subunits themselves and the interactions of associated proteins, suggests potential for substantial tightening of the conformation following rotation. Moreover, the carboxyl terminus of the activator WASp binds to both Arp2/3 and p21 to generate a cooperative system in which actin filaments (F-actin) enhance the affinity of WASp for the complex and further stimulate nucleation (*11*).

The structure of the Arp2/3 complex provides, for the first time, insights into how dendritic branches of actin filaments are assembled. After triggering by WASp, the elementary steps of branched filament growth are the assembly of Arp2/3 complexes and G-actin subunits on F-actin. Activation of WASp family members by various signaling pathways is an essential component of the regulatory process. Both phosphatidylinositol 4,5-bisphosphate and the small ras-related GTP-binding protein Cdc42 are essential for WASp activation, and bind to sequences in the amino-terminal part of this protein. Moreover, Cdc42 must be in its membrane-bound, GTP-containing form (*12*).

The Robinson *et al.* work provides a substantial springboard for further progress. It may be possible to gain structural information about the interplay among all of the components of the Y-branch complex, and about the mechanism that activates dendritic growth. A more ambitious project will be to elaborate the sequence of interactions involving actin depolymerizing factors, capping proteins, and profilin in the recycling of actin subunits. Current evidence suggests a complex series of steps, key to which is whether ATP or ADP is bound to actin (*13*). Future research will unravel how integration of signals at the cell surface mediates the processes that lead to the remodeling of the branched filament network and how this is translated into coordinated cell movement.

References

1. D. Pantaloni *et al.*, *Science* **292**, 1502 (2001).
2. R. C. Robinson *et al.*, *Science* **294**, 1679 (2001).
3. L. M. Machesky *et al.*, *J. Cell Biol.* **127**, 107 (1994).
4. R. D. Mullins *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6181 (1998).
5. T. P. Loisel *et al.*, *Nature* **401**, 613 (1999).
6. L. M. Machesky, R. H. Insall, *Curr. Biol.* **8**, 1347 (1998).
7. T. M. Svitkina, G. G. Borisy, *J. Cell Biol.* **145**, 1009 (1999).
8. L. Blanchoin *et al.*, *Nature* **404**, 1007 (2000).
9. N. Volkmann *et al.*, *Science* **293**, 2456 (2001).
10. H. Gournier *et al.*, *Molec. Cell*, in press.
11. J. B. Marchand *et al.*, *Nature Cell Biol.* **3**, 76 (2001).
12. H. N. Higgs, T. D. Pollard, *Annu. Rev. Biochem.* **70**, 649 (2001).
13. L. Blanchoin *et al.*, *Curr. Biol.* **10**, 1273 (2000).

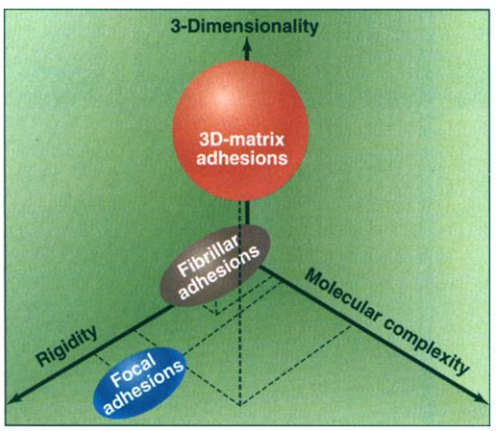
PERSPECTIVES: CELL BIOLOGY

Encounters in Space

Benjamin Geiger

At the end of nearly every talk about cell-matrix adhesions—the structures formed between cultured cells and the substratum on which they grow—someone in the audience invariably asks: "Beautiful pictures, but what is the physiological significance of these adhesions? Aren't they merely artifacts of tissue culture?" Slightly annoyed, the speaker usually mutters something about the general nature of model systems, and highlights similarities between adhesions formed in culture and those formed in vivo. Recent work points to a remarkable molecular heterogeneity in the adhesions formed by cultured cells as they attach to different substrates (*1*). Such studies, however, do not identify which adhesions are akin to those formed in vivo. On page 1708 of this issue, Cukierman and coworkers (*2*) directly address this question and reach some intriguing conclusions.

