Cell, Vol. 75, 1249–1252, December 31, 1993, Copyright © 1993 by Cell Press

R9

The Riddle of Morphogenesis: A Question of Solution Chemistry or Molecular Cell Engineering?

Minireview

Donald E. Ingber
Departments of Surgery and Pathology
Children's Hospital
and Harvard Medical School
Boston, Massachusetts 02115

Analysis of molecules in solution has led to great insights into biochemical mechanisms of cellular control and to our current understanding of the critical influence of molecular genetics in growth and development. Experiments with genetically manipulated microorganisms also have been extremely instructive. However, the problem of morphogenesis, especially the fundamental question of how functional cells and tissues form from individual molecular components, continues to elude mechanistic explanations.

One major limitation of simplified model systems is that they lack the correct structural context; both mechanical influences and normal macromolecular scaffolds are absent. This is a critical point because while chemicals mediate morphogenesis, physical forces often dictate biological pattern. Hemodynamic stresses sculpt blood vessels; compressive loads mold bone; tensile forces shape muscle; and this form of mechanoregulation holds true in plants (e.g., gravitropism) as well as animals (reviewed by Ingber, 1991; Wang et al., 1993). The absence of molecular scaffolds is also important because much of cell metabolism may function in a solid-state: physiologically active membrane receptors, junctional proteins, signaling molecules, regulatory enzymes, and metabolic substrates are often immobilized on insoluble structural elements within the cell and nucleus. Simple examples in the cytoplasm include the colocalization of tyrosine kinases that mediate signal transduction (e.g., $pp60^{v-src}$, $p125^{FAK}$) with structural components of the focal adhesions of the cell (Zachary and Rozengurt, 1992) and the compartmentalization of certain mRNAs via binding of their 3′ untranslated regions to cytoskeletal microfilaments (Kislauskis and Singer, 1992). In the nucleus, DNA packaging and replication enzymes (Pienta et al., 1991), tissue-specific transcription factors (Bidwell et al., 1993), RNA splicing assemblies (Zeitlin et al., 1987), and critical growth regulatory proteins, including the 105 kd retinoblastoma gene product (Hinds et al., 1992) and the viral transforming protein E7 (Greenfield et al., 1991), all physically associate with insoluble nuclear scaffolds. In fact, the nucleus appears to exhibit precise spatial order in terms of chromosome organization (Manuelidis, 1990; Pienta et al., 1991), DNA replication (Hozak et al., 1993), transcription and processing of RNA (Carter et al., 1993; Jimenez-Garcia and Spector, 1993), and translocation of nuclear proteins (Meier and Blobel, 1992).

If a large part of cell metabolism functions in a solid state, then stress-induced changes in scaffold topology or mechanics could provide a mechanism for regulating cellular biochemistry and, hence, efficiently integrating cell structure and function. However, it has been difficult to test this hypothesis using conventional biological approaches. This is because this problem is not based on changes in chemical composition or local binding interactions; rather, it is a question of architecture. To address this type of question, novel technologies need to be developed to induce controlled alterations in scaffold structure and, at the same time, to quantitate changes in both scaffold mechanics and specific molecular functions. In answer to this challenge, molecular cell biologists, biophysicists, and engineers have begun to join together and combine their knowledge and tools. As a result, a new scientific discipline, perhaps best defined as molecular cell engineering, is beginning to emerge and to fill the current gap between genetic engineering and tissue engineering. The purpose of this minireview is to review the critical questions and scientific developments that have led to the emergence of this field and to explore their implications for the future of biology as well as graduate education.

The composition, structure, and function of the insoluble matrices that constitute the skeleton of the cell and nucleus have been debated for many years. In fact, it is clear that the cell is not simply a bag of enzymes: many molecules involved in metabolic regulation are not freely diffusible in the cytoplasm (Gershon et al., 1985), nucleus (Nakayasu and Berezney, 1989; Carter et al., 1993), or mitochondria (Scalettar et al., 1991). Thus, the relevant question is no longer whether these molecular scaffolds exist. Instead, we need to ask whether these structures are responsible for metabolic regulation and, if so, how do these structural cues integrate with chemical signals so that morphogenesis can proceed?

