CELLULAR ENDOCRINOLOGY IN HEALTH AND DISEASE

CELLULAR ENDOCRINOLOGY IN HEALTH AND DISEASE

Edited by

ALFREDO ULLOA-AGUIRRE
National University of Mexico and Instituto Nacional de Ciencias Médicas y Nutrición SZ, México D.F., México

P. MICHAEL CONN
Departments of Internal Medicine and Cell Biology-Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX, USA

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NW1 7BY, UK
225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress.

ISBN : 978-0-12-408134-5

For information on all Academic Press publications visit our website at elsevierdirect.com

Typeset by MPS Limited, Chennai, India
www.adi-mps.com

14 15 16 17 18 10 9 8 7 6 5 4 3 2 1

List of Contributors

Ana Aranda Instituto de Investigaciones Biomédicas "Alberto Sols," Madrid, Spain, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain

Richard Bertram Florida State University, Tallahassee, FL, USA

Andrew A. Bremer Vanderbilt University, Nashville, TN, USA

Maria Luisa Brandi University of Florence, Medical School, Florence, Italy

Sally A. Camper University of Michigan Medical School, Ann Arbor, MI, USA

Nancy Carrasco Yale University School of Medicine, New Haven, CT, USA

Luisella Cianferotti University of Florence, Medical School, Florence, Italy

P. Michael Conn Departments of Internal Medicine and Cell Biology-Biochemistry, Texas Tech University Health Services Center, Lubbock, TX, USA

Constanza Contreras Jurado Instituto de Investigaciones Biomédicas "Alberto Sols," Madrid, Spain, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain

Lique M. Coolen University of Mississippi Medical Center, Jackson, MS, USA

Pascale Crépieux CNRS, Nouzilly, France, François Rabelais University, Tours, France

Francisco Dominguez INCLIVA, Instituto Universitario IVI (IUIVI), Valencia University, Valencia, Spain

Shereen Ezzat University of Toronto, Princess Margaret Hospital, and the Ontario Cancer Institute, Toronto, ON, Canada

Laurine Gagniac CNRS, Nouzilly, France, François Rabelais University, Tours, France

Nathalie Gallay CNRS, Nouzilly, France, François Rabelais University, Tours, France

Peter D. Gluckman University of Auckland, Auckland, New Zealand

Karen Gomez-Hernandez University of Toronto, Princess Margaret Hospital, and the Ontario Cancer Institute, Toronto, ON, Canada

Arturo E. Gonzalez-Iglesias Florida State University, Tallahassee, FL, USA

Robert L. Goodman West Virginia University, Morgantown, WV, USA

Rodolfo Guardado-Mendoza University of Guanajuato, León, Mexico

Florian Guillou CNRS, Nouzilly, France, François Rabelais University, Tours, France

Mark A. Hanson University of Southampton, Southampton, UK

Astrid C. Hauge-Evans King's College London, London, UK

Tomohiro Ishii Department of Pediatrics, School of Medicine, Keio University, Tokyo, Japan

Peter M. Jones King's College London, London, UK

Gerard Karsenty Columbia University Medical Center, New York, NY, USA

Michael N. Lehman University of Mississippi Medical Center, Jackson, MS, USA

Felicia M. Low University of Auckland, Auckland, New Zealand

Olaia Martínez-Iglesias Instituto de Investigaciones Biomédicas "Alberto Sols," Madrid, Spain, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain

Jan M. McAllister Pennsylvania State University College of Medicine, Hershey, PA, USA

Walter L. Miller University of California, San Francisco, CA, USA

Bhavi Modi Virginia Commonwealth University, Richmond, VA, USA

Nicolas Musi Barshop Institute for Longevity and Aging Studies and Geriatric Research, San Antonio, TX, USA

Juan Pablo Nicola Yale University School of Medicine, New Haven, CT, USA

Aurea Orozco Instituto de Neurobiologia, Universidad Nacional Autónoma de México (UNAM), Quéretaro, México

María Inés Pérez Millán University of Michigan Medical School, Ann Arbor, MI, USA

Shanta J. Persaud King's College London, London, UK

Anne Poupon INRA, Nouzilly, France, CNRS, Nouzilly, France, François Rabelais University, Tours, France

Alvin C. Powers Vanderbilt University Medical Center, Nashville, TN, USA

Eric Reiter INRA, Nouzilly, France, CNRS, Nouzilly, France, François Rabelais University, Tours, France

Ludivina Robles-Osorio Facultad de Medicina, Universidad Autónoma de Quéretaro, Querétaro, México

Lidia Ruiz-Llorente Instituto de Investigaciones Biomédicas "Alberto Sols," Madrid, Spain, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain

Carlos Simon INCLIVA, Instituto Universitario IVI (IUIVI), Valencia University, Valencia, Spain

Juan Carlos Solís-S Facultad de Medicina, Universidad Autónoma de Quéretaro, Querétaro, México

Jerome F. Strauss III Virginia Commonwealth University, Richmond, VA, USA

Toru Tateno University of Toronto, Princess Margaret Hospital, and the Ontario Cancer Institute, Toronto, ON, Canada

Manuel Tena-Sempere University of Córdoba, Córdoba, Spain, Instituto de Salud Carlos III, Córdoba, Spain, Instituto Maimónides de Investigaciones Biomédicas (IMIBIC)/Hospital Universitario Reina Sofia, Córdoba, Spain

Judith L. Turgeon University of California, Davis, USA

Alfredo Ulloa-Aguirre Universidad Nacional Autónoma de México and Instituto Nacional de Salud Pública, Mexico D.F., Mexico

Carlos Valverde-R Instituto de Neurobiologia, Universidad Nacional Autónoma de México (UNAM), Quéretaro, México

Michael D. Walker Weizmann Institute of Science, Rehovot, Israel

Dennis W. Waring University of California, Davis, USA

Preface

The last two decades have witnessed tremendous advances in endocrinology – specifically in our knowledge of how endocrine cells govern their own function, as well as the functions of other cells. The intricate mechanisms that control the biosynthesis and secretion of hormones by endocrine cells, as well as the cellular responses to stimuli from a large variety of hormones and chemical signals, are becoming better understood – thanks to improvements in the techniques used to explore cell and molecular biology; these include microscopy, recombinant DNA technology, cell micromanipulation, and all "OMICS" fields. We are now able to identify many of the genetic, biochemical and structural responses that are regulated by environmental cues and endogenous stimuli. We are only starting to understand the intracellular and intercellular networks that maintain homeostasis of the whole organism, but it is clear that that regulatory networks have their genesis in the response of the individual cell.

This book is our attempt to provide an understanding of how endocrine glands function by integrating information resulting in biological effects on both local and systemic levels. The book explores and dissects the function of a number of cell systems, including those whose function as part of the endocrine was not obvious until recently, among these, the bone and the adipose tissue. To this end, the editors selected authors based on their research contributions and their ability to express their thoughts clearly.

The editors want to express appreciation to the authors for providing contributions in a timely fashion and to the staff at Elsevier for helpful input.

Alfredo Ulloa-Aguirre
P. Michael Conn

CHAPTER

1

Thyroid Hormone Receptors and their Role in Cell Proliferation and Cancer

Olaia Martínez-Iglesias, Lidia Ruiz-Llorente, Constanza Contreras Jurado and Ana Aranda

Instituto de Investigaciones Biomédicas "Alberto Sols," Madrid, Spain, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Madrid, Spain

THYROID HORMONE ACTION

The important physiological actions of the thyroid hormones (THs) are mediated by binding to the nuclear thyroid hormone receptors (TRs). The thyroid gland produces predominantly thyroxine (T4), but triiodothyronine (T3) is the most active TH, since it has a higher affinity by the receptors.[1] THs are released by the thyroid gland to the bloodstream and they enter the cells through the adenosine triphosphate (ATP)-dependent monocarboxylate transporters MCT8 and MCT10 and the organic anion transporter proteins (OATPs).[2] The amount of T3 available for binding to the nuclear receptors is regulated by cell-specific expression of selenoenzymes deiodinases (DIOs). DIO1 and DIO2 catalyze the conversion of T4 to T3 in target tissues, increasing intracellular levels of the active hormone, while DIO3 causes hormone inactivation since it converts T4 and T3 by inner ring deiodination to the inactive metabolites reverse T3 (rT3) and T2, respectively.

