Thyroid hormone action

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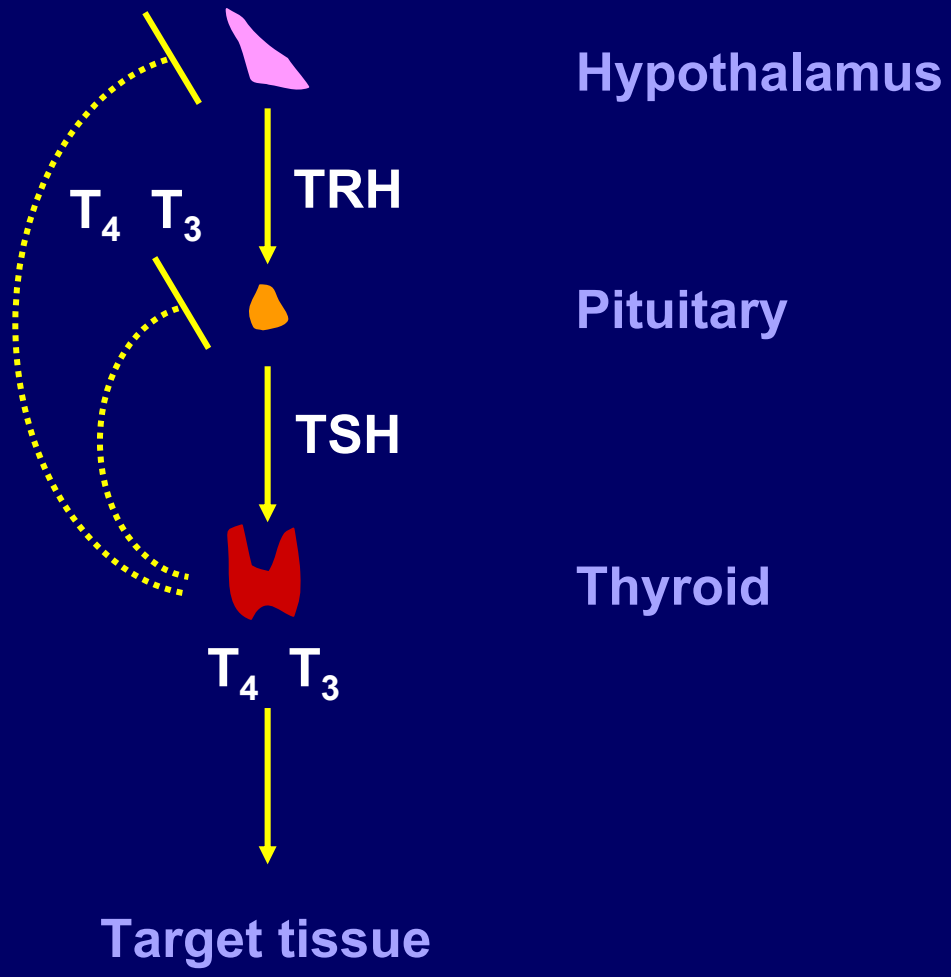
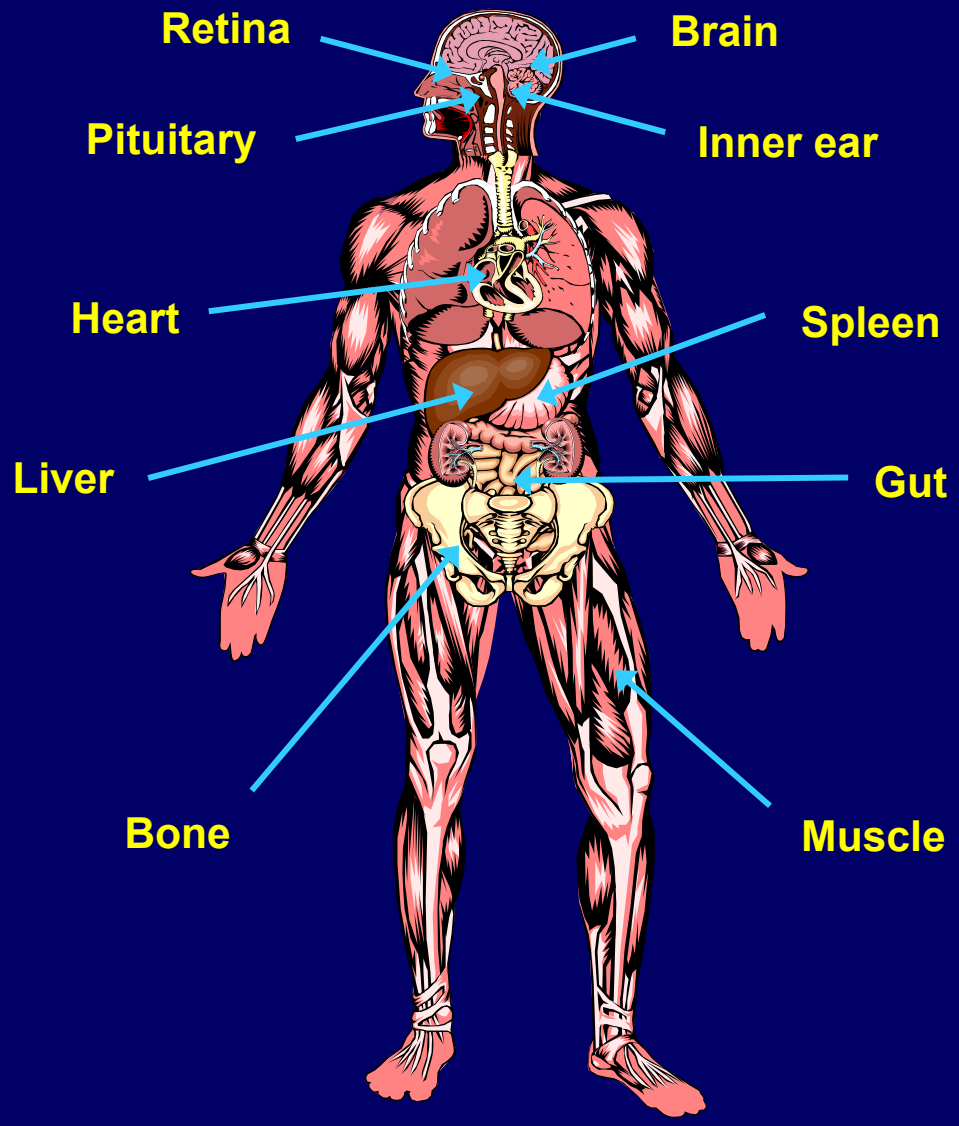
Hypothalamic-pituitary-thyroid axis

