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Tangle analysis of DNA unlinking by the Xer/FtsK system

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要旨：DNA に作用する部位特異的組み換え酵素の働きは、タングルを用いてモデル化され、得られるタングル方程式を数学の結果を用いて解くことにより、部位特異的酵素の働きのトポロジーの特徴付けが出来る。ここでは、Grainge 等 (2007) によって示された部位特異的 Xer/FtsK システムによる DNA 絡み目解消操作の解析を行い、その特徴付けを述べる。特にその作用を特徴付ける主要なタングルが有理タングルとなることを示す。

Abstract : The action of site-specific recombinases can be analyzed using the tangle method, where the reaction is characterized topologically by solving the corresponding tangle equations. We here analyze unlinking of DNA catenanes by the site-specific recombination system Xer/FtsK (Grainge *et al.*, 2007). In particular we show that the key tangle involved in this reaction is rational. Therefore all solutions to the tangle equations can be computed using tangle calculus.

1 Introduction

Topological methods, both computational and analytical, have been successfully applied to the study of enzymes acting on DNA (*e.g.* [2]). Knot theory and low-dimensional topology, and in particular Dehn surgery theory, have been helpful in characterizing topological mechanisms of enzymes such as site-specific recombinases (*e.g.* [4],[6], [7], [9], [19], [21]).

DNA topology is the study of geometrical and topological properties of circular DNA and of the processes that affect them [3]. The two DNA strands in the *Escherichia coli* chromosome are inter-wrapped approximately 420,000 times. The DNA double-helix must be unwound in order to be copied and thus replication of the bacterial chromosome results in two molecules which are non-trivially linked. This creates the topological problem of separating the components of the link prior to cell division. This problem is largely solved by type II topoisomerases, but more recently Grainge *et al.* [12] argued for a role of the Xer-FtsK recombination system in resolution of replication catenanes. We here report on the topological characterization of the mechanism of DNA unlinking by the Xer-*dif*-FtsK system. This work uses a recent result on the twisting of links.

A basic reference of DNA topology is [3]. Applications of knot theory to studies of DNA topology are overviewd in [1], [15]. See [2] for recent studies.

2 Tangle method for site-specific recombination

Certain enzymes are able to change the topology of DNA. These changes can be observed experimentally by incubating circular DNA substrates with the chosen enzyme. The product

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topology is detected experimentally using gel electrophoresis or electron microscopy [3]. Here we use the tangle method to analyze the process of DNA unlinking by Xer recombination when coupled with the translocase FtsK. In a site-specific recombination event two specific sites (short DNA segments) are recognized and bound by the enzyme. The sites are then cut and the ends recombined.

The tangle method is a topological method based on knot theory and low-dimensional topology whereby a site-specific recombination reaction is translated into a system of 2 or more tangle equations [9]. Solutions to the equations correspond to the possible topological mechanisms for the enzymatic reactions. The tangle method has been used to characterize the action of several site-specific recombinases (*e.g.* [6], [19], [21]).

Terminologies of knots, links, and tangles used in this paper can be found in [1], [15]. Here *2-string tangle*, or simply *tangle*, refers to a pair of a ball with two strands inside it. *Rational tangles* are the most simple class of tangles. See the Figure 1 for examples. There is a one-to-one correspondence between the set of rational tangles and $\mathbb{Q} \cup \{1/0\}$. A rational tangle can be untangled by a finite number of twistings.

The *numerator* $N(T)$ of a tangle T is a knot or link obtained by connecting two endpoints of T on the top and two endpoints on the bottom by arcs as illustrated in Figure 2. A new tangle can be obtained from two tangles S and T by connecting two endpoints of S to two endpoints of T as is shown in Figure 2. This is called the *sum* of S and T , and is denoted by $S + T$.

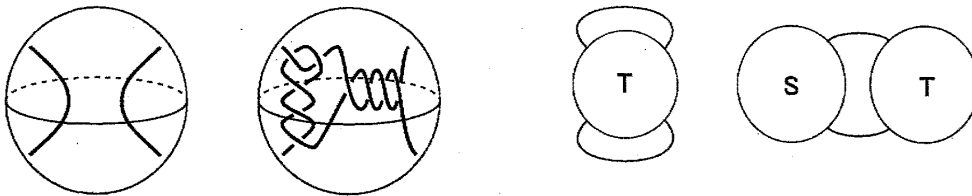


Figure 1: Rational tangles.

Figure 2: The numerator and the tangle sum.

In the tangle method the synaptosome consisting of the enzymes bound to the recombination sites can be modeled as a tangle E . The following biologically reasonable assumptions are needed.