TRs belong to the superfamily of nuclear receptors and act as ligand-dependent transcription factors.[3] Several TR protein isoforms are generated by promoter use or alternative splicing of the primary transcripts of the *TRα* and *TRβ* genes. The TRα1, TRβ1 and TRβ2 are the main hormone-binding isoforms and their relative levels of expression vary among cell types and at different developmental stages, suggesting that they could have organ-specific functions. In the case of TRβ, TRβ1 is more widely expressed, while the expression of TRβ2 is restricted to the anterior pituitary, and some neural cells.[4,5] Studies with genetically modified mice have shown that TRα and TRβ can substitute for each other to mediate some actions of the thyroid hormones but they can also mediate isoform-specific functions.[6]

As shown in Figure 1.1, TRs are composed of several functional domains. The *N*-terminal

Cellular Endocrinology in Health and Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-408134-5.00001-9

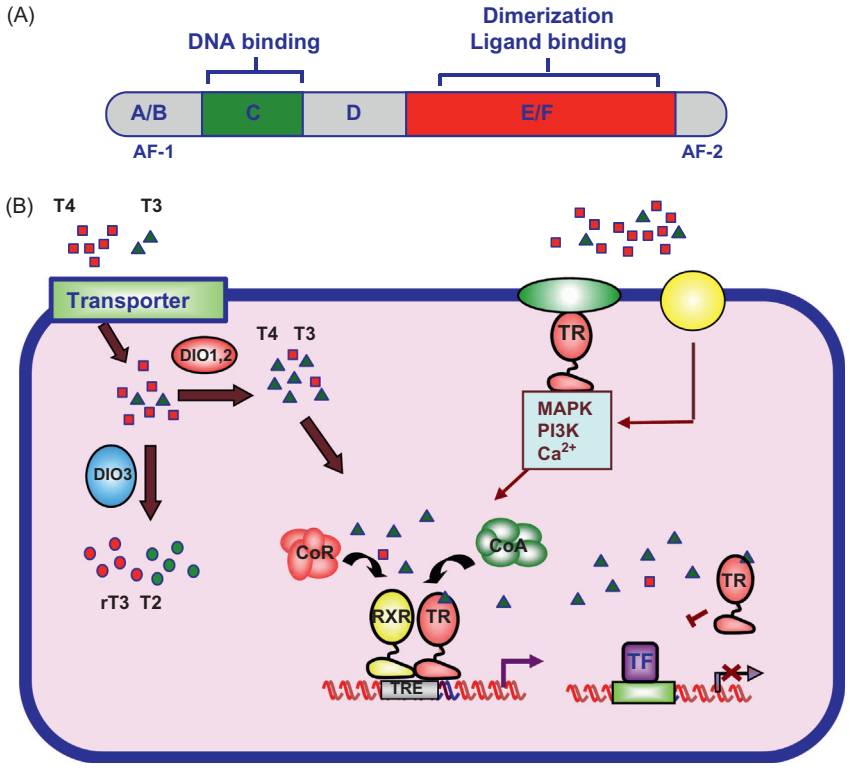

FIGURE 1.1 **Mechanism of action of the thyroid hormone receptors.** (A) Schematic representation of a thyroid hormone receptor, showing the different functional domains. (B) Thyroxine (T4) and triiodothyronine (T3) enter the cell through transporter proteins such as MCT8 and 10 or OATPs. Inside the cells, deiodinases (DIO1,2) convert T4, to the more active form T3. DIO3 produces rT3 and T2 from T4 and T3, respectively. T3 binds to nuclear thyroid hormone receptors (TRs) that regulate transcription by binding, generally as heterodimers with the retinoid X receptor (RXR), to positive or negative thyroid hormone response elements (TREs) located in regulatory regions of target genes. Activity is regulated by an exchange of corepressor (CoR) and coactivator (CoA) complexes. TRs can also regulate the activity of genes that do not contain a TRE through "cross-talk" with other transcription factors (TF) that stimulate target gene expression. Binding of T3 to a subpopulation of receptors located outside the nuclei can also cause rapid "non-genomic" effects through interaction with adaptor proteins, leading to stimulation of signaling pathways. T4 can also bind to putative membrane receptors such as integrin $\alpha V\beta 3$ inducing mitogen activated protein kinase (MAPK) activity.

region (A/B) contains a constitutive ligand-independent transcriptional activation domain, the autonomous activation function 1 (AF-1). This region is followed by the DNA-binding domain (DBD), or region C. The DBD is the most conserved region among the nuclear receptors and is composed of two zinc fingers. In each zinc finger, four invariable cysteines coordinate tetrahedrically with one zinc ion. Amino acids required for discrimination of the thyroid hormone response element (TRE) are present at the base of the first finger in a region termed the "P box," and other residues of the second zinc finger that form the so called "D box" are involved in dimerization. Through the DBD the receptors interact with the major groove of DNA. A hinge domain, or D region, connects the DBD with the E region or ligand-binding domain (LBD), also responsible for dimerization. This hinge domain

contains residues essential for interaction with corepressors. Crystallographic analysis has shown that the LBDs are formed by 12 α-helices, and the *C*-terminal helix (H12) encompasses the ligand-dependent transcriptional activation function, or AF-2.

TRs regulate gene transcription by binding, preferentially as heterodimers with retinoid X receptors (RXRs), to short DNA binding motifs, called thyroid hormone response elements or TREs, which are located in regulatory regions of target genes.[7] TREs are composed of two copies of the AGG/TTCA motif. They can be configured as palindromes (Pal), inverted palindromes (IPs), or direct repeats spaced preferably by four non-conserved nucleotides (DR4). Although TRs can bind to their response elements as monomers or homodimers, heterodimerization with RXR strongly increases the affinity for DNA and transcriptional activity.

Transcriptional regulation by these receptors is mediated by the recruitment of coactivators and corepressors.[3,8,9] In the absence of ligand, TRs can act as constitutive repressors when bound to TREs, due to their association with corepressors such as NCoR (nuclear receptor corepressor) or SMRT (silencing mediator of retinoic and thyroid receptor). NCoR and SMRT belong to multicomponent repressor complexes that contain histone deacetylases (HDACs) and cause chromatin compaction and consequently transcriptional inhibition.[10] NCoR and SMRT are related both structurally and functionally. They contain three autonomous repressor domains (RD) and a receptor interacting domain (the CoRNR motif) located toward the carboxyl terminus. Transcriptional repression by the corepressor-bound receptors appears to be mediated by the recruitment of HDACs to the target gene. HDAC1 or 2 (class I deacetylases) are recruited to the first RD of the corepressors via the adaptor mSin3 protein, and the RD3 has been demonstrated to repress transcription by directly interacting with class II deacetylases (HDACs 4, 5 and 7). In addition, a repressor complex containing the corepressors, HDAC3 and transducin beta-like proteins (TBL1 or TBL1R) appears to be required for repression by TR. Although a receptor CoR box, located within the hinge region, is essential for interaction of receptors with the corepressors, the CoRNR motif does not interact directly with residues in this region, but docks to a hydrophobic groove in the surface of the LBD at H3 and 4.

Hormone binding induces a conformational change in the receptor that allows the release of corepressors and allows the recruitment in a sequential manner of coactivator complexes. The stronger change observed in the receptors upon ligand binding is the position of H12.[11] This helix projects away from the body of the LBD in the absence of ligand. However, upon hormone binding H12 moves in a "mousetrap" model being tightly packed against H3 or 4 and making direct contacts with the ligand. This change generates a hydrophobic cleft responsible for interaction with coactivators.[12] A glutamic acid residue in H12 and a lysine residue in H3, which are conserved throughout the superfamily of nuclear receptors, interact directly with the coactivator and form a charge clamp that stabilizes binding. Consequently, mutation of these residues abolishes coactivator binding and causes the loss of thyroid hormone-dependent transcriptional activation.[13] Since the coactivator binding surface overlaps with that involved in corepressors interaction, coactivator and corepressor binding is mutually exclusive. Some coactivators belong to ATP-dependent chromatin-remodeling complexes, others are part of complexes that induce post-translational modifications of histones, such as acetylation or arginine methylation, and others interact with the basic transcriptional machinery causing the recruitment of RNA polymerase II to the target promoter. Binding of the coactivators causes chromatin decompaction and transcriptional activation.

In addition to causing ligand-dependent transcriptional activation, TRs can also repress gene transcription in a hormone-dependent manner. In some cases, this repression is associated with binding to negative TREs (nTREs). Although the properties of nTREs are not yet well known, these elements are often located very close to the transcriptional start site,[3] and corepressors and deacetylase activity appear to be involved in hormone-dependent negative regulation.[14] TRs can also regulate the expression of genes that do not contain a TRE by positive or negative interference with the activity of other transcription factors or signaling pathways, a mechanism referred to as transcriptional crosstalk.[3] Thus, we have shown that TRs can antagonize AP-1,[15,16] cyclic AMP (cAMP) response element-binding protein (CREB),[17,18] or NF-kB-mediated transcription.[19,20] In this case, the receptors do not bind directly to the DNA recognition elements for these transcription factors in the target gene, but can be tethered to these binding motifs via protein-to-protein interactions. This type of transcriptional crosstalk between transcription factors and nuclear receptors has been shown to be critical for regulation of many cellular functions, including anti-inflammatory and anti-proliferative actions of nuclear receptor ligands.[21–23] Finally, thyroid hormones can elicit rapid non-genomic effects initiated at the cell membrane that can lead to stimulation of kinase pathways. These actions could be mediated by a fraction of membrane-associated nuclear receptors, or by occupancy of putative membrane receptors, such as integrin $\alpha V\beta 3$, which would bind T4 preferentially.[9] Figure 1.1B illustrates the main aspects of thyroid hormone actions on cells.

