

Deconstructing (and Reconstructing) Cell Migration

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ABSTRACT An overriding objective in cell biology is to be able to relate properties of particular molecular components to cell behavioral functions and even physiology. In the “traditional” mode of molecular cell biology, this objective has been tackled on a molecule-by-molecule basis, and in the “future” mode sometimes termed “functional genomics,” it might be attacked in a high-throughput, parallel manner. Regardless of the manner of approach, the relationship between molecular-level properties and cell-level function is exceedingly difficult to elucidate because of the large number of relevant components involved, their high degree of interconnectedness, and the inescapable fact that they operate as physico-chemical entities—according to the laws of kinetics and mechanics—in space and time within the cell. Cell migration is a prominent representative example of such a cell behavioral function that requires increased understanding for both scientific and technological advance. This article presents a framework, derived from an engineering perspective regarding complex systems, intended to aid in developing improved understanding of how properties of molecular components influence the function of cell migration. That is, cell population migration behavior can be deconstructed as follows: first in terms of a mathematical model comprising cell population parameters (random motility, chemotaxis/haptotaxis, and chemokinesis/haptokinesis coefficients), which in turn depend on characteristics of individual cell paths that can be analyzed in terms of a mathematical model comprising individual cell parameters (translocation speed, directional persistence time, chemotactic/haptotactic index), which in turn depend on cell-level physical processes underlying motility (membrane extension and retraction, cell/substratum adhesion, cell contractile force, front-vs.-rear asymmetry), which in turn depend on molecular-level properties of the plethora of components involved in governance and regulation of these processes. Hence, the influence of any molecular component on cell population migration can be understood by reconstructing these relationships from the molecular level to the physical process level to the individual cell path level to the cell population distribution level. This approach requires combining experimental, theoretical, and computational methodologies from molecular biology, biochemistry, biophysics, and bioengineering. *Micros. Res. Tech.* 43:358–368, 1998. © 1998 Wiley-Liss, Inc.

INTRODUCTION

It is relatively easy these days to make systematic alterations in molecular constituents of cells and tissues, employing genetic, pharmacologic, or materials approaches. Such alterations might be manifested in levels of expression or in sequence/structure of what is expressed by, or presented to, a cell. It is immensely more difficult to understand—let alone predict—the effects on cell function and tissue physiology that such alterations may give rise to. The cell function of migration, accompanied by its roles in tissue physiology and pathology, represents a prominent example of this condition (Bray, 1992; Lackie, 1986).

Many and myriad molecular components of cells and their environment have been, and continue to be, identified as being involved in cell migration, leading to intense research efforts devoted to elucidating the mechanisms by which they work together to govern this function. Integrin adhesion receptors appear to be central players in controlling cell locomotion, serving as biomechanical linkages between the intracellular cytoskeleton and the extracellular matrix as well as transducers of biochemical signals influencing force genera-

tion and transmission, among numerous additional possible processes (Huttenlocher et al., 1995; Hynes, 1992; Sastry and Horwitz, 1993). Growth factor receptors seem to be of equivalent importance, transducing biochemical signals that influence a similar range of processes (Chen et al., 1993; Gumbiner, 1996). It is clear now, moreover, that synergistic influences of integrins and growth factor receptors on migration (e.g., Woodard et al., 1998) are carried out via highly interrelated pathways, to a large degree involving common molecular components in the biomechanical and biochemical regulatory mechanisms (Mainiero et al., 1996; Maldano & Furcht, 1995; Plopper et al., 1995).

To ask the question of what role a specific molecular component—be it FAK, MAPK, ras, P13K, or any of a very lengthy list—plays in effecting and/or controlling locomotion behavior of a cell leads immediately to a need to deconvolute a highly complex system. And to

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ask the next question of how to manipulate that component's effect or to control its activity, by means of genetic, pharmacological, or materials intervention leads to the corresponding need to predict the outcome of reconvolution of the system with altered properties. To give merely one very recent instance, Miettinen et al. (1998) have found that P13K stimulation by a chemoattractant is involved in chemotactic migration of a CHO cell population, in a manner that is enhanced by changes in integrin-mediated cell adhesion. As discussed in greater detail elsewhere (Wells et al., 1998), P13K activity may elicit effects on multiple physical processes underlying locomotion, including possibly membrane extension and morphological polarization (Keely et al., 1997) along with cell/substratum adhesion (Shimizu, 1996), and may enhance not only chemotactic migration but random motility as well (Keely et al., 1997). Hence, it is essential to understanding of the roles of any particular molecular component such as P13K to be able to "deconstruct" its effects—breaking them down from observations of gross influence on overall cell population distributions, to studies of their effects on individual cell paths, and then further to their regulation of the physical processes underlying migration. Then, for prediction of what will result from making any genetic or biochemical interventions targeting that component, again for example P13K or molecules that interact with it, it is necessary to integrate, or "reconstruct," the consequent changes in physical processes, up to individual cell paths, and finally up to cell population distributions.

ENGINEERING MODELS FOR CELL MIGRATION

An engineering perspective casts this type of problem in terms of models and parameters, for both analysis of the system ("deconstruction") as well as its synthesis ("reconstruction"). Models break down the operation into underlying processes and permit mathematical description of these processes in terms of simplified and idealized mechanistic assumptions. Parameters characterize the key properties of the actors, the molecular components, in these models. Contributions of the components can thus be elucidated by altering assumptions or parameter values in the models and determining how the computed system behavior should change. Degree of confidence in the resulting predictions must then be tested by experimentally making those alterations as faithfully as is feasible and observing any consequent changes in system behavior.

Our laboratory and others have been attempting to aid conceptual progress in understanding cell migration by applying engineering modeling approaches, in at least two distinct modes. One may be termed phenomenological, in which measureable cell locomotion behavior is broken down into underlying phenomena. The second may be termed mechanistic, in which measureable cell locomotion behavior is broken down into underlying mechanisms. Whether a model is considered "phenomenological" or "mechanistic" largely depends on level of detail, and the distinction is not necessarily important.