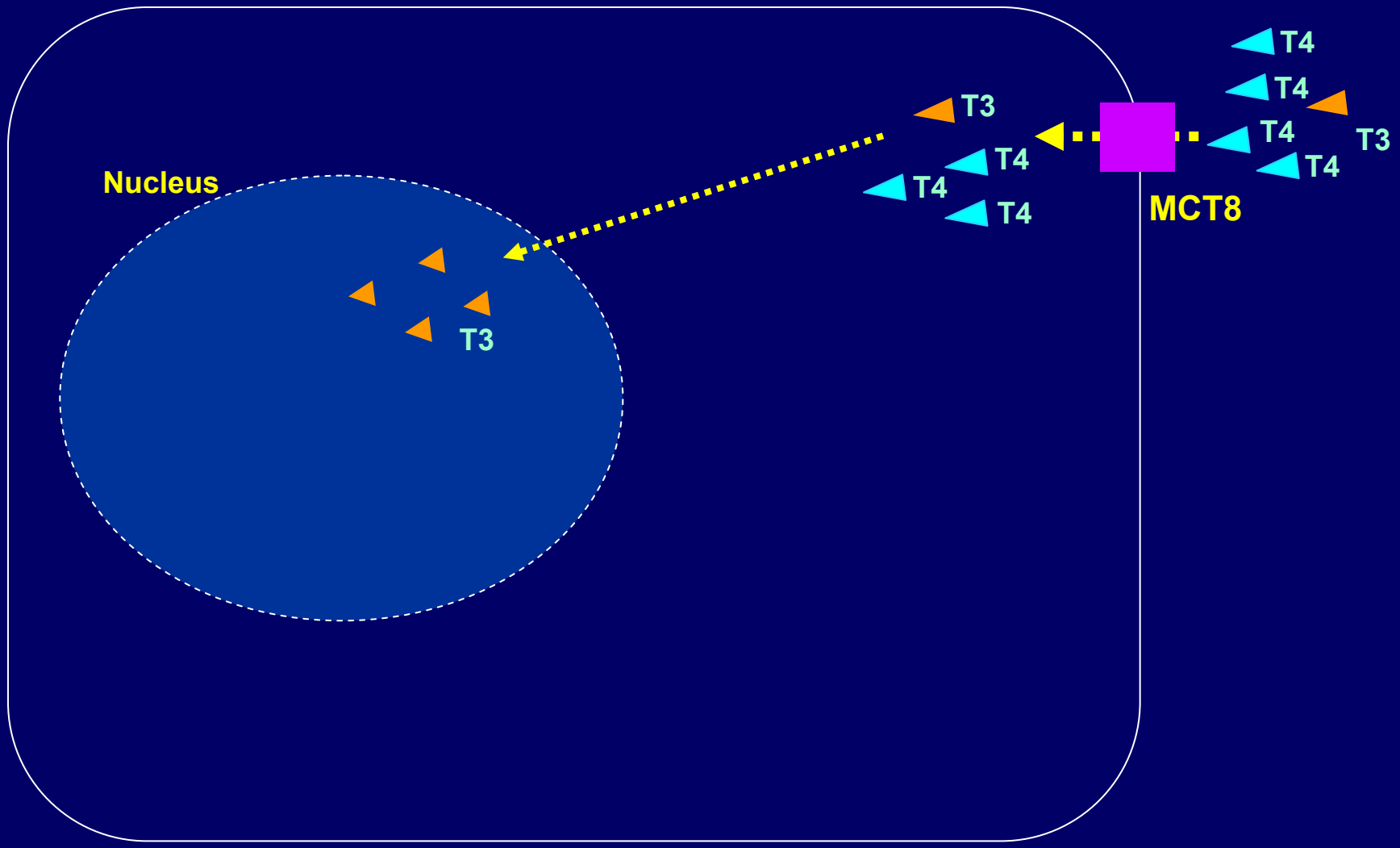


Major T3 target tissues

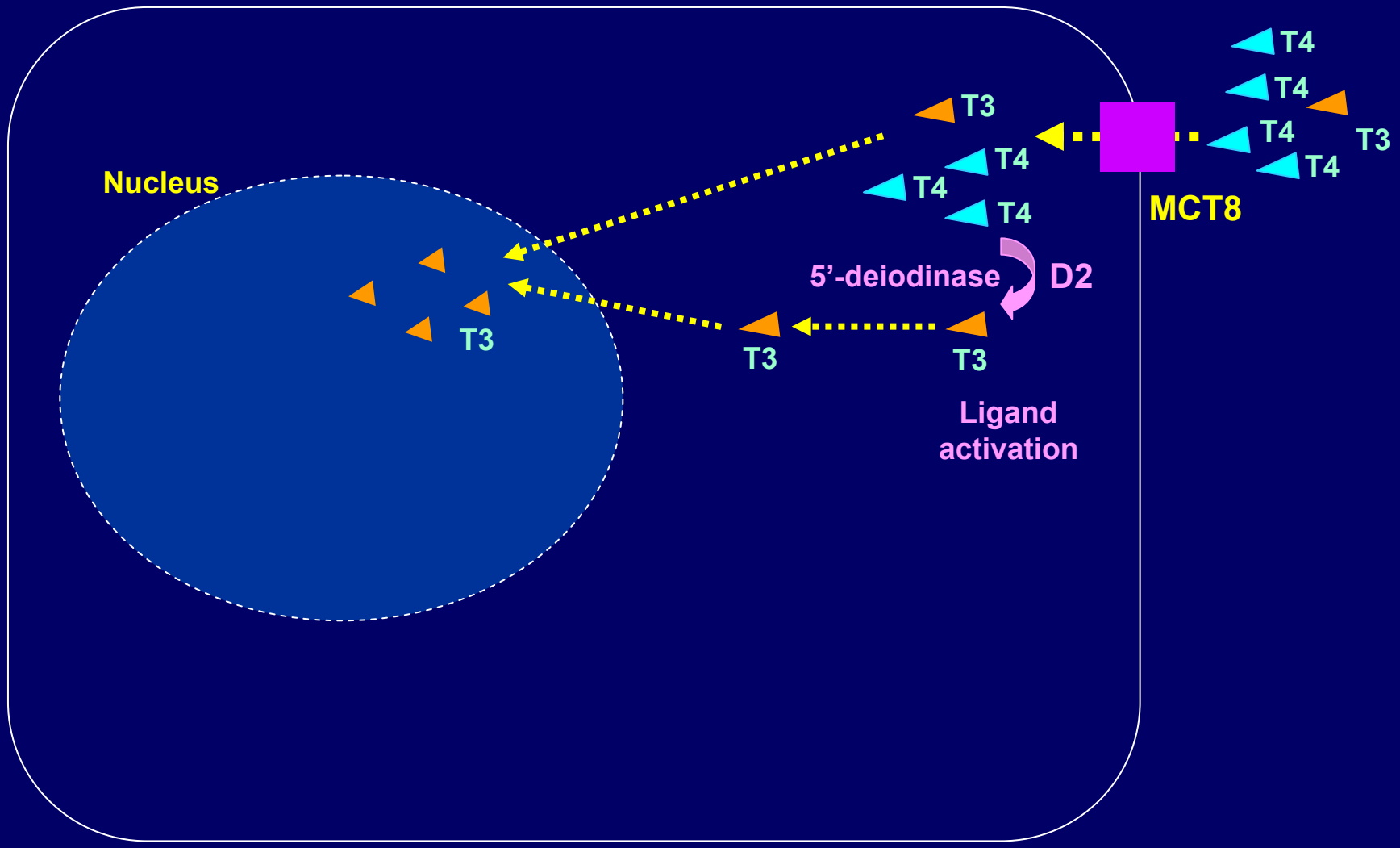


BMR
Thermogenesis
Adipogenesis
Vasculature
Skin
Hair
Bone marrow
Kidney
Lung

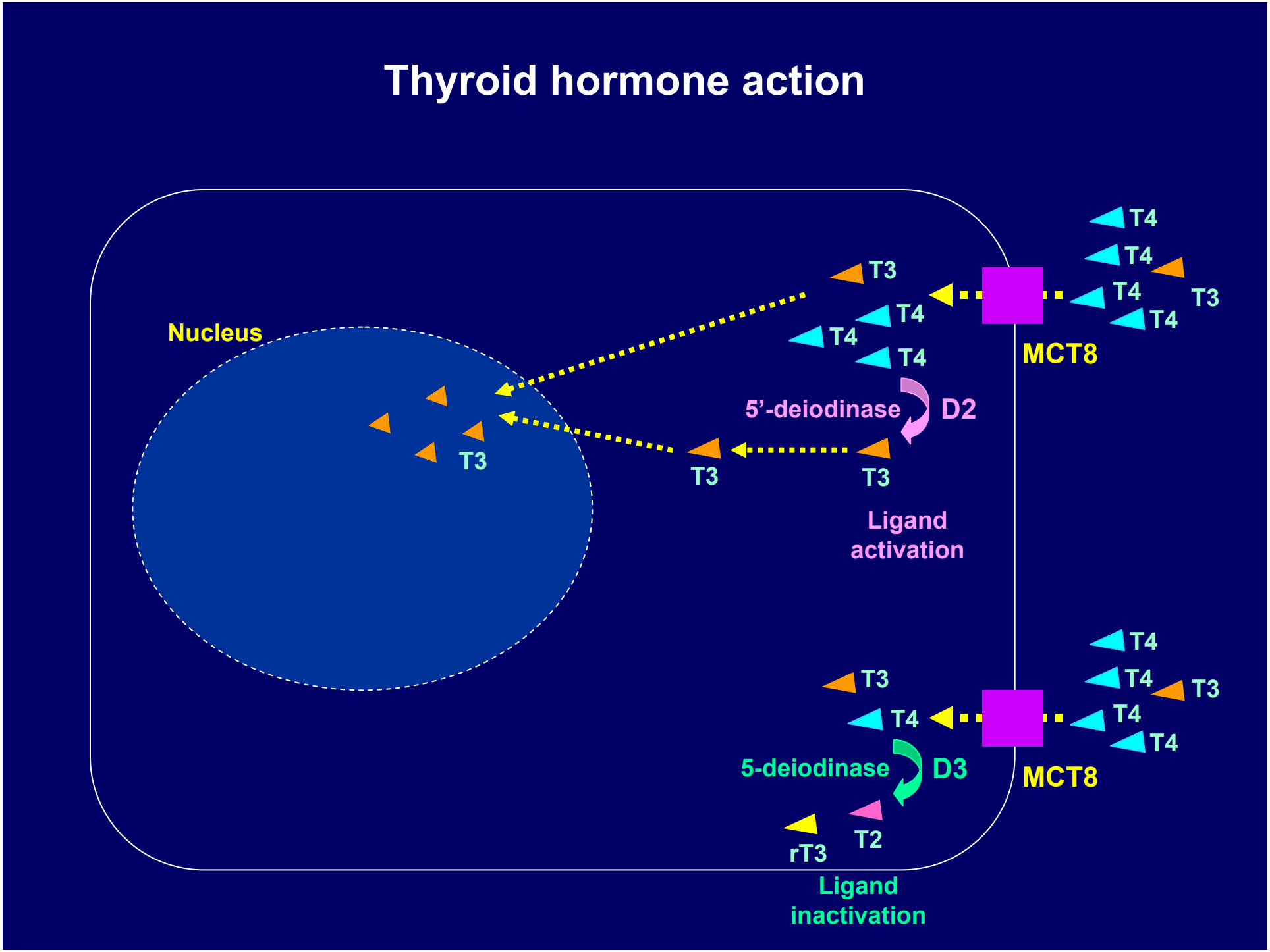
Thyroid hormone action



Thyroid hormone action



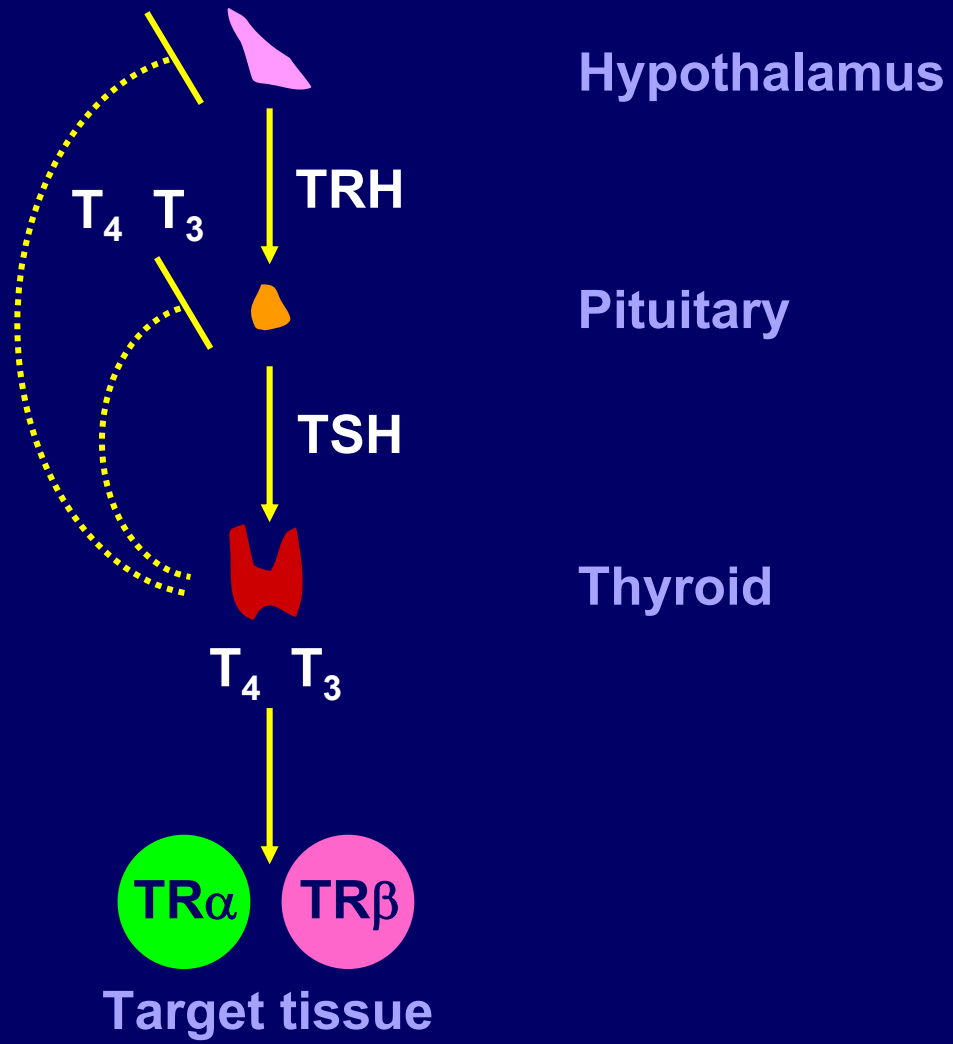
Thyroid hormone action



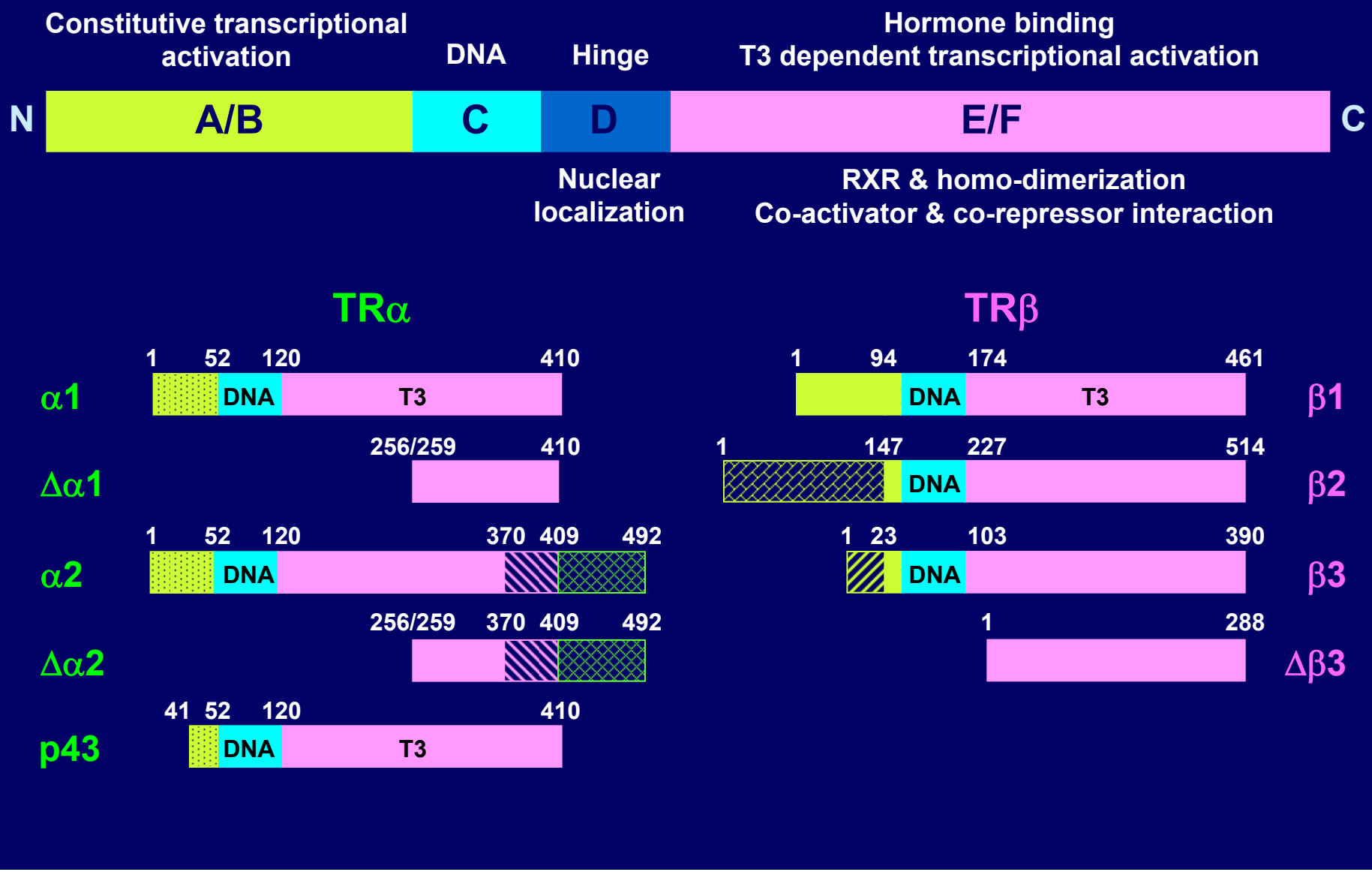
T3 acts via nuclear receptors

- **TRα and TRβ**
  - **Encoded by *THRA* (*NR1A1,* Ch17q11.2) and *THRB* (*NR1A2,* Ch3p24.3)**
  - **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**

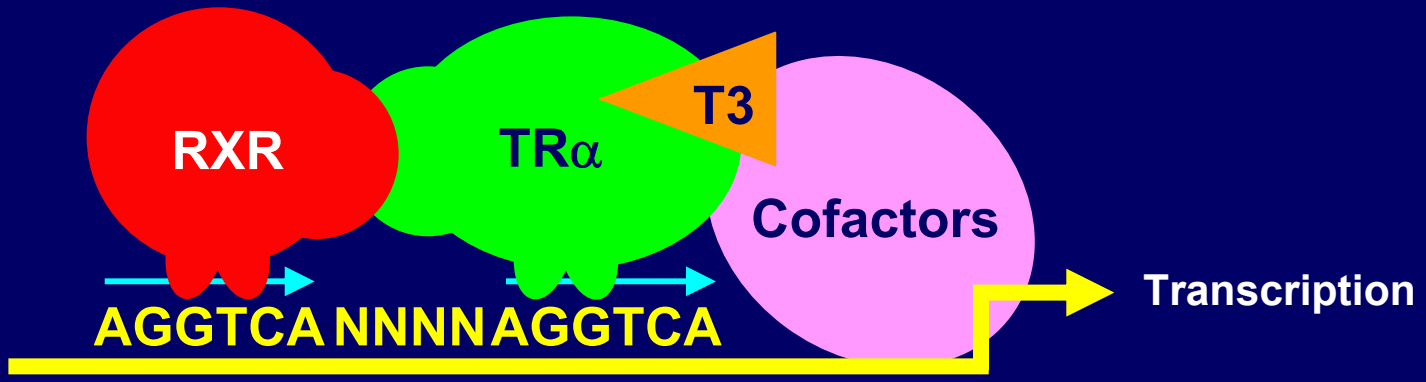
Hypothalamic-pituitary-thyroid axis



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

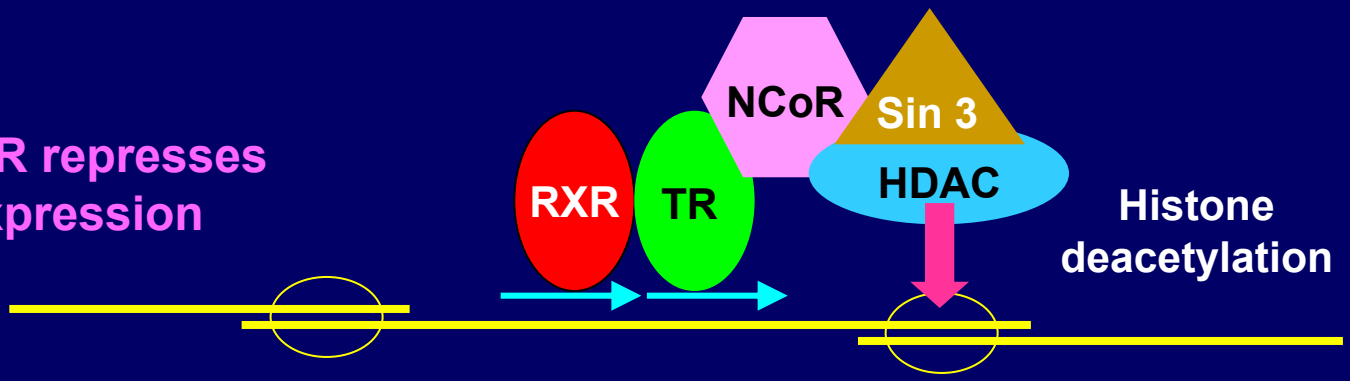
Endogenous TREs

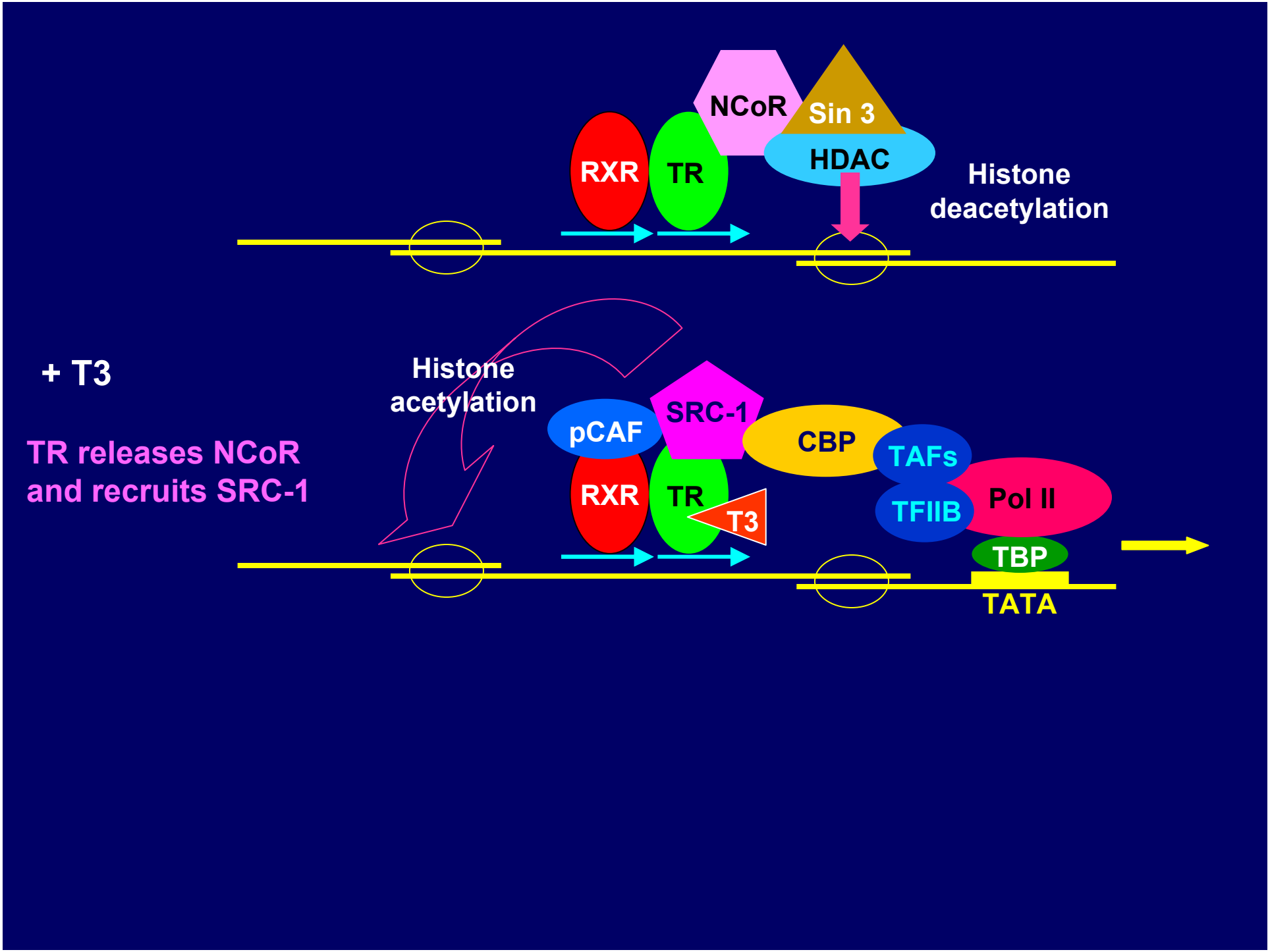
AGGTGA NNNN AGGACA NN AGCCCT — αMHC TRE

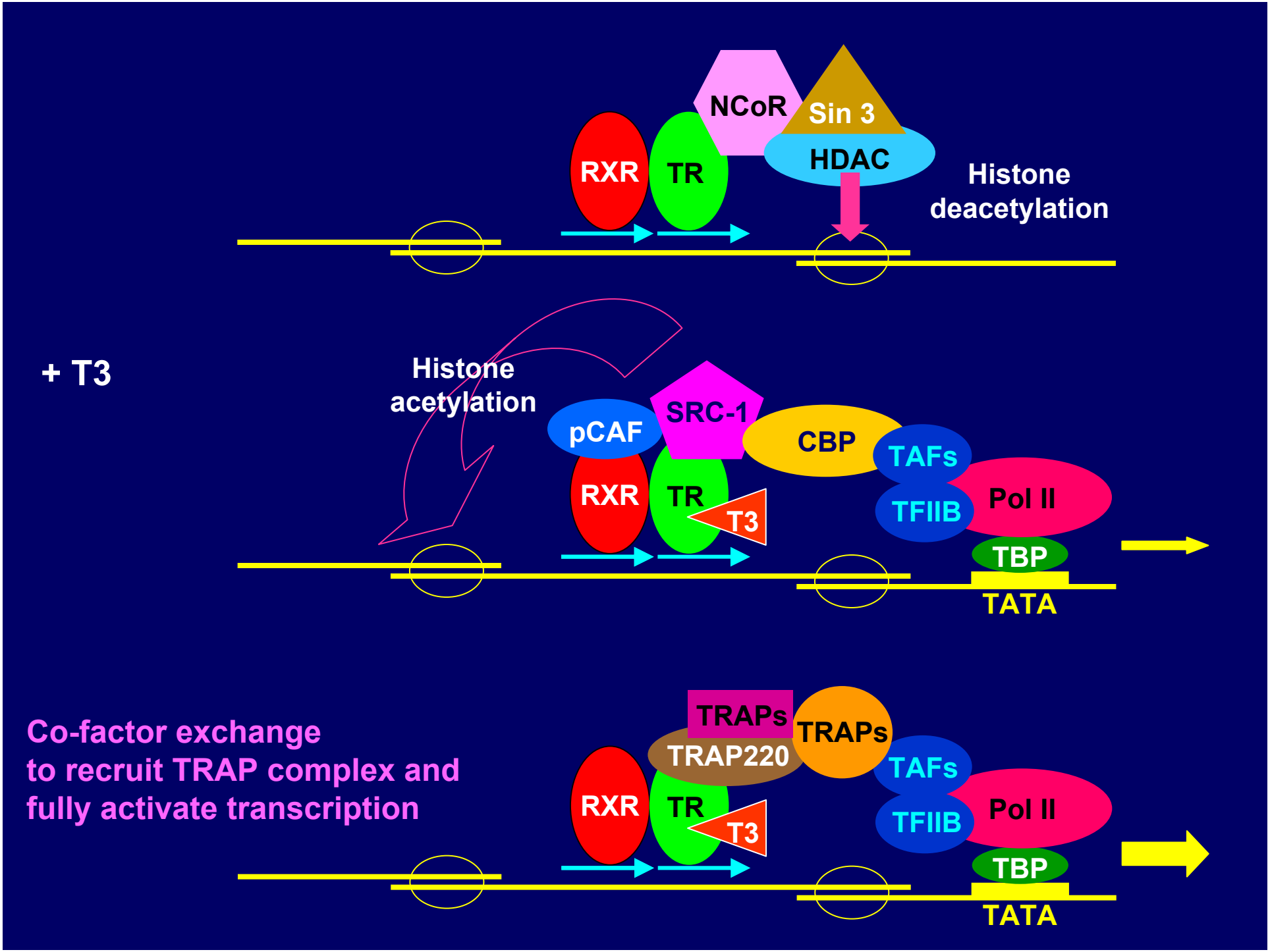
GGGTTA NNNN AGCACA — ME TRE

- T3

Unliganded TR represses basal gene expression



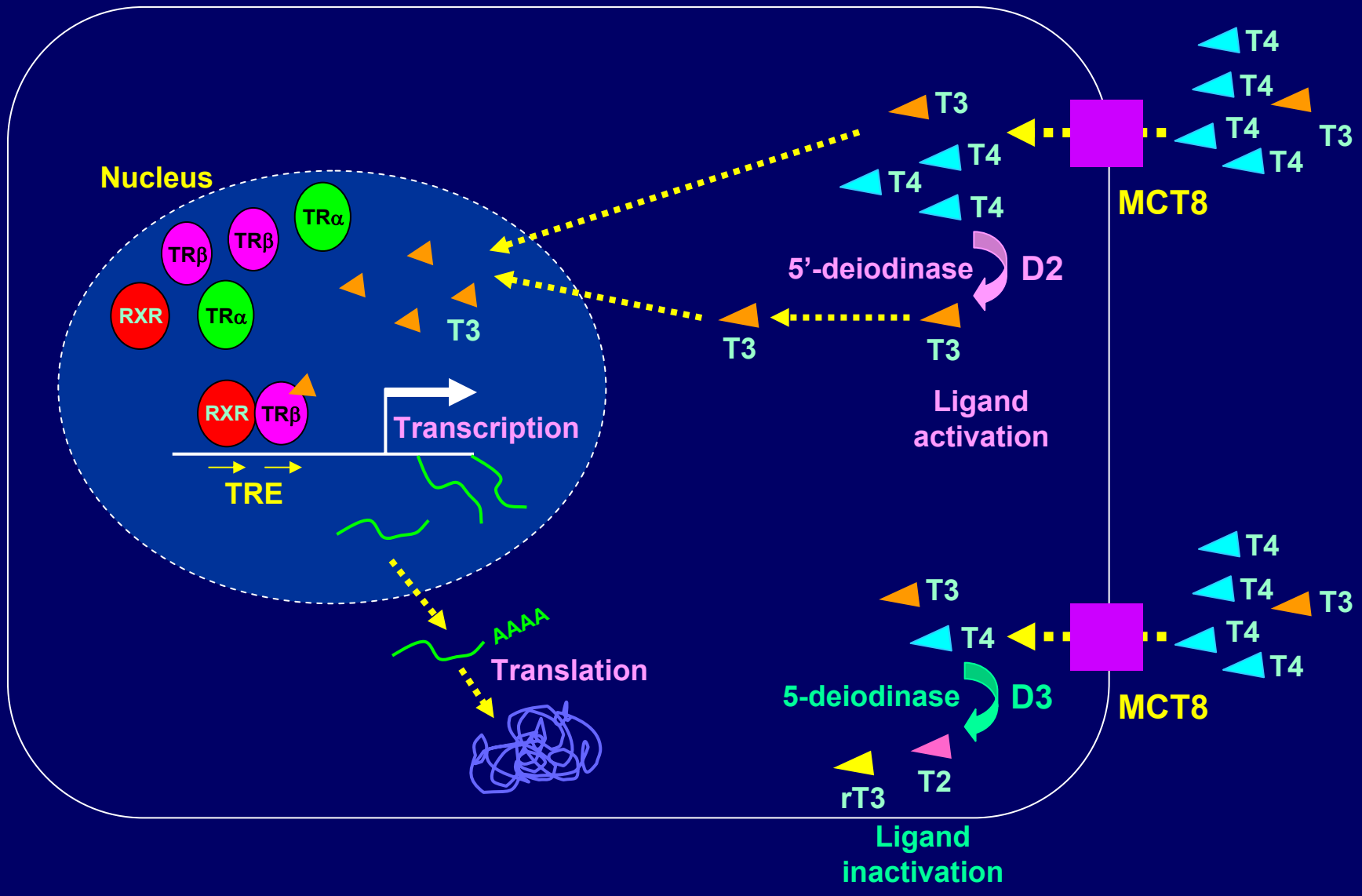

NCoR
Sin 3
HDAC
RXR
TR
Histone
deacetylation
+ T3
Histone
acetylation
TR releases NCoR
and recruits SRC-1
pCAF
SRC-1
CBP
TAFs
RXR
TR
T3
TFIIB
Pol II
TBP
TATA