These investigators grew fibroblasts on shallow 3D matrices derived from other cultured cells or tissues. These 3D matrices are similar to the extracellular matrices produced by cells in vivo, yet are reasonably flat and thus can be readily analyzed by high-resolution fluorescence microscopy. The cultured cell-derived 3D matrix has been extensively used for growing cells under quasi-physiological conditions, yet its capacity to support specific molecular types of adhesions has not been determined (*3*, *4*). In their study, Cukierman and colleagues compare the characteristics of adhesions formed by cultured fibroblasts attached to a cell-derived 3D matrix, a 2D matrix, and a 3D collagen matrix. The rate at which fibroblasts attached to the cell-derived 3D matrix was 6- to 10-fold higher than that measured for all other surfaces. The adhesions formed with this matrix were elongated and morphologically distinct from the focal and fibrillar adhesions typical of cultured cells (*1*). When attached to the cell-derived 3D matrix, fibroblasts became spindle-shaped (like tissue fibroblasts in vivo) and lost their flat morphology. These spindle-shaped fibroblasts proliferated two to three times more rapidly than their counterparts attached to other surfaces.



Close encounters of the 3D kind. The molecular composition, rigidity, (pliability) and topography (three-dimensionality) of a matrix affects the formation of cellular adhesions. Focal adhesions are associated with rigid surfaces and are flat with limited molecular complexity. In contrast, fibrillar adhesions contain the fibronectin receptor and bind to pliable fibronectin fibrils. They are slightly 3D and are of moderate molecular complexity. The adhesions formed between cultured cells and a cell-derived 3D matrix are moderately pliable and probably highly complex, as well as being 3D.

The principal molecule associated with these fibroblast adhesions was integrin $\alpha5\beta1$, the major fibronectin receptor. Function-blocking antibodies against this integrin prevented the formation of 3D-matrix adhesions. The 3D-matrix adhesions contained large amounts of focal adhesion kinase (FAK), paxillin, and vinculin, thus resembling focal adhesions; yet, like fibrillar adhesions, they also contained the fibronectin receptor. Particularly intriguing was the low level of FAK phosphorylation in 3D-matrix adhesions, which contrasted with the highly phosphorylated FAK of focal adhesions.

What are the features of a 3D matrix that make it so different from other substrates? Cukierman and colleagues determined the specific contributions of matrix topography, molecular composition, and mechanical properties (pliability) to the ability of fibroblast adhesions to stimulate appropriate intracellular signaling pathways. They found that the topography (degree of three-dimensionality) of the matrix alone was not sufficient to activate adhesion-mediated signaling, because a 3D collagen matrix could not do the job. Nor was the "proper" molecular composition enough: If the 3D matrix was destroyed by "flattening" there was no signaling activity even with the correct molecular composition. Combining the correct topography and molecular composition was still insufficient, because reducing the

The author is in the Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel. E-mail: benny.geiger@weizmann.ac.il

pliability of the 3D matrix was detrimental to the formation of adhesions that could activate specific signaling pathways. Thus, for adhesions to form properly and for activation of the correct intracellular pathways the 3D matrix must have the full complement of correct features: appropriate topography, molecular composition, and mechanical properties.

How do the properties of a cell-derived 3D matrix compare to those of substrates that induce focal and fibrillar adhesion formation? It is possible that each type of adhesion matrix (whether formed in vivo or in culture) has a unique combination of topography, molecular composition, and mechanical properties. Thus, focal adhesions are associated with a rigid substrate of limited molecular heterogeneity, whereas fibrillar adhesions attach to a matrix of soft, partly 3D fibronectin fibrils (see the figure). In contrast, 3D-matrix adhesions form with a matrix that is 3D, moderately pliable, and complex in its molecular composition.

The contribution of matrix properties to the formation of adhesions is poorly understood. Intuitively, one might think that the molecular composition of the matrix regulates the specific set of integrins and associated molecules recruited to the adhesion site. Pliability of the matrix may be crucial for regulating local tension applied at adhesion sites. This is consistent with the finding that local application of internal or external forces triggers the growth of adhesion sites and the assembly of bundles of actin filaments (stress fibers) (*5*). As the rigidity of the matrix increases, focal but not fibrillar adhesions are formed (*6*). Furthermore, adhesions are dynamic structures that are constantly being reorganized (*7*). A 3D matrix might be able to nucleate (seed) numerous small adhesions, inducing the formation of short actin bundles rather than arrays of large stress fibers.

Are all 3D matrices created equal? Probably not. In fact, it was shown long ago (*8*) that corneal endothelial cells and fibroblasts produce extracellular matrices that differ widely in structure and composition. It thus seems likely that matrices derived from different cells or tissues may each have a unique composition-topography-rigidity "signature" that determines their capacity to "sense" and to respond to the environment. It is noteworthy that certain adhesions formed in vivo resemble the focal adhesions of tissue culture and are associated with stress fibers (*9–12*). Returning to the question of whether adhesions formed in culture are physiological phenomena or merely "tissue-culture artifacts," Cuikerman and coworkers speculate convincingly that the focal and fibrillar adhesions formed in vitro may represent exaggerated precursors (to which I would add "variant forms") of the 3D-matrix adhesions formed in vivo. Their study supports a unified view of how the extracellular matrix exerts its effects on cell structure and fate, and highlights the importance of three-dimensionality, molecular composition, and pliability in this process.

