CELL SIGNALLING

From Wingless to boneless

Human bone mass is the result of a fine balancing act between bone deposition — carried out by bone-forming cells called osteoblasts — and degradation by the osteoclasts. In later life, as bone catabolism supersedes anabolism, the result is often a loss of bone mass (osteoporosis). To prevent this condition, we need to know more about the processes that underlie bone formation, and a report in *Cell* by Matthew Warman and co-workers now takes us a step closer, revealing an intriguing link to the Wingless (Wnt) signalling pathway.

Warman, along with Yaoqin Gong and an international group of clinicians and scientists, started by searching for the gene behind osteoporosis-pseudoglioma syndrome (OPPG) — an autosomal-recessive disorder in which patients have very low bone mass. They studied 28 affected families, and identified six different homozygous frameshift or nonsense mutations in the low-density lipoprotein receptor-related protein-5 (LRP5) gene.

Next the authors looked at expression of mouse *Lrp5* messenger RNA during embryogenesis by *in situ* hybridization on developing skeletal elements. They detected expression in osteoblasts; interestingly, in pluripotent cell lines induced into the osteoblastic lineage by exogenous

growth factors, the authors also found increased expression of LRP5. And this could, they speculate, indicate a role for LRP5 in terminal osteoblastic differentiation.

To delve deeper into the function of LRP5, Warman and co-workers used what is already known about its relatives. The mouse, *Xenopus laevis* and *Drosophila melanogaster* paralogues of LRP5 are involved in the Wnt signalling cascade, so the authors examined the effects of expressing various Wnt proteins *in vitro*.

To do this they used alkaline phosphatase (ALP) as a marker of osteoblast differentiation in two pluripotent mesenchymal cell lines. Whereas WNT3a — which participates in the canonical Wnt signalling pathway — could induce ALP activity in both lines, WNT5a and WNT4 (which use other signalling pathways) could not. WNT3a-induced ALP activity was also reduced when dominant-negative forms of LRP5 that lack the cytoplasmic tail were expressed in these cell lines. Moreover, induction of ALP by WNT3a could be blocked by coexpression with a dominant-negative form of Dishevelled, which acts downstream of WNT3a in the canonical pathway.

Finally, Warman and colleagues studied the effect of culturing bone explants in conditioned media from cells expressing the secreted form of

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Closing the gap

Cell signalling and membrane transport have traditionally been separate fields of cell biology. But they seem to converge more and more, as illustrated by a report in *Science* that describes a new GTPase-activating protein (GAP) for $G\alpha_s$ subunits of heterotrimeric G proteins that seems to also function as a sorting nexin in controlling the lysosomal degradation of epidermal growth factor receptors (EGFRs).

$G\alpha_s$ subunits come in many flavours, and each of them seems to be regulated by a specific GAP of the regulator of G-protein signalling (RGS) family. Farquhar and colleagues set out to find the RGS protein that regulates $G\alpha_s$ — a polyvalent $G\alpha$ subunit that controls various cellular responses, including cell growth, hormone secretion, and learning

and memory. They searched databases for proteins with an RGS domain, and found a sequence that contained such a domain as well as a Phox domain, so they called the protein RGS-PX1.

They showed that RGS-PX1 interacts specifically and directly with $G\alpha_s$, and that it acts as a GAP for $G\alpha_s$ *in vitro*. They also confirmed that the RGS domain of RGS-PX1 attenuates $G\alpha_s$ signalling in HEK293 cells and neonatal rat cardiac membranes.

So, the RGS domain of RGS-PX1 seemed fully functional. What about the Phox domain? Farquhar and colleagues wondered whether RGS-PX1 could function as a sorting nexin — a family of proteins characterized by a Phox domain and involved in membrane transport. They overexpressed RGS-PX1 in HEK293 cells and studied its effect on EGFR sorting. Whereas in control cells, ligand-bound EGFR was rapidly sorted to lysosomes and degraded, in RGS-PX1-expressing cells, its degradation was markedly delayed, which

resulted in sustained activation of downstream targets of EGFR.

Farquhar and colleagues also determined that, like other Phox domains, the PX1 domain could bind to specific phosphoinositides, including phosphatidylinositol 3-phosphate, which is abundant in endosomes. So they tested the localization of RGS-PX1 and found that it is indeed associated with early endosomes, as it colocalized with the early endosomal marker EEA1 in COS-7 cells (see picture).

So, RGS-PX1 is both a GAP for $G\alpha_s$ and a sorting nexin involved in sorting of EGFR. This new link between membrane transport and cell signalling might help us to understand how membrane transport compartmentalizes signal transduction and, conversely, how signal transduction regulates membrane transport.

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References and links

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