

## MOLECULAR BIOLOGY

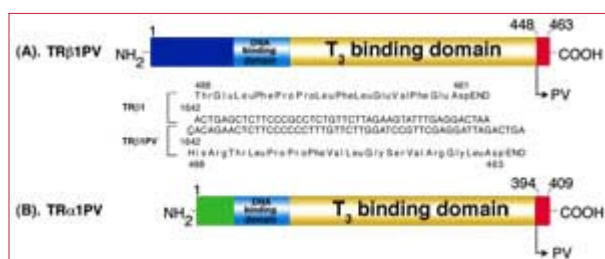
### Mutations of Thyroid Hormone Nuclear Receptors and Disease

Cheng SY. Thyroid hormone receptor mutations and disease: Beyond thyroid hormone resistance. *Trends Endocrinol Metab* 16: 176–82, 2005.

Thyroid hormone nuclear receptors (TRs) are ligand-dependent transcription factors that mediate the biological activities of thyroid hormone (T<sub>3</sub>) in growth, development, differentiation, and the maintenance of metabolic homeostasis. Two TR genes, *TRα* and *TRβ*, located on chromosomes 17 and 3 respectively, encode four major T<sub>3</sub>-binding TR isoforms (α1, β1, β2, and β3). The TRs consist of modular functional structures with the N-terminal A/B, central DNA-binding, and the C-terminal ligand-binding domains. TRα1 and TRβ1 share high sequence homology in the DNA- and ligand-binding domains, but the sequence differs significantly in the A/B domain. The C-terminal region and the A/B domain contain the transcription-activation functions.

Given the critical roles of TRs in cellular functions, it is reasonable to expect that mutations of TRs could have deleterious consequences. Indeed, shortly after the cloning of the *TRβ* gene, mutations of it were discovered to cause the genetic syndrome of resistance to thyroid hormone (RTH). However, whether mutations of the *TRβ* gene cause human diseases other than RTH has been unknown. Likewise, it has been unclear whether mutations of the *TRα* gene could also cause abnormalities.

To address these questions, we used the powerful mouse genetic approach, introducing an identical mutation (*PV* mutation) into the *TRβ* and the *TRα* gene loci. The *PV* mutation was identified in an RTH patient at the NIH. It is a frame-shift mutation in the C-terminal 16 amino acids of TRβ1 (TRβ1PV) and TRα1 (TRα1PV) that leads to the complete loss of T<sub>3</sub> binding activity and transcription capacity (Figure 1).

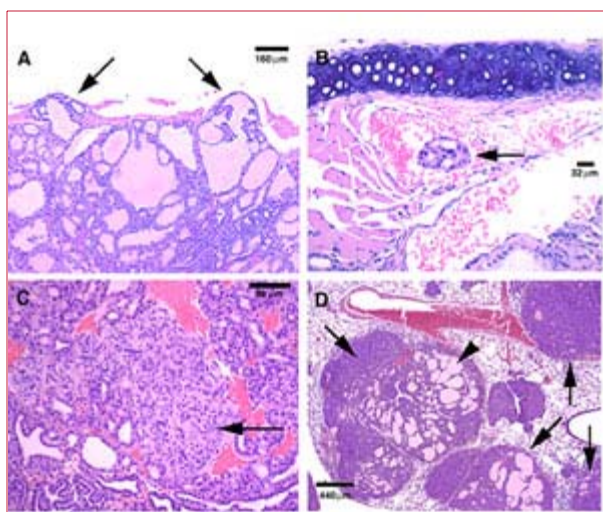


**Figure 1.** The amino acid sequence of PV and its location in the carboxyl terminus of TRβ1

(A) and TR $\alpha$ 1 (B). The PV mutation was identified in a patient with resistance to thyroid hormone. The mutation is from a C-insertion at codon 448 of TR $\beta$ 1, resulting in a frame-shift mutation in the last 16 carboxyl terminal amino acids. The same PV mutation was targeted to the TR $\beta$  and TR $\alpha$  genes to create TR $\beta$ PV and TR $\alpha$ 1PV mice.

