

Research Roundup

All skin and brain

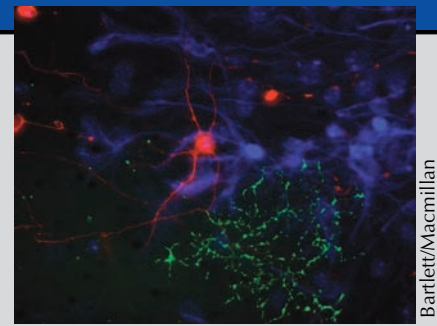
A multipotent skin-derived stem cell, and a purified neural stem cell from the brain have come on the scene at a delicate time in the debate over embryonic stem cell research. Each success in isolating adult stem cells, such as these cells, gives ammunition to opponents of embryonic stem cell research, who point to adult-derived stem cells as an alternative source of transplant-ready material.

The skin cells come from the work of Freda Miller and colleagues at McGill University, Montreal, Canada. Miller was prompted to look for neural precursors in skin based on the fact that certain mechanosensing neurons appear to be born in the skin. She relied on a protocol

used to isolate neural stem cells from the brain, which involves purifying floating clusters (or spheres) of cells away from adherent cells. The purified spheres yielded various cells with characteristics of neurons, glia, smooth muscle cells, and adipocytes, depending on the nature of the added serum. The same range of cell types resulted when single cells were first isolated and grown up before the addition of serum.

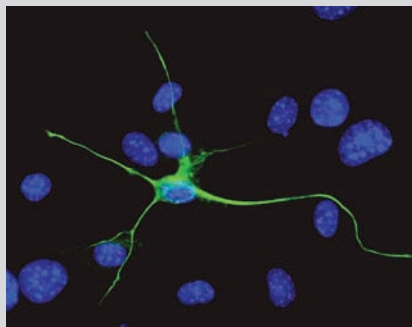
The brain neural stem cells come from Perry Bartlett's group at the Walter and Eliza Hall Institute (Parkville, Australia), after a search in two areas of the brain previously implicated in neural stem cell production. Sorting on the basis of the cells' ability to form neurospheres, they found a cell size and marker phenotype that includes 0.27% of the original cells, but within which 80% of the cells can generate neurospheres. All clones can form neurons, oligodendrocytes, astrocytes, and, when cocultured with a muscle cell line, myocytes and myotubes. Thus, the two studies are in agreement that a single adult stem cell can generate both muscle and neural cells.

For therapeutic purposes, Bartlett's cells can be purified but are still inaccessible. Miller, however, managed to get



Neurons (red), astrocytes (blue), and oligodendrocytes (green) from a single purified stem cell.

Bartlett/Macmillan



Miller/Macmillan

Sensitive skin: nerve cells from a skin stem cell.

significant numbers of her skin-derived stem cells from human scalp. "The fact that we can get anything at all from the tiny scalp samples is very encouraging," she says.

As adult stem cells get purer and reveal greater plasticity, the unique claims of embryonic stem cells to pluripotency (the ability to form all cell types) are being whittled away. But Miller is not ready to declare victory yet. "I think adult stem cells will certainly be a viable option for certain kinds of diseases," she says. "The question is how broad that will be. We are working like crazy to ask how flexible [these stem cells] can be." ■

References: Toma, J.G., et al. 2001. *Nat. Cell Biol.* 3:778–784.

Rietze, R.L., et al. 2001. *Nature.* 412:736–739.

An organelle knockout

By knocking out a single gene for caveolin-1, Teymuras Kurzchalia (Max Planck Institute, Dresden, Germany) and colleagues have ablated a special subset of lipid rafts called caveolae. The resulting mice survive, perhaps surprisingly, but show alterations in signaling that affect cell contractility and proliferation.

Caveolin-1 is the major protein in caveolae, and its oligomerization and cholesterol binding may help form these flask-shaped invaginations. Caveolae have been implicated in both transcytosis and signaling, but the knockout mice seem to retain functional transcytosis in endothelial cells that now lack caveolae. "In the beginning I thought transport through the endothelium would be totally destroyed," says Kurzchalia. "After almost 50 years, this hypothesis needs to be fundamentally revised."

Vasoconstriction and myogenic tone are dysregulated in the



Swimming is a problem despite remaining lipid rafts.

Kurzchalia/AAAS

knockout mice, probably as a result of hyperactive endothelial nitric oxide synthase (eNOS). Kurzchalia suspects that caveolae function as a dumping ground for various signaling molecules, including eNOS, which arrive from other raft systems and are then inactivated upon arrival. Another substrate for this inhibition pathway is inducible NOS (iNOS). Its hyperactivation results in permanent erections for the unfortunate male knockout mice.

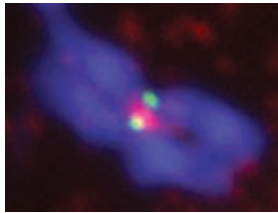
Dysregulation of other signaling pathways may explain the hyperproliferation and fibrosis seen in the lungs of the knockout mice, which leads to poor performance in swimming endurance tests. Kurzchalia hopes to identify the relevant signaling pathways by studying the proliferation of knockout cells in culture. ■

Reference: Drab, M., et al. 2001. *Science.* 10.1126/science.1062688, <http://www.sciencemag.org/cgi/content/abstract/1062688>.

