Role of co-regulators in metabolic and transcriptional actions of thyroid hormone

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Abstract

Thyroid hormone (TH) controls a wide range of physiological processes through TH receptor (TR) isoforms. Classically, TRs are proposed to function as tri-iodothyronine (T_3)-dependent transcription factors: on positively regulated target genes, unliganded TRs mediate transcriptional repression through recruitment of co-repressor complexes, while T_3 binding leads to dismissal of co-repressors and recruitment of co-activators to activate transcription. Co-repressors and co-activators were proposed to play opposite roles in the regulation of negative T_3 target genes and hypothalamic–pituitary–thyroid axis, but exact mechanisms of the negative regulation by TH have remained elusive. Important insights into the roles of co-repressors and co-activators in different physiological processes have been obtained using animal models with disrupted co-regulator function. At the same time, recent studies interrogating genome-wide TR binding have generated compelling new data regarding effects of T_3 , local chromatin structure, and specific response element configuration on TR recruitment and function leading to the proposal of new models of transcriptional regulation by TRs. This review discusses data obtained in various mouse models with manipulated function of nuclear receptor co-repressor (NCoR or NCOR1) and silencing mediator of retinoic acid receptor and thyroid hormone receptor (SMRT or NCOR2), and family of steroid receptor co-activators (SRCs also known as NCOAs) in the context of TH action, as well as insights into the function of co-regulators that may emerge from the genome-wide TR recruitment analysis.

Key Words

- ▶ thyroid hormone receptors
- ▶ nuclear co-repressor
- ▶ steroid receptor co-activator
- ▶ animal models
- ▶ transcription

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Introduction

Thyroid hormone (TH) is essential for normal development, growth, reproduction, and regulation of metabolism and thermogenesis in mammals. Circulating TH levels are tightly controlled by a negative feedback within hypothalamic–pituitary–thyroid (HPT) axis, where thyrotrophin-releasing hormone (TRH) from the hypothalamus stimulates production and secretion of thyroid-stimulating hormone (TSH) by the pituitary, which in turn stimulates synthesis, processing, and

release of both forms of TH by the thyroid gland: predominant thyroxine (T_4) and the active form tri-iodothyronine (T_3). High TH levels signal to suppress TRH and TSH, while a drop in circulating TH will stimulate their synthesis and release (Chiamolera & Wondisford 2009, Costa-e-Sousa *et al.* 2012). Intracellular levels of active TH are also finely regulated by deiodinases and transmembrane transporters such as the monocarboxylate and organic anion transporter families. Type 1 and type 2

5'-deiodinases (DIO1 and DIO2) convert T_4 into active T_3 , and type 3 5'-deiodinase (DIO3) inactivates T_4 and T_3 to form reverse T_3 and T_2 , respectively (Arrojo *et al.* 2013, Charalambous & Hernandez 2013, Gereben *et al.* 2008). Actions of deiodinases and transporters are crucial for normal maintenance of both intracellular and circulating TH levels, as well as function of HPT axis (Bernal *et al.* 2015, Fonseca *et al.* 2013, Hernandez *et al.* 2007, Heuer & Visser 2013, Hoftijzer *et al.* 2011, Schweizer & Kohrle 2013). Physiological effects of TH are largely mediated by regulation of gene transcription by TH receptors (TRs): ligand-dependent transcription factors that belong to the nuclear receptor (NR) superfamily. Two different genes (*Thra* and *Thrb*) encode the THRA and THRB subtypes and produce three main T_3 -binding isoforms THRA1, THRB1, and THRB2, which are expressed in a tissue-specific and developmentally regulated manner (Cheng *et al.* 2010, Hodin *et al.* 1989, Koenig *et al.* 1988, Sap *et al.* 1986, Weinberger *et al.* 1986, Zhang & Lazar 2000). TRs regulate transcription of target genes by binding thyroid hormone response elements (TRE) in their regulatory regions as homodimers or heterodimers with retinoid X receptors (RXR). Soon after the cloning of first TR isoforms, it had become apparent that in addition to T_3 -dependent activation of transcription, TRs possess the capability to silence target genes in the absence of TH (Brent *et al.* 1989, Damm *et al.* 1989). Experiments designed to isolate proteins that interact with either ligand-bound or unliganded NRs confirmed the important role of these receptor-associated proteins in transcriptional regulation by NRs and led to identification and cloning of the first co-activators, such as steroid receptor co-activators 1–3 (SRC1–3, also known as NCOA1–3) and co-repressors: nuclear receptor co-repressor (NCoR or NCOR1) and silencing mediator of retinoic acid receptor and thyroid hormone receptor (SMRT or NCOR2) (Baniahmad *et al.* 1995, Cavailles *et al.* 1994, Chen *et al.* 1997, Chen & Evans 1995, Halachmi *et al.* 1994, Horlein *et al.* 1995, Onate *et al.* 1995, Torchia *et al.* 1997, Voegel *et al.* 1996). Since then, over 300 nuclear receptor co-regulators have been identified (<https://www.nursa.org/nursa/molecules/index.jsf>) representing very diverse group of proteins, which are broadly defined by their role in regulation of transcription and inability to directly bind DNA. Co-regulators may serve as a bridge between NRs and basic transcriptional machinery, but most importantly play a key role in recruitment and function of complexes that mediate ATP-dependent chromatin-remodeling or histone modifications, such as acetylation, methylation, ubiquitylation, and others, which change chromatin structure and accessibility and ultimately lead

to activation or repression of transcription (Kouzarides 2007, Millard *et al.* 2013, Strahl & Allis 2000, Suganuma & Workman 2008). Interactions between co-regulators and NRs are mediated by specific receptor-interacting domains (RIDs) that are characterized by a presence of LXXLL motif, termed NR box, in co-activators (Heery *et al.* 1997) and a LXXH/IIXXXI/L CoNR box in co-repressor proteins (Hu & Lazar 1999, Nagy *et al.* 1999, Perissi *et al.* 1999). The specificity of interactions between co-regulators and NRs is thought to be encoded by the differences in the co-regulator RIDs and surrounding amino acid sequence. Co-repressors and co-activators are recruited to the same surface on the ligand-binding domain (LBD) of NRs, in a mutually exclusive manner (Darimont *et al.* 1998, Feng *et al.* 1998, Nolte *et al.* 1998, Wang *et al.* 2006, Xu *et al.* 2002): generally co-repressors are recruited to unliganded or antagonist-bound NR, while ligands induce a conformational change in helix 12 of LBD that favors recruitment of co-activators (Millard *et al.* 2013, Nagy *et al.* 1999, Perissi *et al.* 1999, Watson *et al.* 2012).

Based on this, the bimodal switch model of TR action has been proposed, where TRs are postulated to bind the DNA independent of the hormone. On a positively regulated target gene, co-repressors are recruited to unliganded TRs and bring enzymatic complexes, importantly histone deacetylases (HDACs), which modify histones and chromatin structure to actively repress transcription. Hormone binding induces a conformational change in the receptor that leads to the dismissal of co-repressors and their replacement by co-activators that bring enzymatic complexes with opposing activities, such as histone acetylases (HATs), to activate transcription (Glass & Rosenfeld 2000, McKenna & O'Malley 2002). The importance of transcriptional repression by unliganded TRs is underscored by the fact that neonatal hypothyroidism presents with much more severe phenotype than mouse models deficient in all TRs and the fact that athyroid mice can be rescued by TR deletion (Flamant *et al.* 2002, Flamant & Samarut 2003). At the same time, the mechanism of transcriptional regulation of negative TR targets, such as *Tshb* gene, which are activated by unliganded TR and repressed upon the T_3 binding, is less clear (Darling *et al.* 1989). It has been suggested that the co-repressors and co-activators may play opposite functions on this type of target genes, but precise molecular mechanism underlying these effects is still lacking. Inappropriate recruitment of CoA and CoR is also thought to play a role in the phenotypic presentation of the syndrome of resistance to thyroid hormone (RTH) as discussed below.

In this review, we will focus on the data regarding the *in vivo* role of co-regulators in TR action obtained from animal models and also incorporate some insights from recent genome-wide TR chromatin occupancy studies. Since the discovery of the SRC family and NCoR/SMRT, many cofactors have been shown to bind and modulate TR activity (at least *in vitro*), including co-activators thyroid hormone receptor-associated protein 220 (TRAP220 or MED1) (Ito & Roeder 2001), peroxisome proliferator-activated receptor gamma (PPARG) co-activator-1a (PGC-1a or PPARGC1A) (Wu *et al.* 2002), and co-repressors receptor interacting protein 140 (RIP140 or NRIP1) (Moore *et al.* 2004), and ligand-dependent co-repressor (LCOR) (Song *et al.* 2012), reviewed by Moore and Guy (2005) and Yao (2014). However, we will limit the review to SRC/p160 family of co-activators and co-repressors NCoR and SMRT, as they are the best studied *in vivo* in the context of TR signaling.

