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# Cortical Flow in Animal Cells

D. BRAY AND J. G. WHITE

**A concerted flow of actin filaments associated with the inner face of the plasma membrane may provide the basis for many animal cell movements. The flow is driven by gradients of tension in the cell cortex, which pull cortical components from regions of relaxation to regions of contraction. In some cases cortical components return through the cytoplasm to establish a continuous cycle. This cortically located motor may drive cell locomotion, growth cone migration, the capping of antigens on a lymphocyte surface, and cytokinesis.**

OUR PURPOSE IN WRITING THIS ARTICLE IS TO SUGGEST how many surface movements of animal cells may be integrated. Observations of the migration of fibroblasts, amoebae, and white blood cells over surfaces; the growth of axons; the capping of antibodies on a lymphocyte; and the changes in shape as cells divide all point to the existence of a concerted flow of actin-containing structures within a cortical region subjacent to the plasma membrane. The movements originate at a specific region of the cell surface—the leading edge of a migrating cell, for example, or the polar region of a dividing cell—and carry material back over the cell surface to a more proximal position. (Why these movements arise at a particular region of the cell surface will not concern us here but may depend on the disposition of cytoskeletal elements such as microtubules in the cytoplasm.) In most cases, it seems necessary to postulate a compensatory forward flow of actin-containing components within the cytoplasm giving rise to a "fountain flow" of cortical components within the cell. These cortical movements are probably a consequence of the contractile nature of the cell cortex and have a number of important implications for the behavior of animal cells, especially in response to contact with solid substrata.

The idea of a concerted flow of surface structures is not new. Suggestions of a similar nature date back to the latter part of the 19th century, when light microscopic observations of freshwater amoebae of the *Amoeba proteus* type revealed the active streaming of cytoplasm that accompanies pseudopodial extension. These observations gave rise to a model for the locomotion of giant amoebae in which the flow of centrally located, more fluid cytoplasm into a pseudopodium is driven by a contraction of cortical cytoplasm at the tail of the cell. With the development of tissue culture methods in the present century, it became possible to examine the locomotion of the much smaller cells from vertebrate tissues, and the suggestion arose that they too possess a "superficial plasmagel," although no layer could be discerned directly by light microscopy. Lewis was one of the earliest protagonists of this view, and in a prescient article published in 1939, he postulated that regional contractions in a cortical layer in white blood cells could provide a driving force for both cell locomotion and cell division (*1*). A similar suggestion is a central tenet of this article.

Despite their venerable origins, global aspects of cell behavior are often overlooked with the contemporary emphasis on detailed molecular mechanisms. We believe that it is useful to reexamine the question of cortical flow in light of information acquired in the intervening years. Many detailed observations of cell movements not considered by Lewis and his contemporaries, such as the migration of fibroblastic cells over planar surfaces, are now available. Certain molecular mechanisms that may power their large-scale movements can now be identified.

## The Actin-Rich Cortex of Animal Cells

Lewis's "superficial plasmagel layer" is now known to consist of a complex network of actin filaments and associated proteins attached to the inner face of the plasma membrane (*2*). This cortical layer has both elastic and viscous properties and maintains an isotropic tension that resists deformation, providing a resilient framework for the otherwise flimsy lipid bilayer (*3*). The cell cortex has the capacity to undergo local contractions, which are seen most clearly in the waves of contraction that travel over the surfaces of many eggs (*4*). More generally, local contractions together with changes in the structure and composition of the cortical layer and the generation of actin-containing extensions on the cell surface form the basis of cell locomotion, cell division, phagocytosis, and the changes in cell shape that accompany tissue morphogenesis.

