structure. Indeed, it might be that the protein acts primarily as a protective host for the two co-substrates, coelenterazine and molecular oxygen, which are held in appropriate orientation relative to each other and are stabilized by the protein matrix. Oxidation to the hydroperoxy anion could thereafter occur quite spontaneously because of relatively facile intramolecular generation of a reactive carbanion from the coelenterazine (Fig. 1b).

Formation of the carbanion is the central process in activation, and is probably facilitated by a side chain of a basic amino acid in the protein (for example by a histidyl side chain, as shown in Fig. 1a). Head *et al.* discuss potential roles for (apparently) key histidine residues in aequorin. But in this paper they do not touch on the question of what function, if any, cysteinyl (thiol) moieties play; reversible chemical modification of thiols in *Aequorea* leads to reversible loss of Ca^{2+} -sensitive bioluminescence.

Coelenterazine is also the substrate for non-photoprotein luciferases found in diverse marine species such as *Renilla*, *Ptilosarcus* and *Gaussia*. These enzymes show no sequence similarity to the photoproteins and are not activated by Ca^{2+} ; and

Figure 2 *Aequorea aequorea* — a shining light of biology.

although they are relatively slow compared with most enzymes, their turnover rates are much higher than that of aequorin. Yet these non-photoprotein luciferases also require only oxygen and coelenterazine for bioluminescence, implying that the catalytic mechanism is similar to that in aequorin. Presumably there are also similar substrate-binding motifs — equivalent, maybe, to the common nucleotide-binding motifs found in proteins of dissimilar sequence.

Despite the availability of this tertiary structure, the mechanistic basis for Ca^{2+} triggering of bioluminescence in aequorin is not yet solved. Head *et al.* speculate reasonably that relatively small and local Ca^{2+} -induced conformational changes would disrupt the tyrosine (phenol)–hydroperoxide hydrogen bond (Fig. 1c), thereby facilitating nucleophilic attack by the hydroperoxy anion on the reactive carbonyl of the substrate (Fig. 1d). Thereafter the intramolecular reaction sequence depicted in Fig. 1 would proceed readily. Given the energetics of hydrogen bonding, thermally driven structural fluctuations should suffice to trigger Ca^{2+} -independent light emission in aequorin. They indeed do so, although the rate of such emission in *Aequorea* is less than a millionth of light emission triggered by Ca^{2+} binding².

Interestingly, other divalent and trivalent cations can evoke aequorin bioluminescence, some less so and some more so than Ca^{2+} . The Ca^{2+} -binding sites known as EF-hands, such as those in *Aequorea*⁵, bind a variety of multivalent cations which can sometimes act as isomorphous replacements for Ca^{2+} . Such promiscuity supports

Cell biology

Centrioles go for a stroll

Centrioles are arguably the most enigmatic cell organelles. In most animal cells, a pair of centrioles resides in the centrosome — a macromolecular complex that organizes the microtubule system (part of the cell's internal skeleton). The two centrioles, a mother and a daughter, "are positioned at right angles to each other". This quote from a popular textbook epitomizes the idea of a fixed arrangement of the two bodies within the centrosome (see part a in the figure: at the top is an end-on view of one of the centrioles, while at the bottom is a longitudinal view of the other centriole). Deviations from a close apposition are considered exceptional.

Not so, say Matthieu Piel and colleagues (*J. Cell Biol.* **149**, 317–330; 2000). They have been following the behaviour of centrioles throughout the cell cycle (the time between successive cell divisions) in living cells. Centrioles are difficult to see by standard light microscopy, so Piel *et al.* made use of the fluorescent tag green fluorescent protein (GFP). They fused GFP to centrin, a small calcium-binding protein present in the lumen of the centriole. Fluorescence — a marker of the amount of GFP-tagged centrin — is always more intense in the mother centriole. So, the intensity of fluorescence seems to be indicative of the maturity of a centriole. This allowed the authors to show that a daughter centriole remains 'immature' until it duplicates and itself becomes a mother. The observation also indicates that one centriole may not be able to replace the other functionally, but this needs to be confirmed.

The most striking observation, however, is that the two centrioles exhibit dramatically different behaviour. For several hours after cell division, the daughter centriole roams extensively through the body of the cell, separating from the mother by many micrometres. Motility gradually subsides at the time of centriole duplication, the start of which coincides with the beginning of DNA replication in the nucleus. But these movements also take place — and are more pronounced — in the absence of nuclei. An example of these movements over a period of 20 minutes is shown in b and c of the figure. While the daughter (blue) runs free, the mother centriole (red) resides in the centre of the microtubule array (d in the figure).

Splitting of the centriole pair has previously

been observed under certain experimental conditions or in specialized cell types, but what the work of Piel *et al.* implies is that centriolar excursions are a normal event during a considerable portion of the cell cycle. Why one centriole should roam the cell after completion of cell division is unknown, as is the mechanism underpinning these movements. But the observations shake the myth of a stereotypical, right-angled orientation of centrioles, and suggest that these curious organelles might have some hitherto unsuspected functions associated with their wanderings.

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