Cohesin's cohesion

Cohesin was a long sought-after protein complex: a glue for sister chromatids that is dissolved at the onset of anaphase thanks to cleavage of its SCC1 subunit by the protease separase. In budding yeast, cohesin lived up to its billing as a complex that was destroyed at anaphase onset.

But in human cells its behavior was less convincing. The vast majority of cohesin comes off human chromosomes in prophase and prometaphase, coincident with the dissociation of sister chromatid arms. Only after a hard look is it apparent that a little cohesin is left at the centromere. Now Jan-Michael Peters and colleagues (Research Institute of Molecular Pathology (IMP), Vienna, Austria) have used a noncleavable SCC1 to



Peters/AAAS

Cohesin (red) keeps centromeres together.

show that cleavage of this residual SCC1 is indeed required for correct chromosome segregation and cytokinesis.

"Because so little SCC1 is cleaved in human cells it was unclear whether this would be of physiological relevance," says first author Silke Hauf. Yet the noncleavable SCC1 induces many errors in chromosome segregation, causes many cleavage furrows to regress, and results in the formation of micronuclei and polyploid cells. Based on the aftermath of the aberrant mitoses, faithful sister chromatid separation is not necessary for mitotic exit or DNA re-replication.

Frank Uhlmann's group (Imperial Cancer Research Fund (ICRF), London, UK), meanwhile, has shown that in yeast separase is doing more than cleaving SCC1. Separase is also cleaving a protein called Slk19, whose cleaved form is necessary to stabilize the anaphase spindle. Uhlmann suggests that the absence of cleaved Slk9 is one reason why triggering anaphase by artificial cleavage of SCC1 results in an unstable spindle. ■

References: Hauf, S., et al. 2001. *Science*. 293:1320–1323.
Sullivan, M., et al. 2001. *Nat. Cell Biol.* 3:771–777.

Rafts to the front; rafts to the rear

T cells assemble different rafts at the front and rear of the cell as they gear up to move. This is the conclusion of Santos Mañes (Universidad Autónoma de Madrid, Spain) and colleagues, who believe that the distinct rafts help T cells to do two very different things at the front and back of the cell.

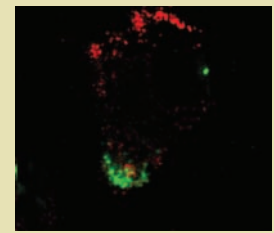
Mañes concentrated on two gangliosides called GM1 and GM3—both markers for rafts and, in fibroblasts, both located at the leading edge of the moving cell. In T cells, however, Mañes found that GM3 was at the front of the cell, whereas GM1 was at the back.

The different segregation of the gangliosides and associated proteins makes sense, based on the biology of the two cell types. Fibroblasts grip tightly to their substrate as they crawl forward, and therefore have most of their cell–cell adhesion proteins at the front of the cell. The back of a fibroblast is essentially a passive tail.

By contrast, T cells skim over the surface, and have little need for cell–cell adhesion at their front end. The back of a T cell, the so-called uropod, recruits bystander cells using intercellular adhesion molecules (ICAMs). It is this need to act as a bipolar sensor that distinguishes T cells.

The differential localization of GM1 and GM3 requires an intact actin cytoskeleton. How the polarization is initiated, or how the different raft proteins are sorted from one another, is not yet known. At the front of the cell, activated chemokine receptors are the best candidates for initiating polarization. ■

Reference: Gómez-Moutón, C., et al. 2001. *Proc. Natl. Acad. Sci. USA*. 98:9642–9647.



Mañes/NAS

GM3 (red) and GM1 (green) at the opposite ends of a T cell.

Squeezing in with sugar

A proteoglycan helps zebrafish cells orient, elongate, and squeeze between each other to lengthen the early embryo, according to work by Lilianna Solnica-Krezel (Vanderbilt University, Nashville, TN) and colleagues.

The cells are participating in a process called convergent extension as part of gastrulation. They first orient and elongate along the ventral to dorsal axis;

these processes create enough space for the cells to squeeze between each other, thus narrowing the dorso–ventral dimension and lengthening the embryo.

Solnica-Krezel and colleagues find that this process is defective in zebrafish that are mutant for the *knypek* gene. Cells lacking *Knypek* can perform other embryonic movements at normal speeds, but cells that would normally undergo convergent extension are less elongated than normal and poorly polarized.

The *knypek* locus encodes a heparan sulfate proteoglycan, glypican, that is probably anchored

to the cell membrane. Complex interactions with a *wnt11* mutation suggest that glypican may help in transmission of the Wnt11 signal, perhaps by stabilizing the Wnt11 ligand on the cell surface.

Mutant phenotypes suggest that glypican may also be involved in a Wnt5 pathway of head cartilage formation that, like convergent extension, involves cell elongation and packing. Glypicans "might be very specific to certain pathways," says Solnica-Krezel. "It is very intriguing that they may be factors contributing to specificity of signaling." ■

Reference: Topczewski, J., et al. 2001. *Dev. Cell*. 1:251–264.



Solnica-Krezel/Elsevier

Glypican is needed to lengthen the zebrafish embryo.