NCoR
Sin 3
HDAC
RXR
TR
Histone
deacetylation
+ T3
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**
  - **Multiple TR isoforms**
    - **TRα1 and TRβ1, β2, β3 are true receptors**
    - **TRα2, Δα1, Δ α2 and TRΔβ3 may act as antagonists**
  - **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**
  - **Organic anion transporting polypeptides (OATP)**
    - **OATPC1 has high affinity for T4 and rT3 and facilitates influx and efflux**
    - **May regulate T4 transport across blood-brain barrier**
  - **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**

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

Control of T3 action – metabolism

- Iodothyronine deiodinases
  - D1
    - Main source of plasma T3 in hyperthyroidism
  - D2
    - Paracrine pathways of D2-mediated T3 production and action control cochlear development and hormone action in brain
  - D3
    - Controls T4 and T3 clearance and prevents intra-cellular T3 production (eg pregnancy)
    - High expression causes consumptive hypothyroidism

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

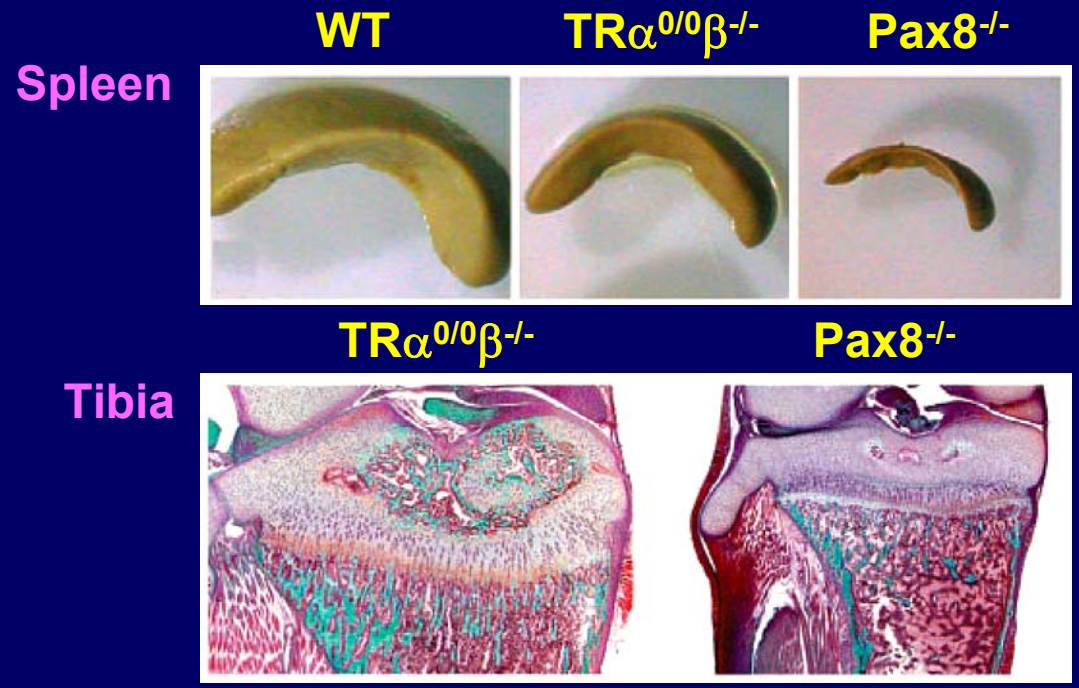
Control of T3 action – TRs and human disease

- 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 not described
- Cancer
  - Somatic mutation or aberrant expression of *THRB* identified in thyroid, liver and renal cell tumours

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

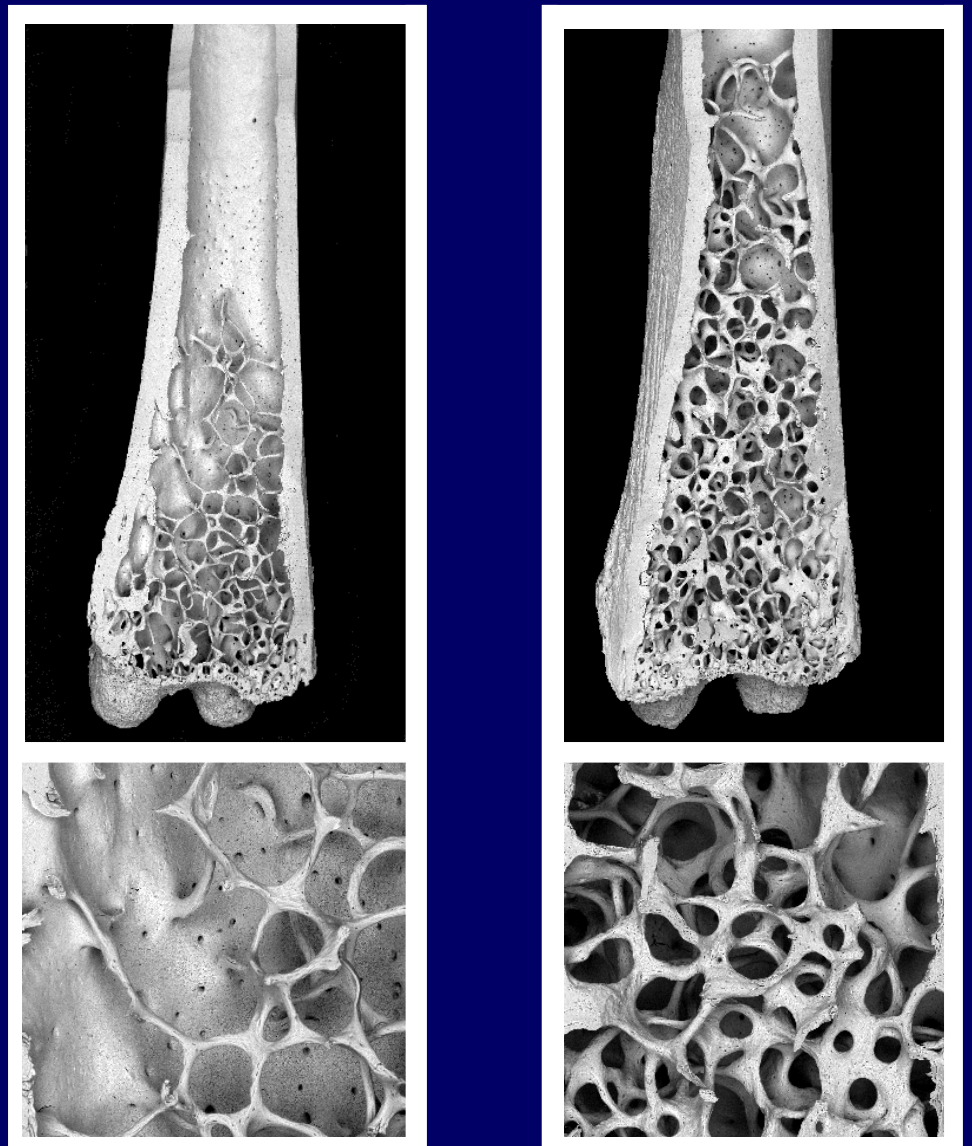
- **Deletion of TRα in Pax8$^{-/-}$TRα$^{0/0}$ compound mutants ameliorates Pax8$^{-/-}$ phenotype**
- **ApoTRα1 plays an important role during development**

Flamant et al 2002 Mol Endocrinol 16:24-32

Bone is a TRα target tissue

WT

$TR\alpha^{0/0}$



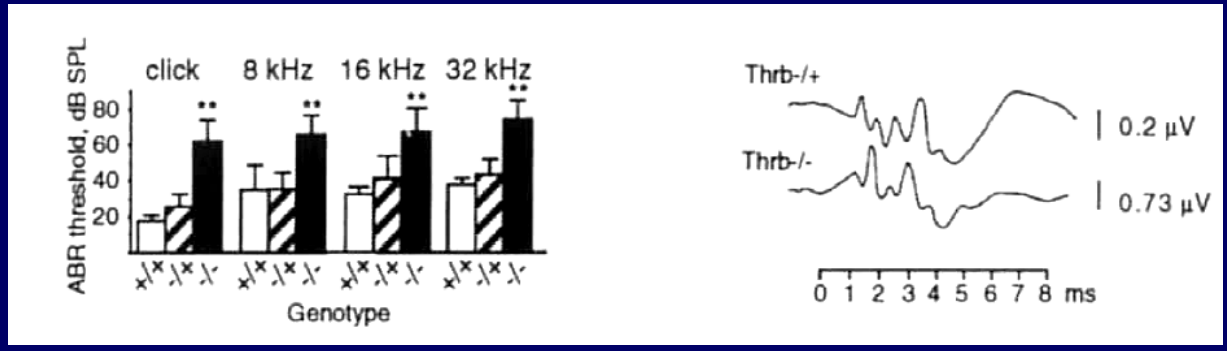
Deletion of TRα

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

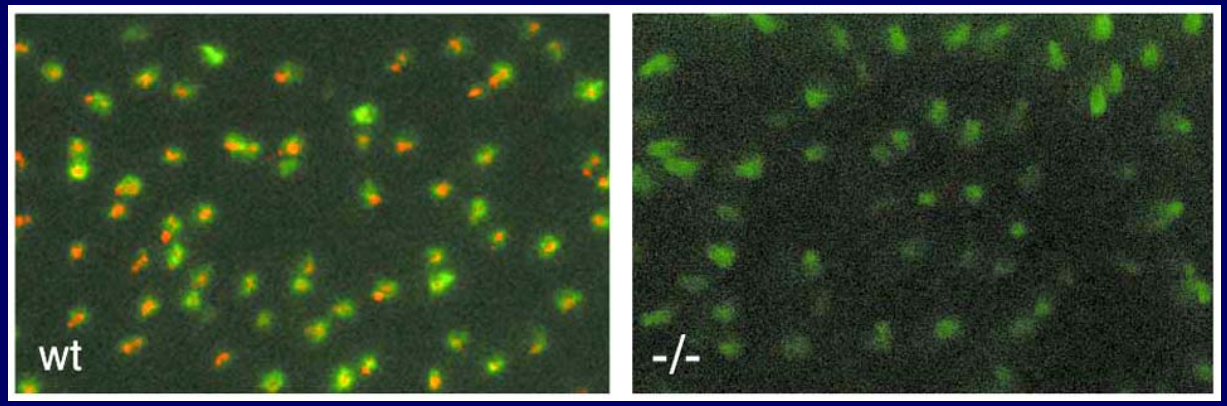
Bassett et al 2007 Mol Endocrinol 21:1095-107