Cell and molecular biologists have primarily used two approaches to address questions of structure. The first is to develop methods, often based on membrane permeabilization techniques, whereby intracellular macromolecular scaffolds can be isolated free of soluble components and be shown to retain normal function in vitro. The second method involves reconstitution experiments in which complex cellular structures, such as whole nuclei (Almouzni and Wolffe, 1993), are reassembled from molecular components and shown to exhibit specific and regulated functional activities. Both approaches have led to the same interpretation: higher order structure is required for normal function in metazoan cells. For example, cytoskeletal preparations obtained after saponin permeabilization continue to exhibit channeling of protein synthesis (Negrutskii and Deutscher, 1992), a solid state regulatory mechanism in which immobilized aminoacyl tRNA is passed directly from its synthetase to elongation factor and to the ribosome. Preparations of intracellular scaffolds obtained by different detergent extraction methods can differ in terms of the efficiency at which different proteins are retained. However, the important point is that insoluble scaffolds obtained by different methods retain the same critical functional activities. For instance, two different procedures for removing membranes and soluble cytoplasmic compo-

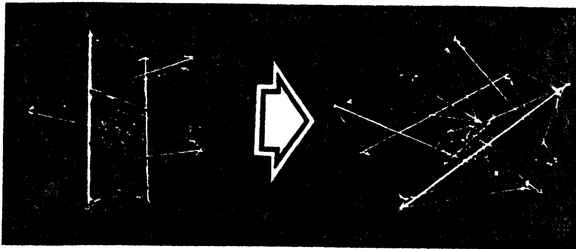

Figure 1. Tensegrity Cell Model

Mechanical stresses associated with the formation of basal cell–substratum adhesions cause structural elements throughout the depth of a nucleated tensegrity cell model to rearrange in a coordinated manner. Similar changes occur in living cells when they attach to extracellular matrix. Use of tensegrity architecture by cells could provide a mechanism to distribute mechanical stresses to regulatory molecules and metabolic enzymes that are immobilized on cytoskeletal and nuclear scaffolds and thereby to integrate cell structure and function (for further discussion see Ingber, 1993; Wang et al., 1993).

nents from whole cells produce the identical result: isolated nuclear–cytoskeletal lattices retain all of the enzymes necessary to organize initiation sites in normal patterns and, in fact, continue actively to replicate DNA (Nakayasu and Berezney, 1989; Hozak et al., 1993). Further isolation of nuclear matrices (the nonchromatin structure of the nucleus) confirms that the spatial distribution and high efficiency of DNA replication (Pienta et al., 1991), RNA splicing (Zeitlin et al., 1987), and transcriptional regulation of tissue-specific genes (Bidwell et al., 1993) observed in vivo all require the presence of an internal nuclear scaffold. In addition, structural interconnections between the cytoskeleton and nucleus (Fey et al., 1984), possibly due to binding interactions between intermediate filaments and nuclear lamins (Djabali et al., 1991), remain mechanically functional after membrane permeabilization; addition of ATP, which drives actomyosin filament sliding, results in coordinated changes in cell, cytoskeletal, and nuclear forms (Sims et al., 1992).

Based on these approaches, it has become clear that the rules by which growth is regulated in animal cells differs greatly from that observed in lower organisms, even though nearly identical molecular machinery is used in both. In contrast with lower organisms, metazoan origins of DNA replication require chromatin structure and nuclear organization in addition to specific DNA sequences (DePamphilis, 1993; Almouzni and Wolffe, 1993). Furthermore, nuclear architecture and function may be controlled mechanically. For example, cell binding to growth factors and the extracellular matrix is not sufficient for growth. Normal cells must also anchor to a substratum that can resist cell tension and physically extend themselves and their nuclei to enter S phase (reviewed by Ingber, 1991). This is especially interesting given that the mammalian cell cycle checkpoint R and the start control of yeast are nearly identical in terms of their dependence on cyclins and related kinases. Furthermore, in many cells, preventing cell extension also switches on differentiation, even though the same growth factor and integrin signaling mechanisms are activated (Ingber, 1991). Thus, extracellular matrix-sensitive response elements found within the promoters of differentiation-specific genes in the nucleus (Schmidhauser et al., 1990) also must be sensitive to changes in matrix mechanics and related alterations in cell structure. In other words, while cell physiology is driven by chemical reactions, changes in cell mechanics may function as the throttle.