Structural and functional domains of co-regulator proteins

The SRC/p160 family of co-activators consists of three closely related proteins: SRC1, -2, and -3 (NCOA1, 2 and 3, respectively), with a molecular weight around 160 kDa, and overall sequence similarity of 50–55%, which contain three main functional domains (Dasgupta *et al.* 2014, Johnson & O'Malley 2012, Xu & Li 2003) (Fig. 1A). The N-terminal basic helix-loop-helix-Per/ARNT/Sim (b-HLH-PAS) domain is the most conserved among the family members. It is not necessary for co-activation of NRs, but interacts with several other transcription factors, contains a nuclear localization signal, and renders SRCs sensitive to proteasome-dependent turnover (Li *et al.* 2007, Onate *et al.* 1995). The central part of the SRC molecule contains three copies of the LXXLL motif (NR box), which are responsible for direct interactions with NRs (Chen *et al.* 1997, Heery *et al.* 1997, Voegel *et al.* 1998). Mutational analysis and *in vitro* interaction studies have demonstrated that distinct amino acid sequences of LXXLL motifs and adjacent portion of the protein dictate specificity of NR–LXXLL motif interactions, so that different co-activators or NR boxes within the same co-activator show preferential binding to certain NRs (McInerney *et al.* 1998, Moore *et al.* 2004). It has also been suggested that SRCs can bind the N-terminal portion of THRB isoforms through noncanonical NR-binding domains in a T₃-independent manner, which may potentially play an important role in the upregulation of *TRH* and *TSH* genes in the absence of hormone (Iwasaki *et al.* 2006, Yang & Privalsky 2001). Finally, two activation domains

AD1 and AD2, located in the C-terminus, are responsible for recruitment of additional chromatin-modifying co-regulator complexes such as CREB-binding protein (CBP)/p300 possessing HAT, co-activator-associated arginine methyltransferase 1 (CARM1), and protein arginine N-methyl transferase 1 (PRMT1) (Chen *et al.* 1999, Dasgupta *et al.* 2014, Koh *et al.* 2001, Li *et al.* 2000, Onate *et al.* 1998, Voegel *et al.* 1998, Xu & Li 2003). AD1 domain also contains three LXXLL-like motifs; however, interaction studies have shown that they are not involved

Figure 1

Schematic representation of the structure of wt SRC family members (A), wt NCoR and SMRT (B), and various genetically modified co-repressor mutants discussed in this review (C). Locations of bHLH/PAS domain, activating domains (AD), receptor-interacting domains (RID), repression domains (RD), and deacetylase activation domain (DAD) are indicated by boxes within the co-regulator molecules. Interactions between co-regulators and NRs, and co-repressors and HDAC3 are shown. Regions of other cofactor recruitment are indicated by arrows. RID3 of SMRT is shown in lighter color to reflect the fact that the predominantly expressed SMRT isoform contains only RID2 and RID1.

in recruitment of SRCs to DNA-bound TR (Takeshita *et al.* 1998). In addition, SRCs have weak intrinsic HAT activity located in their C-terminal domain (Feng *et al.* 2009).

NCoR and SMRT are highly homologous (40% amino acid identity) large proteins of 270 kDa, containing conserved functional domains (Fig. 1B). For comprehensive review of co-repressor structure and complexes, see Millard *et al.* (2013), Perissi *et al.* (2010), and Watson *et al.* (2012). Three repression domains (RD1–3) are located within the N-terminal portion of NCoR and SMRT and mediate transcriptional repression through recruitment of a multiprotein repression complex (Alland *et al.* 1997, Heinzel *et al.* 1997, Nagy *et al.* 1997). Located in the N-terminus is also deacetylase activation domain (DAD), the region of the co-repressors responsible for recruitment and activation of histone deacetylase 3 (HDAC3), which deacetylates histone tails leading to chromatin remodeling and transcriptional repression (Guenther *et al.* 2001, Guenther *et al.* 2000, Ishizuka & Lazar 2003, 2005, You *et al.* 2013). HDAC3 appears to be the main enzyme responsible for the repressive activity of SMRT and NCoR, because it is the protein that associates in the most stable and reproducible way with both co-repressors. Although co-repressor complexes are heterogeneous and context-specific, some proteins are regularly found in stoichiometric association with NCoR/SMRT and appear to be essential for their repressive function. In addition to HDAC3, these partners include G protein pathway suppressor (GPS2) and transducin β -like 1 (TBL1 or TBL1X) and its homolog, TBL-related 1 (TBLR1 or TBL1XR1), which all together form the core repression complex (Oberoi *et al.* 2011). Both TBL1 and TBLR1 are responsible for ubiquitylation-dependent dismissal of the CoR complex, while GPS2 appears to be structurally required for maintenance of this complex (Oberoi *et al.* 2011, Yoon *et al.* 2003). The C-terminus of co-repressor proteins contains three RIDs that are characterized by a presence of CoRNR box, which is essential for NR-binding as outlined above (Hu & Lazar 1999, Nagy *et al.* 1999, Perissi *et al.* 1999). The exons encoding RIDs are subject to alternative splicing that produces different co-repressor isoforms, which are expressed in a tissue-specific manner (Faist *et al.* 2009, Goodson *et al.* 2011, Malartre *et al.* 2004). Interestingly, amino acid sequences of specific RIDs dictate the preferential binding to different NRs, with most N-terminal of the RIDs (N3 in NCoR and S3 in SMRT) having the highest avidity for TR, while S2 has demonstrated preference for the retinoic acid receptor (RAR) (Cohen *et al.* 2001,

Cohen *et al.* 2000, Makowski *et al.* 2003, Webb *et al.* 2000). Because of these differences in binding of specific RIDs to the TRs, it has been hypothesized that NCoR and SMRT play nonredundant roles in the TR actions *in vivo*.

Animal models of co-regulator function

Mice deficient for individual members of the p160 family have first proved the importance of SRC proteins for full physiological response to sex steroids and growth hormone *in vivo*. Decreased growth of organs highly responsive to steroids (testis, uterus, and mammary gland) was observed *Src1*^{-/-} and *Src2*^{-/-}, and accompanied by reduced reproductive function in *Src2*^{-/-} mice (Gehin *et al.* 2002, Xu *et al.* 1998). Defective growth hormone signaling and estrogen production resulting in short stature and reduced female reproductive function were reported in *Src3*^{-/-} mice (Wang *et al.* 2000, Xu *et al.* 2000). These findings established that p160 co-regulators possess certain functional specificity *in vivo* (recently reviewed in Dasgupta *et al.* 2014, Dasgupta & O'Malley 2014, Stashi *et al.* 2014b). Data obtained from these animal models pertinent to TH signaling and metabolic regulation is discussed below in the sections 'Roles of co-regulators in peripheral tissues' and 'Roles of co-regulators in the function of HPT axis and RTH'.

At the same time, the first attempts to elucidate *in vivo* roles of co-repressors revealed that NCoR and SMRT play nonredundant roles in mammalian development and have physiologic functions beyond NR signaling, as germline knockout of either co-repressor resulted in embryonic lethality (Jepsen *et al.* 2000, Jepsen *et al.* 2007). *NCoR*^{-/-} animals have defects in thymocyte and neuronal differentiation and definitive erythropoiesis, which result in embryonic death by E15.5 (Jepsen *et al.* 2000). Knockout of *Smrt* revealed its critical role in heart development through its interactions with a forkhead protein FOXP1. Furthermore, interactions of SMRT with RAR and Notch signaling pathway are responsible for normal forebrain development and maintenance of the neural stem cell state (Jepsen *et al.* 2008). A different whole-body SMRT-deficient mouse model was also found to be embryonic lethal, and analysis of *Smrt*^{+/-} animals revealed that SMRT plays an important role in adipogenesis (Sutanto *et al.* 2010). To better understand physiological function of NCoR and SMRT in adult animal and their role in specific NR signaling, several conditional knockout and mutant knock-in models were generated.

A conditional *NCoR* null allele that can produce complete deletion of *NCoR* in a tissue-specific manner was described and used to generate muscle-, adipocyte-, and liver-specific *NCoR* knockout mice (Jo *et al.* 2015, Li *et al.* 2011, Sun *et al.* 2013, Yamamoto *et al.* 2011). A conditional *Smrt* knockout allele has also been generated, and liver-specific and adult whole-body *Smrt* knockout models have been published (Shimizu *et al.* 2015, Sun *et al.* 2013).

Presence of distinct functional domains in the CoR proteins makes them ideal targets for genetic manipulations that affect one specific function (Fig. 1C). As outlined above, both *NCoR* and *SMRT* have three C-terminal RIDs, that preferentially bind different NRs (Cohen *et al.* 2001, Cohen *et al.* 2000, Faist *et al.* 2009, Goodson *et al.* 2011, Makowski *et al.* 2003, Malartre *et al.* 2004, Webb *et al.* 2000). This feature makes targeting of specific RID an attractive strategy to address the role of co-repressors in the action of specific NRs as well as physiological significance of different co-repressor isoforms. To this end, mice bearing a conditional allele encoding for a mutant *NCoR*, termed *NCoRΔID*, that is lacking the two N-terminal RID domains and is unable to interact with TR and liver X receptor (LXR) isoforms were generated (Astapova *et al.* 2008). Recently, Goodson *et al.* (2014) reported a mouse model (*NCoRω^{-/-}*) with a mutation introduced into a splicing site, which results in the inability to produce the full-length *NCoRω* isoform, and global expression of *NCoRδ*, containing only RID2 and RID1. In a similar approach, the *Smrt^{mRID}* knock-in mouse model was created, where mutations were introduced into RID1 and RID2 domains, so that the resulting mutant *Smrt^{mRID}* loses its ability to interact with RAR, TR, and PPAR isoforms (Nofsinger *et al.* 2008). In *Smrt^{mRID1}* mice, the mutation that abolishes binding to NRs was only introduced into one of the two RIDs, shown as RID2 in Fig. 1B. This leads to an enhanced recruitment of *SMRT* to NRs that preferably utilize the remaining C-terminal RID (Fig. 1C), particularly PPARs (Fang *et al.* 2010, Reilly *et al.* 2010).

As the CoRs are thought to exert much of their repressive actions by recruiting and activating HDAC3 (Guenther *et al.* 2001), mice bearing inactivating mutation in DAD of *NCoR* (*N-DADm*) and *SMRT* (*S-DADm*) were also generated and characterized (Alenghat *et al.* 2008, You *et al.* 2013). As was shown later, while this mutation eliminates HDAC3 activity, it does not completely abolish recruitment of HDAC3 by co-repressors, which preserves nonenzymatic repressive functions of HDAC3 (Sun *et al.* 2013).

