich cortex of the cell and then blebs balloon out into a hemispheric form, presumably forced to bulge away from the cell cortex by the normal hydrostatic pressure of the cytoplasm.

Motility next enters a new phase; instead of retracting, some blebs protrude farther. The microfilamentous barrier at their base breaks down and cytoplasm full of organelles flows into the bleb from the cell body. This not only extends the bleb, giving it a long, blunt finger-like form; it also decreases the size of the cell body and thus represents the first small phase of cell movement, a big event. The cell begins to displace by literally pouring cytoplasm into the extending bleb, which we now called a lobopodium because of its change in form and its function as an organ of locomotion. As the lobopodium continues to extend, the cell moves forward. This mode of cell movement obviously resembles one aspect of classical amoeboid movement, namely, extensive cytoplasmic flow. In some cases, the lobopodium adheres to the surface of the syncytial layer beneath and spreads on it, forming a lamellipodium. This locomotory organelle is a flattened, spreading, adherent extension of the cell’s margin. It forms as a dominant structure in the direction of cell movement, e.g., if a cell were moving from left to right, the right end of the cell would have the largest lamellipodium. Lamellipodia are, in effect, the feet of many moving cells, in animals and in artificial culture conditions. Lamellipodia can also attach to a substratum or another cell, and contract, thus pulling the cell forward. With the development of these locomotory structures, the deep cells begin a massive
random displacement during the blastula stage, before gastrulation. This was at first a surprising discovery, but not once we thought about it. Cell movement begins before gastrulation, but, of course, muscles contract and move before a leg and a runner moves. It seems that gastrulation commences in Fundulus in two phases. First, the cells somehow differentiate the mechanisms for movement in the blastula. Second, the now moving cells respond to certain directional cues, as gastrulation begins, and move toward one site, where they congregate to form the embryo proper. Before moving directionally, you first have to learn how to move.

Shortly after these adventures and discoveries with time-lapse cinemicrography, a young woman named Cheryll Tickle arrived from Britain to work in my laboratory as a postdoctoral fellow. She had taken her doctorate from my friend Adam Curtis at the University of Glasgow and elected to do her postdoctoral research work with me in order to continue her studies of cell sorting. Adam might well have sent her to work with Steinberg or Moscona, but she chose to come to my laboratory. I neither encouraged nor discouraged her in this research, in accord with my practice of giving postdoctoral fellows maximum independence. Gradually, on her own, she lost interest in cell sorting, an intelligent and wise move in my opinion, confirming my impression that this petite and rather shy woman was very bright. This left room for something else in that penetrating intellect. Sure enough, her inquisitiveness and good instincts came up with a very good idea, namely to see if Fundulus deep cells become more extensible or deformable commensurate with the onset of the movements of gastrulation.

I had previously observed that gastrula cells more often than not flatten on a glass substratum in vitro whereas blastula cells remain rounded and do not attach. I had suggested in print that this flattening is due to increased surface adhesiveness. Cheryll thought otherwise—that flattening could as well be due to increased deformability of the cell surface or a combination of increased adhesiveness and increased deformability. Yes, indeed; I hadn’t thought of that. So she constructed a Rube Goldberg machine for exerting negative pressure or suction on the cell surface. The idea was to thrust a micropipette against the cell with a micromanipulator and measure the amount of negative pressure required to suck a protrusion of a given length from the cell surface into the pipette.

With this equipment, I took Cheryll to the MBL to test the hypothesis and to give her the “Woods Hole experience.” On finding our lab, I affixed our names to the door—“Tickle Trinkaus.” Ordinarily I hadn’t bothered to label the lab door with names, but this time I couldn’t resist. Sure enough, a few days later Joel Brown stuck his head in to inquire, “Is that in the imperative?” We all had a good laugh. We quickly got our equipment set up and went to work. I prepared the cells, dissociating them and culturing them in a depression slide. She applied the negative pressure. Her job was the tough part. The seal between the pipette and the cell surface had to fit perfectly, of course, with no leakage. Since there
was often leakage this was often an exasperating operation. One day, after a particularly trying series of leakages, a friend dropped in, looked around and asked, “What in the hell does that contraption do?” “It sucks!” said Cheryll.

With skill, determination, humor, and the legendary British stiff upper lip, Cheryll succeeded in establishing unambiguously that less negative pressure is required in vitro to deform early gastrula cells than cells from early blastulae. This finding correlated nicely with the normal behavior of deep cells in vivo, in the blastoderm: early blastula cells, as noted, are rounded, form blebs, and do not engage in locomotion; whereas, early gastrula cells form extended lobopodia and lamellipodia and engage actively in locomotion. The increased deformability of the gastrula cells also correlates with the breakdown of the microfilament barrier at the base of each bleb and the consequent flow of inner cytoplasm into the bleb. Possibly, a liquification of the cortical cytoskeleton, due to depolymerization of actin-rich microfilaments, is the basis of the commencement of locomotion.

