INTERNATIONAL PEACE FOUNDATION

(Feb. 5 -12, 2004) Bangkok

THE DISCOVERY OF BASE FLIPPING

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DNA methyltransferases play important roles in many biological processes. In eukaryotic cells there are many enzymes that form 5-methyl-cytosine in DNA and they appear to play an important role in a variety of epigenetic phenomenon. For instance, the human DNA methyltransferase, Dnmt1, methylates cytosine residues within the dinucleotide CpG and when these sequences are located within promoter elements, this methylation can lead to gene silencing. My laboratory has been long interested in the DNA methyltransferases that form a part of restriction-modification systems. Here the function of the methylation is to protect the bacterial DNA against the action of its own restriction enzyme. In this case, three different kinds of methylation have been found to provide protection, either 5-methylcytosine, N6-methyladenine or N4-methylcytosine. In the late 1980's, we and several other laboratories cloned the genes for a variety of C5-methyltransferases and determined their sequences. We found that these enzymes contained a series of well-conserved protein sequence motifs that reflected the shared catalytic ability of these enzymes. In addition though, we found a region that was quite variable among these enzymes, which we inferred and later proved, was responsible for DNA sequence recognition. We were curious as to how these enzymes, which act as monomers, could physically interact with the DNA duplex in order to achieve methylation.

We embarked on a program to obtain a crystal structure for a representative example of this class of enzymes. We tried unsuccessfully to crystallize M.MspI and M.HpaII (recognition sequence: CCGG). We were successful in obtaining crystals of M.HhaI (recognition sequence: GCGC) and in a collaboration that involved Saulius Klimasauskas, Sanjay Kumar and Xiaodong Cheng, we were successful in obtaining crystals and eventually a number of high resolution crystal structures of M.HhaI, both alone, in combination with S-adenosyl-methionine, the methyl donor and various DNA substrates. Much to our surprise, when we examined the crystal structure of M.HhaI binding to DNA we found that the cytosine that was to be methylated within the recognition sequence was flipped 180 degrees right out of the DNA helix and into an active side pocket in the enzyme. In retrospect, this provided an elegant means for the catalytic site of the enzyme to access the cytosine residue, which otherwise would have been buried within the DNA helix and would have required some other, perhaps unwieldy, distortion to allow the chemical reaction to take place. Such base flipping had not been previously predicted or seen in any other system.

Although the methyltransferases that form N6-methyladenosine or N4-methylcytosine differ quite considerably from the C5-methyltransferases, we immediately hypothesized that those two would also use base flipping in their interaction with DNA and this was later proved to be the case in several other systems. We also imagined that this distortion of the helix might be used by other enzymes such as DNA glycosylases that perform chemistry on DNA bases and this too, has now been shown to be correct. An interesting feature of the reaction is that it requires no external energy source. It is easy to imagine that because Nature discovered the value of DNA methylation fairly early on in evolution, then the use of base flipping might be quite prevalent in other cases where proteins need to interact with DNA. One attractive possibility is that some nucleic acid polymerases might also use base flipping to gain entry to the DNA helix during the initiation of replication or transcription. This would contrast with the current proposed mechanisms for the initiation of replication or transcription, which is envisioned to involve the melting of specific AT-rich regions.