STRUCTURAL BIOLOGY

Another green revolution

Steven G. Boxer

FOR most scientists, the first glimpse into the world of green-fluorescent protein (GFP) came early in 1994 with the publication of a striking image of a glowing, green nematode. The picture accompanied a paper by Chalfie and colleagues[1], which pointed to the stunning prospect of using fluorescence-imaging microscopy with GFP as a label for tracking the expression and location of other proteins within organisms as diverse as bacteria and nematodes. The three-dimensional structure of GFP just solved by Ormö *et al.*[2] and Yang *et al.*[3], and reported in *Science* and *Nature Biotechnology* respectively, is equally stunning. The structure of GFP is so perfectly suited for its function it looks as if it were manufactured in a machine shop.

In nature, GFP is made by the jellyfish *Aequorea victoria*, which is widely found in the northwest Pacific Ocean. In the jellyfish, GFP functions in concert with the calcium-ion-activated protein aequorin. In response to shaking or attack, calcium-ion levels change and aequorin generates an excited state. The blue light from excited aequorin transfers to GFP, which then emits a bright green flash, presumably blinding the attacker. The jellyfish aequorin–GFP system is also activated by ships moving through the water leaving a trail of light. This explains the long-standing interest of the US Office of Naval Research in GFP and bioluminescent organisms in general, a perfect example of science stimulated by military interest ultimately having a much wider impact.

Over the past 15 years, a group of scientists including William Ward at Rutgers University and Frank Prendergast at the Mayo Cancer Center has laboured in relative obscurity to characterize GFP biochemically. Most coloured proteins contain a bound prosthetic group, such as retinal bound to opsin in the visual pigments, chlorophylls in photosynthetic systems, and haem which gives blood its red colour. These pigments can be extracted from their protein host; however, specific pigment–protein interactions alter the colour substantially, and this is often a key to their biological function. GFP is unusual as its pigment is derived from the post-translational oxidation of a bit of the polypeptide itself[4] (Fig. 1).

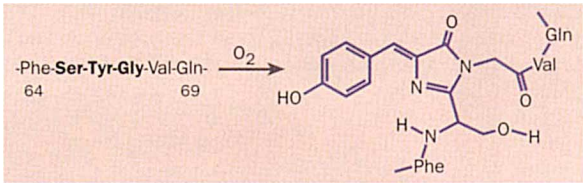

FIG. 1 Autocatalytic oxidation of GFP amino acids leads to the chromophore shown on the right. The green fluorescence requires further interactions of the chromophore with other parts of the protein.

The peptide sequence concerned (serine–tyrosine–glycine) is found in other proteins, but does not undergo the chemical transformation needed to form the pigment. This led some to suspect that the jellyfish possesses an elaborate enzymatic machinery to catalyse the oxidation; but this is not the case, as became clear when Chalfie and co-workers[1] demonstrated that GFP could be expressed and the pigment developed in a wide variety of organisms. In fact, the chemical transformation that generates the pigment is an autocatalytic oxidation that can occur so long as most of the GFP polypeptide sequence is expressed and oxygen is present. No prosthetic group needs to be added.

Therein lies the power of the GFP system as a fluorescent label. Microscopists and pathologists routinely stain complex tissues to highlight particular features. During the past two decades, microscopy has been revolutionized by a remarkable collection of dyes used as *in vivo* indicators of physiologically important quantities such as pH, transmembrane potential or the concentration of calcium ions[5]. Proteins can be stained by binding to specific antibodies that can be imaged by a variety of techniques. All these methods, however, involve the addition of an exogenous agent. By contrast, the DNA sequence coding for the GFP polypeptide[6] can be fused to that of any protein whose expression or location is of interest. Under favourable conditions, the expressed GFP domain folds independently of the protein to which it is fused