As often happens in cell research, when you are looking for something particular to happen and you are watching carefully, you may find the cells reacting in unexpected ways. One day Cheryll called me over to watch as she brought a micropipette against the surface of an early blastula cell preparatory for testing its deformability. A few seconds later the cell formed a bleb on its opposite side. We soon found that if the cell was in tight contact with another cell then the second cell would also bleb, a few seconds later than the first. Nudging a cell causes it to form a bleb and then communicate some sort of signal to an adjacent cell. This result so fascinated us that, even though we didn’t yet have an explanation for these observations, we published them in a short letter to the British journal *Nature*. Later, after Cheryll had returned to England, I continued nudging cells myself (it was great fun and easy, again instant research). I found that if the nudged cell were in close contact with a chain of five or more cells all in tight contact, each cell blebbed sequentially, on down the line, after the first one was nudged. The time (in seconds) of formation of a new bleb on each cell varied directly with its distance from the first cell.

The results of these arcane experiments provide evidence for transmission from cell to cell of a stimulus for a cell surface activity that lies at the basis of the commencement of their locomotion. In the tightly packed blastoderm, once one of these cells forms a bleb, it may pass a stimulus for bleb formation to cells adjacent to it and thus increase profoundly the efficacy of the surface activity leading to cell movement. Once movement has begun, this could be a major factor in the coordinated mass cell movements of gastrulation.

Incidentally, deep cells of *Fundulus* had been shown to be electrically coupled in vivo and in vitro and James Hogan in our lab found that gap junctions occur between deep cells. These communicating intercellular junctions might allow the passage of a signal for blebbing, i.e., by a low molecular weight
messenger, which passes from cell to cell through these low-resistance, aqueous channels. However, this would provide only part of an explanation. Electric coupling is essentially an instantaneous process and, in consequence, far faster than the coupling of these cells for blebbing, which usually takes several seconds. Thus electrical coupling might be involved as the first step, with the final end effect on the cell surface being delayed in the cytoplasm by the additional time required for activation of the cytoskeleton in the blebbing mechanism. Also, of course, these cells at this stage of development might be on the verge of forming a bleb normally and need only one final push to go into operation.

Later, in 1977, I had the pleasure of reporting these results at the celebration of the 500th Anniversary of the renowned University of Uppsala in Sweden. One of the joys of science is that, being so international, it often takes us to other countries and other peoples to discuss our work. My trip to Sweden gave me, for example, the opportunity to meet with and enjoy the company of a legendary experimental embryologist, Professor Sven Hörstadius, a hero from my student days because of his famous, gorgeous microsurgical experiments on early sea urchin embryos. He and his wife were charming people and the four of us had a delightful time together. At our departure he presented me with a copy of his book on sea urchin development. A few months later, Hörstadius and his wife visited us at Yale and spent the night in my study-guesthouse in Guilford.

Cell Motility in Culture

In the same years as this research on Fundulus deep cell motility was going on in Woods Hole different lines of research were being followed in our lab in New Haven. Although my main interest was to study cells in the embryo as they engage in morphogenesis, much was to be learned from working with cells in tissue and cell culture, where they are under optimal optical conditions and can be manipulated manually and chemically with relative ease. The subject of most of the work on cell movement in tissue culture in those days was the chick embryo fibroblast, a connective tissue cell that thrives in various culture media. However, most of the work on fibroblast movement was unsystematic and anecdotal until a certain English embryologist entered the field. In the mid-1950s, Michael Abercrombie at University College London published with Joan Heaysman a few papers that systematically established how fibroblasts at the edge of a chunk of tissue move away from it out onto the glass substratum and thereby form an ever widening zone of “outgrowth.” Their method was statistical analysis of cell to cell contact and motile behavior as observed in time-lapse films. When a fibroblast contacts another it adheres to it and stops moving. They called this contact inhibition of cell movement. Then the free margin of the cell begins motile activity and with this the cell soon breaks away,
moving even farther from the tissue mass until it contacts another cell, is contact inhibited and so on. This work showed that one could explain the mass behavior of a myriad of cells in culture by close, careful observation of the motile behavior of individual cells, concentrating systematically on single aspects of the movement. Afterward, they followed this work by combined light microscopic and electron microscopic studies of the morphology of a fibroblast in different phases of its movement. These papers of Abercrombie and colleagues were of seminal importance to our laboratory. Thanks to them, instead of gazing with wonder at these marvelous moving cells, as we had done so often in the past, the study of cell movement had become a more disciplined science.

I count my association with Michael Abercrombie to be among the richest experiences of my life. It began in 1960, when I first visited him at University College London. There, he showed me films of some of the very cells on which he and Joan Heaysman had founded the concept of contact inhibition. Two years after that, I persuaded Jim Weston to do his postdoctoral research with Michael. About 10 years later, Albert Harris, certainly one of the brightest and most creative of my graduate students, chose on his own to pass his postdoctoral years with Michael (before he accepted a faculty position at the University of North Carolina). And, finally, Norman Wessells, one of my best early students and by then a member of the faculty of the Biology Department at Stanford, chose to spend a sabbatical year with Abercrombie. In addition, Michael and I personally drew constantly closer in our research interests and enjoyed each other’s company frequently at various international meetings from Philadelphia to London to Moscow to Glasgow. Abercrombie was a profoundly intelligent man and a thorough, penetrating scholar, with a generous but modest personality. I learned from Albert Harris that Michael’s father was a distinguished English poet and Professor of Poetry at Oxford. Robert Frost lived with the Abercrombies, for a year or so, just before World War I, before Frost published his books of poetry that made him famous. Michael never mentioned any of this to me. He didn’t like to talk about personal issues. Although Michael and I were quite different in our social demeanor, we nevertheless got along famously.