Figure 1 illustrates a possible way to think about how alterations in molecular components affect cell migration, from this sort of engineering modeling perspec-

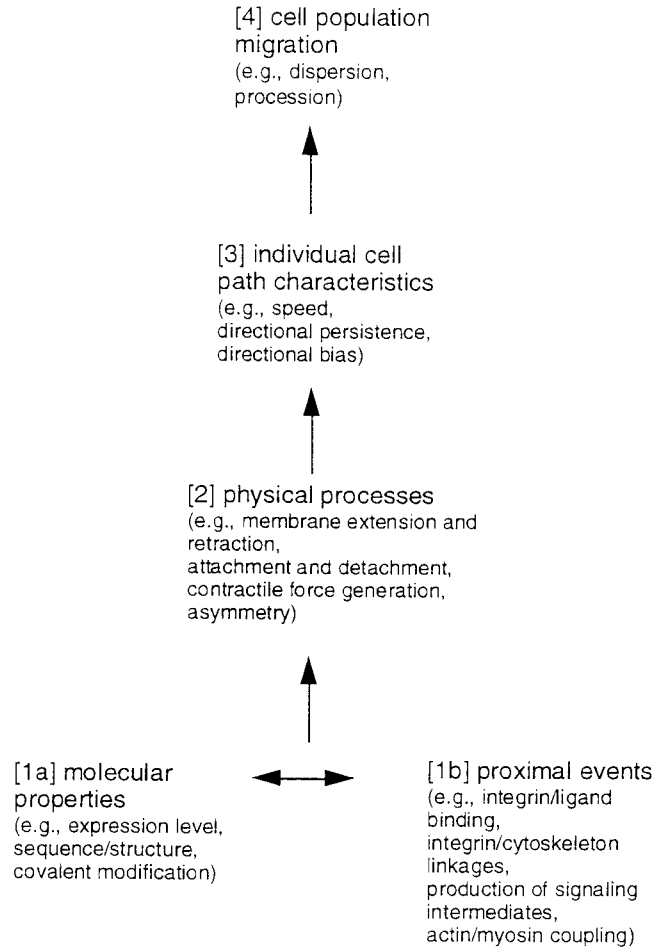


Fig. 1. Outline of hierarchical aspects of cell motility needing "deconstruction" (from cell population behavior down to molecular properties) and "reconstruction" (from molecular properties up to cell population behavior).

tive. The dispersion of a cell population, such as metastatic spread of tumor cells or of leukocytes during an inflammatory response, depends on the net sum of paths taken by the many individual cells. By the same token, the precession of endothelial cells during angiogenesis, or of keratinocytes during epidermal wound healing, likewise depends on a similar summation. Mathematical models have been constructed that relate movements of cell populations to the characteristics of individual cell trajectories (see Lauffenburger and Linderman, 1993). Parameters that quantitatively represent cell population movement properties include a random motility coefficient (analogous to a molecular diffusion coefficient) and a chemotaxis/haptotaxis coefficient (analogous to a fluid flow velocity). Moving down a level in the hierarchy of description, the path taken by an individual cell may be presumed to arise from a combination of these properties: (1) translocation speed, (2) proclivity for changing direction, and (3) tendency to bias that direction toward some reference axis. Mathematical models have been constructed that describe how these properties integrate to yield typical cell paths. In these models, parameters such as speed,

persistence time, and chemotactic/haptotactic index respectively, are used to represent these cell properties quantitatively. Of crucial significance, then, is that the parameters representing cell population movements can be predicted theoretically from the parameters representing individual cell path properties—thus relating the two levels of hierarchy.

Next, each of these cell properties can, in turn, be examined in terms of the physical processes giving rise to it. Translocation speed, directional persistence, and directional bias may all be presumed to be governed by a combination of cell membrane extension, attachment to the movement substratum, cellular force generation and transmission, and detachment from the movement substratum, though with different spatial reference points. Mathematical models have been constructed, to varying degrees of detail, that describe how these processes might integrate to yield typical values of speed, persistence, and/or bias (see Lauffenburger and Linderman, 1993). This allows, for instance, speed to be predicted from measurements of membrane extension, cell/substratum adhesion, cell contractile force, and front-vs.-rear asymmetry.

Finally, at the deepest level of currently-pursued detail, models can be constructed for how the physical processes are influenced by quantifiable properties of particular classes of molecular components. Relevant properties might include thermodynamic (e.g., concentrations, interaction affinities), kinetic (e.g., interaction rate constants), transport (e.g., diffusion coefficients), and mechanic (e.g., elastic moduli, viscosities) categories. Hence, if the physical processes can be measured experimentally, effects of alterations in molecular components can be studied in terms of their influence on each of these. Conversely, if the effect of an alteration in a molecular component on each of these processes is known, its resulting effect on the phenomena of speed, persistence, and bias can be predicted.

Accordingly, experimentally observed cell paths can be analyzed to obtain parameter values characterizing speed, directional persistence, and directional bias, so that effects of alterations in molecular components can be studied in terms of their influence on each of these phenomena. Conversely, if the effect of an alteration in a molecular component on each of these phenomena is known, its resulting effect on cell paths can be predicted. Thus, cell paths can be ultimately analyzed in terms of their governance by particular molecular components by moving down this hierarchy of models. In turn, cell paths can ultimately be predicted from effects of particular molecular components by moving up the hierarchy. Ambitious is this approach, certainly, but the engineering analysis/synthesis perspective has proven highly effective over the past decades for advances in science and technology across a wide spectrum of other fields.

