Hedgehog in growth…

Mutations that activate the Hedgehog (Hh) signalling pathway have been linked to tumour formation, but it's not been clear how. The discovery of a direct link between Hh signalling and key regulators of the cell cycle might now provide the answer.

Wei Du and colleagues were studying eye development in Drosophila melanogaster. The expression pattern of Hh during this process, just posterior to cells entering S phase, indicated that reception of the Hh signal might be needed for entry to S phase. To test this, the authors looked at what would happen if Hh signalling was blocked during eye development. They found that second mitotic wave cells with mutated smoothed (smo), a gene that is required for Hh signalling, do not enter S phase. By contrast, overexpression of Cubitus interruptus (Ci) — the transcription factor that mediates Hh signalling — drove G1-arrested cells to enter S phase.

One protein that promotes S phase is Cyclin D. During eye development, the highest expression of Cyclin D overlaps with that of Ci — so could Ci promote the expression of Cyclin D? Support for this idea came from the observation that levels of Cyclin D are reduced in smo-mutant clones, and also that overexpression of Ci induces high levels of Cyclin D messenger RNA and proteins.

As well as promoting entry into S phase, Cyclin D induces cell growth. Du and co-workers therefore wondered whether Hh might also regulate growth, so they studied the effects of overexpressing either Ci or Patched (Ptc; an inhibitor of Hh signalling) in clones of undifferentiated wing-disc cells. Whereas Ptc overexpression clones were considerably smaller than controls, Ci overexpression clones were much larger, which indicates that Hh signalling not only promotes S phase, but that it also regulates cell growth.

Cyclin E also promotes S phase, and reduced or increased levels of this protein could be detected with loss of smo or overexpression of Ci, respectively. The authors then looked at how Hh signalling might induce the transcription of Cyclin E. They identified several sequences in the Cyclin E promoter with homology to the consensus Ci-binding site, and used chromatin immunoprecipitation to show that Ci indeed binds these sites in vivo. Hh signalling therefore seems to promote S phase by...
Hogness and colleagues have elucidated how Hedgehog signals in frogs. They used Xenopus laevis embryos to study how Hedgehog (Hh) signals, which promote cell proliferation and growth, can be coupled to DNA replication.

The authors investigated how Hh signals are coupled to DNA replication, using Xenopus embryos. They found that Hh signals can directly induce the expression of Cyclin E, which is known to promote DNA replication. This suggests that Hh signalling might be a key regulator of replication in cancer cells.

In conclusion, the study provides evidence for a direct link between Hh signalling and DNA replication, raising the possibility that this pathway might be targeted in cancer therapy.

References:
- Almouzni, G. et al. The molecular basis of nucleosome assembly. Nature Rev. Mol. Cell Biol. 3, 565–575 (2002).
- Jiang, Y. et al. Crystal structure of the K+ channel KCNQ1. Nature 417, 515–522 (2002).
- MacKinnon, R. & Alaimo, P. The structure of potassium channels. Nature 417, 507–514 (2002).

By comparing the structure of the K+ channel with that of an unbound RCK domain from an Escherichia coli K+ channel, the authors gained insight into how the gating ring converts the free energy of Ca2+ binding into mechanical changes in the pore. They propose that Ca2+ binding to the cleft reshapes it, so that the rigid units tilt and expand the diameter of the gating ring. This, in turn, pulls open the inner helices of the pore (see dashed lines) and lets ions pass through.

HIGHLIGHTS

**STRUCTURE WATCH**

**Open the gate...**

Ion channels open and close in response to a stimulus that 'gates' the channel. But how does gating occur, and how do pores open? In *Nature*, new insights have now been provided by two papers from the MacKinnon group.

In the first paper, MacKinnon’s group presents the structural basis of ligand gating in a K+ channel that opens in response to intracellular Ca2+. They determined the 3.3-Å resolution crystal structure of MthK from *Methanobacterium thermoautotrophicum* in its Ca2+-bound, open conformation. The channel is tetrameric, and the subunits that form the pore (top of figure) are each made up of two transmembrane segments. Each subunit also has a ‘regulator of K+ conductance’ (RCK) domain at the intracellular surface, although MthK actually has eight RCK domains (bottom of figure), as four RCK domains join the complex from the intracellular solution. The RCK domains form a ‘gating ring’ through a pattern of alternate ‘fixed’ and ‘flexible’ interfaces, which actually makes four rigid units (RCK-domain dimers joined by the fixed interface). The flexible interfaces form ligand-binding clefts between RCK domains, and two Ca2+ ions (yellow circles) — which are directly correlated with channel gating — bind to each of these clefts.

By comparing the structure of the Ca2+-bound RCK domain of MthK with that of an unbound RCK domain from an *Escherichia coli* K+ channel, the authors gained insight into how the gating ring converts the free energy of Ca2+ binding into mechanical changes in the pore. They propose that Ca2+ binding to the cleft reshapes it, so that the rigid units tilt and expand the diameter of the gating ring. This, in turn, pulls open the inner helices of the pore (see dashed lines) and lets ions pass through.

**...and the pore**

In the second paper, the group investigated how a pore opens by comparing the ‘open’ MthK structure with the known ‘closed’ structure of KcsA — a K+ channel from *Streptomyces lividans*. Although the region around the ion selectivity filter is similar in both channels, the authors noticed large structural differences in the inner helices of the two pores.

The helices are almost straight in KcsA, and form a bundle that closes the pore near its intracellular opening. However, in MthK, the helices are bent and splayed open, which produces a wide pore. The bending point corresponds to a glycine — the most flexible amino acid — that is located deep inside the membrane, and MacKinnon’s group found that this ‘hinge’ residue is conserved in a wide range of K+ channels.

These observations fit neatly with the gating mechanism described above — ligand binding reorganizes the gating ring, which exerts a radial force that focuses at the hinge and causes the inner helices of the pore to bend outwards, thus opening the pore.

**REFERENCES**
- Jiang, Y. et al. Crystal structure and mechanism of a calcium-gated potassium channel. Nature 417, 515–522 (2002).
- Jiang, Y. et al. The open pore conformation of potassium channels. Nature 417, 523–526 (2002).