We became in effect the senior members of an extended family, he the chief and I the deputy. Three of my best students became also three of his best and his fine entourage merged readily and happily with me and mine, in particular a robust young Englishman from Yorkshire named Graham Dunn, who collaborated with Albert Harris. Curiously, although much admired and even loved for his consummate professional and personal fairness, Michael almost always insisted on being the leading author of all his collaborative publications. Nobody seemed to mind this quirk much but it seemed out of character so none of us could figure it out.
I chaired a 1979 Gordon Conference in New Hampshire on Cell Contacts and Movements, a truly great honor. Although I had invited Abercrombie and eagerly looked forward to visiting with him again, our most distinguished anticipated participant was sorely missed. Michael Abercrombie had shockingly died of lung cancer only a few months earlier. He had smoked a lot when he was younger. He was also cured of Hodgkin's disease by irradiation in the 1930s (a rare cure at that time) and this may have contributed to his lung cancer. As Chairman of the Conference I dedicated the meeting to this great man, to the scientific spirit he exemplified, and to the warmth of his character that so inspired all who knew him.

Teiichi Betchaku and I got into the swing of the important lessons learned from Michael Abercrombie. We began to study contact inhibition in great morphological detail. We showed that the ruffling motile activity of the spread leading lamellipodium of a moving fibroblast is paralyzed locally, precisely at the point of contact with the surface of another cell. This suffices to stop temporarily the contacting cell’s movement until it forms a new ruffling lamellipodium elsewhere on the cell surface. This made such a pretty picture that I used a stylized version of it for the cover of my book Cells into Organs (Figure 11.1). More importantly to our laboratory, I persuaded several of my students to devote their graduate research to studying cell movement in tissue culture. There were three good reasons for this push on my part. First, the importance of the questions currently begging answers. Second, the impossibility for graduate research to be limited to summer research at Woods Hole. Third, as had been my practice for years, I didn’t believe graduate student research should be a segment of the professor’s personal research program.

We had a lot of fun in my lab in the late 1960s and early 1970s. There were a lot of students around and some of them were pretty funny. I was having a wonderful time working on my own research and with the motley crew of good junior colleagues while serving Yale as Master of Branford College. I was usually in a good mood and pretty much on top of my game (Figure 11.2). Let me give you an example of the fun we had. Albert DiPasquale, a junior graduate student, worked for a time in my lab alongside Albert Harris, who was, at the time, putting the finishing touches on his dissertation research. Two Alberts in the lab at one time created a nicknaming problem. Albert H has a great sense of humor and Albert D was an extremely reserved, Italian American, who most assuredly had led a sheltered, very Catholic life, graduating from Villanova before coming to Yale. Naturally, Albert H quickly dubbed Albert D, Al “The Biologist” DiPasquale as if Al The Biologist were a torpedo for the Mafia. I recall that at the time, there was a lot of news about a Mafioso in New York with the nickname “Big Tuna.” The sobriquet for Albert D was utterly ironic, because Al The Biologist was reserved and shy, the antithesis of a button man. By the
way, Al The Biologist studied the movement of epithelial cells in culture, did a nice dissertation, and later went on to medical school.

Figure 11.2. The author in 1970, in a relaxed pose in his office on the tenth floor of Kline Biology Tower, on top of his game. Photograph courtesy of Kurt E. Johnson.

Albert Harris worked on the role of the adhesion of fibroblasts to the substratum to gain the traction necessary for cell movement and after achieving his doctorate went to Abercrombie's lab in England, where he studied cell surface movement during the spreading of fibroblasts. Michael was very grateful to have had Albert (and earlier Jim Weston) working in his lab for a while and thanked me feelingly. Albert collaborated extensively with Graham Dunn, who worked in Abercrombie's lab at the same time.

What a pair of characters Harris and Dunn were! Albert Harris is tall, about six feet, six inches and imposing (physically and intellectually). He has impressive verbal skills, both in technical and jocular matters. Being raised by parents who were cultured, his mother a psychiatrist and his father a well-known professional painter (of paintings not houses), Albert didn't drink, smoke, or swear. He was the sort of man who would laugh at the Mexican pronunciation of the Spanish word for "virgin," which comes off sounding like "beer hen." In contrast, Graham Dunn was much shorter, spoke with a thick Yorkshire accent, was bearded, and drank, smoked and cursed a lot. They both shared an appreciation for the twisted comedy of the brilliant English comedy troupe
Monty Python, and both had aggressive, critical personalities. In short, they were a Mutt-and-Jeff-like pair who regularly terrorized their scientific competitors, playing together like a wrestling tag team. I took great pleasure in sitting back and enjoying the fray at several important international convocations.