CELL POPULATION MIGRATION

The number density of migratory cells distributed in time and space (simplified here to a one-dimensional axis) is reasonably well described by the partial differ-

ential equation (Alt, 1980; Lauffenburger, 1983):

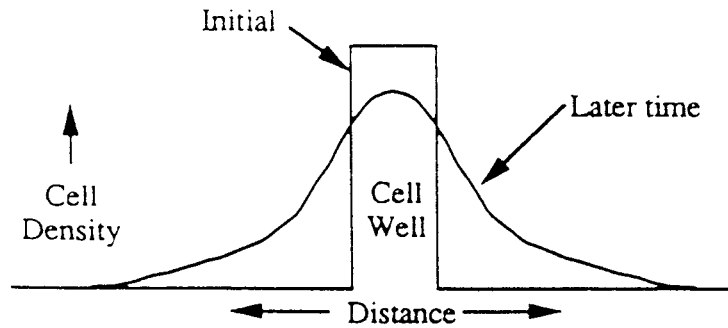
$$\frac{\partial c}{\partial t} = \mu \frac{\partial^2 c}{\partial x^2} - \frac{\partial}{\partial x} \left[\left(\chi - \frac{1}{2} \frac{d\mu}{da} \right) \frac{\partial a}{\partial x} \right] c + R_c \quad (1)$$

Here, $c(x,t)$ is the cell number density as a function of spatial position, x , and time, t , so that the term on the left-hand side of Eqn. 1 quantifies the rate at which cell number density at position x increases or decreases with time. The final term on the right-hand side of Eqn. 1, R_c , is the net rate of production or loss of cells at position x by mechanisms other than migration (e.g., proliferation or death). The first term on the right-hand side quantifies the change in cell number density due to dispersion, arising from random locomotion; μ is the cell random motility coefficient, characterizing how cells would disperse from higher to lower concentration similarly to molecular diffusion. The second term on the right-hand side quantifies the change in cell number density due to spatial gradients of environmental stimuli, such as those stimulating chemotactic or haptotactic responses. The group in brackets essentially represents a net "directional flow" of cells in the stimulus gradient. χ is the chemotactic/haptotactic coefficient, characterizing biased directional movement in response to a spatial gradient, $\partial a/\partial x$, a is the concentration of stimulus. (The additional factor in this bracketed group quantifies net cell procession in a stimulus concentration gradient caused solely by effects of the stimulus on cell speed or directional persistence, i.e., chemokinesis or haptokinesis, having nothing to do with directional orientation bias per se.) Figure 2 illustrates the contributions these terms make to an example cell population number density distribution.

Since a large portion of experimental studies of cell migration employ cell population assays (e.g., Boyden chamber, nucleopore filter, monolayer wounding, under-agarose), it should be appreciated that cell distributions are influenced to varying extents by random motility, chemo/hapto-kinesis, and chemo/hapto-taxis, governed by the relative magnitudes of the parameters, μ , χ , and $d\mu/da$ along with the experimentally variable stimulus gradient, $\partial a/\partial x$. Readers are referred to a number of reports demonstrating how to determine these parameter values from typical experimental assays (Buettner et al., 1989; Farrell et al., 1990; Tranquillo et al., 1988a). Computer-based image analysis methodologies can be employed to measure cell number densities as functions of spatial position and time following some initial distribution, and Eqn. 2 fit to the experimental distribution. The stimulus gradient must be known as a function of position and time, of course, in order to determine the cell parameter values. This can be accomplished by mathematical analysis of attractant diffusion under transient conditions (Buettner et al., 1989; Tranquillo et al., 1988a) or by elegant design of assay chambers permitting stable attractant concentration gradients (e.g., Fisher et al., 1989; Zicha et al., 1991; Zigmond, 1977).

Parameter values that have been reported for a couple of representative cell types may be usefully summarized here for reference. For neutrophil leukocytes migrating in an under-agarose assay in response

a. Uniform chemical concentration: Random motility only (μ)



b. Migration in a chemical attractant gradient:
Random motility (μ), chemokinesis ($\frac{d\mu}{da}$) and chemotaxis (χ)

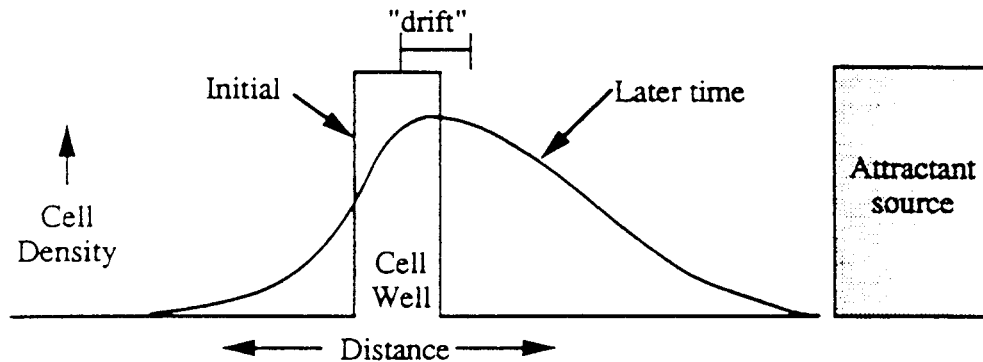


Fig. 2. Illustration of evolution of a cell population number density distribution with time from an initial distribution of cell number density (localized in a cell "well") to a more dispersed (due to random motility) and perhaps "processed" distribution (due to chemotaxis

and/or chemokinesis in a gradient of soluble attractant ligand; similar effects could be seen for haptotaxis and/or haptokinesis in a gradient of immobilized adhesion ligand).

