assembled? In a word, the answer is flexibility. The advantage of 'combinatorial' behavior is fully appreciated in both chemistry and biology. Given the huge and entirely unpredictable types of DNA damage that might be encountered (including unanticipated modern insults to DNA), the repair machinery must have the flexibility to respond to all types of DNA damage. In the event that the wrong DNA repair complex assembles on any type of DNA damage, the complex can disassemble and dissociate, allowing a different subset of repair enzymes to act on the lesion. The second feature of the flexibility argument is that intermediates of repair processes have the option to proceed down different end-pathways, depending on the state of the cell[18]. For example, the ssDNA produced as part of DSBR can be handled in several different ways: it can be annealed to a complementary sequence; it can invade a homologous duplex sequence as part of a recombination mechanism; or it can be repaired by a replicative pathway[19]. Biochemically, the path chosen depends on the nature of the complex that assembles after the RPA–ssDNA complex — Rad52 promotes annealing and Rad51 promotes DNA strand invasion. Finally, since the sites of DNA damage are randomly distributed around the chromosome, the repair machinery must have the capacity to find them. Pre-assembled factories, while efficient, simply cannot move as quickly as individual components that are assemble on site. Thus, the dynamic nature of biological structures facilitates their relocation.

In conclusion, despite the anxiety associated with the phrase, 'some assembly required', many processes in biology benefit from the flexibility associated with an ordered, temporal assembly and exchange/disassembly of structural and functional intermediates. By 'handing-off' components in a specific manner that is dictated by the structures of, and interactions among, early intermediates, the cell assures that later steps of complex processes can progress.

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Going green

The green fluorescent protein (GFP) is one of those tools, like the polymerase chain reaction, that it is hard to imagine living without. Tagging proteins with GFP has revolutionized biology, making it possible to visualize particular cells in living organisms, such as *Caenorhabditis elegans*, and to watch molecules move during biological processes, such as cell division.

The development of GFP as a molecular tool has its roots in the study of bioluminescence, a phenomenon found most commonly in marine organisms such as jellyfish, corals, and dinoflagellates. Bioluminescence differs from fluorescence in one important aspect. In the former, light is produced by an inherent chemical reaction whereas in the latter, radiation of a particular wavelength is absorbed by a fluorescent molecule, and subsequently light of a different wavelength is emitted by that molecule. It was discovered many years ago that certain organisms use bioluminescent proteins and fluorescent proteins in tandem to emit light of a particular wavelength, although the biological advantage of emitting such light is not known.

In the early 1960s, Shimomura *et al.*[1] purified the first light-producing protein from the jellyfish *Aequoria victoria*. They found that this protein, known as aequorin, produced a blue light when activated by calcium — not the green light that was observed in the animals naturally. It was thought that another factor, perhaps a fluorescent molecule, might participate to create the green light. In support of this idea, in the 1950s it had been reported that *A. victoria* produces a greenish fluorescence when subjected to long-wavelength UV irradiation[2]. The situation was later clarified by a number of experiments on several related systems (reviewed in ref. 3). Aequorin is associated with GFP in the light-producing cells and energy transfer from activated aequorin to GFP results in the emission of green light.

It had been shown that the chromophore of GFP is formed from its primary amino acid sequence (see ref. 3 for a review). Nevertheless, it was unclear if GFP would be capable of fluorescing in heterologous cells, as it was thought that perhaps some jellyfish-specific enzyme might be required for assembly of the chromophore. Experiments using *Escherichia coli* and *C. elegans* settled this issue in 1994, showing that GFP expressed from a cDNA construct could indeed fluoresce in other organisms[4].

Research continues today on bioluminescent organisms, which have yielded other photoproteins and fluorescent molecules for use as biological tools. The structure of one such molecule, the red fluorescent protein known as DsRed, is described on page 1133 of this issue.

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