Imperial College
London

BSc ENDOCRINOLOGY

Lecture: Molecular biology of thyroid hormone secretion and action

Lecturer: Graham Williams

LEARNING OBJECTIVES

Students should aim to be able to:

- Understand and discuss negative feedback regulation of the hypothalamic-pituitary-thyroid axis
- Understand and discuss cellular uptake and metabolism of thyroid hormones
- Understand and discuss the nuclear receptor superfamily
- Describe the thyroid hormone receptor variants and their mechanism of action
- Understand the functional difference between unliganded and ligand-bound thyroid hormone receptors

Suggested further reading

Flamant F, Gauthier K, Samarut J. Thyroid hormones signaling is getting more complex: STORMs are coming. Mol Endocrinol. 2007 21:321-33.

Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. J Clin Invest. 2006 116:2571-9.

Beck-Peccoz P, Persani L, Calebiro D, Bonomi M, Mannavola D, Campi I. Syndromes of hormone resistance in the hypothalamic-pituitary-thyroid axis. Best Pract Res Clin Endocrinol Metab. 2006 20:529-46

Friesema EC, Jansen J, Milici C, Visser TJ. Thyroid hormone transporters. Vitam Horm. 2005;70:137-67.

Harvey CB, Williams GR Mechanisms of thyroid hormone action. Thyroid. (2002) 12: 441-446.

Imperial College
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BSc ENDOCRINOLOGY 2007-2008

Lecture: Thyroid hormone action in a peripheral tissue

Lecturer: Graham Williams

LEARNING OBJECTIVES

Students should aim to be able to:

- Discuss the relationship between circulating and tissue thyroid status
- Discuss the physiological implications of differences in the tissue distribution of thyroid hormone receptor isoforms
- Understand and discuss regulatory roles of thyroid hormones in development
- Discuss in detail the role of thyroid hormones and thyroid hormone receptors in skeletal development and in adult bone
- Appreciate the pathophysiological importance of thyroid hormone effects on bone

Suggested further reading

1. Nicholls JJ, Brassill MJ, Williams GR, Bassett JHD (2012) The skeletal consequences of thyrotoxicosis. J. Endocrinol. 213:3,209-21. PMID:22454529

2. Waung JA, Bassett JHD, Williams GR (2012) Thyroid hormone metabolism in skeletal development and adult bone maintenance. Trends Endocrinol. Metab. 23:4,155-162. PMID:22169753

3. Bassett JHD, Williams GR (2009) The skeletal phenotypes of TRα and TRβ mutant mice. J. Mol. Endocrinol.42:269-282. PMID:19114539

4. Bassett JHD, Williams AJ, Murphy E, Boyde A, Howell PGT, Swinhoe R, Archanco M, Flamant F, Samarut J, Costagliola S, Vassart G, Weiss RE, Refetoff S, Williams GR (2008) A lack of thyroid hormones rather than excess TSH causes abnormal skeletal development in congenital hypothyroidism. Mol. Endocrinol. 22: 2, 501-512. PMID:17932107

5. Bassett JHD, O'Shea PJ, Sriskantharajah S, Rabier B, Boyde A, Howell PGT, Weiss RE, Roux JP, Malaval L, Clément-Lacroix P, Samarut J, Chassande O, Williams GR (2007) Thyroid hormone excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. Mol. Endocrinol. 21: 5, 1095-1107. PMID:17327419

6. O'Shea PJ, Bassett JHD, Cheng S-y, Williams GR (2006) Characterization of skeletal phenotypes of TRα1PV and TRβPV mutant mice: implications for tissue thyroid status and T3 target gene expression. Nuc. Rec. Signal. 4:e011. http://www.nursa.org/article.cfm?doi=10.1621/nrs.04011 PMID:16862217

Thyroid hormone action

Graham R. Williams

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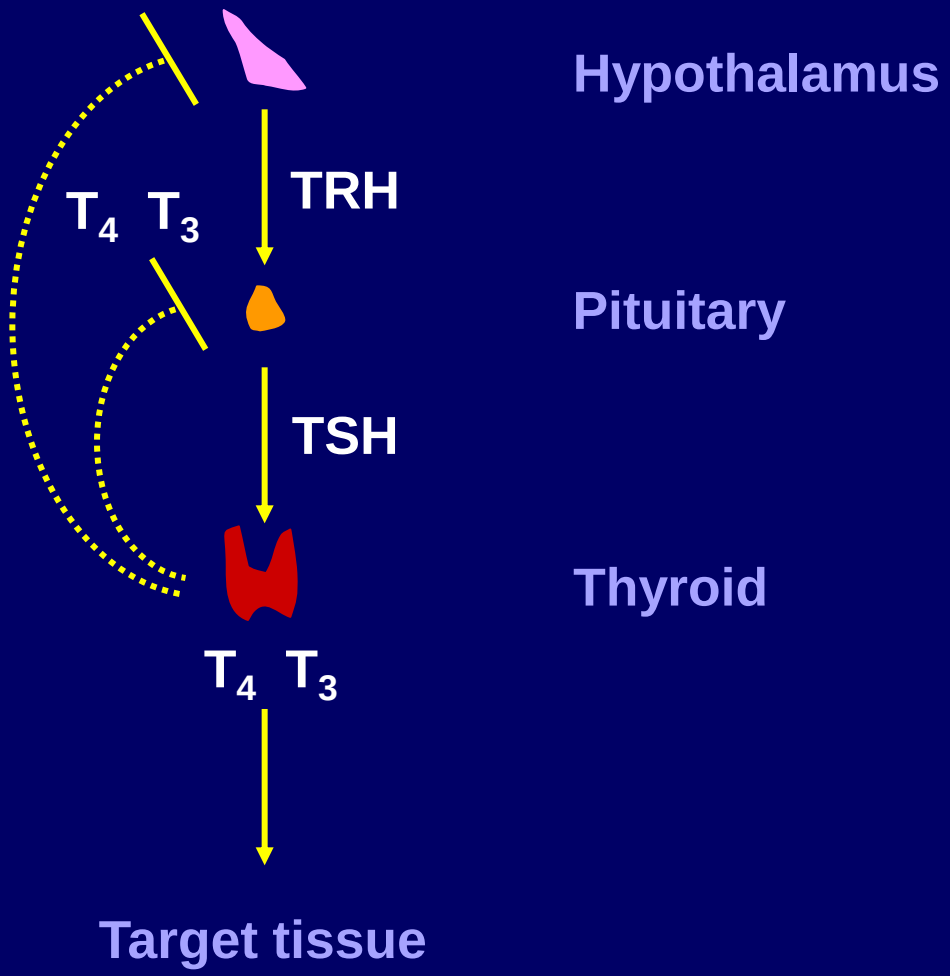
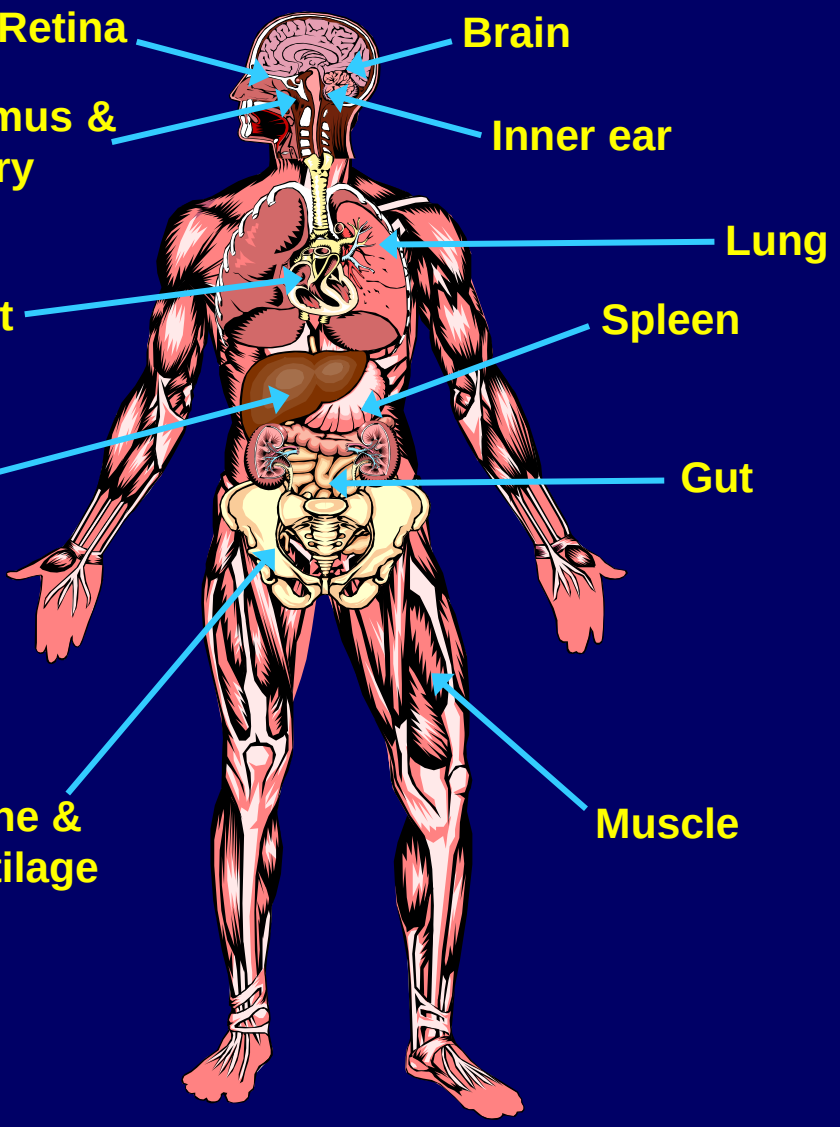
Imperial College London

