


Hairless is a nuclear receptor corepressor essential for skin function

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The activity of nuclear receptors is modulated by numerous coregulatory factors. Corepressors can either mediate the ability of nuclear receptors to repress transcription, or can inhibit transactivation by nuclear receptors. As we learn more about the mechanisms of transcriptional repression, the importance of repression by nuclear receptors in development and disease has become clear. The protein encoded by the mammalian *Hairless (Hr)* gene was shown to be a corepressor by virtue of its functional similarity to the well-established corepressors N-CoR and SMRT. Mutation of the *Hr* gene results in congenital hair loss in both mice and men. Investigation of *Hairless* function both *in vitro* and in mouse models *in vivo* has revealed a critical role in maintaining skin and hair by regulating the differentiation of epithelial stem cells, as well as a putative role in regulating gene expression via chromatin remodeling.

Received October 20th, 2009; Accepted December 11th, 2009; Published December 31st, 2009 | **Abbreviations:** HR: Hairless protein; N-CoR: nuclear receptor corepressor; ROR: retinoic acid receptor-related orphan receptor; SMRT: silencing mediator of retinoic acid and thyroid hormone receptors; TH: thyroid hormone; TR: thyroid hormone receptor; VDR: vitamin D receptor | Copyright © 2009, Catherine C. Thompson. This is an open-access article distributed under the terms of the Creative Commons Non-Commercial Attribution License, which permits unrestricted non-commercial use distribution and reproduction in any medium, provided the original work is properly cited.

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Introduction

Nuclear receptors regulate developmental and physiological processes by regulating the expression of specific target genes. Nuclear receptors typically activate transcription, some without the benefit of a ligand. Some nuclear receptors can repress transcription. A subset of nuclear receptors, including receptors for thyroid hormone, retinoic acid and vitamin D, repress transcription in the absence of their ligand [Jepsen and Rosenfeld, 2002; Mangelsdorf and Evans, 1995; Privalsky, 2004; Smith and O'Malley, 2004]. In general, less is known about the mechanisms of transcriptional repression, but a number of studies indicate the importance of repression by nuclear receptors in development and disease [Privalsky, 2004].

Repression by nuclear receptors occurs through their association with specific proteins that include corepressors. To date, corepressors are far less numerous than their counterparts, the coactivators. Corepressors include proteins that mediate basal repression by unliganded receptors (N-CoR, SMRT) and those that inhibit the activity of ligand-bound receptors (RIP140, L-CoR) [Cavaillès et al., 1995; Fernandes et al., 2003; Steel et al., 2005].

The first nuclear receptor corepressors to be identified were Nuclear receptor Co-Repressor (N-CoR) and Silencing Mediator for Retinoic Acid and Thyroid hormone receptors (SMRT) [Chen and Evans, 1995; Horlein et al., 1995; Sande and Privalsky, 1996]. N-CoR and SMRT were originally shown to mediate repression by unliganded TR and RAR, and were subsequently shown to play a role in transcriptional repression by other factors, as well [Privalsky, 2004]. Both N-CoR and SMRT bind to retinoic acid receptors (RAR) and thyroid hormone

receptors (TR) in the absence of ligand, and mutant receptors that lack interaction with N-CoR and SMRT no longer repress [Chen and Evans, 1995; Horlein et al., 1995].

Similar to coactivators, N-CoR and SMRT are part of large multiprotein complexes that function via associated enzymatic activities that modify chromatin structure, such as histone deacetylation. For N-CoR and SMRT, functional domains and interacting protein complexes have been characterized in great detail (reviewed in [Perissi and Rosenfeld, 2005; Privalsky, 2004]). N-CoR and SMRT are broadly expressed.