A central challenge of contemporary cell biology is to explain cell movements and shape determination in terms of the molecules involved. Toward this end, several dozen proteins able to bind to actin and modify its properties have been identified; the majority of these proteins are present in the cell cortex (*5*). Some actin-binding proteins affect the ability of actin monomers to polymerize into

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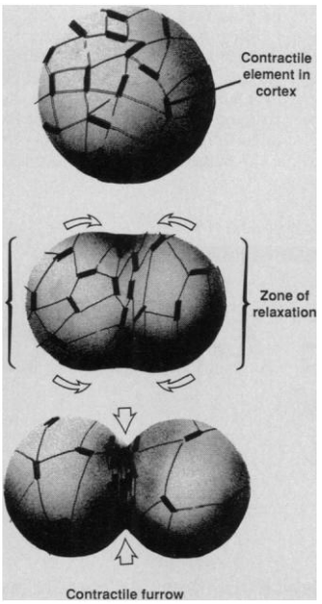


**Fig. 1.** Astral relaxation model of cytokinesis. The cell cortex is represented by a network of thin contractile elements stretched over the surface of a deformable balloon; the elements are free to move laterally but pull neighboring elements by their contraction. Relaxation of the network at the two poles of the cell, possibly due to an influence spreading from the mitotic spindle, leads to an accumulation of cortical elements in the equatorial region. Computer simulations based on these properties generate self-sharpening contractile furrows similar to those observed in living cells (*14*).

filaments or to regulate the lengths of these filaments; others cause actin filaments to assemble into bundles or three-dimensional networks. Various types of myosin interact with actin filaments to produce movement, converting chemical energy in the form of metabolically produced adenosine triphosphate into the contraction of actin bundles or networks.

Despite our detailed understanding of the action of many actin-binding proteins at the molecular level, we remain ignorant of the way in which they interact as an ensemble. Actin-rich extracts from motile cells can undergo gelation, solation, contraction, and localized streaming in response to changes in concentration of intracellular signaling molecules—such as $Ca^{2+}$, adenosine 3′,5′-monophosphate (cAMP), and phosphatidylinositol-bisphosphate—as well as to mechanical stress (*6*). But how this repertoire of behavior is harnessed to allow changes in one region of a cell to be coordinated with changes in another region is not known. It is in this respect that, we believe, the concerted flow of cortical components provides a unifying perspective.

## Cytokinesis

The viscoelastic properties of the cell cortex have been estimated by measuring the deflection of the surface in response to a known force, such as that produced by a suction pipette, or an electrically controlled miniature probe (*3, 7*). Changes associated with physiological events have been observed. For example, during cell division in very large cells, such as sea urchin eggs, there is initially a global increase of tension, which may be related to the tendency of even small vertebrate cells to become round before division, followed by a relaxation at the two poles (*8*). Surface stiffness remains high in the equator of the cell, leading to an indentation of the surface in a circumferential furrow. This "cleavage furrow" becomes progressively deeper, pushing organelles to one side or the other by mechanical force, until it eventually divides the cell into two.

The ultrastructural correlate of the contractile furrow is the "contractile ring": a bundle of actin filaments and associated myosin molecules lying just beneath, and associated with, the plasma membrane (*9*). Although actin and myosin are present throughout a dividing cell, these proteins occur in much higher concentration in the contractile ring and may be drawn there from other regions of the cell surface. Thus, cells of the cellular slime mold *Dictyostelium discoideum* have a uniform distribution of randomly oriented myosin rods in the cell cortices during interphase, and when these cells cleave, myosin rods become concentrated in the equatorial regions of the cell and circumferentially aligned (*10*). Furthermore, a concerted flow of surface-associated structures into the cleavage furrow has been demonstrated in living macrophages exposed to fluorescently labeled concanavalin A, a reagent that binds to and cross-links many surface glycoproteins (*11*).