Roles of co-regulators in peripheral tissues

While efforts have been made to address the specificity of co-regulator–NR interactions, in many *in vivo* models where co-regulator function has been manipulated, it may be difficult to attribute the physiologic effects to a specific transcription factor. TR isoforms and co-regulators are widely expressed and control multiple metabolic pathways (Brent 2012, Cheng *et al.* 2010, Dasgupta *et al.* 2014, Mottis *et al.* 2013, Obregon 2014). In many tissues, including liver, muscle, white and brown adipose tissues, and brain, there is a significant interplay between different NRs. For example in the liver, enzymes involved in fatty acid, carbohydrate, cholesterol, and bile acid metabolism are controlled by various NRs, including TRs, LXRs, PPARs, liver receptor homolog-1 (LRH-1 or NR5A2), Rev-ErbA (NR1D1), and others. The mechanisms of interplay may include: binding of different NRs to specific response elements located in the promoter/enhancer regions of the same gene; regulation of TH metabolism by other NRs; competition for dimerization partners and co-regulators (squenching); and competition for binding to the same NR response element, particular for LXRs, which share the same preferred response element: direct repeat with a 4 nucleotide spacer (DR-4) (Brent 2012, Cheng *et al.* 2010, Christoffolete *et al.* 2010, Hashimoto & Mori 2011, Maglich *et al.* 2004, Miao *et al.* 2015, Qatanani *et al.* 2005). Furthermore, recent genome-wide recruitment studies demonstrated that even NRs with different preferred DNA-binding elements share a great percentage of the binding sites under nonstimulated conditions in the liver (Boergesen *et al.* 2012). Therefore, we will briefly summarize the results of the *in vivo* co-regulator studies with a focus on TH signaling through the TR (Table 1).

Src1 knockout mice

Mouse knockout models identified SRC co-activators as essential regulators of energy homeostasis as they modulate expression of multiple enzymes in different metabolic pathways and tissues (Dasgupta *et al.* 2014, Dasgupta & O'Malley 2014, Stashi *et al.* 2014b).

Src1 knockout mice demonstrate increased weight gain when fed high-fat diet, presumably due to reduced energy expenditure (EE) and defective adaptive thermogenesis likely resulting from the loss of PPARGC1A co-activation by *Src1* (Picard *et al.* 2002). At the same time, lack of SRC1 also leads to hypoglycemia in fed and fasting states, at least partly due to impaired hepatic gluconeogenesis, as SRC1 is involved in activation of transcription of key

Table 1 Summary of phenotypes of mouse models discussed in the review as related to TH signaling in peripheral tissues.

Mouse model	Phenotype	TF responsible for the phenotype as discussed in original paper	References	Possible TR involvement, if not the primary focus of original paper
<i>Src1^{-/-}</i> (<i>Ncoa1</i>)	Obesity on high-fat diet due to reduced EE and defective adaptive thermogenesis	PPARGC1A	Picard <i>et al.</i> (2002)	Possible: TRs control EE and expression and activation of uncoupling protein 1 (UCP1).
	Impaired hepatic gluconeogenesis	CEBPA	Louet <i>et al.</i> (2010)	Possible: TH regulates expression of glucose-6-phosphatase
	Perturbed amino acid metabolism		Tannour-Louet <i>et al.</i> (2014)	Unclear
	Hyposensitivity to TH in the liver; dysregulated control of heart rate	TRs	Sadow <i>et al.</i> (2003a); Vella <i>et al.</i> (2014)	
<i>Src2^{-/-}</i> (<i>Ncoa2</i>)	Lean, increased glucose tolerance, and insulin resistance on high-fat diet. Increased basal and stimulated lipolysis, elevated EE	PPARG	Picard <i>et al.</i> (2002)	Unlikely: the phenotype is opposite of what would be expected in case of lack of TR co-activation
	Impaired hepatic glucose release	RORA	Chopra <i>et al.</i> (2008)	Possible: TH regulates expression of glucose-6-phosphatase
	Diminished fat absorption due to downregulation of bile salt export pump	FXR	Chopra <i>et al.</i> (2011)	Possible: TR regulates expression of bile salt export pump
	Impaired circadian regulation of lipid and glucose metabolism genes	ARNTL, CLOCK	Stashi <i>et al.</i> (2014a)	Possible: TRs regulate expression of lipogenic and gluconeogenic genes
<i>Src3^{-/-}</i> (<i>Ncoa3</i>)	Decreased adipocyte differentiation and expression of PPAR- γ 2	CEBPA	Louet <i>et al.</i> (2006)	Unclear
	Improved glucose and lipid metabolism, resistance to diet-induced obesity, and increased EE	PPARGC1A	Coste <i>et al.</i> (2008)	Unlikely: the phenotype is opposite of what would be expected in case of lack of TR co-activation
	Impaired metabolism of long chain fatty acids			
<i>NCoR^{-/-}</i> (<i>Ncor1</i>)	Embryonic lethality, impaired erythropoiesis, thymocyte, and neuronal differentiation	RAR, possibly TRs	Jepsen <i>et al.</i> (2000)	
<i>Smrt^{-/-}</i> (<i>Ncor2</i>)	Embryonic lethality, defects in heart development, unrestricted neural stem cell differentiation, and impaired forebrain development	FOXP1, RAR, Notch	Jepsen <i>et al.</i> (2007, 2008)	Unclear
<i>Smrt^{+/-}</i>	Obesity with higher number of smaller adipocytes on high-fat diet	Possibly PPARG	Sutnato <i>et al.</i> (2010)	Possibly: TRs regulate adipogenesis, lipogenesis, and lipolysis
Liver-specific <i>L-NCoRΔID</i>	Increased expression of hepatic TR targets in both hypo- and euthyroid conditions	TRs, LXRs	Astapova <i>et al.</i> (2008)	
<i>NCoRΔID</i>	Upregulation of LXR target genes			
	Increased sensitivity to TH in some peripheral tissues, increased EE	TRs	Astapova <i>et al.</i> (2011)	
<i>NCoRΔID</i>	Ameliorates to some degree peripheral presentations of TRB ^{PV/+} and TRA1 ^{PV/+} RTH		Fozatti <i>et al.</i> (2011); Fozatti <i>et al.</i> (2013)	
	Normalized sensitivity to TH in the liver	TRs	Vella <i>et al.</i> (2014)	

(Continued)

Table 1 (Continued)

Mouse model	Phenotype	TF responsible for the phenotype as discussed in original paper	References	Possible TR involvement, if not the primary focus of original paper
<i>NCoR-DADm</i>	Abnormal circadian behavior and expression of lipid metabolism genes, reduced fat mass, increased insulin sensitivity Derepression of TR targets in hypo- and euthyroid states Impaired TH-induced autophagy and fatty acid β -oxidation in the liver	Lack of HDAC3 recruitment to: Rev-Erba TRs TRs	Alenghat et al. (2008) You et al. (2010) Sinha et al. (2012)	Possible: TRs regulate expression of lipogenic genes
<i>NCoR/Smrt-DADm</i>	Upregulation of lipogenic genes in the liver, mild hepatic steatosis	General lack of HDAC3 activation	You et al. (2013)	Very likely, among other NRs
Liver-specific <i>Hdac3^{-/-}</i>	Hepatomegaly and severe liver steatosis Dysregulation of carbohydrate and lipid metabolism genes	General lack of HDAC3	Knutson et al. (2008) , Feng et al. (2011)	Very likely, among other NRs
Liver-specific <i>NCoR^{-/-}</i>	Upregulation of genes in NADPH and lipid synthesis, lipid sequestration, fatty acid oxidation. Significant hepatosteatosis	LXRA, PPARA, ESRRB, Rev-Erba	Sun et al. (2013) , Jo et al. (2015)	Very likely: TRs regulate lipid synthesis and fatty acid oxidation
Adipocyte-specific <i>NCoR^{-/-}</i>	Increased obesity, improved glucose metabolism, decreased adipose tissue inflammation on high-fat diet. Increased expression of PPAR- γ targets	PPARG	Li et al. (2011)	Possibly: TRs regulate adipogenesis, lipogenesis, and lipolysis
<i>NCoRω^{-/-}</i>	Pro-adipogenic: increased obesity, improved glucose metabolism, hepatic steatosis on high-fat diet		Goodson et al. (2014)	Possibly: TRs regulate adipogenesis, lipogenesis, and lipolysis
Muscle-specific <i>NCoR^{-/-}</i>	Increased muscle mass and exercise endurance, increased oxidative metabolism	MEF2, PPARG, ERRs	Yamamoto et al. (2011)	Possibly: TH promotes fatty acid oxidation
Liver-specific <i>Smrt^{-/-}</i>	No major phenotype. Upregulation of a few genes in retinoic acid metabolism	RARs	Sun et al. (2013) ; Shimizu et al. (2015)	Unlikely
Liver-specific <i>L-NCoRΔID Smrt^{-/-}</i>	Cumulative effect on derepression of TR target genes and lipid synthesis and sequestration. Mild hepatic steatosis	TRs, LXRs	Shimizu et al. (2015)	
<i>Smrt^{mRID}</i>	Increased fat mass, induction of adipogenic genes, glucose intolerance, reduced EE Impaired differentiation of type I pneumocytes and respiratory distress syndrome at birth	TRs, PPARG	Nofsinger et al. (2010) Pei et al. (2011)	
<i>Smrt^{mRID1}</i>	Premature aging, metabolic syndrome, reduced mitochondrial function. Obesity, hepatosteatosis, insulin resistance, and decreased EE on high-fat diet	TRs, RARs, PPARGs	Fang et al. (2010) ; Reilly et al. (2010)	

gluconeogenic enzymes by CCAAT/enhancer-binding protein (CEBPA) (Louet *et al.* 2010). It has also been reported that *Src1* is important for normal amino acid metabolism in the liver (Tannour-Louet *et al.* 2014).

When TH function was first assessed in these animals, overt resistance to TH was apparent at the level of HPT axis (Weiss *et al.* 1999). Intact *Src1* function is also required for full TH sensitivity at least in some peripheral tissues as upregulation of positive TH targets in response to T_3 is blunted in the liver of *Src1*^{-/-} mice. However, normal regulation of gene expression was observed in the heart (Vella *et al.* 2014). TR isoform-specific *Src1* function was also assessed in compound *Thra*/*Src1* and *Thrb*/*Src1* knockout mice during TH deprivation and TH treatment. While lack of *Src1* alone has no effect on the growth rate or body weight, in the absence of either THRA or THRB, SRC1 becomes important for normal growth and weight gain, cooperatively regulating these parameters with each TR subtype in the absence of the other. SRC1 is also essential for maintaining normal heart rate, as its absence alone or combined with the knockout of either of TRs leads to significant decrease of the heart rate. *Thra*^{0/0} *Src1*^{-/-} mice have the most dramatic phenotypes in terms of heart rate, probably related to the fact that THRA is the predominant TR isoform in the heart (Sadow *et al.* 2003a). Overall, while metabolic effects of SRC1 have been attributed to signaling by various transcription factors, it is clear that SRC1 is necessary for TH signaling in peripheral tissues, suggesting that abnormal TR action may potentially play a role in metabolic phenotype found in *Src1*^{-/-} animals.

