

# The T Protein: Vertebrae Fit to a T

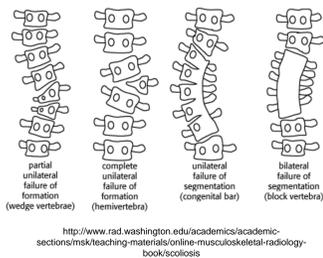
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## Abstract

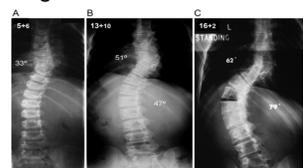
Vertebral malformations (VMs) comprise a group of spinal abnormalities present at birth that include alterations in vertebral shape or number. Evidence suggests VMs have a genetic link, possibly resulting from mutations in multiple genes. One candidate gene is *T*. T protein, a transcription factor found in a variety of animals including humans, is essential for correct embryonic development and guides the development of bone and cartilage from embryonic mesodermal tissue. T protein accumulates in the nuclei of notochord cells, interacts with DNA at specific genes, and acts as a genetic switch to activate the genes. T protein binds to the major and minor grooves of DNA as a dimer. Mutations in T (turning “off” the T protein switch) are hypothesized to result in defects in spinal development. The Cedarburg SMART (Students Modeling A Research Topic) Team has designed a partial model of T protein using 3D printing technology to investigate its structure-function relationship, focusing primarily on the residues important for dimerization of T (Pro125, Asp126, and Pro128) and for binding DNA (Arg67). A 3D model could indicate how the location of the mutations may impact the function of T. T could consequently be a potential target for the development of treatment or prevention options. Program supported by a grant from NIH-CTSA.

## Congenital Vertebral Malformations

Vertebral malformations (VMs) are abnormalities in the spine that are present at birth. They may happen in isolation or as a part of an underlying chromosome anomaly. Although most clinical cases appear randomly, genetic transmissions of VMs have been recorded and the sibling frequency rate of VMs is high enough to suggest that mutations in genes can cause VMs.

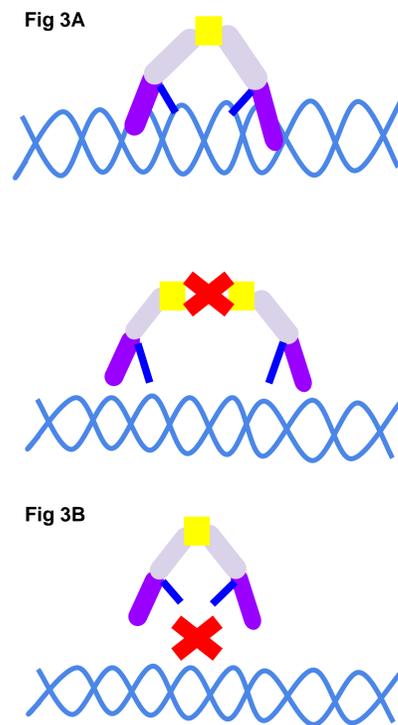


The *T* gene codes for the T protein that helps in the development of the mesoderm. A variant of T, ala338val, that replaces the amino acid alanine with valine at position 338 of T protein has been identified from three of 50 patients with VMs<sup>(2)</sup>. The mutant protein is associated only with patients with VMs, putatively may have altered structure and/or function, and increases the risk of VM in the study patients. In each patient’s family a phenotypically unaffected parent has the mutant allele. Because T is expressed throughout the notochord, it possible for the phenotype to manifest itself anywhere along the vertebral column.



<http://bjps.org/content/bjpsam/95/11/972/F3.large.jpg>

## T Protein Mutations Prevent Proper Function as a Transcription Factor

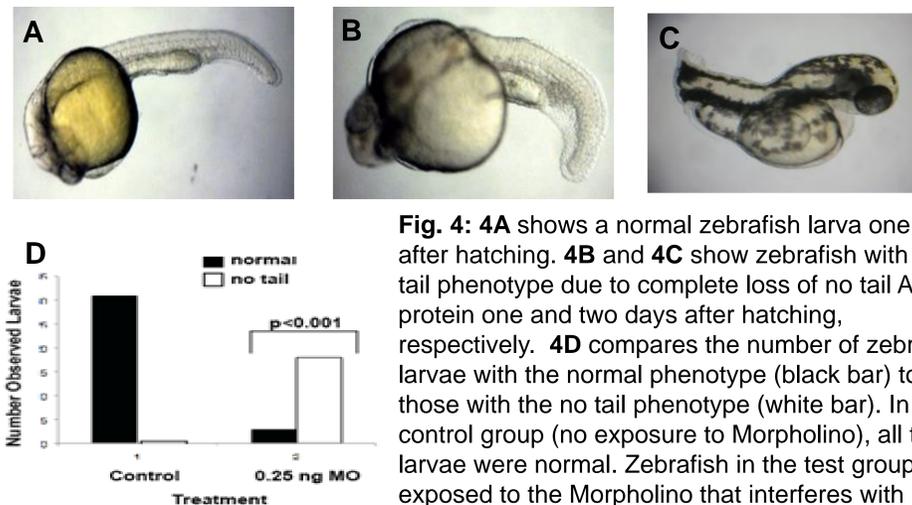


**Fig 3A:** *T* gene codes for T protein. T protein functions as a transcription factor to initiate synthesis of other proteins important in mesoderm development. Transcription factors help “regulate” the process of transcribing DNA into RNA. Functional T protein dimerizes and then binds to DNA. Pro125, Asp126, and Pro128 (yellow) are important for dimerization. Arg67 (blue line) forms a hydrogen bond with a guanine in the major groove of DNA. The C-terminal alpha helices in T protein (deep purple) bind in the minor groove of the DNA through hydrophobic interactions. This is different from the typical helix-turn-helix (HTH) motifs seen in other DNA binding proteins, where the interaction occurs in the major groove.