TRS AND CANCER

The first evidence linking TRs with cancer was the finding that TRα is the cellular counterpart of the v-erbA oncogene of the avian erythroblastosis virus (AEV), a retrovirus that causes erythroleukemia and sarcoma in chickens. v-ErbA acts as a constitutive dominant-negative of TRs since it contains mutations that abolish ligand binding, recruitment of coactivators and hormone-dependent transcriptional stimulation, while maintaining the ability to bind corepressors.[24] There is also evidence that reduced TR expression and/or alterations in TR genes are common events in human cancer.[25] In particular, decreased TR levels as well as somatic mutations in TR genes are frequently present in breast cancers and aberrant TRs have been found in more than 70% of human hepatocarcinomas. Most of these mutants have been shown to act as dominant-negative inhibitors of TR activity,[26] suggesting that the native receptors could act as tumor suppressors and that loss of expression and/or function of this receptor could result in a selective advantage for cell transformation and tumor development. In agreement with this idea it has been shown that TRs could function as tumor suppressors in a mouse model of metastatic follicular thyroid carcinoma.[27]

INHIBITION OF TUMOR CELL PROLIFERATION BY THE THYROID HORMONE RECEPTORS

T3 blocks proliferation of N2a neuroblastoma cells which express TRβ1 (N2a-β cells). Our results have shown that T3 coordinately regulates the expression of several genes that play a key role in cell cycle control. Thus, the hormone induces a rapid down-regulation of the c-*myc* gene, a decrease of *cyclin* D1 transcription, and an induction of the cell cycle inhibitors p27Kip1 and p21Cip.[28–30] Furthermore, gene expression analysis indicates a decreased expression of other cyclins (F, T1, D1 and B2), cyclin-dependent kinase 4

(CDK4) and other cell cycle components such as Wee1 and Cdc20 after T3 treatment.[31]

The c-*myc* oncogene plays an important role in cell cycle progression and different signals that arrest cell growth suppress expression of c-Myc. Transcription of the c-*myc* gene is controlled by several promoters, and a block in transcriptional elongation appears to be essential in the regulation of c-*myc* gene expression. Sequences known to function as a polymerase II pausing region are located immediately downstream of the P2 promoter, and a binding site for the transcriptional repressor CTCF maps precisely within this region of polymerase II pausing and release. Interestingly, we have demonstrated the existence of a nTRE in this region. This element binds TR-RXR heterodimers and is adjacent to the TCTF binding site. Furthermore, a c-*myc* promoter fragment containing binding sites for both transcription factors confers repression by T3 when located downstream of an heterologous promoter, indicating that the receptor in cooperation with CTCF causes premature termination of transcription, decreasing c-*myc* mRNA levels.[32]

One of the molecular events required for cell cycle progression is the inactivation by hyperphosphorylation of retinoblastoma protein family. Accordingly, we found that T3-mediated growth arrest of neuroblastoma cells is associated with hypophosphorylation of the retinoblastoma proteins pRb and p103. This modification is catalyzed by cyclin-dependent kinases (CDKs), whose activity is regulated by different mechanisms including their association with cyclins and with cyclin kinase inhibitors (CKIs). As indicated above, p27Kip1 and p21Cip levels increase upon incubation of N2a-β cells with T3. The strong and sustained increase of p27Kip1 by the hormone is secondary both to augmented levels of p27Kip1 mRNA and to a longer half-life of the CKI, indicating that transcriptional and post-transcriptional mechanisms are involved in T3-induced CKI induction. The increased levels of p27Kip1 lead to a significant increase in the amount of CKI bound to CDK2 and to a marked inhibition of the kinase activity of the cyclin/CDK2 complexes. As a consequence of these changes, retinoblastoma proteins are hypophosphorylated in T3-treated N2a-β cells and progression through the restriction point in the cell cycle is blocked[30] (Figure 1.2).

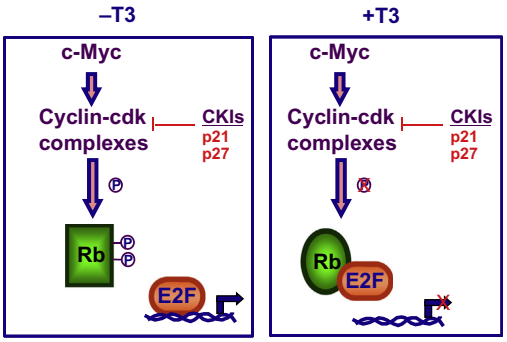

FIGURE 1.2 **Inhibition of proliferation of neuroblastoma N2a-β cells by T3.** In the absence of T3, retinoblastoma proteins (Rb) are phosphorylated by cyclin–cdk complexes and E2F transcription factors are free to bind to genes important for progression through the cell cycle. T3 inhibits expression of the c-*myc* protooncogen and reduces expression of genes encoding several cyclins, among them the *cyclin* D1 gene, while reducing expression of cyclin-dependent kinases such as CDK4. In addition, the hormone increases the levels of the cyclin kinase inhibitors p21 and p27. These changes lead to a reduced activity of cyclin–cdk complexes and to hypophosphorylation of Rb. Under these conditions E2F factors remain bound to Rb and cell cycle progression is blocked.

THE THYROID HORMONE RECEPTOR ANTAGONIZES RAS-INDUCED PROLIFERATION

Ras oncoproteins are small guanosine triphosphate (GTP)-binding proteins that play a crucial role in normal and malignant cell proliferation. Oncogenic mutations in the *ras* gene result in a constitutively active protein that is present in at least 30% of human tumors and can efficiently transform most immortalized rodent cells.[33]

Ras activation induces, among others, the activation of the Ras/mitogen-activated protein kinase (MAPK) signaling pathway, which is a key mediator for mitogenic signaling and Ras-induced transformation.[34] In this pathway, activation of the MAPK extracellular signal-regulated kinase 1/2 (Erk1/2) leads to phosphorylation of transcription factors of the Ets family, or to activation of downstream kinases such as Rsk or Msk,[35,36] which then phosphorylate other transcription factors, among them b-Zip factors of the CREB/ATF family.

Cyclin D1 is one of the main targets for the proliferative, transforming and tumorigenic effects of the *ras* oncogene.[37] In N2a-β cells expression of oncogenic Ras increases Cyclin D1 levels and T3 reverses significantly this induction. In parallel, Ras increases proliferation of N2a-β cells and T3 inhibits this response. Furthermore, the inhibitory effect of T3 on proliferation is significantly reversed after overexpression of Cyclin D1, showing that the repression of Cyclin D1 expression by T3 plays an important role in the mechanism by which the hormone represses Ras-mediated proliferation.[28] In transient transfection experiments with reporter genes containing the *cyclin* D1 gene promoter we have observed that T3 blocks induction of *cyclin* D1 promoter activity by *ras* not only in neuroblastoma cells but also in human hepatocarcinoma cells, in murine fibroblasts, and in rat tumor pituitary cells, indicating that T3-dependent repression on Ras-mediated transcription is a rather general effect. The v-*src* oncogene also stimulates transcription of *cyclin* D1 in a Ras-dependent manner and T3 also antagonizes this response. T3 represses expression of the *cyclin* D1 gene in response to the *ras* oncogene through proximal promoter sequences that do not contain a TRE but contain a CRE (cyclic AMP response element). The CRE constitutively binds b-Zip factors such as CREB and ATF-2 and, accordingly, neither Ras nor the hormone alters the abundance of the factors that bind this motif. However, activation of these transcription factors by Ras is blocked by TR in a T3-dependent manner (Figure 1.3). In addition to antagonizing the MAPK pathway and the activation of downstream kinases such as Rsk2 or Msk, TRs can interact directly with ATF-2 and with CREB inhibiting its phosphorylation.[17]

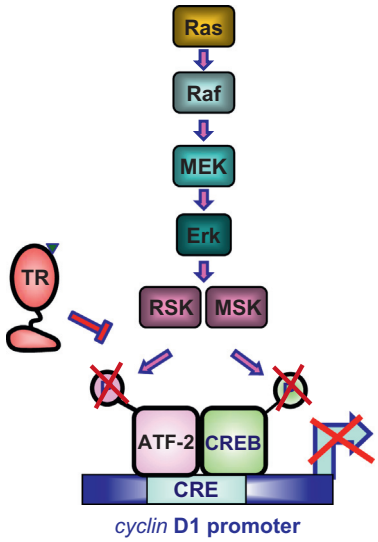

FIGURE 1.3 **Model of the regulation of *cyclin* D1 transcription by *ras* and the thyroid hormone receptors.** Oncogenic Ras stimulates the Ras-MAPK pathway leading to activation Rsk or Msk kinases, which phosphorylate transcription factors such as CREB or ATF-2. These factors bind to a CRE motif located in the proximal promoter of the *cyclin* D1 gene, and their activation stimulates transcription. The liganded thyroid hormone receptor antagonizes activation of the MAPK pathway and interacts with the b-Zip factors blocking their activation by Ras and transcription of the *cyclin* D1 gene.

THE THYROID HORMONE RECEPTORS ANTAGONIZE TRANSFORMATION AND TUMORIGENESIS BY ONCOGENIC RAS

Since TRs can inhibit Ras-dependent proliferation and *cyclin* D1 transcription, we also examined the possibility that they could repress Ras

mediated cellular transformation and tumor growth. To prove this hypothesis, we analyzed formation of transformation foci in NIH-3T3 fibroblasts transfected with oncogenic Ras in the presence or absence of TRs. The results obtained showed that the transforming capacity of the *ras* oncogene is strongly decreased in TR-expressing fibroblasts, although TRβ1 appears to have a stronger anti-transforming activity than the α1 isoform. Not surprisingly, TRs were also able to antagonize fibroblast transformation by v-*src*. Furthermore, the inhibition of transformation by TRβ1 was lost after *cyclin* D1 over-expression, indicating that downregulation of this cyclin is also involved in the anti-transforming effects of the receptor. To analyze whether TRs could act as suppressors of tumor formation by the *ras* oncogene in mice, NIH-3T3 cells expressing in a stable manner oncogenic Ras alone or in combination with TRα1 or TRβ1 were injected into the flanks of immunodeficient nude mice. Whereas large tumors developed in mice injected with fibroblasts expressing Ras alone, tumor formation was blocked in mice injected with fibroblasts co-expressing the oncoprotein and TRβ1. Co-expression of oncogenic Ras with TRα1 abolished tumor formation but caused a strong delay in the appearance of tumors.[28] Furthermore, the tumors formed in the presence of TRα1 presented a more differentiated phenotype, as demonstrated by an increased presence of collagen and a more fusiform morphology of the cells. Therefore, TRs could play a relevant role as suppressors of *ras*-dependent tumors, and although both isoforms suppress tumor growth, TRβ1 appears to exert a stronger anti-tumorigenic effect *in vivo*.

