

# In Search of Cellular Control: Signal Transduction in Context

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**Abstract** The field of molecular cell biology has experienced enormous advances over the last century by reducing the complexity of living cells into simpler molecular components and binding interactions that are amenable to rigorous biochemical analysis. However, as our tools become more powerful, there is a tendency to define mechanisms by what we can measure. The field is currently dominated by efforts to identify the key molecules and sequences that mediate the function of critical receptors, signal transducers, and molecular switches. Unfortunately, these conventional experimental approaches ignore the importance of supramolecular control mechanisms that play a critical role in cellular regulation. Thus, the significance of individual molecular constituents cannot be fully understood when studied in isolation because their function may vary depending on their context within the structural complexity of the living cell. These higher-order regulatory mechanisms are based on the cell's use of a form of solid-state biochemistry in which molecular components that mediate biochemical processing and signal transduction are immobilized on insoluble cytoskeletal scaffolds in the cytoplasm and nucleus. Key to the understanding of this form of cellular regulation is the realization that chemistry is structure and hence, recognition of the importance of architecture and mechanics for signal integration and biochemical control. Recent work that has unified chemical and mechanical signaling pathways provides a glimpse of how this form of higher-order cellular control may function and where paths may lie in the future. *J. Cell. Biochem. Suppl.* 30/31:232–237, 1998. © 1998 Wiley-Liss, Inc.

**Key words:** cytoskeleton, mechanotransduction, integrins, cell architecture, tensegrity

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Molecular cell biology is guided by the desire to understand how cells and tissues develop their unique organic qualities, including the ability to change shape, move, and grow. Once we understand the molecular controls that govern these behaviors, we will be in a position to develop more rational drug therapies and to create artificial tissues for organ repair and replacement. The first question I would like to address in this essay is: are we moving along the correct path?

Many of the fundamental questions of control in cell and developmental biology relate directly to the mechanism of signal transduction: how an external signal produces an intracellular response. This is true whether the stimulus is a soluble hormone, an insoluble extracellular

matrix (ECM) molecule, an adhesive contact with a neighboring cell, or an external mechanical stress. Thus, to evaluate the correctness of our path in this search for cellular control, we must explore whether we truly understand how cells transduce information.

The experimental approaches used to analyze cell signaling are varied. In general, they involve challenging cultured cells with a controlled stimulus while simultaneously quantitating changes in cellular biochemistry or gene expression. For example, the stimulus might be a mitogen which increases growth in a dose-dependent manner. Alternatively, it might be a secretagogue that induces protein secretion or a vasoagonist that stimulates cell contractility. Stimulus-response coupling can be similarly analyzed in suspended cells immediately after they bind to immobilized ECM components (e.g., ECM-coated microbeads). The common theme is that the effects of all these stimuli are mediated by binding of a ligand to specific transmembrane receptors on the cell surface. When the ligand is bound, the receptor molecule activates

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a “signaling cascade” inside the cell. This is a series of chemical and molecular transformations which eventually results in changes in a particular biochemical function (e.g., gene transcription, translation, secretion, ion channel activation).

Using this experimental approach, the general goal is to identify the critical transduction molecule responsible for a particular stimulus-response coupling. This is often interpreted as the molecule that negates the normal response when deleted or inactivated and that restores the response when its activity is regained. However, the process of signal transduction and cellular control is clearly much more complicated than any single signaling molecule, or even any individual transduction pathway. In living tissues, cells receive many simultaneous inputs that activate numerous signaling cascades in parallel. For example, in a healing wound, cells simultaneously sense the binding of soluble mitogens and insoluble ECM components as well as the pull of the surrounding tissue. Yet, one cell will proliferate in this microenvironment while its neighbors, only microns away, respond by remaining quiescent, differentiating, or even dying in different regions of the same tissue. In fact, it is establishment of this type of local growth differential that drives differential tissue expansion and pattern formation during morphogenesis in all growing tissues.

