Research Roundup

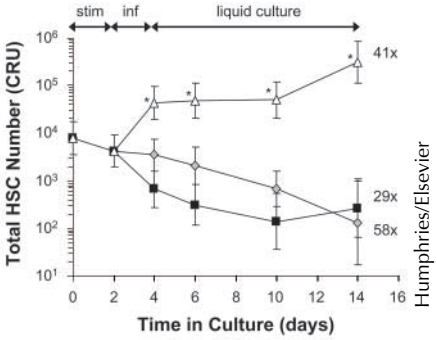
Blood cells expand their numbers

Scientists in the field of blood cell development have found a protein of expansive abilities. According to two new papers, HoxB4 is able to promote self-renewal of hematopoietic stem cells (HSCs) both in vivo and in vitro.

HoxB4 is a homeotic gene whose expression in vivo has been shown previously to enhance HSC regeneration. Now, Jennifer Antonchuk, Guy Sauvageau, and Keith Humphries (Terry Fox Laboratory, Vancouver, BC) demonstrate that the number of adult HSC can be expanded in vitro when bone marrow cells are transduced with *HoxB4*. Compared with untransfected cells, the HoxB4-expressing cells showed a 40-fold net increase after 14 d in culture.

"So far, this [method] stands out uniquely as a means to expand stem cells," says Humphries. "The stem cells are normal in terms of being able to differentiate, and still have a potent ability to regenerate stem cell compartments," as shown by the ability of the in vitro–expanded cells to engraft in irradiated mice. The use of HoxB4-expressing cells could help prevent significant losses of the important HSCs in culture during gene therapy. Additionally, Humphries hopes the technique will improve treatments for the effects of chemotherapy and radiotherapy, by increasing the number of a patient's own stem cells that can be used for transplantation.

HoxB4 is also able to induce embryonic cells to become HSCs, according to a second publication by Michael Kyba, Rita Perlingeiro, and George Daley (Whitehead Institute for Biomedical Research, Cambridge, MA). Expression of *HoxB4* in yolk sac cells or in embryonic stem cells led to the differentiation of these cells into mature blood cells. Additionally, the HSCs generated in vitro were able to engraft in both primary and secondary recipients. The signal transduction pathways induced by HoxB4 that lead to HSC expansion remain to be determined. ■



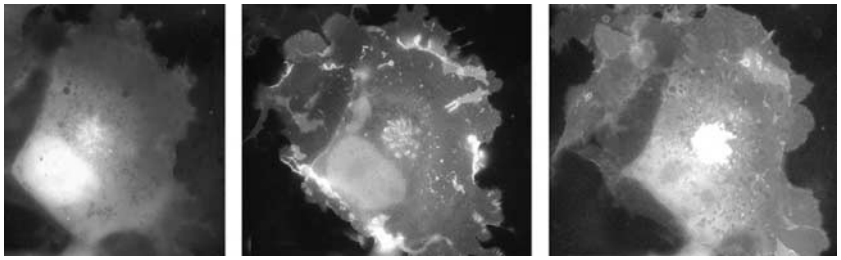
HSCs expressing HoxB4 (triangles) increase in number.

References: Antonchuk, J., et al. 2002. *Cell.* 109:39–45.
Kyba, M., et al. 2002. *Cell.* 109:29–37.

Ras on the inside

The plasma membrane has been considered the exclusive platform from which Ras GTPases signal. But recent results from Vi Chiu, Trever Bivona, Mark Philips (New York University School of Medicine, New York, NY), and colleagues demonstrate that Ras proteins are also active on internal membranes.

Ras GTPases are molecular switches that transduce mitogenic signals by activating downstream effectors, particularly in the MAPK pathway, when GTP is bound. Previous results have led to the belief that Ras is only active when bound to the cytosolic face of the plasma membrane. Several isoforms of human Ras exist, including K-Ras, N-Ras, and H-Ras, but the biological need for this diversity has been unclear. "If all three lead to MAPK activation, why have so many different isoforms?" says Philips. It has been hypothesized that the isoforms are alternatively regulated based on their differential localization within specific microdomains of the plasma membrane. Now, there may be an additional explanation—subcellular localization to specific internal membranes.



Ras (white) is activated on both the plasma membrane and the Golgi (left to right).

Philips' group used fluorescent-tagged reporters to reveal the distribution of active Ras within cells. They showed that mitogens can activate H-Ras and N-Ras on the ER and Golgi as well as on the plasma membrane. K-Ras, however, was active only on the plasma membrane. Ras constructs targeted to different membrane compartments activate specific downstream signals based on their subcellular localization. Although Golgi-localized H-Ras strongly activates Erk and Akt, H-Ras on the ER more efficiently activates Jnk. These differences may allow therapeutic intervention targeted to specific isoforms, such as K-Ras, the most common isoform affected in lung and colon cancers.

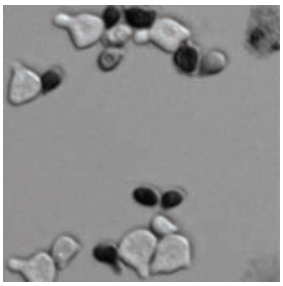
Since vesicular transport is not necessary for mitogens to activate internal membrane-associated Ras, a signal must diffuse from the plasma membrane to the Golgi or ER. Philips hypothesizes that the signal may be a guanine nucleotide exchange factor that either moves from the plasma membrane or is activated at the ER or Golgi in response to a diffusible second messenger, such as Ca^{2+}. ■

Reference: Chiu, V., et al. 2002. *Nat. Cell Biol.* 10.1038/ncb783.

