Chapter 8

OUTLOOK

The high resolution structural data provided by the cocrystal structure of the $(Z\alpha)_2$/Z-DNA complex [6] and the solution structure of unbound Zα, in conjunction with the functional data obtained from scanning mutagenesis and biochemical binding studies, open up the door for further experiments. The role of the second Z-DNA binding domain at the N-terminus of ADAR1, Zβ, for binding to substrate DNA is not well understood. In vitro binding assays showed that Zβ alone possesses only low affinity to Z-DNA, but native or biosynthetic Zα-Zβ constructs bind Z-DNA as tight as Zα alone [15,211]. Thus, further structural and cell biological studies are required to shed light on the role of Zβ for determining DNA substrate specificity, and for potential effects on the regulation of ADAR1-mediated RNA editing *in vivo*.

Presently, dynamic DNA and RNA structures, such as Holiday junctions and antisense triplexes, are intensively studied to improve the efficiency of homologous recombination and antisense therapy. The molecular mechanism of the transition from B- to Z-DNA is of interest because it provides insight into how supercoiling and twisting affects the dynamics of double-stranded nucleic acids. The Zα mutant Y177A showed an intermediate B- to Z-DNA conversion in CD experiments (fig. 26). Thus, a suitable Zα mutant, such as Y177K, which does not reduce the affinity to Z-DNA as strongly as Y177A, may be useful for trapping the transition state between B- and Z-DNA, thereby allowing high resolution structural analysis by X-ray crystallography. This strategy is similar to the usage of transition state antibodies for organic chemical catalysis.

The structural and functional information about the Zα/Z-DNA interaction can also be used to guide *in vivo* experiments. Commonly, the biological function of a novel protein domain or gene is investigated by loss-of-function mutants. Such a mutation or deletion has to be highly selective for the potential function under study because otherwise intricate non-informative phenotypes will arise. In the case of Zα, residues N173 and W195 diminished the affinity to Z-DNA most dramatically when mutated to alanine, rendering them prime candidates for loss-of-function mutations. W195 is difficult to replace because it plays a pivotal role for stabilizing the hydrophobic core of Zα. The structural information allows one to substitute W195 rationally so that the hydrophobic core of Zα is retained. This may be used to design an „intelligent“ mouse knock-out which will ultimately clarify the biological relevance of the Z-DNA binding domains of ADAR1.

In a second step, the Zα domain may be engineered to bind to B-DNA rather than Z-DNA, as all of its homologues do. According to the aforementioned model of steric hindrance, helix α1 of Zα can be shortened and the prehelix can be deleted in order to abrogate steric hindrance. This would confirm or dismiss the model of steric hindrance, and allow one to retarget ADAR1 within the nucleus of a cell, thereby testing the hypothetical model that links RNA editing and targeting of ADAR1.

Another approach to identify binding sites of Zα *in vivo* is UV cross-linking which has been shown to be successful with anti-Z-DNA antibodies in permeabilized nuclei [100]. It is key for substrate specific cross-linking that the chemically active moieties are close to the substrate, but not to other material within a cell. Therefore, Y177 and W195 of Zα, which form an edge-to-face contact with a guanine base of Z-DNA [6], are suitable targets for photo-activated cross-linking. It has been shown that tryptophan can be biosynthetically replaced with hydroxy-tryptophan to customize the UV-absorbance profile of a protein. By a similar biosynthetic route, photo-reactive groups, such as radical starters, may be introduced to Y177 or W195 of ADAR1 making them highly specific for bound substrate DNA and responsive to mild UV laser doses. This cross-linking approach may uncover new RNA editing templates for ADAR1, or alternatively, other sites of action or storage for ADAR1.

Finally, the knowledge about the structure and function of crucial Z-DNA contacting residues can be utilized to design an „intelligent“ training set for searches in both structural and genomic databases. This may lead to a prediction program for Z-DNA binding protein domains, and thus to the discovery of further Z-DNA binding protein domains. New Z-DNA binding proteins open up the door for new biological test systems which may ultimately allow one to provide compelling evidence for biological roles of Z-DNA *in vivo*.