synthesis in the mushroom bodies upon robust and chronic developmental expression as one would expect, although defects in axonal structures were observed. Thus, the RICIN^{CS} transgene used may lack the potency necessary for making strong conclusions about the requirement for normal protein synthesis. A control experiment performed to test the potency of RICIN^{CS} in blocking mushroom-body protein synthesis using the KAEDE reporter is questionable for two reasons. It remains unknown whether KAEDE expression offers a good surrogate for the hoped-for effect on the many endogenous proteins that are involved in LTM. In addition, the inhibition of KAEDE expression was monitored only in the cell bodies of the mushroom bodies. Protein synthesis underlying LTM is complex, being required in multiple cellular compartments, including the cell body, axons and dendrites. Whether the RICIN^{CS} toxin had sufficient efficacy across all compartments is unknown.

Finally, neurons are plastic and redundant. A large body of evidence has indicated that olfactory memory in insects is distributed across multiple nodes of the olfactory nervous system. There exist approximately 5000 mushroom body neurons, but only two DAL neurons. Mushroom bodies are remarkably resilient to insults. A genetic lesion that completely removes the vertical lobes leaves learning and memory after massed conditioning completely intact; one that removes the horizontal lobes leaves learning and memory after both spaced and massed conditioning intact [15]. Redundancy and/or plasticity of the mushroom bodies may allow insults to many of these neurons before phenotypic effects are observed, whereas insults to a node of the system comprising only two neurons would have immediately observable effects.

We believe that past and current evidence from multiple insect species favors a model in which cellular consolidation, including the requirement for new protein synthesis, occurs at multiple nodes within the olfactory nervous system, with systems consolidation — which has not yet been demonstrated in *Drosophila* — overlaying consolidation at the cellular level [16]. The discovery by Chen *et al.* [5] that the DAL neurons comprise one of the nodes in the olfactory nervous system required for olfactory memory in the fly could prove to be an important contribution to our understanding of the circuitry underlying LTM, but prior studies showing that these neurons are important for heat sensation [17] cast doubt on their specificity for memory formation. Most importantly, the negative result with regard to mushroom bodies requires additional experiments. Until then, as the Spanish proverb says, ''those you claim to be dead are in perfectly good health''.

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DOI: 10.1016/j.cub.2012.02.060

Homologous Recombination: How RecA Finds the Perfect Partner

How do two identical DNA sequences find each other during homologous recombination, amidst a 'sea' of unrelated DNA? New studies reveal how RecA promotes the search for homology by sampling DNA in three dimensions.

Kevin Hiom

Homologous recombination is an essential cellular process required, amongst other things, for the completion of DNA replication, the faithful repair of DNA damage and, in meiosis, for the genetic re-assortment that occurs during the production of gametes. In humans, defects in homologous recombination are directly associated with diseases, such as early onset breast and ovarian cancer [1], the haematological disease Fanconi's anaemi [2] and the premature ageing disorder Werner's syndrome [3].

The central component in homologous recombination is

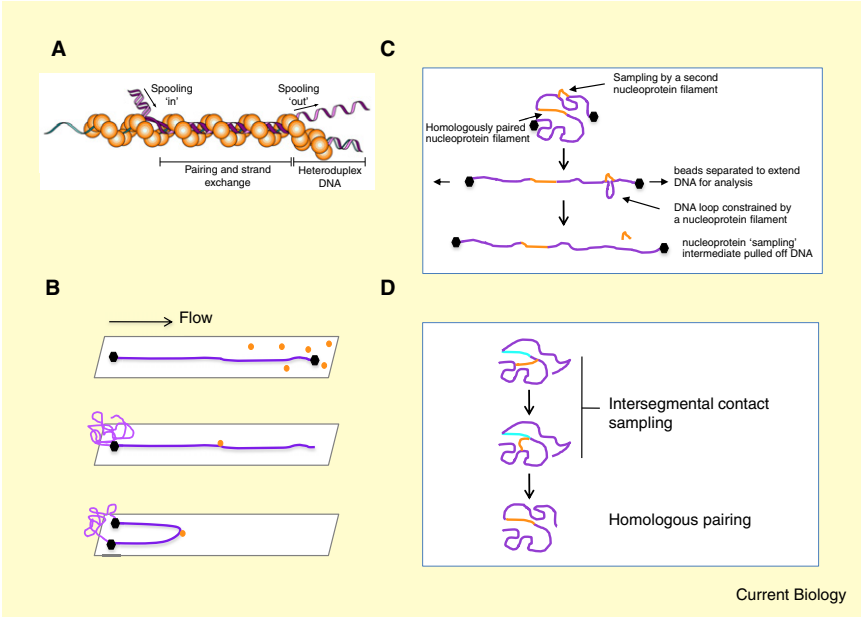

Figure 1. Homology searching by RecA.