The knockin mouse that harbors the TR $\beta$ PV gene (TR $\beta$ PV mouse; **Figure 1, part A**) recapitulates human RTH by exhibiting dysregulation of the pituitary-thyroid axis, reduced weight, abnormally accelerated bone development, hypercholesterolemia, and hyperactivity. This mouse model allowed an elucidation of the molecular basis of RTH unattainable otherwise. TR $\beta$ PV manifests its dominant-negative activity *in vivo* via competition with wild-type TRs in binding to the promoters of T3-target genes. The variable phenotypic expression in RTH patients is dictated by the tissue-dependent abundance of TR isoforms and modulated by multiple combinatorial cellular factors.

Remarkably, homozygous TR $\beta$ <sup>PV/PV</sup> mice spontaneously develop follicular thyroid carcinoma, indicating that the deleterious effect of TR $\beta$  gene mutations is not limited to RTH. The pathologic progression from hyperplasia to capsular and vascular invasion, and eventually to distant metastasis in TR $\beta$ <sup>PV/PV</sup> mice, is similar to human follicular thyroid cancer (**Figure 2**). Thyroid carcinomas are the most common endocrine neoplasms in humans, with a globally increasing incidence. However, little is known about the molecular genetic events underlying their development. This first mouse model of follicular thyroid carcinoma allows one to discern the genetic alterations contributing to thyroid carcinogenesis and to identify potential molecular targets for prevention and treatment. Indeed, analysis of altered gene expression profiles by cDNA microarray indicates a complex alteration of multiple signaling pathways is associated with thyroid carcinogenesis. One pathway, the peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ )-mediated signaling, was found to be repressed by PV during thyroid carcinogenesis. Further molecular study of this pathway led to the identification of PPAR $\gamma$  as a tumor suppressor, thus raising the possibility that PPAR $\gamma$  is a potential target for treatment. Indeed, activation of PPAR $\gamma$ -mediated signaling by treating TR $\beta$ <sup>PV/PV</sup> mice with PPAR $\gamma$  agonists significantly delays the development and progression of thyroid carcinogenesis. These results suggest that PV could act as an oncogene by inhibiting the tumor suppressor functions of PPAR $\gamma$  in thyroid carcinogenesis.



**Figure 2.** Morphological features in thyroid glands and metastasis of  $TR\beta^{PV/PV}$  mice. Histological sections from tissues of  $TR\beta^{PV/PV}$  mice stained with hematoxylin (blue) and eosin (pink) show evidence of capsular invasion (*A*) (arrows) and vascular invasion in thyroid (*B*) (arrow), spindle cell anaplasia within the thyroid shown at higher magnification (*C*) (arrow), and a cardiac metastasis (*D*) (arrow). Capsular and vascular invasion are the pathologic features used in the diagnosis of human neoplastic thyroid tumors. The pathologic progression of thyroid cancer in  $TR\beta^{PV/PV}$  mice is similar to that in humans.

The manifestation of the oncogenic actions of *PV* is not restricted to the thyroid.  $TR\beta^{PV/PV}$  mice also spontaneously develop thyroid stimulating hormone (TSH)–secreting pituitary tumors (TSH-omas). TSH-omas represent about 2% of all pituitary adenomas in humans, affecting vision and causing headaches and other endocrine disorders. The molecular genetics underlying their pathogenesis is largely unknown. Using  $TR\beta^{PV/PV}$  mice as a model, we uncovered a novel mechanism by which *PV* could function as an oncogene in TSH-omas. *PV* acts as a constitutive activator of the expression of cyclin D1, a well-known tumor promoter, by tethering to the cyclic AMP response element binding protein (CREB) on the cyclin D1 promoter. These findings suggest that mutation of *TRβ* is one of the genetic events underlying the pathogenesis of TSH-omas.