Hypothalamic-pituitary-thyroid axis

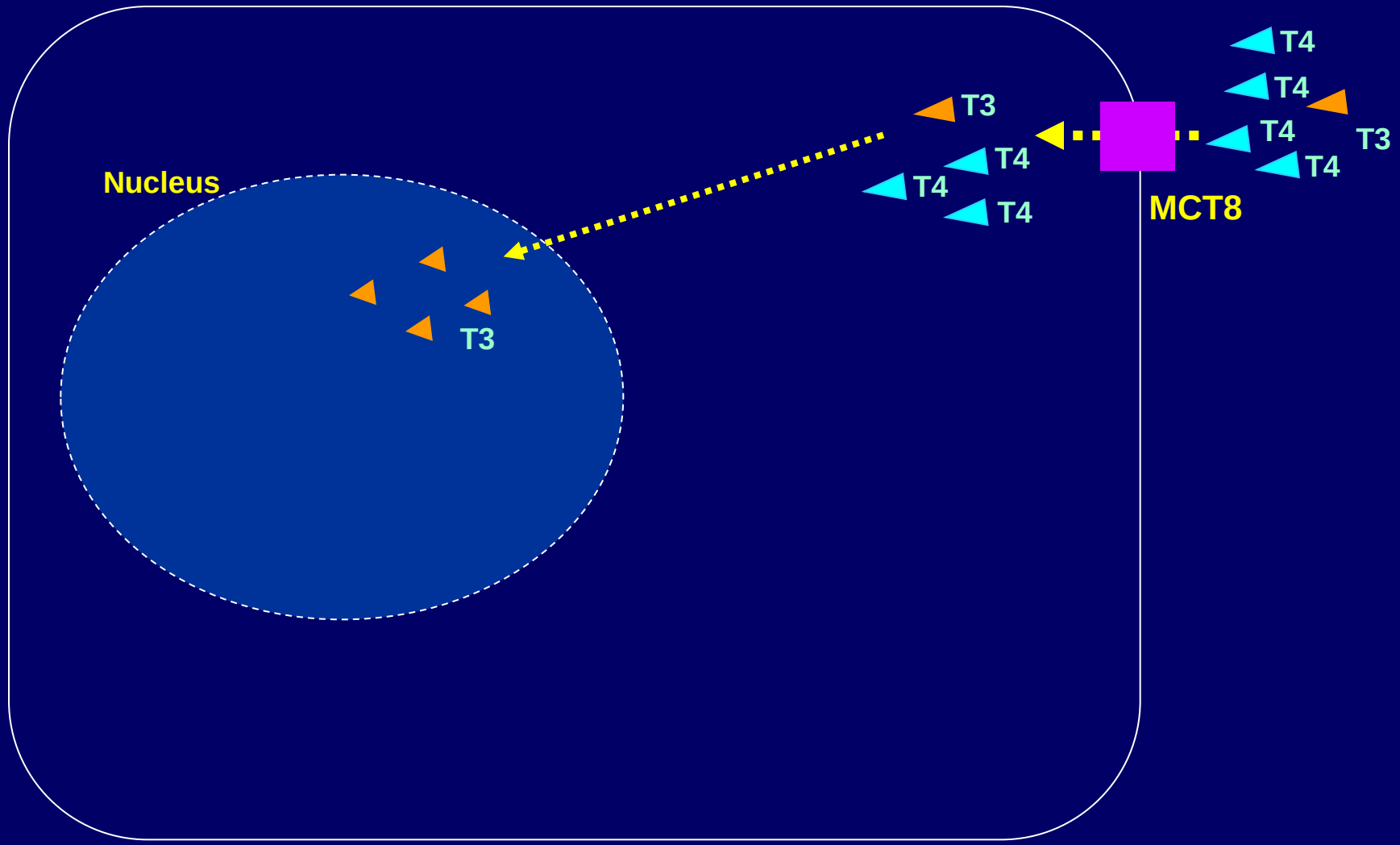


Major T3 target tissues

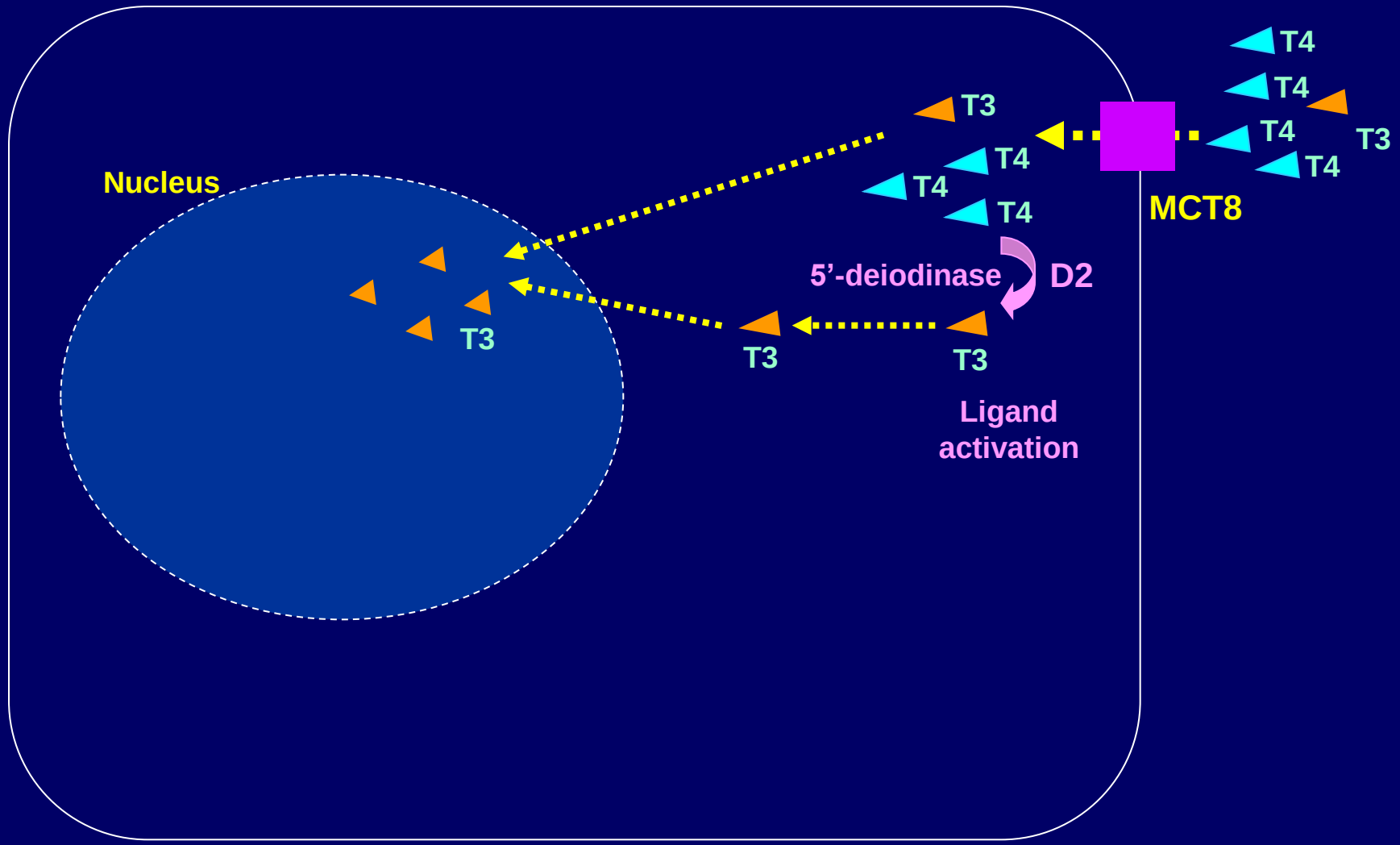


BMR
Thermogenesis
Adipogenesis
Vasculature
Skin
Hair
Bone marrow
Kidney

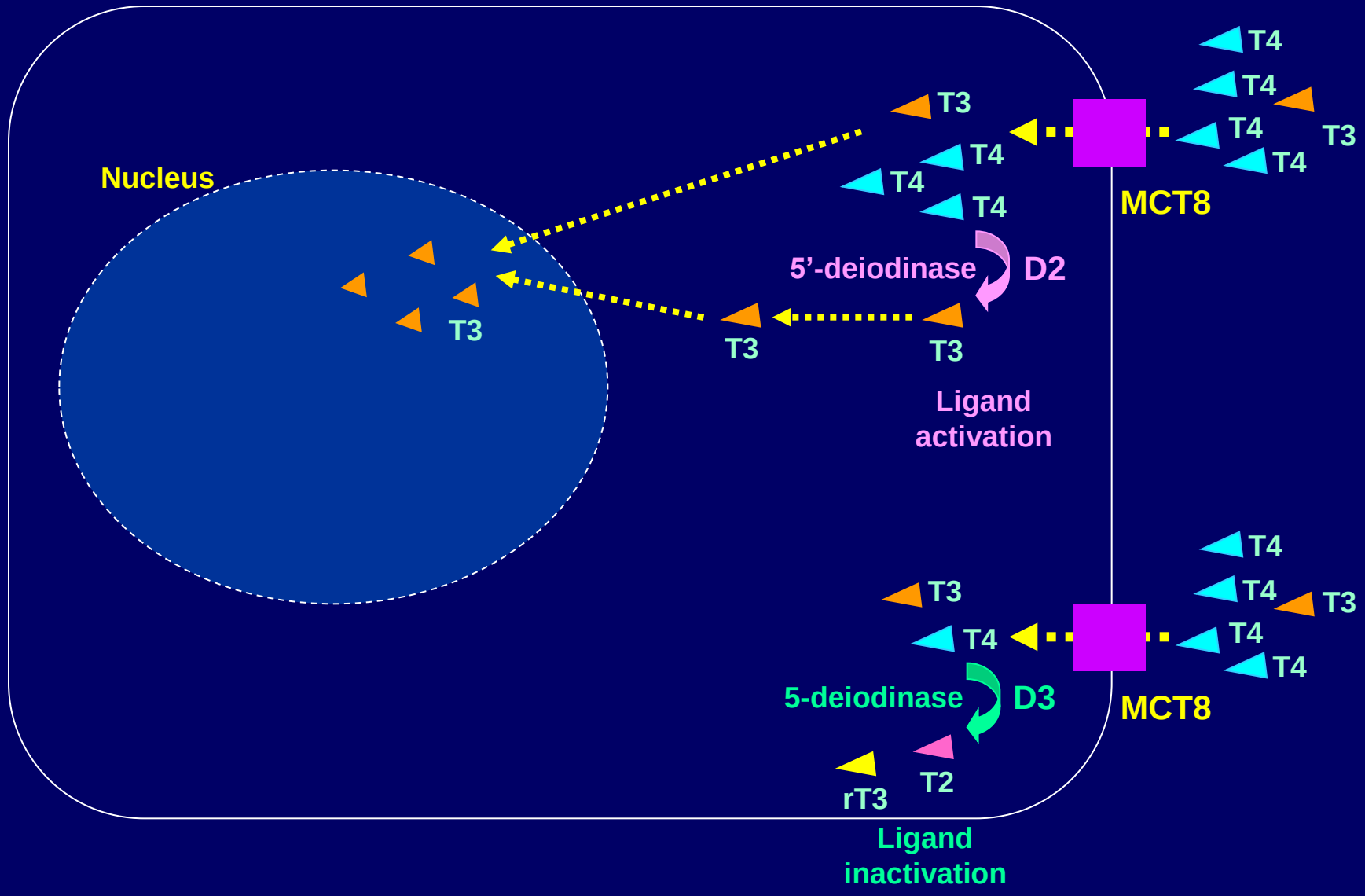
Thyroid hormone action



Thyroid hormone action



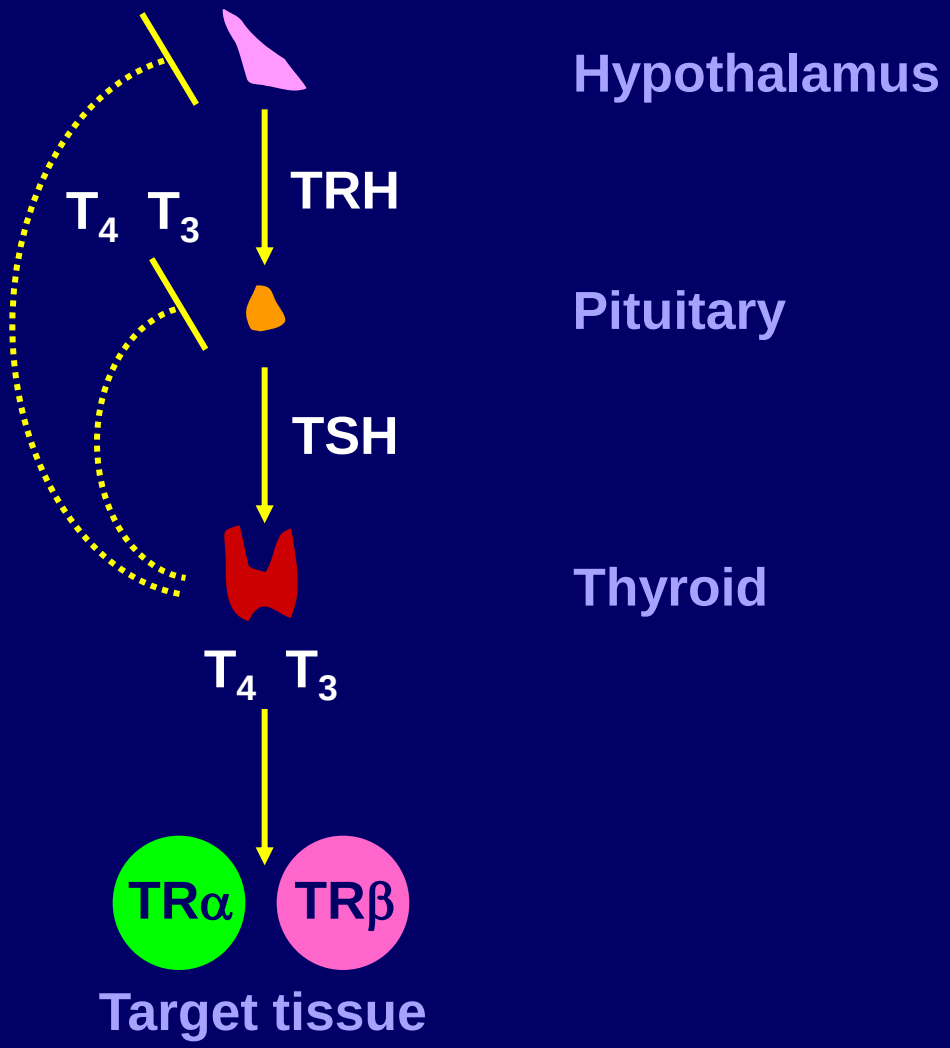
Thyroid hormone action



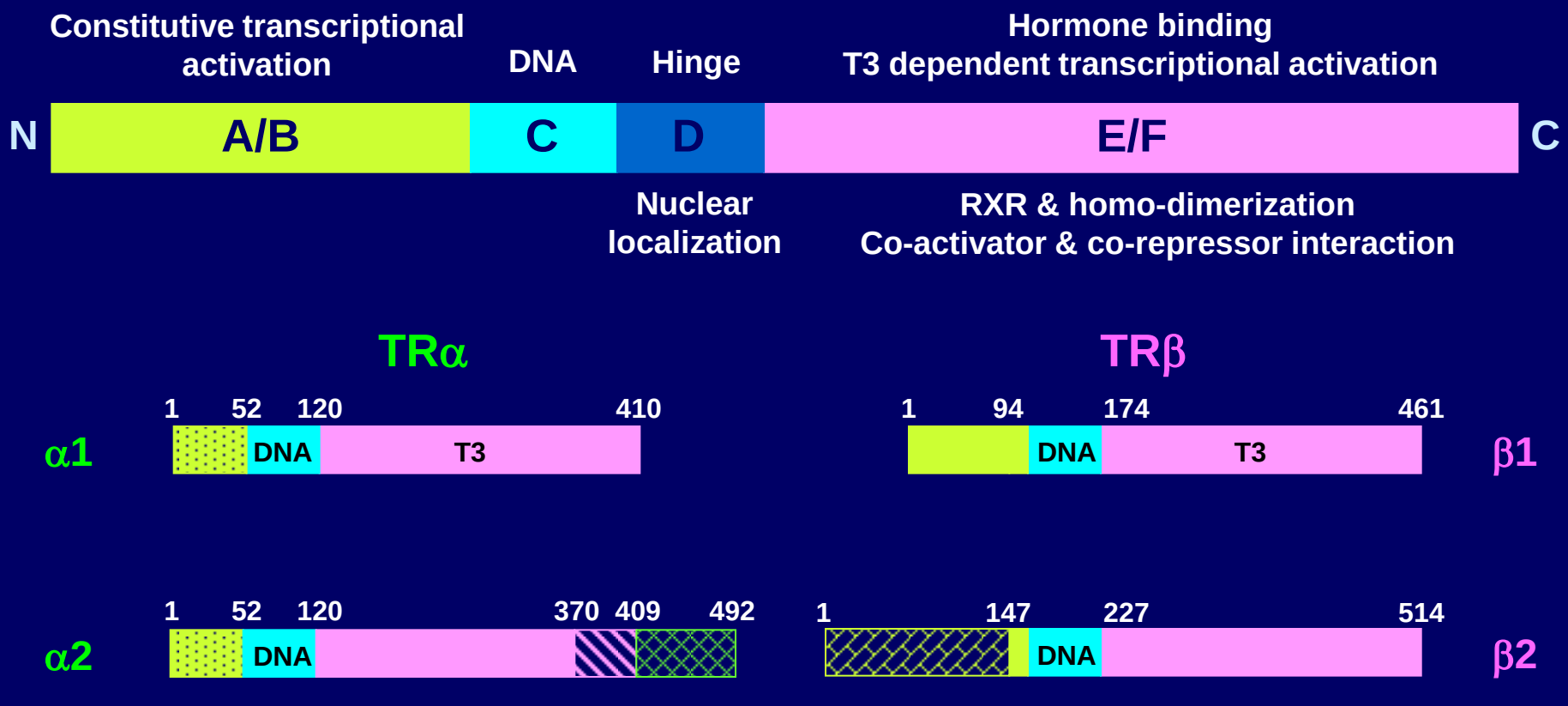
T3 acts via nuclear receptors

- TRα and TRβ
  - Encoded by *THRA* (*NR1A1,* Ch17q11.2) and *THRB* (*NR1A2,* Ch3p24.3)
  - Do not bind T4 under physiological conditions (T4 is a prohormone)
  - Bind T3 with high affinity (K_d 0.1nM)
  - Temporo-spatial regulation of expression during development
  - Expression levels vary between tissues, but TRα is ubiquitous, TRβ more restricted
  - Nuclear localization

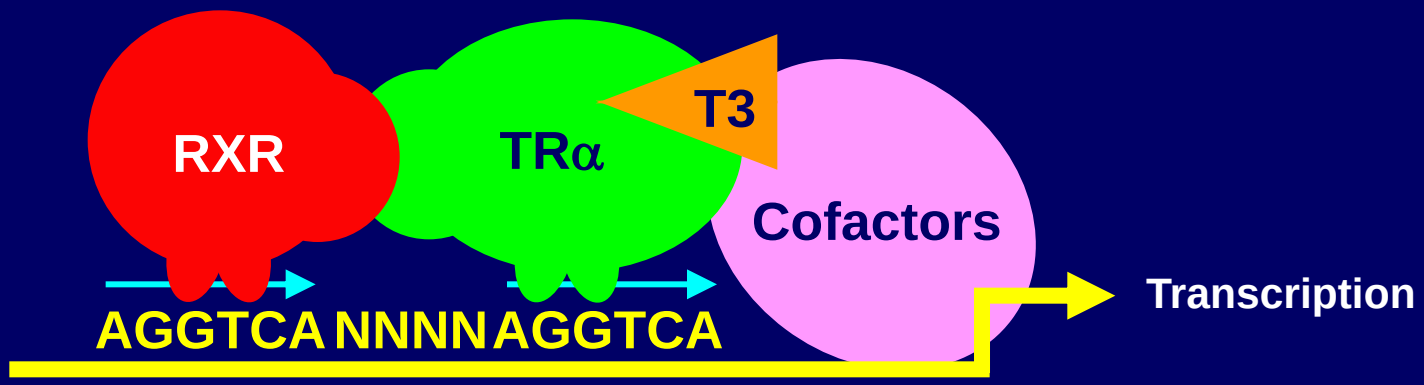
Hypothalamic-pituitary-thyroid axis



Thyroid hormone receptor isoforms



TRs bind TREs in target gene promoters



Consensus TREs

AGGTCATGACCT — Palindromic TRE

AGGTCA NNNN AGGTCA — TRE DR+4

TGACCT NNNNNN AGGTCA — TRE Inverted Pal+6

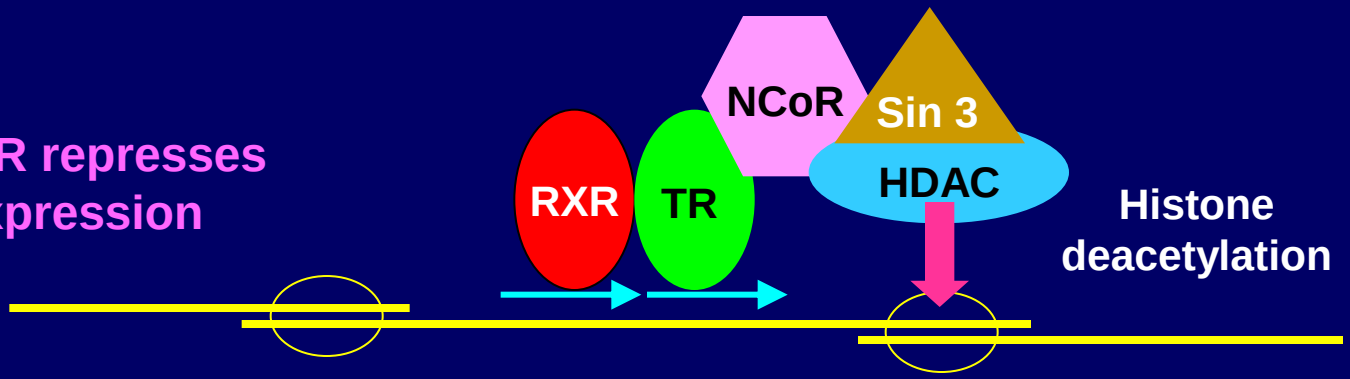
Endogenous TREs

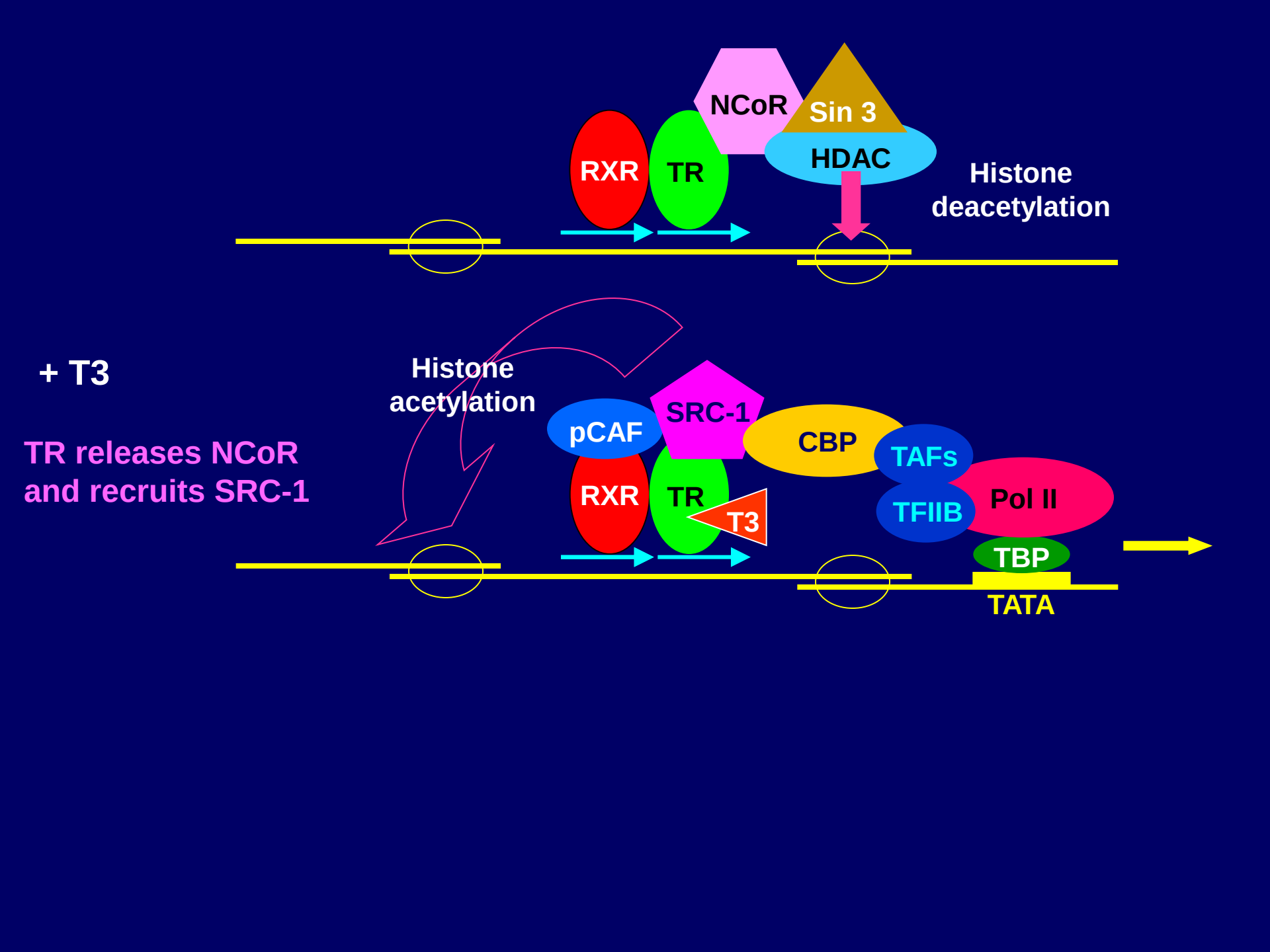
AGGTGA NNNN AGGACA NN AGCCCT — αMHC TRE

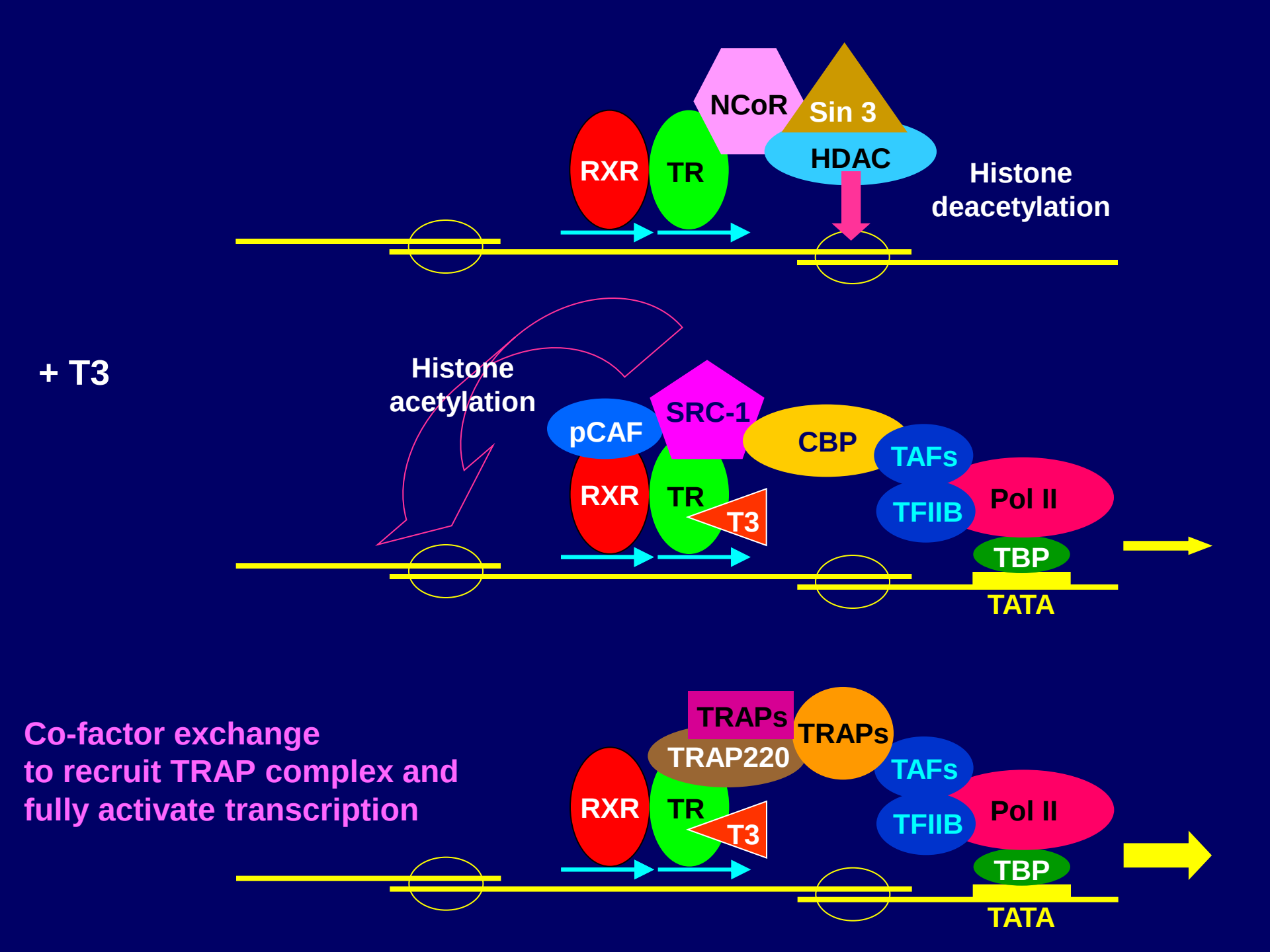
GGGTTA NNNN AGCACA — ME TRE

- T3

Unliganded TR represses basal gene expression




NCoR
Sin 3
HDAC
RXR
TR
Histone
deacetylation
+ T3
Histone
acetylation
TR releases NCoR
and recruits SRC-1
pCAF
SRC-1
CBP
TAFs
RXR
TR
T3
TFIIB
Pol II
TBP
TATA


