

THAP9 transposase cleaves DNA via conserved acidic residues in an RNaseH-like domain

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Abstract

Background: The catalytic domain of most 'cut and paste' DNA transposases have the canonical RNase-H fold which is also shared by other polynucleotidyl transferases like the retroviral integrases and the RAG1 subunit of V(D)J recombinase. The RNase-H fold is a mixture of beta sheets and alpha helices with three acidic residues (Asp, Asp, Glu/Asp - DDE/D) that are involved in the metal-mediated cleavage and subsequent integration of DNA. Human THAP9 (hTHAP9), homologous to the well-studied *Drosophila* P-element transposase (DmTNP), is an active DNA transposase that, although domesticated, still retains the catalytic activity to mobilize transposons.

Results: In this study we have modelled the structure of hTHAP9 using the recently available cryo-EM structure of DmTNP as a template to identify an RNase-H like fold along with important acidic residues in its catalytic domain. Site-directed mutagenesis of the predicted catalytic residues followed by screening for DNA excision and integration activity, has led to the identification of candidate Ds and Es in the RNaseH fold that can be a part of the catalytic triad in hTHAP9.

Conclusions: Many DNA transposases execute DNA excision via a catalytic domain, which has a canonical RNase-H fold. Despite the similar nature of the catalytic domain, these transposases exhibit mechanistically different strategies of transposition. We identify a potential RNase-H fold in hTHAP9 with conserved DDE motif required for cutting DNA. Additionally, we have found a residue, which when mutated, leads to an increase in hTHAP9's transposition activity. Such hyperactive transposase mutants can be exploited as tools in genome engineering and gene therapy. This study has helped widen our knowledge about the catalytic activity of a functionally uncharacterised transposon-derived gene in the human genome.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and [accessed as a PDF](#).

Figures

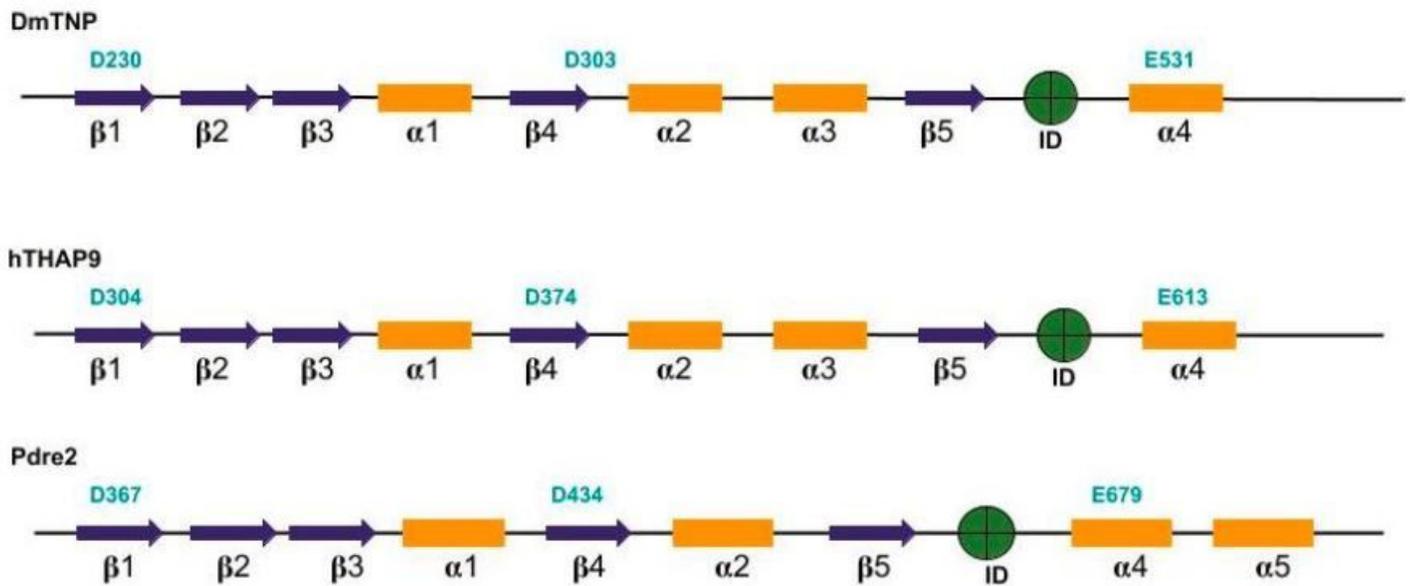


Figure 1

Predicted RNase-H fold in *Drosophila*, human and zebrafish THAP9. DDE motifs for each homolog are represented in bold (cyan) above its putative location. Blue arrows indicate beta sheets and yellow bars represent alpha helices. Insertion domains for each homolog [DmTNP (residues 339-528) (12), hTHAP9 (residues 415-604), Pdre2 (residues 475-670)] are shown in green circles.