These changes of surface stiffness that take place during cell division can be shown experimentally to be controlled from the cytoplasm by the mitotic spindle, which may either induce contraction of the cortex at the equatorial region (*12*) or cause relaxation in the vicinity of the mitotic poles (*13*). In either case, a gradient of cortical tension will develop, which could, in principle, drive the movement of cortical components into equatorial regions. In a recent computer simulation of this process, surface tension is assumed to be generated by linear contractile elements in the cell cortex that are free to move in the plane of the surface while still maintaining tension and exerting force (*14*). As localized changes in surface tension occur (mediated in this model by a relaxing influence spreading from the two mitotic spindles), the cortical elements move from regions of lowest tension (polar) to regions of highest tension (equatorial), changing as they do so from a random orientation to alignment with the equator of the cell. A suitable physical analogy is that of an elastic net stocking drawn tightly around the cell, which is loosened at either pole by cutting some of the threads. The tension of the remaining network then draws the remaining strands together around the middle of the cell while at the same time changing their orientation until they are circumferential (Fig. 1). Computer simulation of these properties generates a sequence of cleavage morphologies that are extremely close to those observed in living cells. The agreement includes not only normal symmetrical divisions but also a variety of unusual cases in which division is asymmetrically placed (*15*).

Morphological and mechanical changes in dividing cells are therefore consistent with the suggestion of a concerted redistribution of cortical actin, driven by the contraction of the cortex and directed by a localized relaxation at the polar regions of the cell. Given a mobile, tension-generating network, the movements become self-perpetuating and self-sharpening once certain threshold requirements have been attained.

Contraction of the equatorial region of the cell cortex has one additional consequence. Examination of the contractile ring sectioned for electron microscopy at successive stages of cell division reveals that its volume, and hence its mass, decreases as the diameter of the ring grows smaller (*16*). Contraction is thus accompanied by dispersal, a feature that may be important in the migration of cells.

## Neutrophil Migration

A concerted flow of cortical materials is also an essential part of the migration of cells over surfaces, as illustrated by the crawling motion of white blood cells or leukocytes (*17*). In the body, polymorphonuclear leukocytes (neutrophils) and macrophages move into tissues in response to inflammatory stimuli or infection; lymphocytes, the cells responsible for immunity, migrate from their site of origin to various peripheral lymphoid tissues. These movements can be observed either in tissue culture or, under favorable conditions, in living tissues, and are accompanied by the production of undulating sheets of cytoplasm, or "lamellipodia" on the cell surface. Some lamellipodia extend smoothly outward over the substratum to establish the leading margin of the cell; others fail to

attach and move back over the cell surface in a wavelike motion known as "ruffling." Closely following the advance of the leading lamellipodium, a flow of cytoplasm—detectable by its content of organelles—carries the bulk of the cell forward; its pear-shaped tail, or "uropod," periodically detaches and retracts into the cell body. These movements are paralyzed by the actin-binding drug cytochalasin and also occur in small fragments of neutrophils lacking a nucleus and cytoplasmic microtubules (*18*).

Lamellipodia are 0.2 μm or so in thickness. They contain a thin meshwork of actin filaments that in whole-mount electron micrographs appears to radiate from focal points on the perimeter. The concentration of actin in lamellipodia of cultured fibroblasts may be as high as 30 mg/ml, and immunofluorescence staining reveals the presence of actin and actin-binding proteins such as α-actinin, fimbrin, and talin (*19*). Since the surface protrusions of neutrophils are supported by a framework of actin filaments and associated proteins, it seems inescapable that as the surface protrusions move toward the rear of the cell over its dorsal surface, they must carry with them their associated actin molecules. Moreover, this movement of actin is probably not confined to the surface extensions but is likely to include deeper regions of the cortex, as seen in the waist-like "constriction rings" that form periodically around the cell as it migrates (*17, 20*). Constriction rings appear as indentations of the cell surface that emerge from the flurry of ruffling activity at its leading end. The rings remain stationary with respect to the substratum, and as the cells migrate they appear to "move through" the rings. Electron micrographs show that the waist-like constrictions are part of the actin-rich cortex and that they can sometimes deform the nucleus (*21*). Staining of migrating lymphocytes with rhodamine phalloidin, a dye specific for filamentous actin, reveals a local accumulation of actin filaments in a ring around the cell at the site of the constriction (*22*). Although this distinctive mode of locomotion may assist the cell to squeeze through the interstices of a three-dimensional fibrillar matrix, it is important to note that constrictions form and move rearward even in cells that are suspended in fluid medium.

