that the decrease in differentiation efficiency due to genotoxic stress is fully reversible, and is not caused by programmed cell death of treated myoblasts.

This extremely interesting work raises many questions and opens an entirely new direction of research. So far, it appears that the differentiation checkpoint is MyoD dependent, and is either activated by the cell cycle checkpoint or is independently induced by DNA damage. The article does not exclude either of these possibilities. However, the article shows that the growth arrest of myoblasts, caused by genotoxic agents, induces the expression of p53, whereas the differentiation checkpoint itself is p53 independent. Moreover, the c-Abl kinase, which is typically inactive in the early G1/G0 cells, in the presence of active Rb (Liu et al., 1996), appears to be the key regulator of the myogenic differentiation checkpoint. These data argue for the differentiation checkpoint mechanism to be independent of the cell cycle, and indeed to become activated in cells that have already entered into the early differentiation program (see Figure). Elucidation of these points is very important, as well as validation of these findings in primary cells. In many cases, the primary cells and tissue precursors in vivo show patterns of proliferation and differentiation that are very different from established cell lines.

The significance of this work is that it offers a novel concept of how cells control the integrity of genetic information prior to terminal differentiation. It remains to be seen whether the analogous checkpoints are regulating other differentiation processes, including osteogenesis, hematopoesis, and adipogenesis. Many tissues appear to possess populations of primitive precursor cells, also termed adult stem cells (Orkin and Zon, 2002; Seale et al., 2001), which potentially participate in tissue maintenance and repair. It is important to find out whether differentiation checkpoints exist in early tissue-specific progenitors, and regulate their developmental progression. Similarly, committed precursors such as satellite cells in adult muscle may manifest similar checkpoints regulating their commitment and differentiation. Conceivably, this mechanism is involved in the formation of tumors from undifferentiated precursors, and it is very interesting to speculate whether the poor differentiation of rhabdomyosarcomas reflects the inappropriate activation of the differentiation checkpoint.

Anna Polesskaya and Michael A. Rudnicki
Ottawa Health Research Institute
501 Smyth Road
Ottawa, Ontario K1H 8L6
Canada

Selected Reading

Liu, Z.G., Baskaran, R., Lea-Chou, E.T., Wood, L.D., Chen, Y., Karin, M., and Wang, J.Y. (1996). Nature *384*, 273–276.

Orkin, S.H., and Zon, L.I. (2002). Nat. Immunol. *3*, 323–328.

Puri, P.L., Bhakta, K., Wood, L.D., Costanzo, A., Zhu, J., and Wang, J.Y. (2002). Nat. Genet. Published online November 4, 2002. 10.1038/ng1023.

Sabourin, L.A., and Rudnicki, M.A. (2000). Clin. Genet. *57*, 16–25.

Seale, P., Asakura, A., and Rudnicki, M.A. (2001). The potential of muscle stem cells. Dev. Cell *1*, 333–342.

Wang, J.Y. (2000). Oncogene *19*, 5643–5650.

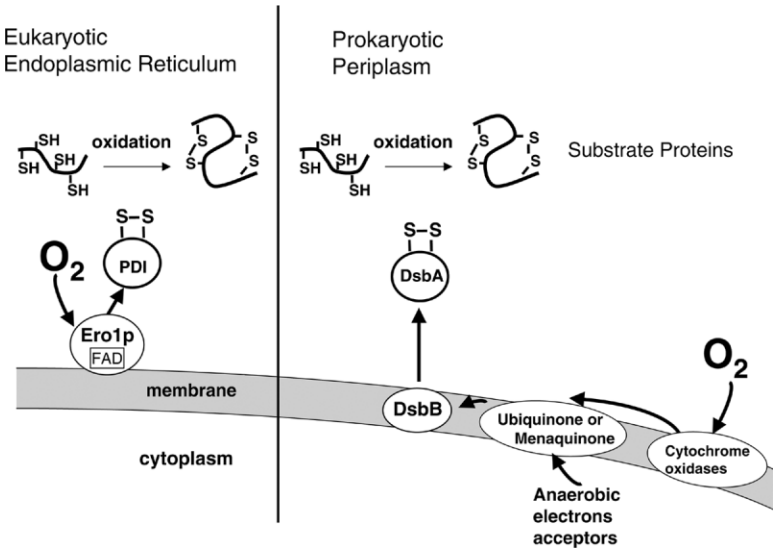
Disulfide Bond Formation, a Race between FAD and Oxygen

The long-running race to find the source of oxidizing potential for disulfide bond formation is over. The winner is one of the first contestants to enter: oxygen.

