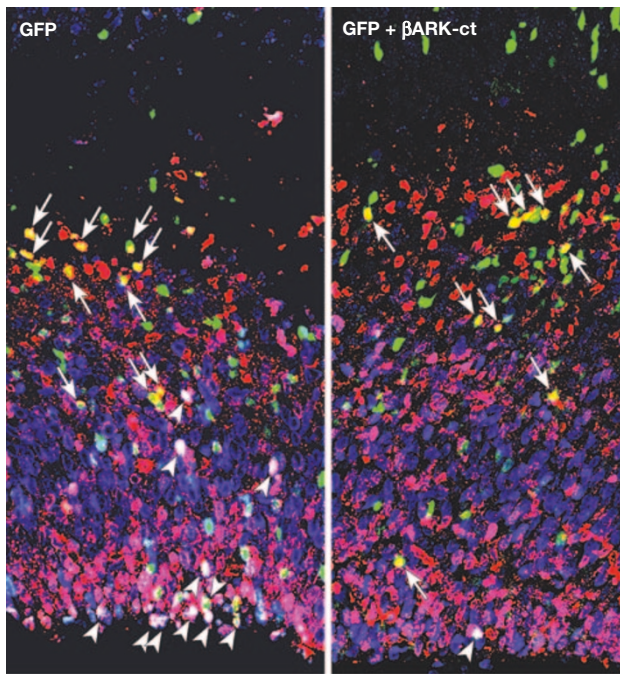


NEUROGENESIS

Lining up with destiny



Brain sections stained with antibodies against the DNA synthesis marker BrdU (red), the cell cycle marker Ki67 (blue) and green fluorescent protein (GFP; green). GFP-positive cells labelled with both BrdU and Ki67 (white; arrowheads) are active in the cell cycle, whereas GFP-positive cells labelled with BrdU but not Ki67 (yellow; arrows) have exited the cell cycle. Image courtesy of L.-H. Tsai, Howard Hughes Medical Institute, Harvard Medical School, Massachusetts, USA.

Orientation of cell-cleavage planes relative to the ventricular zone in the mammalian neocortex is important for the differentiation of progenitor cells into projection neurons, but little is known about how it is regulated. Reporting in *Cell*, Sanada and Tsai show that G protein $\beta\gamma$ subunits ($G\beta\gamma$) are important for regulating mitotic spindle orientation and cell fate determination during neurogenesis in the cerebral cortex.

Progenitor cells at the ventricular zone are highly polarized along the basal–apical axis, and it is thought that determinants of neuronal fate might be asymmetrically located at the basal side. When progenitors divide with the cleavage plane perpendicular to the ventricular surface (vertical cleavage plane), the two daughter cells have the same cellular determinants. Before neurogenesis, this symmetrical division produces two progenitors and expands the pool of cells that will ultimately give rise to neurons. However, when progenitors divide with the cleavage plane parallel to the ventricular surface (horizontal cleavage plane), the determinants are predominantly segregated into the basal daughter

cells, and thereby induce asymmetric cell fates.

In this study, the researchers first established that about 50% of the dividing cells in the neocortex of mouse embryos had the vertical cleavage plane. This percentage increased to 72% when an inhibitor of $G\beta\gamma$, the carboxy-terminal region of β -adrenergic receptor kinase (β ARK-ct), was overexpressed. Overexpression of $G_{\alpha i}$, which is known to sequester free $G\beta\gamma$ and thereby inhibit its signalling, had a similar effect.

Interestingly, overexpression of either β ARK-ct or $G_{\alpha i}$ resulted in a significant increase in the number of differentiated cortical neurons, which indicates that altered orientation in cleavage planes led to changes in cell fate. Further analysis showed that there was an increase in the percentage of progenitors that exited the cell cycle, which was correlated with a decrease in the number of mitotic cells.

Which G-protein activators are responsible for mitotic spindle orientation? Classically, ligands that bind to G-protein coupled receptors (GPCRs) initiate the cascade

SENSORY SYSTEMS

Bittersweet symphony

The neuropeptide cholecystinin (CCK) is thought to be involved in mediating the sensation of bitter taste. Now, a research team led by Herness has shown that neuropeptide Y (NPY) is also expressed by most of the CCK-expressing cells in taste buds and has the opposite physiological effects to those of CCK. These findings might shed new light on the intricate orchestration of chemical signals that are important for the tongue's ability to differentiate bitter and sweet tastes.

Taste buds are clusters of 50–100 differentiated epithelial cells. Sensory afferent nerve fibres connect each bud to the brain to transmit signals about taste. Only a minority of cells in each bud form synaptic contacts with these fibres, and how other cells send signals to the brain has been the focus of intense academic curiosity.

The researchers believe that neuropeptides might be part of the answer. Having established the role of CCK in taste transduction, they moved on to study other neuropeptides and found that NPY was also expressed in a subset of taste bud cells at both the mRNA and protein levels. This prompted them to test the physiological effects of NPY on taste bud cells isolated from rat tongues.

In contrast to CCK, NPY enhances inwardly rectifying potassium currents (Kir) in some taste bud cells and has no effect on intracellular calcium concentrations. The NPY-mediated Kir enhancement can be mimicked by NPY1 receptor agonists and is blocked when the cells are treated with NPY1 receptor antagonists, indicating that the effect of NPY might be mediated by the NPY1 receptor subtype.