One of the important discoveries of Abercrombie was that a certain strain of cancer cells did not show contact inhibition. The cells appeared to move over fibroblasts, criss-crossing as it were. Both Paul Bell and Carol Erickson, graduate students in my lab, checked this on two different cancerous cell lines. They found by careful microscopy, to Michael's dismay, that the criss-crossing of these cells with normal cells is not due to cells crawling over one another, but instead by crawling under them. Harris and others had shown that moving cells adhere to the substratum only at the marginal lamellipodia at the leading edge and at the firmly stuck trailing edge. My old mentor, Professor H. B. Goodrich first described these cells in the 1920s. He called them "canoe cells" because of their shape. So another cell can crawl under the unattached middle of the cell. In addition, Carol Erickson and I studied the source of surface membrane in a spreading fibroblast with scanning electron microscopy (SEM) and found that it comes from a reserve of surface already present in blebs and microvilli of rounded mitotic cells. This observation was an exciting piece of research for it demonstrated that a fibroblast needs to manufacture little or no new surface in order to spread so vastly as it settles on the substratum prior to beginning locomotion. These new discoveries also provided a useful model for Betchaku and me when studying the epibolic expansion of the yolk syncytial layer in Fundulus.

Lessons from Tissue Culture

All this intensive study of the movement and contact behavior of normal fibroblasts and cancerous cells by my graduate students and by Betchaku and myself provided me with a sophisticated education on how tissue cells move in vitro. This education was much more than I would have gained by simply reading the literature—a perfect application of the old instructive maxim promulgated by Louis Agassiz, "Study nature not books." With this background and with new technology, I turned back to the movement of Fundulus deep cells in gastrulation. This time my collaborator was not a first year graduate student but a sophisticated young investigator, Carol Erickson, who had completed a splendid dissertation on the contact behavior of cancerous cells in vitro and was therefore an expert on morphology and motile behavior of cells in culture. She was by then on the faculty of the University of California at Davis.

With the limitations of bright-field microscopy, our first observations of deep cell movement could only go so far. With extreme good luck for us, two techniques for observing cells in far more detail soon became available:
Nomarski optics (DIC), for observing living cells within a three dimensional system like an embryo, and the scanning electron microscope for observing cells at high magnification in whole embryos after fixation. Carol was, and still is, a person of boundless energy, with a smart inquiring mind and a passionate interest in knowing how cells move, interact and change during development. Also, she didn’t hesitate to differ with me—often—openly—refreshingly. It is warming for me to recall what fast friends Carol and Cheryll became, both to one another and to me, when they were both working in my lab.

What Carol and I found was that *Fundulus* embryonic cells *in vivo* do not flatten on their yielding living substrata, the yolk syncytial layer or the underside of the enveloping layer, the way fibroblasts do *in vitro*, on their hard inanimate substrata of glass or plastic. But this difference is trivial. In other respects most moving deep cells *in vivo* closely resemble fibroblasts *in vitro*. They form large dominant lamellipodia and very fine filopodia (fine-pointed marginal extensions) at their leading motile edge. These surface protrusions adhere firmly to the substratum (Figure 11.3). Their trailing edge, which also adheres tightly to the substratum, is pulled out to form a long pointed tail as the leading edge spreads forward, just like that of a moving fibroblast. As the spreading leading edge advances, the cell becomes more and more elongated.

Figure 11.3. A scanning electron micrographic landscape of *Fundulus* deep cells moving on the YSL after the blastoderm has been removed. Reprinted with permission of John Wiley & Sons, Inc.
until the trailing edge finally breaks away from the substratum. Whereupon, the leading edge springs forward, several micrometers per second, just as it does in a moving fibroblast, shortening the cell considerably. Then, the leading edge moves forward again or the cell remains immobile for a while until a new filolamellipodium forms and the cell then moves in its new direction. These results were very encouraging because they demonstrated that the movement of fish embryonic cells *in vivo* resembles in important details the movement of the far more studied chick or mammalian fibroblast-like cells *in vitro*. Our findings consequently established for the first time that much if not all that we learn about the mechanism of the movement of cells in tissue culture can be extended and applied to cells moving *in vivo*, including cancer cells. As we find ways of inhibiting cell movement *in vitro*, the same therapy might conceivably be applied with profit to inhibiting the movement of cancer cells *in vivo*.

For unknown reasons we have only occasionally been able to get *Fundulus* deep cells to move in cell culture, no matter what media or substrata we tried. Our attempts to do so used up a lot of time and movie film as we studied lovely normal cells that seemed about to move but rarely did. It was tantalizing. Paradoxically, this may be the only case in which more is known about how cells move *in vivo* than *in vitro*. You see, research on cell movement is not always as dynamic and exciting as I have portrayed. There are, as in life, genuine depressing disappointments along with the exhilarating successes. Our failure in this case was an intensely frustrating experience.

