Changes in the Topology of DNA Replication Intermediates: *In vivo* vs *In vitro*

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Abstract

Most of the methods used to analyze DNA, including electrophoresis, electron microscopy or atomic force microscopy, involve de-proteinization, and it is well known that the removal of proteins affects DNA topology. After de-proteinization *in vitro*, the topology of replication intermediates changes significantly. A comprehensive analysis of the topological changes introduced during DNA isolation (de-proteinization) is important to get a better understanding of DNA topology *in vivo*.

The topology of replication intermediates examined by electrophoresis, electron microscopy or atomic force microscopy *in vitro* does not necessarily represent the situation *in vivo*.

The un-replicated and replicated regions as independent topological domains *in vivo*

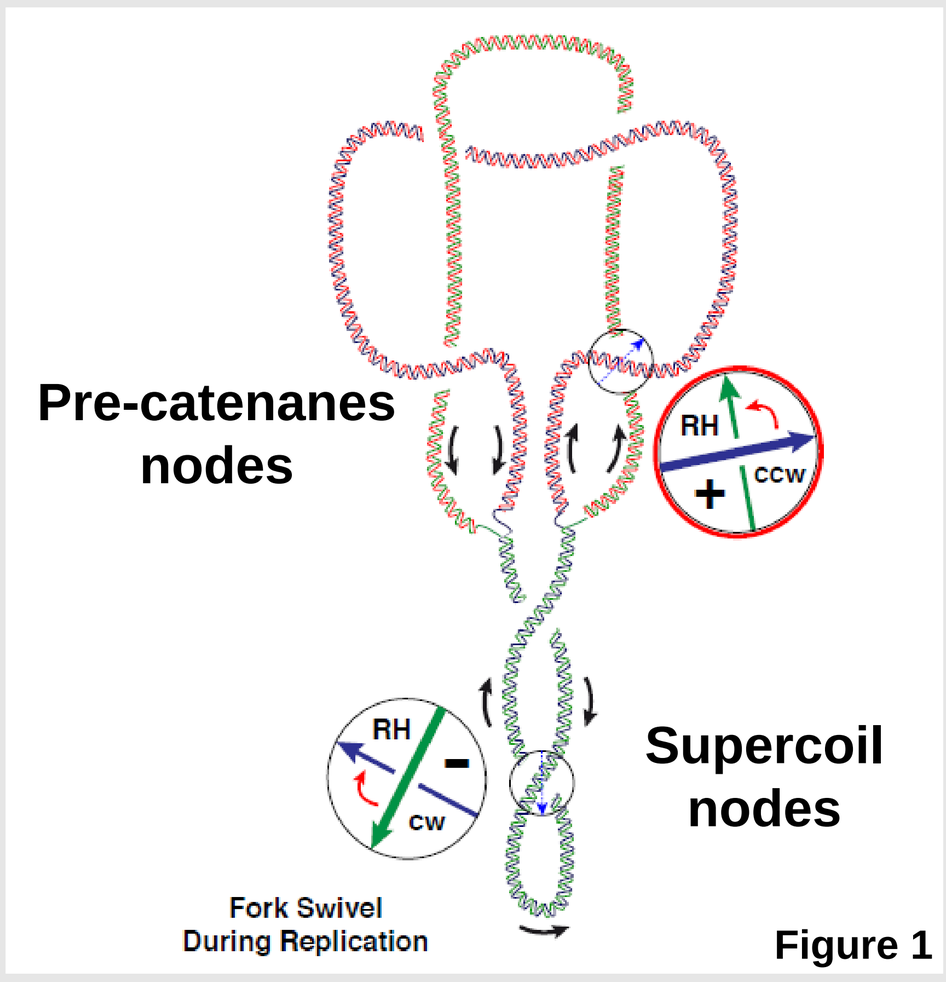

Figure 1

In partially replicated bacterial plasmid the LH supercoil crossings that transiently accumulate immediately ahead of the forks migrate to the replicated region as RH pre-catenane crossings. In the un-replicated region, the combined action of Topo IV, DNA gyrase and swivelling of the forks always kept the un-replicated region with RH supercoiling (**Figure 1**).

The topology of replication intermediates changes after de-proteinization *in vitro*