But how does each cell “know” what to do when confronted by a specific stimulus? The answer is that the same stimulus can produce an entirely different response depending on the cellular context. A simple analogy would be a light switch at the top of a flight of stairs: it either can turn the lights on or off, depending on position of the switch on the floor below. In a similar manner, raising the concentration of growth factor in culture medium results in a dose-dependent increase in growth when cells are plated on a highly adhesive ECM substrate whereas varying the density of the immobilized ECM component can produce similar dose-dependent control of cell growth under conditions in which growth factor levels are optimal [Ingber and Folkman, 1989; Ingber, 1990; Ingber et al., 1990]. Furthermore, cells can be stimulated by optimal growth factors and an optimally adhesive ECM, yet downstream signaling events and cellular behavior can vary dramatically (growth vs differentiation vs apop-

toxis), depending on the degree to which the cell mechanically stretches or retracts [Ingber and Folkman, 1989; Singhvi et al., 1994; Chen et al., 1997; Huang et al., 1998]. In other words, the cellular response is dependent on both the chemical and the mechanical context in which signal transduction proceeds. Thus, the key question is not which signaling molecule is activated, as currently dominates existing approaches. Rather, it is how all these different signaling pathways are integrated inside the cell.

This new challenge in the field of signal transduction is not unlike the multibody problem in physics and it similarly requires a new frame of reference. We tend to teach physics in terms of two body problems because they are readily amenable to analysis, however, the reality is that the two body problem is the rare exception, rather than the rule, in the real world. Similarly, we prefer to describe biological mechanisms in terms of what we can measure, without considering the structural complexity in which these pathways must function in the living cell. This was a sound approach when we had no way to deal with cellular complexity. However, this is rapidly changing and it is clearly our challenge for the future.

Further insight into the mechanism of signal integration has come from analysis of the process by which ECM and mechanical stresses regulate cellular form and function. Binding of ECM molecules to their own cell surface receptors, known as integrins, can activate many of the same intracellular signaling pathways that are stimulated when growth factors bind to their receptors [Clark and Brugge, 1995]. Given that most normal (anchorage-dependent) cells simultaneously require both adhesion to ECM and soluble mitogens for their own growth and survival, much may be gained by understanding how these two signals are integrated inside the cell.

Work in the integrin signaling field has shown that part of the mechanism of signal integration and cellular control is based on the spatial organization of signal transducing molecules inside the cell. Although most analyses of signaling proteins are carried out in solution, many of these molecules normally function when immobilized on insoluble cytoskeletal scaffolds. For example, when cells bind to microbeads coated with ECM molecules that induce integrin receptor clustering, a specialized cytoskeletal struc-

ture, known as the focal adhesion complex (FAC), forms at the site of integrin binding [Plopper and Ingber, 1993] and physically connects the internal actin cytoskeleton to integrins and thus to ECM on the outside the cell [Wang et al., 1993]. Importantly, many key signaling molecules are simultaneously recruited to the FAC [Plopper et al., 1995; Miyamoto et al., 1995a,b]. These signaling components include protein kinases (FAK kinase, c-src, MEK, ERK, JNK, Raf), inositol lipid kinases, small G proteins, ion transporters ( $\text{Na}^+/\text{H}^+$  antiporter) and a subset of high-affinity growth factor receptors, to name a few.

Elements of different signaling cascades may directly interact, and hence integrate, when they are brought into close proximity within the FAC. One example of signal integration based on this form of "solid-state" signaling is the finding that integrin clustering activates synthesis of the inositol lipid substrate, phosphatidylinositol-bis-phosphate (PIP<sub>2</sub>), whereas its breakdown (and the associated release of the downstream signaling molecules, diacylglycerol and inositol-tris-phosphate) is controlled by growth factor clustering-dependent activation of the membrane-associated enzyme, phospholipase C- $\gamma$  [McNamee et al., 1993; McNamee and Ingber, 1996]. In fact, this is a beautiful example of how growth factors and ECM work hand-in-hand to control cell form and function. Cell binding to ECM activates synthesis of this signaling substrate, however, in the absence of a soluble stimulus, signaling proceeds no further. Similarly, growth factor binding may activate phospholipase C, yet if no PIP<sub>2</sub> substrate is available, there would be no release of downstream signaling molecules. However, when the cell both adheres to ECM and binds to growth factors, full activation of downstream signaling cascades occurs and cell behavior is altered.

The important point is that the inositol lipid kinases that mediate the effects of ECM and the phospholipase C that is activated by growth factors appear to colocalize within the same insoluble cytoskeletal microdomain (i.e., FAC) as integrins and growth factor receptors [McNamee et al., 1996; Plopper et al., 1995]. It is the spatial positioning of these signaling components that facilitates signal integration. ECM and growth factors also interact to control other signaling mechanisms (e.g.,  $\text{Na}^+/\text{H}^+$  exchange, expression of growth response genes), but in this case the integrin and growth factor recep-

tor signaling cascades are distinct and additive [Ingber et al., 1990; Schwartz et al., 1991; Dike and Ingber, 1996]. Yet, again it is the proximity between these different signaling components and their colocalization with the FAC that likely optimizes signal processing and integration in these cells.