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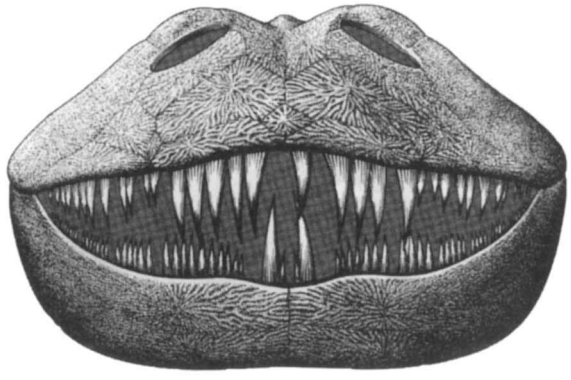
PALAEONTOLOGY

The 365-million-year-old grin



WHAT is the connection between a failed attempt to reach the North Pole by balloon, the political status of Greenland and the earliest amphibians? All is made clear in the long-awaited monograph on the Devonian tetrapod *Ichthyostega* by Erik Jarvik, published as an issue of *Fossils and Strata* (40, 1–213; 1996). Although this animal has featured in textbooks for decades, this is, remarkably, the first detailed, complete description.

Jarvik's colleague, Gunnar Säve-Söderbergh, wrote a preliminary account of the fossil material in 1932, Jarvik undertaking the full description after Säve-Söderbergh's early death in 1948. After a long and distinguished career (he has been publishing since 1935), he has saved the best until last. Not everyone will agree with his interpretations, but the monograph will stand as a palaeontological resource for all time. *Ichthyostega*'s welcoming grin (above) appears on the front cover.

The fossils might have eluded discovery entirely were it not for the disappearance, in 1897, of Andrée Salomon's balloon-borne North Pole expedition. The search for survivors took geologists to East Greenland, where they found plenty of fossils, but no balloon. According to Jarvik, the discovery of *Ichthyostega* by a Danish–Swedish expedition may have been instrumental in the ruling in 1933, by the International Court of Justice in the Hague, to award Greenland to Denmark, rather than Norway. Henry Gee

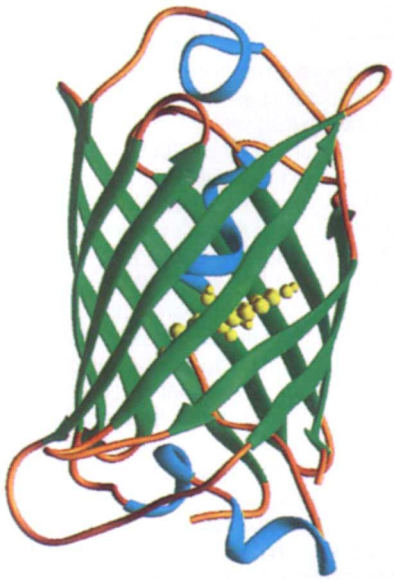


FIG. 2 The three-dimensional structure of wild-type GFP, adapted from Yang *et al.*[3]. The 11 β-strands comprising the barrel are shown in green; the caps at the top and bottom of the barrel are shown in tan; and the chromophore (yellow) is found in the middle of the barrel along the central helix shown in blue.

and accompanies it *in vivo* as a covalently attached fluorescent label.

The new three-dimensional structure of GFP is beautiful and unique (Fig. 2). Most of the polypeptide is folded into a tight, cylindrical, barrel structure consisting of a highly regular series of 11 β-strands. The outer diameter of the barrel is about 30 Å; the inner surface is quite polar and can accommodate the single α-helix found neatly packed inside. The sequence from which the chromophore is derived is contained right in the middle of this α-helix. The barrel is capped on both top and bottom, so the chromophore is packaged in an environment entirely comprised of the protein and some structured interior water molecules, completely protected from the surroundings.

Despite the nearly perfect engineering achieved by the jellyfish, several investigators have sought to improve upon the properties of GFP for applications in biotechnology. Native GFP has two absorption maxima, a strong peak at 395 nm and a weaker peak around 477 nm. These peaks appear to be associated with two conformational isomers of the chromophore that can interconvert both in the ground and excited state. On exposure to light, the form corresponding to the peak at 395 nm photoconverts into the form absorbing around 477 nm. The excited-state process appears to involve ultrafast proton transfer followed by much slower solvation[7].