(A) RecA (orange) forms a helical nucleoprotein filament on ssDNA. The ssDNA is stretched to 1.5 times its normal length to facilitate the search for homology. The nucleoprotein filament interacts with naked duplex DNA until homology is found, then strand exchange is initiated, producing a region of heteroduplex DNA. (B) Homologous pairing reactions were performed in a flow cell with double-stranded DNA tethered at both ends by a polystyrene bead (black hexagon) in an extended form (upper panel), singly tethered dsDNA, which can form a coiled conformation when the flow is stopped (middle panel) and dsDNA with ends tethered at various distances to enable three-dimensional conformation (dumbbell DNA; lower panel). Homologous pairing was indicated by the stable association of fluorescently-labeled RecA–ssDNA nucleoprotein filament with the dsDNA (orange dot). (C) Nucleoprotein filaments (orange) interact transiently with heterologous DNA at distant sites to form a 'sampling intermediate'. These intermediates may constrain loops within the dsDNA (purple) that are pulled off as the dsDNA is extended for analysis. (D) Model for intersegmental contact sampling. Nucleoprotein filaments interact with dsDNA to sample DNA sequences, which may be distant on the linear DNA molecule. These interactions are weak and transient. Iterative sampling by nucleoprotein filament leads to the identification of a region of DNA homology (blue) where homologously pairing occurs.

a recombinase enzyme, called RecA in bacteria and RAD51 in complex organisms. Whilst defects in RecA are often compatible with life, loss of recombinase function in higher organisms causes chromosome fragmentation and cell death [4]. RecA and Rad51 proteins are structurally and biochemically very similar, reflecting the highly conserved mechanism of homologous recombination in bacteria and man. Consequently, many of the paradigms for recombinase function have been established through study of RecA from *Eschericia coli* (reviewed in [5,6]).

The functional unit of recombinase is not monomeric RecA (or Rad51) but rather a helical nucleoprotein filament formed when RecA binds cooperatively to a region of single-stranded (ss)DNA [6]. This filament is assembled in a 5′ to 3′ direction along ssDNA and may eventually extend into regions of surrounding double-stranded (ds)DNA. Within the nucleoprotein filament, DNA becomes under-wound, or 'stretched' (approximately 1.5-fold), into a more open conformation [7].

Recombination is driven by the incorporation of a homologous DNA duplex into the nucleoprotein filament and the exchange of base pairing so that resident ssDNA becomes paired with the complementary strand of the incoming dsDNA to form a heteroduplex (duplex DNA made from DNA strands of different origin). This process is known as strand exchange and can continue along the nucleoprotein filament into neighbouring duplex DNA, where the reciprocal exchange of DNA strands results in the formation of a Holliday junction (Figure 1A). The completion of recombination involves the movement of the Holliday junction along DNA to extend the region of heteroduplex (branch migration) and its cleavage (resolution) into recombinant duplex DNA products (reviewed in [8]). These later steps require additional factors and will not be discussed further.

Up to this point our description of recombination has ignored a key question, which has remained unanswered for several decades. How does recombinase find homologous DNA with which to recombine, amongst the overwhelming excess of unrelated (heterologous) DNA sequences? A recent study by Forget and Kowalczykowski [9] has shed new light on this process, demonstrating that RecA promotes homologous pairing by three-dimensional sampling of DNA sequences.

Forget and Kowalczykowski [9] took advantage of a well-characterised strand-exchange reaction, mediated by RecA *in vitro*, to investigate homologous pairing on individual DNA molecules. Using fluorescently labelled ssDNA they assembled short RecA–ssDNA nucleoprotein filaments *in vitro* and mixed them with long pieces of dsDNA containing a small region of homology. Homologous pairing was visualised by tethering the dsDNA to the surface of a flow-cell through biotin molecules attached to the end of each molecule. In this way most of the dsDNA molecules were drawn into a linear conformation that was maintained in the presence or absence of flow. After washing away free nucleoprotein filaments Forget and Kowalczykowski [9] observed stable co-localization of fluorescent ssDNA at discreet locations on the DNA duplex that corresponded to regions of homology, confirming that homologous pairing had taken place.