Cochlea and retina are TRβ target tissues

Cochlea



Retina

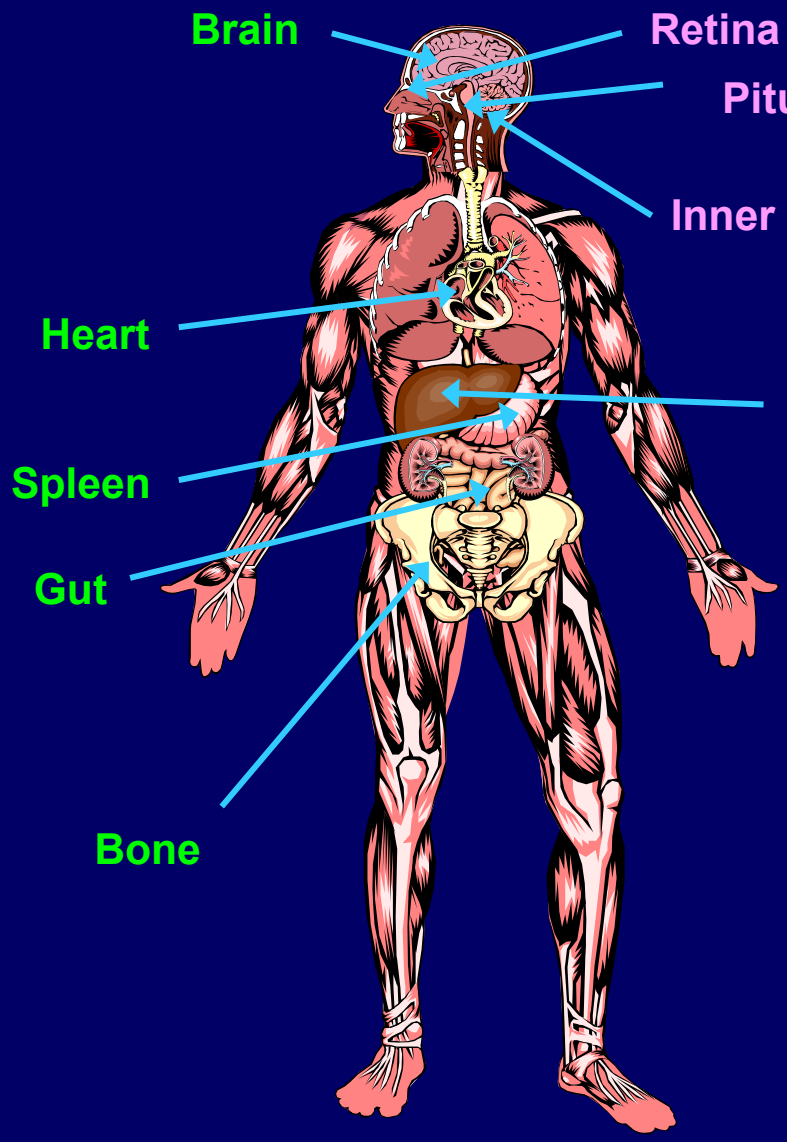


Deletion of TRβ

- **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

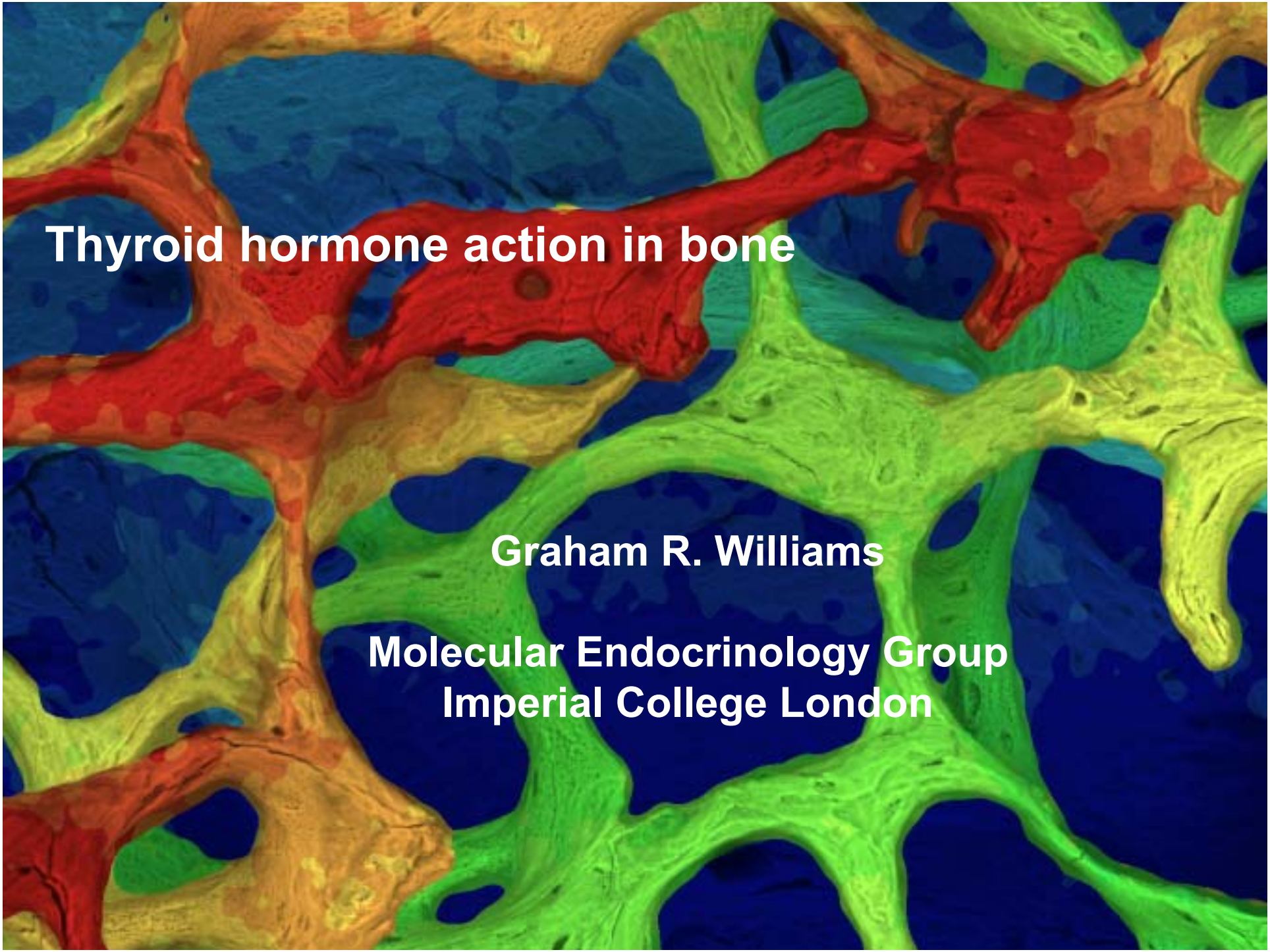


TRα

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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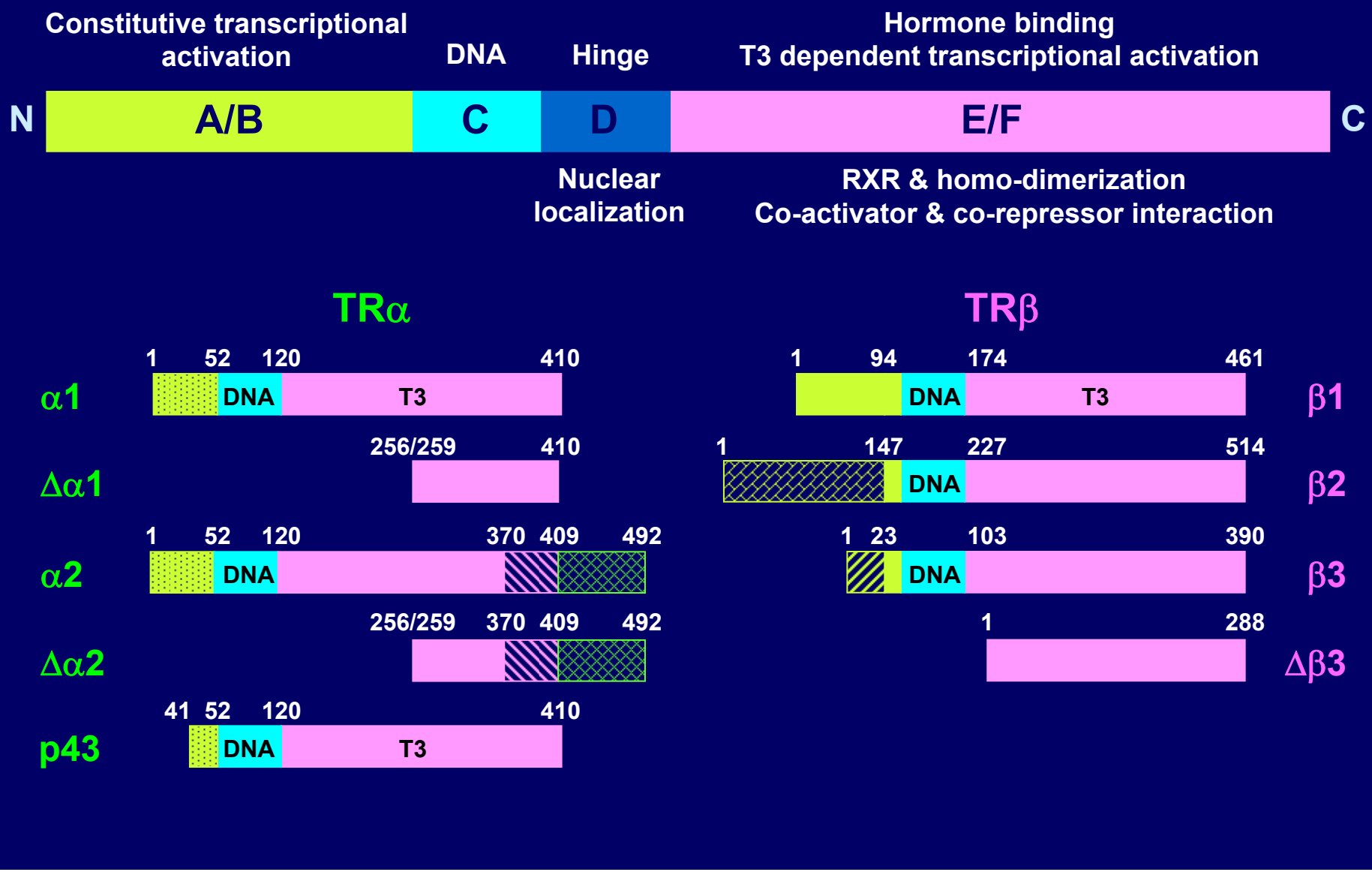
Thyroid hormones and bone

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

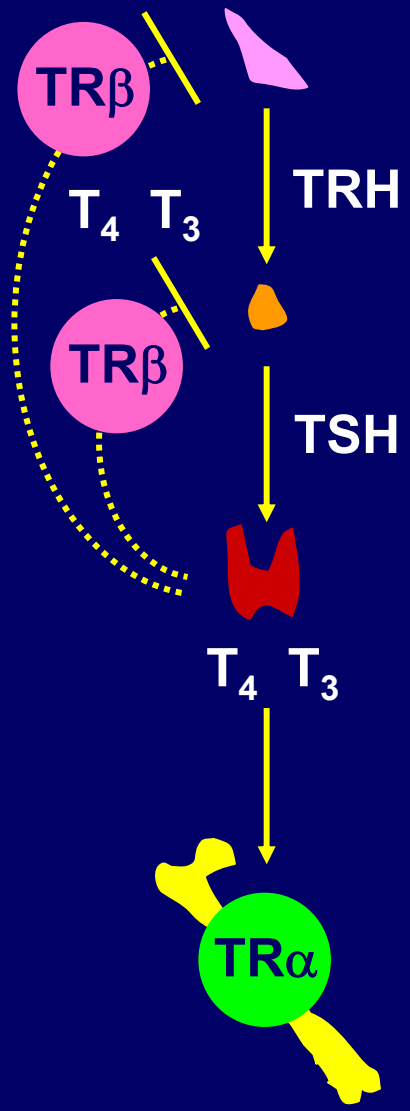
Thyroid hormones are essential for skeletal development and regulate bone mass and mineralization in adults

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

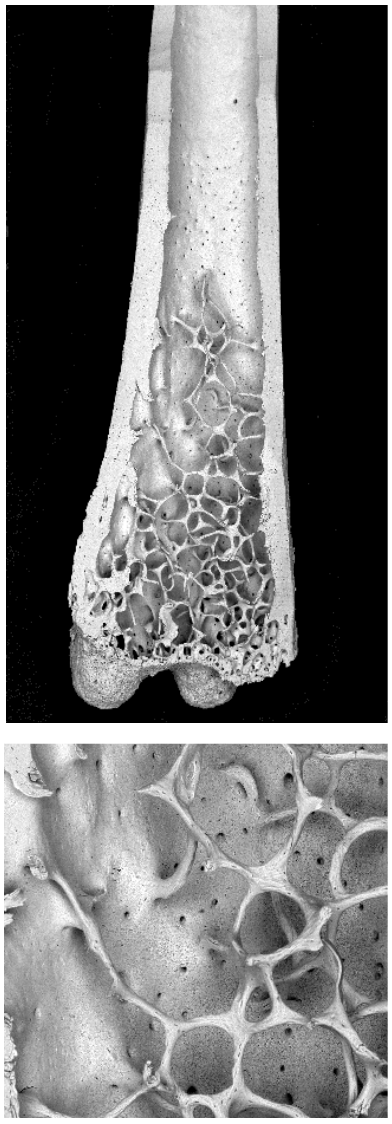
Thyroid

Bone

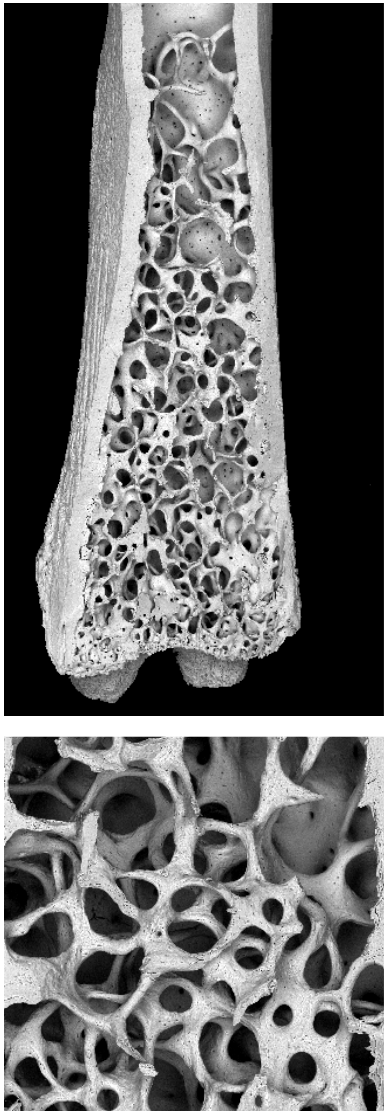
Bassett et al 2007
Mol Endocrinol 21:1095-107
Bassett et al 2007
Mol Endocrinol 21:1893-904

Bone is a TRα target tissue

WT

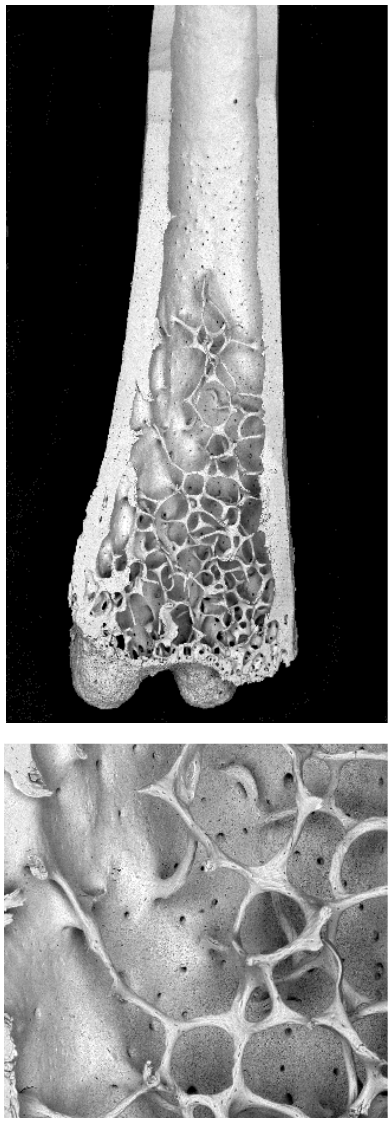


TRα$^{0/0}$

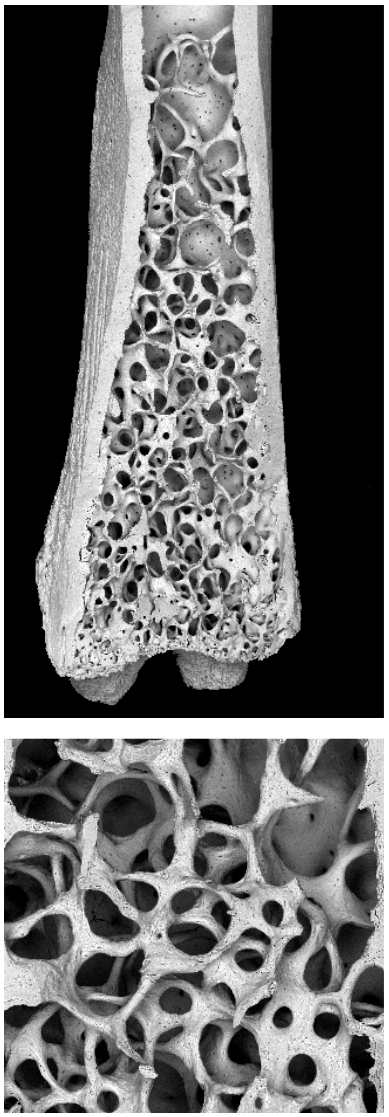


Deletion of TRβ causes an opposite phenotype

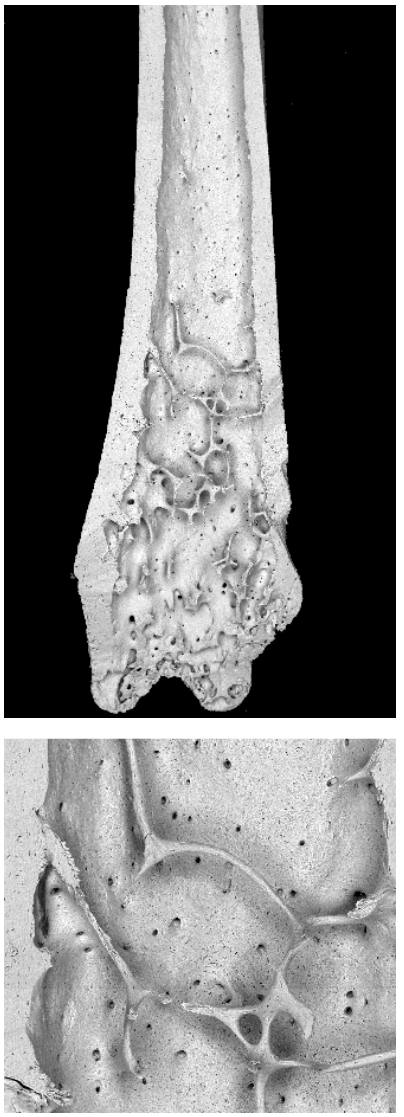
WT



TRα$^{0/0}$



TRβ$^{-/-}$

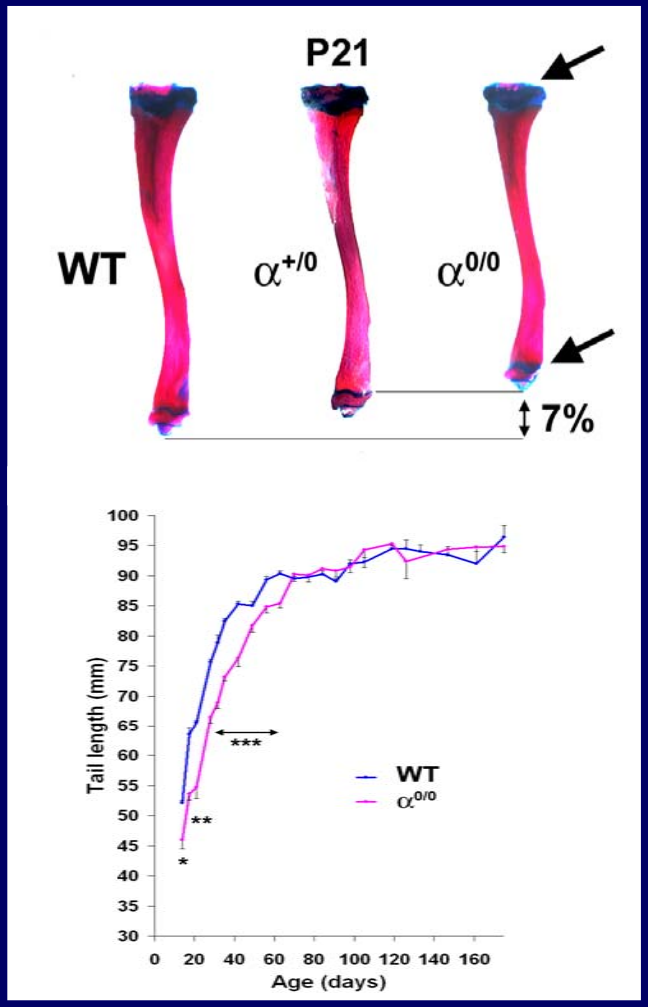


TRα and TRβ knockout mice

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

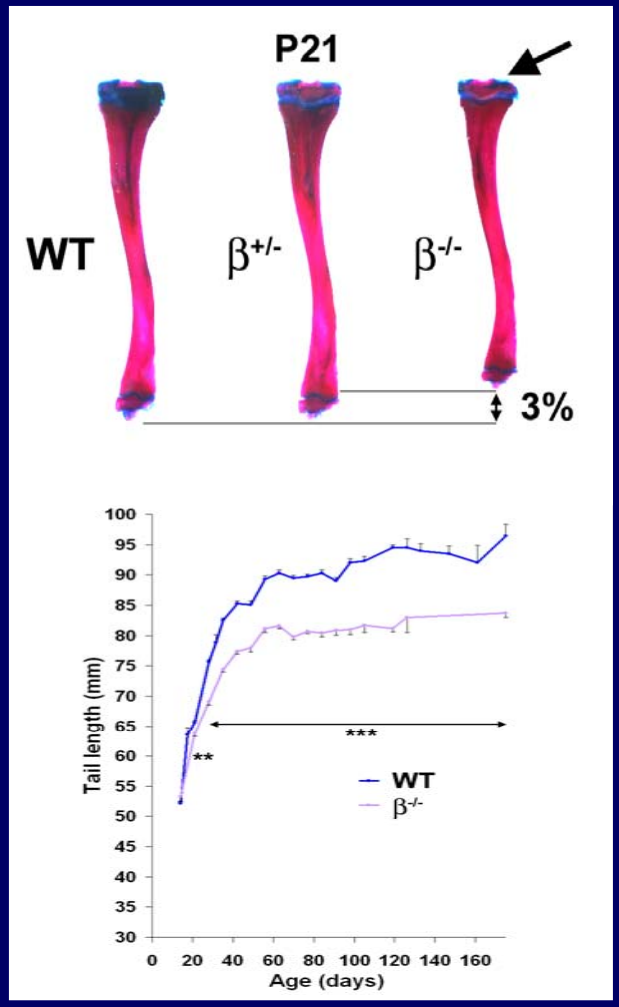
	T4 (μg/dl)	T3 (ng/ml)	TSH (mU/L)	
WT	3.8±0.1	8.4±0.3	25±3.0	Euthyroid
$\alpha^{0/0}$	0.9x	1.2x	0.9x	Euthyroid
$\beta^{-/-}$	4x	6x	12x	RTH