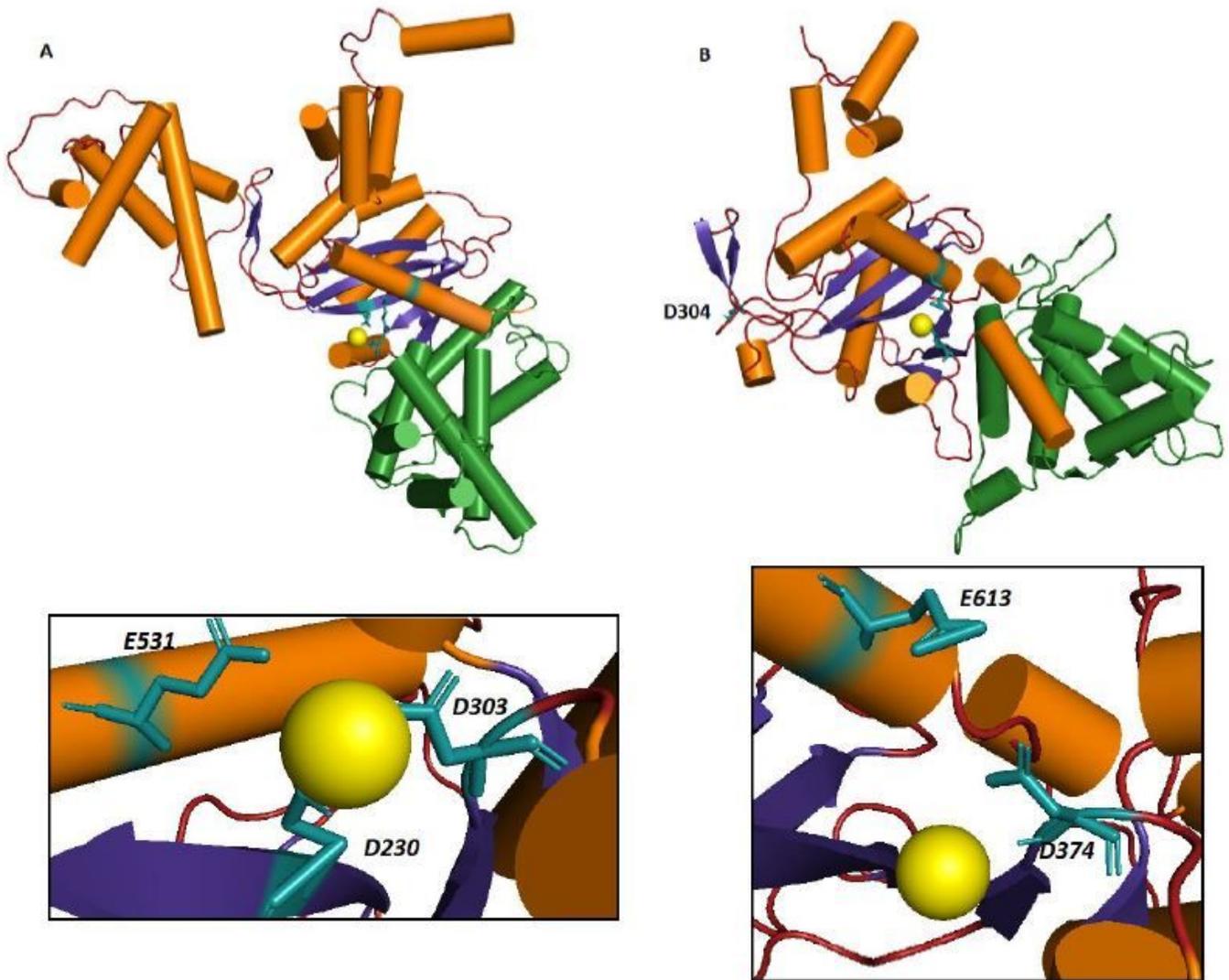


Figure 2

Catalytic triad shown in 3D structure of hTHAP9 built using homology modelling (A) DmTNP structure with beta sheets (blue arrows), helices (orange rods) and insertion domain (green) (PDB ID - 6P5A(12)). The active site is shown in the inset with DDE motif (D230, D303, E531) in close proximity to the Magnesium ion (light yellow). (B) Homology model of hTHAP9 with magnesium ion. Active site shown in the inset has D374 and E613 close to magnesium ion (yellow). D304 does not adopt any secondary structure but follows immediately after a beta sheet.



Figure 3

Sequence conservation in the putative RNaseH-domains of DmTNP, hTHAP9 and Pdre2. Amino acid sequences of DmTNP, hTHAP9 and Pdre2 aligned using Clustal Omega and visualized using MView. The Ds and Es that are conserved in the putative RNaseH-domains of all three homologs are highlighted in black boxes and labeled (in bold on top of the sequence) with the corresponding DmTNP residue number. Coloring scheme of alignment is based on conserved physicochemical properties of amino acids (Colored by identity - Palette P1 of MView, refer to Supplementary Fig.1 for key).