To investigate homologous pairing in real time, Forget and Kowalczykowski [9] performed similar reactions but this time within the flow-cell, adding nucleoprotein filaments to dsDNA that was already tethered within the chamber (Figure 1B). Disappointingly, when the flow was stopped, the authors were unable to detect any homologous pairing. However, luck favours the prepared mind. They noticed that whilst most of the dsDNAs in the flow

cell were tethered at both ends, some were attached to the surface at only one end. When the flow was stopped to score the pairing of the doubly-tethered DNA, the singly-tethered DNA adopted a coiled conformation (Figure 1B, middle). Remarkably, when the flow was restarted it became apparent that these molecules, but not the extended DNA, had undergone homologous pairing with nucleoprotein filament. Seemingly, by extending linear duplex to almost 80% of its full length during the tethering process, Forget and Kowalczykowski [9] had interfered with an important component of the homology search. This result suggested that homologous pairing required DNA to be in a more three-dimensional conformation.

Forget and Kowalczykowski [9] modified their experiment to look at homologous pairing on dsDNA molecules with varying degrees of three-dimensional conformation (Figure 1B, bottom). They used dsDNA molecules with a 1 μM polystyrene bead attached at each end (dumbbell DNA) in a specialized flowcell and optical 'tweezers' to clamp the beads at varying distances apart, enabling the intervening DNA to fold into three-dimensional conformations of varying tightness. Sure enough, they found that when the beads were positioned only 2 μM apart, more than 90% of dumbbell DNA molecules had undergone homologous pairing. However, the efficiency of pairing diminished to almost zero as the distance between the termini of the 48 kilobase dumbbell DNA was increased to 8 μM. In their original experiment, where the duplex was almost linear, the distance between the DNA ends was 13 μM. Clearly the three-dimensional nature of the dsDNA alters the ability of RecA–ssDNA filament to find homologous DNA sequences. Also important is the length of the nucleoprotein filament. The rate of pairing for a nucleoprotein filament containing 1762 nucleotides of ssDNA being 3.8 times faster than a filament containing 430 nucleotides.

Forget and Kowalczykowski [9] were also able to detect *unstable* interactions formed between the nucleoprotein filament and its dsDNA partner. These interactions occurred outside the known regions of homology, persisted for only a few seconds and dissociated as the dsDNA molecules were moved between the flow cell and observation reservoir. In addition to the homologously-paired nucleoprotein filament, some dsDNA appeared to have a second heterologously bound filament at a distant site. Furthermore, as the beads were separated to extend the dsDNA molecules for analysis, the authors detected an abrupt jump in the homologously-paired fluorescent spot associated with release of the heterologous filament. They inferred that these 'unstable' nucleoprotein filaments probably bridge distant sites on the DNA transiently, constraining a loop in the DNA, which was broken as the DNA was extended (Figure 1C).

To account for their results, Forget and Kowalczykowski [9] propose a model in which the RecA nucleoprotein filament searches for homology by sampling local DNA sequences, which may be distant along the linear sequence of the dsDNA, but which are considerably closer in three dimensions. Presumably this search is iterative, with unstable interactions being made and lost until homologous sequences are found, and stable pairing and strand exchange ensue. They named this process 'inter-segmental contact pairing' (Figure 1D).

Interestingly, Charles Radding [10] outlined a similar mechanism more than twenty-five years ago. He found that homologous pairing between nucleoprotein filaments (referred to as pre-synaptic filaments) was promoted by the addition of long heterologous duplex DNA and suggested that co-aggregation of the RecA filaments with dsDNA in a small volume accelerated the search for homology by local sampling. He referred to this mechanism as 'facilitated diffusion'. Stephen Halford [11] invoked a similar mechanism to explain how restriction enzymes find their specific binding sequence, commenting that a one-dimensional translocation probably has utility within a region of 50 base pairs or less. The prescience of Radding's observations is now confirmed by the elegant single-molecule studies of Forget and Kowalczykowski [9], which establish the importance of three-dimensional sampling in the search for homology.

It is interesting to speculate how such a homology search is achieved at the level of the atomic bond. Does sampling involve complete exchange of many several base pairs of DNA within a stretch of nucleoprotein filament (akin to strand exchange) or does it involve intermediate bonding, formed simultaneously between the bases of both the incoming duplex and the unpaired ssDNA? It will also be of interest to determine the consequences of non-complementary bases positioned within an otherwise homologous sequence on the efficiency of the homology search.