***Src2* knockout mice**

In contrast to *Src1* knockout mice, *Src2*^{-/-} animals display a favorable metabolic phenotype characterized by resistance to diet-induced obesity, increased glucose tolerance and insulin sensitivity, higher basal and stimulated lipolysis rates, and elevated EE, at least partially due to a loss of its interactions with PPARG (Picard *et al.* 2002). *Src2* deficiency also results in diminished hepatic glucose release due to the loss of co-activation of RORA that leads to decreased expression of glucose-6-phosphatase, resulting in hepatic glycogen accumulation similar to human genetic von Gierke's disease (Chopra *et al.* 2008). SRC2 also plays an important role in regulation of fatty acid absorption from the gut by activating expression of bile salt export pump through interactions with farnesoid X receptor in response to reduced energy availability (Chopra *et al.* 2011). Recently, SRC2 was also identified as

a co-activator for the two key transcription factors in the clock machinery that control circadian rhythms: BMAL1 (ARNTL) and CLOCK (Stashi *et al.* 2014a). Genomic recruitment of SRC2 in the liver was found to significantly overlap with ARNTL during the light phase (ZT2), and target expression of many metabolic genes, particularly those involved in glucose metabolism and fatty acid synthesis pathways.

***Src3* knockout mice**

SRC3 has been identified as a critical regulator of white adipocyte development. *Src3*^{-/-} mouse embryonic fibroblasts (MEFs) are characterized by decreased adipogenic potential, and *Src3*^{-/-} animals have reduced body weight, decreased adipose tissue mass, and significantly decreased expression of PPARG2; a master regulator of adipogenesis. These effects are mediated by the loss of co-activation of CEBPA in the regulation of *Pparg* gene expression (Louet *et al.* 2006). In addition, *Src3*^{-/-} mice are resistant to diet-induced obesity with improved glucose and lipid serum profiles, and increased EE due to activation of PPARGC1A in brown adipose tissue (Coste *et al.* 2008). SRC3 has also been implicated in control of long chain fatty acid metabolism by directly regulating carnitine/acyl-carnitine translocase (CACT or SLC25A20) gene expression. The phenotype of *Src3*^{-/-} closely resembles phenotype of CACT genetic deficiency in humans, which presents with major metabolic dysregulation leading to hypoketonemia, hypoglycemia, hyperammonemia, and impaired neurologic, cardiac, and skeletal muscle performance (York *et al.* 2012). Consistent with human cases of CACT deficiency, *Src3* knockout mice can be rescued by diets containing only short chain fatty acids. These results point to SRC3 as a key regulator of B-oxidation.

NCoRAID mice

Mice that express a mutant co-repressor NcoR missing RID3 and RID2 (NCoRAID) have been used to address the physiologic role of NCoR-TR interactions (Fig. 1C) (Astapova *et al.* 2008). Analysis of hepatic gene expression in mice with liver-specific expression of NCoRAID (*L-NCoRAID*) clearly demonstrated that NCoR-TR interactions play a role in TR-mediated repression *in vivo* in hypothyroid state. Indeed, 16% of positive TH targets that are repressed in hypothyroid wt mice were significantly derepressed in *L-NCoRAID* animals. This is

in agreement with recent genome-wide hepatic TR recruitment data suggesting that active repression that involves DNA binding by the unliganded TR and co-repressor complex may not occur at all TR-regulated genes: instead, T₃-dependent recruitment of TR may be implicated in the regulation of a significant proportion of positive TR targets (Grontved *et al.* 2015, Ramadoss *et al.* 2013). At the same time, expression of less than 1% of negative TH targets (transcripts that were significantly upregulated in hypothyroid wt animals) was affected in *L-NCoRAID* mice, showing limited, if any, role of NCoR in negative regulation by TH in the liver. Even more importantly, significant upregulation of a number of positive TH targets was observed in *L-NCoRAID* livers in the euthyroid state. These results demonstrate that NCoR plays an important role in repression of positive TR targets during hypothyroidism and determines the sensitivity and responsiveness of gene expression to TH in euthyroid state.

Surprisingly, mice with global expression of NCoRAID (Astapova *et al.* 2011) are born at expected Mendelian ratios and appear phenotypically normal demonstrating that RID3 and RID2 are not required for normal development. Despite a 30% decrease in circulating TH levels compared with wt littermates, *NCoRAID* mice do not present with typical symptoms of hypothyroidism, as they grow normally, have normal body temperature and increased EE, and either normal or increased expression of positive TR targets in peripheral tissues and the pituitary. These findings confirm that NCoR plays an important role in establishing sensitivity to TH in peripheral tissues.

Deacetylase activation domain mutant mice

Similar to global *NCoRAID* mice, animals bearing a mutation in HDAC3-activating domain of NCoR (*N-DADm* mice) are viable and have no gross abnormalities (Alenghat *et al.* 2008). Elimination of DAD-mediated HDAC3 binding and activation leads to upregulation of Rev-Erba (NR1D1) targets and disruption of normal circadian behavior. Alterations in the oscillatory expression patterns of several metabolic genes implicated in lipid metabolism in liver and adipose tissue in *N-DADm* mice lead to a favorable metabolic phenotype characterized by reduced fat mass, elevated EE, and protection from diet-induced obesity and insulin resistance. Similar to *NCoRAID* animals, euthyroid and hypothyroid *N-DADm* mice showed derepression of several positive TH target genes in the liver (You *et al.* 2010). Moreover, *N-DADm* mice have a defect in TH-induced autophagy in the liver, which is a necessary

step in TH-mediated increase in fatty acid mobilization and B-oxidation (Sinha *et al.* 2012). Overall, these data demonstrate that activation of HDAC3 by NCoR plays a crucial role in the regulation of circadian and metabolic physiology through various NRs including Rev-Erba and TR isoforms.

Mice carrying DAD mutations in both NCoR and SMRT (*NS-DADm*) were also generated, and found, as expected, to have almost no detectable HDAC3 activity, increased histone acetylation around HDAC3 recruitment sites and reduced, but not completely abolished, genomic HDAC3 recruitment (You *et al.* 2013). Surprisingly, the phenotype of global *NS-DADm* mice was much less severe than *Hdac3* knockout, which is embryonic lethal (Bhaskara *et al.* 2008), or even a liver-specific *Hdac3* knockout (Feng *et al.* 2011, Knutson *et al.* 2008). Abrogation of HDAC3 activity in *NS-DADm* mice caused upregulation of fewer genes involved in lipid metabolism in the liver, and consistent with that, a much milder hepatic steatosis compared with hepatocyte-specific *Hdac3* knockout. This suggests that while NCoR and SMRT DADs are necessary for HDAC3 activity and local histone deacetylation, these actions are not important for embryonic development (as demonstrated by comparison to germline knockouts of *NCoR*, *Smrt*, and *Hdac3*). It also appears that deacetylase activity contributes relatively modestly to the total effect of HDAC3 protein, as long as it can still be recruited to DNA by NCoR and SMRT, which was further confirmed in experiments using HDAC3 deacetylase-dead mutants and liver-specific *NCoR* knockout mice (Sun *et al.* 2013).

Liver-specific NCoR knockout

In agreement with observations made in mutant *NCoR* knock-in animal models (Alenghat *et al.* 2008, Astapova *et al.* 2008), liver-specific *NCoR* knockout mice demonstrated derepression of genes involved in NADPH and lipid synthesis and sequestration, which are known to be controlled by LXRs, TRs, and Rev-Erba. However, these mice develop significant hepatosteatosis, similar to what is seen in liver-specific *Hdac3* knockout mice, which was not present in the knock-in animals and is supposedly due to the complete loss of genomic HDAC3 recruitment (Sun *et al.* 2013). Paradoxically, expression of fatty acid oxidation and mitochondrial function genes, which are under transcriptional control of PPARs and estrogen-related receptors (ERRs), was also elevated in liver-specific *NCoR* knockout mice (Jo *et al.* 2015). Interestingly, affinity of NCoR to different groups of

NRs may be manipulated in the liver by insulin-induced protein kinase B/Akt-mediated phosphorylation on S1460. Phosphorylation at this site results in diminished binding affinity of NCoR to LXRA and selectively derepresses its target genes resulting in increased lipid synthesis. At the same time, phosphorylated NCoR binds PPARA and estrogen related receptor alpha (ERRa or ESRRA) with higher affinity to attenuate expression of oxidative metabolism genes in the liver when insulin signaling is activated, for example, in the fed state. This insulin-dependent phosphorylation allows for a switch of recruitment of NCoR between different groups of NRs and provides a mechanism for specific modulation of NR signaling depending on physiological conditions (fed vs fasted). While the effect of the S1460 phosphorylation on NCoR–TR interactions has not been studied yet, it could potentially also have important ramifications for TR signaling, as TH has been shown in different experimental paradigms to promote both lipid synthesis and fatty acid oxidation in the liver (Feng *et al.* 2000, Flores-Morales *et al.* 2002, Jackson-Hayes *et al.* 2003, Oppenheimer *et al.* 1991).

Liver-specific *Smrt* knockout

Surprisingly, hepatocyte-specific deletion of *Smrt* revealed that SMRT alone, in contrast to NCoR, plays no role in the regulation of positive or negative TH targets or the lipogenic pathway in general (Shimizu *et al.* 2015, Sun *et al.* 2013). At the same time, SMRT appears to be important for RAR signaling and regulation of RAR target genes (Shimizu *et al.* 2015), confirming the *in vitro* data on preferential interactions between specific RIDs and NRs (Cohen *et al.* 2001, Cohen *et al.* 2000, Makowski *et al.* 2003, Webb *et al.* 2000). Interestingly, the combination of a hepatic SMRT knockout and L-NCoRAID expression demonstrated that SMRT works coordinately with NCoR to regulate lipid synthesis and sequestration, and the regulation of some of TH targets. While SMRT knockout alone has no effect on expression of genes implicated in these pathways, the combination of the two results in further upregulation of these genes compared with L-NCoRAID mice, and significant triglyceride accumulation in the liver. This suggests that a certain hierarchy in the recruitment of co-repressors competing for the same binding site on the TRs, and potentially other NRs, exists: NCoR is clearly the principal co-repressor for TRs and the lipogenic pathway in the liver in general, and is normally recruited to these genes, which is why removal of SMRT alone has no effect on their expression.

