then most lines thereafter crossed out. Madame Auclair, the French violinist, had a gentler approach. Out of central casting, as they say: dark glasses, cigarette dangling, hoarse, accented voice. A friend went for a special violin lesson, and asked whether he might tape record this important event in his life. "Of course, my boy." He played a bit and she said: "Very nice. There are some good things about your playing, very good. Now turn off the tape recorder."

When I am struggling over yet another of my obscurely written drafts I sometimes recall: amateurs play music 'in general'; professionals play each note. And so I present to a tough-minded friend one paragraph — just one — and when that is reported to be transparent I go on to the rest. But even if I have followed the rules I mentioned above, and even if that first paragraph seemed fine at the time, now, in view of what else I have written, that first paragraph might have to go, or be seriously recast. Each paragraph is an experiment — you might not know for some time whether it is any good.

There is a theme here, beautifully expressed by a friend who was going through the agonies of the "just the first paragraph" method in attempting to re-write a book. I hadn't heard from him in a while and began to worry — had I been too tough? — and he wrote: "The only reason I hadn't sent it (the new paragraph) already is that I didn't want to disappoint you. But I realize that the only way you can help me is if I continue to disappoint you. So here it is..." All my teachers, whatever their methods, were trying to help me, and I love them for it. Heifetz I wouldn't be so sure about. Rules are one thing, but in the end communication is all: at the end of a pleasant interview with a fine scientist of foreign extraction she shook my hand and said: "Its been a pleasure talking to me."

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Quick guide

RecA

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What is RecA protein? The bacterial RecA protein is the founding member of a class of proteins with homologs across all domains of life: RadA in archaea, and Rad51 and Dmc1 in eukaryotes. In *Escherichia coli*, RecA is essential for recombinational repair of DNA breaks, induction of the DNA damage-induced 'SOS' response, and activation of translesion DNA synthesis. The functional form of RecA protein in these processes is the nucleoprotein filament, the structure formed by assembly of RecA protein on DNA (Figure 1), generally single-stranded DNA. During homologous recombination, the RecA nucleoprotein filament catalyzes the pairing and exchange of complementary DNA strands between homologous regions of DNA. In response to DNA damage, the RecA nucleoprotein filament activates the SOS response by catalyzing the auto-cleavage (co-protease activity) of the LexA repressor, leading to derepression of over 40 unlinked genes involved in DNA repair, including the *recA* gene itself. And through both cleavage (of the UmuD subunit) and direct binding, the RecA nucleoprotein filament activates DNA polymerase V ($UmuD'_2C$ protein), a lesion by-pass DNA polymerase, to synthesize DNA at otherwise irreparable lesions, resulting in a mutagenic form of DNA repair known as translesion synthesis.

What is the RecA nucleoprotein filament? The active form of RecA and of all its homologs is the ATP-bound nucleoprotein filament formed on DNA. The protein forms a polymorphic right-handed helix around the DNA with approximately six monomers per turn and a pitch of ~9.5 nm, in which the DNA is extended to about 150% of its B-form length. This quaternary organization is responsible for the catalytic properties of the protein. Formation of the nucleoprotein filament occurs by a mechanism similar to that of other self-associating proteins,

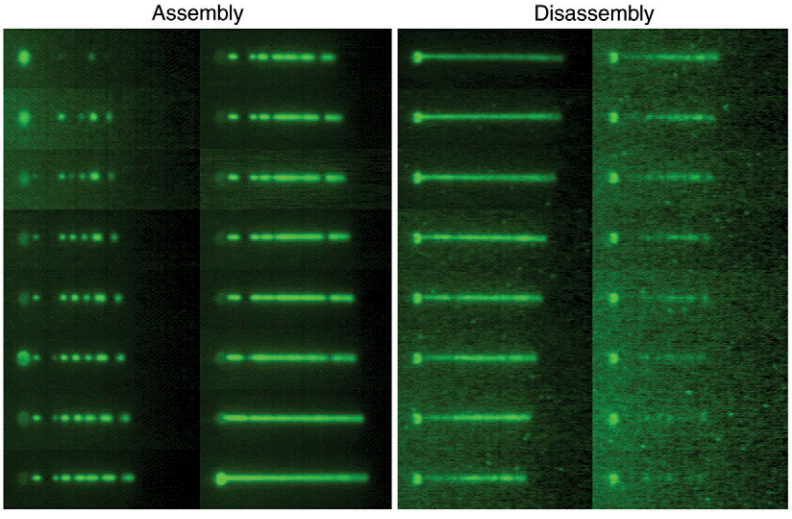

Figure 1. Assembly and disassembly of a RecA nucleoprotein filament formed on an individual double-stranded DNA molecule.