**Directional Cell Movement**

With our new understanding of the morphology of the movement of gastrulae cells, we were in strong position to tackle gastrulation itself. Marking studies by both Jane Oppenheimer and me, showed that cells in the marginal germ ring move dorsally from the right and the left to converge to form the embryonic shield, out of which the embryo proper is fashioned. Do all the cells move continuously? Do they converge in a straight line or do they meander toward the shield, stopping occasionally or even reversing direction from time to time? What is the role of cell division in convergent movement? We were well-equipped to seek answers to these and other questions but didn’t get around to it until some years later. Other research intervened.

This is a good place to mention an important change that occurred in my laboratory when I was in mid-career. During a brief period around 1974 President Richard Nixon froze all grant funds and I had to fire my assistant. Coincidentally, Madeleine lost her teaching job because her school eliminated the French program. So, I had no assistant and she had no job. After some thought, I decided to hire her. Why not? She was very intelligent [having graduated at the head of her class at *Hautes Etudes Commerciales* in Paris (the top school for economics)], full
of energy and devotedly conscientious. Anyone with those qualities could learn to do well, given a little patience, what I required of an assistant. Also, we agreed that I would be boss at the lab and she the boss at home. This arrangement has worked out beautifully for both of us to this very day.

I mention this here because in addition to being my assistant, Madeleine later became a full collaborator. When I turned my attention to the convergence of cells in the germ ring during gastrulation, she joined me and Rachel Fink on the project. Rachel was my last postdoctoral fellow. Among her superb human qualities of high intelligence, good sense, warmth, and humor, she is a technical genius with equipment, quite unlike me. She convinced me to shift from cinematography to videography. When we arrived in Woods Hole this time it was my collaborator who put our names on the door—“Trink and Fink.”

Although some of the most impressive and best studied morphogenetic cell movements during vertebrate embryogenesis are the spreading and folding of cell sheets, it must be noted that cells also move as individuals and as clusters within flowing cell streams. Since the movements of individual cells are often hidden from the eye, either because of the opacity of the embryo or because the cells in question are deeply embedded within, direct study of the detailed motile behavior of living individual cells and small cell clusters is largely precluded. What was needed were highly lucid or transparent eggs in which the cell movements in question take place at the surface of the egg. The converging germ ring of *Fundulus* is perfect for this. Also, because the egg of *Fundulus* is fairly large, 1.8 mm in diameter, its cells are less densely packed and thus more readily observed as individuals than in smaller eggs, like those of the zebra fish.

“By taking advantage of the favorable optical properties of the *Fundulus* egg, the low population density and the approximate monolayered state of the germ ring, we have been able to answer a number of questions concerning the motile and contact behavior of cells moving directionally in a cell stream during a massive movement of convergence during gastrulation. Since movement in cell streams is widespread during both embryogenesis and the spread of cancer and because, insofar as we know, this is so far the only detailed study of this type of morphogenetic cell movement in a living embryo, we believe that our results should have a certain general interest.” So we wrote in the Discussion of our paper. Bluntly, our observations were unique. By the bye, among the zebra fish people, our big paper on convergence came to be referred to as “Trink, Trink and Fink.”

So what were these observations? *All* cells in the marginal region of the blastoderm are motile and, amazingly, *all* move toward and into the embryonic shield. This is a 100% efficient directional morphogenetic movement. Actually, these movements would not need to be 100% efficient to be effective. But they are. Constant directional forces are obviously at work. But no cells move toward the shield in a direct line. They all wander or meander considerably, moving
forward and backward and to the side and even stopping, but their net movement is unidirectional. Cells move mainly by means of filolamellipodia and less frequently by blebs. Actually, however, there is very little movement by cells as individuals; all cells are almost always in adhesive contact with other cells in moving cell clusters. These clusters vary constantly in size, continually aggregating with other cells and other clusters and splitting from them. The cellular displacement process is exceedingly dynamic. Significantly, the cells show contact inhibition of movement (one of the few cases where this has been observed in vivo). Nevertheless, they move and do so directionally, possibly partly because of this.

An unexpected and highly significant observation emerged from our cell-by-cell and minute-by-minute tracings. Cells and clusters nearer the shield move faster toward their target than those farther away. There is a gradient in rate of movement. The net rate increases the closer they are to their target. This is not due to an increase in cell motility, as might be expected, but to a decrease in meandering. As they move closer to the shield they meander less, some moving almost in a straight line. We reason from this that some factor(s) in the environment influences the protrusive activity of the cells such that they show more motile activity on the side of the cell closest to the shield. We also found that this putative directional factor(s) acts in a gradient whose high point is in the embryonic shield. Consistent with this, the most ventral cells and cell clusters, i.e., those farthest from the shield, meander the most and show the least directionality. We do not yet know the nature of this gradient of attractivity. It could be a chemotactic signal emanating from the embryonic shield, but we do not yet know.