FUNCTIONAL DOMAINS INVOLVED IN TR ANTAGONISM OF Ras RESPONSES

To analyze the mechanisms and receptor domains involved in the antagonism of Ras-induced transcription, we examined the effect of various TRβ1 mutants on *cyclin* D1 promoter activity in HepG2 and NIH-3T3 fibroblasts[13] (Figure 1.4A). Mutants E457Q in H12 or K288I in H3 still presented a significant activity to antagonize the activation of the *cyclin* D1 promoter by oncogenic Ras and to reduce Cyclin D1 protein levels. This indicates that residues in H3 and H12 that are essential for coactivators recruitment and ligand-dependent transactivation on a TRE are not required for inhibition of Ras responses by the receptor. TRβ1 mutants in the hinge domain were used to analyze the role of corepressors in this antagonism. A receptor containing the triple mutation AHT-GGA in the CoR box that abolishes interaction with corepressors[38] and ligand-independent repression on a TRE, did not block *cyclin* D1 promoter stimulation by Ras, indicating that corepressors could play a role in the transcriptional

(A)

(B)

TRβ1	Wt	K288I	E457Q	AHT	P214R
Coactivators binding	+	-	-	+	+
Corepressors binding	+	+	+	-	-
Transactivation	+	-	-	+	+
Transrepression	+	+	+	-	-
Inhibition of transformation	+	+	+	-	-
Inhibition of tumorigenesis	+	n.a	+	-	n.a

FIGURE 1.4 **Thyroid hormone receptor domains involved in blocking the responses to the *ras* oncogene.** (A) Scheme of TRβ1 showing the position of the mutations used. (B) Effect of the different receptor mutants on T3-dependent in vitro recruitment of coactivators and corepressors in ''pull-down'' assays, transactivation of a reporter plasmid containing a TRE in transient transfection asssays, transrepression of the response of the *cyclin* D1 promoter to oncogenic Ras, inhibition of formation of transformation foci in NIH-3T3 fibroblasts transfected with the oncogene and inhibition of tumor growth in nude mice injected with Ras-transformed fibroblasts. n.a, not analyzed.

antagonism of TRβ1. In addition, mutation P214R in a residue preceding H1, homologous to that present in a v-erbA transformation deficient mutant (td359),[39] also blocks interaction with corepressors and is unable to antagonize Ras-mediated transcription in a T3-dependent manner (Figure 1.4). Both corepressor mutants were, however, capable of inducing T3-dependent transcription, demonstrating that Ras antagonism is mechanistically different from classical TR actions on transcriptional stimulation. The hinge mutants that were unable to mediate transrepression of the *cyclin* D1 promoter also lost the ability of antagonizing ATF-2 activation by the oncogene, reinforcing the notion that corepressors play a role in the antagonistic effects of TRβ1 on Ras-dependent transcription. This is supported by the finding that overexpression of NCoR or SMRT further represses T3-dependent *cyclin* D1 transcription by Ras.

Transformation assays in NIH-3T3 fibroblasts showed a strict parallelism between the antagonism of Ras-induced transcription and the inhibition of transformation by the TRβ1 mutants. Thus, the number of transformation foci was significantly reduced by T3 in fibroblasts coexpressing Ras and the wild-type receptor and this action was maintained in the E457 and K288 mutants, while the AHT and P214R mutants were unable to mediate T3-dependent antitransforming effects (Figure 1.4B), suggesting again the importance of the hinge domain and the corepressors in the antagonism of Ras-dependent responses. This was confirmed by the finding that the inhibition of formation of transformation foci by T3 was more marked after co-transfection of TRβ1 with SMRT or NCoR. The relevant role of endogenous corepressors on the antitransforming effects of TRβ1 was demonstrated with siRNA experiments that revealed that NCoR downregulation counteracted to a significant extent the inhibitory effect of T3 on cellular transformation, whereas SMRT depletion had a weaker effect. Furthermore, NCoR knockdown increased foci formation by Ras in the absence of the transfected receptor, indicating that this corepressor can act as an endogenous inhibitor of fibroblast transformation.[13] By contrast, the oncogenic TR mutants such as v-erbA or PV that act as dominant-negatives blocking the antiproliferative effects of the wild-type receptors do not suppress Ras function although they bind corepressors constitutively, indicating that ligand binding is also required for this receptor action. This is further suggested by the finding that another dominant-negative receptor, a mutant TRα in three conserved lysine residues in the carboxy-terminal extension of the DBD that, unexpectedly, displays a strongly reduced ligand binding affinity, is unable to antagonize transformation by the *ras* oncogene in a T3-dependent manner.[40]

To analyze *in vivo* the role of corepressors and coactivators on suppression of tumor formation by TRβ1, nude mice were injected with NIH-3T3 fibroblasts expressing the oncoprotein alone or in combination with wild-type TRβ1 or the AHT and E457Q mutants. Tumor formation was blocked in mice injected with cells co-expressing Ras and either the native receptor or the E457 mutant, while the AHT mutant did not inhibit tumor development. These results suggest that corepressors (but not classical coactivators) are essential not only for the anti-transforming effects of TRβ1, but also for inhibiting tumor formation in *vivo* (Figure 1.4B).

TRβ1 SUPPRESSES TUMOR INVASION AND METASTASIS

To analyze the role of TRs in tumor progression and metastatic growth, we re-expressed TRβ1 in hepatocarcinoma SK-hep1 (SK) cells and breast cancer MDA-MB-468 (MDA) cells, which have lost receptor expression, generating the SK-TRβ and MDA-TRβ cell lines. The competence of cancer cells to proliferate in the absence of a solid substrate is required for

the acquisition of an invasive and metastatic phenotype and we observed that TRβ1 inhibited colony formation by these cells in soft agar and blocked cell growth in suspension under rocking conditions. This could decrease survival of TRβ1-expressing cancer cells in the bloodstream. In addition, TRβ1 expression strongly reduced invasion in matrigel assays and this could inhibit cell intravasation and extravasation of the tumor cells. Proliferation, survival and cell invasion also depends on the response of the tumor cell to autocrine and paracrine growth factors. We found that epidermal growth factor (EGF) or insulin-like growth factor-1 (IGF-1) induced proliferation of parental hepatocarcinoma and breast cancer cells and that this response was lost in cells expressing TRβ1. The receptor blocks the mitogenic action of these growth factors by reducing expression of IGFIR, EGFR or ErbB3 receptors and by suppressing activation of ERK and PI3K signaling pathways that are critical for cell proliferation and invasion.[41] In accordance with our observations that TRs can inhibit Ras-mediated responses downstream of ERK, TRβ1 expression blocked the activation of transcription factors such as ELK1 or ATF-2 by EGF and IGF-1 in hepatocarcinoma and breast cancer cells. Furthermore, TRβ1 inhibited TGFβ-dependent proliferation. Many actions of this transforming growth factor are mediated by SMAD activation, but TGFβ can also increase MAPK and PI3K activity, and TRβ1 blocked stimulation of these pathways by TGFβ. Therefore, TR antagonizes stimulation of signaling pathways by growth and transforming growth factors that play a key role in invasion and tumor progression.

Genes that are relevant for metastatic progression have been identified. Among them, high levels of expression in primary tumors of the prostaglandin-synthesizing enzyme cyclooxygenase 2, the transcriptional inhibitor ID1, the chemokine receptors CXCR4, CCR6 and CCR1, the protooncogen c-Met, or some metalloproteases is associated with high risk of metastasis and poor prognosis in patients. Strikingly, we found that TRβ1 coordinately downregulated the expression of these prometastatic genes, suggesting a common molecular mechanism for this receptor action. The finding that inhibitors of MAPK or PI3K inhibit their expression in parental cells suggests that antagonism of these pathways by TRβ1 could participate in their transcriptional repression,[41] although the gene elements and molecular mechanism of repression by the receptor remain to be determined.