The collage shows the major steps in the entire cycle of RecA nucleoprotein filament assembly and disassembly as visualized by single-molecule detection. The DNA, which is invisible in these images, is bound to a polystyrene bead (leftmost 'spot'). The RecA is fluorescently labeled, permitting visualization of the DNA-bound protein. From top to bottom and left to right, snapshots of RecA assembly from multiple nuclei to generate complete filaments (left-most two columns), followed by images of disassembly promoted by ATP hydrolysis (right-most two columns).

such as actin and tubulin, except that polymerization typically occurs on the DNA. The first step is the formation of rate-limiting nuclei that require four or five RecA monomers to achieve stability on DNA. This nucleus then grows via addition of ATP-RecA. Although addition at one end (the 3′-end) of the DNA-bound cluster can be preferred, assembly of RecA at the opposite end can also occur, resulting in bi-directional growth of the nucleoprotein filament, albeit with a net 5′→3′ assembly direction.

How does it work? Kinetically, the preferred substrate for nucleoprotein filament formation is single-stranded DNA. In the prototypic DNA strand-exchange reaction, the RecA nucleoprotein filament forms on single-stranded DNA (presynapsis). Subsequently, the nucleoprotein filament repeatedly binds double-stranded DNA non-specifically, while it 'searches' for homologous regions within the duplex. During this homology search, the double-stranded DNA is topologically unwound, but strand separation does not occur. Such unwinding of the DNA likely activates the normally stable duplex DNA for the subsequent steps of DNA strand exchange. Upon recognition of a homologous region of about 15–40 base pairs in the double-stranded DNA (synapsis), the RecA filament promotes switching of base pairs, resulting in homologously paired 'joint' DNA molecules. Continued pairing and extension of the nascent DNA heteroduplex joint (DNA heteroduplex extension) leads to the final products of DNA strand exchange, where several thousands of base pairs of DNA can be exchanged.

Although single-stranded DNA is the preferred substrate, active RecA filaments can also be formed on double-stranded DNA. The duplex DNA in this nucleoprotein filament is also stretched to ~150% of its B-form length and it is underwound by about 50%. This nucleoprotein filament assembled on double-stranded DNA is active by a number of criteria, but the most compelling is that it can promote DNA strand exchange with single-stranded DNA; it can also promote strand exchange with single-stranded RNA. Being the directional opposite of the canonical reaction, DNA strand exchange initiated with double-stranded DNA nucleoprotein filaments is called 'inverse' DNA strand exchange.

What controls RecA nucleoprotein filament dynamics? *In vitro*, assembly of RecA nucleoprotein filaments is spontaneous, and does not require any accessory proteins. Regulation of the cycle of assembly and disassembly is achieved through the binding and hydrolysis of nucleotide cofactors. Assembly of the active nucleoprotein filaments requires ATP; both non-hydrolyzable ATP analogs (ATPγS) and mimics of nucleoside triphosphates ($ADP{\bullet}AlF_3$) also enable formation of active nucleoprotein filaments. Thus, ATP hydrolysis is not a requisite step in the mechanism of DNA strand exchange. This is because the binding of ATP to RecA is sufficient to induce a state of the protein with high affinity for DNA, and this state is active in homologous pairing, DNA strand exchange, and co-protease function. To disassemble the filaments, ATP hydrolysis is needed. ATP hydrolysis both destroys the effector ligand, ATP, and produces ADP, which destabilizes and inactivates the nucleoprotein filament. The dynamic behavior of RecA protein under conditions of ATP hydrolysis is thus conceptually similar to that of other NTP-hydrolyzing, self-associating proteins, such as actin and tubulin. Although the rate constants for each of the key assembly and disassembly intermediates are still unknown, the preferred end for addition of ATP-RecA monomers is typically the 3′-end of the filament and the ADP-induced dissociation of RecA commonly occurs at the 5′-end. The net behavior, however, is known to be influenced by a wide range of solution conditions resulting in, at times, seemingly contradictory reports regarding RecA protein function.

Furthermore, ATP hydrolysis is required for dissociation of RecA from the heteroduplex product of DNA strand exchange. Indeed, in the absence of ATP hydrolysis — in the presence of ATPγS or $ADP{\bullet}AlF_3$ — pairing and exchange of DNA strands is efficient, resulting in the production of joint molecules. The subsequent phase of DNA heteroduplex extension is, however, blocked. Also, hydrolysis of ATP is a required step in the bypass of heterologous sequences. RecA nucleoprotein filaments can catalyze DNA strand exchange with duplex DNA where the homologous regions are separated by up to ~140 base pairs of an intervening non-homologous sequence. The requirement for ATP hydrolysis in DNA heterology bypass points to a crucial need for a dynamic nucleoprotein filament, with dissociation and redistribution of bound RecA playing a pivotal role.