1. The enzymatic mechanism is constant and independent of the topology of the substrate. The action of binding and strand-exchange occurs in the tangle E . In the model $E = O_b + P$, where O_b is determined by the binding, and recombination (strand-exchange) happens inside P . The tangle O_f , which is the exterior of E , and O_b are unchanged during the recombination. Let $O = O_f + O_b$ be the constant outside tangle.
2. The tangle P is rational, and recombination converts P into another rational tangle R .

Thus a recombination reaction can be considered as a rational tangle surgery of knots and links. Furthermore, the piece of DNA contained in the tangle P is short (32bp for Xer system [18]), which implies that the tangles P and R cannot be very complicated, thus justifying the assumption 2.

Suppose the substrate has the knot or link type K_1 , and the product is of type K_2 . Then we have the following tangle equations as in Figure 3:

$$N(O_f + O_b + P) = N(O + P) = K_1$$

$$N(O_f + O_b + R) = N(O + R) = K_2$$

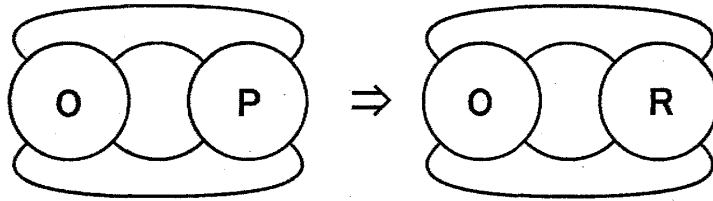


Figure 3: A tangle model of recombination.

If we consider the double branched covering of these rational tangle surgeries, we have Dehn surgeries on knots in 3-manifolds. In general, substrates and products of recombination are 2-bridge knots or links, and thus their double branched covering spaces are lens spaces. For this reason, results on Dehn surgeries such as [5], [13] are valuable in the analysis of site-specific recombination reactions [9], [19], [21].

3 Unlinking DNA catenane by Xer/FtsK system

In [12], the Xer-*dif*-FtsK system was shown to efficiently unlink DNA catenanes formed *in vivo* by DNA replication. There is experimental evidence that right-handed $T(2, m)$ torus links ($2m$ -cats) are unlinked by a stepwise reaction which gradually converts the $2m$ -cat into the unlink, namely, $2m$ -cat, RH $(2m - 1)$ -torus knot, $(2m - 2)$ -cat, \dots , RH trefoil, Hopf link, trivial knot, trivial link, as in Figure 4 [12].

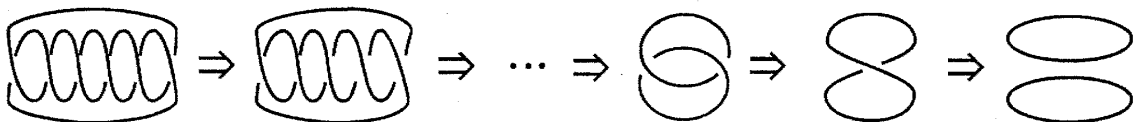


Figure 4: Stepwise unlinking model of a 6-cat by XerCD-FtsK.

4 Iterative recombination and tangle analysis

In this section we introduce an iterative recombination model and report on the topological characterization of unlinking using that model. It is widely accepted that tyrosine recombinases such as those in the Xer system do not act processively. Here we assume “resetting steps” after each strand-exchange which allow the Xer system to act iteratively. In this model the 0-tangle is changed into some integral tangle, i.e. a tangle of horizontal twists.

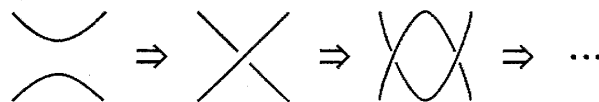


Figure 5: Iterative recombination. After each step a resetting step occurs and the Xer/FtsK system acts iteratively.

The tangle model of iterative recombination can be obtained by setting $P = (0)$ and $R = (k)$, where k is some integer. By assuming this iterative recombination, we give a topological characterization of unlinking by Xer/FtsK system.

Theorem. *Suppose $N(O + P)$ is a $2m$ -cat $T(2, 2m)$ and $N(O + R)$ is a trivial link. If $P = (0)$ and $R = (k)$ for some integer k , then O is rational.*

The detailed explanation of this iterative recombination and the proof of this theorem will appear elsewhere [17].

In the proof we use a method developed by Kobayashi[14] and deep theorems by Gabai[10][11].

Knowing O rational allows to compute all the possible solutions to the Xer-FtsK tangle equations by using mathematical software such as TangleSolve [20][16] or TopoICE-R [8] as shown in [12].

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