References

1. E. Zamir *et al.*, *J. Cell Sci.* **112**, 1655 (1999).
2. Cukierman *et al.*, *Science* **294**, 1708 (2001).
3. I. Vlodavsky *et al.*, *Dev. Biol.* **93**, 285 (1982).
4. I. Vlodavsky, *Curr. Protocols Cell Biol.* **1**, 10.4.1 (1999).
5. B. Geiger *et al.*, *Nature Rev. Cell Biol.*, **2**, 793 (2001).
6. B.-Z. Katz *et al.*, *Mol. Biol. Cell* **11**, 1047 (2000).
7. E. Zamir *et al.*, *Nature Cell Biol.* **2**, 191 (2000).
8. R. Fridman *et al.*, *Exp. Cell Res.* **158**, 461 (1985).
9. H. R. Byers, K. J. Fujiwara, *J. Cell Biol.* **93**, 804 (1982).
10. G. E. White *et al.*, *J. Cell Biol.* **97**, 416 (1983).
11. K. A. Rogers *et al.*, *Biochem. Cell Biol.* **67**, 553 (1989).
12. J. Bateman *et al.*, *Curr. Biol.* **11**, 1317 (2001).

PERSPECTIVES: EPIDEMIOLOGY

Predicting the Unpredictable

Graham F. Medley

Disease predictions have reached epidemic proportions (*1–5*). Predicting the course of a disease in a population certainly fulfills a morbid fascination, but predictions that vary by two or three orders of magnitude are, for all intents and purposes, meaningless. Three papers published this week by *Science* (*1–3*) attempt to predict patterns of disease caused by the infectious agent responsible for bovine spongiform encephalopathy (BSE) in cattle and sheep, and its counterpart in humans called variant Creutzfeldt-Jakob disease (vCJD). To date, there have been 111 confirmed cases of vCJD in the UK. In their analyses of the UK vCJD epidemic, Huillard d'Aignaux *et al.* (page 1729) (*1*) and Valleron *et al.* (page 1726) (*2*) use statistical approaches to predict future numbers of cases. Both groups predict a long incubation period, with the numbers of predicted cases varying from several hundred (*2*) to hundreds of thousands (*1*). In their study of BSE in sheep, Kao and colleagues (*3*) "retrodict" the past epidemic, their principal interests being the extent to which this epidemic has increased human exposure to the BSE agent and its current prevalence in sheep. They predict fewer than 20 clinical cases of BSE in sheep this year (assuming a maternal transmission rate of 10%), but retrodiction of the peak number of infections varies between 25 and 25,000 (*3*). In each study, the width of the confidence intervals (or range of outcomes from different scenarios) can only be described as unhealthy. Why can't we do better?

In essence, the calculation is simple. The numbers of vCJD cases diagnosed during 2002 will be a convolution of the time of infection and the incubation period distribution (IPD), expressed as [(number infected in 1987) × (probability of progressing to disease during 15th year after infection)] + [(number infected in 1988) × (probability of progressing to disease during 14th year after infection)] + (etc.). Age, sex, and genetic predisposition to infection are among the factors that might complicate this relationship, but they do not change it.

Knowledge of any two of these three quantities (time of infection, IPD, and number of cases) allows the other to be estimated. For the current vCJD epidemic, we know neither the numbers infected nor the IPD in humans. In order to make predictions, one must be able to estimate these values simultaneously from case data, which is clearly impossible. For example, the current case data could have arisen from a small number of infections and a short IPD (predictions will be small) or a large number of infections and a long IPD (predictions will be large). Pick a prediction, and a suitable choice of infection rate and IPD will justify it. In contrast, accurate predictions of AIDS in the UK were possible because the pandemic was asynchronous. In that case, estimates of the IPD were available from cohort studies in which the time of infection of individuals was known or could be imputed, and infection times predated the UK epidemic [e.g. (*6*)].

Several approaches have been adopted to overcome these problems (*1, 2, 7*), but they require that strong assumptions be made. First, universally, the IPD is described as a parametric function. More cautious investigators have used extremely flexible functions (with large numbers of parameters) and have performed sensitivity analyses to extend the range of their predictions. However, any IPD estimate is conditional on the observed data, and the IPD is an (unsupported) extrapolation with no supporting data (*8*). Second, demography curtails the upper end of the prediction range: More than 5 million people have died in the UK since 1990 from non-vCJD causes, but some of them would have been infected.

The author is in the Ecology and Epidemiology Group, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK. E-mail: graham.medley@warwick.ac.uk