How is the RecA nucleoprotein filament regulated? The complex interplay of ATP binding and hydrolysis provides a simple but dynamic mechanism for regulating the steady-state level of RecA nucleoprotein filaments. Not surprisingly, *in vivo*, this assembly process is regulated. The first level of regulation is controlled by the single-stranded DNA binding protein, SSB. SSB competes with RecA for DNA binding, and thereby represses unwanted RecA nucleoprotein filaments; this inhibition, for example, prevents spurious filament formation on the lagging strand single-stranded DNA gaps formed during DNA replication. Consequently, there are proteins that promote assembly of RecA nucleoprotein filaments on SSB–DNA complexes. One of the other key enzymes of recombinational DNA repair, the RecBCD helicase/nuclease, actually 'loads' RecA protein onto single-stranded DNA produced by the RecBCD enzyme. This loading of RecA protein ensures that the processed broken chromosomal fragment acquires sufficient RecA to be repaired by recombination in a timely manner. A second regulator of RecA nucleoprotein filament formation is the RecOR complex, which mediates the exchange of the DNA-bound SSB for RecA protein. A third regulator is the RecFOR complex; these three proteins

bind to the junction of duplex DNA and single-stranded DNA, and recruit RecA protein to the SSB–DNA complex. Although the mechanism of these loading and recruitment steps is not completely understood, it is likely that the rate-limiting nucleation of RecA protein is being stimulated via transient, direct interactions with these proteins. Filament disassembly is also regulated. For example, UvrD helicase (helicase II) disassembles RecA nucleoprotein filaments that formed inappropriately. Thus, the regulation of the site and timing of RecA filament dynamics is achieved via coordination with the repair machinery.

Why does RecA form a nucleoprotein filament? The broad spectrum of biological functions attributable to RecA protein, ranging from the homology search of DNA recombination to the activation of proteins *via* its co-protease activity, cannot be attributed to the action of an individual monomer. Rather, it is evident that the catalytic unit of the RecA function is the highly organized filament that assembles on DNA. Formation of a highly ordered filament on its DNA substrate provides a large surface where catalysis can occur ('surface catalysis'), where homology can be measured and matched to a potential complementary partner, and where DNA structural transitions can be cooperatively transmitted over long distances. This requirement for a regular RecA/Rad51 nucleoprotein filament also permits a broad spectrum of regulatory control that is needed for biological function.

Where can I learn more?

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Essay

Marr's vision: Twenty-five years on

Andrew Glennerster

It is twenty-five years since the posthumous publication of David Marr's book *Vision* [1]. Only 35 years old when he died, Marr had already dramatically influenced vision research. His book, and the series of papers that preceded it, have had a lasting impact on the way that researchers approach human and computer vision.

A review at the time of publication predicted that "Even if no single one of Marr's detailed hypotheses ultimately survives ... [his] lifework will have been vindicated when neuroscientists cannot understand how it was ever possible to doubt the validity of his theoretical maxims". Twenty-five years on, most would agree that Marr's recipe for investigating human vision and, in particular, his strategy of dividing the problem into different levels of analysis, has become unquestioned. At the time, Binford, Horn, Minsky, Papert, Rumelhart and others had been advocating computational modelling as a key to understanding the brain's operation but Marr brought a number of different approaches together, made testable predictions, provided a framework for tackling challenging neuroscientific questions and inspired a generation of young scientists to study the brain and visual processing.

Born in Essex, England, Marr studied mathematics at Trinity College, Cambridge, before doing his Ph.D. in what would now be called 'computational neuroscience' with Professor G.F. Brindley. His doctoral work, expressed in a series of three important papers [2–4], tied together detailed anatomical data on the cerebellum, neocortex and hippocampus within a computational framework. These are fundamental papers in the field, especially his paper on the cerebellum, but Marr now changed his focus to vision. He wanted to consider specific algorithms, and the constraints of the real world that made them tractable, rather than the processing of neural signals in general.

One of the central and best known ideas in his book is the suggestion that the visual system generates a sequence of increasingly symbolic representations of a scene, progressing from a 'primal sketch' of the retinal image, through a '2½D sketch' to simplified three-dimensional models of objects. In a paper with Ellen Hildreth [5], he proposed that information from cells tuned to different spatial frequencies (or scales) is combined into 'tokens' that are likely to correspond to real-world entities such as an edge. Although there is no convincing evidence that the particular type of combination Marr advocated is carried out in the visual system (other proposals have more experimental support [6]), it is a good example of Marr's approach. "In the theory of visual processes, the underlying task is to reliably derive properties of the world from images of it; the business of isolating constraints that are both powerful enough to allow a process to be defined and generally true of the world is a central theme of our inquiry" [1]. Today, this approach is normal practice in computer vision and at least a widely accepted mantra in biological vision research.

The tokens comprising the primal sketch were, Marr argued, then used as input to further processes such as object recognition [7]. Object recognition is one of several areas in which Marr's specific ideas about implementation have not survived well. The current focus in both computer and biological vision is on matching of high dimensional view-invariant descriptors of image features [8,9], taking a quite