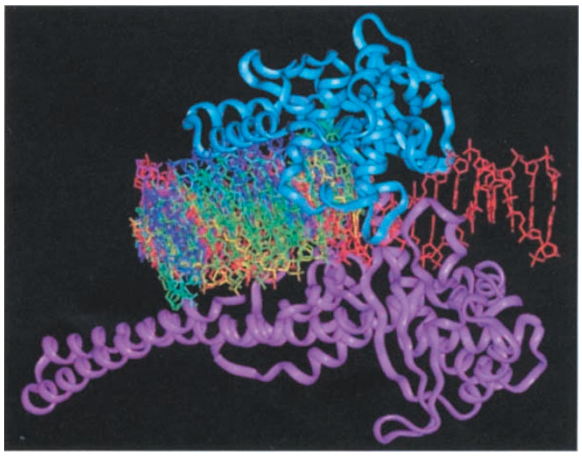
A long unwinding road



DNA would become a tangled mess during cellular processes such as transcription and replication without the aid of the topoisomerase enzymes, which affect supercoiling by breaking and resealing DNA. Monomeric type I enzymes nick only one DNA strand and change the linking number in single steps whereas the dimeric type II enzymes break both DNA strands, and thus allow linking number changes in steps of two. Yet, within the type I group, the prokaryotic and eukaryotic enzymes do not have sequence or structural similarities, and their reaction mechanisms differ in several ways, including their template requirements. Recently, three structures of active fragments of human topoisomerase I have been reported in both noncovalent and covalent complexes with a 22 base pair DNA duplex — complexes that either do not begin the nicking or do not complete the resealing reaction because mutants of either the protein or the DNA respectively were used (Redinbo *et al.* Science **279**, 1504–1513 (1998); Stewart *et al.* Science **279**, 1534–1540, (1998)). In addition to being the first eukaryotic topoisomerase I to have its structure determined, this protein is the sole target of the camptothecin familiy of anticancer drugs which act by stabilizing the nicked reaction intermediates, which in turn presumably block transcription and replication complexes.

Human topoismerase I wraps completely around the DNA. The picture (kindly provided by M. Redinbo, L. Stewart, J.J. Champoux & W.G.J. Hol) depicts a noncovalent complex, showing the catalytic domain (magenta) with the extended linker helices coming off to the left, the cap domain (blue), the DNA upstream of the cleavage site (red; as observed in the three crystal structures), and the DNA downstream of the cleavage site (shown in a multitude of colors with each color representing the duplex rotated in 30° increments). However, the DNA probably cannot rotate freely around only one of the five rotatable bonds in the sugar phosphate network near the cleavage site because, according to their model, steric conflicts between the enzyme and the DNA would occur. Instead, these groups suggest that the DNA is flexible and can rotate in smaller, more 'controlled' steps around many bonds (one type of bond at a time), to avoid negative interactions with the protein. In the picture, to mimic this flexibility, they allow the DNA to rotate around a pseudobond centered roughly on the 3' bridging oxygen atom of a phosphate on the intact strand near the cleavage site.

How is the downstream DNA rotation regulated in this model? The enzyme–DNA contacts mainly involve phosphates and are limited to the 10 base pairs surrounding the central cleavage site. However, although they do not interact directly with the DNA, the DNA-proximal portions of the linker helices are highly positively charged, as are the DNA-proximal sides of the two small 'nose cone' helices (visible on the left of the cap domain in blue above the multicolored rotating DNA). Thus, perhaps the various rotation events are stabilized by the positively charged linker and 'nose cone' helices. Such participation of the linker region in this 'controlled rotation' model is reasonable since the linker enhances but is not required for activity. **TLS**

A road not taken

I enjoyed the editorial sketching the history of model building[1]. However, I must set the record straight about Alwyn Jones's ill fated sojourn to Chicago. I was awakened by a phone call on the Sunday he was to have arrived and was surprised to hear Alwyn's voice as I expected him to be en route to Chicago. When I asked him why he decided not to travel, he responded in that inimitable Welsh accent, "Paul, I have fallen in love with a girl from Munich." Of course, I forgave Alywn *on the spot!* Who am to oppose such forces? So, you see, the rumor [that I have not forgiven him for abandoning his plans to do postdoctoral research with me] is not true. Creating Frodo probably did more for structural biology than what we had planned to do, that is to solve the folding problem. Ah, the best laid plans.

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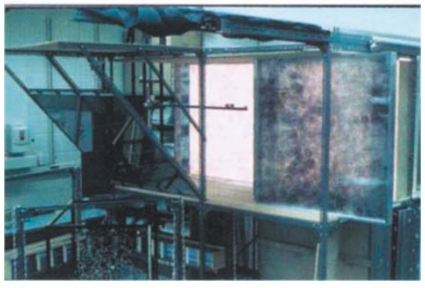

Fig. 1 An example of an optical comparator, the devices — often called 'follies' — used to project density maps so that molecular models could be built using wire. These were the immediate precursors to Frodo, the program written by Alwyn Jones that allowed computer modeling. Shown here is the Yale 'folly'. In addition to the model, lights and mirror, the mounting of the map to permit easy selection of the sections to be viewed can be seen. The whole device was quite inexpensive compared to the computers which replaced it. However, the lab floor space required to accommodate it and the equivalent space for a PC are quite different. At $300–$400 per foot, part of the price difference is removed by the space charges.

1. Editorial, *Nature Struct. Biol.* **4**, 961–964 (1997).