When NCoR is absent, SMRT can only partially substitute its function, and moderate activation of the gene expression can be seen. Removal of both co-repressors results in a full derepression of the pathway leading to a phenotype with marked lipid accumulation. These effects are likely mediated by different NRs including TRs, LXR, and Rev-ErbA. These experiments again confirmed that neither NCoR nor SMRT plays a role in the negative regulation by ligand-bound TRs in the liver.

NCoR in adipose tissue: adipocyte-specific and NCoR ω knockout

Adipocyte-specific deletion of *NCoR* has shown that this CoR represses PPARG signaling pathway in adipocytes (Li *et al.* 2011). Adipocyte-specific *NCoR* knockout mice fed high-fat diet demonstrate phenotypic resemblance to animals treated with PPARG agonists, which includes obesity, improved whole-body glucose tolerance, and changes in adipocyte morphology and function, diminished macrophage infiltration, and inflammation. Indeed, PPARG target genes were upregulated in adipose tissue of *NCoR* knockout animals, and these mice were refractory to further PPARG-stimulation with rosiglitazone, in agreement with the notion that deletion of *NCoR* increases sensitivity to endogenous PPARG ligands.

It has been shown that adipogenesis in mice is associated with a switch in co-repressor splicing from NCoR ω carrying all three RID domains, which represses adipogenesis, to NCoR δ where only RID2 and RID1 are present, which appears to support a pro-adipogenic program (Goodson *et al.* 2011). Consistent with the adipocyte-specific *NCoR* knockout phenotype, whole-body expression of the pro-adipogenic NCoR δ isoform results in protection from glucose intolerance despite increased weight gain and adiposity, as well as hepatic steatosis on a high-fat diet (Goodson *et al.* 2014). These results indicate that adipocyte-specific change in *NCoR* splicing during development helps drive normal adipocyte differentiation, while the presence of the full-length NCoR splice variant prevents excessive fat accumulation in the liver. They also suggest that since *NCoR ω* ^{−/−} is missing RID3, which is critical for NCoR–TR interactions, TR signaling is likely to contribute to the phenotype of these and, possibly, adipose-specific *NCoR* knockout animals in addition to PPARG.

Muscle-specific *NCoR* knockout

Muscle-specific deletion of *NCoR* has demonstrated that it plays a role in repression of fatty acid oxidation in

the muscle (Yamamoto *et al.* 2011). The lack of NCoR expression in the muscle results in higher mitochondrial activity and number and overall higher oxidative capacity leading to improved exercise endurance. These effects were attributed to increased activity of transcription factors MEF2, PPAR δ , and ERR isoforms, but no observations related to TR activity were made, even though TH is also known to promote fatty acid oxidation. It was also demonstrated that NCoR expression is decreased in physiological conditions that are associated with an increase in fatty acid oxidation, such as aging, fasting, high-fat feeding, or after endurance exercise.

SMRT receptor interacting domain mutant mice (*Smrt^{mRID}* and *Smrt^{mRID1}*)

Smrt^{mRID} mouse model bears mutations in two SMRT RIDs (RID1 and RID2) (Fig. 1C), which abolish its interactions with RAR, TR, and PPAR isoforms (Nofsinger *et al.* 2008). *Smrt^{mRID}* mice are born at expected ratios, but have slightly reduced viability on a mixed C57BL/6-Sv129 genetic background. Adult animals display a number of metabolic disturbances: significantly increased adiposity with reduced body weight, decreased EE, and RER, increased basal hepatic glucose production, and diminished insulin-stimulated glucose disposal leading to elevated fasting glucose and glucose intolerance. MEFs isolated from *Smrt^{mRID}* mice demonstrate increased PPAR γ signaling and adipogenic potential suggesting that SMRT, by repressing signaling by PPAR γ and potentially other NRs, plays an important role in determining the adipogenic set point. Hypothyroid *Smrt^{mRID}* mice demonstrate significant derepression of some TH target genes in the liver and improvement of hypothyroidism-induced hypercholesterolemia, indicating that SMRT plays a role in repression of positive TR targets in the absence of hormone. This is contrary to what was seen in the liver-specific *Smrt* knockout (Shimizu *et al.* 2015), and whether the differences are due to the whole-body vs liver-specific deletion or the ability of mutant SMRT^{mRID} to still bind HDAC3 and other components of the repression machinery remains to be clarified.

Interestingly, on a pure C57BL/6 background, close to 99% of *Smrt^{mRID}* pups die due to a defect in the development of type 1 pneumocytes that leads to previously unknown respiratory distress syndrome (Pei *et al.* 2011). The *Smrt^{mRID}* pups could be rescued by treating the pregnant dams with antithyroid drugs propylthiouracil or methimazole, which led to a complete restoration of expression of type 1 pneumocyte markers and normal survival. It has

been determined that the lethal phenotype is due to downregulation of expression of a negative TH target Krüppel-like factor 2 (*Klf2*) in neonatal *SMRT^{mRID}* lungs. These observations demonstrate that normal development of neonatal lung and animal survival are dependent on expression of transcription factor KLF2, which is activated by TR through its interactions with SMRT in a ligand-independent manner.

Smrt^{mRID1} mice carry a mutation only in RID1 (Fig. 1C), which changes the interaction patterns, so that while RID1-mediated interactions are abolished, the interactions mediated by the most C-terminal RID (RID1 in Fig. 1) are enhanced leading to increased repression by PPARs and potentially some other NRs (Fang *et al.* 2010, Reilly *et al.* 2010). *Smrt^{mRID1}* mice have decreased lifespan accompanied by premature aging and related metabolic disease. They are also characterized by repression of genes in the oxidative metabolism pathway and reduced mitochondrial function, potentially due to enhanced repression of these genes by PPARs. Similar to *Smrt^{mRID}* mice, these animals are more susceptible to diet-induced obesity and development of insulin resistance, and have decreased RER. However, unlike *Smrt^{mRID}* mice they are refractory to glucose-lowering effects of thiazolidinediones and 5-amino-4-imidazolecarboxamide ribose and also display marked hepatic steatosis. The common features of these two SMRT mutant mouse strain phenotypes are likely due to the derepression of NRs interacting with the similarly mutated RID, while the differences are the result of increased repression through PPAR isoforms in *Smrt^{mRID1}* animals.

Overall, multiple animal models discussed above have demonstrated that in peripheral tissues co-repressors attenuate expression of positive TR and other NR target genes both in the absence and presence of ligand, while co-activators are necessary for full activation of positively regulated genes in response to the hormonal signal. However, possible role of co-regulators in ligand-dependent negative regulation remains much less clear.

Role of co-regulators in the function of HPT axis and RTH

The HPT axis has developed as an elegant feedback system that controls circulating TH levels. In this system, elevated concentrations of TH negatively regulate production of both TRH in the hypothalamus and TSH in the pituitary, while a drop in serum TH would induce synthesis and secretion of both TRH and TSH (Chiamolera & Wondisford 2009). This regulation is highly sensitive,

so that circulating TSH level is the most common TH function test in humans. Hormone-bound TRs potently represses both *TRH* gene expression in the paraventricular nucleus of hypothalamus and expression of TSH subunits, encoded by *CGA* and *TSHB* genes, in the pituitary, making these genes a classic example of negative TH targets, but exact molecular mechanisms underlying this hormone-dependent negative regulation remain unclear (Abel *et al.* 2001, Abel *et al.* 1999, Hollenberg *et al.* 1995, Sugrue *et al.* 2010, Wood *et al.* 1989).

Classic RTH is an inherited syndrome characterized by various degrees of peripheral and central hyposensitivity to TH, such that high serum levels of free T_4 and T_3 are accompanied by inappropriately elevated or normal TSH, lack of most usual symptoms of thyrotoxicosis and often a goiter (Beck-Peccoz & Chatterjee 1994, Refetoff *et al.* 1967, Refetoff *et al.* 1993). This type of RTH is most commonly caused by mutations in the *THRB* gene that have been identified in affected subjects belonging to more than 450 families. In majority of cases, peripheral hyposensitivity to TH is compensated for by high circulating TH levels, with some variability between individuals. Thyroid status also varies between different tissues, most likely as a result of tissue-specific expression of *THRA* vs *THRB*, leading to a simultaneous presentation of symptoms of hypo- and hyperthyroidism (lack of negative feedback in HPT axis and delayed growth vs tachycardia and hyperactivity) (Dumitrescu & Refetoff 2013). Most mutations found in subjects with classic RTH are located in the activation function-2 (AF-2) and reduce its affinity for the hormone. Such mutants interfere with the function of the wt *THRB* in a dominant-negative manner (Adams *et al.* 1994, Hayashi *et al.* 1995). Some mutant *THRB*s were found to have impaired interaction with one of the cofactors involved in the regulation of TH action (Collingwood *et al.* 1997, Collingwood *et al.* 1998, Liu *et al.* 1998, Tagami & Jameson 1998, Yoh *et al.* 1997).

Recently, a few families with mutations in *THRA* have also been identified, with clinical presentations very different from classic *THRB* RTH. The affected individuals have slightly abnormal thyroid function tests with low to normal free T_4 , slightly elevated free T_3 , reduced rT_3 , and normal TSH levels, but display clear phenotypic characteristics of hypothyroidism with growth and developmental retardation, skeletal dysplasia, marked constipation (diarrhea in one case), and cognitive impairment. Identified mutations in *THRA* gene led to expression of either a truncated *THRA* completely unable to bind T_3 or mutations decreasing affinity to T_3 and transcriptional activity showing

dominant-negative features (Bochukova *et al.* 2012, Espiard *et al.* 2015, Moran *et al.* 2014, Moran *et al.* 2013, Tylki-Szymanska *et al.* 2015, van Mullem *et al.* 2012). Cases of RTH in the absence of mutations in the *THRB* or *THRA* genes were also identified suggesting that mutations in co-regulator protein might be involved; however, human subjects with this type of RTH are yet to be identified (Reutrakul *et al.* 2000, Weiss *et al.* 1996).