NCoR
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TR
Histone
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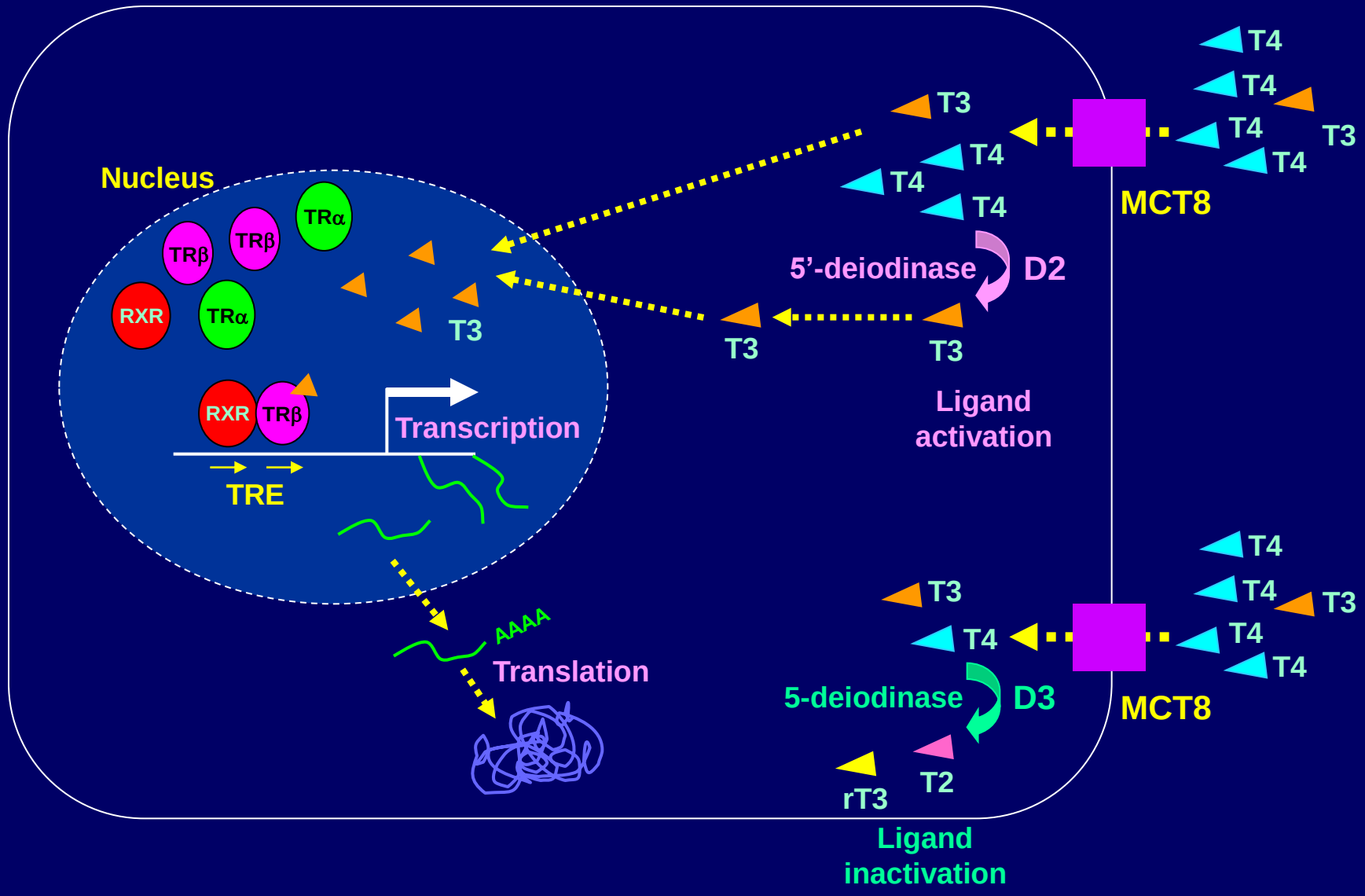
Histone
acetylation
pCAF
SRC-1
CBP
TAFs
RXR
TR
T3
TFIIB
Pol II
TBP
TATA
Co-factor exchange
to recruit TRAP complex and
fully activate transcription
TRAPs
TRAPs
TRAP220
TAFs
RXR
TR
T3
TFIIB
Pol II
TBP
TATA

TRs act as repressors and T3-inducible transcription factors

- **TRα and TRβ**
  - **TR isoforms**
    - **TRα1 and TRβ1 bind T3 and are true receptors**
    - **TRα2 does not bind T3 and its function is unknown**
  - **Nuclear localization is constitutive**
  - **Bind to TREs of varying structure**
  - **Interact with co-repressors, co-activators and other nuclear proteins that may be tissue-specific**
  - **Unliganded apoTR is a repressor**
  - **T3-stimulated positive or negative regulation of T3 target gene transcription**
    - **Positive TREs in *GH*, *DIO1*, *ME*, *MHC* genes**
    - **Negative TREs in *TRH*, *TSHB* genes**

Thyroid hormone action



Control of T3 action – ligand availability

- **Thyroid hormone transport (redundancy)**
  - **MCT8**
    - High affinity for T4, T3, rT3, T2
    - Widely expressed
    - *MCT8* mutations cause severe X-linked psychomotor retardation with elevated serum T3, slightly low T4 and normal TSH
  - **MCT10**
    - Transports T3 in preference to T4
    - Widely expressed, high levels in cartilage
  - **Organic anion transporting polypeptides (OATP)**
    - OATPC1 has high affinity for T4 and rT3 and facilitates influx and efflux
    - Restricted expression pattern
    - Regulates T4 transport across blood-brain barrier
  - **L-type amino acid transporters (LAT1, LAT2)**
    - Transport T4 and T3
    - Widely expressed

Friesema et al (2003) J Biol Chem 278:40128-35
Dumitrescu et al (2004) Am J Hum Genet 74:168–75
Friesema et al (2004) Lancet 364:1435–7
Visser et al (2011) Mol Endocrinol 25:1-14

Control of T3 action – metabolism

- **Iodothyronine deiodinases**
  - **D1**
    - Main source of plasma T3 in hyperthyroidism
    - Role in euthyroid individuals uncertain
    - Up regulated by thyroid hormone
  - **D2**
    - Controls intracellular supplies of T3
    - Essential regulator of T3 action in target cells
    - Paracrine pathways of D2-mediated T3 production and action control cochlear development and hormone action in brain
    - Down regulated by thyroid hormone, rapid turnover of protein
    - Very efficient enzyme
  - **D3**
    - Controls T4 and T3 clearance and prevents intra-cellular T3 production (e.g. development and pregnancy)
    - High expression causes consumptive hypothyroidism
    - Stable efficient enzyme
    - Expression decreased in hypothyroidism

Bianco & Kim (2006) J Clin Invest 116:2571-9
Bernal (2007) Nat Clin Pract Endo Metab 3:249-59
Bianco (2011) Endocrinology 152:3306-11

Control of T3 action – TRs and human disease

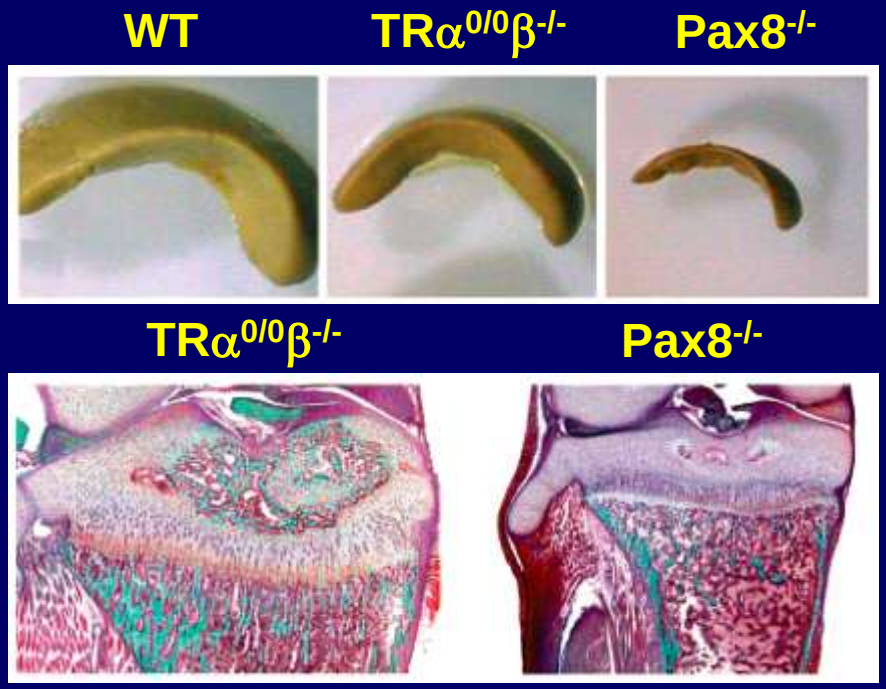
- **RTH**
  - **Autosomal dominant *THRB* mutations cause RTH, in which negative feedback regulation of TSH is disrupted**
    - **Mutations interfere with T3 binding, co-repressor release or co-activator recruitment**
    - **Mutant TRβ acts as dominant-negative antagonist**
    - **Phenotype is variable**
  - ***THRB* mutation absent in 15% of cases**
  - ***THRA* mutations first described in 2012**
    - **3 patients described**
    - **Mutations affect only TRα1 and interfere with T3 binding and co-repressor release**
    - **Mutant TRα1 acts as a dominant-negative antagonist**
    - **Major effects on skeleton and GI tract**
- **Cancer**
  - **Somatic mutation or aberrant expression of *THRB* identified in thyroid, liver and renal cell tumours**

Dumitrescu & Refetoff (2012) Biochim Biophys Acta (in press)
Bochukova et al (2012) NEJM 366:243-9
Van Mullem et al (2012) NEJM 366:1451-3

Tissue specific TR action

- Lessons from murine gene targeting
  - ApoTR
  - TRα
  - TRβ
  - Physiological relationship between TRα and TRβ responsive tissues

ApoTR – hormone deficiency is worse than receptor deficiency



Phenotype of congenitally hypothyroid Pax8$^{-/-}$ mice is more severe than mice lacking all TRs

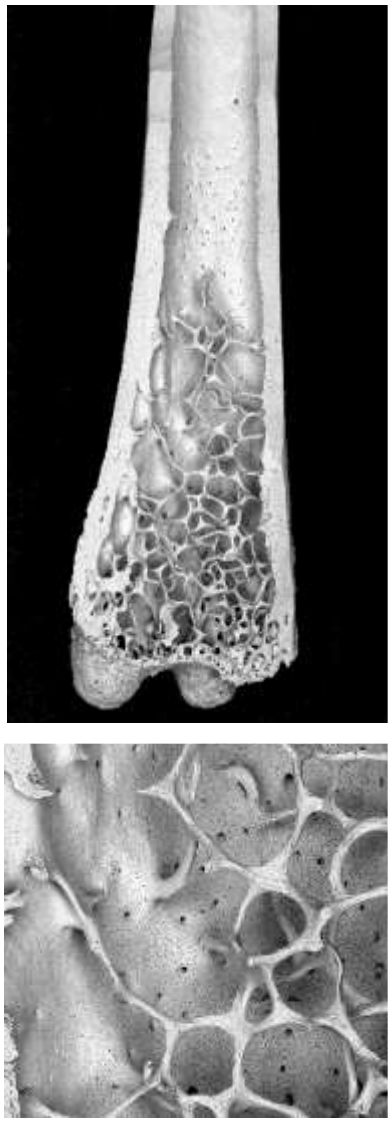
- **Deletion of TRα in Pax8$^{-/-}$TRα$^{0/0}$ compound mutants ameliorates Pax8$^{-/-}$ phenotype**
- **ApoTRα1 plays an important role during development**

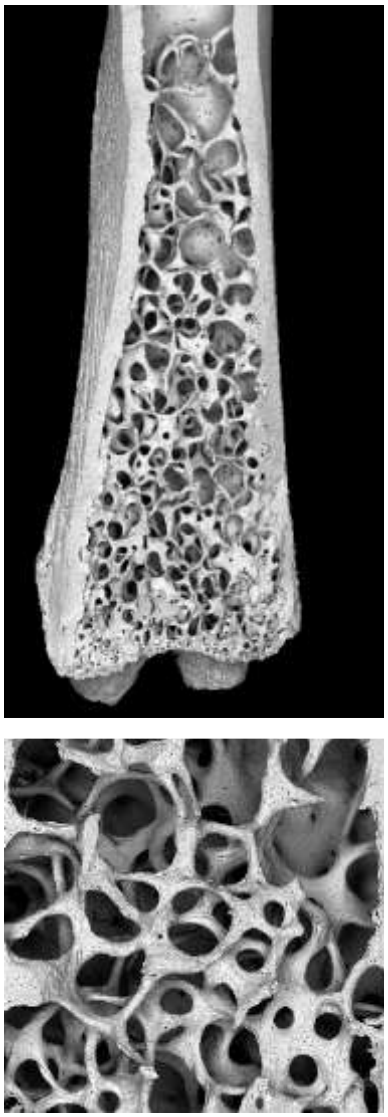
Flamant et al 2002 Mol Endocrinol 16:24-32

Bone is a TRα target tissue

WT

$TR\alpha^{0/0}$





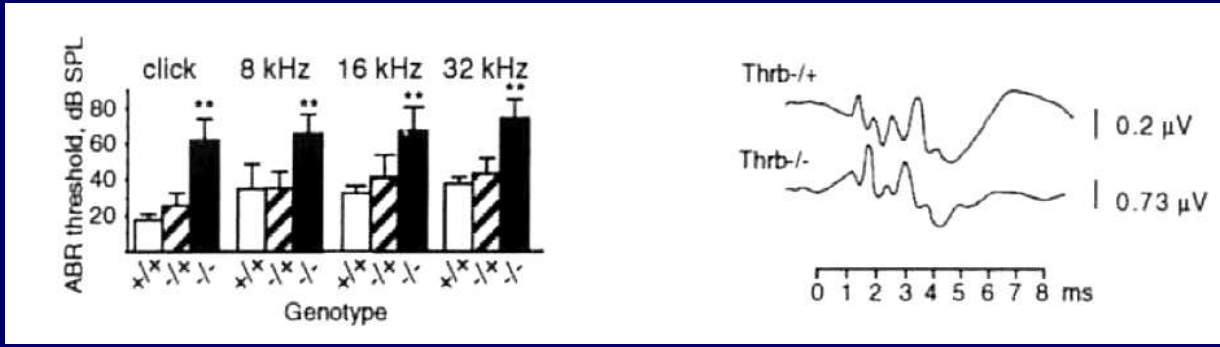
Deletion of TRα

- **Growth retardation**
- **Delayed ossification**
- **Impaired bone resorption**
- **High bone mass**

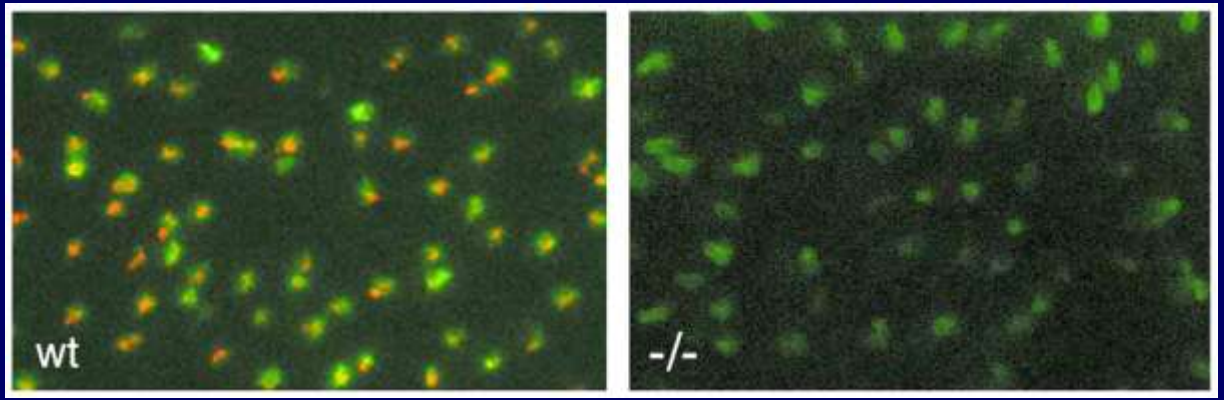
Bassett et al 2007 Mol Endocrinol 21:1095-107

Cochlea and retina are TRβ target tissues

Cochlea



Retina



Deletion of TRβ

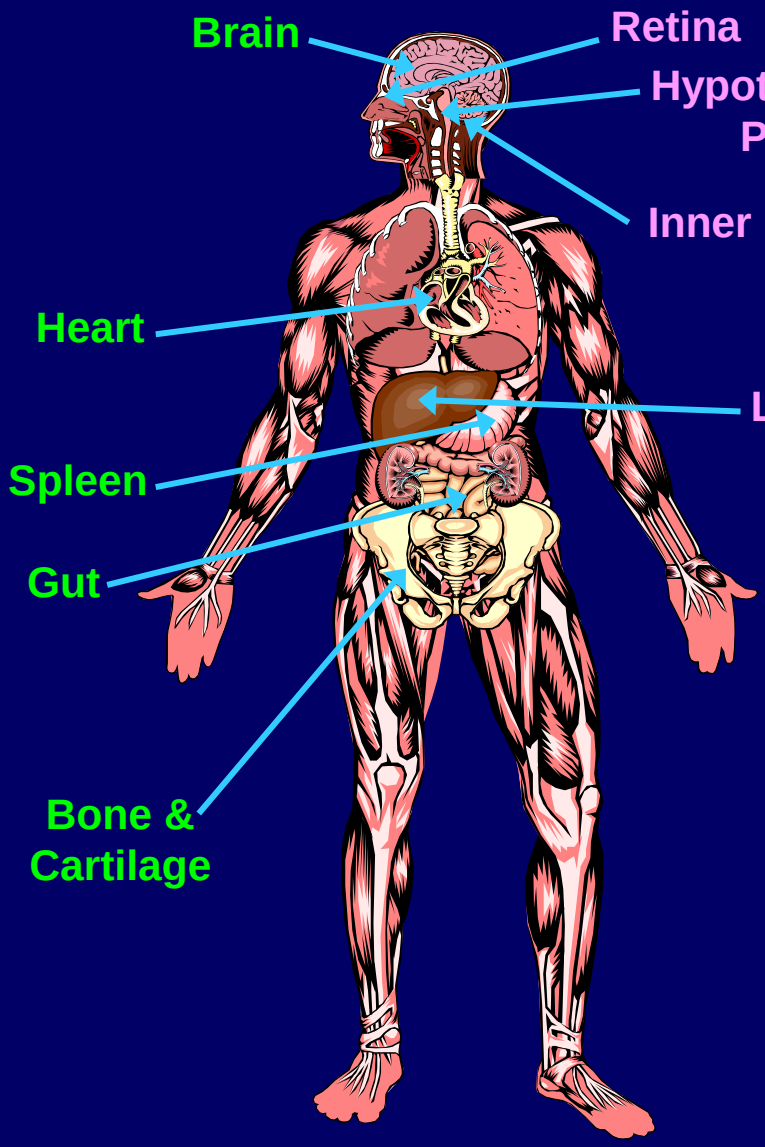
- **Impaired auditory evoked brainstem response**
- **Absent M-opsin and redistribution of S-opsin cones in retina**