During all of this convergent movement, there is active cell division. As one of my graduate students, Kurt Johnson, showed for amphibian gastrula cells, cell division temporarily stops cell motile activity cold. The same is true of Fundulus cells and for Metazoan cells in general. There is apparently an antagonism between cell movement and cell division. We were, therefore, mildly surprised to observe how frequently these very actively motile cells divide, for, as expected, they do indeed cease locomotion when they divide. It turns out, however, that the total duration of the mitotic cycle averages only 20-25 minutes plus 2-5 minutes for cytokinesis itself before movement of the daughter cells begins. While in one sense this delay is a considerable period, it is small compared to the many hours during which cells are actively converging. Moreover, only a small proportion of the cells is dividing at any one time. We conclude, therefore, that cell division does not impede convergence in any significant way. It takes the cells out of their dorsad trek toward the embryonic shield only briefly. Then, at the termination of cytokinesis, the daughter cells rapidly join the throng and begin moving with them, quite as their mothers were doing before.
One day when Rachel Fink and I were dechorionating *Fundulus* eggs to prepare to do some experiment, she observed something remarkable. Deep cells in the yolk sac of a very advanced gastrula were moving toward an accidental wound in the enveloping layer at the surface of the egg, where they slowly aggregated into a dense cluster. Something in the wound was attracting them. We became quite excited because we immediately thought of galvanotaxis. Two facts suggested this to us. Wounds in biological systems have long been known to generate an electric current, and cells in tissue culture will move selectively toward one pole or the other in an artificially constructed electric field. This movement is called galvanotaxis, and has often been proposed but has been difficult to prove as a means of directing cell movement in developing embryos. We were especially elated because we had a beautiful set-up for studying in detail the potential for operation of a galvanotactic directional cell movement during embryonic development: a highly transparent embryo with the cell movements in question taking place at the egg surface just beneath the very thin EVL, with its glass-like transparency. All we had to do was puncture the EVL with a fine needle and watch and videotape the cells moving toward the tiny wound. The response was both rapid and widespread; cells from as far away as 800 µm responded as quickly as those nearby and by 100 minutes after wounding up to 90% of the blebbing cells within this radius had moved to the wound site and aggregated around it. The attraction was clearly powerful because the cells moved quite directly toward the wound with little meandering. Aside from our interest in the mechanism of the phenomenon, this is a striking example of the quick reaction of the embryo to protect itself from any outside physical insult (Figure 11.4).

We naturally took advantage of this opportunity to study details of the cells’ motile behavior. For example, when a stationary cell finds itself under the influence of the factor emanating from the wound, its locomotory activity begins by forming a bleb on the side of its surface facing the wound. When cells have aggregated at the wound they keep milling about in spite of their close contacts. Thus they are not contact inhibited. Along with these detailed observations and several others our main interest, however, was to determine if the cells were moving directionally in an electrical field. To this end we approached Lionel Jaffe, a friend and colleague at Woods Hole, who was at the time the world’s authority on bioelectric fields and their biggest fan. Lionel was convinced that we had a beautiful case of galvanotaxis and gave us access to his equipment and staff to see if we could prove it. First, we confirmed that, indeed, wounding does set up an electric field. But that was not enough. We had to show that the cells were responding to the field rather than, say, a chemotactic signal or some other stimulus emanating from the wound. To check this possibility, we used microelectrodes to inject current through the EVL, into the subepithelial space. We were attempting to block the normal wound current by
injecting current of equal magnitude and opposite polarity. To our great disappointment, this technique did not work, except for a few times. So we were left with an excellent system for inducing and studying directional cell movement during embryogenesis, but no evidence for the cause of the directionality. But we survived. We did learn much about details of directional cell movement and had an amusing and sometimes exhilarating time working at it. We published a paper on our findings, and Rachel used part of our footage for her beautiful and popular video series, *A Dozen Eggs*.

In the 1960s, I turned myself into a cell biologist in order to be a better embryologist and subsequently suggested to a number of graduate students that they study cell motile behavior in tissue culture. None did so more successfully than a young Chinese student from Taiwan who came to Yale from a small college in Boston and spoke broken English. Since his American compatriots made no effort to pronounce his Chinese name, they named him Jake, Jake Chen. I quickly put an end to that, insisting that everyone address him by his given name, Wen-Tien. Because of his language problem, I had no accurate idea of how good he was. He turned out to be a sleeper. After the usual slow start, Wen-Tien Chen revealed himself to be an exceptionally creative researcher with an added bonus—a lively sense of humor. I gave him a very straightforward problem: explain how the taut, pointed tail of a moving fibroblast detaches and retracts

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**Figure 11.4.** Photographs of deep cells moving directionally toward a wound in the *Fundulus* EVL. Left, six minutes after wounding, right, 75 minutes after wounding. Notice the aggregation of deep cells at wound site. Scale bar equals 100 μm. Tracings of cells' tracks to wound site revealed highly directional movement. 
Reprinted with permission of John Wiley & Sons, Inc.
during the course of the cell’s jerky, crawling movement. He not only did an excellent job at that but also showed outstanding serendipity. How many of us have watched the snap retraction of the tail of a moving fibroblast without paying any attention to the leading lamella during the process. Well, Wen-Tien did pay attention and made a remarkable discovery that generated a good deal of excitement at the time. As the trailing tail retracts, either spontaneously or with the aid of a microneedle, the leading lamella, that had been moving the cell forward, undergoes a quick spurt in spreading, 10-30 fold as fast as normally within eight seconds of the onset of the retraction. This surge in protrusive activity moves the cell farther forward. This observation was important for three reasons. First, measurements showed that retraction of the trailing edge provides new surface material for spreading at the leading edge. Second, by reinforcing this spreading, it explains why a fibroblast tends to move in the same direction for a while. Third, it provides a ready means of inducing spreading: simply disrupt the adhesion of the trailing tail with a microneedle and observe directly the increased spreading of the leading lamella as the trailing edge retracts.

