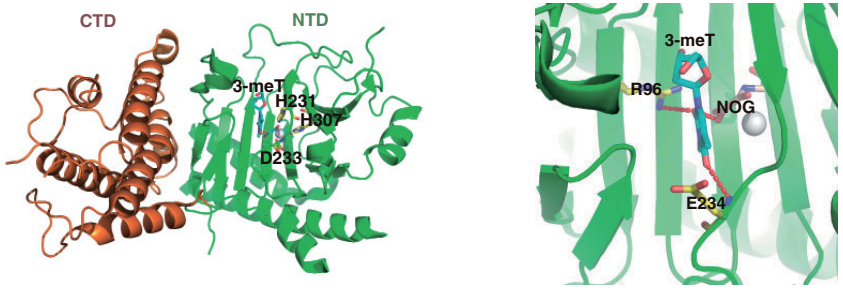
Structural Basis for FTO Substrate Selection

Recent studies have unequivocally established the link between the fat mass and obesity-associated (*FTO*) gene and obesity in human and mice. FTO protein is a DNA/RNA demethylase. We have determined the crystal structure of human FTO in complex with the mononucleotide 3-meT. Structural analysis reveals that a loop conserved in FTO family protein is important for its preference of single-stranded DNA and RNA. In addition, biochemical and structural studies show that the C-terminal domain of FTO plays a role in regulating the activity of FTO. The crystal structure of FTO may serve as a foundation for the rational design of FTO inhibitors as potential anti-obesity agents.

Obesity poses a serious threat to health in the world. In 2007, genome-wide association studies have shown a strong correlation of a single nucleotide polymorphism (rs9939609) in the first intron of the FTO gene with obesity risk. People being homozygous in the FTO gene are more susceptible to obesity. A mechanistic link between FTO and obesity has been demonstrated through knockout and mutagenesis experiments (1, 2). Sequence analysis predicts that FTO protein is a DNA/RNA demethylase. Biochemical studies have revealed FTO strongly prefers 3-meT/3-meU in single-stranded (ss)DNA /RNA than double-stranded (ds)DNA/RNA (3, 4). To unravel the underlying molecular basis, we solved the structure of FTO in complex with the mononucleotide 3-meT (5).

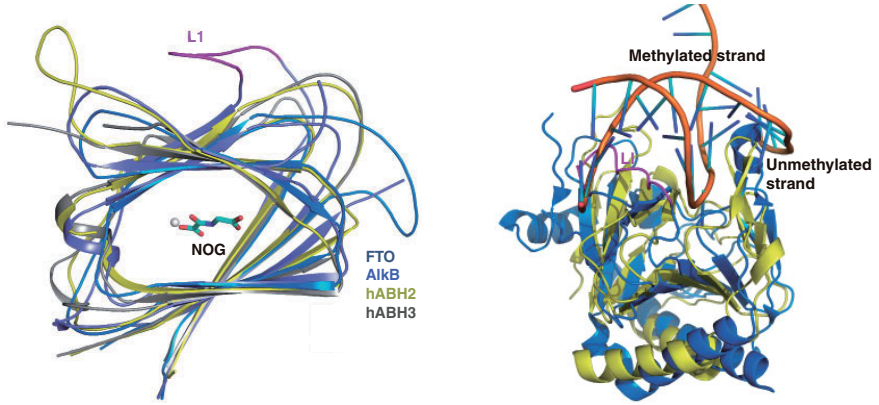
The structure of FTOΔ31 contains two well-defined domains: an N- (residues 32–326) and a C- (residues 327–498) terminal domain (referred to as NTD and CTD,respectively) [Fig. 1(a)]. As predicted, the catalytic core of the NTD is mainly composed of a distorted double-strand β-helix dubbed as a jelly-roll motif which is characteristic of AlkB-family DNA/RNA demethylase. No known structure was found to share significant homology with the CTD. Structural analysis show that extensive hydrophobic contacts are made between the NTD and the CTD, which suggests that the CTD plays a role in stabilizing the conformation of the catalytic core and is therefore important for FTO catalytic activity. Indeed, deletion of the CTD resulted in a catalytically dead FTO, whereas co-expression with the CTD rescued the enzymatic activity of NTD via their interaction with each other. Taken together, these results demonstrate the CTD has an important role in FTO enzymatic activity.

A database search using DALI showed that the NTD is most similar to the jelly-roll motif of the AlkB, with a root mean squared deviation (r.m.s.d.) of 2.3A over 182 Ca atoms. The highly conserved residues His 231, Asp 233 and His 307 from this motif are coordinated to Fe^{2+} [Fig.1(a)]. In addition to chelating Fe^{2+} , NOG also forms salt bonds with Arg 316 and Arg 322. One side of the jelly-roll motif is buttressed by two α-helices, whereas the other side is covered by a long loop which is absent in other AlkB members [Fig. 2(a)] but highly conserved among FTO proteins from different species. Structural comparison between FTO-3-meT and ABH2–dsDNA complex showed that this loop selectively competes with the unmethylated strand of the DNA duplex for binding to FTO [Fig. 2(b)], suggesting that it has an important role in FTO selection against double-stranded



(a) (b)

Figure 1
The overall structure of FTO.
(a) FTO contains two well-defined domains. The NTD (residues 32–326) and CTD (residues 327–498) of FTO are coloured in green and orange, respectively. Fe (grey sphere) and 3-meT (cyan stick) and NOG(tint stick) are shown. (b) Detailed interaction between FTO and 3-meT.



(a) (b)

Figure 2
The L1 loop of FTO is important for its selection against dsDNA.
(a) Structural comparison of FTO with AlkB, human ABH2 and ABH3 around the jelly-roll motif. (b) Structural alignment of FTO (residues 25–326) with ABH2-dsDNA complex and the L1 loop(residues 213–224) which is distinctive for FTO is shown in magentas.

nucleic acids. The result also supports a previous proposal that suggests that structure elements other than the region surrounding Phe102 in human ABH2 (important in ABH2 preference for dsDNA over ssDNA) can also contribute to substrate specificities of AlkB family proteins.

In complex structure, the thymine portion of 3-meT, which is well defined in the electron density map, is deeply anchored in a narrow substrate recognition cleft of FTO.Although interactions of 3-meT with FTO involve both hydrophobic contacts and hydrogen bonds, the two hydrogen bonds (one between O2-3-meT and Arg 96 and the other between Glu 234 and O4-3-meT) appear to be mainly responsible for specific recognition of 3-meT by FTO [Fig. 1(b)]. Indeed, mutations of these residues in FTO expected to disrupt these hydrogen bonds resulted in no detectable FTO activity. This observation not just demonstrates the essential role of hydrogen bonds in 3-meT recognition by FTO, but also offers an explanation why other AlkB members are less active toward 3-meT. The methyl group at C5-3-meT, which is lacking in uracil, does not contribute to interaction of 3-meT with FTO. This probably explains the observation that FTO has a similar activity towards 3-meU in ssRNA. These results also imply that DNA/RNA, in particular methylated rRNA, may be substrates of FTO *in vivo*.

Overall, the information derived from the crystal structure of FTO not only explains the structural basis for FTO substrate selection but also provides a foundation for the rational design of FTO inhibitors as potential anti-obesity agents.

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