to the formyl peptide attractant FMLP, μ was found to increase ~ 30 -fold from a base value of 3×10^{-8} cm²/second to a maximum of 8×10^{-7} cm²/second for FMLP concentrations in the range of 3×10^{-9} M to 3×10^{-8} M (Tranquillo et al., 1988a). χ was determined to be $\sim 2-4 \times 10^2$ cm²/second-M over this intermediate FMLP concentration range, near its K_D value of $\sim 2 \times 10^{-8}$ M. The contribution of the chemokinesis term was always the smallest of the three terms in the right-hand side of Eqn. 1 over the course of the experiment. One way to evaluate chemotactic sensitivity in this cell/attractant system is to calculate the quantity $\chi a/\mu$, essentially the ratio of chemotactic to random motility, yielding $\sim 2-4$ for FMLP concentrations in the range 10^{-9} to 10^{-7} M. For microvessel endothelial cells responding to aFGF in the same type of assay, μ was much smaller and almost constant, varying only between $0.5-1 \times 10^{-8}$ cm²/second across the full concentration spectrum (Stokes et al., 1990); thus, again the chemokinesis term was negligible. χ was found to be $\sim 2-4 \times 10^{-9}$ cm²/second-M in the aFGF concentration

range near its K_D value of $\sim 5 \times 10^{-10}$ M. The ratio $\chi a/\mu$ for this cell/attractant system yields $\sim 10-30$ for aFGF concentrations in the range 10^{-11} to 10^{-9} M, indicating, perhaps surprisingly, that the endothelial cell population chemotactic sensitivity to aFGF is actually stronger than that of neutrophils for FMLP.

It is important to emphasize that, in some cases, apparently small effects of stimulus gradients on cell number distributions may, in fact, be found to reflect quite strong cell migration responses, so that analysis of assay data in terms of the intrinsic cell population migration parameters μ , χ , and $d\mu/da$ is recommended when more than a "yes/no" answer is desired. The comparison noted above between chemotactic responses of slow-moving cells such as endothelial cells and fast-moving cells such as neutrophils provides an excellent example of the need for quantitative analysis. Movement of the leading front of an endothelial cell population over a 72-hour period in the under-agarose chemotaxis assay was found to be increased only by $\sim 30\%$ in response to an FGF gradient from a source

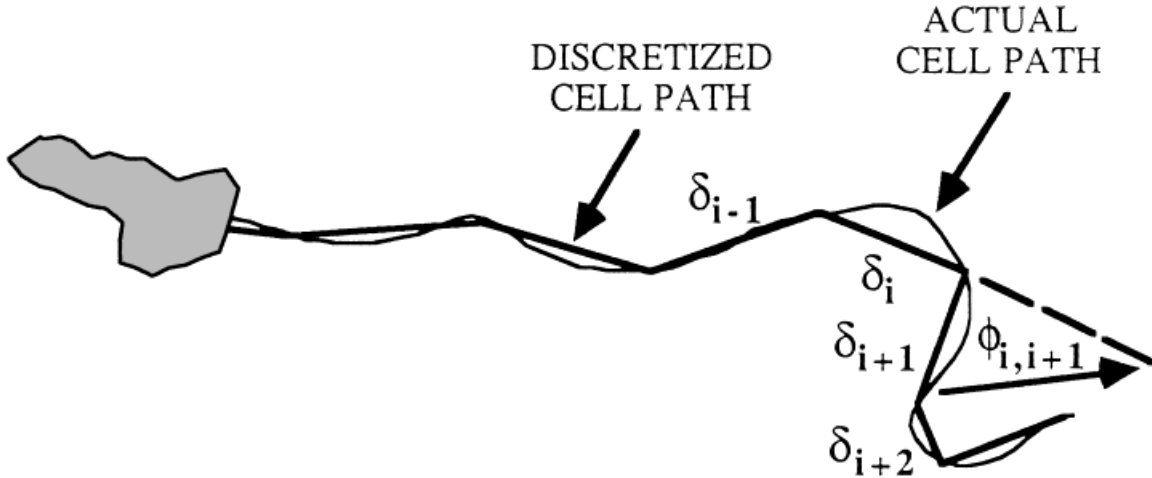


Fig. 3. Example of discretization of an individual cell path. δ_i is the position of the cell centroid at time t_i , so the displacement Δx for any time period Δt is equal to $\delta_i - \delta_j$. $\phi_{i,i+1}$ is the angle between successive displacements.

concentration of $\sim 20 \times K_D$ (Stokes et al., 1990), an apparently much less dramatic effect than the $\sim 500\%$ increase in leading front distance observed for neutrophils over a 4-hour period in response to an FMLP gradient from a source concentration of $\sim 20 \times K_D$ (Tranquillo et al., 1988a), yet the actual chemotactic sensitivity of the endothelial cells is at least as strong as that of the neutrophils. And in more complicated situations, this sort of mathematical analysis should be of critical value even in understanding qualitative features of experimental observations. The fascinating recent findings by Foxman et al. (1997) regarding net migration behavior of neutrophil populations in the face of multiple competing chemoattractant gradients arise from quantitative combination of comparative sensitivities to the various attractants and magnitudes of the attractant concentration gradients present. Some results, such as the observation that cells migrate against a proximal gradient but towards a more distal competitive gradient, can be best resolved by reference to the quantitative analysis offered by Eqn. 1 extended to include two different attractants, α_1 and α_2 , each associated with corresponding cell motility parameters.

Also of great significance is that these cell population parameters are intimately related to parameters characterizing individual cell path properties. This point will be explained in the next section.

CELL LOCOMOTION PATHS

Numerous investigators have undertaken mathematical treatments of individual cell paths, because experimental observations of these typically can reveal greater insights into cell locomotion behavior than can be obtained by cell population assays. Typically, individual cell path data are broken down into measurements of displacement, Δx , of a cell centroid during a time-period Δt (see Fig. 3). For locomotion in the absence of any directional bias (i.e., lacking any chemotactic or haptotactic responses) the most widely used model of cell paths describes the mean value of the square of the displacement, $\langle (\Delta x)^2 \rangle$, averaged over many such time-periods (and, often, many cells) as a function of the

time-period:

$$\langle (\Delta x)^2 \rangle = nS^2P [(\Delta t) - P(1 - \exp [-(\Delta t)/P])] \quad (2)$$

n is the number of dimensions accessible to cell translocation; i.e., 2 for locomotion observed on a two-dimensional substratum (Dunn and Brown, 1987; Gail and Boone, 1970). S is the translocation speed, essentially the speed of locomotion over any straight-line portion of the trajectory between direction changes:

$$S = \lim_{(\Delta t) \rightarrow 0} \frac{\langle (\Delta x)^2 \rangle^{1/2}}{(\Delta t)} \quad (3a)$$

