100 YEARS AGO

It is only sixty years ago since George Catlin wrote his "North American Indians," and graphically described the vast herds of bison, numbering millions of individuals, travelling for days together across the rolling prairies; yet we have seen these disappear, like the aborigines, and their places usurped by the "cow-boy," and by countless herds of domestic cattle. If we could only wind the clock of Time still further backwards, and make him disclose, with moving-photographic-vividness, some of those earlier Mesozoic scenes on the American continent, or even in our own little island, for that matter, we should find, not herds of bison, but far other cattle, though some wore horns, and were big and ugly enough in all conscience; yet they were mostly harmless, and herbivorous in diet, but belonging to patterns now entirely obsolete, like the old "brown-bess" of our grandfathers' days, only more so. ... One of the most curious points about these mediæval animals is, that they make their earliest appearance in the Triassic period, and were first known in North America some sixty years ago, not by their bones or teeth, but by their footprints. – "Dinosaurs."

From *Nature* 18 March 1897.

50 YEARS AGO

The sudden widespread interest in the Antarctic, aroused by the announcement that no less than twelve nations are organising expeditions to that region, has resulted in a spate of articles which has been chiefly remarkable for inaccurate rumours and speculations. Supposed rich deposits of uranium and other valuable minerals have been depicted as the prize for the winners of an international race to stake out territorial claims. ... What is now happening is the realization on the part of all concerned that the economic development of this frozen continent is technically within sight – providing there is anything there which is worth the trouble of development.

From *Nature* 22 March 1947.

Many more extracts like these can be found in *A Bedside Nature: Genius and Eccentricity in Science, 1869–1953*, a 266-page book edited by Walter Gratzer. Contact David Plant (e-mail: subscriptions@nature.com).

ever, the sequence of the lone γ-subunit did not show any tripartite character and yet all three of the enzyme sites needed to interact with it to function. These facts led Boyer to propose a radical idea; namely that the binding-change mechanism did not correspond to allosteric activation of three sites in sequence. Instead, by analogy with the flagellar motor, the α- and β-subunits literally turned with respect to an axially located γ-subunit, bringing each of the three sites into play, one after the other[10]. This model was dubbed 'rotational catalysis'.

At first, few people believed this idea. Perhaps the γ-subunit had greater symmetry (or mobility) than imagined. Perhaps there was an allosteric explanation. Perhaps the enzyme just oscillated back and forth. But when the crystal structure of the F_1-ATPase was eventually solved[5], it looked every bit like a three-piston rotary motor, with a hexagonal ring of α–β pairs surrounding a drive shaft made up of the γ-subunit (Fig. 1 of Noji *et al.*, page 300). Armed with a detailed knowledge of the structure, two groups soon weighed in with experimental data in support of rotational catalysis.

Duncan *et al.*[11] genetically engineered a cysteine residue on the β-subunit, at a location near to a corresponding cysteine residue of the γ-subunit. This allowed them to form a reversible disulphide crosslink between one of the three β-subunits and the γ-subunit. Such a linkage stops the F_1-ATPase in its tracks, presumably by welding the drive shaft to the engine block. After forming the linkage, complexes were chemically dissociated, then reconstituted, but this time the two unlinked βs were substituted with radioactively labelled subunits. The disulphide crosslinks were reduced, unfettering the enzyme, which was then supplied with ATP. After many rounds of catalysis, the crosslink was re-established by oxidation, and dissociation of the complex revealed an admixture of linked β–γ-subunits — some radioactive and others not — that was quantitatively consistent with a random redistribution of the linkage sites among all three β-subunits. Although this did not prove that an ordered, sequential rotation occurs, it confirmed that the γ-subunit could visit each β-subunit freely, consistent with rotational catalysis.

Sabbert *et al.*[12] took advantage of another cysteine residue on the γ-subunit drive shaft to link up a maleimide-coupled eosin dye. By exciting the dye with a flash of polarized laser light from one direction, then recording the anisotropy change for the subsequent absorption of continuous polarized light coming from an orthogonal direction, they obtained signals that decayed over time and were consistent with rotation of the bound eosin chromophore through at least 200°. These signals depended on ATP, and the time constant for the decay was roughly compatible with the known rates of ATP hydrolysis.

However, nothing is quite so compelling as seeing rotation with one's own eyes, and this is exactly what Noji *et al.*[1] have done. Once again, the crystal structure served to identify likely sites of attack. First, histidine tags were genetically engineered into the β-subunits on their cytoplasmic faces, so that F_1 complexes could be attached stereospecifically to a Ni^{2+}-coated surface, with their F_0-binding faces pointing up, away from the surface. Second, a cysteine was engineered at position 107 of the γ-subunit and used to couple a short, fluorescently labelled actin filament (about a micrometre in length) to the drive shaft. Third, isolated complexes were bound at low surface-densities inside microscope flow-cells coated with Ni^{2+}, and viewed in fluorescence mode. In the presence of ATP, some of the actin filaments were driven round and round like microscopic propellers, powered by hydrolysis in the F_1 complex. The handedness of this rotation could be predicted in advance because (viewed from above) the catalytic sites are arrayed clockwise in the order: ATP-binding, ADP-binding and empty. So the reverse rotation induced when the F_1-ATPase splits ATP (rather than when the H^+-ATP synthase makes it) should carry the drive shaft around anticlockwise — and all of the spinning actin filaments indeed turned anticlockwise (Fig. 1, page 300).

Curiously, the angular speeds of filament rotation were rather constant, showing little tendency to slow down or speed up three times per revolution, contrary to what might be expected for a motor with only three 'pistons'. This might be due to thermal motion, but it could also be a clue to the molecular mechanism: more data are needed on speeds, torques and fluctuations. The flagellar motor also turns remarkably smoothly, despite having just eight force generators[3,13]. The reported angular speeds for the F_1-ATPase were relatively low (< 4 Hz) — solution hydrolysis rates would predict speeds nearer to 20 Hz. Enormous mechanical loads imposed by viscous drag on the actin filaments doubtless slowed things down. Noji *et al.*[1] estimated this drag to be over 90 pN nm Hz^{-1} for a 2.6-μm-long filament (Fig. 2, page 300), and the (unknown) proximity of filaments to the nearby surface may raise the value threefold; perhaps more.

A filament rotating at 0.5 Hz, therefore, dissipates an energy in excess of $(2\pi) \times (90\ \text{pN nm Hz}^{-1}) \times (0.5\ \text{Hz}) = 280$ pN nm per revolution. This should be compared with the chemical free energy available from hydrolysis, which is roughly 80 pN nm per molecule of ATP. Assuming tight coupling of rotation to hydrolysis, and exactly three ATPs per revolution, the chemical-energy input would come to 240 pN nm. So either the F_1-ATPase is operating at 100 per cent