Deletion of TRα or TRβ affects growth



TRα$^{0/0}$

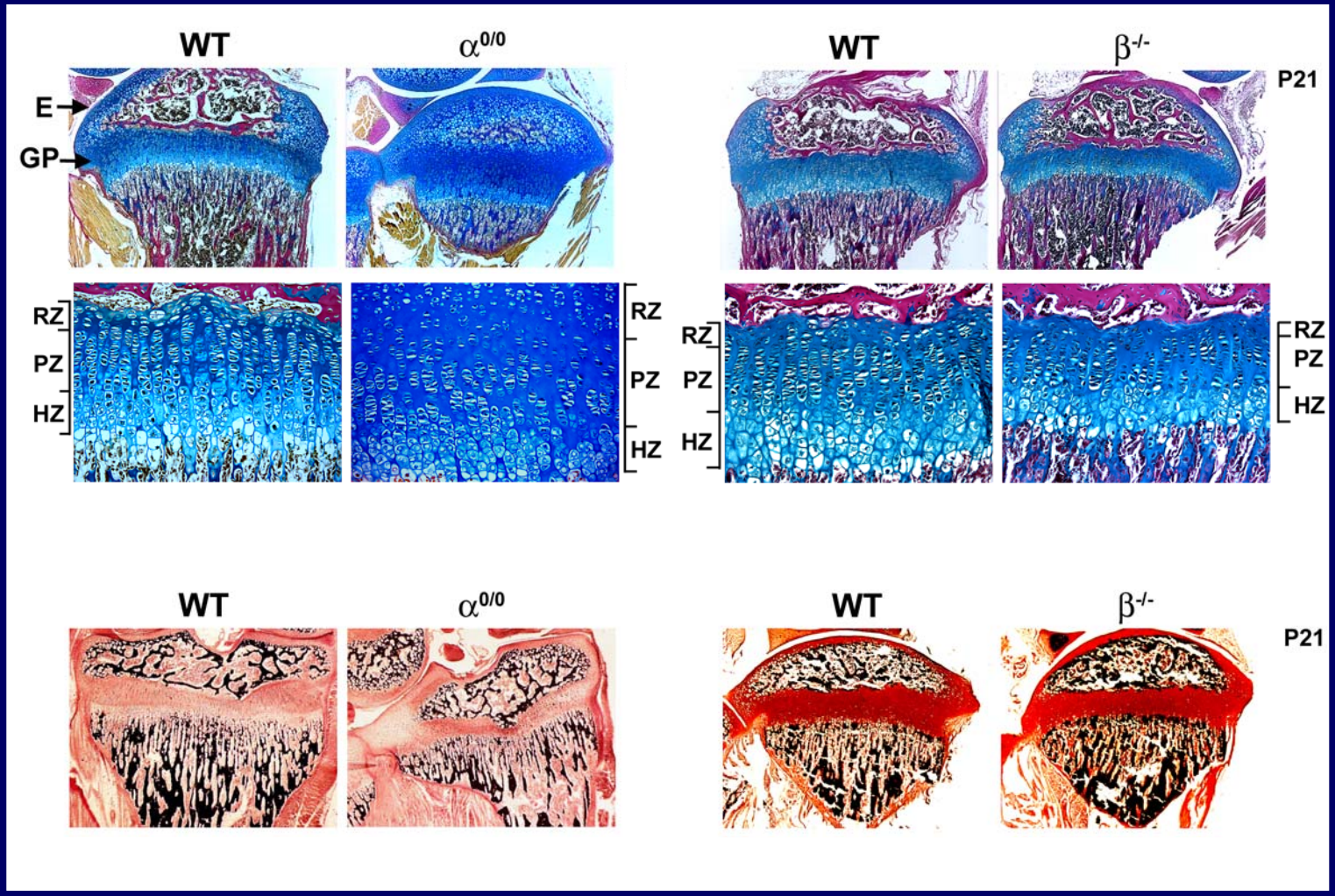
Transient growth delay



TRβ$^{-/-}$

Persistent short stature

Deletion of TRα or TRβ affects ossification



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

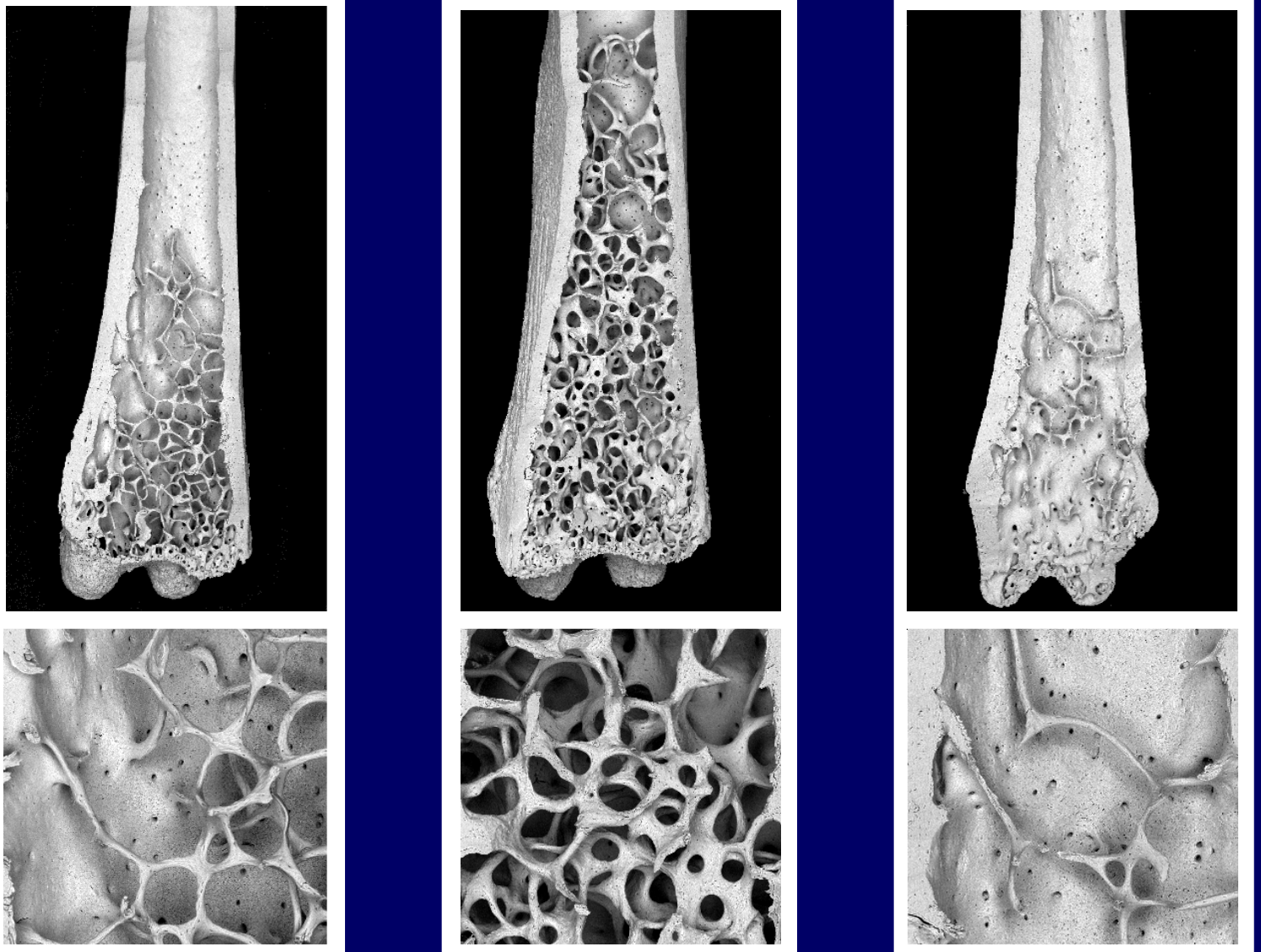
TRβ$^{-/-}$
Advanced endochondral ossification

Deletion of TRα or TRβ affects bone mass

WT

$\alpha^{0/0}$

$\beta^{-/-}$



Osteosclerosis

Osteoporosis

Bone mineralization density by qBSE

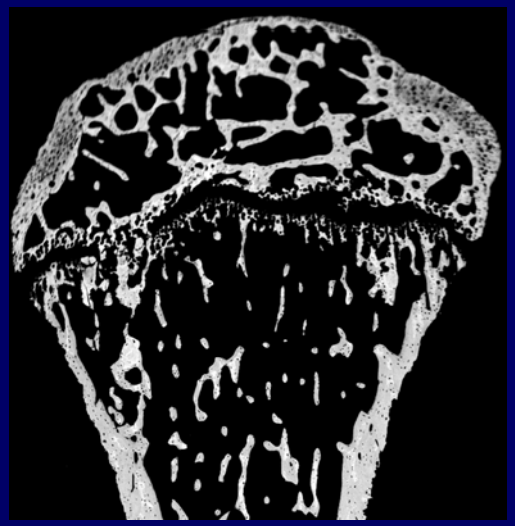
Tissue fixed in 70% ethanol

Embedded in poly-methyl-methacrylate

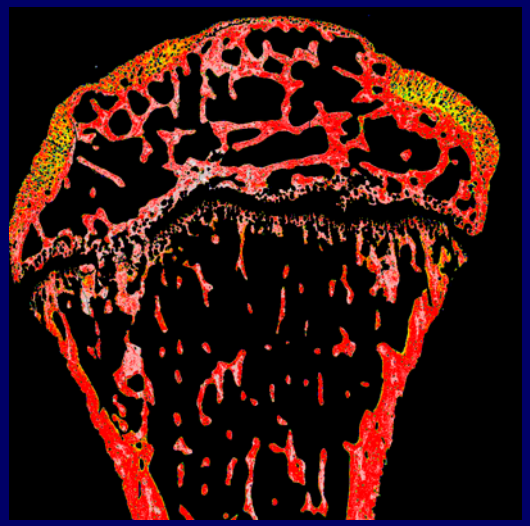
Blocks were diamond micro-milled until optically flat

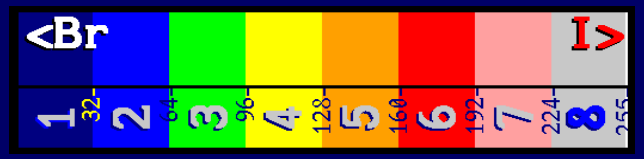
Digital SEM (Zeiss DSM962) with solid state BSE detector

qBSE has a 0.46µm resolution (DXA 200µm and µCT 10µm)

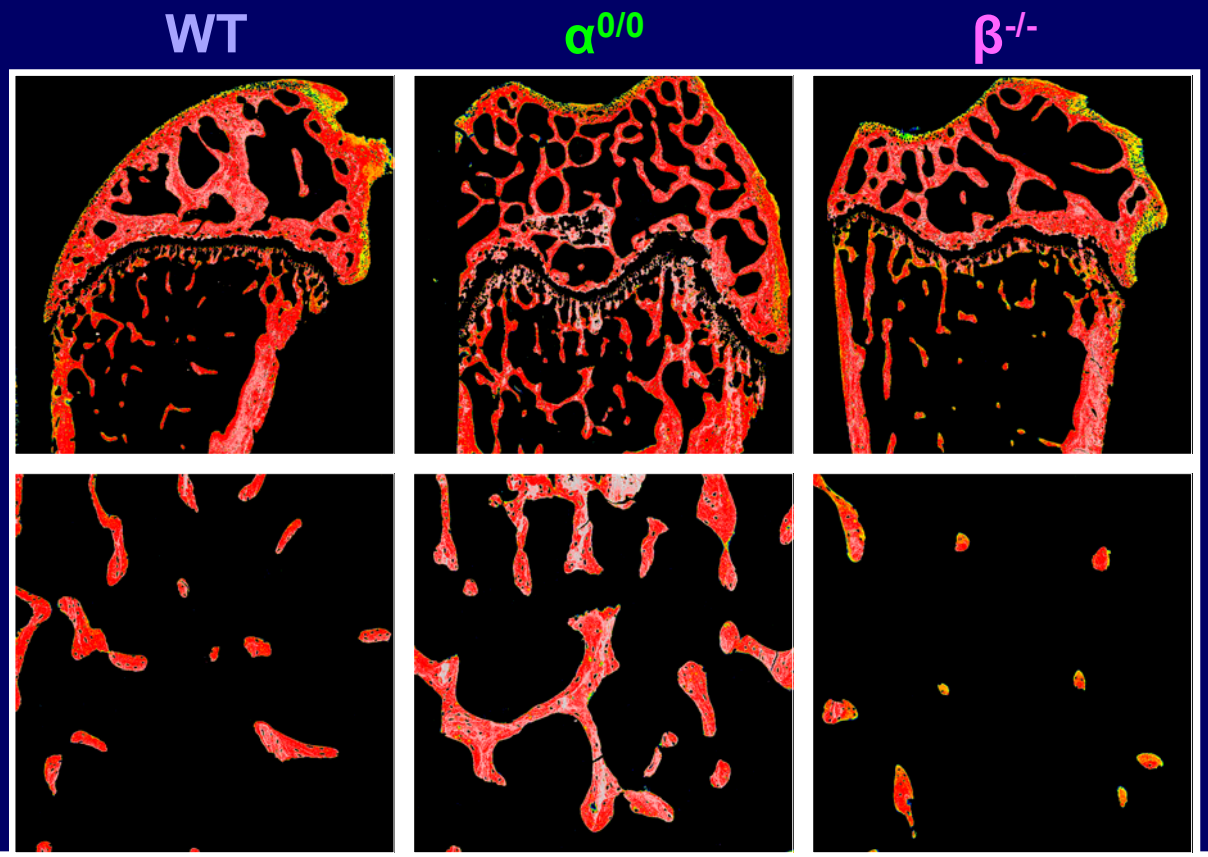


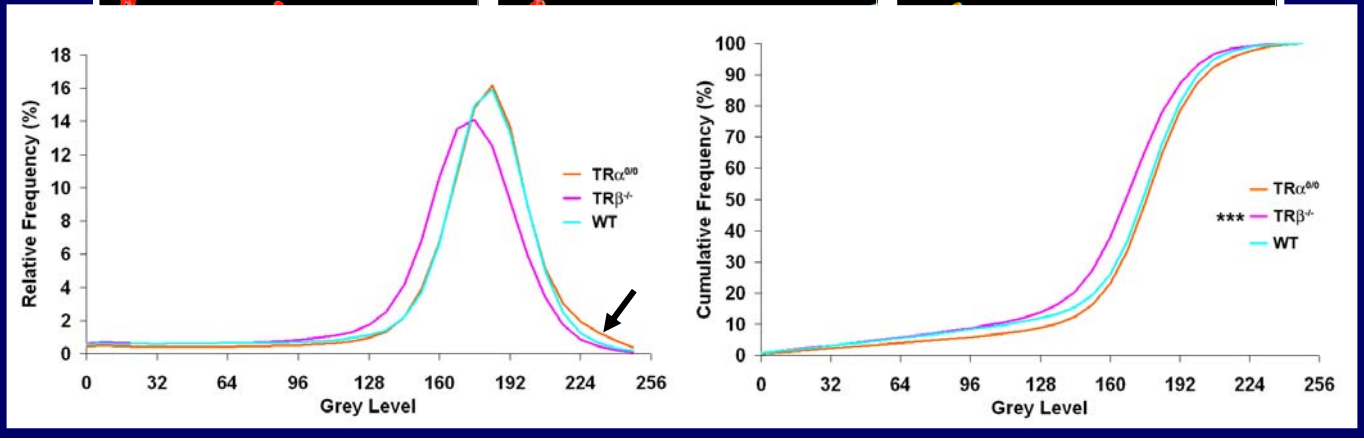
Calibration using halogenated dimethacrylate standards



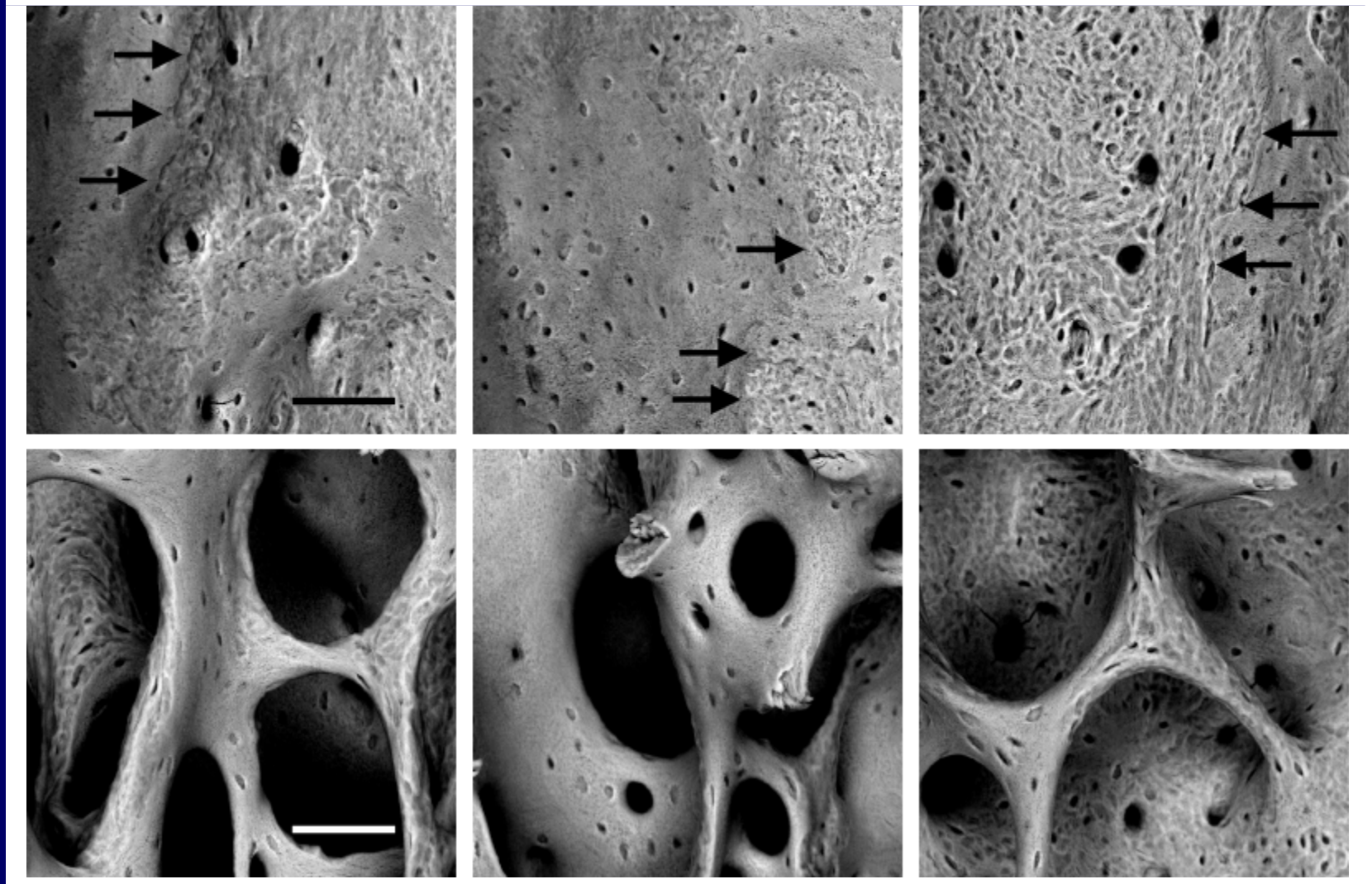


Deletion of TRα or TRβ affects mineralization





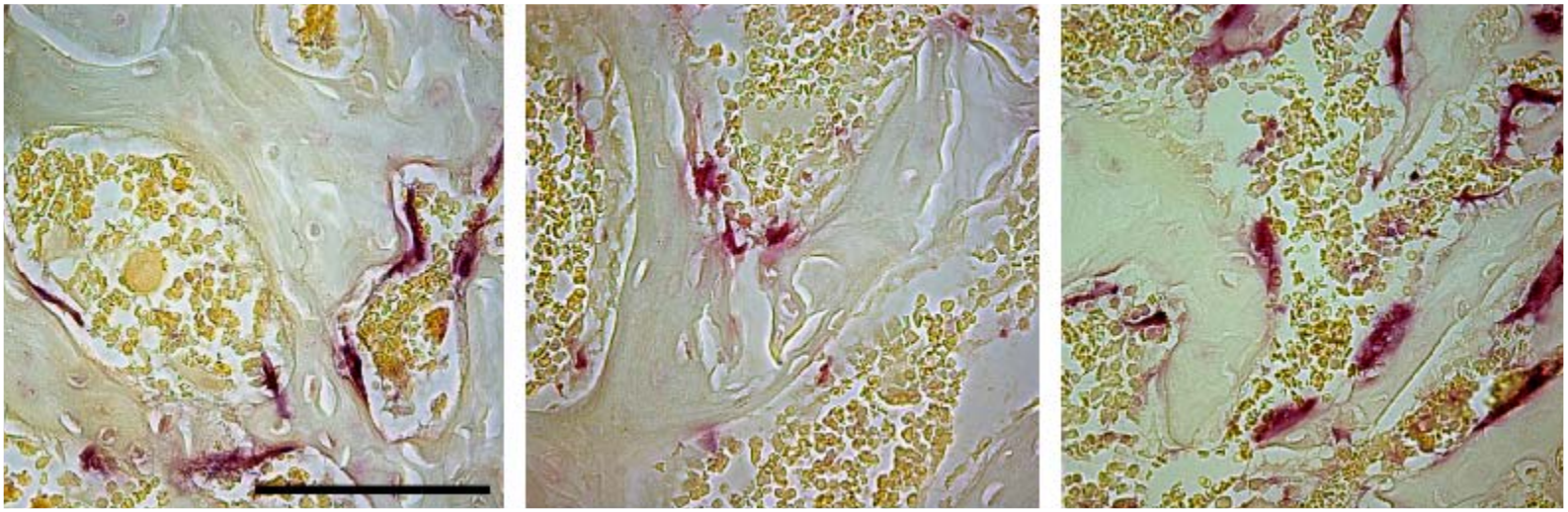
Deletion of TRα or TRβ affects bone resorption