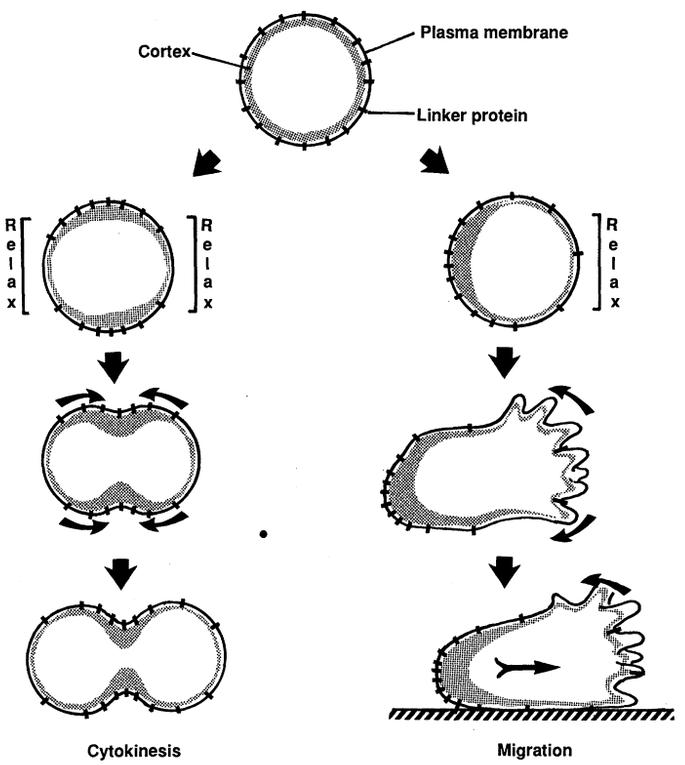


**Fig. 2.** Analogy between cortical movements during cytokinesis and cell migration. In this diagram the cortex is represented in cross section, lying close to the inner face of the plasma membrane to which it is attached through special linker proteins. The linker proteins are connected to molecules on the external surface of the cell, such as those concerned with adhesion to a substratum. During cytokinesis, relaxation at the polar regions (or, what is equivalent for the present purposes, contraction at the equator) leads to the accumulation of actin and associated protein in a band around the equator of the cell (Fig. 1 shows an alternate view of this process). In a migrating cell, only one region is relaxed and cortical components are pulled in a unidirectional flow toward the region of greatest tension. Here components of the cortex are dispersed and carried forward through the cytoplasm to complete the cycle. Although the flow continues in a cell suspended in fluid medium, contact with a solid substratum permits attachments to be formed with the cortical linker proteins; the cortical flow will consequently drive the cell forward, as shown.

We see therefore that the migration of lymphocytes and neutrophils over planar surfaces is accompanied by a continual flow of actin-containing structures, produced at the leading margin of the cell and carried back to its trailing uropod. There is consequently a formal similarity between migration and cytokinesis, underscored by the sporadic generation in a migrating leukocyte of rings of actin filaments similar in size and form to the contractile rings formed during cell division. Evidently a cortical network with the properties mentioned earlier—able to move in the plane of the cell surface while still maintaining tension—could drive a cell over a surface. If we start with a cell enclosed in a tensile cortical network, as before, and now imagine a localized relaxation in only one location (rather than two opposite positions, as in cytokinesis), then we see that the cortex will be pulled unidirectionally toward the opposite region of the cell. In a cell suspended in fluid medium, surface components will move toward the rear of the cell in a symmetrical flow. If the cell is then placed in contact with a solid surface, the cortical flow in the region of contact will drive the cell forward over the substratum (Fig. 2).

