the integrin signaling pathway. Their findings point to focal adhesion kinase (FAK) as a critical mediator of integrin signaling in this process. FAK is a nonreceptor tyrosine kinase localized in focal adhesions, which are formed by the interaction of integrins with the extracellular matrix and cytoskeletal proteins. FAK is a major mediator of integrin signaling events that control a variety of cellular processes including cell spreading, migration, survival, and cell cycle progression (*11*). However, Palazzo *et al*. discovered that several known targets of FAK, including paxillin, Cas, and the Src family of kinases, are not involved in the regulation of Rho coupling to mDia and localized microtubule stabilization. It will be interesting to determine which FAK targets are responsible for mediating microtubule stabilization by the integrin-FAK signaling pathway. Likewise, other pathways downstream of integrins but independent of FAK may also be involved. From the plasma membrane perspective, there is the question of whether lipids in the rafts are the only components bound by Rac and Rho. Del Pozo *et al*. suggest that this is unlikely and that other components (perhaps proteins) may also contribute to Rac localization. Investigating events both downstream of integrins and upstream of Rac targeting to the plasma membrane should provide a more complete picture.

The two new studies both clearly show that raft distribution is regulated by integrin signaling, however, neither study examines whether rafts themselves are colocalized with integrins in focal adhesions. Thus, the process of signal propagation from the focal adhesions to the rafts remains to be elucidated, although protein-protein and protein-lipid interactions are likely to be key. Other parts of the puzzle to be resolved include the fate of internalized Rac and Rho in detached cells (for example, whether they remain associated with internalized rafts) and the relation between membrane targeting and activation (by GEFs) or inactivation (by GAPs) of the Rho GTPases. Finally, recent studies suggest that the differential localization of rafts is important in the control of cell migration (*12*–*14*). It will be interesting to determine directly whether the integrin-regulated local coupling of Rac and Rho to their effectors through raft-mediated targeting to the plasma membrane is critical for the control of cell migration.

Although the overall themes and conclusions of both studies are similar (and indeed complement each other), there are important differences between them. Del Pozo *et al*. showed internalization of cholesterol and GPI-linked proteins along with G_{M1} upon cell detachment. However, Palazzo *et al*. found that G_{M1} but not other rafts markers—caveolin 1, cholesterol, or green fluorescent protein fused to a GPI tail—had a differential distribution in FAK-deficient cells versus cells that expressed FAK. G_{M1} is the common marker for lipid rafts, which are a collection of membrane domains of heterogeneous composition and size. This raises the interesting possibility that different types of rafts may be involved in the regulation of Rac and Rho coupling to their effectors. If so, it is possible that signaling pathways other than the integrin-FAK signaling pathway highlighted by Palazzo *et al*. may regulate Rho coupling to mDia and perhaps Rac coupling to PAK. Despite differences in details, these two papers provide new insights into the mechanisms by which integrins control signal transduction in a temporally and spatially restricted manner. Given that other signaling pathways may be affected by lipid rafts (*1*, *2*), integrin regulation of raft distribution in the plasma membrane may enable cell adhesion to modulate other signaling events in a spatially restricted manner.

References

1. D. A. Brown, E. London, *J. Biol. Chem.* **275**, 17221 (2000).
2. K. Simons, D. Toomre, *Nature Rev. Mol. Cell. Biol.* **1**, 31 (2000).
3. M. A. del Pozo *et al.*, *Science* **303**, 839 (2004).
4. A. F. Palazzo, C. H. Eng, D. D. Schlaepfer, E. E. Marcantonio, G. G. Gundersen, *Science* **303**, 836 (2004).
5. R. Hynes, *Cell* **110**, 673 (2002).
6. A. Hall, *Science* **279**, 509 (1998).
7. C. K. Miranti, J. S. Brugge, *Nature Cell Biol.* **4**, E83 (2002).
8. M. A. del Pozo *et al.*, *Nature Cell Biol.* **4**, 232 (2002).
9. M. A. del Pozo *et al.*, *EMBO J.* **19**, 2008 (2000).
10. A. F. Palazzo *et al.*, *Nature Cell Biol.* **3**, 723 (2001).
11. J. T. Parsons, *J. Cell Sci.* **116**, 1409 (2003).
12. S. Manes *et al.*, *EMBO J.* **18**, 6211 (1999).
13. P. K. Ghosh *et al.*, *Nature Cell Biol.* **4**, 894 (2002).
14. L. M. Pierini *et al.*, *J. Biol. Chem.* **278**, 10831 (2003).