Chen called this retraction-induced spreading (RIS) and I arranged for him to publish his findings quickly as a Rapid Communication in the Journal of Cell Biology. We had just learned that our good friend and colleague, Graham Dunn, in Abercrombie's lab in Cambridge had simultaneously made the same discovery and, revealing a little competitive spirit, we didn’t want Wen-Tien to be scooped. It was a joint discovery by two very talented young investigators. Graham’s contribution was appropriately noted in Wen-Tien’s paper.

But how does the cell surface and its associated cytoskeleton manage this quick spectacular shift? With this question in mind, I sent Chen to do postdoctoral work with my old friend and fellow of Branford College, Jonathan Singer, to find out. Jon had transferred to the University of California at San Diego and was certainly one of the best molecular and cellular biologists anywhere working on the cell surface and the cytoskeleton. It was he who provided the final evidence for the fluid mosaic structure of the plasma membrane, a truly major advance. I thought that if anybody would have some good ideas as to how to attack the mechanism of the RIS it would be Singer. But no. Jon was not interested. I learned later that everyone in Singer's lab works on projects that he suggests, which are usually very good. After Wen-Tien left La Jolla, Jon told me that if I have anyone else as good as Chen I should please send him out. Incidentally, when Jon left Yale, he told me that his main regret was leaving the Branford College Fellowship.

All of this naturally brought me back to Fundulus gastrulation. Does RIS operate during convergence to reinforce cell directionality in vivo as in does in vitro? To get the best quality images of moving cells, I prevailed on Rachel Fink, by then on the faculty of Mount Holyoke College, to help me with her state-of-the-art, computer-controlled video equipment. It was not easy to find
cells in the germ ring isolated long enough from their neighbors to give the required information, but I found some cells moving by themselves and, by God, they definitely exhibited retraction-induced spreading. When the taut trailing tail ruptured and retracted, the leading edge surged forward. Thus RIS could be a factor reinforcing directional movement toward the embryonic shield. How significant it is for gastrulation remains for future investigation.

Curiosity

Back in May, 1980, Madeleine and I went to the famous Station Biologique de Roscoff in Brittany to study epiboly in another fish egg. Considering the enormous taxonomic variability among the teleosts, it occurred to me that I really ought to try to see if epiboly operates in the same way in another teleost, one somewhat different from Fundulus. I chose the lumpfish because of its rather large eggs (lumpfish caviar). The collectors of the station dutifully provided me with lumpfish eggs, but, sadly, they were unsatisfactory. I found it impossible to remove their membranes.

What to do? I discussed the problem with Alain Maron, one of the excellent marins (collectors) of the Station. After some brief contemplation he came up with a suggestion—the blenny (Blennius pholis), a strange looking little teleost that hangs around the intertidal zone and deposits its eggs on the underside of rocks of almost any size, from pebbles to enormous boulders. He showed me some eggs. What I saw was a very lovely translucent embryo with a beautiful pigment pattern, especially in the pectoral fins, reminiscent of Fundulus. Clearly, I had to see the early stages. The next step was to crowd a bunch of blennies in the aquarium in my little laboratory in the old original building of the Station (with a direct view of a charming walled garden). I added a pile of flat rocks, fed the blennies chopped frozen fish (they preferred mackerel, but in fairness, I should have fed them chopped lumpfish or caviar) and waited. Not long. With my well-known luck, there were hundreds of eggs on the underside of one of the rocks the very next morning. This carpet of eggs was fascinating of itself. Each egg was deposited by the female literally cheek by jowl in a precise monolayer on a common community adhesive pad. Extraordinary! With little effort, individual eggs could be dissected off the pad and their membranes quite easily removed. They were of a nice size (about 1.2 mm in diameter) and sufficiently lucid to reveal cell motility. So, I went to work to follow early development using an antique dissecting microscope lent me by the Station.

Exploring new beautiful material like this is captivating. In addition, being on leave from my duties at Yale gave me plenty of time to delve into my own little biological world by myself and at my leisure. Fortunately the Blennius egg develops slowly, in keeping with the temperature of the local seawater,
which hovers around 12° C. in the month of May. What struck me quickly soon after gastrulation had begun, was something totally unexpected. Two big black spots slowly appeared just lateral to the prospective trunk region on either side of the embryonic axis. What in the world were they doing there so precociously? The neural crest was surely just beginning to form. Naturally I was curious; indeed, I was entranced. So I postponed studying epiboly.