Mouse models carrying mutations in *Thrb* gene found in patients with RTH, such as *Thrb^{PV}* and *Thrb^{T337D}*, were generated and found to recapitulate classic dominantly inherited RTH (Hashimoto *et al.* 2001, Kaneshige *et al.* 2000). Mice expressing *Thrb^{E457A}* mutation that abolishes co-activator binding and homozygous *Thrb* knockout animals also demonstrate various degrees of resistance to TH (Ortiga-Carvalho *et al.* 2005), reviewed in Flamant and Samarut (2003). *Thra* knock-in mouse models carrying mutations analogous to those found in patients with *THRB* RTH were generated before identification of patients with *THRA* gene mutations (*Thra^{PV}*, *Thra^{R384C}*, *Thra^{L400R}*, *Thra^{P398H}*, *Thra^{R384C}*) and display varying phenotypes, with some of them being similar to patients with *THRA* RTH (Kaneshige *et al.* 2001, Liu *et al.* 2003, Quignodon *et al.* 2007, Tinnikov *et al.* 2002), reviewed in van Mullem *et al.* (2014) and Vennstrom *et al.* (2008). It has been suggested that while inability to recruit co-activators, such as SRC1, would worsen *THRB*-mediated RTH, loss of co-repressor binding to TRs would at least partially rescue symptoms of RTH, which phenotypically present as hypothyroidism, with the effect on the hyperthyroid symptoms depending on the drop of circulating TH. Lack of co-repressor recruitment in the *THRA*-mediated RTH may potentially have an even bigger effect, as in the presence of normal circulating TH levels (as opposed to high in *THRB* RTH), ability of TR to recruit co-repressors should be higher. Data regarding the role of co-regulators in the function of HPT axis and presentation of RTH obtained using genetically modified mouse models, are summarized in Table 2.

Co-activator knockout models

The *Src1* knockout model demonstrated for the first time *in vivo* that SRC1 is necessary for normal function of the HPT axis (Weiss *et al.* 1999). Mice deficient in *Src1* display RTH with phenotypic presentation similar to classic human RTH: elevated serum TSH levels accompanied by high serum free T_4 and T_3 . These mice demonstrated reduced sensitivity to TH at thyrotrope level, as suppression of TSH by T_3 is severely blunted compared with wt animals,

Table 2 Summary of phenotypes of mouse models discussed in the review as related to regulation of HPT axis.

Mouse model	Phenotype	References
<i>Src1</i> ^{-/-} (<i>Ncoa1</i>)	RTH similar to TRB RTH: high T ₃ , T ₄ , and unsuppressible TSH Worsening of <i>Trb</i> ^{PV/+} and TRB ^{E457A/E457A} RTH	Weiss et al. (1999) Kamiya et al. (2003); Alonso et al. (2009)
<i>Src2</i> ^{-/-} (<i>Ncoa2</i>)	No phenotype at the level of HPT axis	Weiss et al. (2002)
<i>Src1</i> ^{-/-} / <i>Src2</i> ^{-/-}	Worsening of RTH compared with <i>Src1</i> ^{-/-} alone	Weiss et al. (2002)
<i>NCoRA1D</i> (<i>NCoR1</i>)	Decreased circulating T ₄ and T ₃ , normal TSH; normal response to dynamic changes in TH levels; decreased responsiveness of the thyroid to TSH	Astapova et al. (2011)
Postnatal <i>NCoRA1D</i>	Partial improvement of RTH in <i>Thrb</i> ^{PV/+} and <i>Thra1</i> ^{PV/+} models Decreased circulating T ₄ and T ₃ , normal TSH similar to global <i>NCoRA1D</i> animals	Fozzatti et al. (2011); Fozzatti et al. (2013) Costa-e-Sousa et al. (2012)
Pituitary-specific <i>Cga-NCoRA1D</i>	Decreased circulating T ₄ , T ₃ , and TSH. Blunted upregulation of TSH during hypothyroidism	Costa-e-Sousa et al. (2012)
<i>NCoR-DADm</i>	Normal T ₄ and T ₃ levels, elevated TSH; normal TSH response to dynamic changes in TH levels	You et al. (2010)
<i>Smrt</i> ^{mRID} (<i>NCoR2</i>)	Slightly reduced T ₄ on C57/BL6 background	Pei et al. (2011)
Postnatal <i>Smrt</i> ^{-/-}	No effect on HPT axis	Shimizu et al. (2015)
<i>NCoRA1D Src1</i> ^{-/-}	Normalized HPT function	Vella et al. (2014)

both in baseline 'euthyroid' and in hypothyroid state. This suggests that SRC1 behaves as a co-repressor *in vivo* when it interacts with a gene that is negatively regulated by TH as was observed previously *in vitro* (Tagami et al. 1997). The elevated circulating TH in *Src1*^{-/-} animals appears to be driven by TSH, as suppression of TSH by the administration of supraphysiological doses of T₃ results in reduction of endogenous T₄. Notably, the absence of *Src1* did not affect the full ligand-independent stimulation of TSH, suggesting that other co-regulators may play a co-activator role in this case. Overall, *Src1*^{-/-} mice demonstrate RTH phenotype at the level of HPT axis comparable to the *Thrb*-null mice, but less severe than the TH resistance present in total *Thrb* or compound *Thrb* and *Thra*-deficient animals (Takeuchi et al. 2002, Weiss et al. 1999).

The specific role of SRC1 in activation of TH-regulated gene transcription by different TR isoforms in the pituitary was also assessed using compound *Src1/Trb*- and *Src1/Thra*-deficient mice (Sadow et al. 2003b). Interestingly, *Src1/Thrb* mice have more severe TH resistance than either of the single knockouts, implying that SRC1 has a role in TH action independent of THRB. At the same time while *Thra* knockout mice are hypersensitive to TH action at the thyrotrope level, *Src1/Thra*-deficient mice demonstrate TH resistance presented as inefficient suppression of TSH. Consistent with previous observations (Xu et al. 1998), *Src1* deficiency was found to lead to elevation of expression of *Src2* and -3 in pituitary, which could be a mechanism of partial compensation for *Src1*. This is also supported by the data obtained in compound *Src1* and -2 knockout

mice (Weiss et al. 2002). While *Src1*^{-/-} mice are resistant to TH, *Src2*^{-/-}, *Src2*^{+/-}, and *Src1*^{+/-} mice displayed normal thyroid function tests. Surprisingly, double heterozygous *Src1/Src2* mice presented with a RTH phenotype similar to the *Src1* homozygous knockouts. Deletion of both *Src1* and -2 resulted in marked increases of serum TH and TSH concentrations, showing dramatic resistance to TH, surpassing that of total *Thrb*^{-/-} mouse model. This demonstrates partial functional redundancy of SRC1 and -2 in the regulation of HPT axis by TH. However, in this context, SRC1 is clearly the preferred co-activator for TRs, while SRC2 becomes important only when the levels SRC1 are decreased or abrogated, which is also associated with an increase in SRC2 as discussed above. These data clearly demonstrate that co-activator recruitment to TRs is crucial for normal regulation of HPT axis.

Co-repressor-deficient models

Mice with global expression of *NCoRA1D* generated through germline mutation were used to address the potential role of co-repressors in the regulation of HPT axis *in vivo* (Astapova et al. 2011). *NCoRA1D* mice have low circulating T₄ and T₃ levels with inappropriately normal serum TSH and expression of TSH subunit mRNA in the pituitary, suggestive of central hypothyroidism. However, *NCoRA1D* mice do not appear hypothyroid due to enhanced sensitivity to TH in peripheral tissues. At the same time, the sensitivity of HPT axis is not altered, as changes in circulating TH produce normal response in TSH levels and expression. Interestingly, these animals

have reduced intrathyroidal levels of free T_4 and T_3 , and the ability of the thyroid gland to secrete TH in response to TSH stimulation is significantly attenuated. Therefore, disruption of NCoR–TR interactions leads to increased sensitivity to TH in peripheral tissues, which is compensated for by a reset HPT axis where the thyroid produces less TH, while pituitary and possibly hypothalamus recognize lower TH levels as normal. Importantly, mice where global expression of NCoRAID was induced in adulthood using an inducible ubiquitous Cre recombinase, demonstrated an identical phenotype, confirming that the alterations in HPT axis are not a result of a developmental defect (Costa-e-Sousa *et al.* 2012). Moreover, expression of NCoRAID specifically in the pituitary using a Cre recombinase driven by the glycoprotein A-subunit promoter led to a phenotype characterized by low TH levels and decreased TSH production. Remarkably, the rise of TSH during hypothyroidism was also blunted in these animals, in contrast with *Src1* knockout mice. While serum T_4 concentrations were slightly reduced in *Smrt^{mRID}* mice on C57/BL6 background (Pei *et al.* 2011), postnatal global ablation of SMRT had no effect on circulating T_4 levels or TSH subunit expression in the pituitary, confirming that similarly to the liver, specificity of co-repressor recruitment exists in HPT axis, and NCoR is the preferred TR co-repressor (Shimizu *et al.* 2015).

A somewhat different phenotype related to dysregulation of HPT axis was found in *N-DADm* mice (You *et al.* 2010). These mice were characterized by significantly elevated serum TSH and TSH subunit expression in the presence of normal serum T_4 and T_3 . Manipulations with circulating TH levels, produced normal response in terms of serum TSH; however, pituitary expression of TSH and type 2 deiodinase remained modestly but significantly elevated in all conditions, suggesting that in these animals HPT axis is reset to recognize normal TH levels as low, opposite to what is seen in *NCoRAID* mice. This discrepancy is potentially due to the fact that while NCoRAID simply does not bind TRs potentially increasing their ability to recruit other cofactors, in *N-DADm* mice NCoR does not activate HDAC3, but is still recruited competing with co-activators for the binding site.