Text By Nicole LeBrasseur
lebrasn@rockefeller.edu

Suicidal yeast

Programmed cell death (PCD) in multicellular eukaryotes is a form of altruism, in which one cell activates its own death for the greater benefit of the organism. But it is more difficult to envision a benefit for a single-celled microbe. Now, Fedor Severin and Anthony Hyman (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany) demonstrate that PCD is indeed a normal physiological process for yeast cells during mating.



Severin/Elsevier

Yeast cells self-destruct (stained cells) when they cannot mate.

Mating in yeast is initiated by *a* and α mating pheromones. High levels of, or prolonged exposure to, the mating factor is toxic to the cells. The group's results now show that the toxicity is due to the onset of PCD. Exogenous addition of α factor to *a* cells induced several markers of PCD, including production of reactive oxygen species and initiation of DNA degradation, in ~30% of *a* cells. The cell death was also sensitive to an inhibitor of apoptosis.

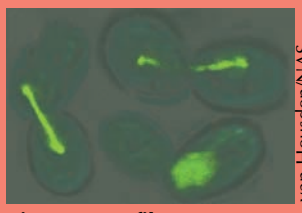
PCD also occurred in native conditions with mixed *a* and α cells. Successful mating prevented mating factor-induced cell death, indicating that yeast may use the process to weed out old or otherwise damaged cells. "When yeast cells mate, this is the only moment in time when [they] show communal behavior," says Severin. "If a particular cell is surrounded by mating partners and still cannot mate, it determines that it is weak, and for the sake of the community, the cell commits suicide." ■

Reference: Severin, F., and A. Hyman. 2002. *Curr. Biol.* 12:R233–R235.

A new member joins the yeast filament party

A novel yeast filament protein has been found. Martijn van Hemert, Paul van Heusden (Leiden University, Leiden, Netherlands), and colleagues have identified Fin1, which forms a filament only at a specific point in the cell cycle.

The group identified Fin1 in a screen for interaction partners of the 14-3-3 family of regulatory proteins. In nondividing cells, Fin1 is undetectable, whereas in small budded cells it localizes to the nucleus. As the cell cycle progresses, Fin1 forms a filament between the nuclei of dividing cells, extending between the spindle pole bodies. Fin1 also forms an intermediate filament in vitro and seems to act independently of tubulin. "It is the first time that someone has found a filament between spindle poles that is different from microtubules," says van Heusden.



van Heusden/NAS

Fin1 (green) filaments span the nuclei of dividing cells.

The only other known yeast intermediate filament is the Mdm1 filament involved in mitochondrion inheritance. The function of Fin1 is unknown, as the *fin1* mutant is viable. van Heusden speculates that the filament may organize proteins in the nucleus during cell division, and he plans to identify synthetic lethal mutants to address this possibility.

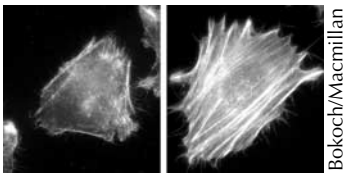
Unlike mutation of *Fin1*, protein overexpression is lethal in haploid cells. The filamentous structures that develop in these cells and their detrimental effects on cell growth resemble the accumulation of the tau protein in Alzheimer's disease. Fin1 may therefore provide a model for the study of tau filament formation. ■

Reference: van Hemert, M., et al. 2002. *Proc. Natl. Acad. Sci. USA.* 10.1073/pnas.072556099.

Exchange is necessary for migration

Mira Krendel, Frank Zenke, and Gary Bokoch (Scripps Research Institute, La Jolla, CA) have identified a bridge between microtubule assembly and actin polymerization that links these processes during cell migration.

During migration, microtubule polymerization at the leading edge leads to increased Rac GTPase activity and the formation of lamellipodia via actin polymerization. Conversely, microtubule depolymerization in the cell body activates Rho and leads to myosin contractility. Now, it seems that a guanine nucleotide exchange factor (GEF) is "the missing link between microtubule activation and RhoGTPase regulation of the actin cytoskeleton," according to Bokoch.



Bokoch/Macmillan

Free GEF-H1 (right) promotes actin stress fiber formation.

Bokoch found that GEF-H1 activates Rho GTPase, but only when the exchange factor is not associated with microtubules. Disassembly of microtubules in the cell body of a migrating cell frees GEF-H1, which activates Rho to promote myosin contractility and actin stress fiber formation. The assembly of microtubules at the leading edge leads to low GEF-H1 activity, and therefore low Rho activity, allowing lamellipodial assembly.

GEF-H1 exchange activity is specific to Rho. Bokoch speculates, however, that a similar mechanism may regulate Rac activity. A different exchange factor could be activated by microtubule binding, up-regulating Rac at leading edge of the cell.

According to Bokoch, microtubule-regulated GEFs could be involved in more than just cell motility. They may promote growth cone stability during axonal pathfinding, or regulate Rho activity and actin assembly during cell division, when microtubules direct positioning of the division furrow. ■

Reference: Krendel, M., et al. 2002. *Nat. Cell Biol.* 4:294–301.