Forrest et al 1996 Nat Genetics 13:354-7
Ng et al 2001 Nat Genetics 27:94-8

TR isoform-specific target tissues

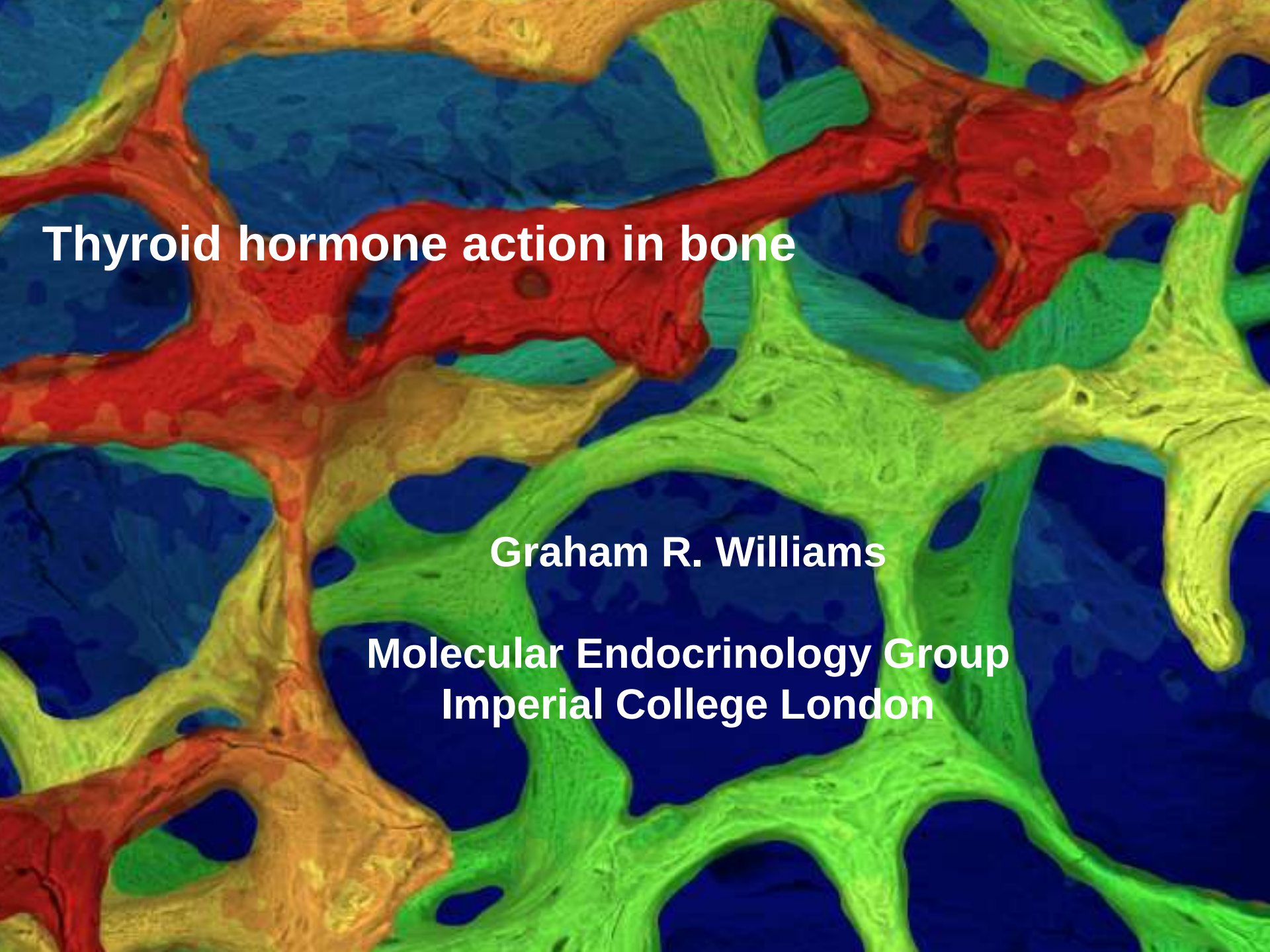
TRα

TRβ



Mixed TRα and TRβ or unknown

- BMR
- Thermogenesis
- Adipogenesis
- Vasculature
- Skin
- Hair
- Muscle
- Bone marrow
- Kidney
- Lung



Thyroid hormone action in bone

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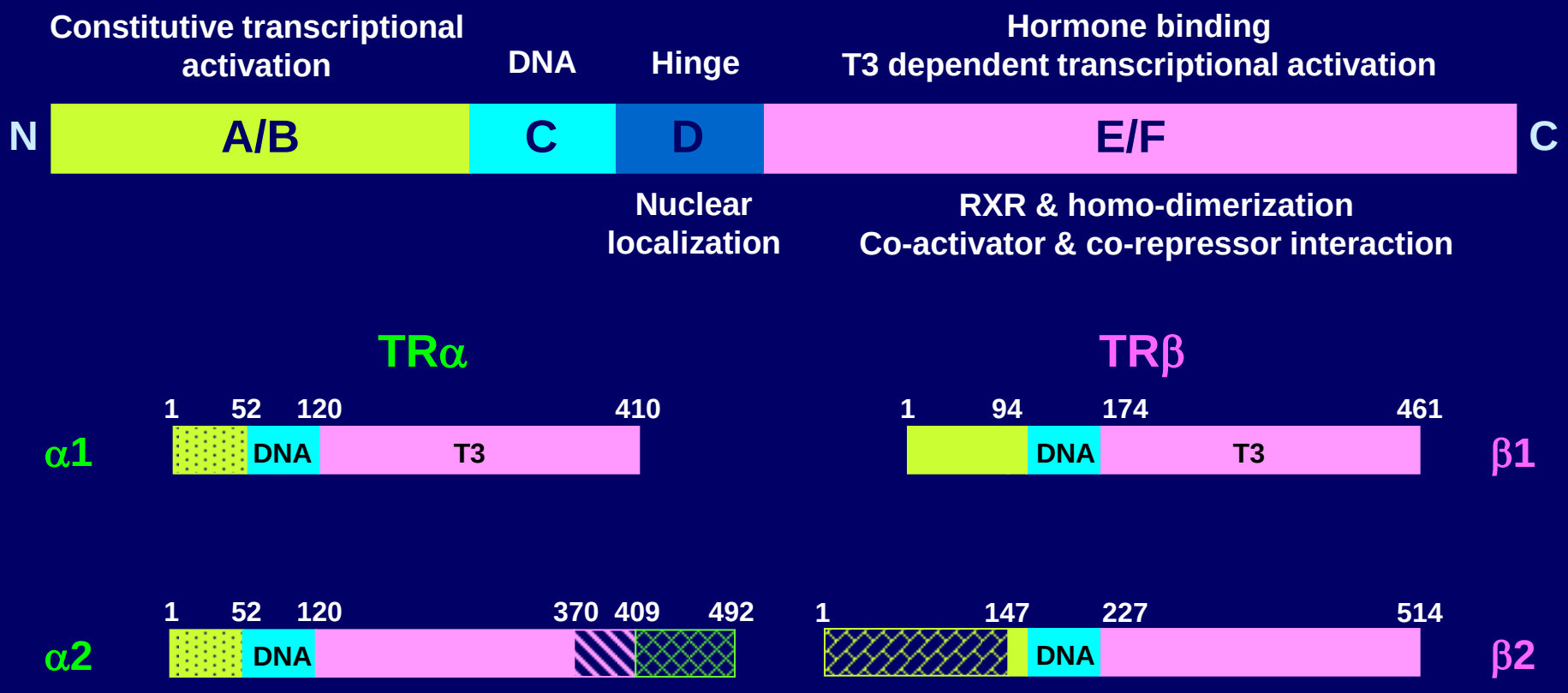
Thyroid hormones and bone

- **Children**
  - **Hypothyroidism**
    - **Growth arrest, delayed bone age, epiphyseal dysgenesis, immature body proportion**
  - **Thyrotoxicosis**
    - **Accelerated growth, advanced bone age, short stature, craniosynostosis**
- **Adults**
  - **Hypothyroidism**
    - **Increased fracture risk in population studies**
  - **Thyrotoxicosis**
    - **Accelerated bone loss, osteoporosis with increased susceptibility to fracture**

Thyroid hormones are essential for skeletal development and regulate bone mass and mineralization in adults

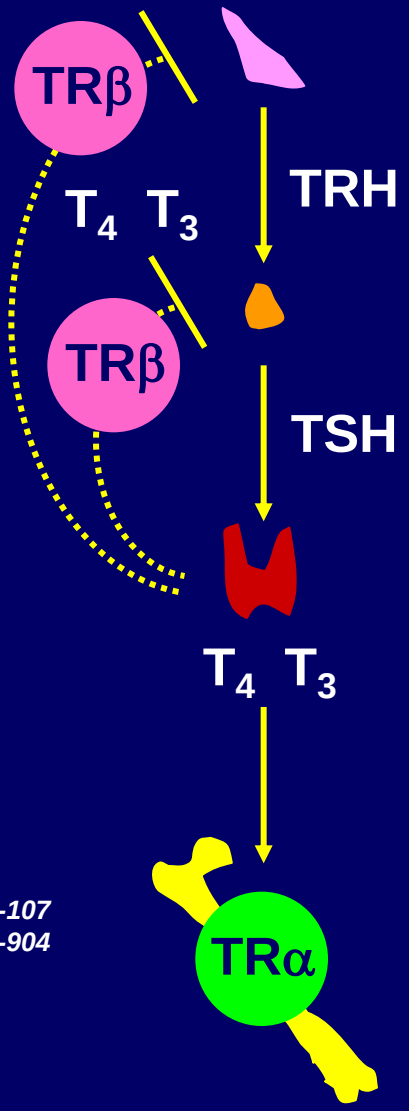
Waung et al (2012) Trends Endocrinol Metab 23:155-62

Thyroid hormone receptor isoforms



Relationship between TRα and TRβ

Forrest et al 1996 EMBO J 15:3006-15
Abel et al 2001 J Clin Invest 107:1017-23



Hypothalamus

Pituitary

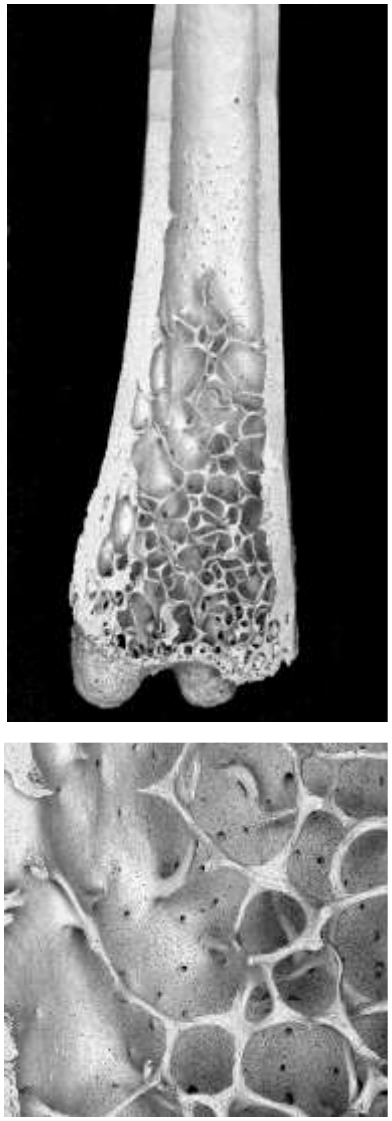
Thyroid

Bone

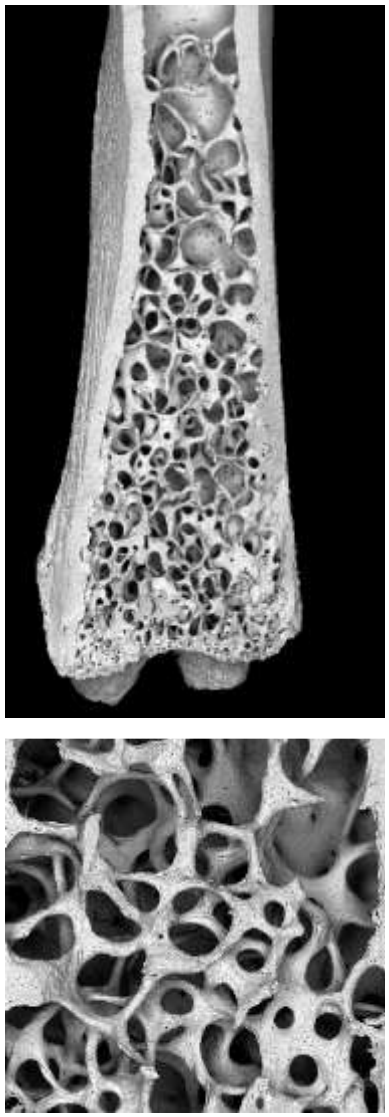
Bassett et al 2007 Mol Endocrinol 21:1095-107
Bassett et al 2007 Mol Endocrinol 21:1893-904

Bone is a TRα target tissue

WT

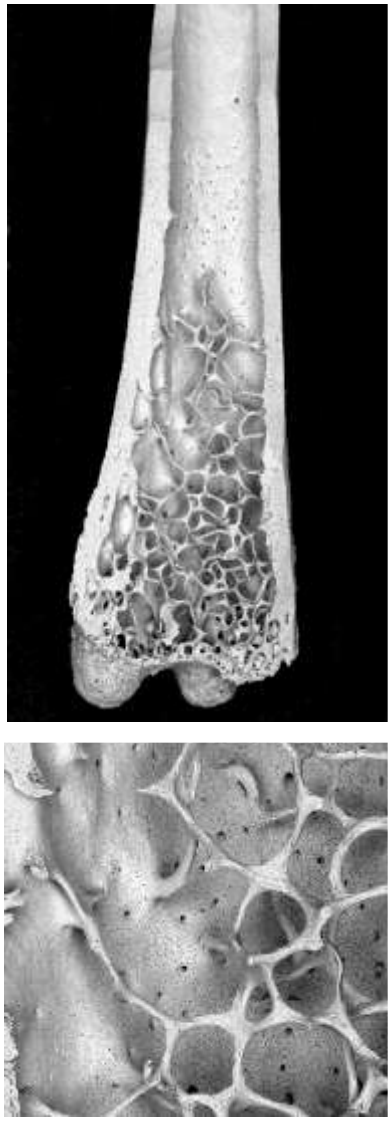


$TR\alpha^{0/0}$

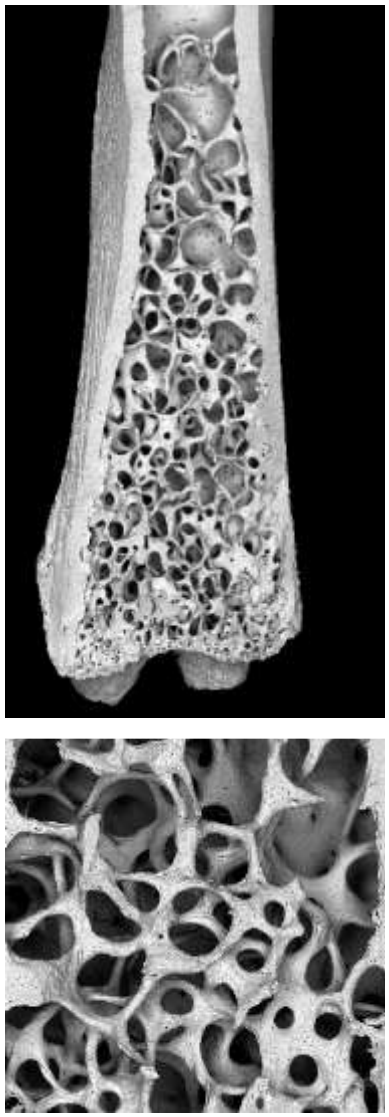


Deletion of TRβ causes an opposite phenotype

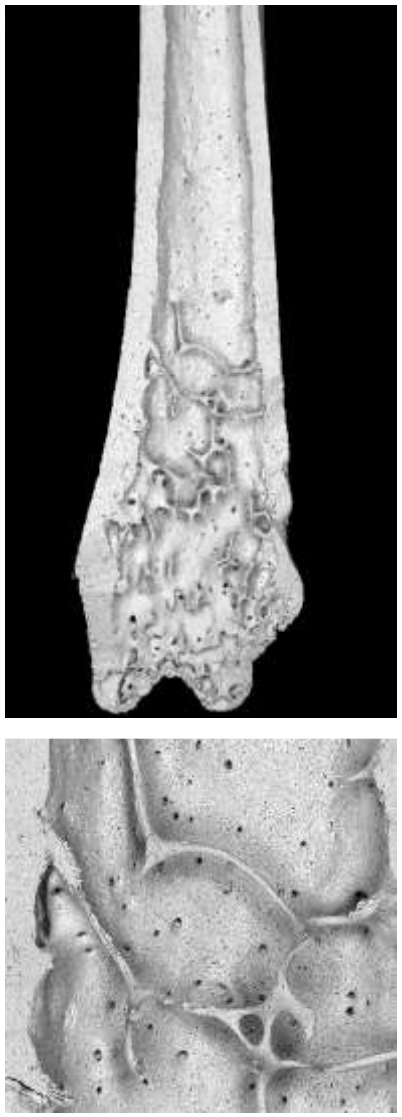
WT



TRα$^{0/0}$



TRβ$^{-/-}$



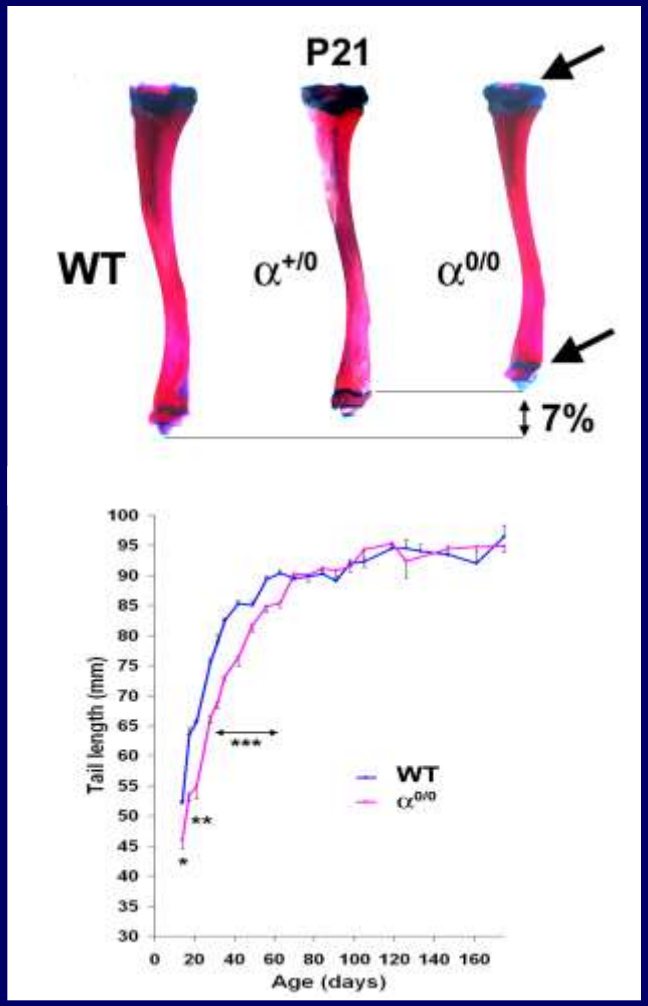
TRα and TRβ knockout mice

Thyroid status of TR$\alpha^{0/0}$ and TR$\beta^{-/-}$ mice

	T4 (μg/dl)	T3 (ng/ml)	TSH (mU/L)	
WT	3.8±0.1	8.4±0.3	25±3.0	Euthyroid
$\alpha^{0/0}$	0.9x	1.2x	0.9x	Euthyroid
$\beta^{-/-}$	4x	6x	12x	RTH

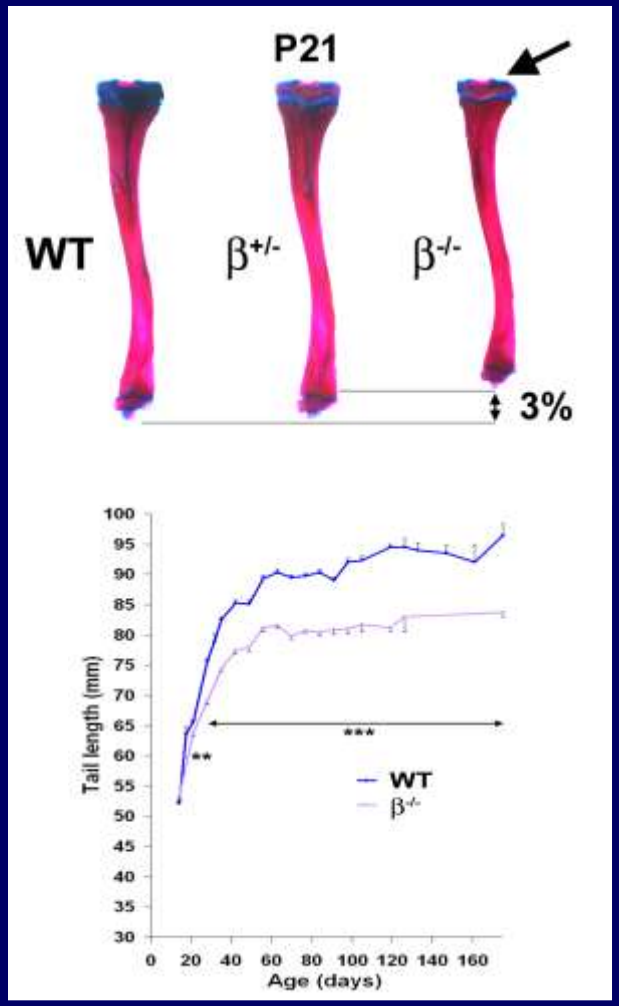
Bassett et al 2007 Mol Endocrinol 21:1095-107