P is the persistence time, which is inversely related to the proclivity of a cell to change directions while locomoting. In the context of this particular model, P has a precise mathematical definition:

$$P = \lim_{(\Delta t) \rightarrow 0} \frac{2(\Delta t)}{\langle \phi^2 \rangle} \quad (3b)$$

where ϕ is the angle between successive displacements, so that $\langle \phi^2 \rangle$ is the mean of its value squared over many time-periods. Thus, one way to think about P is that it takes on the value of $\sim 80\%$ of the average time-period needed for a cell to turn by $\sim 90^\circ$. Another way to consider it is in terms of correlation of movement direction. After a time-period equal to the value of P , an ensemble of cell displacements will exhibit only a $\sim 37\%$ correlation of their current direction with their previous direction.

Experimentally, values of cell centroid displacement can be measured for a series of time-periods by using computer-based image analysis of cell paths (Dow et al., 1987). The resulting values of $\langle (\Delta x)^2 \rangle$ can be plotted vs. their corresponding values of (Δt) . S and P can then be determined from such data by any of a variety of alternative procedures. Most simply, S can be estimated using Eqn. 3a, from a plot of root-mean-squared displacement, $\langle (\Delta x)^2 \rangle^{1/2}$ vs. time-period, (Δt) , and followed by extrapo-

lation to shorter time-periods. With S in hand, P can then be fit from the plot of $\langle(\Delta x)^2\rangle$ vs. (Δt) . Or, P can be estimated using Eqn. 3b, from a plot of $(\phi^2)^{-1}$ vs. (Δt) , again extrapolating to shorter time-periods. Here, with P in hand independently, S can then be fit from the plot of $\langle(\Delta x)^2\rangle$ vs. (Δt) . Most commonly, S and P are fit simultaneously from the plot of $\langle(\Delta x)^2\rangle$ versus (Δt) (Dickinson and Tranquillo, 1993a).

As examples of this type of determination, values of S and P for microvessel endothelial cells have been measured to be on the order of $\sim 30 \mu\text{m}/\text{hour}$ and 3–5 hour, respectively (Stokes et al., 1991), and $\sim 5 \mu\text{m}/\text{min}$ and ~ 1 minute, respectively, for neutrophil leukocytes (Dow et al., 1987). It must be emphasized, however, that quantitative values for these individual cell parameters, like those for cell population parameters, can be strongly influenced by environmental conditions such as substratum and soluble ligand types and concentrations.

An alternative quantity for characterizing directional persistence, essentially a rate of change of directional angle over a movement time period, has been more recently proposed and utilized in individual cell tracking studies (Farina et al., 1998). The relationship of this quantity to a mathematical treatment of cell movement paths is at present unclear but could be examined theoretically.

Some investigators have pursued cell locomotion path analysis to an even greater degree of complexity. Mathematical models have been developed to further characterize the change in the angle between consequent displacements using parameters such as angular velocity which describes the angular turning and an independent "jump process," which characterizes sudden turns (Alt, 1989). In the past few years, microscopic techniques have been developed that now permit the tracking of cell paths through three dimensional matrices (Friedl et al., this issue; Dickinson et al., 1994; Mandeville et al., 1997; Noble, 1987; Parkhurst and Saltzmann, 1992).

Referring now to the previous hierarchical level of modeling, that at the level of cell populations, the cell population random motility coefficient, μ , is related by mathematical theory to the individual cell population parameters S and P, as follows:

$$\mu = \frac{1}{n} S^2 P \quad (4)$$

That is, for time-periods very long compared to the directional persistence time, the mean-squared cell displacement, $\langle(\Delta x)^2\rangle$, is equal to $\mu(\Delta t)$. Hence, if S and P are measured from individual cell paths, cell population dispersion can be predicted.

Cell paths in the presence of an environmental stimulus gradient can be analyzed in similar fashion. Over time-periods long compared to the directional persistence time, a chemotactic (or haptotactic) index, CI, can be defined as the ratio of the net displacement of cell centroid along the stimulus gradient axis, (Δx_{axis}) to the total cell path length, L:

$$CI = \frac{(\Delta x_{\text{axis}})}{L} \quad (5a)$$

Purely random locomotion should yield a value $CI = 0$, whereas locomotion perfectly directed along the stimulus gradient axis should yield a value $CI = 1$. An observed value of CI, of course, depends on the magnitude of the chemo/hapto-tactic ligand gradient as well as on the intrinsic cell/ligand chemo/hapto-tactic sensitivity.

This expression must be corrected when the experimental data are taken over time-periods of magnitude similar to the persistence time (Othmer et al., 1988):

$$CI = \frac{(\Delta x_{\text{axis}})}{L} \left\{ 1 - \left(\frac{(\Delta t)}{P} \right)^{-1} [1 - \exp \{- (\Delta t)/P\}] \right\} \quad (5b)$$

As with random motility, chemo/hapto-tactic behavior of a cell population can be predicted from observations of individual cell paths. The population chemo/hapto-tactic coefficient is theoretically related to the individual cell parameters of S and CI, given that the stimulus gradient in the individual-cell assay, $\partial a/\partial x$, is known:

$$\chi = \frac{CI * S}{\left(\frac{da}{dx} \right)} \quad (6)$$

As noted in the previous section, it is crucial to have quantitative information on the attractant concentration gradient in the assay.

These relationships between individual cell path properties and cell population migration parameters have been experimentally verified, at least for the system of macrophage responses to the chemoattractant C5a (Farrell et al., 1990). Measured values of $S = \sim 2 \mu\text{m}/\text{minute}$ and $P = \sim 30$ minute corresponded nicely to the measured value of $\mu = \sim 1-2 \times 10^{-8} \text{ cm}^2/\text{second}$, and a value of $CI = \sim 0.2$ measured in a C5a gradient of roughly $10^{-7} \text{ M}/\text{mm}$ agreed well with a measured value of $\chi = \sim 10^2 \text{ cm}^2/\text{second-M}$.

