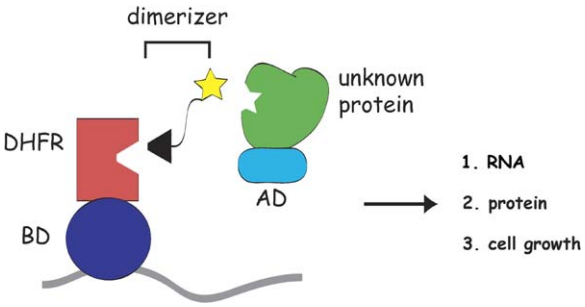

Figure 1. Schematic of a Yeast Three-Hybrid System

Transcription factor DNA binding (BD, dark blue) and activation (AD, light blue) domains are fused to DHFR (red) and an expressed library of proteins (green), respectively. A library of chemical dimerizers comprised of methotrexate (black) and a candidate small molecule (yellow) joined by a linker has the potential to bind to both DHFR and the unknown, target protein. Binding reconstitutes the transcription factor activity leading to expression from a reporter gene promoter (gray) and an observable phenotype. Figure provided by L. Szewczak.

activities (binding affinities and target proteins) will create a fingerprint against which future molecules may be compared. One can envisage the optimization of kinase inhibitors for activity against a specific subset of kinase targets or a particular signaling pathway that has been associated with the biological effect of interest. Importantly, these affinity-based techniques are not limited to compounds that bind to an enzyme active site, but in principle could be extended to allosteric inhibitors and inhibitors of proteins that lack a catalytic function.

Affinity chromatography and Y3H are complementary, since each is subject to different limitations including the use of cell lysate versus intact live cells, the use of mammalian versus yeast cells, and very likely different influences attributable to protein expression levels. Both techniques are limited by the requirement to modify chemically or to immobilize the biologically active probe molecule. Y3H shares an advantage with display cloning, namely that identification of the target protein is linked to identification of the corresponding gene, facilitating its identification and subsequent protein overexpression. The visual read-out from Y3H (cell growth), and its potential for automation at many steps, offers considerable potential for parallel screens against different proteomes, multiple protein classes, or mutants of the same protein. The selection of compounds matching a specific binding profile from a library of potential inhibitors also is conceivable. The future extension of three-hybrid technology to mammalian cells will expand the scope of compounds that can be used, increase the potential for competition assays, as well as provide a more direct relationship to phenotypic data from mammalian cells. As such powerful tools become increasingly available, it no longer will be sufficient to focus on a small number of proteins in evaluating inhibitor selectivity. Chemical tools will have to meet a higher standard of characterization, and we all may have to be more circumspect in our use of the word "selective."

Peter L. Toogood
Pfizer Global Research and Development
Michigan Laboratories
2800 Plymouth Road
Ann Arbor, Michigan 48105

Selected Reading

1. Caligiuri, M., Becker, F., Murthei, K., Kaplan, F., Dedier, S., Kaufmann, C., Machl, A., Zybarth, G., Ricahrd, J., Bockovich, N., et al. (2005). Chem. Biol. *12*, this issue, 1103–1115.
2. Cohen, P. (1999). Curr. Opin. Chem. Biol. *3*, 459–465.
3. Manning, G., Whyte, D.B., Martinez, R., Hunter, T., and Sudarsanam, S. (2002). Science *298*, 1912–1934.
4. Davies, S.P., Reddy, H., Caivano, M., and Cohen, P. (2000). Biochem. J. *351*, 95–105.
5. Bain, J., McLauchlan, H., Elliott, M., and Cohen, P. (2003). Biochem. J. *371*, 199–204.
6. Sche, P.P., McKenzie, K.M., White, J.D., and Austin, D.J. (1999). Chem. Biol. *6*, 707–716.
7. Fabian, M.A., Biggs, W.H., Treiber, D.K., Atteridge, C.E., Azimioara, M.D., Benedetti, M.G., Carter, T.A., Ciceri, P., Edeen, P.T., Floyd, M., et al. (2005). Nat. Biotechnol. *23*, 329–336.
8. Knockaert, M., and Meijer, L. (2002). Biochem. Pharmacol. *64*, 819–825.
9. Daub, H., Godl, K., Brehmer, D., Klebl, B., and Muller, G. (2004). Assay Drug Dev. Technol. *2*, 215–224.
10. Licitra, E.J., and Liu, J.O. (1996). Proc. Natl. Acad. Sci. USA *93*, 12817–12821.
11. Kley, N. (2005). Chem. Biol. *11*, 599–608.
12. Fields, S., and Song, O. (1989). Nature *340*, 245–246.
13. Chien, C.T., Bartel, P., Sternglanz, R., and Fields, S. (1991). Proc. Natl. Acad. Sci. USA *88*, 9578–9582.
14. Becker, F., Murthi, K., Smith, C., Come, J., Costa-Roldán, N., Kaufmann, C., Hanke, U., Degenhart, C., Baumann, S., Wallner, A., et al. (2004). Chem. Biol. *11*, 211–223.
15. Shim, J.S., and Kwon, H.J. (2004). Expert Opin. Ther. Targets *8*, 653–661.
16. Hart, C.P. (2005). Drug Discov. Today *10*, 513–519.