As to the molecular basis of cell locomotion, an attractive possibility is that it is driven by a local contraction of the cortex caused by an interaction of actin and myosin. Consistent with this notion, the distribution of myosin rods in the cortex of a *Dictyostelium* cell shifts to the trailing uropod during migration over a surface, rather as it shifts into the contractile ring of a dividing cell (*10*). However, *Dictyostelium* cells in which the high molecular weight species of myosin is either defective or absent nevertheless continue to migrate over the substratum (*23*), so this species of myosin cannot be solely responsible for directed migration.

Cell migration seems more complex than cell division in at least one respect. Since it persists for many hours, even in the absence of protein synthesis, the cortical components drawn to the tail must in some manner be returned to the advancing edge in a continual cycle. The cytoplasmic streaming that accompanies neutrophil migration and the migration of fibroblasts and giant amoebae may be evidence of this retrieval mechanism.

## Lymphocyte Capping

A movement of cortical structures in lymphocytes also forms the basis of antibody capping (*24*). If cells are exposed to polyclonal antibodies that bind to certain membrane components, the antigen collects into small aggregates or "patches" on the cell surface. Similar aggregates can also be produced by means of concanavalin A and other lectins. When a lymphocyte that has been induced to patch is further incubated at 37°C for several minutes, then the small patches congregate into a single large assembly, or cap, at one region of the cell. Usually the cap forms at the posterior uropod of the lymphocyte.

Capping differs from patching in that it requires metabolic

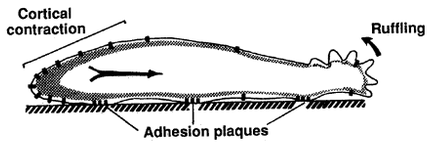


**Fig. 3.** Modulation of cortical flow by adhesion to a substratum. In the flattened fibroblast represented here, the central region of the cell cortex is immobilized by its anchorage to the substratum. The advancing margin and the retracting tail are the only locations at which cortical movements are possible.

energy. Furthermore, there is evidence that capping, and not patching, involves the peripheral membrane-associated cytoskeleton. Capping is inhibited by cytochalasin, and analysis of the region of cytoplasm just beneath an antibody-induced patch reveals an accumulation of actin and actin-binding proteins (*25*). Other cytoskeletal proteins such as tubulin or intermediate filament proteins are not markedly enriched in this region. The suggestion is, therefore, that the patches are carried into the cap by an actin flow similar to that occurring during cell locomotion.

Capping and cell locomotion also entail a concerted movement of membrane components. Lipid molecules and integral membrane proteins move from regions of exocytosis to regions of endocytosis in cycles similar to those followed by cortical components. Indeed, it has been proposed that this flow of lipid, by sweeping membrane proteins and attached cytoskeletal proteins in its path, could provide the ultimate driving force for cell locomotion (*26*). However, we are persuaded that the principal agent of mechanochemical transduction in a eukaryotic cell must be the cytoskeleton. In our view, capping and cell locomotion are driven by the development of tension in the cortex: lipid flow is an ancillary phenomenon.

Lectins and antibodies to any of a wide variety of surface antigens provoke cap formation whereas monovalent antibodies or lectins do not. The lack of specificity in the response, and its dependence on reagents able to cross-link surface proteins, lead to the notion that the physical size of the aggregate on the cell surface rather than its composition is the critical feature leading to an association with actin filaments. A similar feature is encountered in the response of cultured cells such as fibroblasts to a solid substratum.