Taken together, these findings suggest that somehow the generic molecular machinery and master switches that are found in all cells have been placed under the control of a higher authority: cell and nuclear architecture. This realization has attracted the interest of many engineers because they realize that any change in structure, regardless of scale, must result from the action of a force acting on a mass. This perspective offers another way to approach the question of cell regulation, namely, by exploring how mechanical forces influence cells. To accomplish this, mechanical engineers have collaborated with molecular cell biologists and biophysicists. Through their combined efforts, multiple devices have been developed and used to apply controlled mechanical stresses to cells and tissues. Entire cells and surface membranes can now be strained, compressed, sheared, poked, pulled, and exposed to suction. Most recently, it has even become possible to twist specific transmembrane receptors mechanically (Wang et al., 1993) and to pull individual molecules out of lipid bilayers (Evans, 1993) without producing generalized changes in cell form.

Using these engineering approaches, mechanical forces have been shown to regulate many cell functions, including DNA synthesis, transcription, translation, secretion, and ion transport as well as a number of different chemical signaling pathways (reviewed by Ingber, 1991). By proceeding further and analyzing the molecular basis of these phenomena, entirely novel regulatory mechanisms have been uncovered. One recent example is the sequencing of a cis-acting fluid shear stress responsive element that is present in a number of different of genes and is responsible for their transcriptional activation in response to mechanical perturbation (Resnick et al., 1993).

Another strength of the engineering approach is that it permits quantitation of changes in cell structure and mechanics that result from experimental manipulation. Measurements of this sort have revealed that mechanical signals that are responsible for changes in cell structure are transmitted across the cell surface by specific transmembrane receptors (integrins) and that the cytoskeletal response to stress (an increase in mechanical stiffness) involves higher order structural interactions among all three filament systems: intermediate filaments, microfilaments, and microtubules (Wang et al., 1993). Further, it appears that the entire cytoskeleton responds to stress as a single, tensionally integrated (tensegrity) structure (Figure 1), that is, a molecular continuum of mechanically interdependent struts and tensile elements that rearrange, rather than deform locally, in response to stress (Wang et al., 1993; Ingber, 1993). This mechanism of structural stabilization provides one explanation for why all structural support elements, from the cell surface to the nucleus, are mechanically coupled and for how changes in the cellular mechanical force balance produce distinct molecular patterns (e.g., actin stress fibers versus polygonal nets) within

the cytoskeleton. It also explains why the contractile function of an entire cell and tissue (e.g., myocardium) may be sensitive to changes in the mechanics of a single type of cytoskeletal filament (microtubules), even though it is not directly involved in generating the force that drives contraction (Tsutsui et al., 1993). Perhaps most important for the design of future experiments, critical structural elements (e.g., F-actin filaments) no longer exhibit the mechanical properties and functions of interest when isolated free from this type of prestressed network and studied in vitro.

How could stress-induced changes in the structure or mechanics of an intracellular scaffold alter biochemical function? First of all, it is important to note that while signal transduction events that involve ligation of a receptor or protein modification (e.g., tyrosine phosphorylation) are referred to as examples of chemical signaling, they effect changes in molecular function by producing dramatic alterations in the structure, shape, and mechanics (e.g., elasticity) of the target protein (Urry, 1992; Lauffenburger and Linderman, 1993). Mechanical stress-induced changes in cytoskeletal mechanics (e.g., stiffness, permanent deformation) and topology may similarly alter the function of the structural molecules that comprise the cytoskeleton and nucleus, including critical regulatory proteins. For example, consider stretch-responsive ion channels, which mediate sensitivity of animal and plant cells to many types of mechanical stimuli. While isolated stretch-sensitive channels can be activated in liposomes via direct mechanical perturbation of the membrane, the normal high mechanosensitivity and adaptive behavior of these channels requires mechanical coupling with the underlying cytoskeleton (Hamill and McBride, 1993).

Altering structural arrangements within the cytoskeleton and nuclear matrix may expose or obscure internal molecular binding sites, release mechanical constraints for molecular remodeling events, or change the porosity of the lattice. For example, changes in the arrangement of cytoskeletal filaments may expose sequestered RNA molecules to cytoplasmic enzymes to which they are usually resistant (Negrutskii and Deutscher, 1992) and thus reduce their stability. Transmission of mechanical stress between the cytoskeleton and nucleus also could explain why nuclear pores physically expand and nucleocytoplasmic transport rates increase in spreading cells (Feldherr and Akin, 1993) and hence provide a structural basis for shape-dependent growth control.