When parental, as well as TRβ-expressing MDA and SK cells, were inoculated into nude mice, it was observed that TRβ1 reduced tumor proliferation (Figure 1.5) retarding tumor growth, and enhanced expression of epithelial markers while reducing expression of mesenchymal markers. In addition, TRβ1 increased the necrotic area of the tumors and significantly reduced angiogenesis. These changes are compatible with reduced invasion and tumor growth. Indeed, whereas tumors formed by the parental hepatocarcinoma and breast cancer cells are highly infiltrative, TRβ1-expressing cells caused the appearance of tumors to be more compact and surrounded by a pseudocapsule of collagen and inflammatory cells (Figure 1.6). Most of these tumors do not infiltrate the adjacent tissues and they do not originate distant nodular metastasis. To analyze the effect of the receptor in formation of experimental metastasis, parental and TRβ1-expressing cells were injected into the tail vein of nude mice. Examination of the lungs at necropsy showed that TRβ1 had a potent inhibitory effect on metastasis formation (Figure 1.7). The incidence of metastasis, their size, the number of metastases per lung, and the area of the lungs affected by metastatic growth were markedly reduced in mice injected with TRβ1-expressing cells. Extravasation was also strongly decreased in TRβ1-expressing cells, indicating that the receptor has anti-metastatic

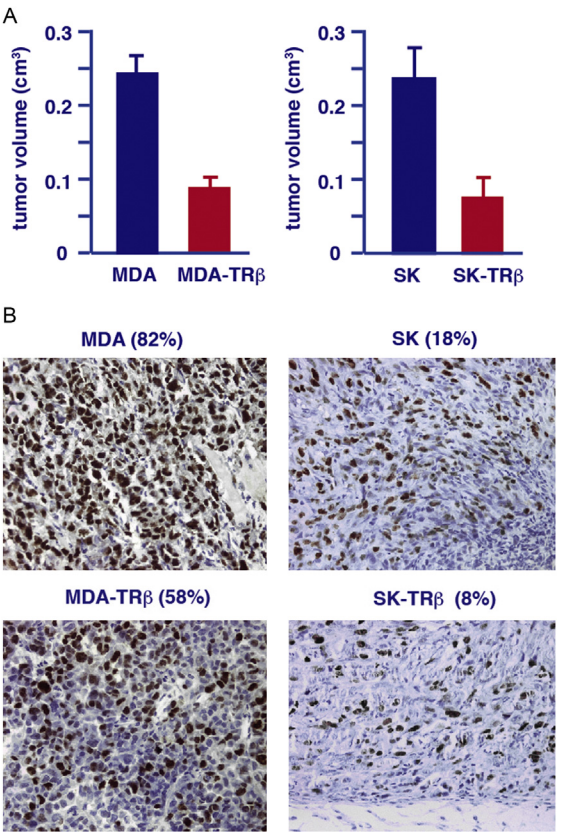

FIGURE 1.5 **TRβ1 inhibits tumor growth.** MDA-MB-468 breast cancer cells and SK-hep1 hepatocarcinoma cells were stably transfected with an empty vector or with TRβ1 and inoculated into the fat mammary pad or the flank of nude mice, respectively. (A) Mean tumor volume 25 days after inoculation. (B) The tumors were excised and tumor cell proliferation was assessed by immunohistochemistry with the proliferation marker Ki67.

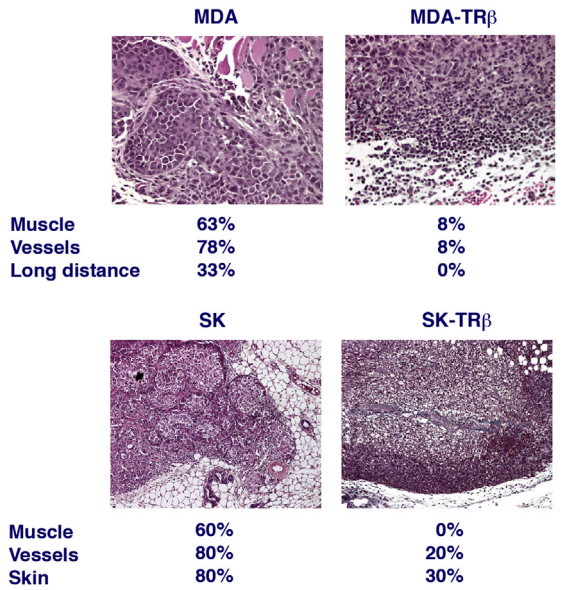

FIGURE 1.6 **TRβ1 inhibits tumor invasion.** Hematoxylin and eosin staining of the tumors formed by parental MDA-MB-468 breast cancer cells and SK-hep1 hepatocarcinoma cells shows that most tumors infiltrate surrounding tissues. In contrast, tumors formed by TRβ1-expressing cells are more compact and less infiltrative. The percentage of tumors invading the indicated tissues and forming spontaneous long distant metastasis is indicated.

activity by blocking not only the ability of cancer cells to proliferate and colonize the lung parenchyma but also by reducing cancer cell extravasation.[41] The inhibitory effect of TRβ1 on tumor invasiveness and formation of metastasis observed in the mice is compatible with the reduced invasion and the repression of expression of pro-metastatic genes observed in the cultured cells.

HYPOTHYROIDISM RETARDS TUMOR GROWTH BUT INCREASES METASTASIS DEVELOPMENT

In contrast with the well-accepted role of TRs as tumor suppressors, no clear association between thyroidal status and cancer has been found in humans. Thus, hypothyroidism is more prevalent in hepatocarcinoma patients and might be a possible risk factor for liver cancer,[42] but hypothyroid patients have been reported to present both a higher and a reduced incidence of breast carcinomas.[43,44] These confounding effects could be secondary to the important metabolic changes associated with hypothyroidism rather than to direct

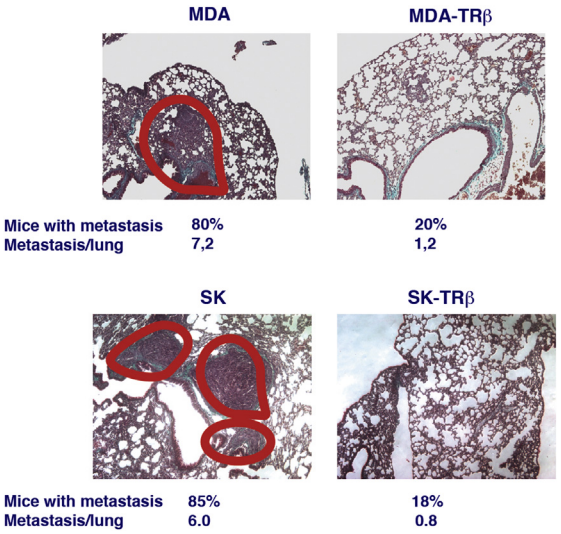

FIGURE 1.7 **TRβ1 inhibits formation of experimental metastasis.** Parental and TRβ1-expressing cells were injected into the tail vein of nude mice and formation of lung metastasis was analyzed 30 days later. The figure illustrates representative hematoxylin and eosin staining of lungs showing the appearance of large nodular metastasis (delineated by a continuous line) in mice injected with parental MDA-MB-468 or SK-hep1 cells, which are absent in the TRβ1-expressing cells. The percentage of injected animals bearing metastatic lesions in the lungs, as well as the mean number of metastasis/lung is indicated.

binding of the thyroid hormones to TRs in the tumor cells, as they often present inactivating mutations. Therefore, we compared tumor growth, invasion, and formation of metastasis in control and hypothyroid nude mice injected with both parental and TRβ1-expressing SK and MDA cells. We found that tumor growth was retarded in hypothyroid mice inoculated with both parental and TRβ1-expressing cells (Figure 1.8). The reduced tumor volume in hypothyroid hosts correlated with a lower proliferation index, reduction of Cyclin E expression and increased necrosis of the tumors. In addition, tumors developed in hypothyroid mice had a strong reduction of epithelial markers and a more mesenchymal phenotype, which could facilitate invasion and dissemination of the tumor cells to distant organs. Indeed, we found that hypothyroidism increased the number of invasion fronts of the tumors, the infiltration to neighboring tissues such as muscle, blood and lymph vessels or skin and the formation of distant metastasis in lung, liver or bone (Table 1.1). Furthermore, formation of lung metastasis after inoculation of the cancer cells into the tail vein of the hypothyroid nude mice was also markedly enhanced with regard to the metastatic growth observed in normal hosts, again both in parental and TRβ1-expressing cells (Figure 1.9). Therefore, hypothyroidism appears to favor a permissive tissue microenvironment for cancer metastasis. The increased malignancy of tumors formed by the parental hepatocarcinoma and breast cancer cells that do not express TRs in hypothyroid mice, suggest that changes in the stromal cells, most likely secondary to the important metabolic changes associated with hypothyroidism, rather than a direct effect of the hormone on the cancer cells, could be responsible for the increased tumor aggressiveness. In summary, normal thyroid hormone levels appear to favor growth of primary xenografts, but they also block tumor cell dissemination and metastasis formation.[45] These divergent effects could help to explain the contradictory reports on the influence of hypothyroidism in human tumors. Furthermore, because our results show that similar effects are observed independently of the presence or absence of TR in the cancer cells, it would be expected that thyroidal status could impact tumor progression, even in tumors in which TRs are deleted or mutated, a common event in human cancer.

DIVERGENT EFFECTS OF TRs ON NORMAL AND TRANSFORMED CELL PROLIFERATION

The actions of THs are highly pleiotropic, and the various TR isoforms might have