The protein encoded by the mammalian *Hairless (Hr)*, formerly *hr* gene was initially shown to be a nuclear receptor corepressor by virtue of its functional similarities to N-CoR and SMRT, including: 1) interaction with nuclear receptors (TR) in the absence of ligand, 2) mediating repression by unliganded TR, 3) multiple independent repression domains, 4) multiple receptor interaction domains comprised of conserved hydrophobic residues, and 5) interaction with histone deacetylases (HDACs) [Guenther et al., 2000; Horlein et al., 1995; Huang et al., 2000; Kao et al., 2000; Li et al., 1997a; Nagy et al., 1997; Ordentlich et al., 1999; Perissi et al., 1999; Wen et al., 2000; Xu et al., 2002]. HR can also inhibit transcription by both activated and orphan receptors.

The *Hr* gene is essential for proper skin function, as both humans and mice with mutations in *Hr* suffer from congenital hair loss and in some cases epidermal abnormalities [Ahmad et al., 1999; Panteleyev et al., 1998b]. This review will summarize the properties of HR protein function as a nuclear receptor corepressor, including its *in vivo* role in regulating epithelial stem cell differentiation.

History of Hairless

Research on Hairless began with the simple discovery of a mouse with no hair [Brooke, 1926; Sumner, 1924]. Studies of these *hairless* (Hr^{hr}) mutant mice showed that initial hair growth is normal, but after the hair is shed it does not grow back. This observation implicated the *Hr* gene as an essential regulator of the hair cycle, the process by which hair follicles are maintained in adult mammals [Mann, 1971; Montagna et al., 1952; Orwin et al., 1967; Panteleyev et al., 1998a; Zarach et al., 2004]. Over 60 years later, the murine *Hr* gene was isolated by cloning the retroviral insertion site in the original Hr^{hr} allele [Cachon-Gonzalez et al., 1994; Stoye et al., 1988]. The rat *Hr* gene was concurrently isolated as a gene that is regulated by thyroid hormone (TH) in neonatal rat brain [Thompson, 1996]. The identification of the rodent *Hr* genes [Cachon-Gonzalez et al., 1994; Stoye et al., 1988; Thompson, 1996] led to the identification of the human *Hr* gene, revealing that *Hr* is mutated in congenital hair loss disorders (alopecia universalis, papular atrichia) that phenotypically resemble the mouse mutants [Ahmad et al., 1998; Cichon et al., 1998].

Identifying the molecular function of HR

The rat *Hr* gene encodes a 130 kDa protein (HR) that at the time did not show significant homology to known structural or functional motifs, other than several cysteine residues proposed to form a zinc finger [Cachon-Gonzalez et al., 1994]. Thus, the primary sequence of HR did not provide insight into its function as a corepressor.

Identifying the function of the HR protein as a nuclear receptor corepressor was facilitated by the surprising observation that HR interacts with TR. Interaction with TR was discovered in a yeast two-hybrid screen for HR-interacting proteins [Thompson and Bottcher, 1997]. The interaction of HR with TR was demonstrated *in vitro* using multiple biochemical assays, and *in vivo* by coimmunoprecipitation with both overexpressed and endogenous proteins [Potter et al., 2001; Potter et al., 2002; Thompson and Bottcher, 1997]. Evidence that HR bound to TR more avidly in the absence of TH suggested that HR might function as a corepressor [Thompson and Bottcher, 1997].

The results of cotransfection assays demonstrated that HR can mediate repression by unliganded TR, while HR has little effect on transcriptional activation by TR [Potter et al., 2001]. Repression by HR requires receptor binding, as both deletion and point mutants of HR that lack TR-binding no longer repress [Potter et al., 2001]. HR can mediate repression by unliganded TR on different TH response elements (TREs) [Potter et al., 2001; Potter et al., 2002]. Notably, HR represses TR-mediated basal transcription most effectively in pituitary-derived cell lines, a cell type in which HR is endogenously expressed, and not in many other cell lines such as CV-1 and COS. This may reflect the presence of tissue-specific factors, or may be due to compensation by N-CoR and/or SMRT expression in most cells [Misiti et al., 1998].

