IN THE NEWS

Keeping time

Some say the biological clock is linked to lunar cycles or sunspots, but a husband and wife team has discovered that a single protein could be Nature's timepiece.

The findings, published in *Biochemistry*, have wide-reaching implications because "…the body's clock affects nearly every bodily activity…", as James Morré, who has been intrigued with the biological clock for over 40 years, told *The Indianapolis Star* (12 January). Together with his wife, Dorothy, he isolated a single, cylinder-shaped protein that apparently directs 12-minute periods each of growth and rest in living cells. The couple propose that the protein has two faces. "One handles cell enlargement. Then the protein 'flips over', allowing the second face to carry out other activities while cell enlargement rests."

The Morrés verified the protein's links to biological clocks by cloning the gene and altering it to produce different period lengths. "The 'day' that the cell experienced was precisely 60 times the period length of the protein's cycle" (*BBC News*,12 January).

Dorothy Morré declared "This could give us new insights into cellular activity, such as cholesterol synthesis, respiration, heart rhythms, responses to drugs, sleep, alertness…", providing a potential wealth of clinical applications. Currently, though, they want to build up a better picture of the protein, adding that "…the practical applications would be best left to drug firms and medical experts."

Katrin Bussell

CELL CYCLE

Polar trek

Bacteria lack the spindle structure that, in eukaryotic cells, allows the segregation of sister chromosomes to opposite poles. Bacterial sister chromosomes segregate from each other in a process whereby the region containing the origin of replication moves towards the cell pole. Whether these origin regions are anchored at the poles is unknown. However, Richard Losick and colleagues now describe, in *Science Express*, a protein — RacA (for remodelling and anchoring of the chromosome) — with a polar anchoring, as well as a chromosome remodelling, function.

The two sister chromosomes in sporulating *Bacillus subtilis* condense and form a structure called the axial filament. Near one of the cell poles, a septum is formed that divides the cell into a smaller forespore and a larger mother cell. Losick and co-workers found that *racA* transcription is switched on during early sporulation and therefore suspected a role for RacA in sporulation. RacA mutants showed delayed septum formation and had a compact DNA mass (nucleoid) in contrast to an extended axial filament in wild-type cells. Also, in ~50% of the cases examined, the forespore lacked DNA.

Using fluorescence microscopy, Losick and colleagues saw that a RacA–green fluorescent protein (GFP) construct was present at the poles, and a diffuse fluorescence haze indicated that RacA–GFP colocalizes with the nucleoid. RacA–GFP expression was transient and specific for early sporulation, as the fluorescent signal disappeared subsequently.

The authors constructed a strain that allowed inducible expression of RacA and RacA–GFP during growth. Following induction, fluorescent foci were detected at the poles and diffuse fluorescence was visible at the nucleoid. In many cells, the nucleoids had moved towards the poles, which did not occur in uninduced cells. So, RacA localization depends on its own expression, and is sufficient to anchor the chromosomes to the cell poles.

But how is RacA bound to the cell poles? A candidate protein is the cell-division protein DivIVA, which is located at the poles where it sequesters the division inhibitor MinC–MinD (MinCD). As growth

DEVELOPMENT

And on your right…

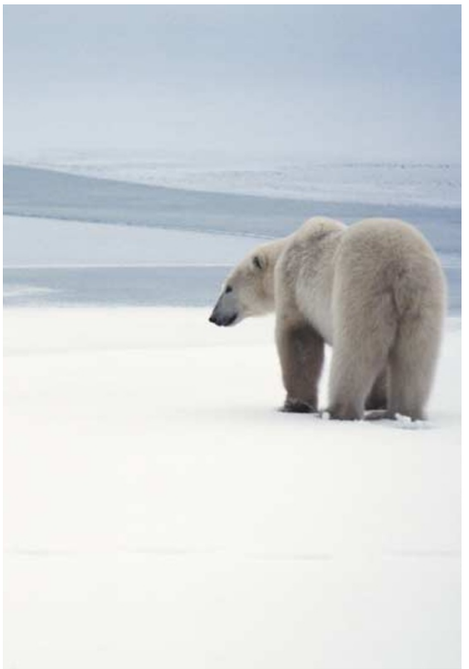
Left–right asymmetry is established early in embryonic development so that your heart, for example, ends up on the left side of your body. Syndecan-2 is known to be involved in this process by transmitting left–right information from the ectoderm to the adjacent migrating mesoderm during gastrulation, but the mechanism for this was largely unknown. Now, however, Yost's group show that the cytoplasmic domain of syndecan is targeted by protein kinase C (PKC)-γ in right, but not left, ectodermal cells in *Xenopus* and that this is one of the earliest steps in left–right development, occurring before the appearance of nodal cilia.

***In vitro*, PKC family members phosphorylate syndecans, so the group hypothesized that a PKC might function in early left–right development. Specific inhibitors and dominant-negative forms of PKC (dnPKC) showed that PKCγ in the ectoderm regulates left–right development during early gastrula stages. Inhibiting PKCγ function specifically in left or right ectodermal lineages indicated that PKCγ is specifically required in cells of the right ectoderm.**

Logically, PKCγ substrates should be present in right ectodermal cells. Immunocytochemical analysis of mid-gastrula-stage embryos showed that syndecan-2 was present in the deep layer of ectoderm that interacts with the migrating mesoderm — the sensorial ectoderm. But phosphorylated syndecan-2 was present only in the right sensorial ectoderm, which directly contacts migrating mesoderm.