Importantly, mechanotransduction is directly overlaid on top of this solid-state signaling mechanism. Mechanical stresses are not transmitted equally across the plasma membrane at all points on the cell surface. Instead, integrins and other transmembrane adhesion receptors that link extracellular attachment scaffolds (ECM, other cells) to the internal cytoskeleton appear to provide preferred paths for transmembrane mechanical signal transfer [Wang et al., 1993; Yoshida et al., 1996; Maniotis et al., 1997; Potard et al., 1997]. The finding that mechanical signals also converge on the FAC and influence the activity of many of the signaling molecules that are immobilized on the cytoskeletal backbone of this complex, raised the possibility that signals from ECM, growth factors, and mechanical distortion may be integrated directly within the FAC at the site of integrin binding [Ingber, 1991, 1997; Chicurel et al., 1998a]. In fact, recent studies show that cell structure, biochemistry, and signal transduction differ depending on the level of mechanical forces balanced across the FAC [Wang et al., 1993; Chen and Grinnell, 1995; Maniotis et al., 1997; Choquet et al., 1997; Chicurel et al., 1998a,b].

In contrast to signaling by soluble agonists and insoluble ECM components, external mechanical signals are always imposed on a pre-existing force balance [Ingber, 1991]. All living cells continually generate mechanical tension within their contractile cytoskeletal microfilaments and they transmit these forces to all the parts of the cell. Thus, this form of signal transduction involves modulation of biochemical events by changing the level of stress in the cell. For this reason, the response of the cell to an external stress may vary depending on cell extension and the initial prestress (internal tension) in the cytoskeleton [Wang and Ingber, 1994], much like how the quality of a musical tone varies when one tunes a guitar string.

The FAC and other membrane adhesion complexes are not the only sites of signal integration. Because cells use a tension-based system of architecture, known as tensegrity, to structure

themselves [Ingber, 1993, 1998], a local stress applied to integrins may promote long-range structural rearrangements throughout the cell and nucleus [Wang et al., 1993; Maniotis et al., 1997]. Coordinated restructuring of the whole cell may help orchestrate the entire cellular response to both chemical and mechanical signals. This dependence on cell architecture may help explain how altering the balance of mechanical forces in a cell and cell shape can induce it to produce entirely different functional outputs (growth vs differentiation vs apoptosis) given the same set of inputs (e.g., growth factors and ECM) [Chen et al., 1997].

How could this work? How could a mechanical distortion alter intracellular biochemistry? The answer comes from molecular biophysics. If the cytoskeletal framework of the cell is abruptly deformed by applying mechanical stresses, and if the framework does not break, at least a subset of the molecules that comprise these scaffolds must physically distort. Changing molecular shape or mechanics alters thermodynamic and kinetic parameters and thus directly influences biochemistry [Ingber, 1997]. An interesting historical point is that the preface of certain biochemistry textbooks published in the early part of this century explained that pressure and volume were to be ignored because it was assumed that all biochemical reactions under study were occurring in a test tube (free in solution). However, the preface also warned the reader to be aware that real living systems would never be understood if viewed as based on a structureless chemistry and thus, that we must return to address this question in the future when techniques become available to deal with structural complexity. Unfortunately, this preface was deleted from subsequent editions, so many readers of these basic textbooks fail to realize the fundamental importance of physical parameters (local changes in pressure and volume and related stress tensors) on biochemical reactions. This link between mechanics and chemistry helps to explain how all living cells respond to mechanical stresses and how they alter their form and function to best accommodate these stresses [Ingber, 1991, 1997].