Disulfide bonds are so important for protein folding that their reduction will often cause proteins to unfold. An initial idea was that disulfide bond formation is a spontaneous process that requires only the presence of oxygen and perhaps an intermediary such as a flavin or metal to speed the process. A flurry of papers (reviewed by Sevier and Kaiser, 2002) reported instead that pathways of enzymes are responsible for the formation of disulfides and their transfer to folding proteins. These enzymes function in the endoplasmic reticulum of eukaryotes and in the periplasm of prokaryotes. Much of this work has focused on disulfide exchange reactions, which neither generate nor destroy disulfides. Recent work has started to illuminate the crucial step where disulfides are created de novo. Ironically, this has brought us full circle; it now appears that oxygen and flavins do indeed play important roles in the formation of disulfide bonds.

In the November issue of *Molecular Cell*, Tu and Weissman (2002) have shown that Ero1p, the key enzyme in eukaryotes that generates disulfides, does so by coupling disulfide bond formation directly to the consumption of oxygen in a FAD-dependent reaction. This is a very satisfying result. Ero1p had previously been shown to oxidize protein disulfide isomerase, which in turn oxidizes folding proteins in the endoplasmic reticulum of eukaryotes (reviewed by Sevier and Kaiser, 2002). Ero1p had also been shown to be a FAD-dependent enzyme (Tu et al., 2000).

The race to discover the primary source of oxidizing power began in the early 1960s when it was realized that disulfide bond formation required an electron acceptor in vivo. The first candidate to enter this race was molecular oxygen. Glutathione became a strong contender in 1992, when high levels of oxidized glutathione were detected in the endoplasmic reticulum (Hwang et al., 1992). It remained a favorite candidate until it was suddenly eliminated from competition (Cuozzo and Kaiser, 1999). The colorful flavin FAD entered the race at a very late point (Tu et al., 2000), making a very strong showing. The dependence of Ero1p-catalyzed oxidative folding on excess FAD led to the impression that FAD may become reduced to $FADH_2$ in Ero1p's catalytic cycle. Disulfide bond formation would then consume free FAD. However this was very surprising, as FAD normally functions as a tightly bound cofactor, not as an enzymatic substrate (Massey, 2000). Oxygen, the original contestant, was back up and running strong, with the



O_2 Is the Source of Oxidizing Power for Disulfide Bond Formation in Both Prokaryotes and Eukaryotes

In the eukaryotic endoplasmic reticulum, protein disulfide isomerase (PDI) is the direct donor of disulfides to newly secreted proteins. PDI is kept in an oxidized state by Ero1p, a FAD-containing, membrane-associated protein. Ero1p is then oxidized directly by molecular oxygen. In prokaryotes, DsbA is the direct donor of disulfides to periplasmic proteins, and it is reoxidized by the inner membrane protein DsbB. DsbB is a quinone reductase. Under aerobic conditions it is oxidized by ubiquinone, which is reoxidized by cytochrome oxidases, which in turn are oxidized by molecular oxygen. Under anaerobic conditions, DsbB is reoxidized by menaquinone, which is reoxidized by a variety of anaerobic electron acceptors.

observation that overproduction of the sulfhydryl oxidase Erv2p could complement the *ero1-1* mutant (Sevier et al., 2001). Sulfhydryl oxidases contain FAD as a tightly bound cofactor. They generate disulfides by the conversion of oxygen to hydrogen peroxide (Thorpe et al., 2002). Thus, for at least one pathway of disulfide bond formation, molecular oxygen is the direct source of oxidizing power (Sevier et al., 2001). However, it is unlikely that Erv2p is the chief source of oxidative power for disulfide bond formation in the endoplasmic reticulum—Ero1p is a much stronger contender. For Ero1p, it remained unclear whether oxygen was the main source of oxidizing power, and how the dependence of Ero1p on added FAD could be explained.