## Neuronal Growth Cone

Locomotory activity similar to that seen in neutrophils and lymphocytes is exhibited by other kinds of vertebrate cells, although the details vary considerably. One of the most interesting cases is that of the neuronal growth cone, the motile expansion of cytoplasm found at the leading tip of a growing axon (*27*). Both in tissue culture and in the developing embryo this "leukocyte on a leash" (*28*) displays lamellipodia and thin actin-containing filopodia that undergo ruffling movements similar to those described above for neutrophils. Detailed analysis of advancing growth cones shows that filopodia form most frequently at the central apex of the growth cone and are lost most readily from its lateral regions (*29*). Balancing this net addition and loss is the movement of filopodia that swing rearward across the surface of the substratum or through the fluid culture medium. Particles of debris picked up by the growth cone as it migrates are carried rearward and deposited in the "palm" of the growth cone.

Although the movements of a neuronal growth cone bear a family resemblance to those of a neutrophil, they differ in that the advance of the growth cone is coupled to the elongation of the axon or the dendrite. Cortical components delivered to the growing tip are used to make the cortex of the growing axon. On an appropriately sticky surface, not all filopodia are detached at the end of their cycle but some may remain tethered to the local region of substratum (*26*). As the growth cone moves forward, these tethered filopodia remain in position, becoming lateral projections of the axon. A similar pattern of movement is also shown by small particles on the axonal surface that are left behind as the growth cone advances, indicating that the cortex of the axon is left behind in this region.

## Fibroblast Locomotion

The form of cell migration most intensively studied in recent years is also, perversely, one of the hardest to understand. Although fibroblasts explanted into tissue culture will crawl over the culture substratum, this property is only rarely manifested in the body—usually in the course of wound healing (*17*). Fibroblasts are normally static cells that secrete and maintain the collagen-rich extracellular matrix. They have the capacity both to adhere firmly to collagen fibrils and to pull on these fibrils, thereby developing tension in the collagen network. Therefore, when put into tissue culture, fibroblastic cells adhere strongly to the substratum and pull strongly against it (*30*). However, these phenomena are not directly related to the migration of the cell; indeed, they appear to restrain it (*31*).

Fibroblasts put into tissue culture flatten onto the surface and glide over the surface. As seen by time-lapse video or cinematography, lamellipodia surge forward at the leading margin of the cell with a smooth fluid motion, pausing at irregular intervals for brief retrograde movements during which their leading margin is pulled backward. Rising up over the dorsal surface of the cell, they then collapse like waves at a region some 10 μm back from the leading edge. The continual rearward motion, which also carries particles of debris picked up by the cell (*32*), resembles the ruffling motion already described on the surface of a neutrophil, although it is restricted to a more limited proportion of the cell surface.

As in neutrophils, it is likely that the flurry of surface ruffling in a migrating fibroblast includes not only surface features of the cell cortex but also deeper regions of the cortex. At irregular and unpredictable intervals, crescent-shaped bundles of actin filaments, oriented at right angles to the direction of cell locomotion, detach from the leading margin of the cell and move back to a region closer to the cell nucleus. In the electron microscope, these actin "arcs" can be seen to lie just beneath the dorsal or upper surface of the cell. Evidently, they move back in synchrony with the ruffles (*33*).

Whereas a concerted movement of cortical components similar to that described above for leukocytes is evident at the leading margin of a cultured fibroblast, especially on its upper surface, movements on its lower surface are restrained by the formation of tight adhesions to the substratum (Fig. 3). Here, cortical actin accumulates, not in microspikes and lamellipodia, but in adhesion plaques—regions of close contact with the substratum into which, on the cytoplasmic side, bundles of actin filaments are inserted (*19*). As lamellipodia extend forward, adhesion plaques are seen by interference reflection microscopy to form in contact with the substratum. Like the impression of numerous feet, the plaques remain stationary as the cell moves over them, detaching from the culture substratum when they reach more proximal positions. Adhesion plaques pin the fibroblast down to the substratum and oppose the contraction of the cortex, which would otherwise cause the cell to become round (*30*). Within the cell, tension developed between adhesion plaques causes the cortex to form into distinct bundles of actin filaments, or stress fibers.