By then I was late for dinner and had to leave, else Madeleine would worry. We had no telephone. So I walked quickly home along the harbor. It was low tide and all the boats were up on stilts. At home, I downed a generous scotch to celebrate my bizarre discovery, had dinner and then immediately drove back to the lab. I remember that little trip vividly. It was night; there was dense fog; the streets were empty; and, the Station Biologique was in complete darkness. I opened the gate in the garden wall and suddenly remembered the ornamental fish pool. I never gave it a second thought during the day, except to watch the fish, but now, in the dead of night I realized that I might fall in. Fortunately, I felt the gravel of the path beneath my feet and with this as a guide groped my way to the faintly looming bulk of the laboratory. Upon entry, I once again, for the thousandth time, appreciated the wonderful little illuminated button installed at the entry and at the foot of the stairs, as is the case throughout France. Voilà, I had light.

I was wise to have returned so quickly. The spots had expanded. Their distal periphery now had a ragged look as if they were spreading. So I decided that I’d better stay with my materials. It was a bit eerie being alone in the laboratory at night. It brought me back to my student days, but with a difference. Back then, there were always other night-owls like myself. Here there were none. Maybe the French are different, at least at Roscoff. Anyway, I liked it—"the lonely scientist sequestered with his embryos in his simple nineteenth-century laboratory." Corny, but cosy.

The spots continued to spread and, as the intensity of their blackness decreased, they were assuming the appearance of a typical spreading epithelial sheet in which the cells now seemed to be arranged in a quasi-monolayer. Also, typical of a spreading sheet, rifts that slowly widened to form semicircular gaps began to appear between the submarginal cells, indicating increasing tension in the spreading sheet (Figure 11.5). As spreading continued, the gaps widened and some groups of distal peripheral cells began to break away from the sheet and to spread on their own over the yolk sac away from the sheet and, of course, away from the embryo. This dismembering continued until there were several such directionally moving groups.

Suddenly, it hit me. I had made a discovery! In the excitement I had unconsciously risen and was pacing my little laboratory and talking to myself. "Trinkaus, do you realize that you are observing active directional movement of cell clusters in vivo in the embryo for the first time ever! For the first time in
history!” Pause. “Yes, indeed.” I wanted to tell somebody. But there was not a soul to tell. So I told myself. Then, after a moment of excited reflection, with heart throbbing (I do not exaggerate), I returned to the microscope, and resumed my observations. Where will these clusters go?

Lacking a camera, I had been making drawings as I watched, to record what my simple curiosity was revealing. Now, however, my drawings began to make scientific sense. I had discovered an important phenomenon and I had better record it well and in significant detail. I should point out, not at all incidentally, that I was prepared to recognize the significance of this discovery. John Kolega, one of my graduate students, was at the time in the midst of his doctoral research on the movement of cell clusters in tissue culture. “Nature favors the prepared mind.”

![Image: Two clusters of moving pigmented cells in the yolk sac of a Blennius pholis embryo, recently studied at Station Biologique de Roscoff.](image)

Figure 11.5. Two clusters of moving pigmented cells in the yolk sac of a Blennius pholis embryo, recently studied at Station Biologique de Roscoff. In the lower left cluster of cells, the light spheres are cell nuclei.

I ended up spending much of the night following this remarkable phenomenon. The spots, or “pigmented cell masses,” as I came to call them, continued to spread slowly, breaking up into a dozen or more cell clusters. The more peripheral ones moved directionally, more or less steadily away from the embryo proper, until each side of the yolk sac was dotted with more or less equally spaced black epithelial, monolayered cell clusters. Curiously, movement of all ceased when the most peripheral ones reached the most ventral region, just short of the mid-ventral line, suggesting passage of some kind of a message.

There is much more to tell. Later, the cells of these scattered epithelial clusters transformed into mesenchymal dendritic melanocytes, a dramatic
change in cell type. The clusters then disaggregated in a gradient, beginning with those closest to the embryo and the melanocytes thus liberated migrated directionally as individuals toward the pectoral fin bud on each side of the embryo. As they reached the pectoral fin bud, they penetrated it and eventually formed an intricate beautiful pigment pattern, the very pattern I had seen when I first observed a *Blennius* embryo.

The following spring and the spring thereafter, I returned to Roscoff fully equipped optically and photographically and studied with passion all of this and more with great care. I continued to collect eggs from an aquarium. But since the captive fish did not always cooperate, I had to supplement my supply with frequent trips to the vast intertidal zone (*la grève*) of Roscoff. Here, the two or three capable and jovial *marins* of the Station accompanying me would turn over little and huge boulders to look for blenny eggs underneath. This was really a festive sport, communally enjoying the look and smell of the sea, the rocks, the seaweed, finding the eggs, and feeling as if we were safely embedded in Mother Nature’s womb.

I tell this little story in an effort to convey some of the emotional impact of a simple scientific discovery and the importance of following one’s curiosity. But think, as is often the case, this discovery depended in large part on a striking stroke of good luck. If the cells of those spots were not stuffed with black melanin granules they would never have caught my eye. I also tell this story to try to convey some of the vitality of my love for experimental work on living embryos. Imagine this. I have been fortunate enough to have been doing my kind of science, for a pretty good living, for more than 60 years, having a lot of fun at it, and having received more than my fair share of admiration and even affection from respected colleagues.