Chemistry & Biology, Vol. 12, October, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.chembiol.2005.10.001

Small Molecules Driving Myotube Fission

In this issue of *Chemistry & Biology*, Duckmanton et al. [1] have rigorously studied myotube fragmentation, or "cellularization," triggered by microtubule-disrupting agents. They convincingly demonstrate that cellularization remains integral to myogenic dedifferentiation, but is insufficient for reentry of the mononucleate progeny into the cell cycle.

For decades, researchers have examined in great detail the mechanisms underlying the spontaneous

dedifferentiation of postmitotic cells into malignant cancer cells in an effort to design ways in which to counter their hyperproliferation. Such efforts have led to the development of drugs that target the mitotic spindle [2] or inhibit proteasomes [3] or alter the covalent modification of small GTP binding proteins (i.e., $p21^{ras}$) [4]. These drugs all share the ultimate goal of interfering with cell cycle progression in these proliferating, pathological cells. The antithesis of this line of investigation is that there exist other pathological states (e.g., tissue degenerative diseases, severe wounding, etc.) where it would be of enormous therapeutic benefit to deliberately reactivate the cell cycle within fully differentiated but quiescent cells in order to bring about tissue regeneration. The discovery and development of small molecules to realize this goal is understandably still in its infancy.

Few models of nonpathological cell dedifferentiation exist in higher organisms, with the process of limb regeneration in the urodele amphibian arguably being the best studied. A deliberate and carefully controlled, but nonetheless poorly understood, program of cell dedifferentiation is utilized by urodele amphibians (i.e., newts and axolotls) to begin regeneration of damaged or missing body structures (e.g., limbs, tail, etc.). Fully differentiated, postmitotic muscle tissue and other tissues adjacent to a wound or amputation are induced to reenter the cell cycle to form a rapidly proliferating mass of undifferentiated cells referred to as the "blastema." Over time, the blastema gradually regenerates the missing body structure. The process of limb regeneration had historically been well studied in urodeles both from a phenomenological [5] as well as an anatomical viewpoint [6]—yet only recently has it been investigated at the cellular and biochemical levels.

Jeremy Brockes and coworkers pioneered a shift in the study of cell dedifferentiation from the organism itself into the more easily manipulated realm of the tissue culture dish. Through use of cultured newt muscle cells and injectable markers, they have previously shown not only that newt myoblasts (muscle cell progenitors) could be differentiated in vitro into multinucleate myotubes [7], but also that these same cultivated newt myotubes could be biochemically labeled and surgically implanted into early newt limb blastema to give rise to mononucleate cells in vivo—within the regenerated limb [8]. The question was posed then as to the role of fragmentation, or cellularization, of the multinucleate myotube in the context of dedifferentiation: was cellularization an early and causal step in the process of cell dedifferentiation, or merely one downstream consequence of an upstream signal for dedifferentiation?

Brockes and coworkers reasoned that since urodele regeneration typically requires wounding and hemostasis [9], thrombin might play a role in activating dedifferentiation. Subsequently, in Tanaka et al. they showed that cultured newt myotubes were stimulated to reenter the cell cycle upon application of thrombin-treated serum [10]. This thrombin-activated dedifferentiating activity was present in the blood serum from many vertebrate species, thereby indicating that certain elements of the regeneration program might yet be preserved in mammals. Efforts continue toward the purification and identification this latent serum factor [11].