GENETICS

Robust Interactions

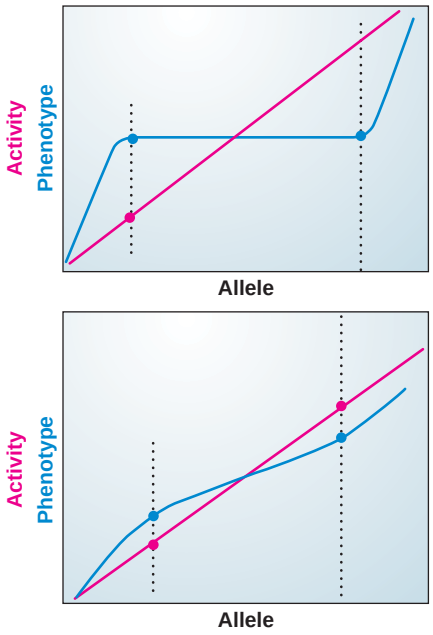
Lee Hartwell

Synthetic lethality in yeast results when two mutations in different genes are each viable as single mutations in the genome but lethal when combined into the same haploid genome. An article by Tong *et al.* (*1*) on page 808 of this issue reports on the beginnings of a global analysis of synthetic lethality in this organism. Why would one care to construct a catalog of all synthetic lethal interactions in yeast? There are two answers. The first relates to the concept of molecular homeostasis—how cells achieve a robustness to perturbations in their environment or in their internal molecular composition. For example, how can a cell with a grossly disturbed genome containing regions of both duplication and deletion not only survive but proliferate, leading to cancer? A second reason is less obvious. It relates to how the enormous genetic variation that exists in outbred populations such as our own manifests itself in phenotypic variation—that is, the relationship of complex genotypes to complex phenotypes. This is a major research focus for the human genome project as it seeks to correlate sequence variation among individuals with health and disease.

Through our ability to manipulate the genes of an organism we have come to appreciate the concept of robustness. Kacser and Burns (*2*) realized that metabolic pathways exhibit robustness if their enzymes follow Michaelis-Menton kinetics and do not become saturated with their substrates. Large decreases in the amount of a particular enzyme in a metabolic pathway have little effect on the flux of substrate through the pathway. Other pathways that incorporate feedback controls (as in, for example, bacterial chemotaxis) are also quite robust to large perturbations in the amounts of their components (*3*). The evolution of this robustness is not difficult to understand because individual cells exhibit strong stochastic differences in the numbers of particular molecules they contain, and their systems must be relatively insensitive to these variations. Thus, when a mutation reduces the function of a component in a system but does not eliminate the component, the system may operate nearly normally.

Tong *et al.* exploited such compromised mutations in their study. However, these researchers also investigated many mutations that were complete gene deletions. The role of such genes in the robustness of the cell is not so obvious. One might imagine that a gene that is "nonessential" under certain laboratory conditions is just not needed in that environment. Surprisingly, this is not correct. In fact, the combination of two single gene deletions, neither of which produces a phenotype on its own, can often produce lethality. Studies in yeast suggest that the majority of "nonessential" genes actually operate under most conditions but are functionally re-

The author is in the Department of Genetics, University of Washington, Seattle, WA 98195, USA. E-mail: lhartwel@fhcrc.org



From complex genotype to complex phenotype. (**Top**) The red line represents the activity in vitro measured for an enzyme encoded by different alleles. The blue line is the phenotype determined by this enzyme in vivo. The robust interval is contained between the dotted lines. (**Bottom**) The robustness of the above example has been reduced by mutation of another gene. Two alleles located at the dashed lines (blue dots) have the same phenotype in the top graph but different phenotypes in the bottom graph.

dundant with other genes in the genome. Of course, genes cannot be completely redundant because natural selection would not maintain two separate genes in the genome doing the same job. Functionally redundant genes are likely to be complementary, contributing to the robustness of the pathway.

In the new work, Tong *et al.* crossed 132 yeast mutants carrying mutations in different query genes with ~4700 yeast mutants carrying viable gene deletions. They then scored the double-mutant progeny for defects in robustness. From their genetic interaction network comprising ~1000 genes and ~4000 interactions, the investigators discovered ~1000 synthetic lethal interactions among the double-mutant progeny. These gene pairs are of interest because they identify interactions that contribute to the robustness of yeast biological pathways. As such, they provide a rich catalog of candidates for future molecular biology studies seeking to understand the biochemical mechanisms of robustness.