Two modifications to the "flattened tent" form of the fibroblast occur as a consequence of migration. At one margin of the cell the protrusive activity generates lamellipodia and occasionally microspikes. The growth of these structures depends, it is believed, on the assembly of actin filaments, which evidence suggests grow in length through the addition of actin molecules to the region closest to the plasma membrane (*34*). The other modification to the static form of the fibroblast occurs at the back of the cell as regions of the cell are drawn into long tails, also known as "retraction fibers," which periodically pull away from the surface, snapping back into the cell (*35*). By a "conservation of mass" argument, the components of the fibroblast tail must return to the cytoplasm and eventually find their way to the newly forming extension at the front end of the cell. Their route and the manner of progress are unknown, but it may be significant that tail retraction is usually followed within a few seconds by a flurry of enhanced activity at the leading edge (*35*). Perhaps tail retraction could cause a dispersal of cortical structures, as seen in the contractile ring.

## Amoeboid Locomotion

Many small amoebae, such as the amoeboid form *Dictyostelium discoideum* and the free-living soil amoeba *Acanthamoeba castellanii*, crawl over surfaces in a fashion similar to the motion of vertebrate tissue cells. But the migration of giant amoebae of the *Amoeba proteus* type—the classically defined "amoeboid movement"—has features that appear to set it apart (*36*). Most notably, the migration of these cells appears to be driven by vigorous cytoplasmic streaming, not visible in the interior of smaller vertebrate cells. Under suitable ionic conditions, cytoplasmic streaming persists even after removal of the plasma membrane, so that its relation to the cortical flow under discussion is, at first, problematic.

In the past 10 years, however, it has become clear that giant amoebae also have a dense filamentous actin-containing cortex (*37*). Depending on its functional state and position in the moving cell, this layer is either associated with the cell membrane or disengaged from it, forming the outer boundary of the region of gelated, organelle-rich cytoplasm known in classical terms as "granular ectoplasm." The cortical layer is always continuous over the entire surface and varies in thickness depending on the cell polarity. At the front of the cell it is thin, with an average thickness of 0.1 μm; in the trailing uroid regions it increases to 0.5 μm or more. Contact of the filamentous layer with the plasma membrane is incomplete at the front end and the rear but mostly continuous at the middle cell region. Whereas all parts of the cortex contain numerous thin actin filaments, thick filaments thought to be myosin are present mainly in the uroid region.

A large body of evidence—morphological, physiological, and behavioral—is consistent with the following model for the locomotion of *Amoeba proteus* (*38*). Contractions of the cortical layer maintain a pressure flow within the cell that drives centrally located, fluid cytoplasm into advancing pseudopodia. At the front end of the cell a relatively thin cortical layer represents a formative region in which new cortex is assembled. At the posterior, or uroid, region of the cell, a thicker cortical layer rich in structures believed to be myosin filaments generates active contractions that lead to the dispersal of the cortex into the amoeboid cytoplasm. In intermediate regions, a flow of cortex from the front to the back of the cell is coupled to the plasma membrane and through it to the substratum, where it drives the forward migration of the cell.

If the above picture is correct, then the migration of giant free-living amoebae conforms to the general pattern of cortical movements already described and has the advantage of being more readily visualized by light microscopy. The forward flow of centrally located cytoplasm, which is a necessary general feature of the cortical flow model of cell locomotion, is seen clearly only in *Amoeba proteus* and its relatives. Furthermore, patterns of movement of both inner and outer layers of the cell as it migrates provide insight into the modulation of cortical flow by adhesion to the substratum (*39*). Thus, when *Amoeba proteus* is attached firmly over most of its lower surface to a substratum, cortical movements occur principally in the advancing tips of pseudopodia and retracting uroid regions—a situation not unlike that described for cultured fibroblasts above. In contrast, when the amoeboid cell is lightly attached, a complete fountain flow of contents is seen, streaming forward in the centrally located cytoplasm and backward in the cortical region. The similarity of this latter form of movement to that of neutrophils and other leukocytes is underscored by the sporadic formation of constriction rings, which form at the tip of the advancing pseudopodium and move backward in concert with the cortical cylinder (*39*).