Tu and Weissman's (2002) demonstration that oxygen is consumed during Ero1p-catalyzed oxidation of RNaseA in vitro is convincing evidence that molecular oxygen can serve as a terminal electron acceptor for Ero1p. Must oxygen serve the role of terminal electron acceptor for Ero1p? Here the judges have still not decided. Yeast grows and appears to form disulfide bonds anaerobically, suggesting that an alternate electron acceptor may function under these conditions. The fact that Ero1p is essential even under anaerobic growth conditions argues that Ero1p can use alternate electron acceptors and that there probably is not an important Ero1p-independent system for anaerobic ER oxidation.

If Ero1p consumes oxygen but not FAD, then how can we explain the dependence on FAD levels for Ero1p function? Ero1p is a FAD-dependent enzyme, but unlike most FAD-dependent oxidases, seems exquisitely sensitive to physiological FAD levels. Since FAD remains tightly bound to Ero1p, Tu and Weissman (2002) postulate a second, weaker affinity FAD binding site that could function as a sensor to allow regulation of oxidative folding in response to cellular FAD levels. The function of this intriguing regulation is unclear, as very little is known about how the levels of free FAD vary. However, it may provide a way for the cell to modulate the rate of disulfide bond formation in response to metabolic cues.

The situation in prokaryotes has many parallels with that in eukaryotes (reviewed by Sevier and Kaiser, 2002), but important differences (see Figure). The protein DsbA directly oxidizes folding proteins in a disulfide exchange reaction that leaves DsbA reduced (Bardwell et al., 1991). DsbA and PDI are functionally and structurally similar. DsbB reoxidizes DsbA. Like Ero1p, DsbB is membrane associated. Here, however, the similarities of the two systems appear to diverge. DsbB is a quinone reductase that uses the oxidizing power of quinones to generate disulfides de novo (Bader et al., 1999). It is not FAD dependent, and does not directly consume oxygen. Under aerobic conditions, the reduced quinones are reoxidized by cytochrome oxidases, which in turn are oxidized by molecular oxygen. This makes disulfide bond formation also an oxygen-consuming process in prokaryotes, aerobically. When oxygen is absent, prokaryotes utilize a backup system where the final electron acceptor can be a number of small molecules, including fumarate. It is unclear whether an anaerobic backup system for disulfide bond formation exists in eukaryotes.

Thus, as the final electron acceptor for disulfide bond formation, oxygen, when available, comes out as the overall winner in all walks of life. This represents a pleasing return to the original idea.

James C.A. Bardwell
Department of Molecular, Cellular,
and Developmental Biology
University of Michigan
Ann Arbor, Michigan 48109

Selected Reading

Bader, M., Muse, W., Ballou, D.P., Gassner, C., and Bardwell, J.C.A. (1999). Cell *98*, 217–227.

Bardwell, J.C.A., McGovern, K., and Beckwith, J. (1991). Cell *67*, 581–589.

Cuozzo, J.W., and Kaiser, C.A. (1999). Nat. Cell Biol. *1*, 130–135.

Hwang, C., Sinskey, A.J., and Lodish, H.F. (1992). Science *258*, 1496–1502.

Massey, V. (2000). Biochem. Soc. Trans. *28*, 283–296.

Sevier, S., and Kaiser, C.A. (2002). Nat. Rev. Mol. Cell. Biol. *3*, 836–847.

Sevier, C.S., Cuozzo, J.W., Vala, A., Aslund, F., and Kaiser, C.A. (2001). Nat. Cell Biol. *3*, 874–882.

Thorpe, C., Hoober, K.L., Raje, S., Glynn, N.M., Burnside, J., Turi, G.K., and Coppodk, D.L. (2002). Arch. Biochem. Biophys. *405*, 1–12.

Tu, B.P., and Weissman, J.S. (2002). Mol. Cell *10*, 983–994.

Tu, B.P., Ho-Schleyer, S.C., Travers, K.J., and Weissman, J.S. (2000). Science *290*, 1571–1574.

The Web and the Rock: Cell Adhesion and the ARP2/3 Complex

Cell locomotion entails functional and structural cooperation between cell surface adhesion and the actin cytoskeleton. A new paper by DeMali et al. provides new insights into the link between actin assembly and integrin adhesion at the leading edges of migrating cells.

Morphogenetic cell movements take many forms. In addition to the commonly discussed translocation of individual cells or whole groups of cells, closely related phenomena include neurite outgrowth (where cell extensions grow without net movement of cell bodies) and the cell upon cell extension that occurs during compaction or epithelial recognition. All these events, though, have a common requirement for intracellular force to be productively coupled to the external environment.