These results hold even greater importance for the investigation of complex genetic traits. Numerous studies show that most quantitative traits in outbred populations are genetically very complex. One of the best studies (*4*) detected over 30 loci that contribute to lung cancer susceptibility differences between two inbred strains of mice. This is a surprising degree of complexity given that two inbred mouse strains contain between them the genetic complexity of a single diploid genome in an outbred mouse. Why is there such complexity? One way to think about this is in relation to the robustness of molecular pathways. Owing to robustness, there will be a range of activities for most proteins over which the organism remains insensitive to variations in protein activity (see the figure). If the phenotype is under selection, then genetic variants that accumulate in the population will be contained within this window of robustness; variants outside of this window would be selected against. Variants within the robustness window would increase in numbers in the population and would not cause disease susceptibility in most genetic backgrounds. However, when a mutation that confers reduced protein activity is combined with a mutation reducing robustness, then disease susceptibility may appear (see the figure). According to this model, disease susceptibility would be expected to result from combinations of at least two loci.

In this context, what is highly significant about the results of Tong *et al.* is the number of potential synthetic interactions for the average gene. Of 143 mutant genes tested, they found an average of 34 interactions per mutant gene. For those interested in uncovering the genetic basis of disease susceptibility in the human population, this result is daunting. Genome-wide association studies would be largely unsuccessful, as indeed they have been, and one would need to focus on the right set of gene candidates. Yeast can provide some guidance in selecting the candidates. In a compilation of the synthetic interactions among genes involved in yeast secretion (*5*), we found that half of the interactions for a particular gene were in the local biochemical pathway, one-quarter were in closely related pathways, and one-quarter were in genes of unclear relationship to the primary gene. Moreover, if the catalog begun by Tong *et al.* were completed, then, as they suggest, the candidates for interactions with each yeast gene would be documented, and these could serve as likely candidates for their orthologs in humans.

References

1. A. H. Y. Tong *et al.*, *Science* **303**, 808 (2004).
2. H. Kacser, J. A. Burns, *Genetics* **97**, 639 (1981).
3. U. Alon *et al.*, *Nature* **397**, 168 (1999).
4. N. Tripodis *et al.*, *J. Natl. Cancer Inst.* **93**, 1484 (2001).
5. J. L. Hartman *et al.*, *Science* **291**, 1001 (2001).

CANCER

Respect Thy Neighbor!

Derek C. Radisky and Mina J. Bissell

The complexity of multicellular organisms necessitates a high degree of coordination among a diverse range of specialized cell types. Maintaining this organization requires a constant and dynamic stream of intercellular communication. Increasing evidence suggests that this organized exchange of information is essential for maintaining the differentiated state of cells, and that sustained disruption of key intercellular signaling pathways can predispose to malignancy (*1*). Epithelial tissue is the source of more than 80% of human cancers, and many studies have focused on identifying the factors that activate signaling pathways involved in the proliferation of epithelial cells. The stromal cells that surround and sustain epithelia have been viewed primarily as a source of oxygen, nutrients, and additional growth stimuli for tumors. However, on page 848 of this issue, Bhowmick *et al.* (*2*) report that defective stromal cells stimulate the development of epithelial tumors, which suggests that normal stromal cells may prevent epithelia from becoming tumorigenic. The investigators found that transgenic mice with stromal fibroblasts unable to respond to the cytokine TGF-β (transforming growth factor–β) rapidly developed lethally aggressive cancers derived from the forestomach and prostate epithelium. These results provide insight into the multifaceted roles of TGF-β, and into the larger question of how stromal-epithelial interactions affect the development of epithelial tumors.

It is well established that cellular tumorigenic potential is profoundly influenced by the microenvironment and that malignant cells can be induced to maintain a differentiated state by growth in an appropriate tissue microenvironment [for a review, see (*3*)]. The classic work of Mintz and colleagues showed that injection of undifferentiated embryonal carcinoma cells into mouse blastocysts suppresses their inherent tumorigenicity, allowing these po-

The authors are in the Division of Life Sciences, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720, USA. E-mail: mjbissell@lbl.gov