Deletion of TRα or TRβ affects growth



TRα$^{0/0}$

Transient growth delay

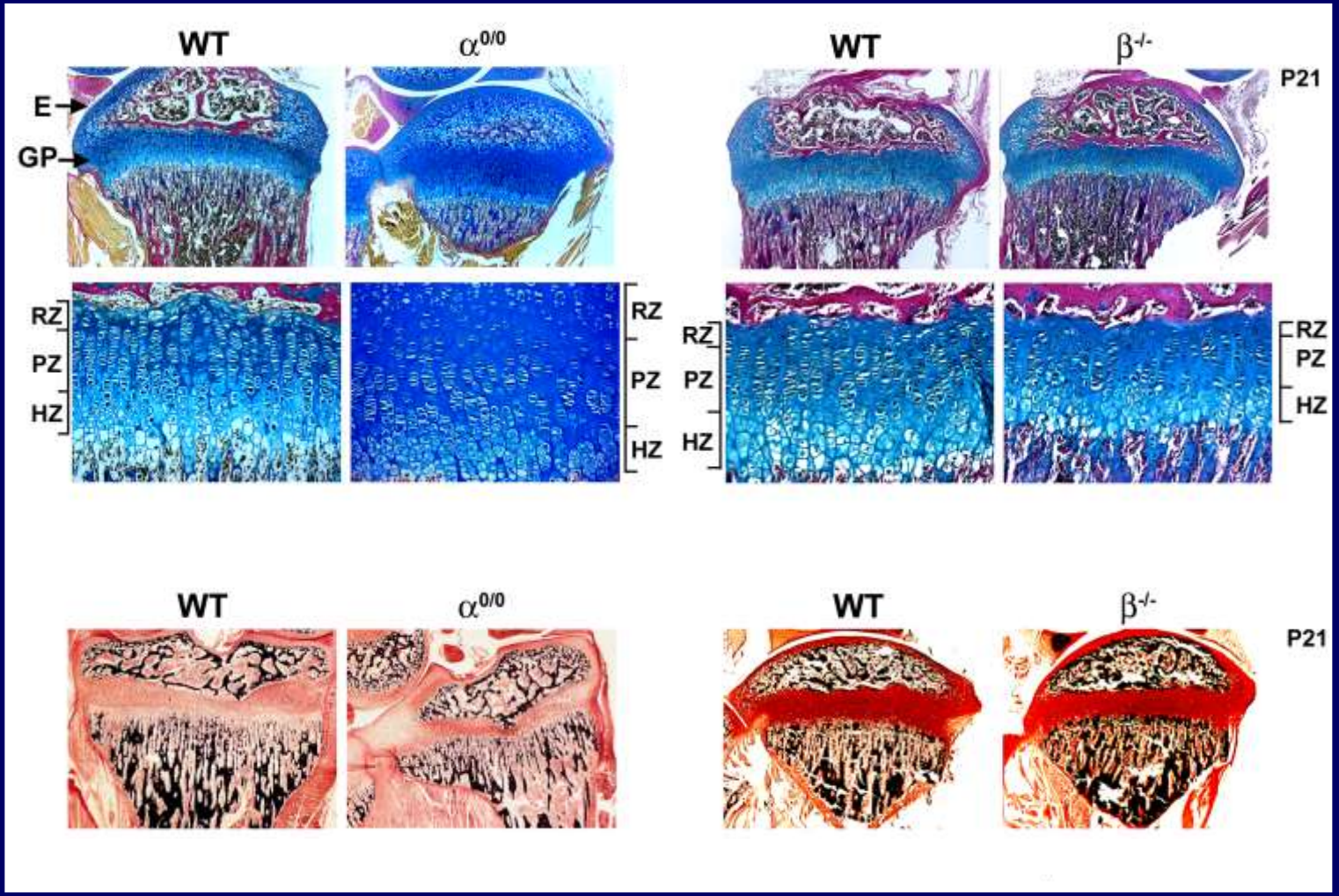


TRβ$^{-/-}$

Persistent short stature

Bassett et al 2007 Mol Endocrinol 21:1095-107

Deletion of TRα or TRβ affects ossification

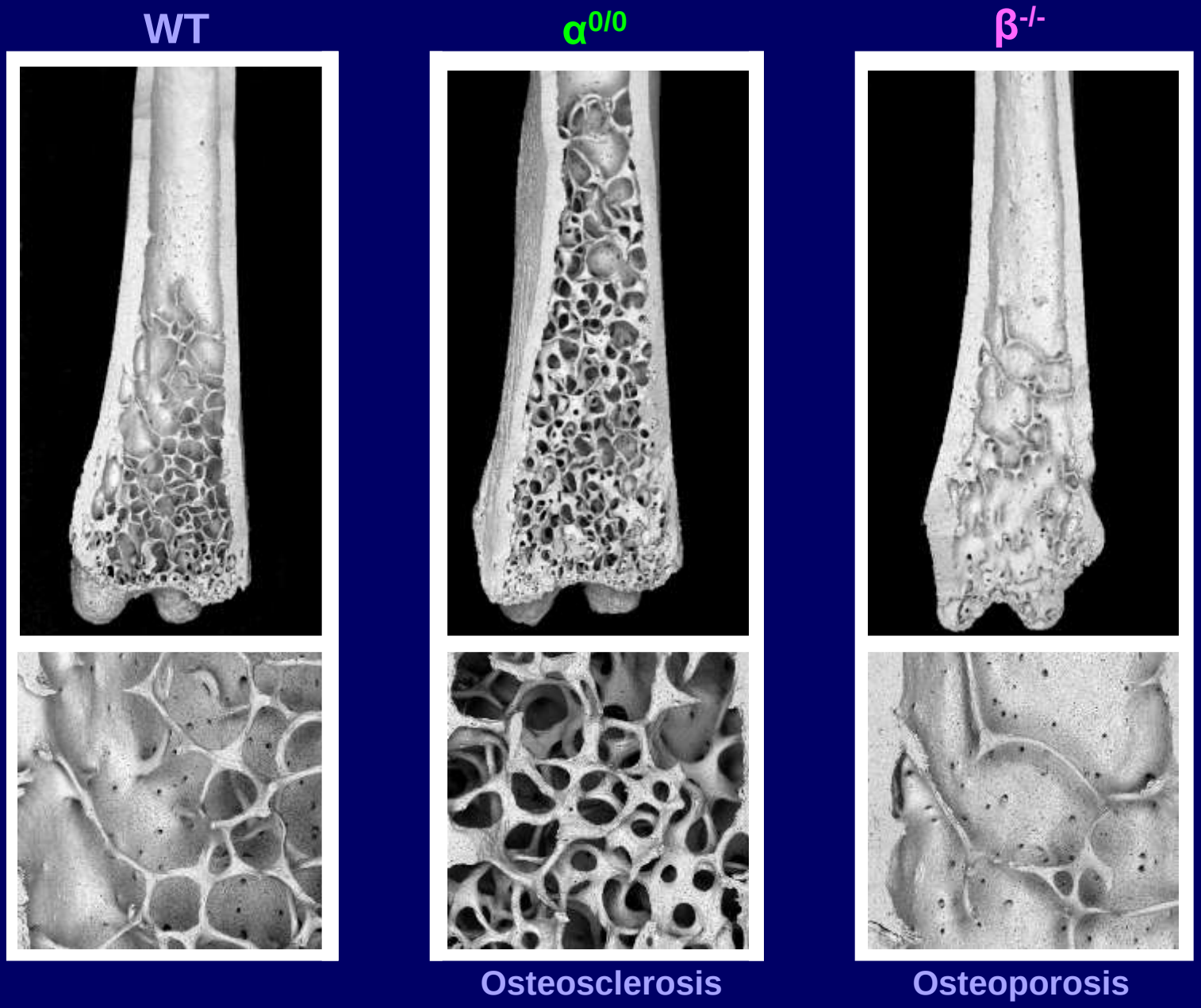


TRα$^{0/0}$
Delayed endochondral ossification

TRβ$^{-/-}$
Advanced endochondral ossification

Bassett et al 2007 Mol Endocrinol 21:1095-107

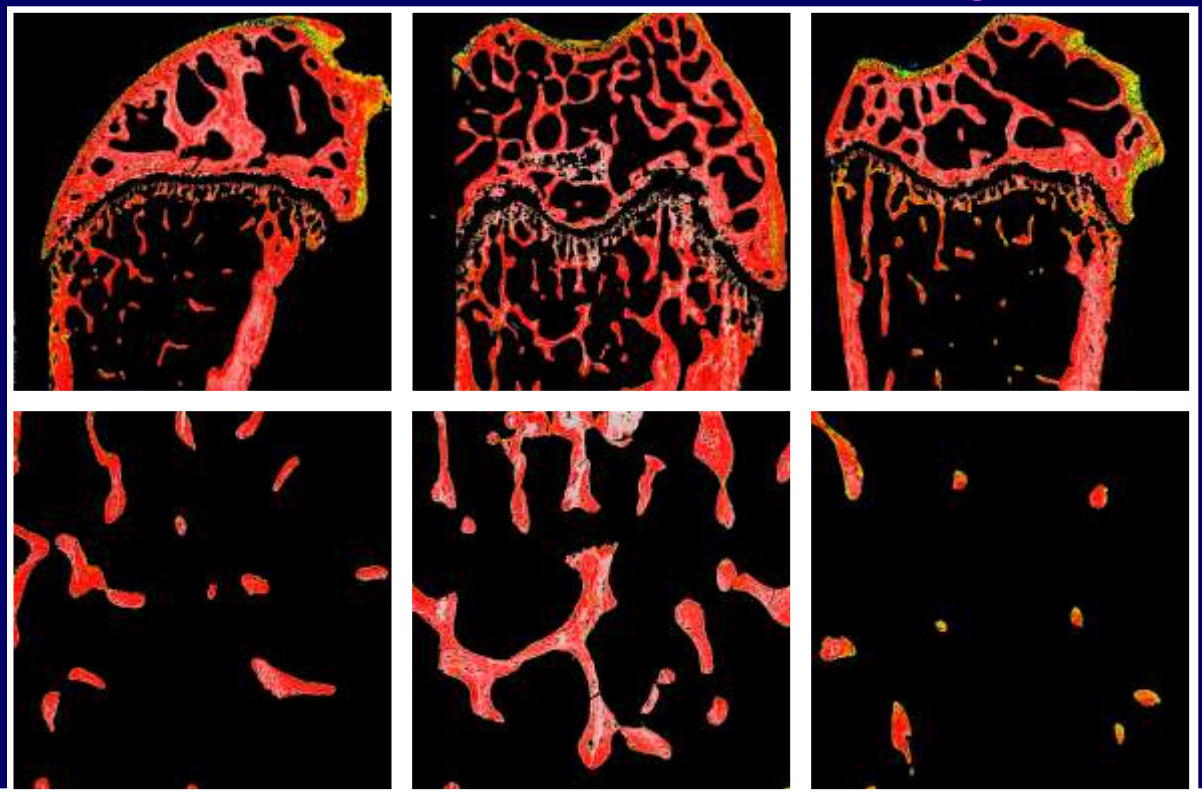
Deletion of TRα or TRβ affects bone mass

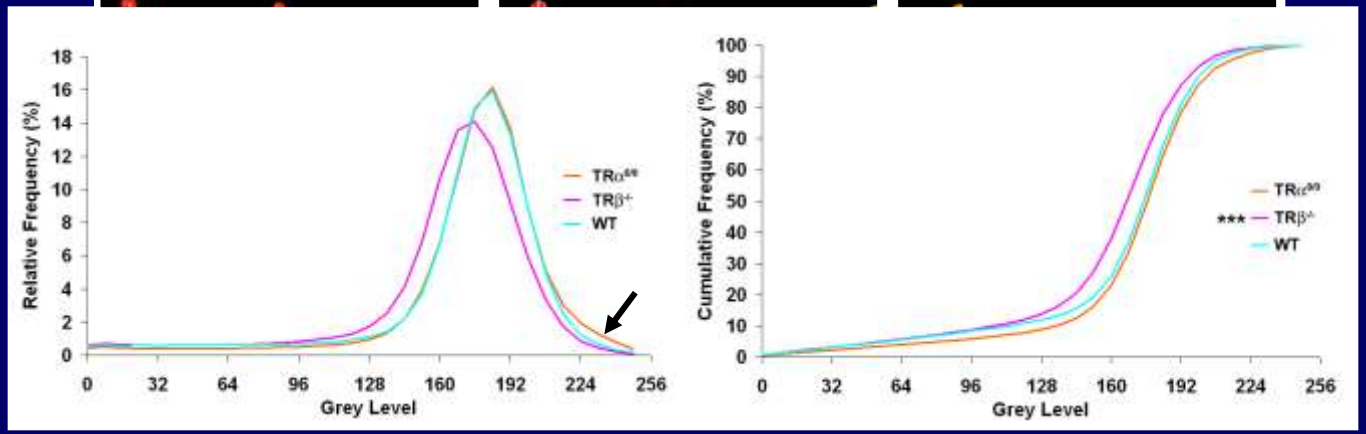


Bassett et al 2007 Mol Endocrinol 21:1095-107

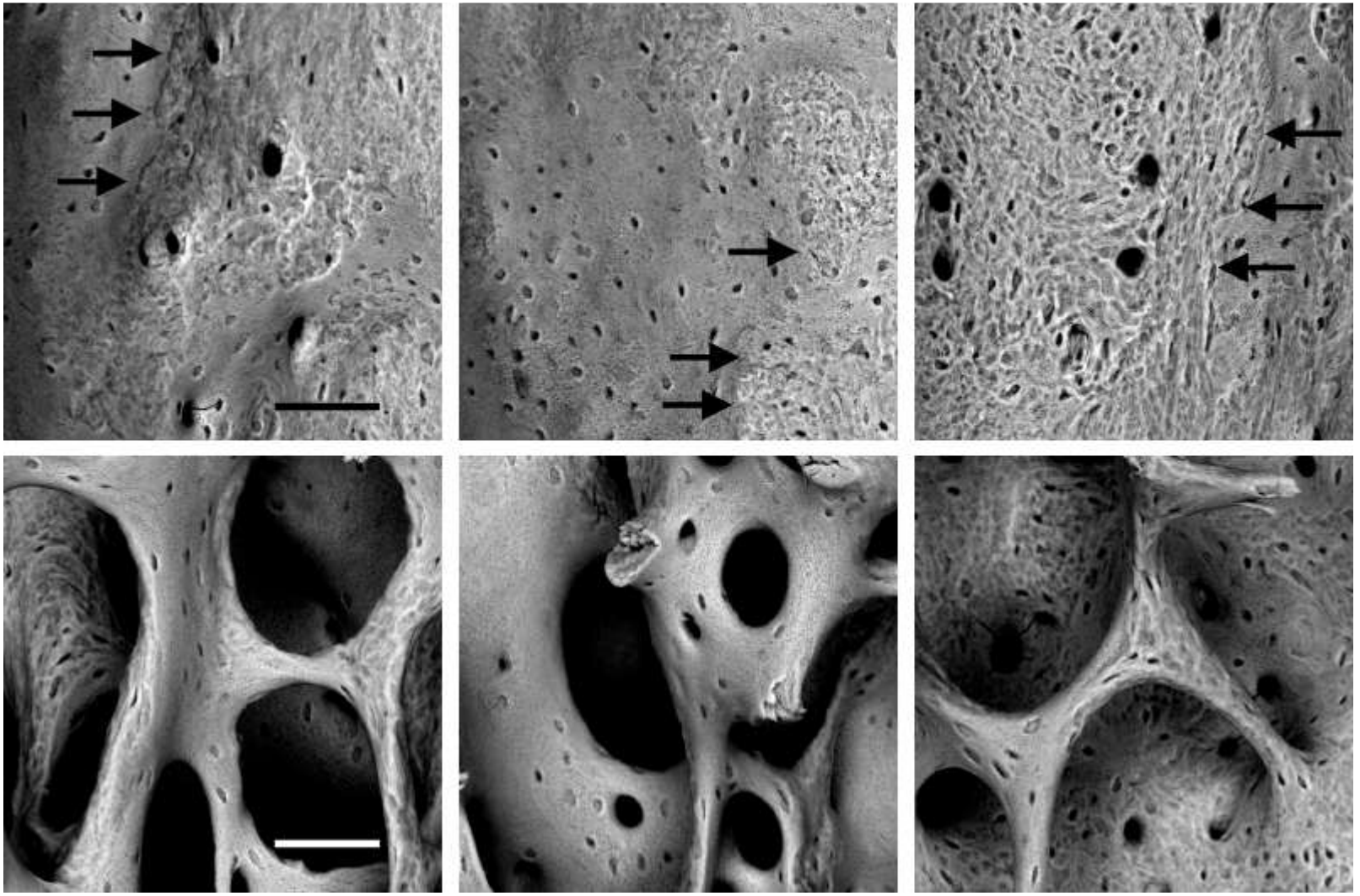
Deletion of TRα or TRβ affects mineralization

WT $\alpha^{0/0}$ $\beta^{-/-}$





Deletion of TRα or TRβ affects bone resorption



WT

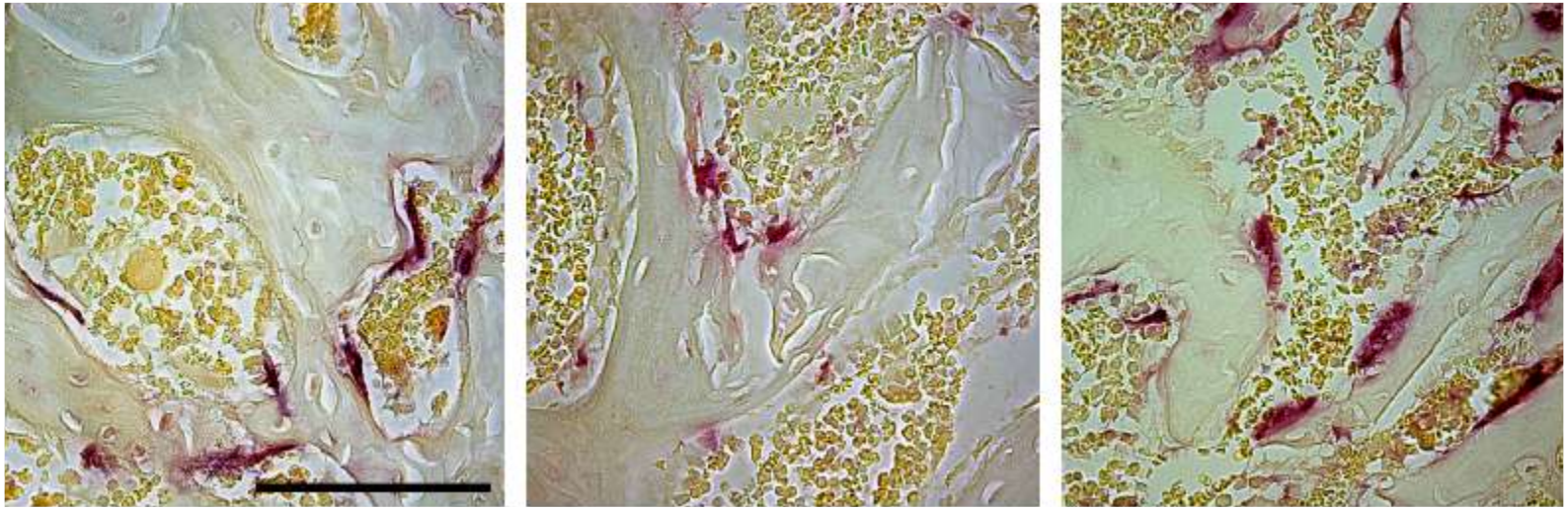
TRα$^{0/0}$

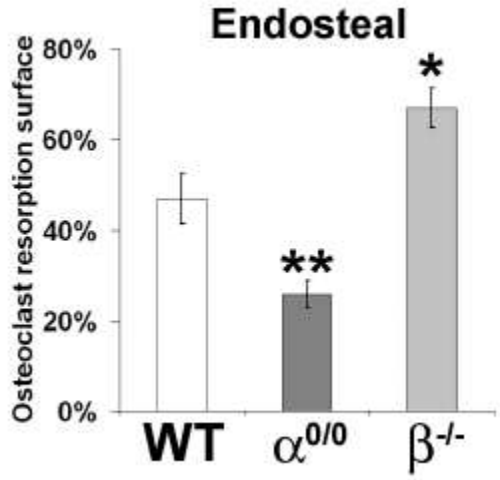
TRβ$^{-/-}$

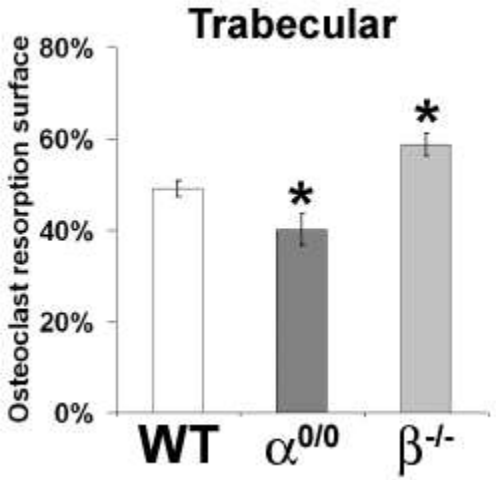
Bassett et al 2007 Mol Endocrinol 21:1095-107