Evidence that HR expression is induced by TH in brain and other tissues suggests that HR would not act as a corepressor for TR *in vivo*. However, HR could function via TR in tissues in which HR expression is not TH-dependent, such as skin and adult brain [Thompson, 1996]. In addition, recent work indicates that HR can suppress TR-mediated transcriptional activation at physiologic TH concentrations [Thompson and Beaudoin, 2006]. Thus, HR may modulate TR/TH signaling in addition to mediating basal repression.

HR functional domains

Repression domains

Analysis of HR deletion derivatives expressed as fusion proteins with the GAL4 DNA binding domain revealed three domains capable of mediating repression [Potter et al., 2001]. Repression domains include a single amino terminal domain (amino acids 236-450; RD1), and two carboxyl terminal domains (amino acids 750-864, RD2; amino acids 864-981, RD3) (Figure 1). The functional organization differs from N-CoR and SMRT, as RD2 overlaps with a receptor interaction domain (see below).

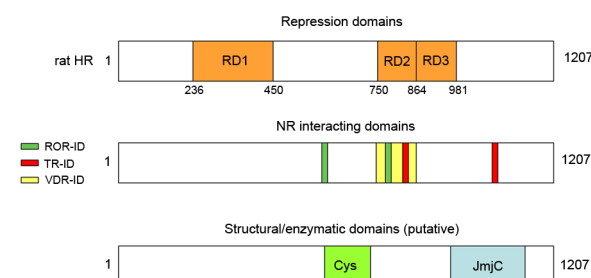


Figure 1. Schematic representation of rat HR structural and functional domains. Repression domains (RD1, 236-450; RD2, 750-864; RD3, 864-981); TR-interacting domains (TR-ID1, 816-830; TR-ID2, 1026-1038); ROR-interacting domains, ROR-ID1, 586-590; ROR-ID2, 778-782); cysteine-rich domain, 587-712; JmjC domain, 964-1175. Note that rat HR is 1207 amino acids, mouse and human HR initiate at an internal AUG (amino acid 27 in rat *Hr*) and are 1182 and 1189 amino acids, respectively.

TR-interacting domains

Analysis of a series of HR deletion derivatives in multiple binding assays (far western, yeast two hybrid, coimmunoprecipitation) showed that HR interacts with TR via two carboxyl terminal regions (between amino acids 750-864 and 980-1084) (Figure 1), with the amino terminal region interacting with higher affinity [Potter et al., 2001]. Specific amino acids required for interaction were identified using site-directed mutagenesis, and essential residues were identified as those that inhibited or abolished interaction by yeast two hybrid and far western analyses [Potter et al., 2001]. Compiling essential residues in the two TR interaction domains (TR-ID1, 816-830; TR-ID2, 1026-1038) revealed a consensus motif for HR binding to TR (I/L-I-X-X-L/V-V) similar to those identified for N-CoR and SMRT binding to TR and RAR, including the L/I-X-X-I/V-I motif termed the CoRNR box [Cohen et al., 2001; Hu and Lazar, 1999; Nagy et al., 1999; Perissi et al., 1999; Webb et al., 2000] and the extended amphipathic α helix predicted and subsequently

demonstrated to mediate corepressor: receptor interaction [Perissi et al., 1999; Xu et al., 2002]. The spacing of hydrophobic residues is conserved in the HR consensus, while the identity of hydrophobic residues is not, suggesting that the identity of specific residues determines the specificity of corepressor: receptor interaction. For example, HR does not bind to RAR, and SMRT shows higher affinity for binding to RAR than to TR [Potter et al., 2001; Privalsky, 2004].

JmjC domain

Subsequent to the initial identification of the *Hr* gene and predicted protein, a JmjC domain was recognized in the HR carboxyl terminus (amino acids 964-1175, rat HR) [Clissold and Ponting, 2001]. The JmjC domain is one of multiple conserved motifs originally identified in the *Jumonji* (JMJ) protein, a transcriptional repressor [Clissold and Ponting, 2001; Kim et al., 2003; Takeuchi et al., 1995; Toyoda et al., 2003]. The JmjC domain is conserved in a number of proteins, but until recently, had no known function [Clissold and Ponting, 2001; Takeuchi et al., 1999; Takeuchi et al., 2006; Takeuchi et al., 1995].