The two forms and the interconversion process can now be explained by specific interactions between the chromophore and amino acids on the inner surface of the β-barrel. The two published structures are actually of slightly different proteins — a mutant, in which the serine at residue 65 is converted to threonine, from Ormö *et al.*[2]; and the wild type from Yang *et al.*[3]. Remarkably, the replacement of serine 65 with threonine greatly simplifies the spectrum to a single peak absorbing at 488 nm (ref. 8). Further engineering of the surrounding interior surface of the barrel, remote in primary sequence from the chromophore, leads to optimization of the chromophore properties for use in fluorescence microscopy. Until now, these changes were largely based on inspired guesses or screening large libraries of mutants, but the palette of colours can now be rationally designed based on the structure.

This last feature and the observation of ultrafast excited-state dynamics[7] suggest that GFP is an ideal object to test and refine computational methods widely used to analyse and re-engineer proteins and to characterize binding sites. The GFP chromophore by itself does not exhibit green fluorescence; rather, a specific set of interactions with the protein 'solvent' leads to the unique spectral and dynamic properties. In fact, GFP can be reversibly denatured with a loss of the green fluorescence[9]. This dependence of the colour on tertiary structure might be turned to advantage for studying protein folding in real time.

Finally, a key problem in biology is discovering which proteins interact at a molecular level during development and self-organization. GFP tags may be useful for identifying which proteins are in close proximity by energy transfer using differently coloured GFPs or fluorescence depolarization. Thousands of laboratories are now reputed to be using GFP for a wide range of clever purposes, so the future looks very green. □

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REPRODUCTIVE BIOLOGY

The vocabulary of the egg

Roger Gosden

At one time, eggs were regarded like the cargo in the hold of a vessel — finally discharged after a voyage by ovulation or, more frequently, shipwrecked in atresia. We now recognize that each egg actively influences the development of its own follicle — it despatches commands affecting the growth and differentiation of the granulosa cells around it, while receiving information and nutrition from them[1]. The extent to which the character or quality of an egg shapes the destiny of its follicle is still open to question. But there is no longer any doubt that the phenotype of the follicle is affected by signals from the oocyte inside, and on page 531 Dong *et al.*[2] report the first identification of such an oocyte factor.

The earliest experimental indication that oocytes influence follicle development was published more than 25 years ago by Andy Nalbandov and his colleagues at the University of Illinois[5]. They showed that when oocytes were removed from Graafian follicles in rabbit ovaries, the granulosa cells became prematurely luteinized — that is, they behaved as though they were post-ovulatory, and secreted progesterone. Any questions about the artefactual nature of these results were quashed by studies showing that oocytes secrete a factor (or factors) that increases the production of oestrogen and decreases that of progesterone by granulosa cells that have been stimulated by follicle-stimulating hormone (FSH) and testosterone *in vitro*[4].

So the idea that oocytes communicate with neighbouring cells is not new, nor should it be particularly surprising in an integrated developmental unit (Fig. 1). But the range of their vocabulary is greater than had been suspected. After removal of the oocyte, the surrounding cumulus granulosa cells within a large follicle can no

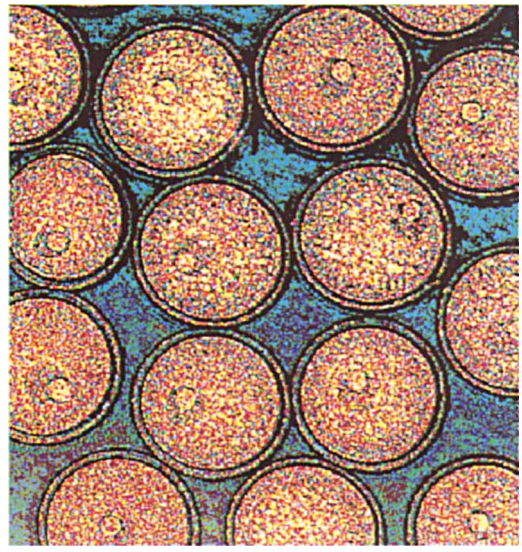

FIG. 1 A cluster of fully grown mouse oocytes: each cell is enclosed by a zona pellucida and contains a prominent nucleus and nucleolus. (Photo: J. J. Eppig, Jackson Laboratory, Bar Harbor, Maine.)