As *Src1*-deficient mice are resistant to the actions of TH but the disruption of interactions between TR and NCoR in *N-CoRAID* mice leads to increased sensitivity to TH, it was hypothesized that RTH in *Src1*^{−/−} mice is caused by unopposed co-repressor action. Remarkably, when *Src1*^{−/−} were crossed with *N-CoRAID* animals, the resulting compound knockout mice had normal circulating TH and TSH levels and normal ability to suppress TSH in

response to T_3 treatment, thus demonstrating reversal of resistance present in *Src1*-deficient mice (Vella *et al.* 2014). Additionally, T_3 -mediated upregulation of positive TR target genes in the liver, which is blunted in *Src1*^{−/−} mice, was restored in *NCoRAID Src1*^{−/−} animals. Interestingly, expression of *Src2* and recruitment of SRC2 to promoters of positive TR target genes was significantly increased in *NCoRAID Src1*. This again suggests a hierarchy in the recruitment of co-regulators to TR: in the absence of SRC1, high affinity binding of NCoR prevents recruitment of SRC2 co-activator even in the presence of ligand. However, removal of NCoR allows to re-establish the co-regulator balance.

Role of SRCs in other RTH models

Inability to recruit co-activators by mutant THRB is thought to play a role in the development of RTH. In *Thrb^{PV}* mutant mice, a model of RTH with a knock-in mutation in the *Thrb* locus that completely abolishes T_3 binding to THRB (Kaneshige *et al.* 2000), lack of *Src1* leads to a worsening of the HPT axis dysregulation and growth abnormalities in *Thrb^{PV/+}* mice but not in *Thrb^{PV/PV}* mice. In *Thrb^{PV/PV}* animals, *Src1* deficiency intensified the pathological progression of thyroid follicular cells to papillary hyperplasia. Lack of *Src1* did not affect serum cholesterol levels in either *Thrb^{PV/+}* or *Thrb^{PV/PV}* mice, but led to dysregulated expression of several T_3 target genes in the pituitary and liver (Kamiya *et al.* 2003).

Mice expressing THRB with E457A mutation in AF-2 that does not affect ligand binding but completely abolishes recruitment of co-activators to LBD also demonstrate profound RTH phenotype at peripheral tissues and HPT axis (Ortiga-Carvalho *et al.* 2005). Surprisingly, disruption of *Src1* in the *Thrb^{E457A/E457A}* mice worsened the degree of resistance to TH, resulting in increased serum T_4 and TSH at baseline. During TH deprivation, simultaneous disruption of AF-2 and *Src1* resulted in a blunted TSH rise, only 50% of what was seen in *Thrb^{E457A}* mutant alone, suggesting that *Src1* can interact with THRB outside of the AF-2 domain and, contrary to previous conclusions from *Src1* knockout mice, is necessary for activation of HPT axis during TH deprivation, likely through interaction with another region of the THRA or THRB. At the same time, ligand-dependent repression of TSH requires recruitment of SRC1 to AF-2 (Alonso *et al.* 2009). These data clearly demonstrate that disruption of co-activator recruitment to TRs contributes to dysregulation of HPT axis present in RTH by a variety of different mechanisms.

Role of co-repressors in RTH

Constitutive recruitment of co-repressors by the mutant TR has been proposed to be one of the molecular mechanisms underlying RTH. Availability of *NCoRAID* mice provided an opportunity to test this hypothesis *in vivo*. The absence of TR-NCoR interactions in *Thrb^{PV}/NCoRAID* animals results in a moderate but significant decrease in circulating T_4 , T_3 , and TSH levels and TSH subunit expression, especially in the presence of only one mutant PV allele (a situation more similar to human RTH) (Fozzatti *et al.* 2011). At peripheral level, weight loss, severe thyroid hyperplasia, and repression of a number of hepatic TR target genes were also reversed. Furthermore, expression of *NCoRAID* could partially ameliorate abnormalities in the HPT axis of *Thra1^{PV/+}* mice that carry the dominant-negative frameshift PV mutation and display a phenotype similar to that found in humans with mutations in *Thra* gene (Fozzatti *et al.* 2013). *NCoRAID/Thra1^{PV/+}* animals also displayed improvement in severe growth retardation, infertility, delayed bone development, and impaired adipogenesis. Thus, aberrant recruitment of NCoR by mutant TRs may contribute to clinical presentation of both THRB and THRA RTH. It appears that abolishing NCoR-TR interaction is effective in terms of correcting the peripheral symptoms of hypothyroidism in both types of RTH, but less effective in correcting the resistance at the level of HPT axis seen in TRB RTH. Therefore, it is unlikely to have a positive effect on clinical presentation of hyperthyroid symptoms seen in certain tissues in patients with classic RTH.

Lessons from genome-wide analysis of TR chromatin recruitment

The bimodal switch model of TR-mediated transcriptional regulation is based on the primarily nuclear localization of TRs (as opposed to sex steroid receptors) and assumption that TR binding to response elements in the target genes is not affected by the presence of TH (Baumann *et al.* 2001, Perlman *et al.* 1982, Wong *et al.* 1995, Xu *et al.* 1999). While preferred TR-binding sites have been established *in vitro*, and a number of TREs have been identified in some target genes, a comprehensive analysis of TR-binding patterns on the genome-wide level has not been performed until recently (Chatterjee *et al.* 1989, Glass *et al.* 1987, Glass *et al.* 1988, Petty *et al.* 1990, Zilz *et al.* 1990).

In two studies, a genome-wide recruitment of tagged overexpressed TRs was assessed in neural and hepatic cell lines using chromatin affinity precipitation (ChAP)-seq (Ayers *et al.* 2014, Chatonnet *et al.* 2013). Chatonnet *et al.*

compared THRA and THRB transcriptomes and cistromes in neural cell lines, where T_3 responsiveness was restored by expression of tagged version of either TR isoform, while Ayers *et al.* (2014) studied THRB genome-wide binding in hepatocellular carcinoma cell line. Both studies found significant enrichment for TR-binding sites in the proximity of genes positively regulated by T_3 . In contrast, no significant enrichment of TR binding was seen at negatively regulated genes, thus providing no statistical confirmation for negative regulation by liganded TRs. TRE half-sites and classical TREs were present at many peaks, and a direct repeat of a NR-binding motif AGGTCA with a 4 nucleotide spacer (DR-4) was established as the consensus TR-binding site for both THRA and THRB in neural cells as expected. It was also demonstrated that genomic THRA and THRB binding had not only some overlap, but also had a great number of unique targets.

Two other studies have investigated genomic recruitment of *THRB1* in the liver under hypo- and hyperthyroid conditions *in vivo* using different approaches: i) using biotin-tagged THRB1 overexpressed in mouse liver by adenoviral delivery to allow for affinity precipitation and ChAP-seq (Ramadoss *et al.* 2013) and ii) using a mouse monoclonal THRB-specific antibody to assess recruitment of endogenous *THRB1* by chromatin immunoprecipitation (ChIP)-seq (Grontved *et al.* 2015).

Ramadoss *et al.* (2013) reported a high number of TR-binding peaks (20,939 peaks in PTU-treated and 15,723 in T_3 -treated livers), with most of the peaks located within the genes. Interestingly, at many sites recruitment of THRB1 was affected by the presence of T_3 . At most sites where TR occupancy changed depending on the presence of T_3 TR binding was increased by T_3 (80% of all changed peaks). These sites were located farther away from transcription start sites (TSS) of known genes, suggesting that hormone-induced changes in THRB1 binding are more likely to occur at distal enhancer elements. Only 2% of T_3 -sensitive peaks were located within the proximal promoters (TSS to -500 bp), while 17% of all peaks identified in hypothyroid and 7% of all peaks identified in hyperthyroid states were present in that region. Moreover, T_3 -induced peaks showed less overlap with DNase hypersensitivity peaks (data obtained from mouse ENCODE project), compared with invariant or peaks that were decreased with T_3 treatment, suggesting that T_3 may promote binding of THRB1 to less accessible chromatin regions and potentially induce chromatin remodeling. Overall, the changes in THRB1 binding correlated with changes in the expression of nearby genes in the same direction; however, some

genes were identified where the expression was regulated in the opposite direction. Therefore, it is possible that diminished THRB1 binding in the presence of T_3 may be the mechanism of negative regulation for some genes. Similar to the findings in the cell lines, significantly more genes upregulated by T_3 treatment were found to have associated THRB-binding sites (73%) as compared with downregulated genes (40%), suggesting that negative regulation by T_3 may be largely indirect. However, the association of negatively regulated genes with THRB1 binding was still significant, thus implying that liganded THRB1 can in some instances function as a repressor. Motif analysis of all THRB1-binding sites has identified DR-4 as the most enriched element, consistent with previous reports. Surprisingly, while DR-4 was also the most represented motif in the peaks where TR binding was increased in the presence of T_3 and associated with elevated gene expression, DR-0 was enriched in the peaks decreased with T_3 treatment and associated with diminished gene expression. This provides an insight into the potential mechanisms as to how TR may mediate positive and negative regulation by employing differential binding at unique target sites.

Finally, [Grontved et al. \(2015\)](#) combined THRB1 ChIP-seq approach with DNaseI hypersensitivity assay to assess potential chromatin remodeling events associated with T_3 signaling. Much fewer TR-binding sites were identified in this study compared with the study by [Ramadoss et al. \(2013\)](#), which could be due to the differences in the abundance of endogenous THRB1 vs overexpressed and/or relatively low avidity of the monoclonal TR antibody used in this study. The obtained data suggest a model, where T_3 greatly increases THRB1 recruitment to chromatin with 864 TR-binding sites identified in hypothyroid and 2186 in hyperthyroid conditions, where majority of the TR-independent peaks overlapped with those found only in the presence of T_3 . *De novo* motif analysis found enrichment for the DR-4 TRE in 60% of hormone-independent TR-binding peaks and 42% of T_3 -facilitated peaks. Interestingly, less than 20% of unique hypothyroid peaks contained TREs, suggesting that these peaks may result from indirect TR binding. Overall, 60% of T_3 -induced genes were found to have at least one TR-binding peak within 50 kb of TSS, while genes negatively regulated by T_3 show little enrichment, suggesting that T_3 -dependent repression may be largely mediated by indirect mechanisms. T_3 was found to induce robust changes in chromatin accessibility. In general, 92% of identified TR-binding sites were localized within the accessible chromatin (either unchanged or