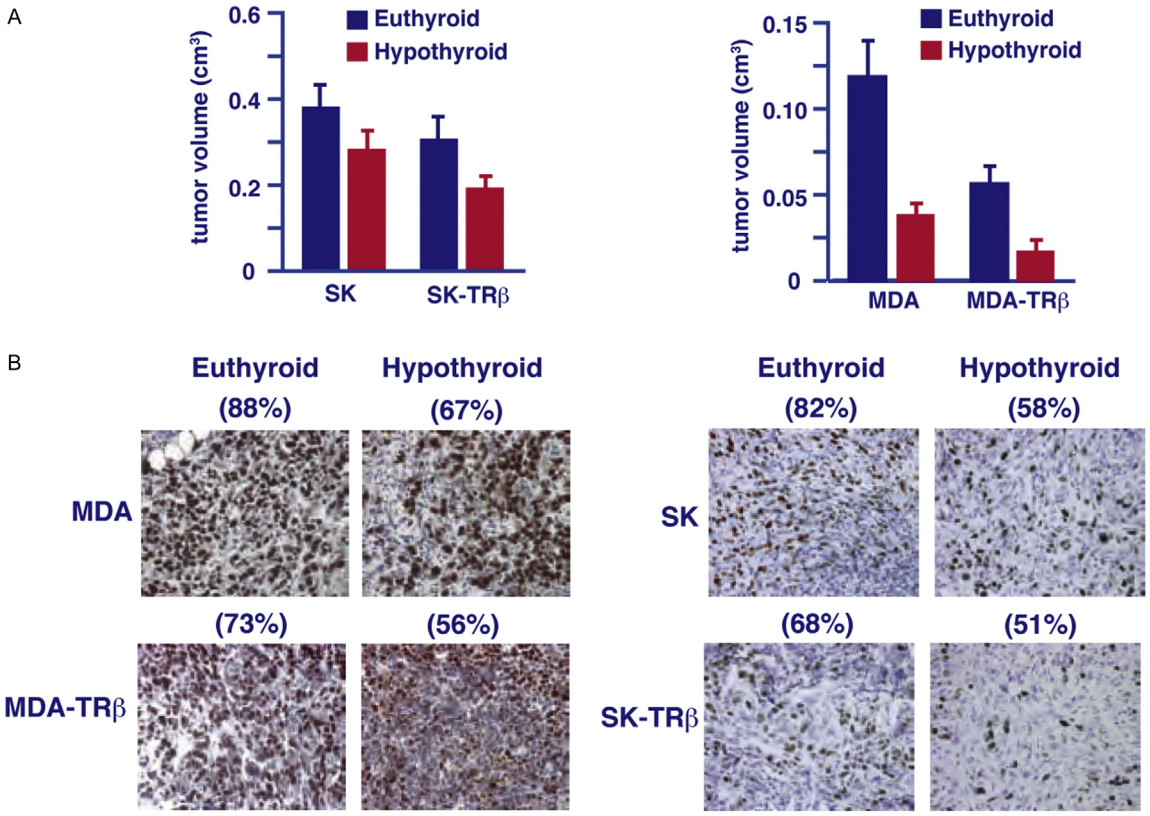

FIGURE 1.8 **Hypothyroidism reduces tumor growth.** MDA-MB-468 and SK-hep1 cells were stably transfected with an empty vector or with TRβ1 and inoculated into euthyroid and hypothyroid nude mice. (A) Mean tumor volume 7 weeks after inoculation. (B) The tumors were excised and tumor cell proliferation was assessed by immunohistochemistry with the proliferation marker Ki67.

opposing effects on cell proliferation depending on the cell type, the cellular context or the transformation status (see ref 5 for a recent review). For instance, in agreement with our results in hepatocarcinoma cells showing that expression of TRβ1 retards tumor growth and inhibits metastasis formation in nude mice, it has been found that THs induce a rapid regression of carcinogen-induced hepatic nodules in rodents, reducing the incidence of hepatocarcinoma and lung metastasis in a TRβ-dependent manner.[46–48] Paradoxically, TH administration can cause liver hyperplasia in animals.[49] Although the adult liver has a low replicative activity, hepatocytes can proliferate in response to partial surgical hepatectomy. It has been shown that hypothyroidism delays liver regeneration after partial hepatectomy,[50] and we have demonstrated that TRβ promotes hepatocyte proliferation in response to hepatectomy, since knockout (KO) mice lacking TRβ or TRα1 and TRβ show a significant retardation in the restoration of liver mass. In the absence of TRs there is a retarded initiation of proliferation accompanied by an important but transient apoptotic response that does not occur in normal mice. These changes are linked to increased nitrosative

TABLE 1.1 Hypothyroidism Enhances Tumor Invasion.

Cells Inoculated	Host Mice	Muscle	Vessels	Bone	Skin	Lung	Liver
MDA	Euthyroid	40%	58%	n.d	n.d	n.d	n.d
	Hypothyroid	60%	82%	30%	n.d	n.d	n.d
MDA-TRβ	Euthyroid	30%	30%	n.d	n.d	n.d	n.d
	Hypothyroid	50%	50%	n.d	n.d	n.d	n.d
SK	Euthyroid	60%	80%	n.d	80%	0%	0%
	Hypothyroid	100%	100%	n.d	100%	30%	30%
SK-TRβ	Euthyroid	0%	20%	n.d	20%	n.d	n.d
	Hypothyroid	0%	50%	n.d	40%	n.d	n.d

Abbreviation: n.d, not detected.
Parental and TRβ1-expressing MDA-MB-468 and SK-hep1 hepatocarcinoma cells were inoculated into euthyroid or hypothyroid nude mice. Tumors were excised 8 weeks later and the percentage of tumors invading the indicated tissues for each condition was scored

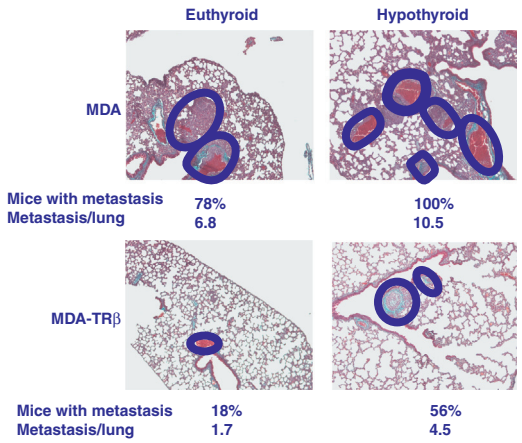

FIGURE 1.9 **Hypothyroidism enhances formation of metastasis.** Parental and TRβ1-expressing MDA-MB-468 cells were injected into the tail vein of nude mice and formation of lung metastasis was analyzed 30 days later. The figure illustrates representative hematoxylin and eosin staining of lungs in the different groups. The percentage of injected animals bearing metastatic lesions in the lungs, as well as the mean number of metastasis/lung is indicated.

stress, resulting from a drop in the levels of asymmetric dimethylarginine (ADMA), a potent physiological inhibitor of nitric oxide synthase (NOS) activity.[51] Therefore, TRs appear to have opposite effects on normal hepatocytes with low proliferative activity and on tumor hepatic cells that proliferate rapidly, although the mechanism responsible for these differences is still unknown.

The skin is also a target for TR-mediated cell proliferation. Thyroid dysfunction is associated with skin pathologies in patients,[52,53] and topical application of TH stimulates epidermal proliferation and dermal thickening and accelerates wound healing in rodents.[54,55] Using genetically modified mice we have observed that the effects of TH on skin proliferation are mediated through interactions with both TRα1 and TRβ.[56,57] We found reduced keratinocyte proliferation and decreased hyperplasia in response to topical application of 12-*O*-tetradecanolyphorbol-13-acetate (TPA) or retinoids in the epidermis of mice lacking TRs and also in hypothyroid mice (Figure 1.10). Reduced proliferation in TR KO mice correlates with increased expression of cyclin-dependent kinase inhibitors (CKIs) in the interfollicular epidermis, with strongly reduced Cyclin D1 expression in the keratinocytes of the basal layer and with increased production of pro-inflammatory cytokines,

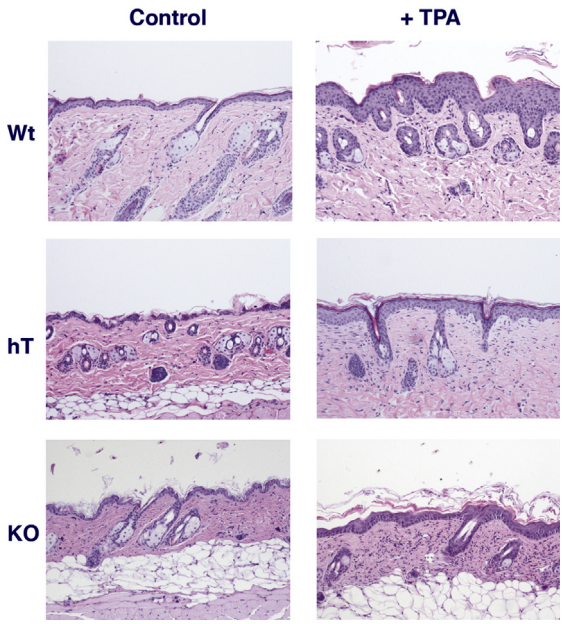

FIGURE 1.10 **The liganded thyroid hormone receptor is required for normal keratinocyte proliferation.** Control mice (Wt), mice made hypothyroid (hT) by treatment with anti-thyroidal drugs and genetically modified mice lacking the thyroid hormone binding isoforms TRα1 and TRβ (knockout, KO), were treated topically for 4 days with the tumor promoter TPA or with the solvent (control). Hematoxylin and eosin staining shows the reduced skin hyperplasia found in KO and hypothyroid animals.