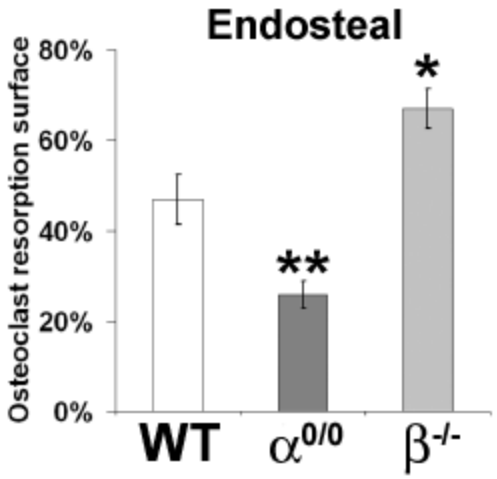


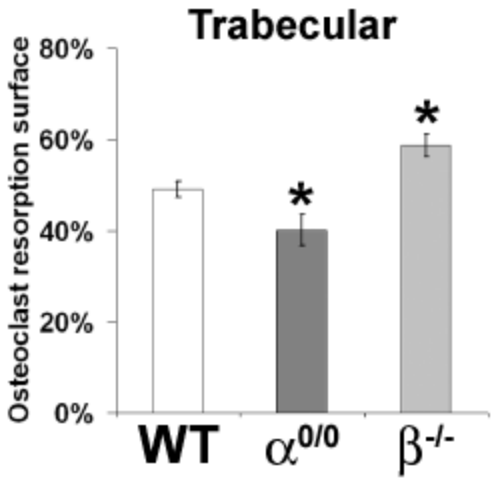
WT TRα$^{0/0}$ TRβ$^{-/-}$

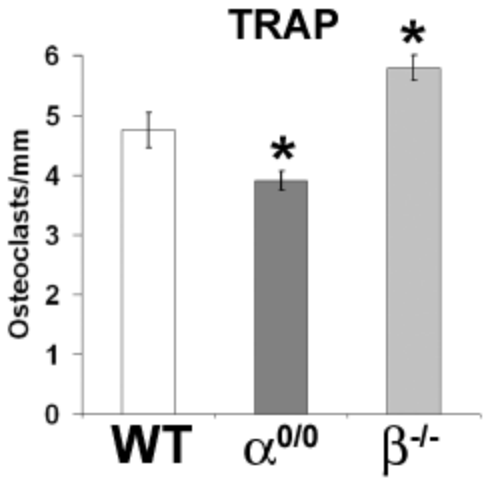
Deletion of TRα or TRβ affects osteoclasts

WT TRα$^{0/0}$ TRβ$^{-/-}$









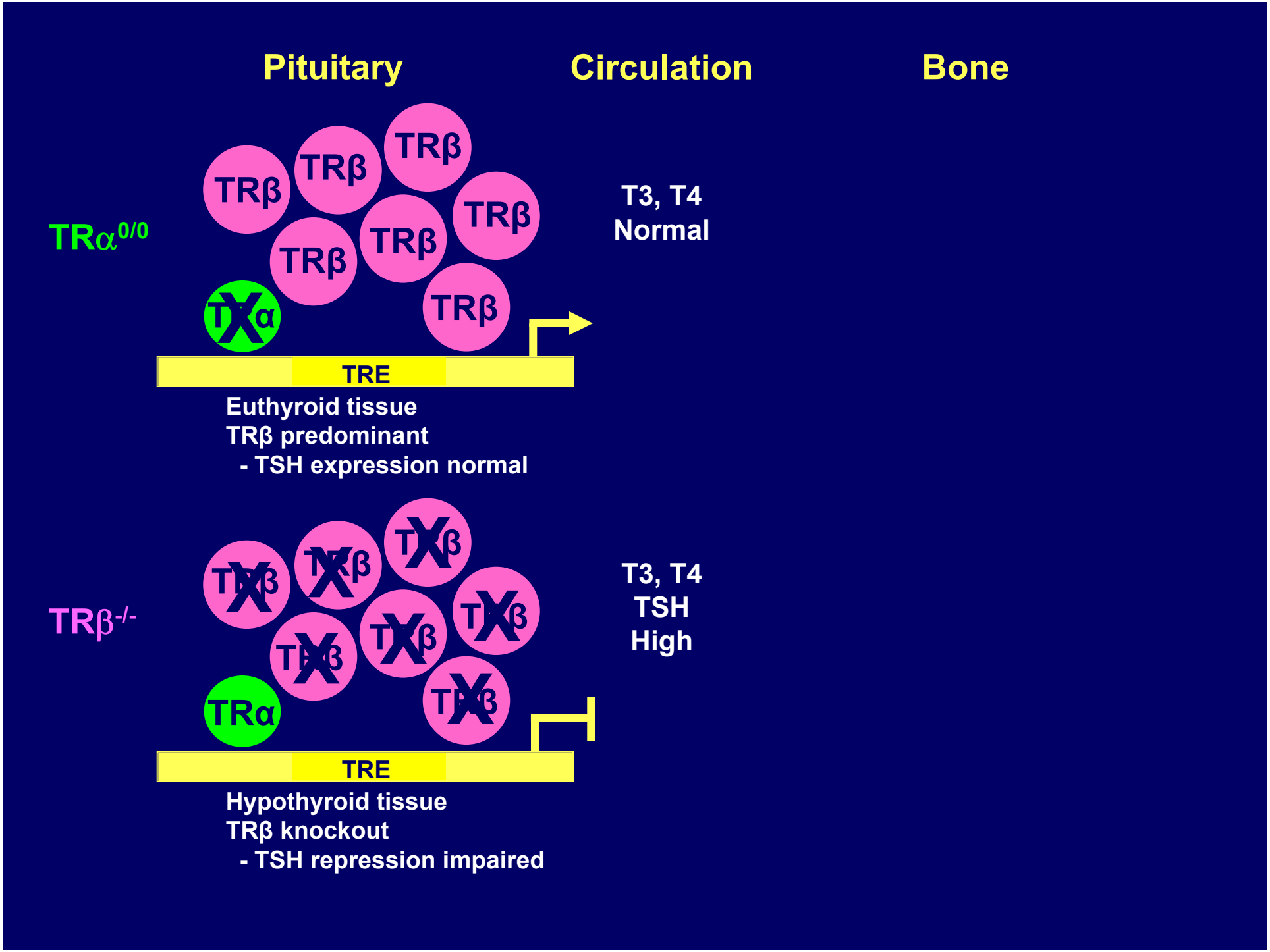
Summary

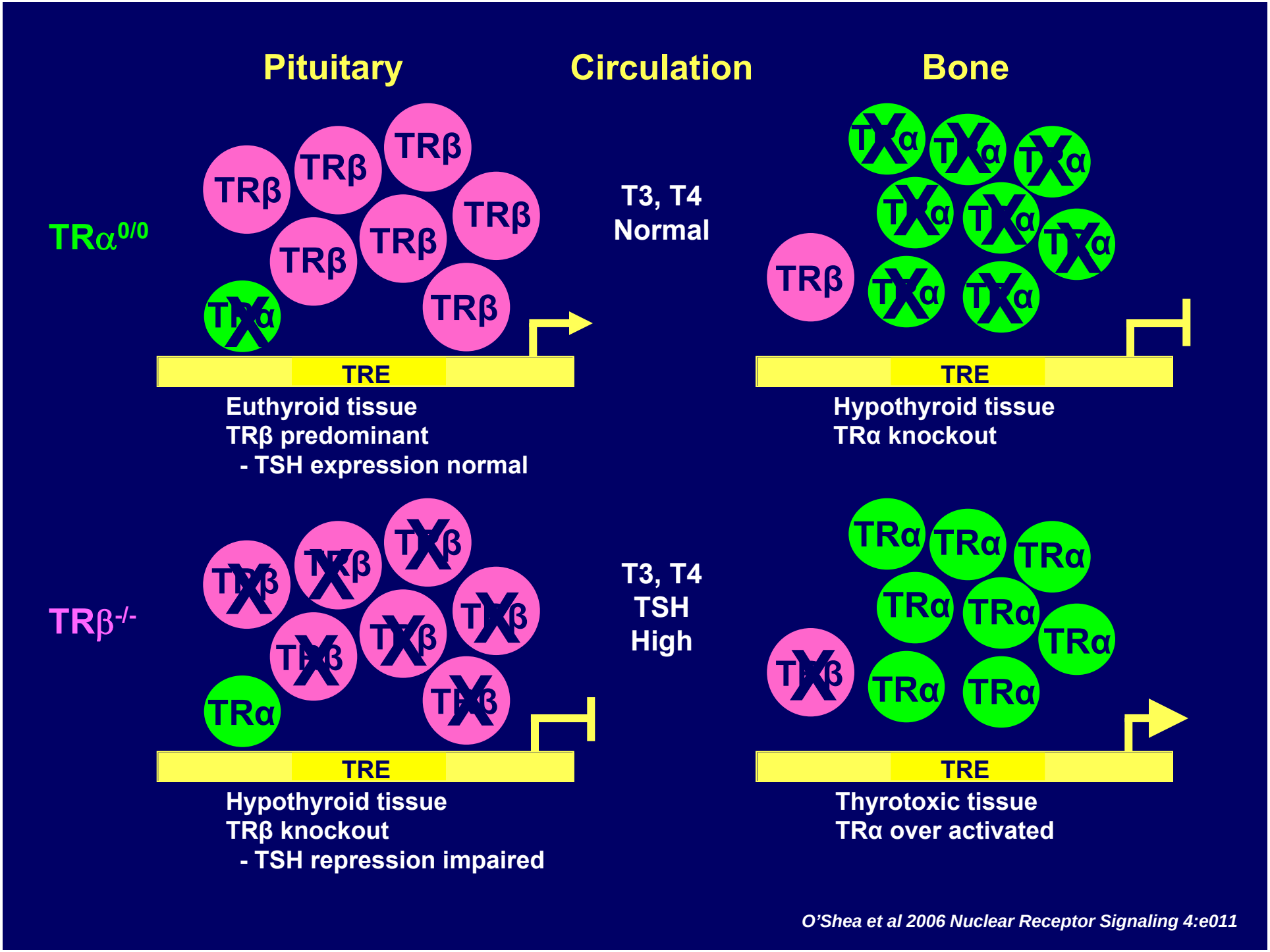
- **TRα$^{0/0}$**
  - ***Delayed*** **ossification, reduced calcified bone and growth retardation**
  - ***Increased*** **adult bone mass (reduced bone resorption)**
- **TRβ$^{-/-}$**
  - ***Advanced*** **ossification, increased calcified bone, accelerated early growth but persistent short stature**
  - ***Reduced*** **adult bone mass and mineralization (increased bone resorption)**

Bassett et al 2007 Mol Endocrinol doi:10.1210/me.2007-0033

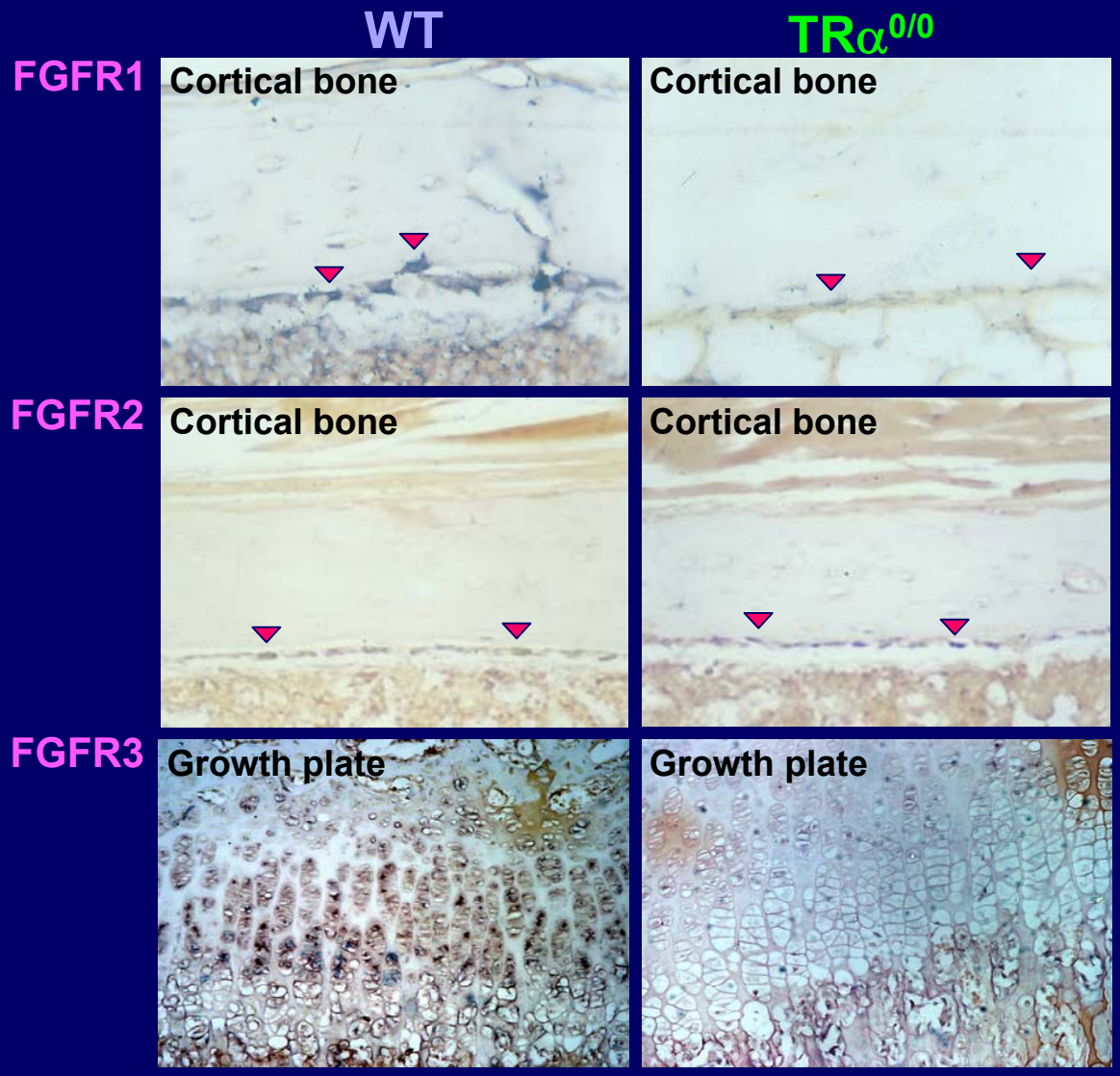
Mechanism of T3 action in bone

in vivo


Pituitary
Circulation
Bone
TRα0/0
TRβ
TRβ
TRβ
TRβ
TRβ
TRβ
TRβ
TRα
TRE
T3, T4
Normal
Euthyroid tissue
TRβ predominant
- TSH expression normal
TRβ-/-
TRβ
TRβ
TRβ
TRβ
TRβ
TRβ
TRα
TRE
T3, T4
TSH
High
Hypothyroid tissue
TRβ knockout
- TSH repression impaired


Pituitary
Circulation
Bone
TRα0/0
TRβ
TRα
T3, T4
Normal
TRE
Euthyroid tissue
TRβ predominant
- TSH expression normal
Hypothyroid tissue
TRα knockout
TRβ-/-
T3, T4
TSH
High
Hypothyroid tissue
TRβ knockout
- TSH repression impaired
Thyrotoxic tissue
TRα over activated
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

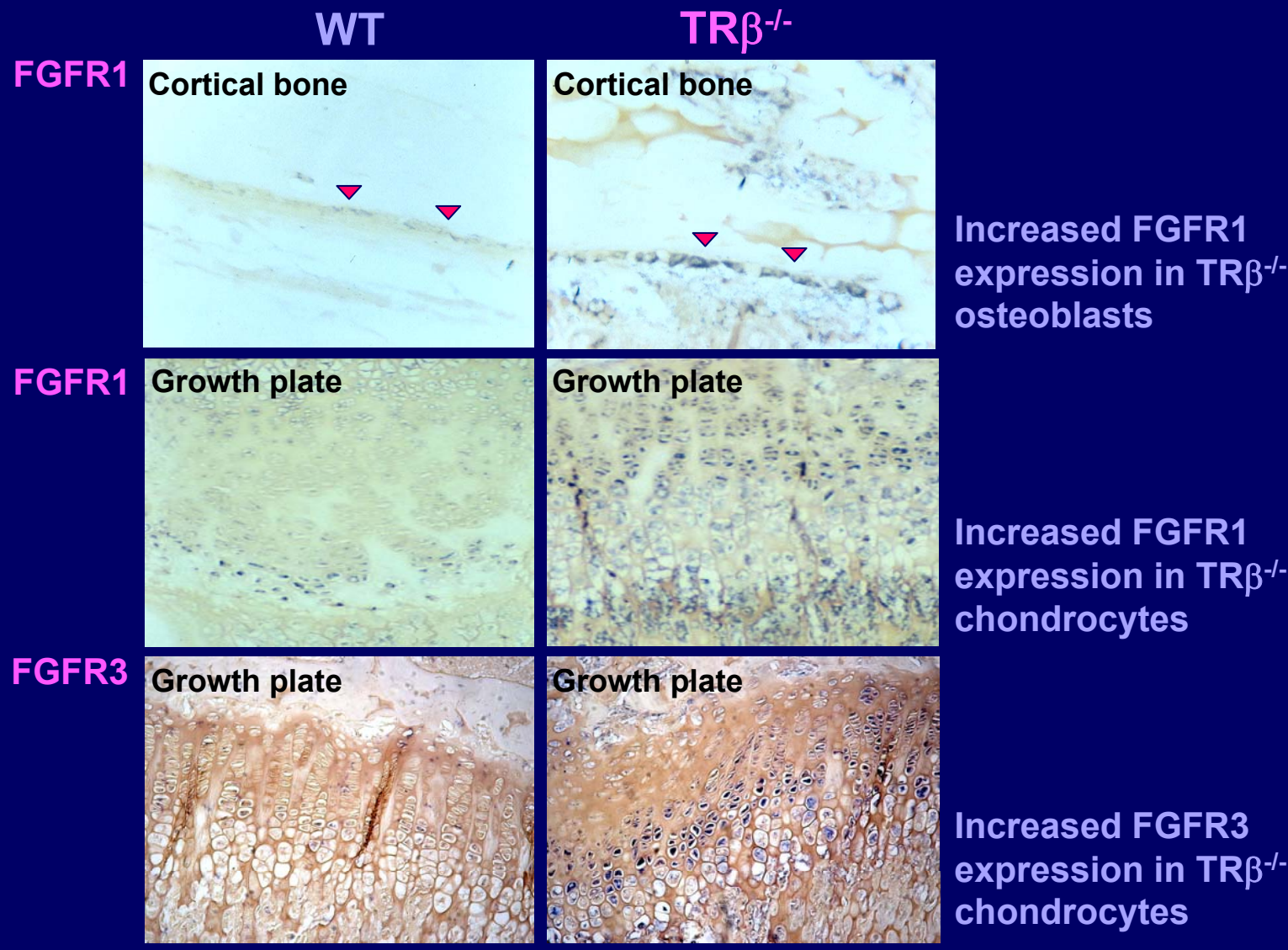
Unchanged FGFR2 expression in TRα$^{0/0}$ osteoblasts