So, is this phosphorylation of relevance to left–right asymmetry? Syndecan-2 mutants in which either or both of two cytoplasmic phosphoacceptor serine residues were changed to alanines showed reversal of the normal position of the heart in 19% and 41% of cases, respectively, when both sides of the ectoderm were targeted at the same time. When targeted individually with the double mutant, the right-side ectoderm showed greater disruption. The converse experiment, using phosphomimetic mutants, showed that syndecan-2 must be phosphorylated on the right, but non-phosphorylated on the left, for normal left–right development. Finally, PKCγ was shown to be upstream of syndecan-2 in the same pathway because phosphorylation of endogenous syndecan-2 depended on PKCγ and phosphomimetic syndecan-2 overcame the loss of PKCγ activity.

What is unclear at present is how serine phosphorylation



in a DivIVA mutant strain is impaired, a MinD DivIVA double-mutant strain was used. RacA–GFP failed to localize to the extreme poles, whereas localization in a MinD mutant was similar to the wild-type strain, which indicates that RacA localization is indeed dependent on DivIVA.

Chromatin immunoprecipitation and subsequent PCR experiments confirmed that RacA colocalizes with the entire nucleoid, and also revealed preferential binding sites for RacA in the replication origin region.

Losick and colleagues have therefore proposed a model in which RacA is a kinetochore-like protein that binds preferentially near the replication origin and anchors the chromosome to the cell pole by binding — directly or indirectly — to DivIVA. In addition, nonspecific binding of RacA throughout the nucleoid allows remodelling of the chromosome into an axial filament structure. A possible role for RacA in polar division requires further investigation.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Ben-Yehuda, S. *et al.* RacA, a bacterial protein that anchors chromosomes to the cell poles. *Science Express* 19 December 2002 (DOI: 10.1126/science.2079914)

WEB SITE

Richard Losick's laboratory: http://mcb.harvard.edu/losick/

influences the inside–out transducing function of syndecan-2 in the ectoderm to enable it to act cell non-autonomously to influence the migrating mesodermal cells. However, there seems to be no shortage of ideas.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Kramer, K. L., Barnette, J. E. & Yost, H. J. PKCγ regulates syndecan-2 inside–out signaling during *Xenopus* left–right development. *Cell* **111**, 981–990 (2002)

FURTHER READING Essner, J.J. *et al.* Conserved function for embryonic nodal cilia. *Nature* **418**, 37–38 (2002)

WEB SITE

Joseph Yost's laboratory: http://www.hci.utah.edu/groups/yost/

IN BRIEF

DEVELOPMENT

Heart regeneration in zebrafish.

Poss, K. D. *et al*. *Science* **298**, 2188–2190 (2002)

In most vertebrates, cardiac injury leads to scar formation. By contrast, zebrafish can regenerate cardiac tissue following mechanical injury, as is now shown by Mark Keating and colleagues. Cardiomyocyte proliferation occurs at the leading epicardial edge, and regeneration is complete within 2 months of 20% ventricular resection. However, zebrafish with a mutation in the Mps1 mitotic checkpoint kinase do not regenerate and form scar tissue.

PROTEIN TRANSLOCATION

Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70.

Young, J. C. *et al*. *Cell* **112**, 1–20 (2003)

The delivery of preproteins from the cytosol to mitochondria is poorly understood. Young *et al.* now report that, in mammals, the cytosolic chaperones Hsp90 and Hsp70 dock onto the import receptor Tom70 at the outer mitochondrial membrane. This interaction is essential for targeting a subset of preproteins to the receptor for subsequent import. However, in yeast, only Hsp70 docking is needed for effective preprotein delivery.

BIOENERGETICS

SRC-1 and TIF2 control energy balance between white and brown adipose tissues.

Piccard, F. *et al*. *Cell* **111**, 931–941 (2002)

Piccard *et al.* found that two members of the p160 coregulator family — TIF2 and SRC-1 — have a function in the energy homeostasis of white and brown adipose tissues. *TIF2*$^{-/-}$ mice are protected against excessive fat accumulation and have increased insulin sensitivity. By contrast, *SRC1*$^{-/-}$ mice are prone to obesity due to reduced energy expenditure. These phenotypes are caused by changes in the expression ratio of TIF2 and SRC-1 leading to an altered composition of coregulator complexes, which, in turn, affects the transcriptional control of fat storage and thermogenesis.

APOPTOSIS

c-MYC apoptotic function is mediated by NRF-1 target genes.

Morrish, F. *et al*. *Genes Dev*. **17**, 240–255 (2003)

Although earlier studies indicated a link with the mitochondrial apoptotic signalling pathway, the precise mechanism by which c-Myc induces apoptosis has remained elusive. Morrish *et al.* now show that c-Myc can stimulate target genes of the nuclear respiratory factor (NRF)-1, a transcription factor for several mitochondrial-related genes, including *cytochrome c*. Under conditions that trigger c-Myc-induced apoptosis, overexpression of NRF-1 sensitizes cells to apoptosis. Also, a dominant-negative NRF-1 mutant inhibits c-Myc-induced apoptosis but not c-Myc-induced proliferation.