**Mechanism for substrate preference of TET with structural analyses**

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**The microreview tells about the recent research about mechanism for the reactions catalyzed by human TET proteins. TET1 and TET2 act more active on 5mC-DNA substrates than on 5hmC/5fC-DNA substrates. The recent research found that substrate recognition does not account for the preference. They proposed a mechanism for the reaction that consists of four steps and the hydrogen abstraction is the rate-controlling step.**

**Introduction**

Ten-eleven translocation (TET) protein is dioxygenase dependent on alpha ketoglutarate and Fe2+, existing in organisms. Its catalytic domain is near the C end including three metal ions, a binding site for alpha ketoglutarate and a zone rich in cystine. TET proteins is an important enzyme in DNA demethylation by iteratively oxidizing 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), while DNA methylation is an important epigenetic modification which regulates a number of biological processes.

The discovery of the biological function of TET protein provides a new understanding of the mechanism for the demethylation of 5mC, because 5hmC may be an important intermediate in the process of 5mC methylation. On one side, 5hmC can be further catalysted by the deamination enzyme AID and become 5-hydroxymethyl uracil (5hmU), which can be recognized and cut by thymine DNA glycosylase (TDG).[1] The site will eventually be converted to cytosine via the base excision repair (BER) pathway. Thus, DNA methylation is realized. On the other side, 5hmC can also achieve 5mC demethylation effect through other ways. Previous research found that 5-hmC can be further oxidized into 5- formyl cytosine (5fC) and 5-carboxyl cytosine (5caC).[2] DNA demethylation can be realized through base excision repair (BER). In a word, with cytosine modified DNA methylation transferase (DNMT), the dynamic balance at the same sites on DNA of three base types C, 5mC and 5hmC is achieved, which can be seen as the mechanism for reversible methylation of DNA.

Human TET protein family has three members, respectively, TET1, TET2 and TET3. TET1 is identified and named when studying a case of T (10; 11) (q22; q23) iectopic leukemia patient. They play an important role in maintaining the polyfunctionality of stem cells. Mutation of TET gene can cause a variety of tumors, especially hematopoietic system tumors.

**Recent Progress**

The article titled “ Structural insight into substrate preference for TET-mediated oxidation ” by Lulu Hu from Fudan University first reported the molecule mechanism for the different catalytic activity of human TET1 and TET2 on three DNA methylated derivatives.[3] For getting the mechanism, following biochemistry experiments were conducted.

First, they performed an in vitro enzymatic activity assay. The results told that human TET1 and TET2 preferred 5mC-DNA to 5hmC/5fC-DNA substrate regardless of the concentration of TET proteins. Also, the product generation at different time points shew that the preference exists at every point detected. Moreover, the steady-state kinetic analyses for the TET2-mediated oxidation of three different DNA substrates indicated that 5mC -DNA is the best substrate for TET2 with the highest enzymatic activity. All the experiments above reflected that human TET1 and TET2 both acted more actively on 5mC-DNA than on 5hmC-DNA/5fC-DNA.

Second, further research is conducted for understanding the mechanism for the preference of human TET1 and TET2 for 5mC-DNA. What they had in mind first is the DNA-binding affinities. Fluorescence polarization measurements, however, showed that the binding affinity of TET2 binding to 5mC/5hmC/5fC-DNA is comparable. The little difference in the association or disassociation constant told that DNA-binding affinity is not the cause of human TET preference. After that, they determined the crystal structure of TET2-5mC-DNA, TET2-5hmC-DNA and TET2-5fC-DNA. Extensive hydrogen bonds and hydrophobic interactions play an important role in the binding of TET2 and 5mC-/5hmC-/5fC-DNA. The conformations of the cytosine portion of 5mC/5hmC/5fC within the catalytic cavity are almost the same. TET2 only specifically recognize CpG dinucleotide of DNA, which is consistent with the genome-wide analyses. In this part, we can get the conclution that substrate recognition does not account for the preference of human TET1 and TET2 on different substrates.

Last but not least, they speculated the mechanism by making analogy between 2-OG/Fe-depedent dioxygenases and human TET1/2 since the mechanism for the former one has been proposed by AlkB,[4] which involves four steps of reactions and the rate-controlling step is hydrogen abstraction. The comparison of reactions using 5mC-DNA and deuterated-5mC-DNA indicated an obvious kinetic isotope effect, standing by the hypothesis that hydroen abstraction is the key step for TET-mediated oxidation of 5mC. Furthermore, they used stopped-flow spectroscopy to measure the formation and decay of the ferryl-oxo intermediate for 5mC-/5hmC-/5fmC-DNA and similar conclusion is drew. Besides, the analysis of homolytic C-H bond dissociation energy was also taken. Although the C-H bond dissociation energy for the hydroxym-ethyl group of 5hmC is the lowest, the C-H bond dissociation energy for the formyl group of 5fC is slightly higher than that of 5mC, which is different from out expectation since previous experimental and computational studies have suggested that the higher homolytic C-H bond dissociation energy of substrates would brought about lower hydrogen abstraction efficiency.[5] Planar conformation of 5fC might result in the abnormity discussed above. What is more, the tendency to form intracellular hydrogen bonds of free 5hmC and 5fC may prevent the hydrogen abstraction, reducing their activity.

In summary, the paper reveals that human TET1 and TET2 show greater preference on the 5mC-DNA substrates to on the 5hmC-DNA and 5fC-DNA substrates. What accounts for the preference is not the substrate recognition but the hydrogen abstraction step. The mechanism the proposed finally is similar to the consensus mechanism for 2-OG/Fe-dependent dioxygenases with four steps of reactions.

**Discussion**

In the part of Recent Progress, the research group from Fudan University tried to explain the reasons of the substrate preference for TET-mediated oxidation. They excluded some possible reasons and proposed their own explanation via analogy, supported by some experimental phenomena. However, I feel that the evidence seems not strong enough since the method of analogy is not rigorous and there also exist some anomalies. There are two channels for the demethylation of 5mC-DNA, as we can see from the Introduction part. This paper proposed a mechanism for one of the two channels, while the other channel remains to be studied.

The research group from Fudan University used almost the same method to analyze autoinhibition and histone H3-induced activation of DNMT3A.[6] In these two studies, high-resolution and clear electron density maps played an significant role in determining the structure of proteins and DNA. That means they can accurately know which specific amino acid or nucleotide the substrate react with. Based on these information could they propose a new mechanism, which is just the progress they made than before.

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