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



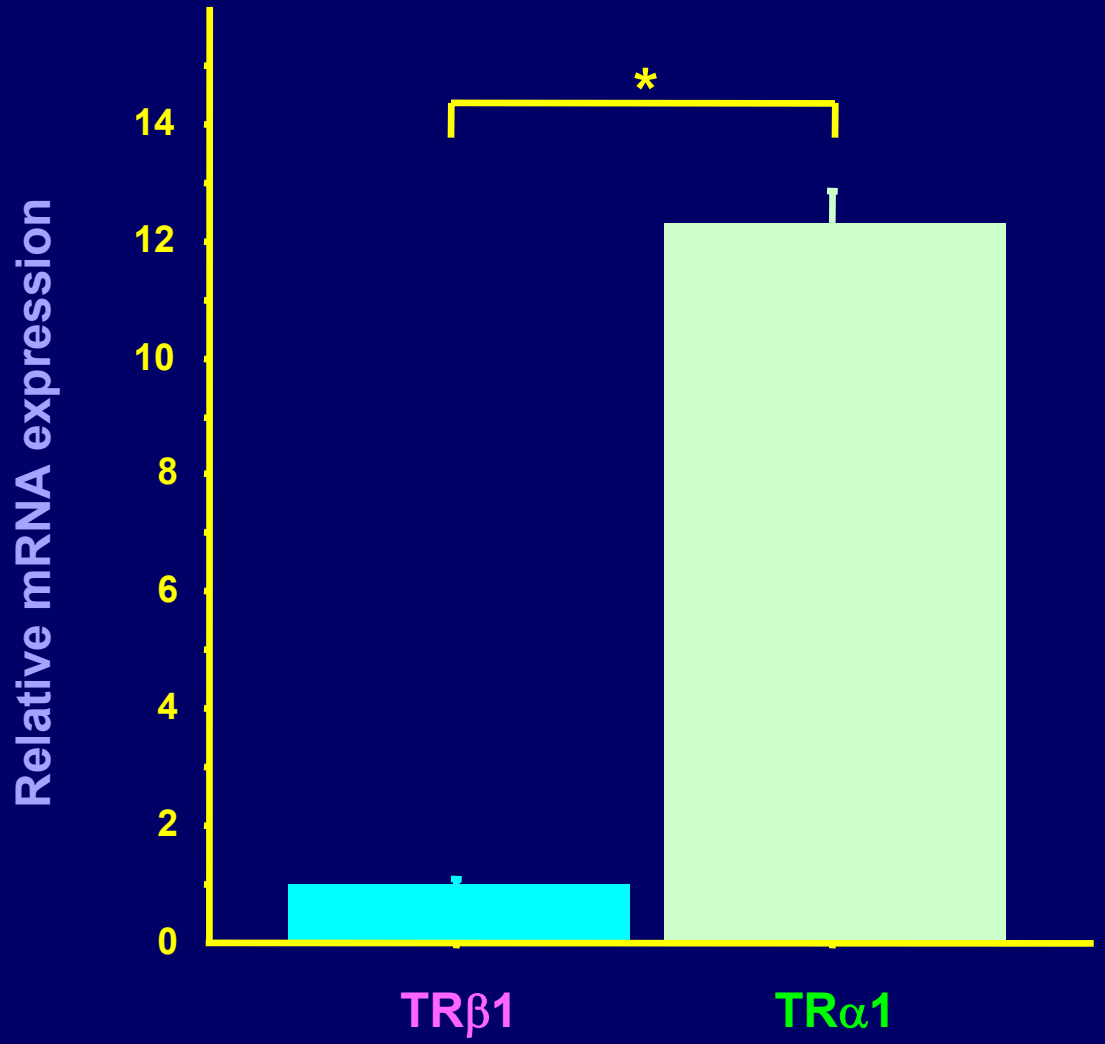
Increased FGFR1 expression in TRβ$^{-/-}$ osteoblasts

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

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

TRβ$^{-/-}$ skeleton is thyrotoxic

Receptor mRNA expression in bone



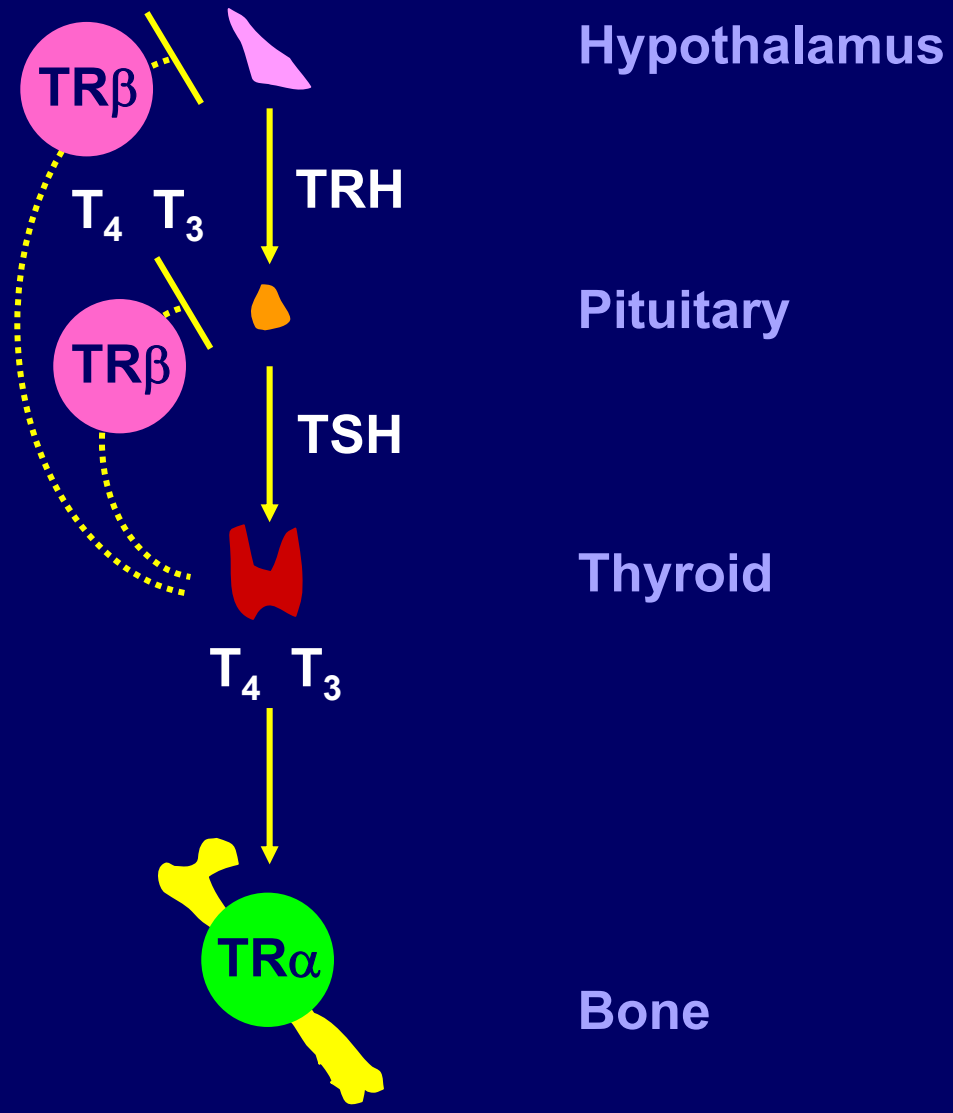
O'Shea et al 2003 Mol Endocrinol 17: 1410-1424

Conclusion

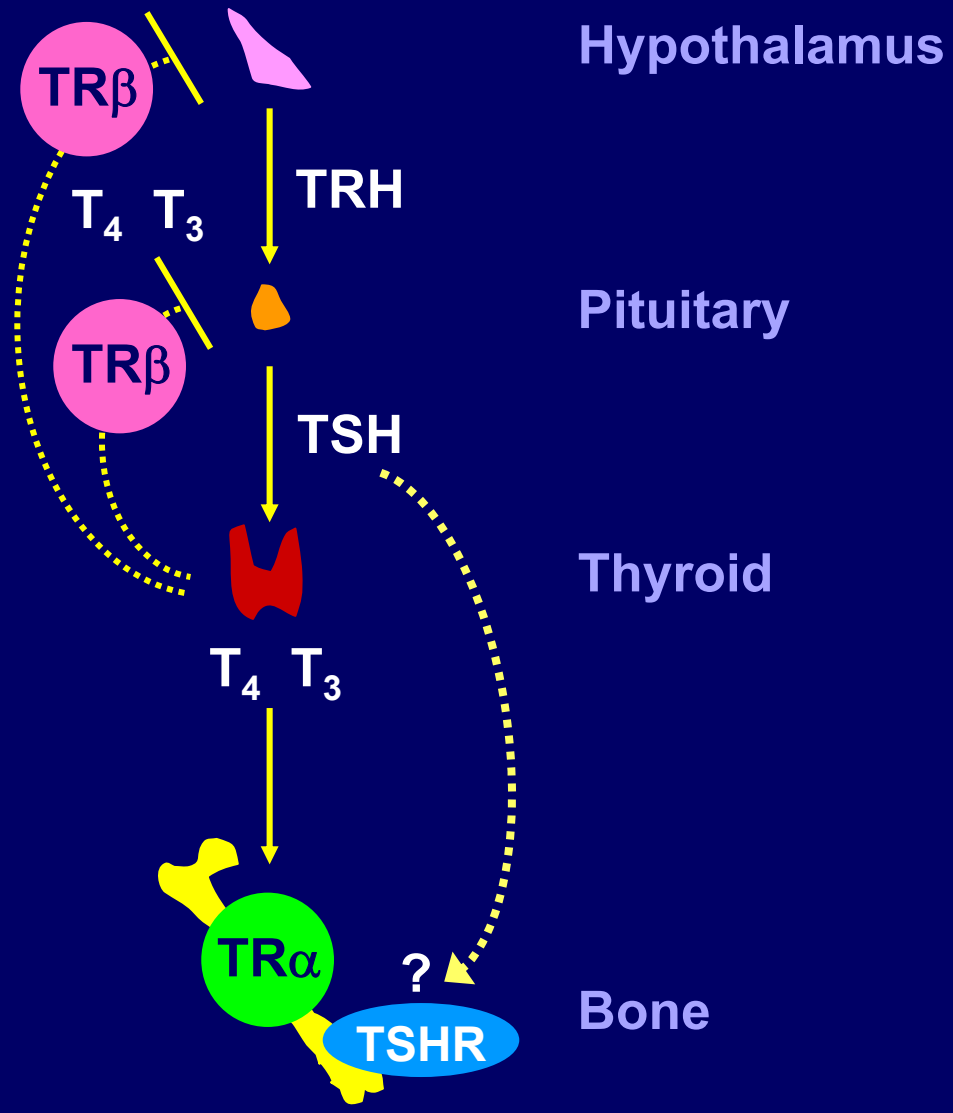
- **Deletion of TRα**
  - **Causes skeletal hypothyroidism despite normal thyroid status because T3 action in bone is disrupted**
- **Deletion of TRβ**
  - **Causes osteoporosis indirectly because elevated thyroid hormones act in bone via TRα**

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

Hypothalamic-pituitary-thyroid axis



Hypothalamic-pituitary-thyroid axis



Thyroid hormone or TSH?

$TSHR^{-/-}$ mice

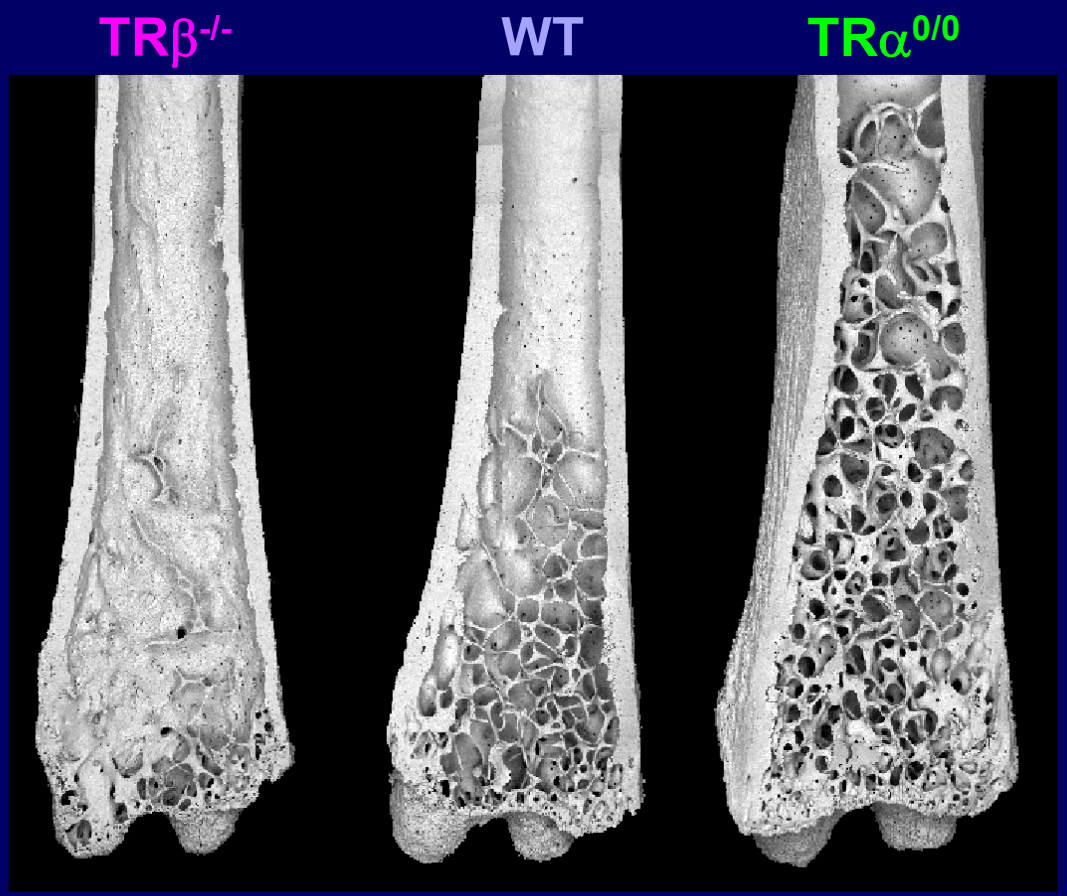
- $TSHR^{-/-}$
  - Thyroid hypoplasia
  - fT_4 & fT_3 undetectable, TSH 500x
  - Severe growth delay
  - Die by 10w unless given TH at weaning
  - High bone turnover osteoporosis (aged 7 weeks)
- $TSHR^{+/-}$
  - Euthyroid
  - Normal growth
  - Intermediate skeletal phenotype
- TSH inhibits
  - Osteoclast 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
- **TR$\beta^{-/-}$ mice**
  - 3x fT4/fT3 and 10xTSH
  - Osteoporotic
- **TR$\alpha^{0/0}$ mice**
  - Euthyroid, TSH normal
  - High bone mass



