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Brief report

RecA-DNA filament topology: The overlooked alternative of an unconventional *syn–syn* duplex intermediate

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ABSTRACT

The helical filaments of RecA protein mediate strand exchange for homologous recombination, but the paths of the interacting DNAs have yet to be determined. Although this interaction is commonly limited to three strands, it is reasoned here that the intrinsic symmetry relationships of quadruplex topology are superior in explaining a range of observations. In particular, this topology suggests the potential of post-exchange base pairing in the unorthodox configuration of *syn–syn* glycosidic bonds between the nucleotide bases and the pentose rings in the sugar–phosphate backbone, which would transiently be stabilized by the external scaffolding of the RecA protein filament.

1. Introduction

Next to the semiconservative mode of replication, the capability of recombining with another molecule of corresponding sequence is a fundamental property of DNA. RecA-like proteins provide the recombinase function for homologous recombination to drive the initial strand exchange reaction in all organisms [1,2]. These proteins belong to the RecA–AAA$^+$ superfamily of ATPases, which self-assemble in the active state with ATP and convert chemical energy into conformational change. RecA of *Escherichia coli* is typical for these recombinases, and most structural work has been devoted to this particular protein.

RecA-type recombinases administer the proverbial search for a needle in the haystack: in trying to locate the perfect match for a given DNA sequence of typically 100 nucleotides or more, they have to shift through potentially all the different sequence tracks of similar length throughout a complex genome. This means that literally thousands of unproductive encounters have to be assessed rapidly and reversibly, before a track of sufficient sequence homology allows the strand exchange reaction to be completed irreversibly.

In vitro, RecA subunits with ATP assemble readily as helical filaments on single-stranded (ss) DNA. In this activated filament, a duplex DNA of any sequence can be incorporated as well. This interaction is transient for nonhomologous sequences, but in the case of a perfect match, strands are being exchanged according to the following formula:

$$\mathrm{IN} + \mathrm{COM{::}OUT} \rightarrow \mathrm{IN{::}COM} + \mathrm{OUT}$$

Therein, the invading strand (IN) from the RecA filament displaces the outgoing strand (OUT) from the homologous

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duplex, and the complementary strand (COM) to either one changes base pairing from one to the other. The substrate and product molecules are commonly referred to as homoduplex and heteroduplex, respectively. In contrast to ssDNA, a single duplex associates with RecA rather slowly, as limited by inefficient nucleation. However, if dsDNA is mechanically stretched beforehand, it rapidly forms a RecA filament as well [3]. Also, once nucleated by 4–5 ATP–RecA monomers, additional subunits assemble more readily on duplex DNA as well [4,5].

So far, the molecular coordinates have been worked out mainly for the RecA protein residues, but the exact paths of the participating strands of DNA in the central void of the filament are not yet known. Early models were subject to multiple degrees of freedom, which subsequently got constrained by new results. In fact, none of the models existing in the current literature appears to be fully compatible with all the relevant experimental data. Hence, some re-evaluation from first principles may be appropriate. Basically, the DNA in the helical RecA filament is right-handed as usual, but it is stretched by 50% and partly unwound by 40% with respect to B-form DNA [6,7]. Each RecA subunit covers three nucleotides, and about six subunits complete a turn of the helical filament. As each subunit only touches its nearest neighbors on either side, a furrow is left open between the turns of the ribbon, through which the central void can be entered from outside. Characteristically, one of the furrow rims is tiled quite smoothly by the protein subunits, while the other rim shows a pronged appearance, as formed by the protruding C-terminal domains of RecA. The stretching imposed upon the DNA effectively unstacks some of the base pairs, which is important for the activated intermediate to transiently disengage from base pairing in the original duplex. The RecA subunits are assumed to contact the sugar–phosphate backbone, rather than the bases [8], and they appear to bind a duplex from the minor groove [9,10].

Well before the era of RecA filaments, an imaginative and far-sighted structure was proposed for the possible strand exchange between two fully intertwined duplexes of DNA, as demonstrated by a space-filling model for the presumable pre-exchange complex [11]. A similar structure was also proposed for the post-exchange complex [12]. However, the remarkable quadruplex symmetry of these structures fell into disregard when it became clear that the RecA-driven exchange reaction primarily operates with three strands of DNA, not four [13]. Instead, subsequent model building concentrated on the assessment of various triple-helix arrangements, as stabilized by supplementary hydrogen bonds [14,15]. Notably, all current models of strand exchange are based on the lateral extrusion of bases from the COM strand. Such a movement within the plane of the original base pair cannot be performed without considerable buckling of the backbone, although part of this strain can be absorbed by changing the puckering of the pleated pentose rings [16]. In the triplex models the invading strand could enter the homologous duplex either from its major groove (preferred originally) or the minor one. This ambiguity seemed to be resolved by the finding that the outgoing strand left the duplex from the major groove [17]. Hence, it was indirectly concluded that the invading strand thus had to enter from the minor groove, which is not readily compatible with the presumed binding of RecA protein to the minor groove as well. This is because a protein domain that binds to DNA across the minor groove is physically blocking that side of the binding site from interacting with another DNA, so that potential strand exchange could only be initiated from the major groove. Furthermore, according to recent analyses of strand exchange kinetics by fluorescense energy transfer, the relevance of hydrogen-bonded triple helixes as recombination intermediates has been questioned altogether, preferring the direct exchange of base pairing instead [18].

Moreover, a very informative experiment revived the possibility that four strands of DNA could simultaneously interact within a given RecA filament, provisionally referred to as recombination *in trans* [19]. When a RecA filament was preassembled on single-stranded DNA of a given sequence and supplemented with a nonhomologous duplex DNA, this duplex was activated for exchange with a homologous fourth strand, which was added last to the established filament. However, it very much depends on the topological arrangement whether the fourth strand actually approaches the duplex from the other side (the opposite groove "*in trans*"), with regard to the first single strand in the filament. According to earlier suggestions, the strongest DNA binding site, which is presumed to accommodate the invading single strand, has intuitively been placed deepest in the central furrow of the RecA filament [1,15]. Both strands of the homologous duplex could then straddle the furrow from rim to rim. In such an arrangement, the extra fourth strand would indeed approach the duplex from the opposite groove, as seen from the first single strand. Careful cross-linking studies, however, have located the single strand in the primary filament close to the smooth rim of the furrow [20]. Hence, the deepest site in the furrow is probably accommodating the complementary strand for the strand exchange reaction. The alternative model given below accounts for this condition, and both single strands mentioned above would approach the duplex from the same side.

2. Base swivelling to a RecA-supported *syn–syn* duplex—a reasonable alternative to the traditional model?

As to the four-fold symmetry of intertwining duplexes [11], a certain aspect is particularly suitable to facilitate the reversible exchange of base pairing in a stretched and unstacked recombinational intermediate. This concerns the glycosidic bonds between the nucleotide bases and the pentose rings in the sugar–phosphate backbones. Notably, all these bonds are diagonally oriented in equivalent positions. Hence, if the interacting strands in a cross-section of the RecA filament were externally constrained to three corners of a square, the complementary strand at the central position could readily assess the possibility of base pairing to either side by swivelling its base residues by 180° around the glycosidic bonds (Fig. 1). In such a configuration, the extra *trans*-strand could take the empty seat at the fourth corner, which should be readily accessible between the furrow rims. Incidentally, the flipping of bases by 180° would alternate between the sterical "*anti*" and