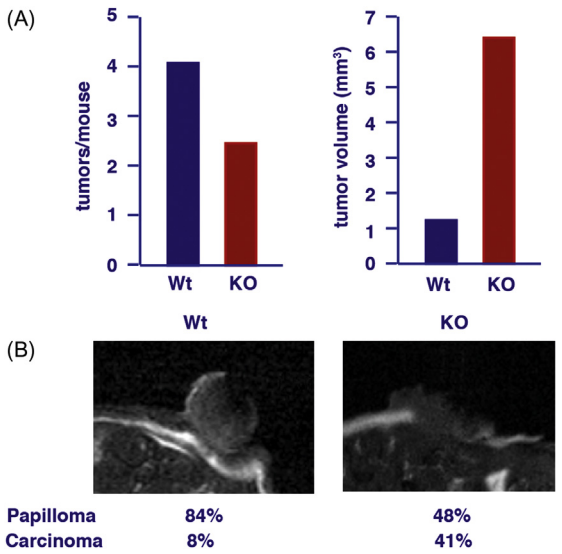

FIGURE 1.11 **Increased malignancy of skin tumors in mice lacking thyroid hormone receptors.** Wild-type mice and TRα1/TRβ knockout mice were subjected to a protocol of two-stage chemical skin carcinogenesis. (A) The mean number of tumors per mouse as well as the mean tumor volume was measured after 30 weeks of treatment with the tumor promoter TPA. (B) Representative nuclear magnetic resonance images showing a benign papilloma in a control animal and a tumor invading the subcutaneous fat layer, histologically classified as a carcinoma, in a TRα1/TRβ knockout mice. The percentage of benign and malignant tumors for each genotype is indicated.

which is associated with enhanced phosphorylation of p65/NF-κB and STAT3 transcription factors.[56,57]

In a protocol of two-stage chemical skin carcinogenesis benign papillomas and malignant tumors can be induced on the backs of mice after exposure to the initiating carcinogen 7,12-dimethylbenzaanthracene (DMBA), and subsequent chronic treatment with TPA. The finding that TR KO mice show a strongly reduced epidermal proliferation in response to the tumor promoter suggests that they may be less sensitive to skin carcinogenesis. Indeed, we have observed that TRα1/TRβ develop fewer tumors than normal mice when subjected to the carcinogenesis protocol. However, after 20 weeks, tumor growth was significantly faster in KO animals and the tumors had a more malignant phenotype (Figure 1.11). Whereas most tumors found in wild-type mice were typical well-differentiated papillomas, in KO mice almost half of the tumors were classified as *in situ* carcinoma or squamous cell carcinomas (SCCs). Therefore, TR deficiency seems to inhibit benign tumor formation at early stages of skin carcinogenesis and increases malignization at later stages, indicating again that these receptors can have divergent effects on cell proliferation and malignant transformation. Supporting the notion that TRβ could act as a suppressor of tumor progression, we also found that TRβ expression can be detected in

normal skin of wild-type mice, but receptor expression was strongly reduced in the papillomas and was totally lost in SCCs.[41]

Acknowledgements

The laboratory of Ana Aranda is supported by Grants BFU2011-28058 from Ministerio de Economía y Competitividad, RD12/0036/0030 from the Fondo de Investigaciones Sanitarias, and S2011/BMD-2328 (TIRONET) from the Comunidad de Madrid.

References

1. Flamant F, Baxter JD, Forrest D, Refetoff S, Samuels H, Scanlan TS, et al. International union of pharmacology. Lix. The pharmacology and classification of the nuclear receptor superfamily: Thyroid hormone receptors. *Pharmacol Rev* 2006;**58**:705–11.
2. Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol Endocrinol* 2011;**25**:1–14.
3. Aranda A, Pascual A. Nuclear hormone receptors and gene expression. *Physiol Rev* 2001;**81**:1269–304.
4. Lazar MA. Nuclear receptor corepressors. *Nucl Recept Signal* 2003;**1**:e001.
5. Pascual A, Aranda A. Thyroid hormone receptors, cell growth and differentiation. *Biochim Biophys Acta* 2013;**1830**:3908–16.
6. Flamant F, Gauthier K. Thyroid hormone receptors: the challenge of elucidating isotype-specific functions and cell-specific response. *Biochim Biophys Acta* 2013;**1830**:3900–7.
7. Yen PM, Ando S, Feng X, Liu Y, Maruvada P, Xia X. Thyroid hormone action at the cellular, genomic and target gene levels. *Mol Cell Endocrinol* 2006;**246**:121–7.
8. Brent GA. Mechanisms of thyroid hormone action. *J Clin Invest* 2012;**122**:3035–43.
9. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. *Endocr Rev* 2010;**31**:139–70.
10. Privalsky ML. The role of corepressors in transcriptional regulation by nuclear hormone receptors. *Annu Rev Physiol* 2004;**66**:315–60.
11. Wagner RL, Apriletti JW, McGrath ME, West BL, Baxter JD, Fletterick RJ. A structural role for hormone in the thyroid hormone receptor. *Nature* 1995;**378**:690–7.
12. Feng W, Ribeiro RC, Wagner RL, Nguyen H, Apriletti JW, Fletterick RJ, et al. Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors. *Science* 1998;**280**:1747–9.
13. Garcia-Silva S, Martinez-Iglesias O, Ruiz-Llorente L, Aranda A. Thyroid hormone receptor beta1 domains responsible for the antagonism with the ras oncogene: role of corepressors. *Oncogene* 2011;**30**:854–64.
14. Wang D, Xia X, Liu Y, Oetting A, Walker RL, Zhu Y, et al. Negative regulation of tsh{alpha} target gene by thyroid hormone involves histone acetylation and corepressor complex dissociation. *Mol Endocrinol* 2009;**23**(5):600–9.
15. Perez P, Palomino T, Schonthal A, Aranda A. Determination of the promoter elements that mediate repression of c-fos gene transcription by thyroid hormone and retinoic acid receptors. *Biochem Biophys Res Commun* 1994;**205**:135–40.
16. Perez P, Schonthal A, Aranda A. Repression of c-fos gene expression by thyroid hormone and retinoic acid receptors. *J Biol Chem* 1993;**268**:23538–43.
17. Mendez-Pertuz M, Sanchez-Pacheco A, Aranda A. The thyroid hormone receptor antagonizes creb-mediated transcription. *Embo J* 2003;**22**:3102–12.
18. Sanchez-Pacheco A, Palomino T, Aranda A. Negative regulation of expression of the pituitary-specific transcription factor ghf-1/pit-1 by thyroid hormones through interference with promoter enhancer elements. *Mol Cell Biol* 1995;**15**:6322–30.
19. Chiloeches A, Sanchez-Pacheco A, Gil-Araujo B, Aranda A, Lasa M. Thyroid hormone-mediated activation of the erk/dual specificity phosphatase 1 pathway augments the apoptosis of gh4c1 cells by down-regulating nuclear factor-kappab activity. *Mol Endocrinol* 2008;**22**:2466–80.
20. Lasa M, Gil-Araujo B, Palafox M, Aranda A. Thyroid hormone antagonizes tumor necrosis factor-alpha signaling in pituitary cells through the induction of dual specificity phosphatase 1. *Mol Endocrinol* 2010;**24**:412–22.
21. De Bosscher K, Vanden Berghe W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-kappab or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev* 2003;**24**:488–522.
22. Rosenfeld MG, Lunyak VV, Glass CK. Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes Dev* 2006;**20**:1405–28.
23. Pascual G, Glass CK. Nuclear receptors versus inflammation: mechanisms of transrepression. *Trends Endocrinol Metab* 2006;**17**:321–7.
24. Wolffe AP, Collingwood TN, Li Q, Yee J, Urnov F, Shi YB. Thyroid hormone receptor, v-erba, and chromatin. *Vitam Horm* 2000;**58**:449–92.
25. Aranda A, Martinez-Iglesias O, Ruiz-Llorente L, Garcia-Carpizo V, Zambrano A. Thyroid receptor: roles in cancer. *Trends Endocrinol Metab* 2009;**20**:318–24.

