STRUCTURE WATCH

A small change makes a big difference

A transcriptional complex — hypoxia-inducible factor-1 (HIF-1) — controls homeostatic responses to oxygen levels, and it is the α subunit of this complex (HIF-1 α) that is regulated by oxygen. In hypoxic conditions, HIF-1 α is stable, but when oxygen is present, HIF-1 α is targeted for proteasomal degradation by an E3 ubiquitin ligase complex that includes the protein product of the von Hippel–Lindau tumour suppressor gene (pVHL). Capture of HIF-1 α by pVHL is regulated by the oxygen-dependent hydroxylation of specific prolines in HIF-1 α , and Jones and colleagues now provide structural insights into this recognition process in *Nature*.

The authors determined the crystal structure of a hydroxylated HIF-1 α peptide bound to a section of the E3 complex that contains pVHL. The HIF-1 α peptide contained the carboxy-terminal oxygen-dependent degradation (CDD) region that has a hydroxyproline (Hyp) at position 564. They found that two sites in the peptide bind to pVHL, although site 1 is the primary site that drives binding. The site-1-binding cavity in pVHL contains a single, conserved pocket that buries Hyp564, and the authors could not detect any stable complexes between pVHL and the unhydroxylated CDD region. They concluded that optimized hydrogen bonding to the buried hydroxyproline enables pVHL to discriminate between hydroxylated and unmodified prolines. As small molecules could be designed to disrupt pVHL–HIF-1 α binding, these results have highlighted a new potential therapeutic target for the treatment of ischaemic diseases.

REFERENCE Hon, W.-C. *et al.* Structural basis for the recognition of hydroxyproline in HIF-1 α by pVHL. *Nature* **417**, 975–978 (2002)

Success at last

On binding a hormone molecule, the glucocorticoid receptor (GR) dimerizes, translocates to the nucleus, and activates or represses the transcription of target genes that are involved in processes such as glucose homeostasis and bone turnover. Given the biological and pharmaceutical importance of GR, there has been great interest in determining the structure of the GR ligand-binding domain (LBD), which, in addition to recognizing ligand, also has a ligand-dependent activation function. But, there has been a problem — the GR LBD has been tricky to express. Now, however, there has been success. Xu and co-workers discovered a single mutation that greatly improves GR LBD expression, so they determined the crystal structure of this domain bound to its ligand (dexamethasone) and to a coactivator motif from the transcriptional intermediary factor 2 (TIF2).

In their work published in *Cell*, the authors show that, despite its structural similarity to other steroid receptors, the GR LBD has a dimer interface unlike that observed for any other nuclear receptor, and that this interface is important for the transcription activation function of GR. In addition, they found that a second, previously unknown ‘charge clamp’ determines the TIF2-binding selectivity of the GR LBD. The structure also showed a distinct hormone-binding pocket, which explains the selectivity of the GR LBD. Together, these data have provided the basis on which to understand the roles of protein–hormone and protein–protein interactions in the GR signalling pathway.

REFERENCE Bledsoe, R. K. *et al.* Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell* **110**, 93–105 (2002)

DEVELOPMENT

Spinning around

For the first time, one of the long-standing questions in developmental biology — that of left–right asymmetry — has some direct evidence to support it. In the mouse embryo, it has been hypothesized that a leftward fluid flow on the ventral side of the embryonic node, caused by vortical motion of cilia (referred to as nodal flow), is crucial in developing left–right asymmetry. But, in *Nature*, Shigenori Nonaka, Hiroshi Hamada and colleagues now provide the first direct evidence to support this model.

The generation of left–right asymmetry during development is a fundamental part of establishing a body plan. Previous work indicated that Nodal and Lefty, members of the transforming growth factor- β family, are part of this process. However, the initial step in breaking symmetry during development is unknown. It has been proposed to involve nodal flow and the rotational movement of the cilia in the node. Several mouse mutants that show *situs inversion* are accompanied by a loss of motility in respiratory cilia. This indicates that the nodal-flow model could be correct, and many developmental biologists propose that the rotational movement of cilia in the node generates a leftward flow that transfers an unknown molecule to the left side of the embryo. This unknown factor would then determine left–right patterning.

Using *in vitro* experiments, Hamada and colleagues developed a culture system (flow culture) in which mouse embryos were allowed to develop under a constant flow of culture medium that was driven by a peristaltic pump. Using this system, the authors could determine whether the embryos received a leftward or rightward flow, and whether this was fast or slow. The flow was adjusted to take into account the initial orientation of the embryo. Under conditions of fast or slow leftward flow the embryos developed normally, as did embryos under conditions of slow rightward flow. However, embryos that were subjected to fast rightward flow developed with a reversed symmetry compared with the initial orientation of the embryo. This indicated that the fast rightward flow was enough to overcome the intrinsic nodal flow of the embryo. Artificial flow (both fast and slow, leftward and rightward) could also direct the left–right patterning of mutant mouse embryos with immotile cilia. These beautiful experiments seem to show that the nodal-flow model is indeed important, although it is still unclear how the flow works to determine asymmetry.

Sarah Greaves, Senior Editor,
Nature Cell Biology

References and links

ORIGINAL RESEARCH PAPER Nonaka, S. *et al.* Determination of left–right patterning of the mouse embryo by artificial nodal flow. *Nature* **418**, 96–99 (2002)

Left–right asymmetry of early embryos is revealed by asymmetric staining of *Pitx-2*. The embryo on the left has reversed situs as a result of reversed (rightward) nodal flow.