Well, then, how does binding of a receptor “activate” intracellular signaling cascades? The conventional answer is that the transmembrane protein autophosphorylates or that it interacts with a cytoplasmic signaling protein

when it binds to its external ligand. However, the reality is that chemistry is structure. Binding of a growth factor to a small region of the extracellular portion of a large transmembrane receptor protein results in higher-order structural transformations that propagate throughout the length of the molecule and across the plasma membrane. This is possible because the protein itself is composed of discrete stiff and flexible regions (e.g.,  $\alpha$ -helices,  $\beta$ -strands, hinge regions) that interact via tensile hydrogen bonding to pull themselves into a stable three-dimensional form. This dependence of the structural stability of individual molecules on internal prestress, continuous tension, and local compression is characteristic of tensegrity architecture, which also guides the organization of living cells and tissues [Ingber, 1998]. Because of tensegrity, a local stress induced by growth factor binding to its receptor results in global structural rearrangements throughout the membrane protein molecule. These changes in molecular shape and mechanics (e.g., stiffness, vibration mode) may result in exposure of previously sequestered catalytic regions within the cytoplasmic portion of the receptor molecule or changes in its binding affinity for different cytoplasmic components. In this manner, a structural remodeling cascade is triggered inside the cell and biochemical changes result.

Another example of how chemistry is structure can be seen in signaling based on protein tyrosine phosphorylation, perhaps the classic paradigm of signal transduction. The conventional approach is to view this as an on/off signal and to focus on the protein sequences that mediate these events. However, from a structural or biophysical perspective, the key signaling event is a change in molecular mechanics. The addition of a phosphorylation moiety to a tyrosine group changes molecular flexibility and shape (e.g., from globular to linear); in fact, this specific type of modification is used to create adhesive substrates for tissue engineering with controlled flexibility [Urry, 1992]. I would suggest that similar cascades of molecular restructuring events occur when tyrosine phosphorylation signaling pathways are activated in living cells. Furthermore, it is likely that it is through these coordinated changes in molecular mechanics and resulting alterations in the flexibility and architecture of larger tensionally coupled supramolecular scaffolds that many different signaling pathways can be simul-

taneously orchestrated, even though they exist in different locations inside the FAC.

It is important to note that this form of mechanochemical regulation is not limited to the FAC [Chicurel et al., 1998a]. Similar integration may occur at lateral cell-cell junctions which also exhibit high levels of cytoskeletal-associated signaling molecules [Yamada and Geiger, 1997], as well as efficient transmembrane mechanical force transfer [Yoshida et al., 1996; Potard et al., 1997]. Furthermore, solid-state biochemistry also plays a key role in other types of metabolic processing events (i.e., other than signal transduction) and in different locations in the cell. For example, many of the biochemical reactions involved in DNA synthesis, RNA processing, protein synthesis, and glycolysis also appear to proceed in a solid-state along insoluble cytoskeletal scaffolds within both the cytoplasm and the nucleus [Ingber, 1993b]. In the case of protein synthesis, intermediates in the process, aminoacyl-tRNAs, are channeled directly from the aminoacyl-tRNA synthetases to the elongation factor to the ribosomes without dissociating into the cellular fluid [Stapulionis and Deutscher, 1995]. Solid-state biochemistry and enzymatic channeling may help explain the high level of efficiency of biochemical reactions that is observed in living cells but often cannot be mimicked in a test tube. It also provides a mechanism by which cell shape modulation and associated cytoskeletal distortion can result in changes in cellular biochemistry and gene expression [Ingber, 1997]. Most importantly, it emphasizes how we will never be able to fully understand cellular control if we only analyze individual molecules in isolation.

In summary, the reductionist experimental approach that dominates current thinking in the field of molecular cell biology clearly has had a major positive impact in terms of increasing our knowledge base. It also has greatly facilitated drug discovery. However, the question remains: is this the correct paradigm for the future? As briefly discussed above, it is unlikely that this solution chemistry approach can provide all the answers we are seeking. Thus, the new challenge is to develop methods, imaging techniques and experimental approaches to study critical biochemical processes within the complexity of the living cell and to controllably vary cell structure and mechanics. A look to the past shows that many of the major breakthroughs were facilitated by the develop-

ment of new theories (and methods to test them) that changed the frame of reference that previously dominated the field. The recent convergence of molecular cell biology with bioengineering has attracted new types of investigators to this field and has led to the generation of entirely new experimental and analytical approaches [Ingber, 1993b]. As these fields merge with computer science and informatics, new advances will likely be accelerated.

Understanding how biochemistry proceeds within the context of the structural complexity of living cells may lead to development of entirely new approaches in medicine, engineering, and materials science that could make functional genomics appear primitive by comparison. This will be a brave new world in which science and technology will be driven by biomimetics rather than genomics, by structure and mechanics rather than chemistry alone, and by fabrication strategies that mimic biological mechanisms of self-assembly and structural remodeling. The challenge for the next millenium is to choose our first steps wisely.

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