P-element superfamily **D230**(~90)**D303**(4)[N/y/h](~30)**D339**(2)H(2)K(2)[R/y][N/t](~190)**E531**(2)[F/n](3)R
hTHAP9 **D304**(~70)**D374**(4)H(~30)**D414**(2)H(2)R(2)RN(~190)**E613**(1)F(4)R
Pdre2 **D367**(~70)**D434**(4)N(~30)**D474**(2)H(2)K(2)RN(~190)**E679**(2)F(3)R

Figure 4

Signature string of P-element superfamily in hTHAP9 and Pdre2. Signature string of P element superfamily (8) along with similar signature strings identified in human and zebrafish THAP9. Conserved

Ds and Es are represented in bold and the numbers represent the spacing between conserved amino acid residues.

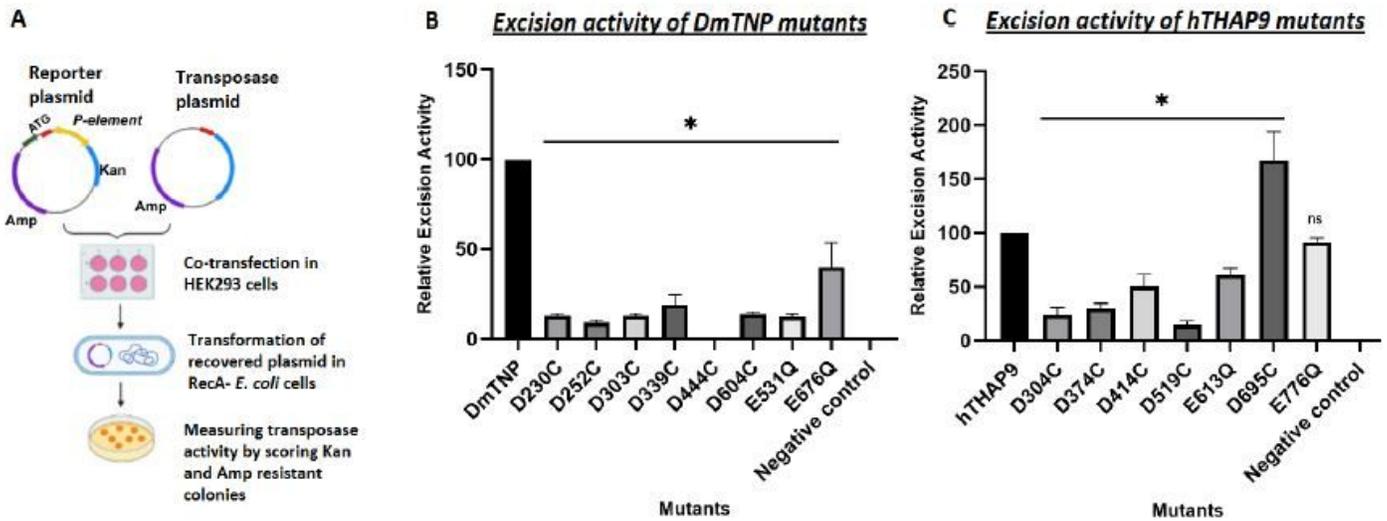


Figure 5

Conserved acidic residues within the catalytic triad and signature string of DmTNP and hTHAP9 are important for DNA excision. (A) Schematic of transposon excision assay. Relative excision activities of (B) DmTNP mutants (compared to wild type DmTNP) (C) hTHAP9 mutants (compared to wild type hTHAP9). All the values are represented as a mean of 4 independent experiments (\pm SEM, n=4) p value <0.05. Negative control (pBluescript empty vector)