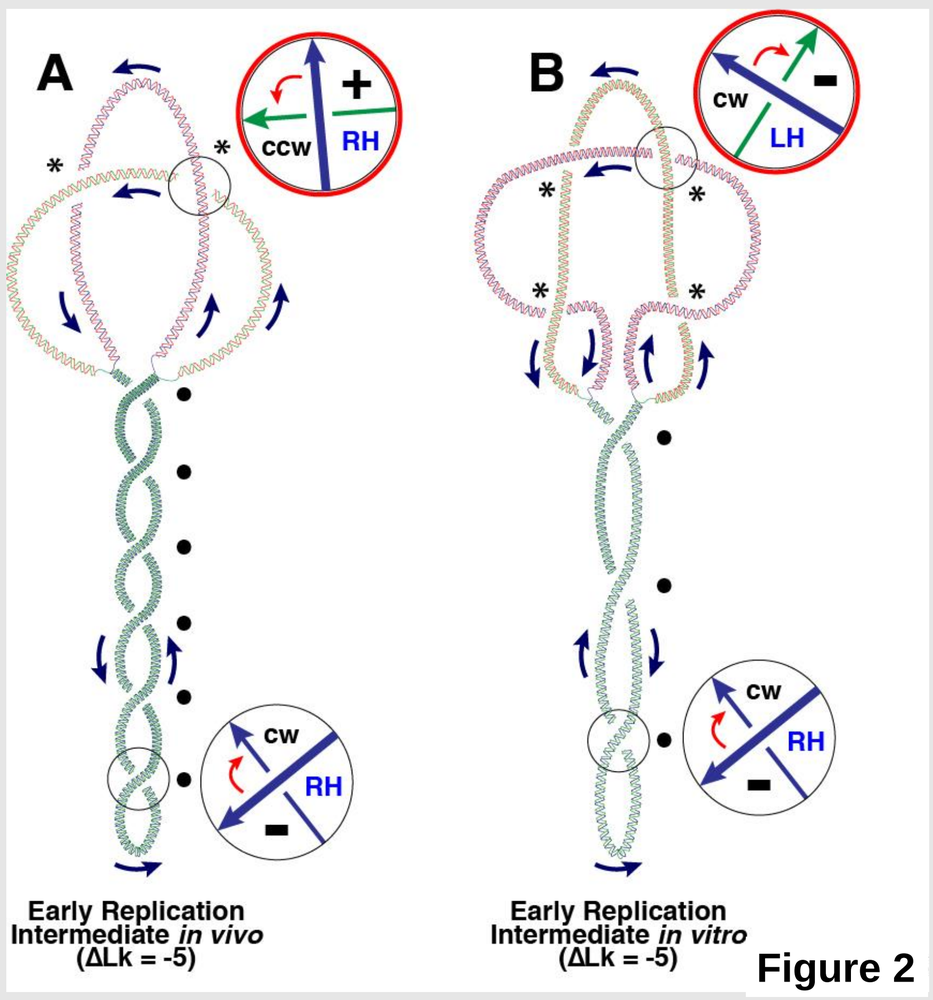

Figure 2

At **early stages** of replication most of the molecule remains un-replicated and negatively supercoiled showing RH crossings with a (-) sign (**Figure 2A**). The small replicated region may show a few RH pre-catenane crossings with a (+) sign. After de-proteinization, once thermodynamic equilibrium is achieved, the molecule ends-up showing RH crossings with a (-) sign in the un-replicated region, and LH pre-catenane crossings with a (-) sign in the replicated one (**Figure 2B**).

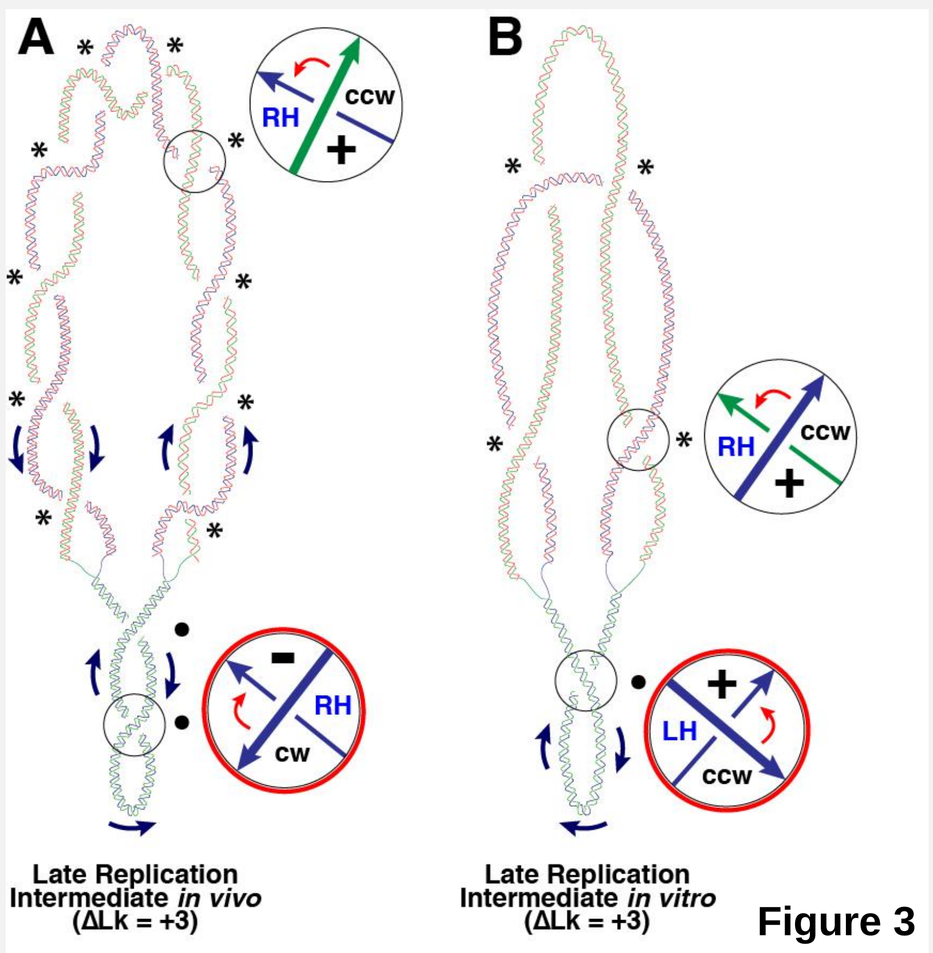

Figure 3

At **late stages** of replication, however, most of the molecule have already been replicated. There are few RH crossings with of a (-) sign in the un-replicated region and most of the crossings are RH pre-catenanes with a (+) sign (**Figure 3A**). When de-proteinized, the molecules may end-up with some LH crossings with a (+) sign in the un-replicated region and RH pre-catenane crossings with (+) signs in the replicated one **(Figure 3B)**.

The topology of DNA replication intermediates after de-proteinization *in vitro* does not reflect the situation *in vivo*. These observations may have important experimental consequences.

It will be of interest to examine how these changes in the topology of replication intermediates after de-proteinization affect the efficiency of type II DNA topoisomerases on partially replicated bacterial plasmids *in vitro*.

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