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