The dependence of many biochemical functions on cell architecture also may be based on a novel form of regulation in which the location and rate of chemical reactions are governed by the geometry of the orienting scaffold. An example of geometric control of biochemical reactions already exists: the vertices of actin nets and not the intervening struts are used as nucleation sites for actin polymerization during filopodial assembly (Ingber, 1993). Force-induced changes in molecular pattern may reorient microtubular tracks upon which molecular motors ride and thus polarize cells. It also could play a role in macromolecular assembly and mechanoelectrical coupling within extracellular matrix.

In this context, specific architectural descriptions of the cell, like the tensegrity paradigm, are important because they open the way for the introduction of computers and sophisticated forms of continuum mechanics analysis into the realm of intracellular structure. This type of approach should be facilitated by the work of engineers and biologists who are beginning to quantitate complex biological phenomena and to describe them in mathematical terms. Individual cell surface molecular binding events (Evans, 1993), receptor-mediated behavioral responses such as cell adhesion and motility (Lauffenburger and Linderman, 1993), relations among focal contact formation, shape, and attachment strength (Davies et al., 1993; Ward and Hammer, 1993), and mechanics of the cytoskeleton in a unicellular organism (Dembo, 1989) have all been measured and described in these terms. A joint engineering–biological approach also resulted in a thermodynamic model of microtubule polymerization that incorporates the mechanical interdependence between different cytoskeletal filaments described above and predicts changes in microtubule assembly that are observed in living cells (Buxbaum and Heidemann, 1988).

From this discussion, it should be clear that it is necessary to recognize the importance of biological structure in studies on gene expression and molecular regulation. To understand its role, novel methods must be developed for applying controlled mechanical stresses to particular molecular scaffolds and simultaneously measuring structural and functional responses. We also must begin to explore how global changes in cell structure and mechanics alter local molecular interactions within different subcellular microenvironments. The recent availability of methods to isolate and reconstitute functional nuclei (Almouzni and Wolffe, 1993) as well as cytoskeletal microdomains that retain normal structure and chemistry (e.g., intact focal adhesion complexes; Plopper and Ingber, 1993) may greatly facilitate this analysis. In fact, by focusing on the regulatory role of the orienting scaffolding rather than on the chemical messenger itself, it may be possible to identify novel molecular targets for pharmacological intervention, including some that are cell type specific. Finally, given the importance of mechanical changes in gene regulation (e.g., torsional strain in transcription, DNA unwinding activity during replication), it will be interesting to determine whether local molecular remodeling events feed back to alter higher order structure and thus to modulate other cell functions. Although these are speculations, they are testable if methods for simultaneous chemical and structural analysis are available.

In conclusion, investigators who come from many different fields are simultaneously coming to realize that we must go back to structure to understand function. However, neither the molecular biologist, the biophysicist, nor the engineer alone currently has the ability to explain fully the role of complex structures in cell physiology. On the other hand, a single investigator trained in molecular cell engineering could have the knowledge and skills necessary to accomplish this goal. He or she also could access computerized design capabilities, now limited to molecular modeling, for analysis of cellular structures and thus be perfectly poised to bring cell biology into the twenty first century. Yet the success of this emerging field clearly

depends on the openness of programs in graduate education. Each discipline, whether in engineering or biology, has its own specialized vocabulary, techniques, and principles of operation. These boundaries need to be broken down when young investigators are still in training. Thus, the time to combine these different approaches is now. The potential for expanding our understanding of how cells and tissues function and for leading to new technologies and therapeutic approaches can not be underestimated.

References

Almouzni, G., and Wolffe, A. P. (1993). Exp. Cell Res. *205*, 1–15.

Bidwell, J. P., van Wijnen, A. J., Fey, E. G., Dworetzky, S., Penman, S., Stein, J. L., Lian, J. B., and Stein, G. S. (1993). Proc. Natl. Acad. Sci. USA *90*, 3162–3166.

Buxbaum, R. E., and Heidemann, S. R. (1988). J. Theor. Biol. *134*, 379–390.