Shortly thereafter, McGann et al. [12] were the first to reveal that differentiated *mammalian* myotubes do indeed possess the capability to reenter the cell cycle as well. Treatment of cultured, isolated mouse myotubes with an extract prepared from newt blastemas not only resulted in detectable incorporation of the nucleotide 5-bromo-2′-deoxyuridine (BrdU) into the nuclei of these myotubes, but also stimulated fission of the multinucleated tubes into mononucleate progeny cells. Although the highly specialized and limiting nature of the blastema starting material has made traditional biochemical purification of the responsible factor(s) a daunting endeavor, the demonstration that mammalian myotubes may undergo dedifferentiation and cellularization similar to their amphibian counterparts was very encouraging. The results of these studies involving blastema extract and thrombin-treated serum have spurred the search for small molecules capable of triggering myogenic dedifferentiation in mammalian systems. The first small molecule identified from screens of chemical libraries was a trisubstituted purine that seemed capable of triggering fission of differentiated murine myotubes into actively proliferating myoblasts [13]. "Myoseverin," as it was aptly named, was found to bind tubulin and disintegrate the highly ordered microtubule cytoskeleton within the myotube, resulting in cytokinesis surrounding the nuclei. Initial studies involving myoseverin also reported that these resulting mononucleates were capable of incorporating BrdU, consistent with the notion that they were able to proliferate following fission [13, 14]. This result supported the view that cellularization might be sufficient to trigger myogenic dedifferentiation, a surprising and somewhat unexpected result that is meticulously revisited in the study described herein [1].

In this issue of *Chemistry & Biology* [1], Duckmanton et al. use time-lapse photography of fully differentiated, isolated mouse myotubes to document that myoseverin, as well as nocodozole and their own newly created triazine-based small molecule, can instigate their cellularization into mononucleate cells. These mononucleates are still competent to re-fuse into myotubes upon withdrawal of the microtubule depolymerizer; however, they are incapable of dividing and proliferating as assessed by BrdU incorporation and centriole examination. Microarray analysis of changes in mRNA levels induced by the active molecules and their inactive isomers detected few consensus increases or decreases in gene expression, suggesting that microtubule depolymerizers are insufficient to cause the full program of dedifferentiation.

The innovation of this report is the considered application of existing technologies to more carefully inspect the effects of this class of molecules. The authors have worked extensively with cultured myotubes of both amphibian as well as mammalian lineage, and they recognized that myoblast to myotube fusion is less than quantitative. Thus, in order to obtain an enriched population of differentiated myotubes, the remaining myoblasts were filtered away using nylon microsieves that selectively retain the myotubes. On top of that, the authors relied on time-lapse photography and staining for myosin heavy chain to unambiguously identify mononucleates as progeny of treated myotubes. These

measures prevented the misidentification of contaminating, undifferentiated myoblasts which quite likely contributed to the confusion regarding the activity of microtubule depolymerizing agents in myogenic dedifferentiation.

More recent studies have identified other agents, which seem more certain to induce dedifferentiation in vitro. "Reversine" is a small molecule that can trigger myoblasts, which are lineage committed, to dedifferentiate into more multipotent progenitor-type cells which are capable of being directed to differentiate not only into osteoblasts but adipocytes as well [15]. Such discoveries have not been limited to small molecules: Chen et al. recently showed that ciliary neurotrophic factor (CNTF) can similarly induce myoblasts to adopt a multipotent phenotype capable of redifferentiating into adipocytes, glial, and neuronal cells [16]. It remains to be determined whether reversine and CNTF share a common mechanism or have an effect on the more differentiated myotube. Perhaps a combination of a microtubule depolymerizer and reversine might accomplish that which the former, and agents like it, alone cannot—complete cellularization and dedifferentiation of mammalian myotubes.