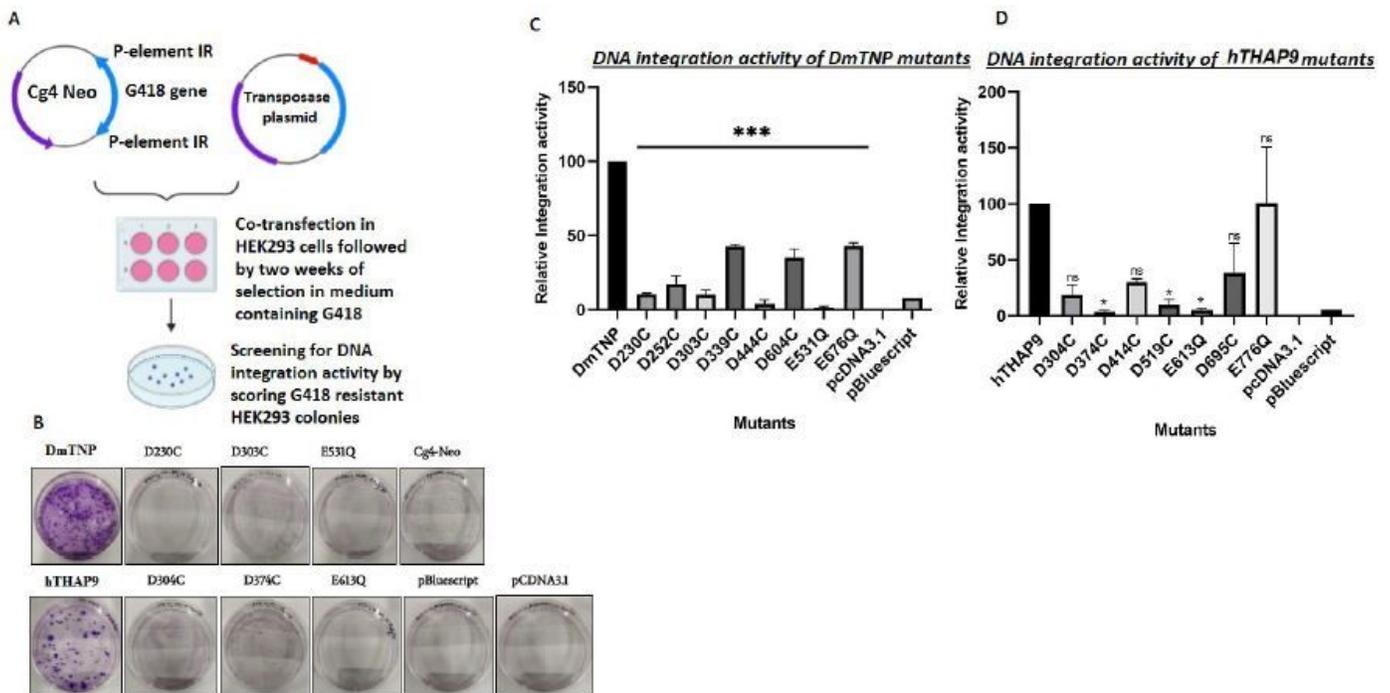


Figure 6

Conserved acidic residues within the catalytic triad and signature string of DmTNP and hTHAP9 are important for DNA integration (A) Schematic of Transposon integration assay. (B) Crystal violet stained G418 resistant colonies of wildtype *Drosophila* and hTHAP9 along with DDE motif mutants and negative controls. Relative integration activities of (C) DmTNP mutants (compared to wild type DmTNP) (D) hTHAP9 mutants (compared to wild type hTHAP9). Empty vectors - pCDNA3.1 (+) and pBluescript were used as negative controls. All the values are represented as mean of two independent experiments performed in duplicates (\pm SEM) p value <0.05.

Supplementary Files

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