The knockin mice harboring the *PV* mutation in the *TRα* gene ( $TR\alpha^{IPV}$  mice; **Figure 1, part B**) exhibit a phenotype distinct from that of  $TR\beta^{PV}$  mice. Homozygous  $TR\alpha^{IPV/IPV}$  mice die very shortly after birth. The heterozygous mice ( $TR\alpha^{IPV/+}$ ) display reduced fertility, increased mortality, delayed bone development, dwarfism, and metabolic disorder, indicating that mutations of *TRα* lead to severe consequences. The contrasting phenotypes of  $TR\alpha^{IPV}$  and  $TR\beta^{PV}$  mice reveal that the actions of TR mutants *in vivo* are isoform dependent. Analysis of transcription regulation of T3-response genes in several target tissues suggests that distinct phenotypic expression is mediated, in part, by the differentially dominant activity of TR isoform mutants *in vivo*.

The  $TR\alpha^{IPV}$  and  $TR\beta^{PV}$  mice have provided a powerful tool to uncover the novel functions

of TR mutants and to elucidate their mechanisms of molecular actions *in vivo*. Accumulated evidence from the study of *TRβ<sup>PV/PV</sup>* mice suggests that *PV*, a *TRβ* mutant, not only causes RTH, but could also function as a new oncogene to contribute to thyroid carcinogenesis and pathogenesis of TSH-omas. Importantly, *TRβ<sup>PV/PV</sup>* mice could be used as a preclinical model to develop new treatment strategies. Considering that human diseases harboring *TRα* mutations are yet to be discovered, *TRα<sup>IPV</sup>* mice could be used as a model to search for and to identify those diseases whose phenotypic manifestation resembles that of *TRα<sup>IPV</sup>* mice.