It can be seen from Eqns. 4 and 6 that cell movement path studies can reveal regulatory effects at a finer level of detail than can cell population assays. Indeed, certain molecular components can influence cell migration in terms of either movement speed or directional persistence in ways not easily discernable from population assays. For example, mutations in integrins affecting cytoskeletal interactions have been determined to give rise to changes in translocation speed but not directional persistence in individual cell movement paths, which could not be distinguished simply from overall population dispersion (Schmidt et al., 1995a).

Now, the next step in ultimately relating molecular components and mechanisms to cell migration behavior is to analyze the cell path characteristics with respect to physical processes underlying motility.

CELL MOTILITY PHYSICAL PROCESSES

Despite differences among diverse cell types, a number of physical processes underlying locomotion are generalizable—though, of course, not universal nor exclusive—as depicted in Figure 4 (Lauffenburger and Horwitz, 1996; Sheetz, 1994): (1) membrane extension,

Forces in Cell Migration

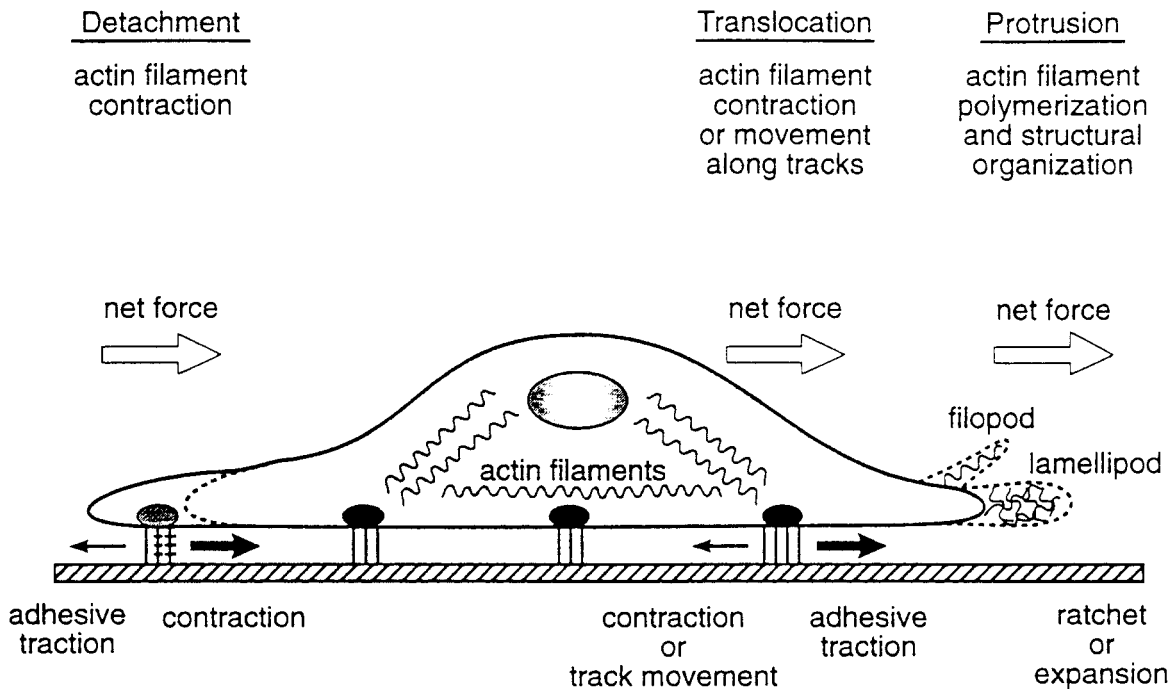


Fig. 4. Schematic view of how various physical processes contribute to the net force for cell locomotion.

(2) attachment formation, (3) contractile force generation, (4) cell rear detachment, and (5) front-vs.-rear asymmetry. The rate of locomotion is not likely to be universally limited over a comprehensive range of movement environments by any single one of these individual motility processes (Lauffenburger and Horwitz, 1996). For instance, in some circumstances migration speed seems to be governed by lamellipod extension and attachment (Wessels et al., 1994), whereas in others rear detachment appears to be limiting (Jay et al., 1995; Marks et al., 1991). Quantitative studies elucidating conditions under which particular physical processes are rate-limiting for cell migration speed have begun to appear recently (Palecek et al., 1998; Maheshwari et al., 1998).

In order to generate predictions concerning how alterations in molecular properties should affect cell migration behavior, this fundamental conceptual model must be cast into a mathematical framework because of the great difficulties encountered in trying to simply intuit the net result of complicated interactions. Early work of this type has focused on speed of cell migration rather than directional behavior, resulting in the development of mathematical treatments that attempt to predict how linear translocation speed varies with molecular properties such as cell receptor number, extracellular-matrix (ECM) ligand concentration, receptor/ligand binding affinity, and cellular properties such as contractile force, membrane protrusion frequency, and front-vs.-rear asymmetry in cell/substratum adhe-

siveness. The mathematical analysis itself can be either fairly rudimentary, yielding algebraic equations whose solution gives average migration speed as a function of these parameters (Lauffenburger, 1989), or much more complex, yielding differential equations whose solution gives similar predictions (DiMilla et al., 1991). The main difference between these two analyses lies in the treatment of cellular mechanics, but their predictions concerning parameter effects on cell migration speed are quite similar.