remodeled upon T_3 treatment), with almost no binding found within the DNase hypersensitive sites (DHS) reduced by T_3 treatment. Most interestingly, 10% of T_3 -induced TR peaks were localized in the regions with DHS formed *de novo* upon T_3 treatment, suggesting that TRs can induce chromatin remodeling in a ligand-dependent manner. This demonstrates that TR can be recruited to chromatin in different scenarios, including hormone-independent binding of unliganded TR to open chromatin and ligand-induced TR recruitment to previously inaccessible chromatin, leaving a question as to how TR discriminates between these types of binding sites, especially in hypothyroid conditions. Motif analysis of identified TR peaks suggests that the presence and strength of the TRE (DR-4) and chromatin accessibility are important determinants of TR recruitment in the absence of T_3 , where unliganded TR preferentially binds to accessible chromatin regions containing strong consensus TREs. When T_3 is available, TR is able to occupy accessible chromatin harboring less stringent TR-binding motifs. Only strong, highly conserved TR-binding motifs and presence of T_3 allow for TR binding at sites within inaccessible chromatin, which are remodeled *de novo* upon T_3 -dependent TR recruitment. These findings suggest that the role of co-activators and co-repressors in the TR-mediated transcriptional regulation may depend on the type of response element and overall chromatin context. Indeed, only genes with nearby T_3 -independent TR-binding peaks can be actively repressed by TR in that state. Examples of such genes include *Gpd2*, *Pdp2*, and *Idh3*, where at least one of the identified TR-binding sites was found to be hormone-independent. In agreement with the previous studies showing that *Gpd2* and *Idh3a* are significantly derepressed in hypothyroid *L-NCoRΔID* animals that lack interaction between TR and NCoR, activation of these genes is not associated with considerable change of TR occupancy or chromatin remodeling, while NCoR and HDAC3 occupancy is reduced in response to T_3 . At the same time, co-activator CBP was found at those TR-binding sites at all times. *Dio1* represents a class of genes, where all nearby TR binding is ligand-dependent and TR recruitment leads to dramatic remodeling of the chromatin surrounding binding sites. The presence of T_3 leads to increased recruitment of co-activator CBP, whereas NCoR and HDAC3 occupancy remains unchanged, suggesting that *Dio1* is not directly repressed by TR in the absence of TH. This is again in agreement with the fact that disruption of NCoR–HDAC3 and NCoR–TR interactions have been reported to have little effect on *Dio1* transcription in the liver.

A few general conclusions can be drawn from these data. All of the studies point to DR-4 as a consensus TR-binding element. Three out of four reports found no significant enrichment in the TR recruitment to the genes negatively regulated by TRs, at least within the proximity limits set in the studies (10–50 kb from TSS), suggesting that negative regulation by TH may be largely mediated by indirect mechanisms or TR recruitment in these cases occurs almost exclusively at distant enhancers. In the report by Ramadoss *et al.* (2013), where TR binding was found to be associated with negatively regulated genes, the enrichment was much lower than for positive genes. While this result still suggests that indirect mechanisms may be responsible for the regulation of a large percentage of negative TH targets, this study also identified DR-0 as a potential ‘negative’ TRE. Finally, the two *in vivo* studies found that, unexpectedly, TR recruitment to DNA is influenced by T_3 , and T_3 -induced TR binding or release

affects target gene expression. Most strikingly, while majority of TR-binding sites were found to reside within open chromatin regions, it was also demonstrated that upon ligand binding TR can bind previously inaccessible chromatin regions and induce chromatin remodeling, similar to other NRs (Boergesen *et al.* 2012). These new insights into different scenarios of TR action imply that the role of co-activators and co-repressors in TR signaling may vary significantly depending on the type of TR-binding site and surrounding chromatin landscape (Fig. 2).

Summary and future perspectives

Mouse models with disrupted co-regulator function overall demonstrate that co-activators and co-repressors play a role in the regulation of transcription by TRs not only in the presence or absence of TH respectively, but are also essential for the normal signaling by TR

A T3-independent recruitment of TR

B T3-modulated recruitment of TR

C Positive regulation through T3-induced recruitment of TR

D Negative regulation through indirect recruitment of TR

Figure 2

Models of TR-mediated regulation of transcription based on the insights obtained from TR ChIP-seq experiments. Possible roles of co-regulators are hypothesized based on *in vitro* binding data, animal models with altered co-regulator function, and a limited number of ChIP-PCR experiments. (A) Positively and negatively regulated genes, where TR is recruited to an open chromatin region independently of T_3 (based on classic bimodal switch model). On a positively regulated target gene, with a consensus DR-4 TR-binding site, in the absence of TH RXR/TR heterodimer or TR/TR homodimer preferentially recruits co-repressor complex to repress transcription. Upon binding of T_3 , a conformational change occurs that favors recruitment of co-activators to activate transcription. On negatively regulated targets, with yet undetermined consensus binding site, co-repressor complexes are recruited in the absence of hormone to activate transcription, while co-activators recruited to the liganded TR, mediate transcriptional repression. (B) Positively and negatively regulated genes, where TR binding to open chromatin is affected by T_3 . On a positive target gene containing a DR-4-binding site, there is weak TR recruitment in the absence of hormone. In the presence of T_3 , TR strongly binds to the response element and brings co-activator complexes to activate transcription. On a negatively regulated gene, TR is recruited to DR-0 response element in the absence of hormone to activate transcription either through ligand-independent recruitment of co-activators or by recruitment of co-repressors, which act as functional co-activators. Binding of T_3 leads to dissociation of TR from DNA and diminished transcription of the target gene. (C) A positively regulated gene located within a region of inaccessible chromatin and containing a strong DR-4 TR-binding motif. TR can only be recruited in the presence of T_3 to initiate chromatin remodeling and bring co-activator complex to activate transcription. (D) Negative TH-dependent regulation mediated by indirect recruitment of TR and co-regulators through yet unidentified transcription factors (TF).

isoforms under a range of TH concentrations. Removal of co-repressor or co-activator function produces a shift in the dynamics of cofactor recruitment and balance of transcriptional activation vs repression that underlies TR actions. Lack of co-repressor recruitment results in a higher percentage of the receptors that are available to bind T_3 and co-activators, thus increasing TR sensitivity and transcriptional response to the hormone. At the same time, co-activator removal not only precludes binding of other components of co-activator complex necessary for maximal transcriptional activation, but also leads to enhanced recruitment of co-repressors, thus 'locking' TRs in the repressive mode.

Studies using animal models have also confirmed that co-regulator specificity does exist *in vivo*, with NCoR and SRC1 being the preferred co-repressor and co-activator for TR isoforms. SMRT and SRC2 appear to be a 'second-tier' co-regulator and can only minimally, if at all, compensate for the absence of NCoR and SRC1 in the context of TH signaling. In fact, the role of SMRT and SRC2 only becomes apparent in the compound knockout models, such as *Src1^{-/-}/Src2^{-/-}* (Weiss *et al.* 2002), *L-NCoRAID/Smrt^{-/-}* (Shimizu *et al.* 2015), and *NCoRAID/Src1^{-/-}* (Vella *et al.* 2014).

At the same time, the role of co-regulators in the control of expression of negative TH targets remains unclear. It has been proposed that co-activators and co-repressors paradoxically play the opposite roles on negative targets. The only definitive *in vivo* proof of co-repressors playing a role in direct transcriptional activation in the absence of TH is activation of expression of *Klf2* in the lung during neonatal period (Pei *et al.* 2011). Most studies so far found little to no role for co-regulators in the negative regulation by TH in peripheral tissues. While many models with disrupted co-activator or co-repressor function demonstrate abnormal regulation of HPT axis to some degree, whether these effects are mediated through direct regulation by TRs remains unclear.

This is further substantiated by genome-wide TR recruitment data, where three out of four studies have found no evidence for direct TR binding near the genes negatively regulated by T_3 (Ayers *et al.* 2014, Chatonnet *et al.* 2013, Grontved *et al.* 2015), and one suggested an entirely new mechanism, where negative regulation is achieved through diminished recruitment of TRs in the presence of T_3 (Ramadoss *et al.* 2013). These results suggest that negative TH targets may still be regulated directly by TR, but TR binding in this case occurs preferentially at very distal enhancer sites and therefore cannot be assigned to the negatively regulated genes by proximity

in ChIP-seq experiments. Alternatively, TH-dependent negative regulation may be indirect, which includes recruitment of TR to DNA through tethering to other transcription factors or regulation by products of genes directly regulated by TRs.

Mouse models have definitively demonstrated that alterations in co-regulator recruitment can lead to resistance or increased sensitivity to TH, and may also modulate phenotypic presentation of RTH caused by mutations in either TR isoform. However, human patients with disturbances in TH signaling due to abnormal co-regulator recruitment, function, and/or expression remain to be identified. Importantly, clinical features of these groups of patients are likely to vary widely and display more phenotypic presentations than the two types of TR-dependent RTH.

Genome-wide interrogation of TR binding also demonstrated that there is a wide repertoire of the TR-binding sites that control expression of TH target genes, and the mode of TR and co-regulator recruitment and function at these sites may vary considerably. Therefore, it is not surprising that *in vivo* functions of co-activators and co-repressors may appear different depending on the target gene. ChIP-seq data on genomic occupancy of some co-regulators are currently available (Feng *et al.* 2011, Stashi *et al.* 2014a, Tannour-Louet *et al.* 2014). However, it would be useful to re-evaluate genomic recruitment of co-regulators as well as distribution of histone modifications indicative of transcriptional activation/repression under different thyroid conditions (hypo-, eu-, and hyperthyroid) and align it with TR-binding sites. Such analysis will help put the genome-wide co-regulator binding data in the context of TR signaling, particularly now, when it became evident that thyroid status may greatly influence genomic TR binding. Moreover, while ChAP-seq and ChIP-seq data regarding chromatin TR binding provided great insight into the mechanisms of transcriptional regulation by TRs, these methods suffer certain limitations. Combining existing TR-binding data with Hi-C performed under different TH conditions, or new ChIA-PET experiments for genomic TR-binding will help to better inform us about TR-regulated distant enhancers, and potentially shed light on the negative regulation by TRs. Furthermore, comparison of TR cistrome with those of other NR prominent in the specific cell-type (e.g. LXR, PPAR, Rev-Erba in the liver) could help better understand the interplay between the different pathways, and how their roles change under different physiological conditions.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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