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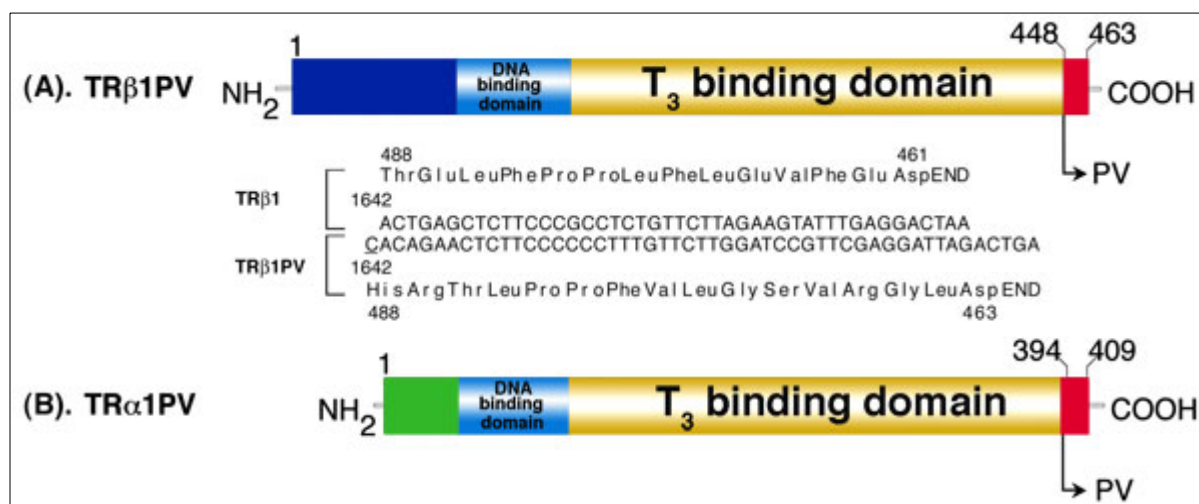
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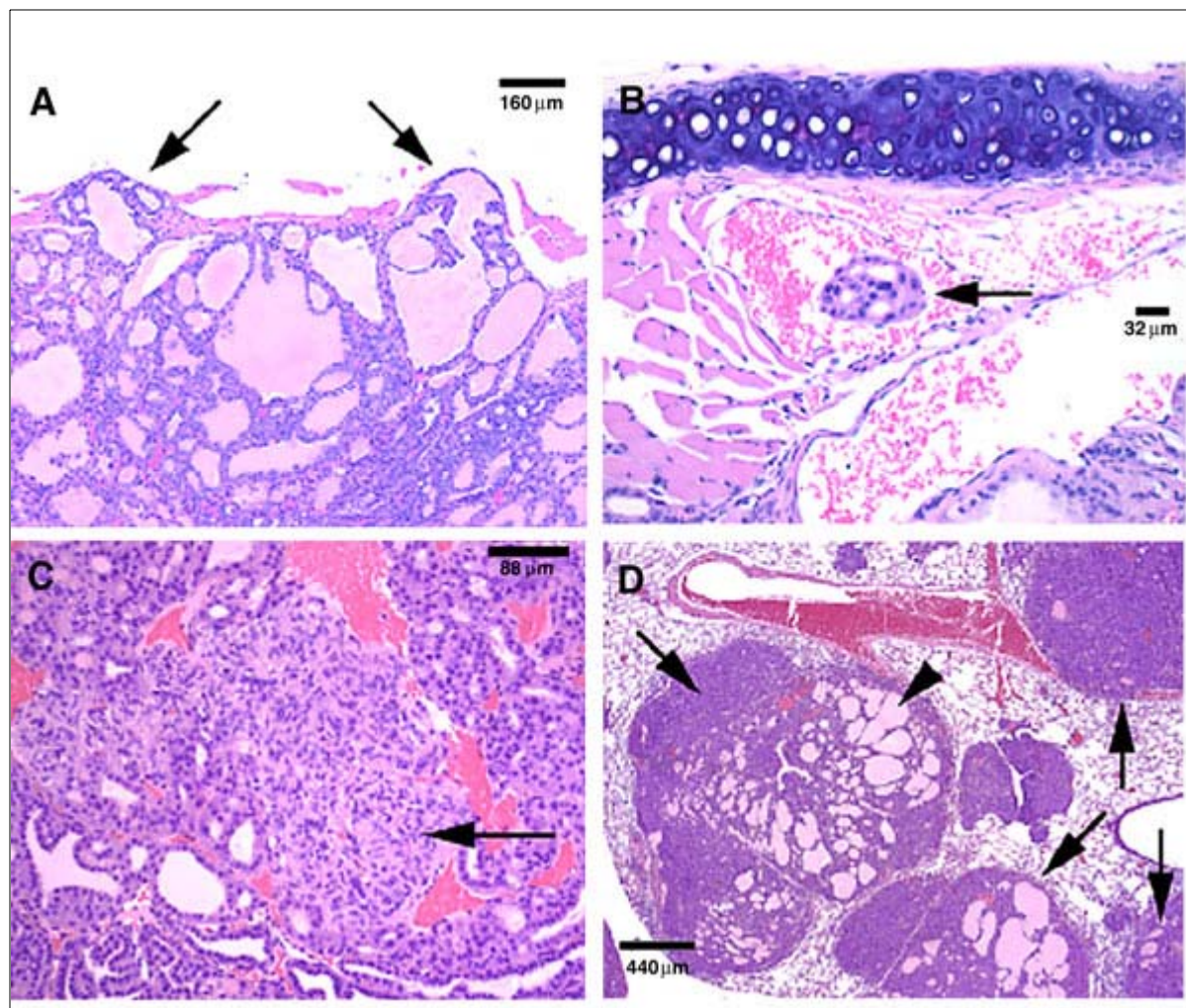
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**Figure 1.** The amino acid sequence of PV and its location in the carboxyl terminus of TRβ1 (A) and TRα1 (B). The *PV* mutation was identified in a patient with resistance to thyroid hormone. The mutation is from a C-insertion at codon 448 of *TRβ1*, resulting in a frame-shift mutation in the last 16 carboxyl terminal amino acids. The same *PV* mutation was targeted to the *TRβ* and *TRα* genes to create *TRβ<sup>PV</sup>* and *TRα<sup>IPV</sup>* mice.



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