A central prediction is: (1) that speed should depend in biphasic manner on the number of receptor/ligand bonds dynamically linked to the force-generating cytoskeleton; this represents the short-term cell/substratum adhesiveness characteristic of dynamic interactions at the front and rear of a migrating cell. This prediction implies that changes in cell receptor number $[R]$, ECM ligand concentration $[L]$, and receptor/ligand binding equilibrium constant, K_A , alter movement speed through their product, $K_A[R][L]$, equal to the number of receptor/ligand bonds. Hence, if speed is measured as a function of ligand concentration, increases in cell receptor number and/or receptor/ligand binding affinity should only shift a plot of speed versus ligand concentration to the left, i.e., to lower ligand concentrations, without changing the maximum speed or the range of ligand concentration over which substantial speed is supported. Additional important predictions are: (2) rear detachment should be rate-limiting at higher ligand concentrations, (3) increased membrane protrusion

sion frequency should increase movement speed at lower ligand concentrations, until rear detachment becomes rate-limiting, (4) increased front-vs.-rear asymmetry in cell/substratum adhesiveness should broaden the range of ligand concentrations over which substantial migration speed is supported, and (5) increased contractile force should primarily act to decrease effective cell/substratum adhesiveness, by decreasing the number of bonds at a given set of $[R]$, $[L]$, and K_A through stress-induced disruption. By speed what is meant here is the average translocation speed of cells across a population, so this quantity might be observed to change because of uniform changes in the individual translocation speeds of all the cells in the population, or, alternatively, because of changes in the proportions of cells exhibiting various different individual translocation speeds.

The most central prediction, (1), has been subjected to rigorous experimental test by independently altering each of the three variables $[R]$, $[L]$, and K_A , and finding excellent agreement of all three cases with the model (DiMilla et al., 1993; Palecek et al., 1997). There have been, of course, a number of earlier experimental reports in the literature demonstrating biphasic dependence of cell migration on molecular properties such as ligand concentration, receptor number, binding affinity, and levels of cytoskeletal components. We have also successfully tested prediction (2), both by direct observation and by showing that a molecular mechanism regulating rear detachment affects movement speed to a much greater extent at higher ligand concentrations than at lower ligand concentrations (Palecek et al., 1998). Predictions (3), (4), and (5) concerning effects of membrane protrusion frequency, front-vs.-rear asymmetry, and contractile force on movement speed have not yet been experimentally tested in rigorous manner to our knowledge, because of the current lack of understanding of how these processes can be altered systematically by molecular-level interventions.

Another relevant model prediction that has been verified by experimental test is that presence of soluble competitors for adhesion-receptor binding should not simply diminish migration under all circumstances, but instead should shift the curve of migration speed vs. matrix ligand concentration toward higher concentrations (Wu et al., 1994). That is, soluble competitors can either inhibit migration or enhance it, depending on the extant balance between cell force generation and cell/substratum adhesiveness. Evidence of this complex but articulatable principle has also been obtained from *in vivo* experiments concerning tumor cell metastasis (Ho et al., 1997).

Understanding this not entirely intuitive concept is important for interpreting a variety of experimental findings; for instance, that sometimes anti-integrin antibodies can result in enhanced capillary tube formation by endothelial cells (Stromblad and Cheresh, 1996), and, that matricellular proteins such as SPARC and thrombospondin can either promote or interfere with angiogenesis, at least in part possibly by multimodal effects on endothelial cell/ECM adhesion (Sage, 1997).

A clear message from these findings is that mechanistic effects of molecular components on cell migration behavior cannot be reliably discerned from comparative experiments under a single environmental condition.

That is, for cells moving on one level of an ECM ligand, altering some signaling pathway component could enhance motility whereas on a different ligand level precisely that same alteration could reduce motility. In order to gain an accurate picture of the effect of any component, its influence on migration over a full range of environmental conditions should generally be sought.

Concerning extrapolation of this model framework to migration through 3-dimensional matrices, data have been generated for neutrophil migration in collagen gels demonstrating influence of matrix mesh structure on cell/matrix adhesive interactions, steric hindrances due to matrix pore restrictions, and contact guidance arising from cell-induced matrix alignment (Dickinson et al., 1994; Kuntz and Saltzman, 1997; Mandeville et al., 1997). Operation of cell-produced matrix proteases may regulate all of these facets (Stefansson et al., 1998). Hence, to the physical processes mentioned above relevant to migration over 2-dimensional surfaces, interactions of cells with matrix geometry in terms of steric hindrances and contact guidance must be added for analysis of 3-dimensional movement behavior. Mathematical modeling combining these new types of interactions has not yet been undertaken.

These sorts of predictions, when and if verified, will provide improved understanding of the roles by which molecular components govern cell migration. However, in order for that to happen, the effect of individual molecular components on these physical processes needs to be determined which then will aid in climbing the hierarchy described in Figure 1. For example, two targets implicated in integrin-mediated signaling, focal adhesion kinase (FAK) (Gilmore and Romer, 1996) and mitogen-activated protein kinase (MAPK) (Klemke et al., 1997), are reported to be regulatory for movement speed, but there is no information regarding the quantitative relationship between their activation levels and migration speed over a range of substratum environments, which should reflect the downstream processes by which these signals act. For example, does an increase in one of these targets alter the right/left shift of the curve of speed vs. ligand concentration (as would occur from modulation of cell/substratum adhesiveness), or does it alter the height of the curve at lower ligand concentrations (as would occur from modulation of membrane protrusion frequency)? And how are the levels of activation of these targets related to integrin/ligand binding?

As another example, detachment of the cell rear appears to be the rate-limiting step for movement speed on the right-hand side of the biphasic curve characterizing the dependence of speed on adhesiveness, and release of integrins from the cell membrane appears to be a mechanism heavily involved in this process. At the same time, one mechanism providing a front-vs.-rear asymmetry in cell/substratum interactions has been identified to be disruption of integrin/cytoskeleton linkages at the cell rear. Is there a regulatory process responsible for both of these phenomena, i.e., the release of integrins during cell rear detachment and the disruption of integrin/cytoskeleton linkages at the cell rear? And can the effect of this process be predicted from the model in terms of the degree of front-vs.-rear asymmetry, by affecting the range of adhesiveness over which substantial migration is supported?