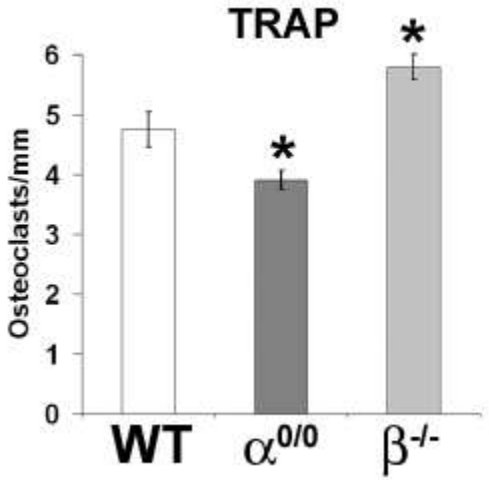
Deletion of TRα or TRβ affects osteoclasts

WT TRα$^{0/0}$ TRβ$^{-/-}$









Bassett et al 2007 Mol Endocrinol 21:1095-107

Summary

- **TRα$^{0/0}$**
  - ***Delayed*** **ossification, reduced calcified bone and growth retardation**
  - ***Increased*** **adult bone mass (reduced bone resorption)**

- **TRβ$^{-/-}$**
  - ***Advanced*** **ossification, increased calcified bone, accelerated early growth but persistent short stature**
  - ***Reduced*** **adult bone mass and mineralization (increased bone resorption)**

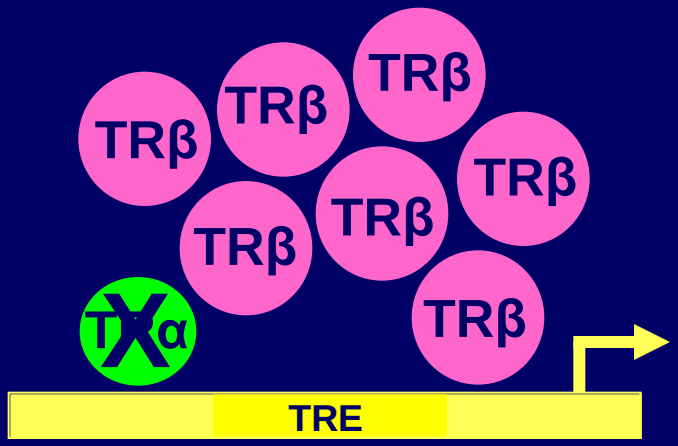
Bassett et al 2007 Mol Endocrinol 21:1095-107

Mechanism of T3 action in bone

in vivo

Pituitary Circulation Bone

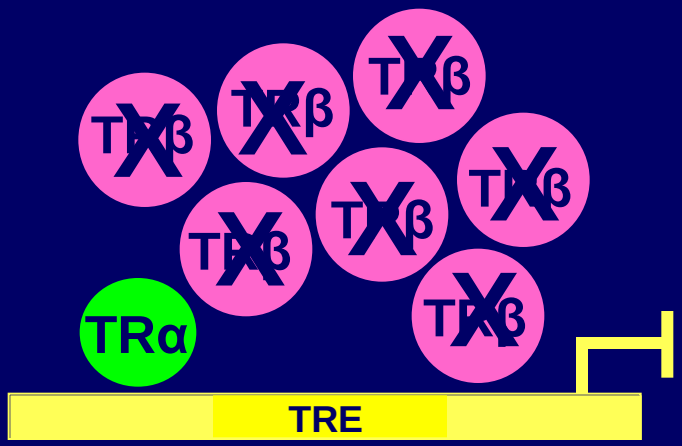
$TR\alpha^{0/0}$



T3, T4
Normal

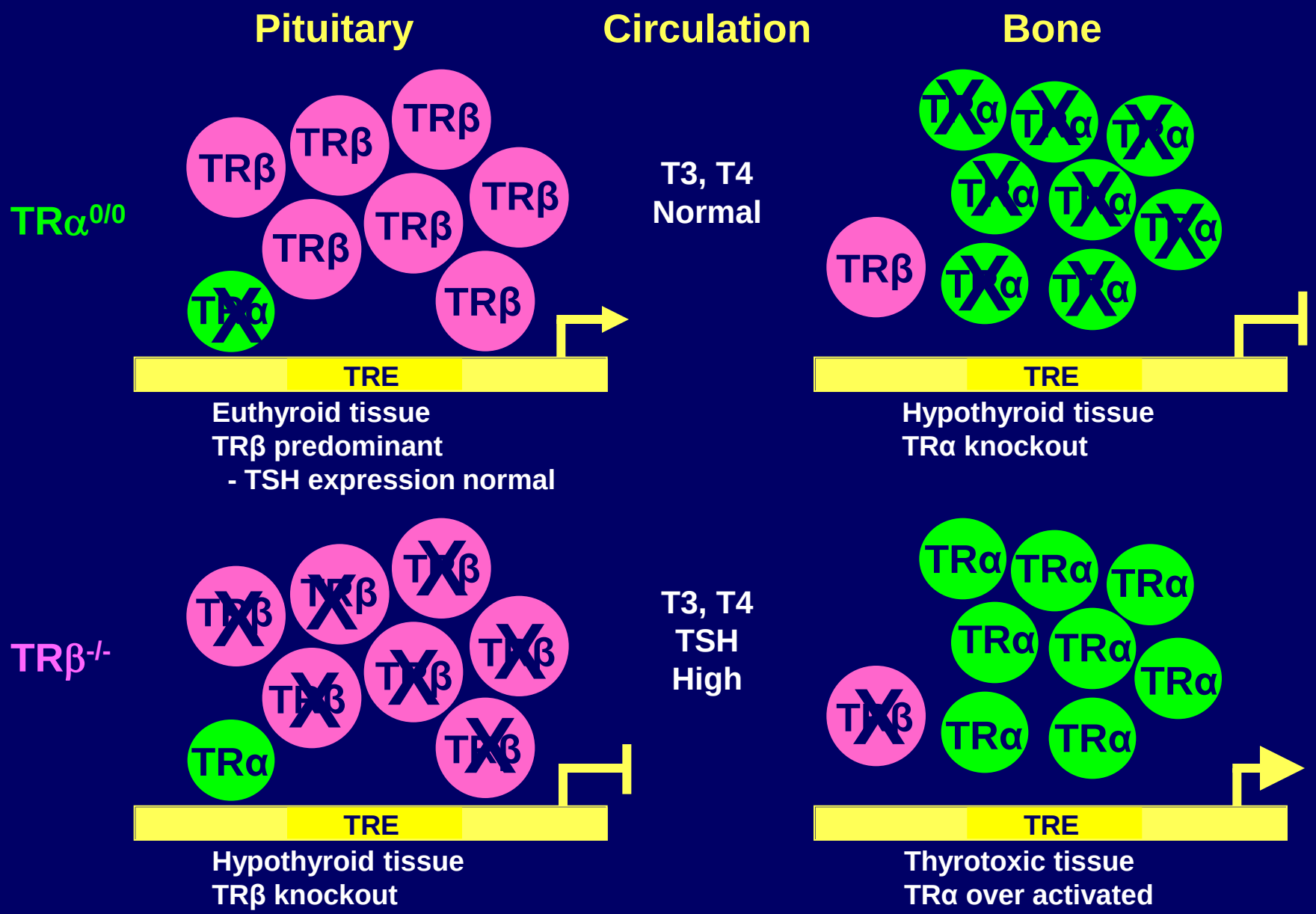
Euthyroid tissue
TRβ predominant
- TSH expression normal

$TR\beta^{-/-}$



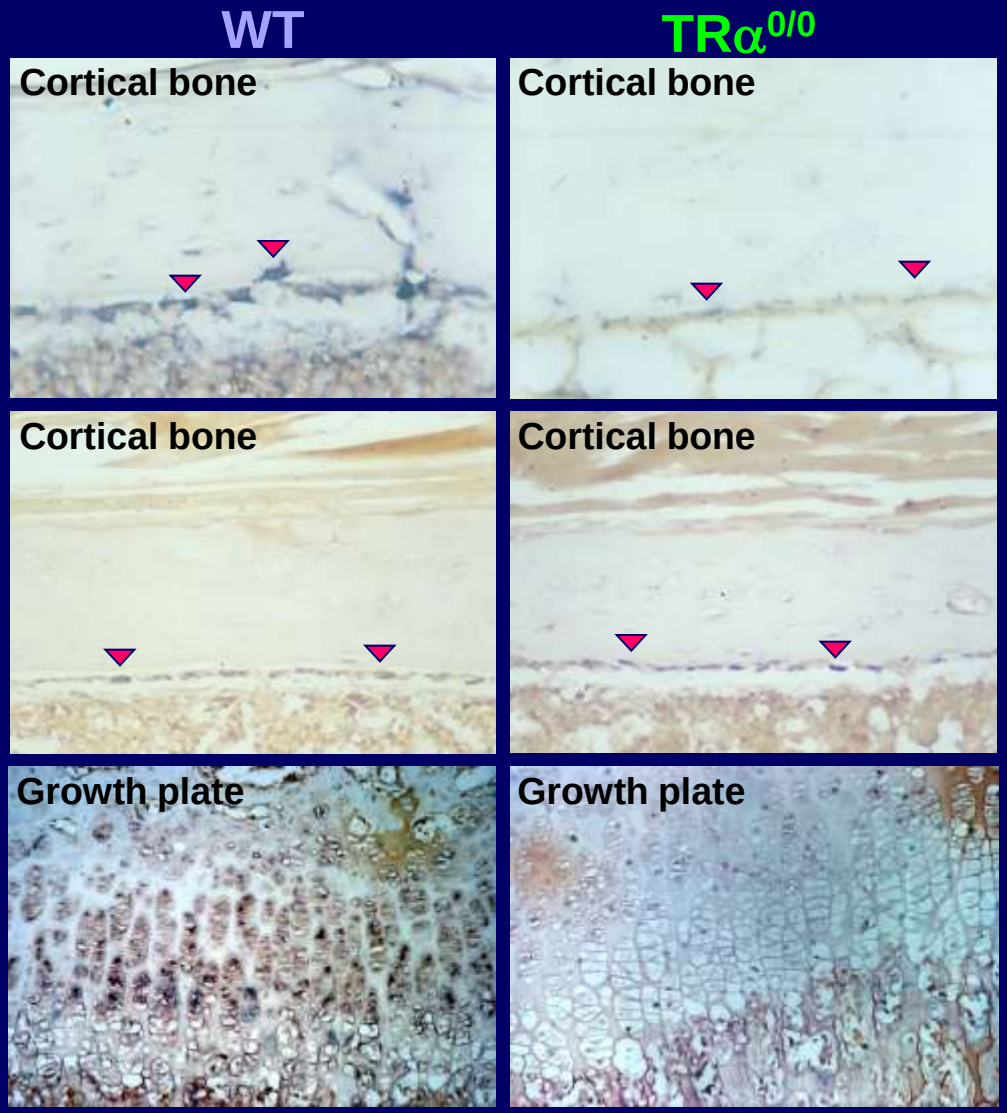
T3, T4
TSH
High

Hypothyroid tissue
TRβ knockout
- TSH repression impaired



O'Shea et al 2006 Nuclear Receptor Signaling 4:e011

FGFR expression in TRα$^{0/0}$ bone



Decreased FGFR1 expression in TRα$^{0/0}$ osteoblasts

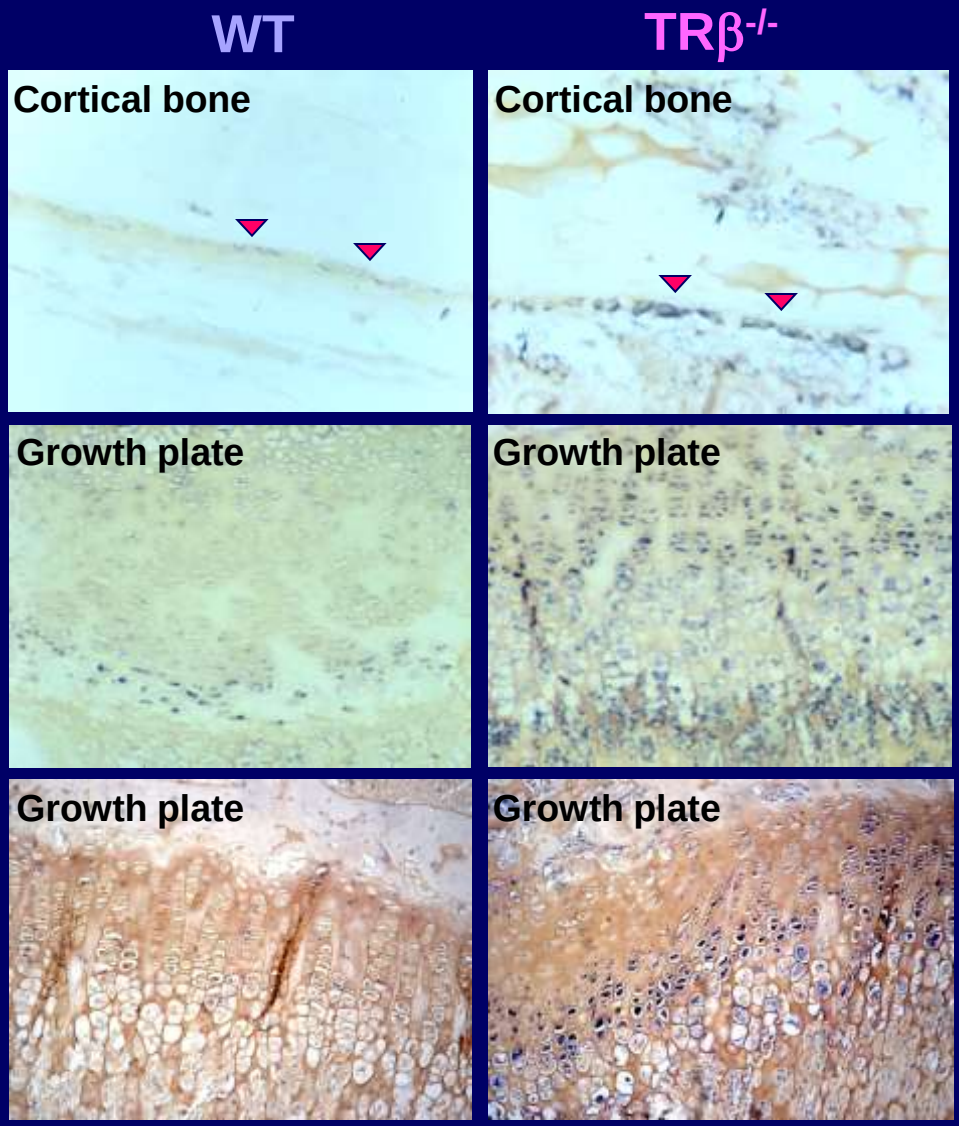
Unchanged FGFR2 expression in TRα$^{0/0}$ osteoblasts

Decreased FGFR3 expression in TRα$^{0/0}$ chondrocytes

TRα$^{0/0}$ skeleton is hypothyroid

Stevens et al 2003 Mol Endocrinol 17: 1751-1766

FGFR expression in TRβ$^{-/-}$ bone



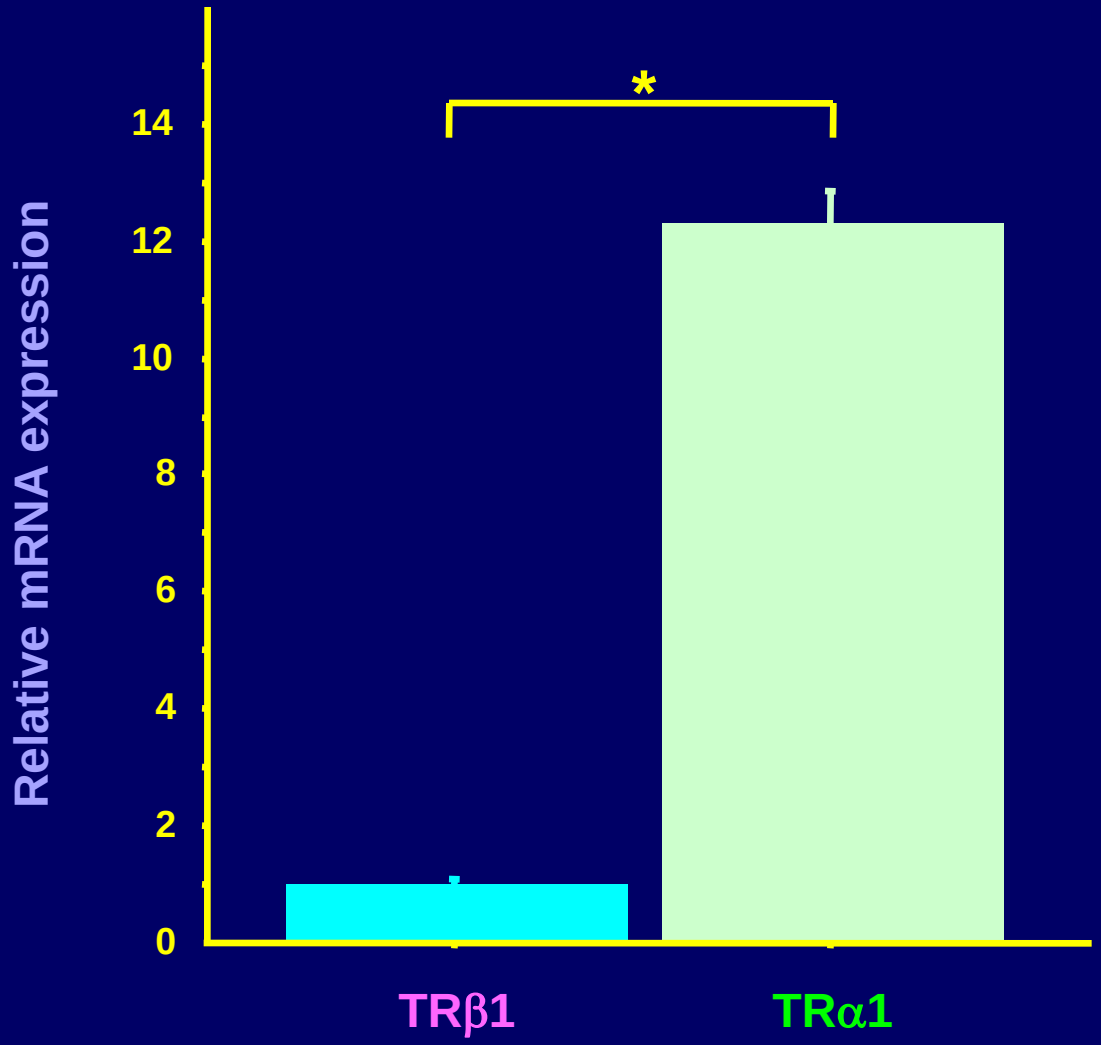
Increased FGFR1 expression in TRβ$^{-/-}$ osteoblasts