If CCK and NPY have contrasting physiological effects, one simple hypothesis

could be that they are expressed by non-overlapping subsets of taste bud cells and are responsible for transducing bitter and sweet tastes, respectively. However, the researchers found that 68% of cells that expressed CCK also expressed NPY, and 95% of cells that expressed NPY expressed CCK. In other words, a subset of taste bud cells releases both neuropeptides.

Therefore, the brain might sense bitter and sweet tastes as a result of two competing signalling pathways: CCK and NPY might excite the transduction of one taste and, at the same time, suppress that of another. Alternatively, the same subset of taste bud cells might release only one neuropeptide in response to either bitter or sweet tastes, despite being able to release both.

Jane Qiu

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WEB SITE

Herness' laboratory: <http://ctoc.osu.edu/herness.htm>

of downstream signalling. However, Sanada and Tsai found that a GPCR-independent activator of G proteins, AGS3, was important for establishing the axis of division in cortical progenitors. AGS3 is expressed by cortical progenitors in mouse embryos, and silencing its expression causes abnormalities in the mitotic spindle orientation that are similar to those caused by disruption of G β signalling.

This elegant study provides the first direct evidence for the molecular mechanism that regulates mitotic spindle orientation in cortical progenitor cells. It will lead to new ways of deciphering the complex events that trigger changes in cell-cleavage plane orientation and asymmetric cell fate choices during neurogenesis.

Jane Qiu

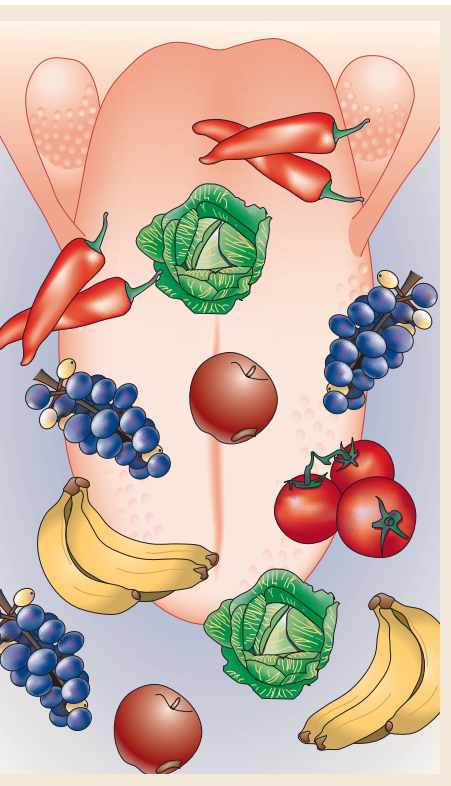
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ION CHANNEL STRUCTURE

Switching on to potassium channels

Our knowledge of the structure of voltage-dependent K⁺ (Kv) channels, which shape the neuronal action potential, has come largely from studies of prokaryotic channels, which can be expressed at high levels in *Escherichia coli*. Now the crystal structure of a mammalian Kv channel has been published, providing important insights into how the structural features of these channels allow them to operate in eukaryotic cells.

Eukaryotic Kv channels are very similar to their prokaryotic counterparts. For example, the sequence of the selectivity filter — the part of the channel that confers specificity for K⁺ — is highly conserved and is likely to produce a similar structure in all K⁺ channels. But eukaryotic channels also have some unique features. To gain a better understanding of the structural basis of Kv channel function in eukaryotes, the researchers, led by Rod MacKinnon, chose to investigate Kv1.2, a member of the *Shaker* family of K⁺ channels.

To obtain crystals of Kv1.2 in its native arrangement, the group used a mixture of lipids and detergent during purification and crystallization. This clever approach helped to compensate for removal of the lipid membrane during isolation of the channel, keeping the voltage sensors correctly positioned with respect to the channel pore. The channel was crystallized in a complex with an oxidoreductase β -subunit, an auxiliary protein that might regulate mammalian Kv channels in their natural environment.

The amino terminus of Kv1.2 is known to form a 'T1' domain inside the cell, which is located over the pore entrance to the cytoplasm. This domain serves as a binding site for the β -subunit, which is thought to be involved in the process of channel inactivation.

Long *et al.* found that the relationships between the membrane-spanning components of Kv1.2, the intracellular T1 domain and the β -subunit were consistent with functional studies of inactivation gating in *Shaker* channels. Interestingly, the structure also pointed to a possible role of the β -subunit as a redox sensor, coupling the redox state of the cell to activity of the channel.

Kv1.2 was crystallized with the pore in the open conformation. Because the normal conformation of the voltage sensors was preserved in this structure, Long *et al.* were able to take a closer look at the way in which the voltage sensor 'paddles', which move through the membrane in response to voltage changes, open and close the pore. They found that the voltage sensors are almost independent domains inside the membrane, being only weakly attached to the pore through 'linker' helices. The positioning of arginine residues in the voltage sensor suggested that a balance between electrostatic and hydrophobic forces is central to the way in which the sensor performs mechanical work on the pore.

The hope is that these fascinating insights into the structure of a mammalian Kv channel in the open conformation will soon be followed by a view of the switch in its closed state.

Rebecca Craven

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