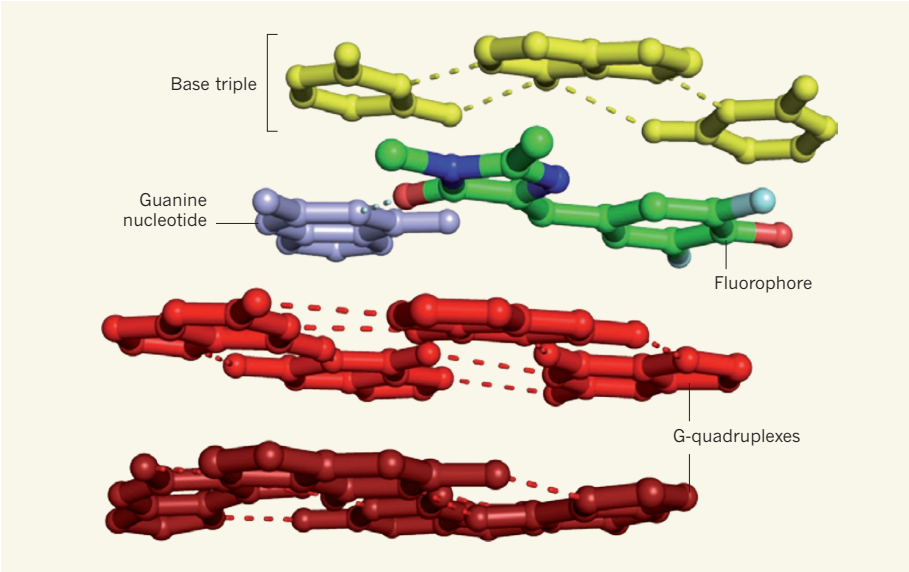

Figure 1 | Spinach sandwich. Two studies[3,4] report crystal structures depicting how the 'Spinach' RNA motif binds its fluorophore — the dye molecule that fluoresces only when bound to Spinach. The structures reveal that the fluorophore binds tightly between a base triple (a structure formed from three nucleotide bases) and two stacked G-quadruplexes (each quadruplex is a coplanar duo of unusual RNA G–G base pairs; G is the nucleic-acid base guanine). The positioning and orientation of the fluorophore are further mediated by a coplanar guanine nucleotide. Broken lines indicate hydrogen bonding. In the fluorophore, oxygen atoms are shown in red, nitrogens in blue, fluorines in cyan and carbons in green. The figure was produced from coordinates for Huang and colleagues' structure[3], using PyMOL software (version 1.7.0.3).

ultraviolet light and fluoresces as bright green light.

Numerous complex cellular processes are controlled and orchestrated by RNA molecules, rather than by proteins. A particularly noteworthy example is RNA interference, in which small RNAs regulate, interfere with or inhibit gene expression. Furthermore, genes are expressed through the intermediate action of messenger RNA, which may be compartmentalized in a cell. The ability to tag and track the intracellular movement of any RNA by means of fluorescent molecules would therefore be of obvious use to biologists.

Unfortunately, nature has not provided a potential RNA tool analogous to GFP. Instead, using a process called *in vitro* directed evolution, biologists can identify RNA motifs that bind to small fluorescent molecules; these molecules are chemically similar to the fluorescent component of GFP and have similar fluorescent properties. Spinach is the most useful of such motifs, and can be fused to many RNAs of interest.

Spinach binds with high affinity to a synthetic dye molecule that resembles GFP's fluorophore. The dye has the invaluable property of becoming fluorescent only when it binds to Spinach, and the further merit (as does GFP) of being non-toxic to cells. The fluorophore thus becomes visible only when it is bound to the RNA and illuminated with ultraviolet light, making it an ideal visualization marker.

The two sets of crystal structures for Spinach reveal a previously unknown fold and fluorophore-binding site — the complexity of which defied prediction by computer programs commonly used to calculate RNA secondary structures. Huang *et al.* obtained their set of structures using an in-house approach[5] in which the RNA was co-crystallized with an antibody. To address the potential criticism that the highly unusual RNA structure might be an artefact resulting from this method, the authors devoted considerable time and effort to providing many reassuring experimental controls.

The fluorophore can exist as four potential isomers, each of which can have multiple binding modes to Spinach. To identify the orientation of the bound fluorophore unambiguously, Huang and colleagues solved the crystal structure of the fluorophore alone, and that of the RNA bound to a bromine-bearing analogue of the fluorophore. The X-ray-absorption properties of the bromine allowed the binding position of the analogue, and therefore that of the original fluorophore, to be pinpointed. Huang and colleagues' heroic undertaking has been unambiguously validated by the subsequent publication of Warner and co-workers' crystal structures, which were obtained using a different (and more standard) crystallization approach.

So what have we learnt from the two sets of structures? Most importantly, the key to understanding how green fluorescent RNA works has been revealed. The fluorophore sits on a platform of two stacked G-quadruplexes (each quadruplex is a coplanar duo of unusual RNA G–G base pairs; G is guanine, a nucleic-acid base). G-quadruplexes are often found at the ends of DNA molecules, but are seldom observed in RNA structures. The fluorophore is sandwiched tightly between the quadruplex platform and a coplanar RNA base triple (a structure analogous to a base pair, but involving three bases; Fig. 1). The binding pocket thus created enforces planarity on the bound fluorophore.

Two interrelated structural effects seem to be responsible for activating fluorescence. First, a negative charge on the oxygen atom attached to the fluorophore's benzene ring is required for fluorescence. That negative charge is stabilized by RNA interactions in the binding site. These include hydrogen bonding to a nearby ribose structure; a 'stacking' interaction formed with the base triple that caps the binding site; and an electrostatic interaction with a nearby bound potassium ion that has a positive charge balancing the negative charge. Second, the large planar surface formed by the G-quadruplex platform provides an opportunity for extensive stacking interactions that greatly enhance fluorescence.

Our understanding of macromolecular structure and function can be put to the test by attempting to design molecules with a given function. Warner and colleagues demonstrated this by developing an improved green fluorescent RNA motif using the insight gleaned from their crystal structures. The resulting molecule is smaller and folds more efficiently than Spinach, and has been dubbed "Baby Spinach" by the authors. It is an ideal candidate marker for the next generation of RNA visualization experiments. ■

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CORRECTION

The News & Views article 'Palaeoanthropology: The time of the last Neanderthals' by William Davies (*Nature* **512,** 260–261; 2014) incorrectly named the modelled overlap period between Neanderthals and modern humans as 470–4,900 years (25–250 generations) instead of 2,600–5,400 years (130–270 generations).