A key step in locomotion is forward extension of the cell margin. In the common case of cells migrating on extracellular matrices, this occurs at the very leading edges of organelles called lamellipodia. Leading edge extension is now commonly acknowledged to rely upon actin assembly driven by the Arp2/3 actin nucleator complex (Pollard et al., 2000). The Arp2/3 complex concentrates at leading edges, where it catalyzes the growth of branched actin networks that are believed to provide the protrusive force for leading edge extension.

However, it has long been recognized that productive leading edge extension also requires that protrusion be coupled to cell adhesion (Mitchison and Cramer, 1996). Without effective adhesion, forward movements of the leading edge would fold back upon themselves, leading to ineffectual ruffling of the cell surface. Indeed, integrin receptors are found at the very leading edges of locomoting cells (e.g., Kiosses et al., 2001), but these have generally been envisaged to serve a passive role, stabilizing newly formed leading edges and serving as precursors for more proximal sites of strong cell-substrate adhesion (focal adhesions).

The interesting new study from DeMali et al. (2002) in *The Journal of Cell Biology* reveals that integrins exert a much more active influence on the actin cytoskeleton than was previously appreciated. In this work, the authors identified a regulated interaction between the Arp2/3 complex and vinculin, a peripheral membrane component of integrin adhesion complexes. Vinculin binds F-actin and is often considered to allow integrins to scaffold onto preformed actin filaments. DeMali et al. now show that vinculin can associate directly with Arp 2/3, via a region of the vinculin molecule that is distinct from the actin binding site. The interaction was dynamic and appeared to principally involve subpopulations of both Arp2/3 and vinculin that localized to the very leading edges of lamellipodia. Interestingly, Arp 2/3 was not seen with vinculin in older, mature focal adhesions, suggesting that the integrin contacts at leading edges might be functionally distinct from mature focal adhesions (Kiosses et al., 2001). Importantly, ablation of the Arp2/3 binding site of vinculin significantly reduced the amount of Arp2/3 that was recruited to newly forming integrin contacts and impaired the ability of cells to form lamellipodia. Although the authors did not directly test the effects of vinculin on actin assembly per se, Arp2/3 localization at the periphery tends to be a reasonably faithful reporter of actin assembly (Schafer et al., 1998). Thus, the current paper suggests that vinculin can serve to mark sites for actin assembly to occur at newly forming integrin contacts.

What functional purpose might be served by such vinculin-dependent recruitment of Arp2/3? The most attractive general explanation is that this interaction contributes to efficient leading edge protrusion, an outcome that could occur by several potential mechanisms. First, direct recruitment of Arp2/3 to newly forming integrin contacts is predicted to concentrate the actin assembly apparatus, thereby allowing actin assembly to be efficiently directed toward forward protrusion of the leading edge. However, vinculin is unlikely to be solely responsible for recruiting Arp2/3 to leading edges. Instead, it is more likely that vinculin cooperates with other signals and linker proteins to promote fidelity of Arp2/3 localization in cells (Pollard et al., 2000).

Second, the association between Arp2/3 and vinculin would support direct mechanical coupling between actin assembly and cell surface adhesion. Not only would this allow immediate stabilization of nascent cell protrusions, but, perhaps more interestingly, it provides the opportunity for integrin adhesions themselves to serve as platforms for efficient force generation. For cell surface protrusion to occur, the forces generated by barbed-end actin assembly must overcome the resistance of the plasma membrane, which itself tends to drive actin filaments backward. Therefore, immobilization of Arp2/3 by vinculin in newly forming integrin contacts would provide traction, preventing backward movement of actin meshworks, thus allowing force generation to more efficiently overcome the resistance of the plasma membrane.

Finally, Arp2/3 is not the only determinant of actin assembly that is involved in leading edge extension. Notably, proteins of the ena/VASP family modulate post-nucleation actin filament growth and influence both leading edge extension and cell locomotion (Bear et al., 2002). Therefore, it is noteworthy that vinculin can also bind VASP, which DeMali et al. show to occur independently of Arp2/3 binding. Vinculin may therefore serve as a scaffold, coordinating the action of several key determinants of actin assembly and leading edge extension.

A key observation in the current paper is that the