direct induction of Cyclin E expression, as well as Cyclin D.

This study shows a direct link between Hh signalling and cell growth (though Cyclin D) and proliferation (through both Cyclin D and Cyclin E). And, as the authors conclude, "constitutive Hh signalling, which promotes deregulated expression of G1–S cyclins that have been associated with diverse forms of human cancer, would promote both cell proliferation and growth in tumours".

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Duman-Scheel, M., Weng, L. & Du, W. Hedgehog regulates cell growth and proliferation by inducing Cyclin D and Cyclin E. *Nature* **417**, 299–304 (2002)

WEB SITE

Wei Du's laboratory:
http://devbio.bsd.uchicago.edu/index3.html?content=faculty/wDu/index.html

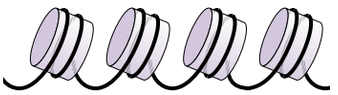
survival of fetal Leydig cell precursors in the interstitium of XY gonads. Again, however, there was no difference in these processes in the presence or absence of DHH signalling.

So how does the DHH pathway affect differentiation? Capel and colleagues think that its main role is in the upregulation of *Scc* in Leydig precursor cells. *Scc* is the target of the steroidogenic factor 1, and there is evidence that this, too, is upregulated in Leydig cells. By upregulating these factors, the DHH pathway could trigger the differentiation of precursors into Leydig cells. However, as the authors point out, not all cells that express *Ptch1* differentiate as Leydig cells, so other signals probably combine with the DHH pathway to specify Leydig cell fate.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Yao, H. H.-C., Whoriskey, W. & Capel, B. *Desert Hedgehog/Patched 1* signaling specifies Leydig cell fate in testis organogenesis. *Genes Dev.* **16**, 1433–1440 (2002)



CHROMATIN

HIRA order

The DNA in chromatin is tightly wound around nucleosomes — but how does it get that way? Although several factors are known to deposit histones on DNA during DNA synthesis, a report in *Molecular Cell* now uncovers an independent chromatin-assembly pathway that is not coupled to DNA replication.

Geneviève Almouzni and colleagues were studying HIRA, a protein that was previously shown to interact with histones in mammals. They first isolated the homologue of HIRA in *Xenopus laevis*, and showed that GST–HIRA could bind to all four core histones (H2A, H2B, H3 and H4) with varying affinities.

The authors next used a supercoiling assay to show that HIRA can facilitate the formation of nucleosomes *in vitro*. They mixed purified histones with a relaxed DNA plasmid in the presence of recombinant HIRA, then monitored the electrophoretic mobility of the plasmid. Nucleosome assembly causes supercoiling of the DNA, and hence faster migration on a gel.

Almouzni and co-workers then studied nucleosome assembly using *Xenopus* egg extracts, which are very efficient at chromatin assembly and are enriched in HIRA. Although these extracts could promote nucleosome assembly, extracts that had been immunodepleted of HIRA could not. However, depletion of HIRA did not affect the chromatin-assembly activity that is coupled to nucleotide-excision repair or to DNA synthesis.

These results, conclude the authors, "show that HIRA is critical in a specific chromatin-assembly process that is not coupled to DNA synthesis". What they also emphasize is that there are at least two distinct nucleosome-assembly pathways.

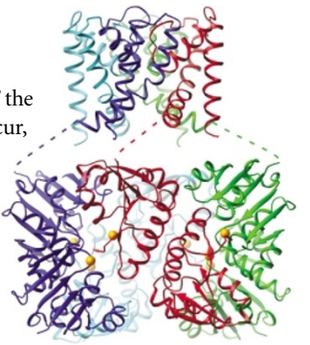
Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Ray-Gallet, D. *et al*. HIRA is critical for a nucleosome assembly pathway independent of DNA synthesis. *Mol. Cell* **9**, 1091–1100 (2002)

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 Ca^{2+}. They determined the 3.3-Å resolution crystal structure of MthK from *Methanobacterium thermoautotrophicum* in its Ca^{2+}-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 Ca^{2+} ions (yellow circles) — which are directly correlated with channel gating — bind to each of these clefts.

By comparing the structure of the Ca^{2+}-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 Ca^{2+} binding into mechanical changes in the pore. They propose that Ca^{2+} 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)