History suggests that the inter-segmental homology search observed for RecA will undoubtedly inform us on the equivalent process mediated by Rad51 in human cells. The inter-segmental model already provides a very clear explanation for why, in diploid organisms, recombination primarily occurs between sister-chromatids, which are brought in close three-dimensional proximity prior to mitotic cell division. Conversely, pairing between homologous chromosomes, which are distant in three-dimensional space, is much less likely, except during meiosis where homologues are brought together to promote crossing-over.

Clearly in the molecular dating game, it appears that a series of dates with random members of the local community is much more efficient in locating the perfect partner than systematic introductions by immediate neighbours.

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DOI: 10.1016/j.cub.2012.03.002

Palaeontology: The 165-Million-Year Itch

New flea-like fossils from China provide a rare, tantalizing glimpse of bizarre insects in the Cretaceous and Jurassic. Possibly the oldest flea-like animals known, they provide a challenge to the functional morphologist to infer which animals they may have targeted.

George O. Poinar, Jr.

Who hasn't been bitten by a flea, whether it came from a dog, cat or some wild animal. Perhaps you were lucky and avoided an allergic reaction, or scratching didn't result in an infection. If you were living in Europe in the 14th century, however, a flea bite could easily have meant an infection of the bubonic plague (Black Death) and your chances of survival would have been slim. This disease claimed the lives of over 75 million humans since it was first recorded in AD 541, more lives than were presumably lost in all previous wars [1]. If you detest our fleas that are only a few millimetres in length, imagine what it would have been like to be bitten by one of the giant Mesozoic flea-like insects reported by Tai-ping Gao and his colleagues in this issue of *Current Biology* [2]. These fossils are surprisingly similar to those recently reported from the same Chinese localities by a separate team of scientists [3].

To Know a Flea

True or modern fleas are small (1–8 mm long), wingless insects with laterally compressed, heavily chitinized bodies. Their legs have enlarged coxae for jumping. They have short three-segment antennae hidden in grooves on the side of their head. All fleas are adapted to feeding on warm-blooded vertebrates: 94% of the over 2,300 known flea species attack mammals while the remainder feed on birds [1]. Some fleas are quite specific for their host species, while others, like the cat flea that also attacks humans, can change hosts readily. The unusual body form of the fossil insects suggests that they also may have attacked a specific group of vertebrates, but which one remains a mystery.

How long have fleas existed? Recognizable true fleas of the Order Siphonaptera have been around for at least 40–50 million years and occur in both Baltic and Dominican amber (Figure 1). However, no fleas with modern features have been found in older Mesozoic (~65–250 mya) deposits. Extant fleas are thought to have evolved from winged scorpionflies (Mecoptera), a group of medium-sized insects with elongated bodies and long tubular mouthparts, the adults of which are considered nectar feeders. However, some Early Cretaceous (100 mya) scorpionflies have long pointed mouthparts bearing rows of fine serrations, suggesting that these lineages already fed on blood [4]. Such scorpionflies may have been an early lineage leading to the true fleas.

The large, soft-bodied flea-like fossils described by Gao *et al.* [2] are wingless, have reduced eyes and short, beaded antennae. Both species look quite similar, even though *Pseudopulex jurassicus* lived in the Jurassic some 165 mya (Figure 2) and *Pseudopulex magnus* survived later in the Cretaceous about 125 mya. Even though these fossils from Inner Mongolia share some characters with true fleas — such as the absence of wings, relatively small thorax and a body covering of stiff, posteriorly-directed setae — there are several significant differences: first of all, the flea-like fossils are much larger than extant fleas, measuring from 17 mm to nearly 22 mm in length (most fleas are under 6 mm in length) and their mouthparts are much larger than those of fleas. The bodies of these fossils are dorsal-ventrally flattened and their antennae are exposed with 14–17 segments, while modern fleas have short, hidden antennae with only three segments. The long legs of the fossils are not modified for jumping and their powerful proboscis is quite broad and coarsely serrated, not narrow and finely serrated as with true fleas. This is why Gao *et al.* [2] label them 'flea-like', placed them in a new family Pseudopulicidae and acknowledged that the order is uncertain (*incertae sedis*) [2].

Figure 1. A true flea.

A modern-looking flea in 20–30 million year old Dominican amber. All fleas from the Tertiary closely resemble modern fleas and can usually be placed in extant families or genera. Note the laterally compressed body, powerful jumping hind legs and concealed antennae (the articles extending down from the small head are the mouth palps).