26. Chan IH, Privalsky ML. Thyroid hormone receptors mutated in liver cancer function as distorted antimorphs. *Oncogene* 2006;**25**:3576–88.
27. Zhu XG, Zhao L, Willingham MC, Cheng SY. Thyroid hormone receptors are tumor suppressors in a mouse model of metastatic follicular thyroid carcinoma. *Oncogene* 2010;**29**:1909–19.
28. Garcia-Silva S, Aranda A. The thyroid hormone receptor is a suppressor of ras-mediated transcription, proliferation, and transformation. *Mol Cell Biol* 2004;**24**:7514–23.
29. Garcia-Silva S, Perez-Juste G, Aranda A. Cell cycle control by the thyroid hormone in neuroblastoma cells. *Toxicology* 2002;**181–182**:179–82.
30. Perez-Juste G, Aranda A. The cyclin-dependent kinase inhibitor p27(kip1) is involved in thyroid hormone-mediated neuronal differentiation. *J Biol Chem* 1999;**274**:5026–31.
31. Bedo G, Pascual A, Aranda A. Early thyroid hormone-induced gene expression changes in n2a-beta neuroblastoma cells. *J Mol Neurosci* 2011;**45**:76–86.
32. Perez-Juste G, Garcia-Silva S, Aranda A. An element in the region responsible for premature termination of transcription mediates repression of c-myc gene expression by thyroid hormone in neuroblastoma cells. *J Biol Chem* 2000;**275**:1307–14.
33. Bos JL. Ras oncogenes in human cancer: a review. *Cancer Res* 1989;**49**:4682–9.
34. Calvo F, Agudo-Ibanez L, Crespo P. The ras-erk pathway: understanding site-specific signaling provides hope of new anti-tumor therapies. *Bioessays* 2010;**32**:412–21.
35. Anjum R, Blenis J. The rsk family of kinases: emerging roles in cellular signalling. *Nat Rev Mol Cell Biol* 2008;**9**:747–58.
36. Arthur JS. Msk activation and physiological roles. *Front Biosci* 2008;**13**:5866–79.
37. Gille H, Downward J. Multiple ras effector pathways contribute to g(1) cell cycle progression. *J Biol Chem* 1999;**274**:22033–40.
38. Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, et al. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* 1995;**377**:397–404.
39. Damm K, Beug H, Graf T, Vennstrom B. A single point mutation in erba restores the erythroid transforming potential of a mutant avian erythroblastosis virus (aev) defective in both erba and erbb oncogenes. *EMBO J* 1987;**6**:375–82.
40. Sanchez-Pacheco A, Martinez-Iglesias O, Mendez-Pertuz M, Aranda A. Residues k128, 132, and 134 in the thyroid hormone receptor-alpha are essential for receptor acetylation and activity. *Endocrinology* 2009;**150**:5143–52.
41. Martinez-Iglesias O, Garcia-Silva S, Tenbaum SP, Regadera J, Larcher F, Paramio JM, et al. Thyroid hormone receptor beta1 acts as a potent suppressor of tumor invasiveness and metastasis. *Cancer Res* 2009;**69**:501–9.
42. Reddy A, Dash C, Leerapun A, Mettler TA, Stadheim LM, Lazaridis KN, et al. Hypothyroidism: a possible risk factor for liver cancer in patients with no known underlying cause of liver disease. *Clin Gastroenterol Hepatol* 2007;**5**:118–23.
43. Beatson G. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment, with illustrative cases. *Lancet* 1896;**2**:104–7.
44. Cristofanilli M, Yamamura Y, Kau SW, Bevers T, Strom S, Patangan M, et al. Thyroid hormone and breast carcinoma. Primary hypothyroidism is associated with a reduced incidence of primary breast carcinoma. *Cancer* 2005;**103**:1122–8.
45. Martinez-Iglesias O, Garcia-Silva S, Regadera J, Aranda A. Hypothyroidism enhances tumor invasiveness and metastasis development. *PLoS One* 2009;**4**:e6428.
46. Ledda-Columbano GM, Perra A, Loi R, Shinozuka H, Columbano A. Cell proliferation induced by triiodothyronine in rat liver is associated with nodule regression and reduction of hepatocellular carcinomas. *Cancer Res* 2000;**60**:603–9.
47. Perra A, Kowalik MA, Pibiri M, Ledda-Columbano GM, Columbano A. Thyroid hormone receptor ligands induce regression of rat preneoplastic liver lesions causing their reversion to a differentiated phenotype. *Hepatology* 2009;**49**:1287–96.
48. Perra A, Simbula G, Simbula M, Pibiri M, Kowalik MA, Sulas P, et al. Thyroid hormone (t3) and trbeta agonist gc-1 inhibit/reverse nonalcoholic fatty liver in rats. *FASEB J* 2008;**22**:2981–9.
49. Francavilla A, Carr BI, Azzarone A, Polimeno L, Wang Z, Van Thiel DH, et al. Hepatocyte proliferation and gene expression induced by triiodothyronine in vivo and in vitro. *Hepatology* 1994;**20**:1237–41.
50. Malik R, Mellor N, Selden C, Hodgson H. Triiodothyronine enhances the regenerative capacity of the liver following partial hepatectomy. *Hepatology* 2003;**37**:79–86.
51. Lopez-Fontal R, Zeini M, Traves PG, Gomez-Ferreria M, Aranda A, Saez GT, et al. Mice lacking thyroid hormone receptor beta show enhanced apoptosis and delayed liver commitment for proliferation after partial hepatectomy. *PLoS One* 2010;**5**:e8710.
52. Paus R. Exploring the "thyroid-skin connection": concepts, questions, and clinical relevance. *J Invest Dermatol* 2010;**130**:7–10.

53. Slominski A, Wortsman J. Neuroendocrinology of the skin. *Endocr Rev* 2000;**21**:457–87.
54. Safer JD, Crawford TM, Holick MF. Topical thyroid hormone accelerates wound healing in mice. *Endocrinology* 2005;**146**:4425–30.
55. Safer JD, Fraser LM, Ray S, Holick MF. Topical triiodothyronine stimulates epidermal proliferation, dermal thickening, and hair growth in mice and rats. *Thyroid* 2001;**11**:717–24.
56. Contreras-Jurado C, Garcia-Serrano L, Gomez-Ferreria M, Costa C, Paramio JM, Aranda A. The thyroid hormone receptors as modulators of skin proliferation and inflammation. *J Biol Chem* 2011;**286**:24079–88.
57. Garcia-Serrano L, Gomez-Ferreria MA, Contreras-Jurado C, Segrelles C, Paramio JM, Aranda A. The thyroid hormone receptors modulate the skin response to retinoids. *PLoS One* 2011;**6**:e23825.

CHAPTER

2

The Molecular Cell Biology of Anterior Pituitary Cells

Arturo E. Gonzalez-Iglesias and Richard Bertram

Florida State University, Tallahassee, FL, USA

INTRODUCTION

> "Do not stop to question whether these ideas are new or old, but ask, more properly, whether they harmonize with nature." ***Marcello Malpighi***

The dawn of neuroendocrinology is coincident with the beginning of the tale of a remarkable gland: the pituitary gland. Around AD 170 Galen postulated that the "pituita" (from the Greek *ptuo*, "to spit," and the Latin *pituita*, "mucus") secreted waste products (phlegm, one of the four humors of the body) from the brain into the nasal cavities. Nineteen centuries later, we have found not only that Galen's concept was far from the truth but also that a fascinating – yet still primitive – understanding of the gland has emerged.

The pituitary is one of the two elements that make up the hypothalamo–hypophysial unit, the joint anatomical structure comprising hormone-producing neurons and cells by which the brain regulates the vital functions of the body. Indeed, a key regulator of body homeostasis during development, stress, and other physiological processes, the pituitary gland acts as a double interpreter, mediating the talk between the brain and the peripheral organs, and integrating their respective cues as well as those of its own (local autocrine and paracrine factors). Being functionally and anatomically connected to the hypothalamus by the median eminence (ME) via the infundibular stalk,[1] the pituitary has two embryologically and functionally distinct divisions: the neurohypophysis (or neural lobe) and the adenohypophysis (anterior pituitary and intermediate lobes). The remarkable molecular and cellular aspects of the biology of the cells that constitute the anterior pituitary is the focus of this chapter.

ANTERIOR PITUITARY: ORGANIZATION, CELL TYPES, HORMONES AND FUNCTIONS

The anterior pituitary is an endocrine gland responsible for secreting hormones that regulate a wide range of functions. These hormones

Cellular Endocrinology in Health and Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-408134-5.00002-0

are synthesized and released by distinct groups of polygonal endocrine cells that are organized as interlacing cords and lined up on an anastomizing web of capillary vessels (the secondary capillary plexus) derived from the hypophysial artery. The cytoplasm of these cells contains granules of stored hormone that are released by exocytosis. The endothelial cells of the capillaries are fenestrated to facilitate the exchange of molecules between the endocrine cells and the blood, which not only bring in the hypothalamic and peripheral factors (through the long portal vessels and hypophysial arteries, respectively) that regulate the activity of the gland but also carry the released pituitary hormones away into the general circulation.[1] In addition, an extensive web of interconnected folliculostellate (FS) cells surround the endocrine cells. These cells regulate both the interaction of neighboring endocrine cells and the exchange of molecules between them and the capillaries. FS cells represent about 5–10% of the anterior pituitary cell population.

The traditional view of the pituitary holds that there are five endocrine cell types that are responsible for synthesizing six anterior pituitary hormones (Table 2.1). For each cell type, several immortalized cell lines have been developed, characterized and used extensively.[2]

Somatotrophs, which synthesize and release growth hormone (GH), are the major endocrine cell type in the anterior pituitary and constitute 40–50% of its cell population. They are localized predominantly to the lateral portions of the anterior lobe. Somatotroph function is primarily regulated by hypothalamic factors: growth hormone-releasing hormone (GHRH) produced by neurons in the arcuate nucleus is stimulatory, whereas somatostatin (STT) produced by neurons in the periventricular nucleus is inhibitory. STT suppresses both basal and GHRH-induced GH release, having no effect on GH synthesis. GH production and secretion also receives inhibitory feedback from the major target of GH, insulin-like growth factor-I from the liver. Somatotrophs express receptors for many other regulators of GH synthesis and release, including ghrelin, pituitary adenylate cyclase-activating peptide, thyroid hormone, glucocorticoids, insulin and endothelins. GH is secreted from the somatotrophs at a pulse frequency of about 1–2 h with a half-life that ranges between 6–20 min, and the pattern exhibits gender differences. In the case of males, the pulses are much larger early at night, whereas in females the pattern is more irregular and the pulses tend to be more uniform throughout the day. The pattern of GH release appears to be driven by the rate at which GHRH is released from the arcuate nucleus neurons.

GH is also called somatotropin (*soma*, "body") because of its profound and widespread anabolic effects throughout the body. In its absence, growth is stunted. Although virtually every tissue responds to some degree, skeletal muscle cells, liver, and chondrocytes (cartilage cells) are particularly sensitive to GH levels. Though the metabolic effects are direct actions of GH, it is now apparent that most, if not all, of the anabolic effects of GH are mediated by the production of a family of peptide hormone intermediaries known as insulin-like growth factors (IGFs) which are secreted by the liver, cartilage, muscle, and other tissues where they can act locally in a paracrine or autocrine fashion. GH, acting through the IGFs, stimulates protein synthesis, cell growth and a positive nitrogen balance, leading to an increase in lean body mass and a decrease in body fat. Many hours must elapse after administration of GH before its anabolic, growth-promoting effects become evident.

Thyrotrophs comprise approximately 5% of the anterior endocrine cell population and are typically spread over the anteriomedial and lateral portions of the gland. Thyrotrophs synthesize and secrete thyroid-stimulating hormone (TSH), also known as thyrotropin, which is controlled by central and peripheral