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

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**

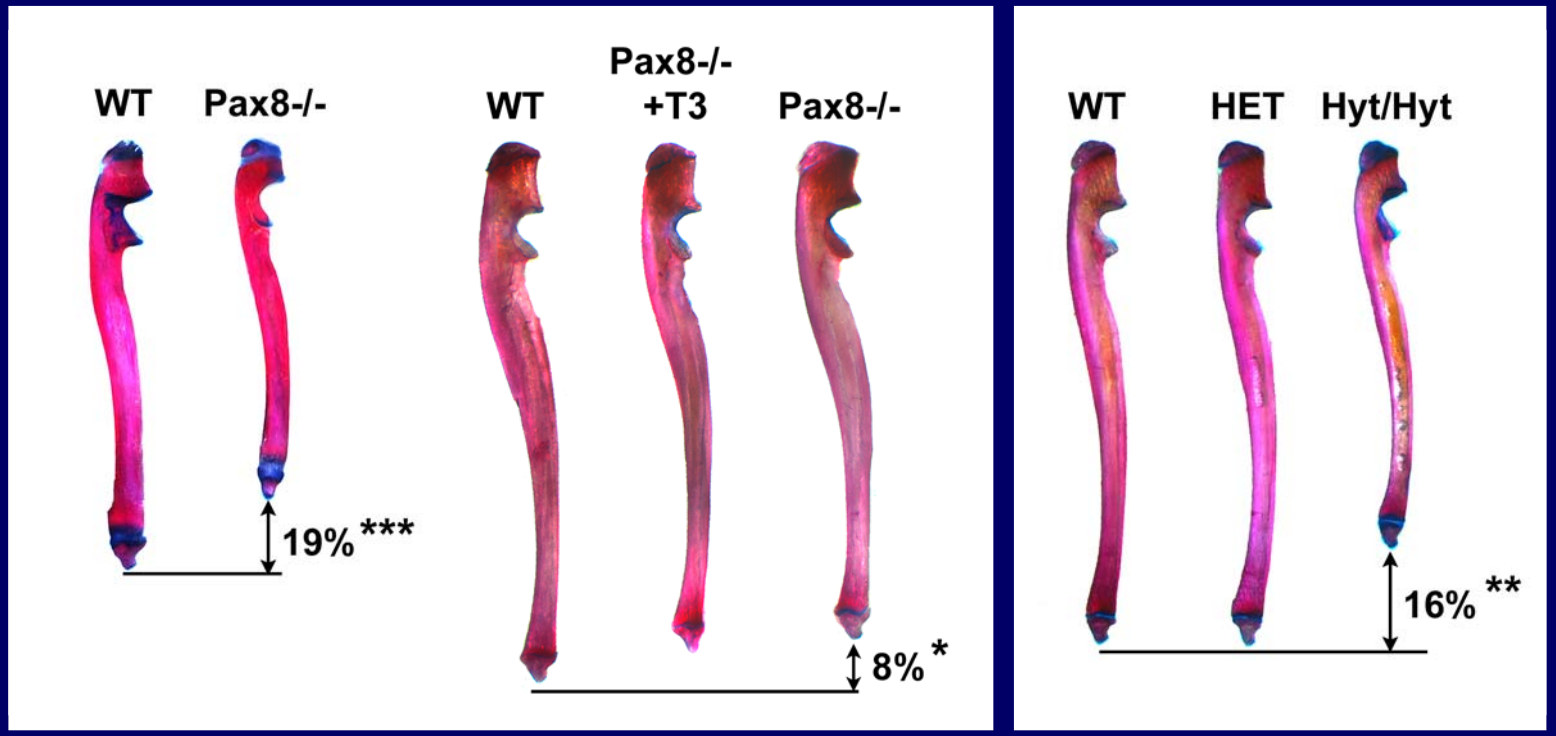
Elevated TSH non-functional TSHR

- **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

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

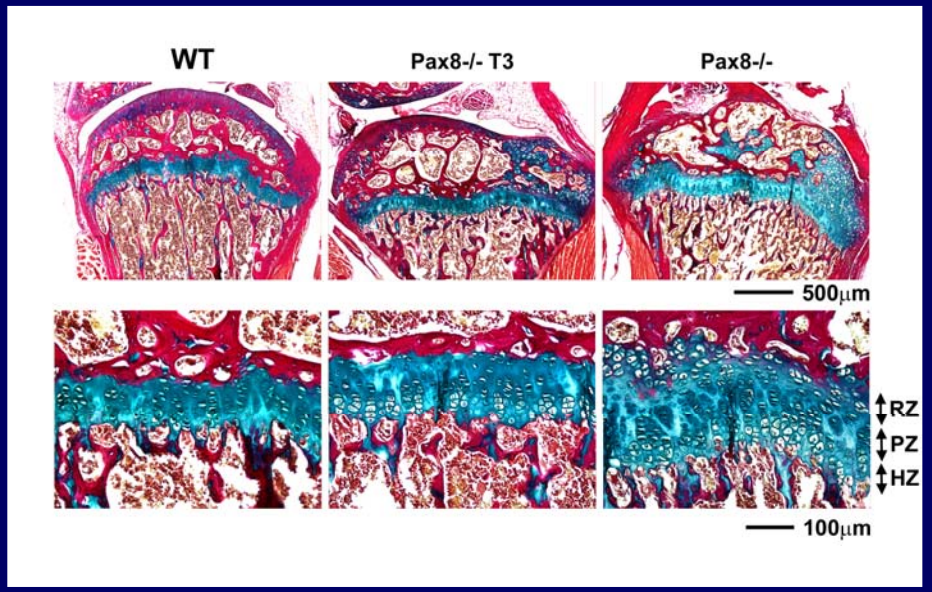


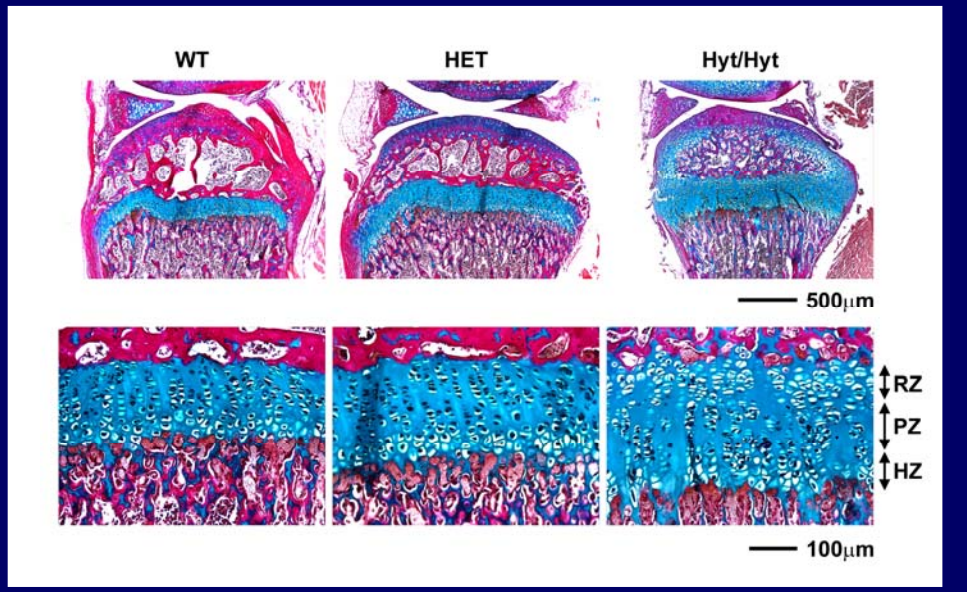
2 weeks

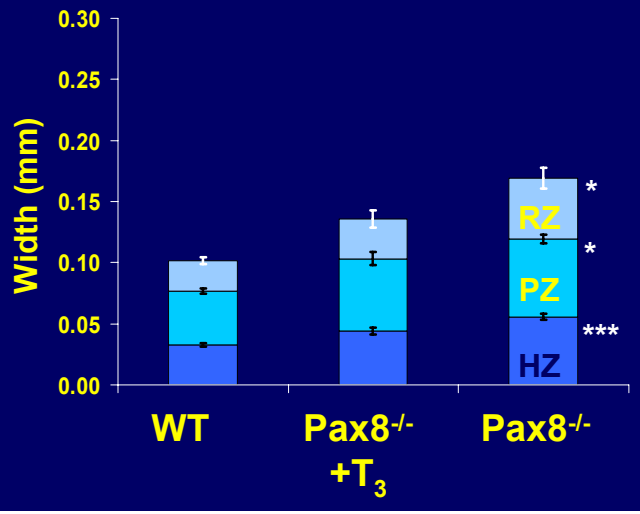
10 weeks

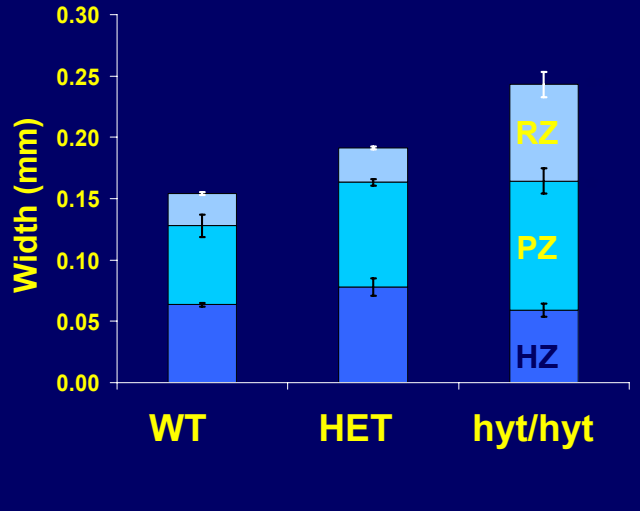
7 weeks

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

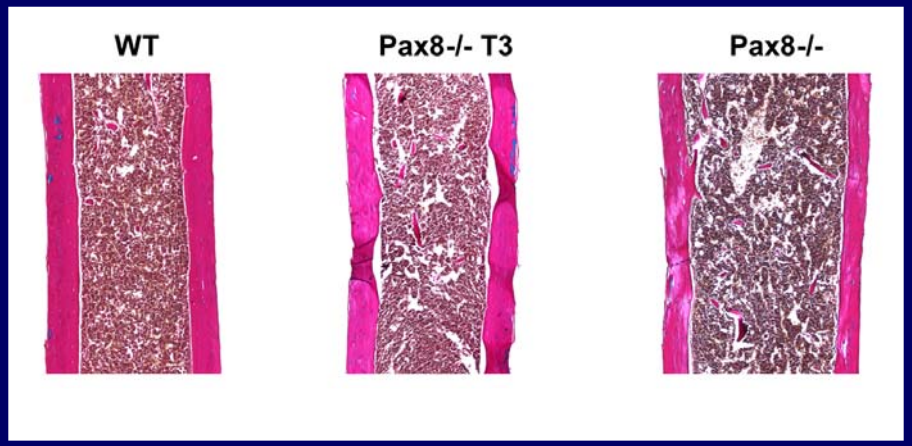


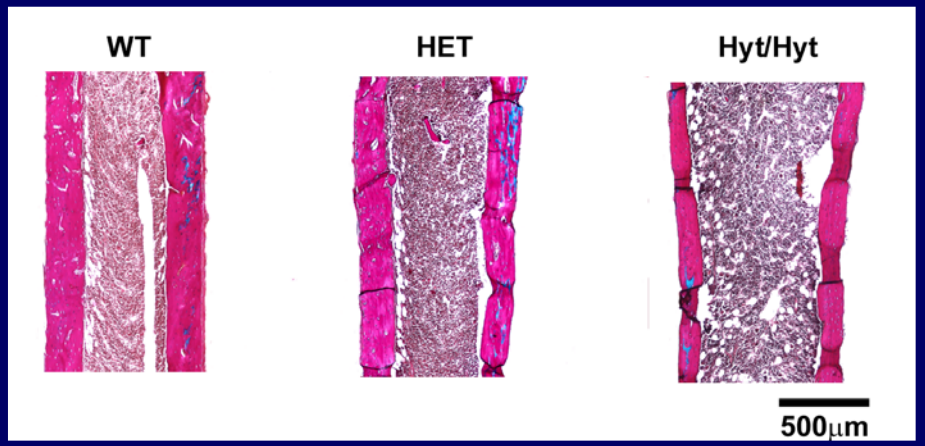


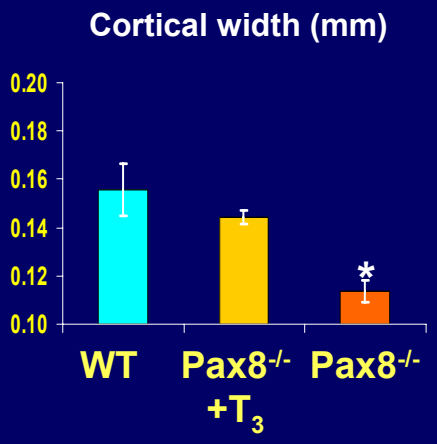


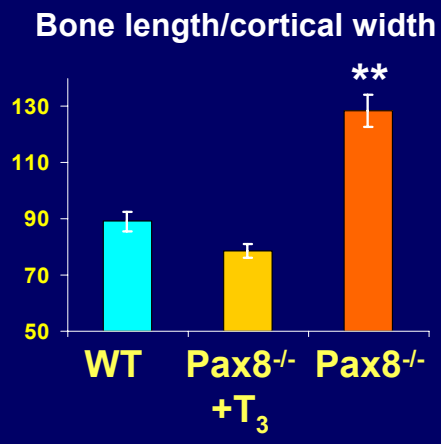


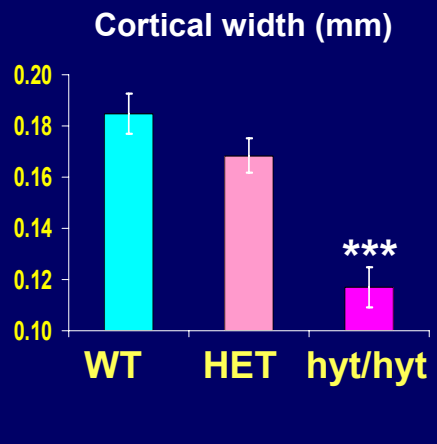
Both hyt/hyt and Pax8$^{-/-}$ mice have reduced cortical bone

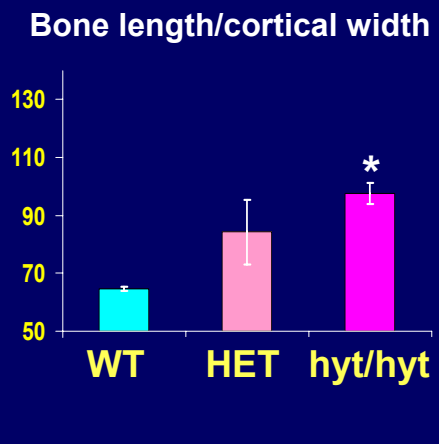




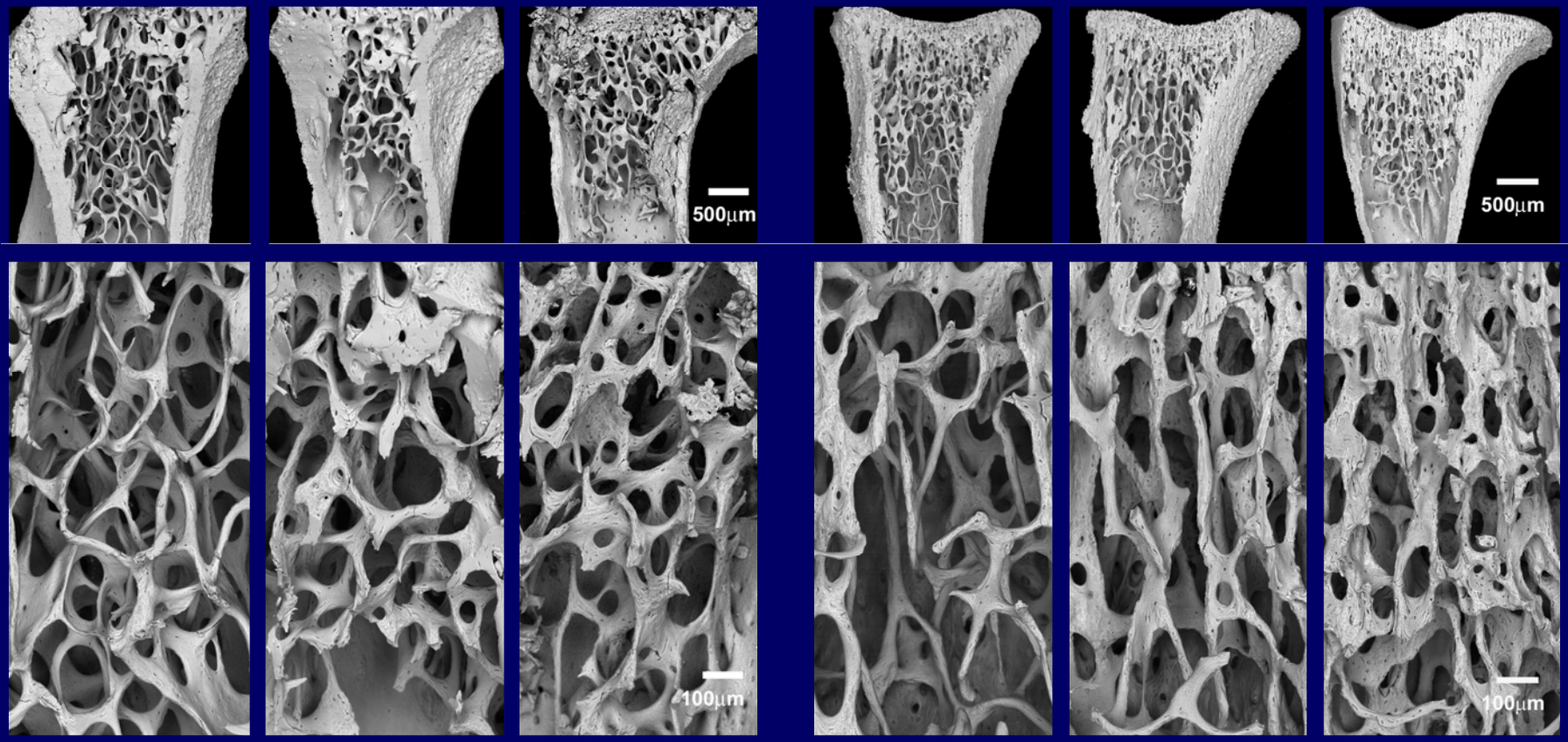




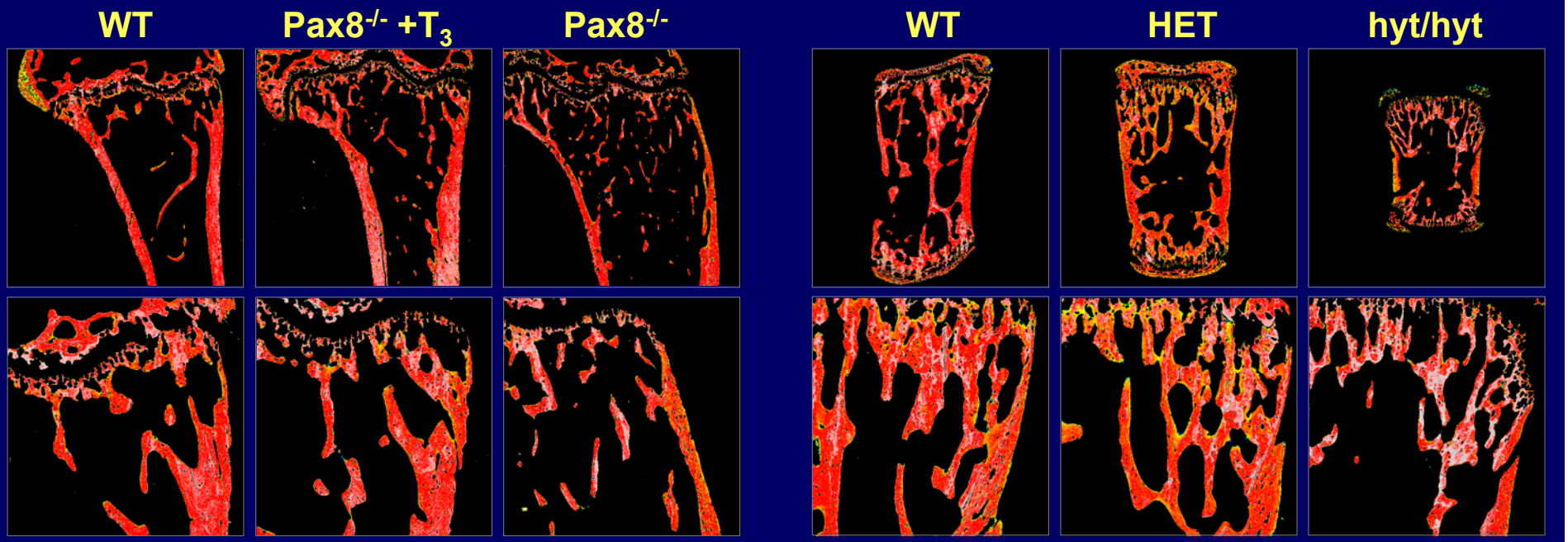


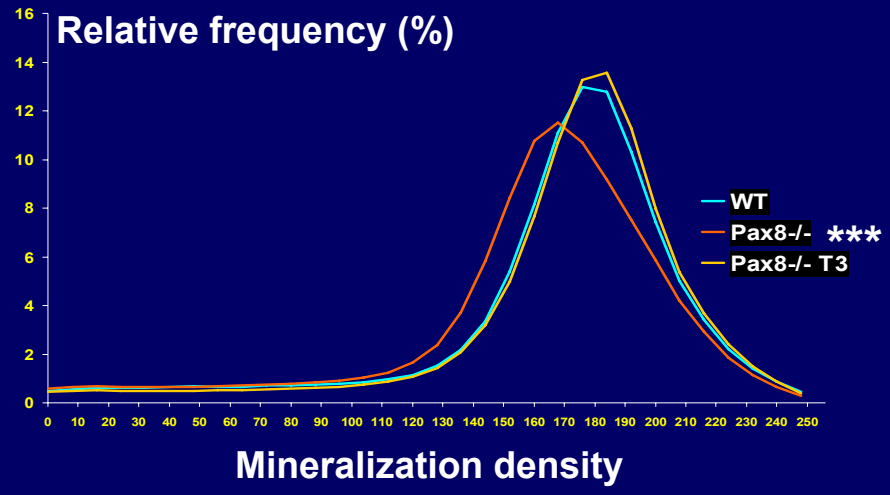


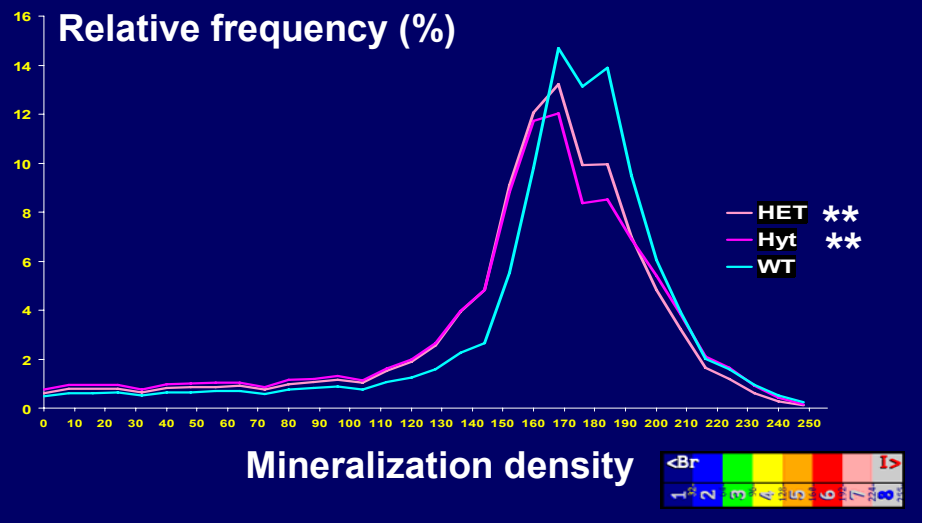
Both hyt/hyt and Pax8$^{-/-}$ mice have similar bone micro-architecture (BSE SEM)



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

Conclusion

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

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