Carter, K. C., Bowman, D., Carrington, W., Fogarty, K., McNeil, J. A., Fay, F. S., and Lawrence, J. B. (1993). Science *259*, 1330–1335.

Davies, P. F., Robotewskyj, A., and Griem, M. L. (1993). J. Clin. Invest. *91*, 2640–2652.

Dembo, M. (1989). Biophys. J. *55*, 1053–1080.

DePamphilis, M. L. (1993). J. Biol. Chem. *268*, 1–4.

Djabali, K., Portier, M. M., Gros, F., Blobel, G., and Georgatos, S. D. (1991). Cell *64*, 109–121.

Evans, E. (1993). In Handbook of Biophysics, R. Lipowsky and E. Sackmann, eds. New York: Elsevier Scientific Press.

Feldherr, C. M., and Akin, D. (1993). Exp. Cell Res. *205*, 179–186.

Fey, E. G., Wan, K. M., and Penman, S. (1984). J. Cell Biol. *98*, 1973–1984.

Gershon, N. D., Porter, K. R., and Trus, B. L. (1985). Proc. Natl. Acad. Sci. USA *82*,5030–5034.

Greenfield, I., Nickerson, J., Penman, S., and Stanley, M. (1991). Proc. Natl. Acad. Sci. USA *88*, 11217–11221.

Hamill, O., and McBride, D. (1993). Biophys. J. *65*, 17–18.

Hinds, P. W., Mittnacht, S., Dulic, V., Arnold, A., Reed, S. I., and Weinberg, R. A. (1992). Cell *70*, 993–1006.

Hozak, P., Hassan, A. B., Jackson, D. A., and Cook, P. R. (1993). Cell *73*, 361–373.

Ingber, D. E. (1991). Curr. Opin. Cell Biol. *3*, 841–848.

Ingber, D. E. (1993). J. Cell Sci. *104*, 613–627.

Jimenez-Garcia, L. F., and Spector, D. L. (1993). Cell *73*, 47–59.

Kislauskis, E. H., and Singer, R. H. (1992). Curr. Opin. Cell Biol. *4*, 975–978.

Lauffenburger, D. A. and Linderman, J. J. (1993). Receptors: Models for Binding, Trafficking, and Signaling, (New York: Oxford University Press).

Manuelidis, L. (1990). Science *250*, 1533–1540.

Meier, U. T., and Blobel, G. (1992). Cell *70*, 127–138.

Nakayasu, H., and Berezney, R. (1989). J. Cell Biol. *108*, 1–11.

Negrutskii, B. S., and Deutscher, M. P. (1992). Proc. Natl. Acad. Sci. USA *89*, 3601–3604.

Pienta, K. J., Getzenberg, R. H., and Coffey, D. S. (1991). Crit. Rev. Eukaryot. Gene Expr. *1*, 355–385.

Plopper, G. and Ingber, D. E. (1993). Biochem. Biophys. Res. Commun. *193*, 571–578.

Resnick, N., Collins, T., Atkinson, W., Bonthron, D. T., Dewey, C. F., Jr., and Gimbrone, M. A., Jr. (1993). Proc. Natl. Acad. Sci. USA *90*, 4591–4595.

Scalettar, B. A., Abney, J. R., and Hackenbrock, C. R. (1991). Proc. Natl. Acad. Sci. USA *88*, 8057–8061.

Schmidhauser, C., Bissell, M. J., Myers, C. A., and Casperson, G. F. (1990). Proc. Natl. Acad. Sci. USA *87*, 9118–9122.

Sims, J., Karp, S., and Ingber D. E. (1992). J. Cell Sci. *103*, 1215–1222.

Tsutsui, H., Ishihara, K., and Cooper, G., IV (1993). Science *260*, 682–687.

Urry, D. (1992). Prog. Biophys. Mol. Biol. *57*, 23–57.

Wang, N., Butler, J. P., and Ingber, D. E. (1993). Science *260*, 1124–1127.

Ward, M. D., and Hammer, D. A. (1993). Biophys. J. *64*, 936–959.

Zachary, I., and Rozengurt, E. (1992). Cell *71*, 891–894.

Zeitlin, S., Parent, A., Silverstein, S., and Efstratiadis, A. (1987). Mol. Cell. Biol. *7*, 111–120.