## Conclusions

Our principal contention is that most animal cells have the capacity to generate a large-scale internal flow of actin-containing components of the cortex. The clearest evidence for this view exists in locomoting cells, where surface extensions containing actin as their principal cytoskeletal element and local accumulations of actin in the cortex can be seen by direct microscopical observation to move backward over the dorsal surface of a migrating cell. Cortical flow in this situation appears to be closely linked to cell migration and, logically, to require a compensatory forward movement of actin (together with actin-binding proteins and associated components of the plasma membrane) through the cytoplasm in order to balance the rearward motion in the cortex. Movement is also observed to take place during lymphocyte capping and is inferred, from less direct evidence, to take place during cytokinesis. It is a direct prediction of our thesis that the introduction of suitably labeled actin molecules into a cell that is locomoting over a surface, or undergoing division, will reveal a cycle of incorporation into the cortex and progressive movement to more proximal (or equatorial) positions.

The force driving cortical flow is most apparent in cell division. At the onset of cytokinesis, the entire surface of the cortex undergoes a contraction, which is later relaxed only at the two polar regions. Given a degree of both mobility and mechanical integrity in the cortex, then a localized relaxation at the polar regions of the cell will allow cortical elements to be drawn to the equator, where they form a contractile ring. A similar underlying mechanism of force generation might also provide a basis for cell locomotion: uniform contraction over the entire surface of the cell, which is relaxed only at localized regions, generating a continual flow of cortical components rearward. Depending on the formation of adhesions to the substratum, this rearward flow would produce surface ruffling and, more important, provide the driving force for cell locomotion.


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## Research Articles

# Three-Dimensional Structure of an Oncogene Protein: Catalytic Domain of Human c-H-*ras* p21

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**The crystal structure at 2.7 Å resolution of the normal human c-H-*ras* oncogene protein lacking a flexible carboxyl-terminal 18 residue reveals that the protein consists of a six-stranded β sheet, four α helices, and nine connecting loops. Four loops are involved in interactions with bound guanosine diphosphate: one with the phosphates, another with the ribose, and two with the guanine base. Most of the transforming proteins (in vivo and in vitro) have single amino acid substitutions at one of a few key positions in three of these four loops plus one additional loop. The biological functions of the remaining five loops and other exposed regions are at present unknown. However, one loop corresponds to the binding site for a neutralizing monoclonal antibody and another to a putative "effector region"; mutations in the latter region do not alter guanine nucleotide binding or guanosine triphosphatase activity but they do reduce the transforming activity of activated proteins. The data provide a structural basis for understanding the known biochemical properties of normal as well as activated *ras* oncogene proteins and indicate additional regions in the molecule that may possibly participate in other cellular functions.**

THE MOST COMMONLY FOUND ONCOGENES ISOLATED FROM human tumors or transformed human cell lines belong to the *ras* gene family (*1*). In the human genome there are three distinct cellular *ras* genes: c-H-*ras*, c-K-*ras*, and N-*ras*, the former two having sequence similarity (*2–5*) to the transforming principles of the Harvey and Kirsten strains of rat sarcoma viruses (*6, 7*), respectively. All proteins encoded by these genes are called p21, have a molecular weight of about 21,000 with 188 or 189 amino acid residues, and have practically identical amino acid sequences, with the exception of the carboxyl-terminal 25 residues (*8*). The difference between the normal *ras* proto-oncogenes and the activated (transforming) *ras* oncogenes is usually a single base change at one of a few critical positions, resulting in a single amino acid substitution in p21 (*9–11*).

In vivo involvement of the *ras* genes in neoplasia was recently shown by the experiment that transgenic mice bearing activated c-

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