Accompanying this modeling perspective must come experimental measurement of the key physical processes. Membrane extension and retraction can be quantified by high-magnification videomicroscopy during cell locomotion, comparing cell contour perimeters at the beginning and end of sequential time periods (Bailly et al., 1998; Segall et al., 1996; Ware et al., 1998). Cell/substratum adhesiveness can be a confounding factor in these studies, because lamellipodia and filopodia can extend and retract over very short time-scales if they are not stabilized by attachment to the movement substratum. So, either the videomicroscopy observations must be made during sufficiently short periods that extensions are counted whether stabilized or not (Alt et al., 1995; Wessels et al., 1994), or the data must be interpreted in terms of both membrane activity and adhesion processes taken together.

Cell/substratum adhesiveness can be quantified by assays in which a well-characterized distractive force is applied to cells adhered to a surface. The two best-characterized approaches are fluid flow chambers (of a variety of configurations including parallel-plate, Hele-Shaw, and radial) and centrifugation (Chu et al., 1994; Piper et al., 1998; Powers et al., 1996; Usami et al., 1993). It is useful to note here that differences in adhesiveness between particular test conditions are best determined by measuring cell attachment or detachment across a range of magnitudes of distractive force. When this force is very low or very high, actual differences in adhesiveness between two test conditions may be masked, whereas the differences will appear only in an intermediate window of distractive force, large enough to affect cells under one test condition but small enough to not affect cells under the other test condition. An example of this has been seen in studies of EGF and fibroblast migration (Ware et al., 1998), in which a centrifuge assay revealed that EGF reduces fibroblast adhesion to an Amgel-coated substratum only when tested with sufficiently high distractive force. It is necessary, further, to estimate the distractive force in all assays in order to have an idea about whether the observed effect might be relevant to cell-generated forces during motility.

Measurement of cell contractile force is more difficult at present. One approach is to observe cells on compliant substrata, with contractile force reflected in the extent to which the underlying surface is deformed (Harris et al., 1980; Oliver et al., 1995). Analyses have been offered that permit quantitative estimation of contractile force from dislocation of substratum markers (Dembo et al., 1996). A very recent, technically sophisticated approach is utilization of microfabrication technology to place moveable levers within the movement substratum. When a cell migrates over such a lever, displacement of the lever due to cell traction can be quantified and translated into a contractile force (Galbraith and Sheetz, 1997). An issue in any of these measurement procedures is that what is measured is the transmitted force rather than the generated force; these may be different due to dissipation of some portion of the generated force in breaking of adhesion linkages.

Finally, there is the issue of the degree of front-vs.-rear asymmetry in a migrating cell, presumably related to the ability of the cell to transform isotropic contrac-

tile forces into a net difference in traction between the front and rear of the cells. A conceptual problem is that it is not clear precisely what cell property would serve accurately as a potential asymmetry to be targeted. Diverse candidates such as, number of integrin adhesion receptors, cell/substratum contact area, strength of integrin/ECM bonds, and strength of integrin/cytoskeleton linkages may be posed (Sheetz, 1994). An asymmetry has indeed been found in this last property in migrating fibroblasts and neuronal growth cones, using laser trap technology (Felsenfeld et al., 1996; Schmidt et al., 1993, 1995b). Integrin binding beads coated with either anti-integrin antibodies or with ECM proteins have been found to be more strongly anchored to underlying cytoskeleton at the front of migrating cells than at the rear, and the extent of this disparity can be influenced by biochemical and structural features of the integrins responsible for binding. Because in some cell types, at least, detachment of cell/substratum can be partially regulated at the integrin/cytoskeleton linkage, development of asymmetry in this property seems to be a plausible process involved in control of cell migration.

Some analogous modeling effort has been devoted to relating physical processes to directional aspects of cell locomotion (Dickinson and Tranquillo, 1993b; Tranquillo et al., 1988b). To date, however, these models have not been explicitly tested by relating changes in cell directional persistence or directional bias corresponding to those in simultaneous measurements of the underlying physical processes, or in response to systematic alterations in molecular components. One counter-intuitive model prediction is that directional orientation bias might be stronger for chemoattractant ligands possessing lower, instead of greater, receptor-binding affinity, because more frequent association/dissociation signal transduction cycles can improve the "signal-to-noise ratio" inherent in sensing ligand concentration gradients (Tranquillo et al., 1988b). Interestingly, this prediction appears to be consistent with data for neutrophil chemotactic responses to interleukin-8 variants (Clark-Lewis et al., 1991), but dedicated experimental examinations analogous to those cited above for testing models of translocation speed are needed to pursue this and other predictions of models for directional persistence and bias.

FUTURE PROSPECTS

As the increasing complexity of cellular mechanisms governing cell migration is revealed with the discovery of new molecules implicated in its regulation, it is essential that an attempt be made to integrate this knowledge into a unified model which describes cell locomotion. One way that this has been attempted is by treating the cell as a black box and then characterizing cell location in terms of the physical processes which are thought to be involved. Future work will involve developing models further identifying the mechanisms that in turn govern these physical processes. For example, we can think of characterizing the strength of the contractile force that the cell is able to generate by identifying the key components that may be involved such as: (1) rate and extent of actin polymerization, (2) strength of the linkage between the cytoskeletal network and the integrins, and (3) strength of linkage

between the nucleus and the cytoskeleton. As and when molecular components affecting one or more of these processes are identified, their effects may be incorporated as variations in the parameters describing the model. As an instance, it has been identified that molecules such as myosin I, myosin II, and α -actinin are involved in maintaining the integrity of the actin meshwork and that overexpression of myosin I impairs cell migration. In order to include the effect of myosin I in our model, we could alter the parameter value characterizing the rate and extent of actin polymerization. This, in turn, would feed into the effect that contractile force generation has on cell locomotion and thus we would be able to describe the effect of myosin I on cell locomotion. In this manner, development and testing of engineering models for cell migration, across the full hierarchy of description levels outlined in Figure 1, should aid in the daunting task of identifying key molecular properties that can be manipulated effectively to obtain desired patterns of cell migration.

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