John Hines
Department of Molecular, Cellular and Developmental Biology
Yale University
266 Whitney Avenue
New Haven, Connecticut 06511

Selected Reading

1. Duckmanton, A., Kumar, A., Chang, Y.-T., and Brockes, J.P. (2005). Chem. Biol. *12*, this issue, 1117–1126.
2. Knick, V.C., Eberwein, D.J., and Miller, C.G. (1995). J. Natl. Cancer Inst. *87*, 1072–1077.
3. Hanada, M., Sugawara, K., Kaneta, K., Toda, S., Nishiyama, Y., Tomita, K., Yamamoto, H., Konishi, M., and Oki, T. (1992). J. Antibiot. (Tokyo) *45*, 1746–1752.
4. Manne, V., Yan, N., Carboni, J.M., Tuomari, A.V., Ricca, C.S., Brown, J.G., Andahazy, M.L., Schmidt, R.J., Patel, D., Zahler, R., et al. (1995). Oncogene *10*, 1763–1779.
5. Tassava, R.A., and Mescher, A.L. (1975). Differentiation *4*, 23–24.
6. Tank, P.W., Carlson, B.M., and Connelly, T.G. (1976). J. Morphol. *150*, 117–128.
7. Ferretti, P., and Brockes, J.P. (1988). J. Exp. Zool. *247*, 77–91.
8. Lo, D.C., Allen, F., and Brockes, J.P. (1993). Proc. Natl. Acad. Sci. USA *90*, 7230–7234.
9. Tassava, R.A., and Loyd, R.M. (1977). Nature *268*, 49–50.
10. Tanaka, E.M., Drechsel, D.N., and Brockes, J.P. (1999). Curr. Biol. *9*, 792–799.
11. Straube, W.L., Brockes, J.P., Drechsel, D.N., and Tanaka, E.M. (2004). Cloning Stem Cells *6*, 333–344.
12. McGann, C.J., Odelberg, S.J., and Keating, M.T. (2001). Proc. Natl. Acad. Sci. USA *98*, 13699–13704.
13. Rosania, G.R., Chang, Y.-T., Perez, O., Sutherlin, D., Dong, H., Lockhart, D.J., and Schultz, P.G. (2000). Nat. Biotechnol. *18*, 304–308.
14. Perez, O.D., Chang, Y.-T., Rosania, G., Sutherlin, D., and Schultz, P.G. (2002). Chem. Biol. *9*, 475–483.
15. Chen, S., Zhang, Q., Wu, X., Schultz, P.G., and Ding, S. (2003). J. Am. Chem. Soc. *126*, 410–411.
16. Chen, X., Mao, Z., Liu, S., Liu, H., Wang, X., Wu, H., Wu, Y., Zhao, T., Fan, W., Li, Y., et al. (2005). Mol. Biol. Cell *16*, 3140–3151.

Chemistry & Biology, Vol. 12, October, 2005, DOI 10.1016/j.chembiol.2005.10.002

The Stereochemistry of Ketoreduction

In this issue of *Chemistry & Biology*, Leadlay and coworkers [1] report overproduction of a number of ketoreductase domains from modular polyketide synthases. These discrete enzymes allow the stereochemistry of polyketide ketoreduction to be studied in isolation.

The well-studied 6-deoxyerythronolide B synthase (DEBS) catalyzes formation of the macrolactone core of erythromycin [2]. DEBS contains a module for each of the six cycles in the chain assembly process. This modular organization is found in many other important polyketide synthases (PKSs) [3]. In a typical module, an acyl transferase (AT) loads an extender unit onto the phosphopantetheine thiol of an acyl carrier protein (ACP). The extender condenses with an acyl chain that is thioester-linked to the active site cysteine of a ketosynthase (KS). The resulting β-ketoacyl-ACP may be reduced to a β-hydroxyacyl intermediate by a ketoreductase (KR) and may be processed further by dehydratase and enoyl reductase enzymes. An entire PKS consists of a series of modules that are housed within large multienzyme polypeptides. A frequently occurring module catalyzes the incorporation of propionate, and reduction of the β-ketone group to an alcohol. The 2-methyl-3-hydroxyacyl thioester product has two new chiral centers. All four combinations of methyl and alcohol stereochemistry [(2*R*, 3*S*), (2*S*, 3*R*), (2*R*, 3*R*), (2*S*, 3*S*)] can appear in nascent polyketide chains. It is unclear how these different stereochemical outcomes are achieved by PKS modules of apparently similar domain composition and sequence.

Detailed studies on DEBS and truncated derivatives have given deep insights into the stereochemistry of polyketide chain extension. All six modules use (2*S*)-methylmalonyl-CoA as a source of activated propionyl extender units [4]. In the cycle catalyzed by DEBS module 2, condensation proceeds with inversion of stereochemistry, so that the initial product is (2*R*)-2-methyl-3-ketoacyl-ACP2 [5]. With DEBS module 1, the