Increased FGFR1 expression in TRβ$^{-/-}$ chondrocytes

Increased FGFR3 expression in TRβ$^{-/-}$ chondrocytes

TRβ$^{-/-}$ skeleton is thyrotoxic

Receptor mRNA expression in bone



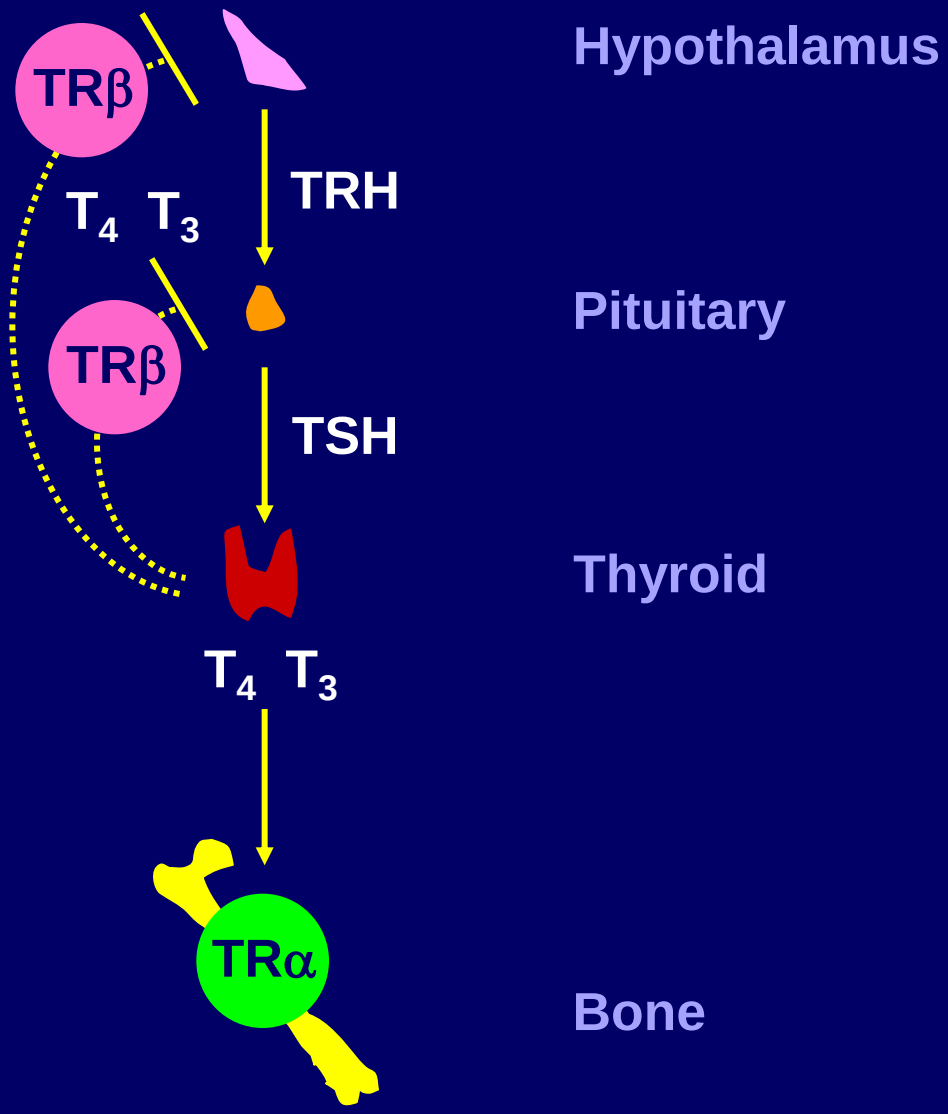
O'Shea et al 2003 Mol Endocrinol 17: 1410-1424

Conclusion

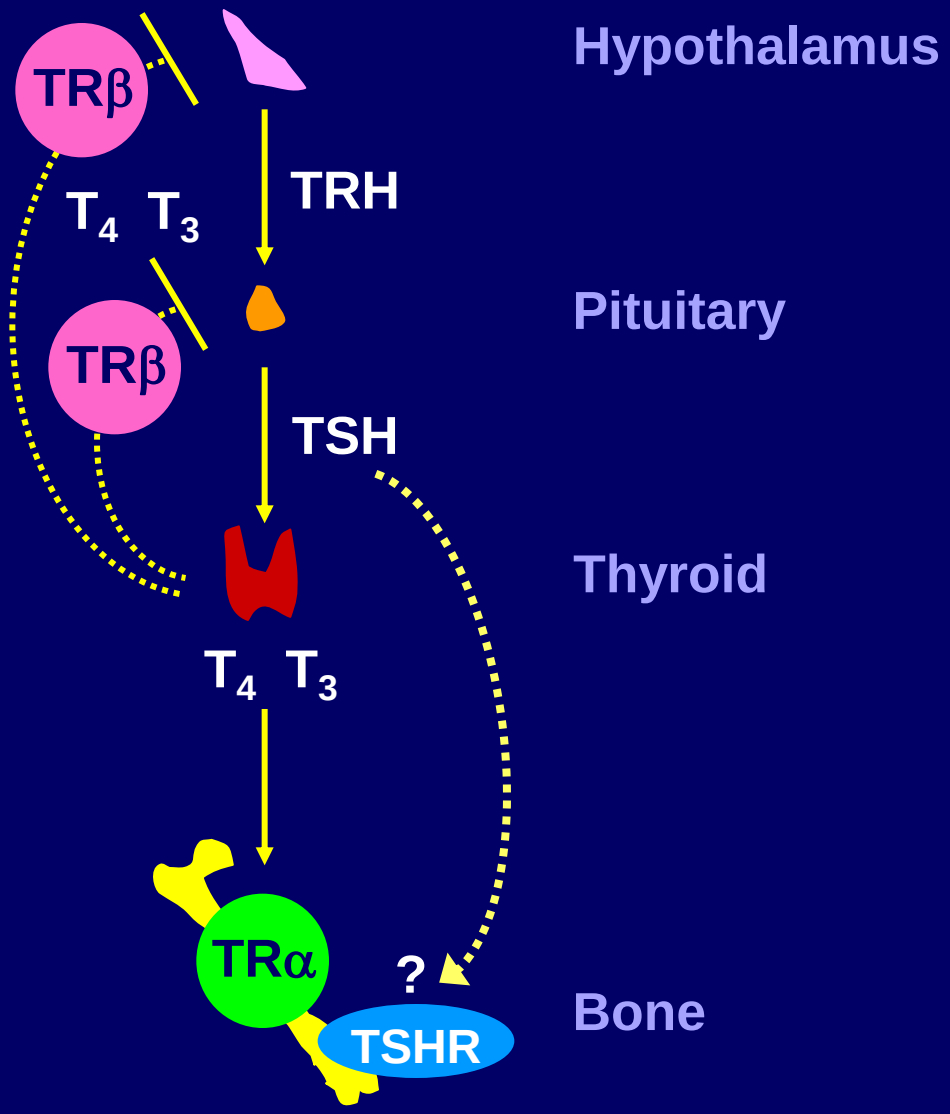
- **Deletion of TRα**
  - **Causes skeletal hypothyroidism despite normal thyroid status because T3 action in bone is disrupted**
- **Deletion of TRβ**
  - **Causes osteoporosis indirectly because elevated thyroid hormones act in bone via TRα**

Systemic and tissue-specific actions of T3 receptors are inter-dependent

Hypothalamic-pituitary-thyroid axis



Hypothalamic-pituitary-thyroid axis



Thyroid hormone or TSH?

Bassett & Williams (2008) Bone 43:418-26

$TSHR^{-/-}$ mice

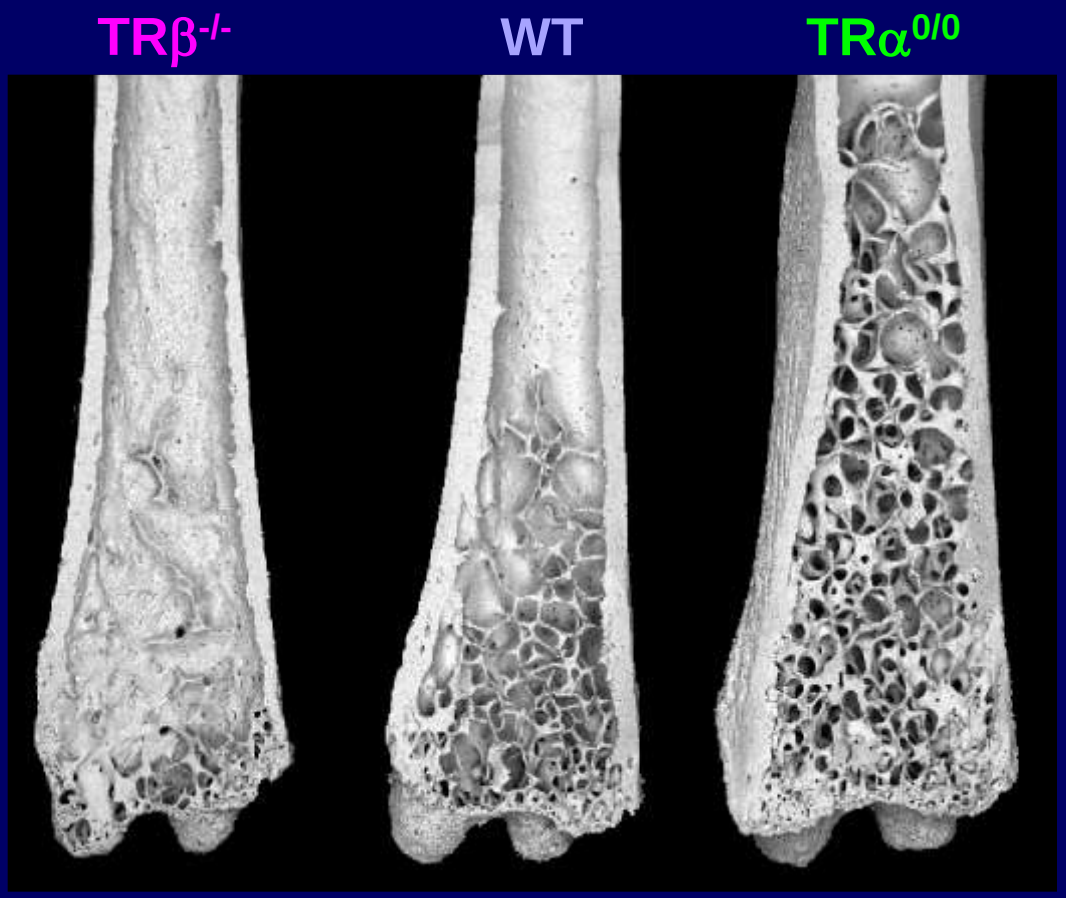
- $TSHR^{-/-}$
  - Thyroid hypoplasia
  - fT_4 & fT_3 undetectable, TSH 500x
  - Severe growth delay
  - Die by 10w unless given TH at weaning
  - High bone turnover osteoporosis (aged 7 weeks)
- $TSHR^{+/-}$
  - Euthyroid
  - Normal growth
  - Intermediate skeletal phenotype
- TSH inhibits
  - Osteoclast formation, osteoblast differentiation

Lack of TSHR results in osteoporosis
TSH preserves bone

Marians RC et al, PNAS 2002 99:15776-81
Abe E et al, Cell 2003 115:151-62

Thyroid hormone or TSH ?

- **TSHR$^{-/-}$ mice**
  - Congenital hypothyroidism
  - Treated with TH from weaning
  - Analyzed during growth
- **Graves' disease**
  - Osteoporosis
  - TSHR stimulating antibodies
- **cAMP responses**
  - TSHR secondary messenger
- **TRβ$^{-/-}$ mice**
  - 3x fT4/fT3 and 10xTSH
  - Osteoporotic
- **TRα$^{0/0}$ mice**
  - Euthyroid, TSH normal
  - High bone mass



Data from TSHR$^{-/-}$ mice inconsistent with clinical observations & with TRKO mice

Bassett & Williams (2008) Bone 43:418-26

hyt/hyt and Pax8$^{-/-}$ mice

- **hyt/hyt**
  - **TSHR $^{Pro}556^{Leu}$ mutation does not bind TSH**
  - **Hypoplastic thyroid & congenital hypothyroidism**
  - **fT_4 0.1x, fT_3 0.05x, TSH 1900x**

Elevated TSH non-functional TSHR

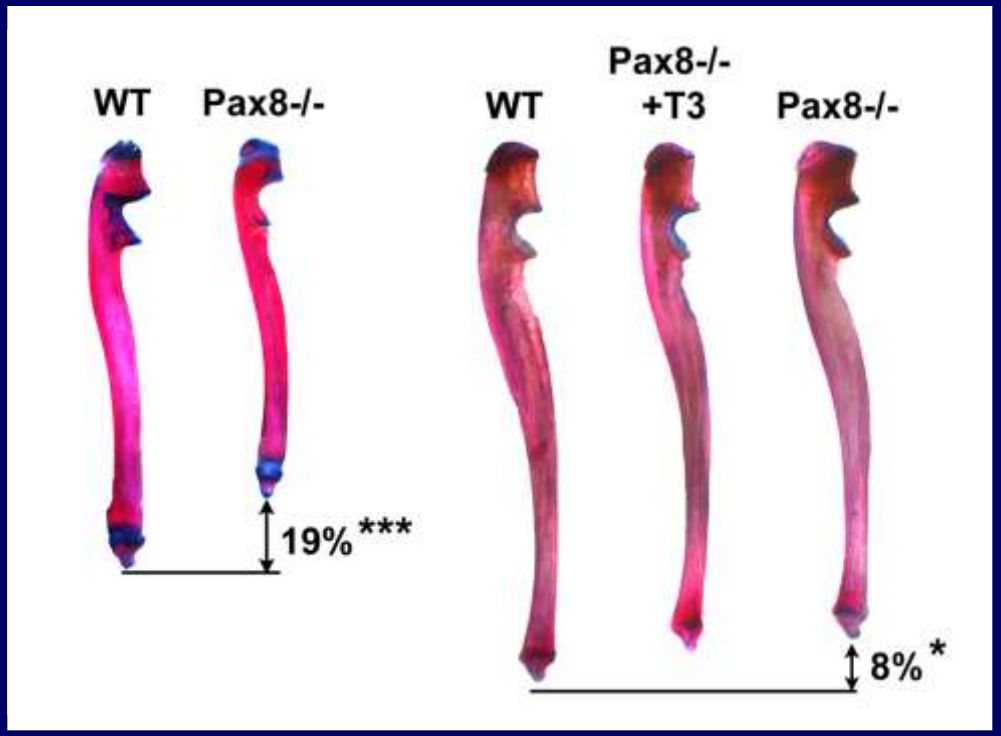
- **Pax8$^{-/-}$**
  - **Thyroid follicular cell agenesis**
  - **Congenital hypothyroidism**
  - **fT_4 & fT_3 undetectable, TSH 2300x**

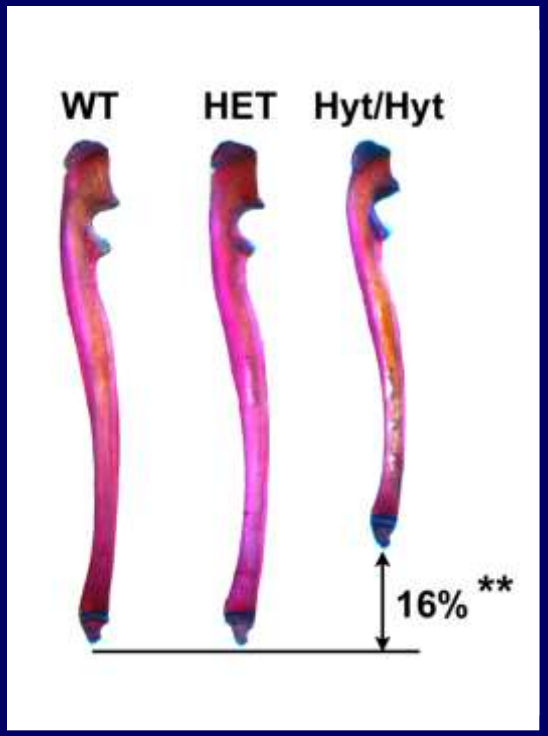
Elevated TSH active TSHR

If TSH is important *in vivo* these mice must have opposite skeletal phenotypes

Bassett et al (2007) Mol Endocrinol 22:501-12

Both hyt/hyt and Pax8$^{-/-}$ mice have growth retardation





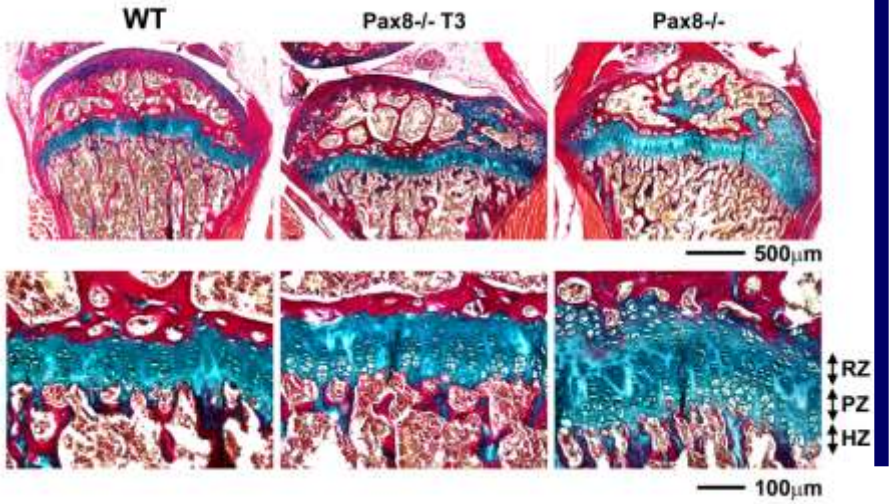
2 weeks

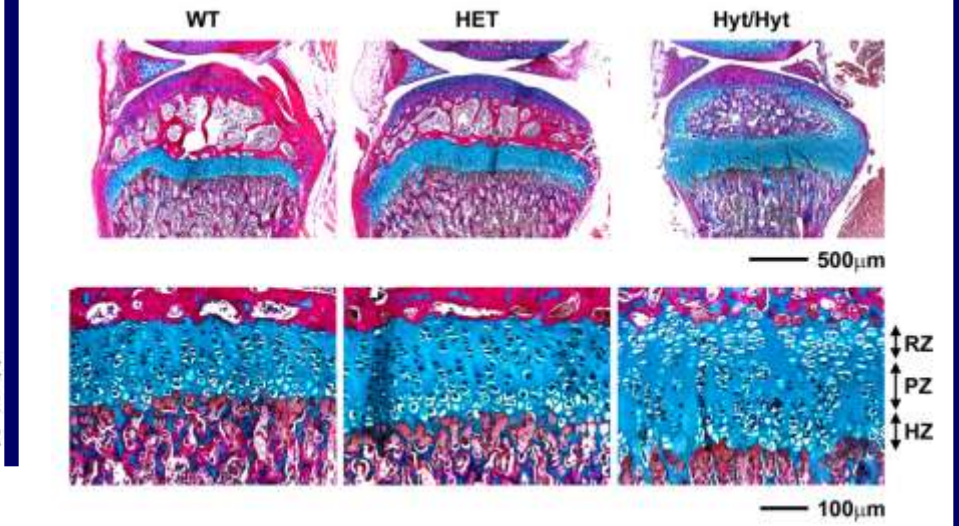
10 weeks

7 weeks

Bassett et al (2007) Mol Endocrinol 22:501-12

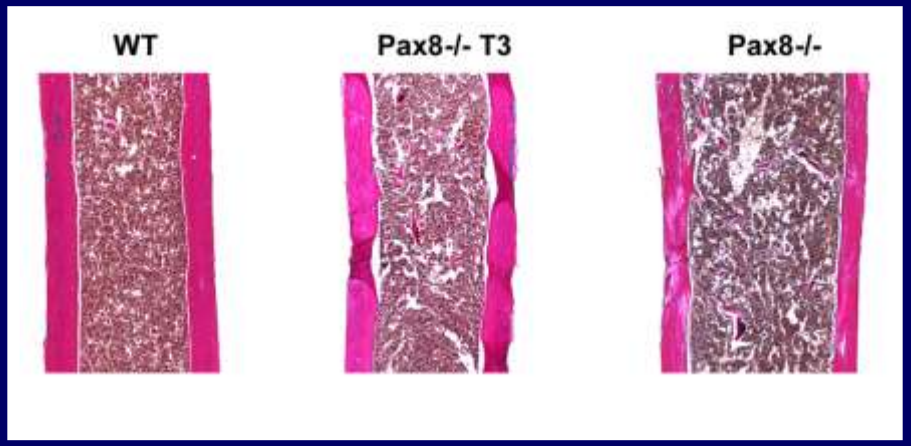
Both hyt/hyt and Pax8$^{-/-}$ mice have delayed ossification