**Fig 3B:** Mutations in the T protein can cause malformations during development of the notochord or vertebrae. It is hypothesized that mutations in T protein either prevent dimerization or prevent the dimerized T protein from binding DNA.

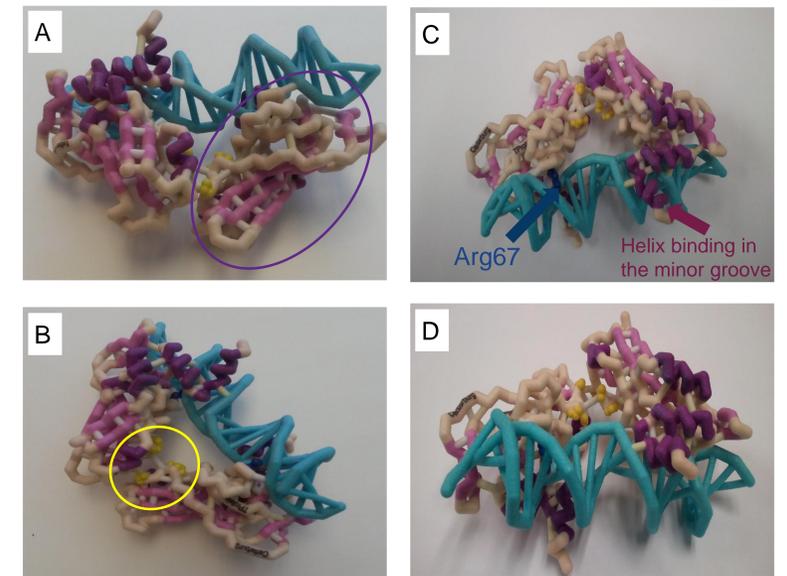
Key	
	Pro125, Asp126, and Pro128
	Alpha Helices
	Arginine
	“Does not bind”
	Beta Sheets
	DNA

## Knockdown studies of a protein corresponding to human T in zebrafish, No tail A, show that No tail A is important for proper development of the tail, trunk, and vertebrae in fish.



**Fig. 4:** 4A shows a normal zebrafish larva one day after hatching. 4B and 4C show zebrafish with the no tail phenotype due to complete loss of no tail A protein one and two days after hatching, respectively. 4D compares the number of zebrafish larvae with the normal phenotype (black bar) to those with the no tail phenotype (white bar). In the control group (no exposure to Morpholino), all the larvae were normal. Zebrafish in the test group were exposed to the Morpholino that interferes with transcription of the *T* gene and prevents synthesis of the T protein. This results in larvae with the no tail phenotype. The ala338val variant has not been investigated in zebrafish to date.

## The structure of the T protein determines how it binds to DNA.



**Fig. 5:** Backbone models of T protein. DNA is colored cyan, alpha helices are colored purple, beta sheets are colored pink. T protein functions as a transcription factor as a dimer. A monomer of T protein is circled in 5A. Pro125, Asp126, and Pro128, colored yellow and circled in 5B, are implicated in T dimerization. 5C highlights regions of T important for binding DNA. The blue arrow points to Arg67 (also colored dark blue), the residue important for binding to a guanine in the major groove of DNA. A novel feature of this protein is the helix that binds to the minor groove (purple arrow). It is hypothesized that when there is a mutation in the T protein, it is not able to serve as an effective transcription factor either because T is unable to form a dimer or is unable to bind to the DNA.

## Conclusion

- T is an important transcription factor during development, and the structure of T enables it to bind to DNA and function as a transcription factor.
- Complete loss of T protein as observed with no tail A in zebrafish results in abnormal trunk, tail, and vertebral development.
- Although mapping the human ala338val T variant identified by Ghebraniou *et al.*, (2008) was not possible due to incomplete crystallization data, based on the current knowledge of T structure, it is speculated that the mutation may alter dimerization of T monomers or T binding to DNA.
- Further studies hope to map the human ala338val T variant to zebrafish and identify consequences of a similar mutation in zebrafish.

References:  
<sup>1</sup>Muller, C. and Hermann, B. (1997). Crystallographic structure of the T domain-DNA complex of the Brachyury transcription factor. *Nature* 389: 884-888.  
<sup>2</sup>Ghebraniou, N., Blank, R., Raggio, C., Staubli, J., McPherson, E., Ivacic, L., Rasmussen, K., Jacobsen, F., Faciszewski, T., Burmester, J., Pauli, R., Boachie-Adjei, O., Glurich, I., and Giampietro, P. (2008). A Missense T(Brachyury) Mutation Contributes to Vertebral Malformations. *J Bone Miner Res* 23:1576-1583  
<sup>3</sup>Schulte-Merker, S., van Eeden, F., Halpern, M., Kimmel, C., and Nüsslein-Volhard, C. (1994). no tail (ntl) is the zebrafish homologue of the mouse T (Brachyury) gene. *Development*. 120(4):1009-15.  
<sup>4</sup>Kispert, A., B. Koschorz, and B. G. Herrmann. The T Protein Encoded by Brachyury Is a Tissue-specific Transcription Factor. *The EMBO Journal*. U.S. National Library of Medicine, 2 Oct. 1995. Web. 12 Mar. 2015.