In This Issue

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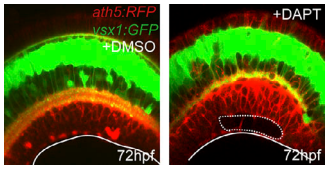
Glia hold it together



Compared with a control group (left), retinas lacking Müller glia (right) are subject to retinal tearing, visible in the area enclosed by the dotted line.

MacDonald et al. show that glial cells within the zebrafish retina hold its layers of neural tissue together.

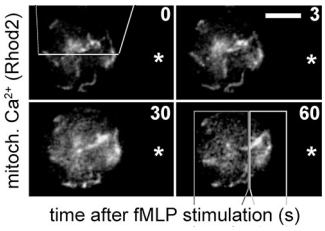
Glia are nonneuronal cells that reside within neural tissues, performing various functions ranging from myelination to immune surveillance. Glia are also thought to provide physical support to neurons by holding notoriously "squishy" nerve cells together into a coherent tissue, but this idea has never been directly tested.

MacDonald et al. investigated this function of glial cells in zebrafish retina, an easily accessible neural tissue. In the vertebrate retina, the development of several cell types including glia depends upon Notch signaling, so the researchers determined the exact time when Notch is critical for genesis of Müller glial cells (MG), the sole glial cell type in the retina. Blocking Notch signaling at this time prevented development of MG, while sparing neurons.

MG cell bodies reside between the retinal photoreceptor layer and the ganglion cell layer, and extend processes to connect both retinal surfaces. Retinas lacking MG were morphologically normal except they developed rips (a condition called retinoschisis) in their ganglion cell layer. Pushing or pulling on retinas using atomic force microscopy showed that tissue lacking MG was abnormally stretchy and soft. MG cell bodies recoiled after laser ablation of MG processes, showing that these processes are normally under stress. This suggests that MG compress retinal tissue and prevent it from tearing. The next questions to ask, says first author Ryan MacDonald, are how MG do this and to what extent MG loss impacts vision.

MacDonald, R.B., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201503115

Mitochondria sense the way forward



Mitochondrial activation occurs at the cell front but not the cell rear in neutrophils polarized toward a chemotactic signal.

Bao et al. reveal how mitochondria act as sensory organelles to direct and coordinate neutrophil chemotaxis.

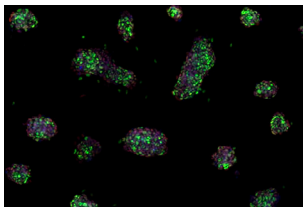
Neutrophils and other immune cells sensing the presence of bacterial products acquire a polarized morphology that facilitates chemotactic migration. During polarization, the serine/threonine kinase mTOR drives actin polymerization at the cell front to facilitate motility, whereas adenylyl cyclase activation at the cell rear promotes uropod formation. Purinergic receptors, which respond to ATP and its breakdown products, also become asymmetrically distributed; nucleotide-responsive P2Y2 receptors signal from the cell front while adenosine-sensitive A2a receptors accumulate at the rear. ATP produced by mitochondria is released to the cell surface, where it and its derivatives promote chemotaxis by stimulating these purinergic receptors.

Bao et al. found that mTOR signaling activates mitochondria at the front of migrating neutrophils, increasing ATP production and initiating an autocrine feedback loop downstream of P2Y2 receptors, resulting in further mitochondrial activation. Meanwhile, A2a receptor signaling blocks mitochondrial activity by inhibiting mTOR signaling at the cell rear. The bulk of the mitochondrial mass is inactivated during neutrophil chemotaxis; only a small number of mitochondria are activated, but this is sufficient to direct migrating cells.

The findings support existing models of chemotaxis that rely upon local excitatory and global inhibitory mechanisms. Senior author Wolfgang Junger now plans to investigate what happens in migrating cells when mitochondrial ATP generation is impaired, such as may occur in the hypoxic environment that accompanies sepsis.

Bao, Y., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201503066

Clearing the way for efficient stem cell differentiation



Treating hPSC with PP1 yields higher numbers of differentiated cells expressing the differentiation marker Brachyury (green). Nuclei in blue and the pluripotency marker Oct4 in red.

Chetty et al. reveal that treating human pluripotent stem cells (hPSCs) with the Src inhibitor PP1 enhances their differentiation.

Efforts to adapt hPSCs for therapeutic purposes have been hampered by the difficulty of obtaining differentiated cell types from stem cell lines. This limits the type and number of studies that can be performed.

Chetty et al. noticed that hPSCs spend little time in the G1 phase of the cell cycle, when cells are most receptive to the signals that drive differentiation; earlier work had shown that exposing stem cells to dimethysulfoxide (DMSO) improves differentiation efficiency by increasing the time cells spend in G1. Because the tyrosine kinase Src regulates cell cycle in many cell types, and Src activity is often up-regulated in rapidly dividing cells including hPSCs, the researchers investigated whether inhibiting Src would also improve the efficiency of stem cell differentiation. Blocking Src activity using the potent inhibitor PP1, or via siRNA-mediated knockdown, dramatically increased the differentiation of a variety of embryonic and induced hPSC lines, and synergized with DMSO to further improve differentiation efficiency.

Enhanced differentiation after Src inhibition was attributable to changes in gene expression brought about by hypophosphorylation of the tumor suppressor retinoblastoma protein. This produced lasting changes in cell behavior that persisted long enough to improve yields even after application of protracted culture protocols aimed at directing stem cells' differentiation toward specific phenotypes. It will be interesting to see whether Src inhibition enhances the functional utility of differentiated hPSCs transplanted in vivo, says first author Sundari Chetty.

Chetty, S., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201502035