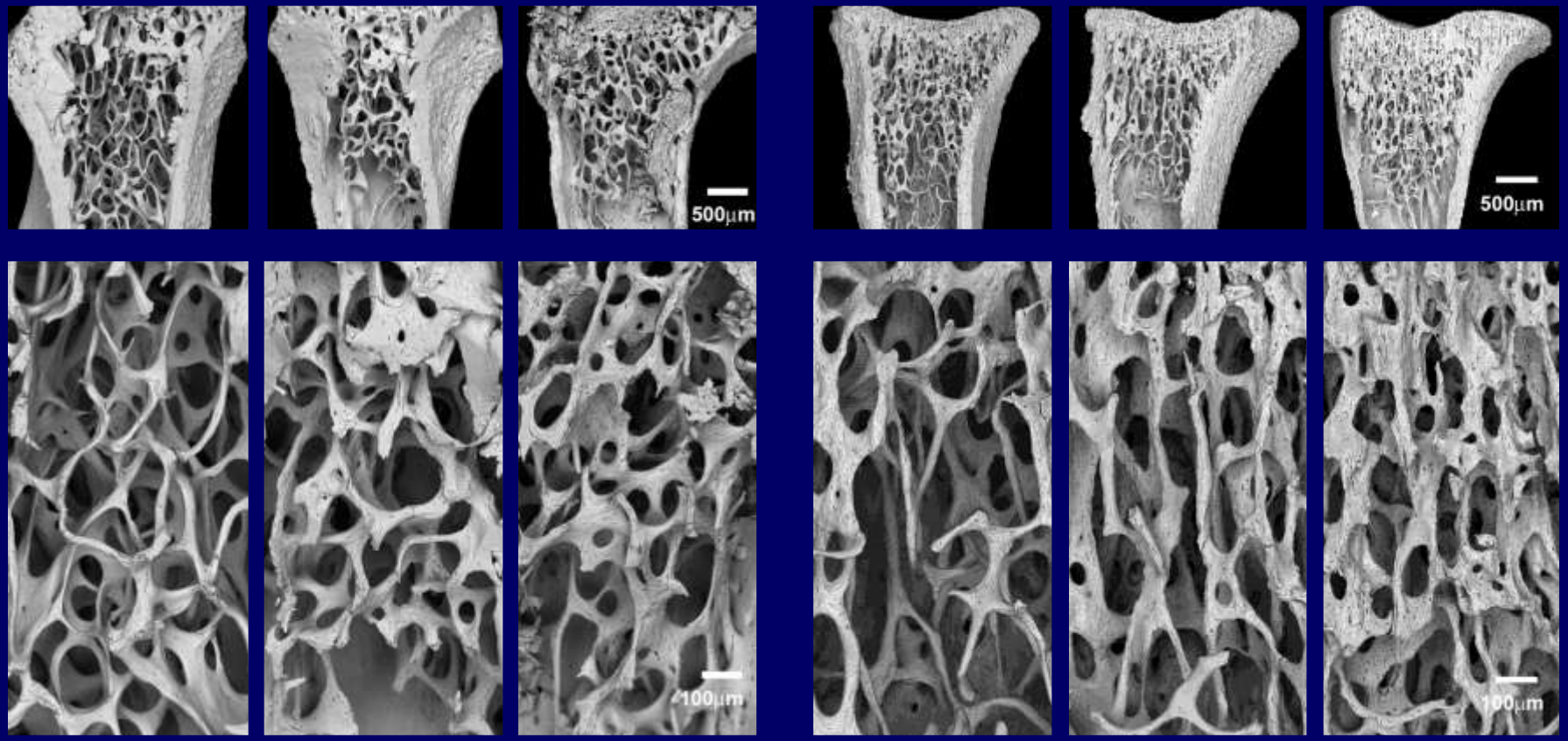
Bassett et al (2007) Mol Endocrinol 22:501-12

Both hyt/hyt and Pax8$^{-/-}$ mice have reduced cortical bone



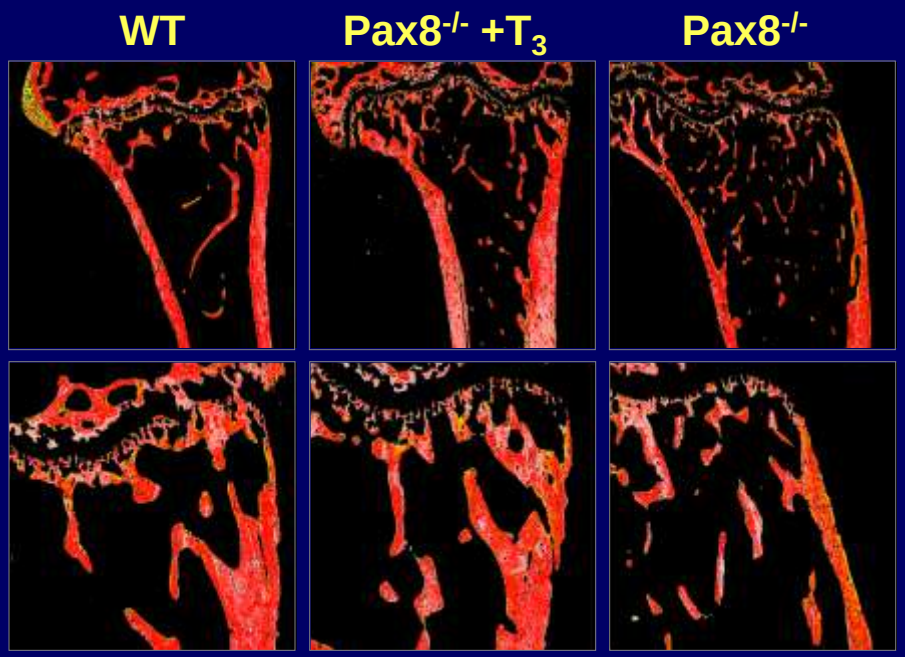
Bassett et al (2007) Mol Endocrinol 22:501-12

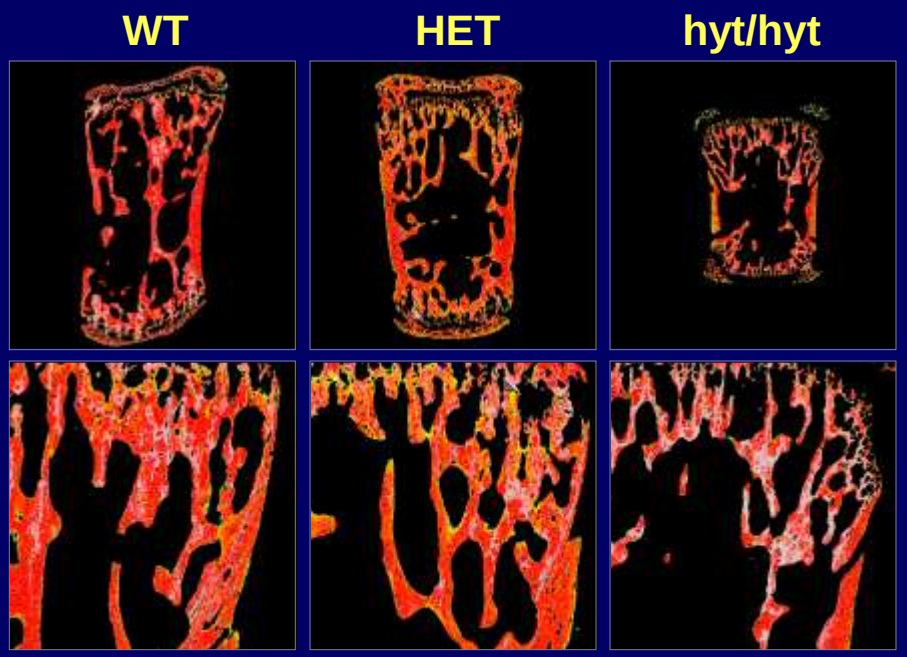
Both hyt/hyt and Pax8$^{-/-}$ mice have similar bone micro-architecture (BSE SEM)

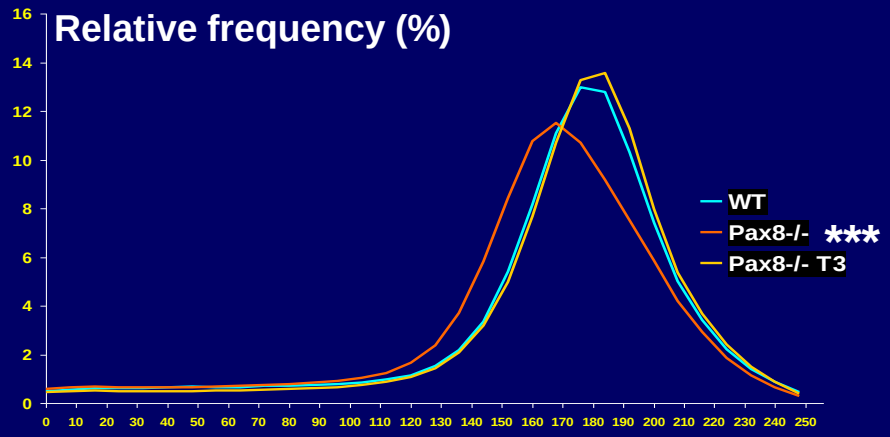


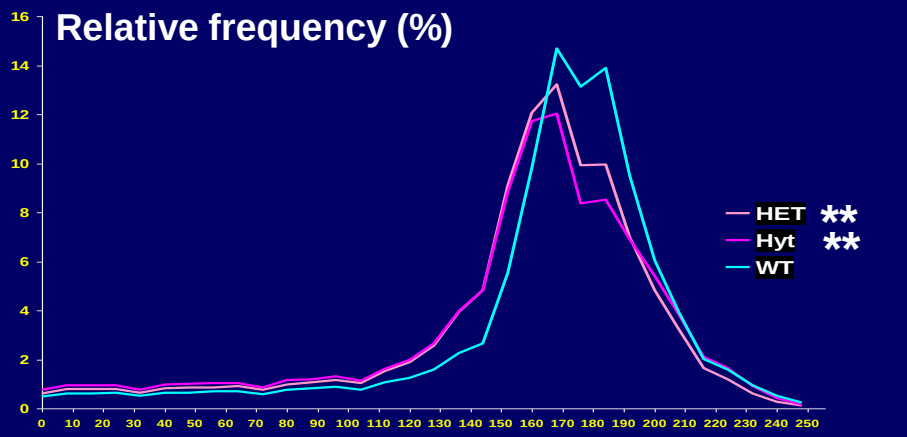
Bassett et al (2007) Mol Endocrinol 22:501-12

Both hyt/hyt and Pax8$^{-/-}$ mice have reduced bone mineralization density (qBSE)









Conclusion

- **Pax8$^{-/-}$ (↑TSH, active TSHR)**
- **hyt/hyt (↑TSH, non-functional TSHR)**

have similar, not opposite, phenotypes

- **Growth retardation & delayed ossification**
- **Reduced cortical bone**
- **Impaired trabecular bone remodelling**
- **Reduced bone micro-mineralization density**
- **Reduced bone volume fraction**

The skeletal phenotype of congenital hypothyroidism is independent of TSH

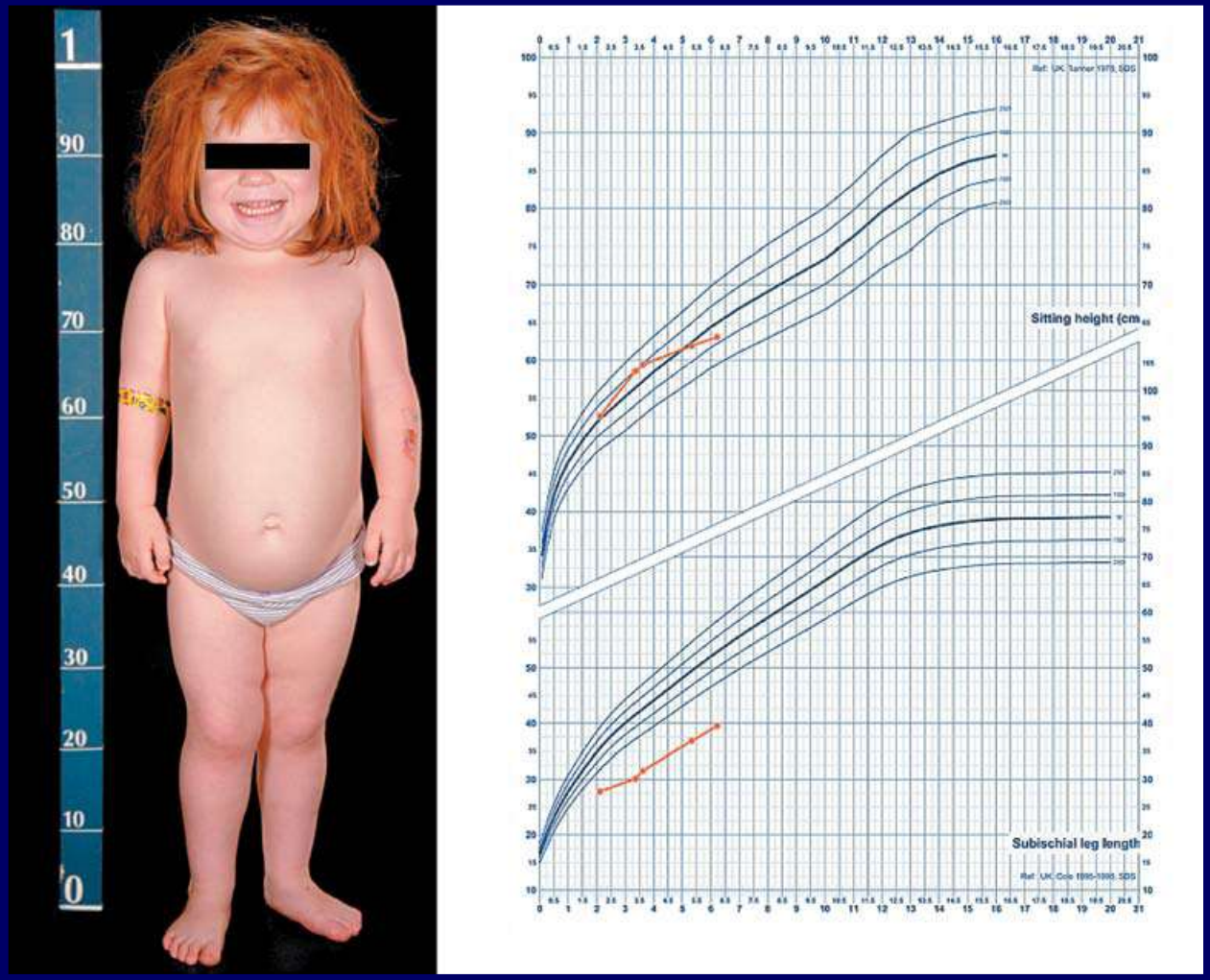
Bassett et al (2007) Mol Endocrinol 22:501-12

Conclusion

- **TRα is the major functional T3 receptor in bone**

The hypothalamic-pituitary-thyroid axis regulates bone via the actions of T3 and TRα

TRα mutations in humans recapitulate phenotypes identified in TRα mutant mice

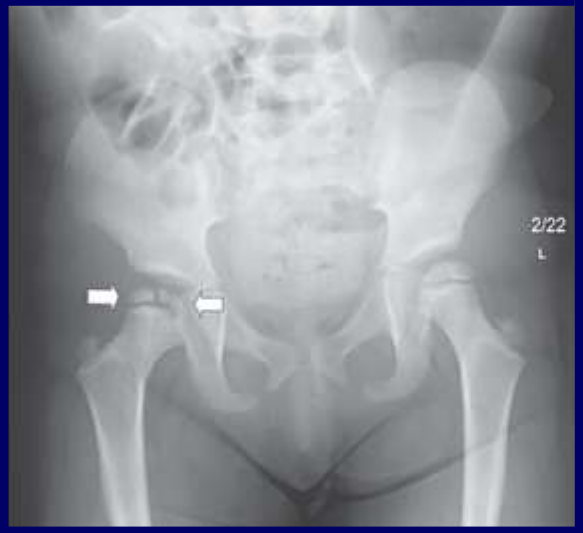


Growth retardation and disproportionate short stature

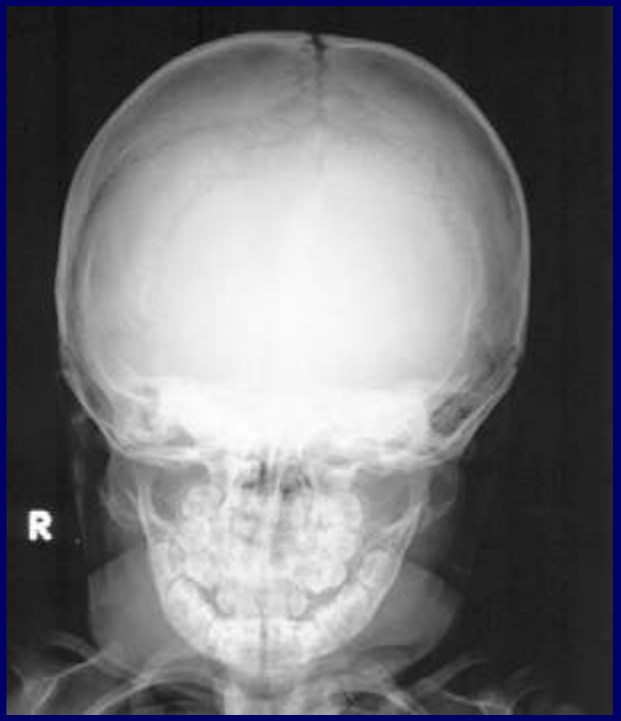
Bochukova et al (2012) NEJM 366:243-9
Van Mullem et al (2012) NEJM 366:1451-3

TRα mutations in humans recapitulate phenotypes identified in TRα mutant mice

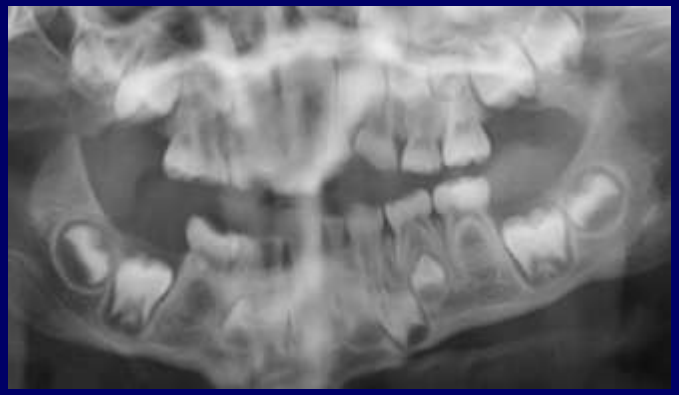
Wormian sutures



Epiphyseal dysgenesis



Delayed closure of skull sutures



Delayed tooth eruption

Bochukova et al (2012) NEJM 366:243-9
Van Mullem et al (2012) NEJM 366:1451-3