Recent work has shown that the JmjC domains in a number of proteins function as histone demethylases [Klose et al., 2006; Tsukada et al., 2006], including one (JMJD2A, also known as JHDM3A, KDM4A) that is found in a complex with N-CoR [Zhang et al., 2005]. Although the HR protein lacks conserved coregulator binding sites found in these proteins, preliminary results suggest that the JmjC domain in HR has a similar enzymatic activity with novel specificity (J. M. Sisk and C.C.T., unpublished observations). If so, part of the repression function of HR and other JmjC-domain containing proteins may lie in providing an additional component of the histone code for regulating transcriptional activity.

Interactions

Transcriptional repression often results from the association of corepressors with histone deacetylases (HDACs) [Burke and Baniahmad, 2000; Glass and Rosenfeld, 2000; Pazin and Kadonaga, 1997]. Multiple HDACs have been shown to interact with corepressors, and biochemical analyses have indicated that HDAC3 is found in an endogenous complex with corepressors [Guenther et al., 2001; Li et al., 2000a; Wen et al., 2000; Yoon et al., 2003]. HR interacts indirectly with several HDACs, including HDACs 1, 3 and 5 [Potter et al., 2001; Potter et al., 2002]. HR corepressor activity is reduced in the presence of an HDAC inhibitor (trichostatin A), suggesting that HDAC activity is partially responsible for repression by HR [Potter et al., 2002].

Biochemical studies have revealed the presence of N-CoR and SMRT in large multiprotein complexes that include HDACs, TBL1, TBLR1, as well as other proteins [Privalsky, 2004]. Although similar biochemical studies have not yet been performed with HR, based on its functional similarities, HR likely functions as part of a multiprotein complex.

HR is a corepressor for multiple nuclear receptors

HR interacts with ROR α , an orphan nuclear receptor important for cerebellar development

Unlike N-CoR and SMRT, HR does not interact with retinoic acid receptor (RAR); however, HR does bind to ROR α (Retinoic acid receptor-related Orphan Receptor α). ROR α is a constitutively-active orphan nuclear receptor that is critical for cerebellar development [Dussault et al., 1998; Hamilton et al., 1996; Matysiak-Scholze and Nehls, 1997]. HR interacts with ROR α , and inhibits transcriptional activation by all ROR isoforms (α , β , γ) [Moraitis et al., 2002]. Further analysis has shown that HR binding protects ROR α from proteasome-mediated degradation [Moraitis and Giguere, 2003]. The domain of HR that interacts with ROR was mapped to a region with two motifs with the LxxLL consensus sequence for coactivator interaction [Heery et al., 1997; McInerney et al., 1998]. Surprisingly, mutation of both LxxLL motifs abolished the ability of HR to repress ROR α activity, while mutation of TR-ID1 had no effect on activity [Moraitis et al., 2002]. Since coactivators interact with the AF-2 domain of nuclear receptors, the requirement for this domain was tested by changing the AF-2 domain of RAR α (which does not interact with HR) to that of ROR α . Remarkably, the specificity of HR corepressor action could be transferred to RAR α by exchanging the AF-2 domain, a change of only 5 amino acids [Moraitis et al., 2002]. These results unexpectedly demonstrated that in the context of ROR, the repressive activity of HR is dependent on coactivator-type binding motifs.

HR interacts with vitamin D receptor, a nuclear receptor important for skin function

The interaction of HR with TR does not easily explain the prominent skin phenotype in *Hr* mutants, as although TH-deficient humans and mice do have some hair and skin defects, none are as striking as the complete hair loss and skin wrinkling observed in *Hr* mutant [Alonso and Rosenfield, 2003]. Targeted deletion of RXR α and β in mice results in a hair loss phenotype [Li et al., 2000b]. RXRs are heterodimeric partners for multiple nuclear receptors, and there is no evidence of direct interaction between HR and RXRs. However, HR does have multiple receptor-interacting domains, suggesting that HR may contact RXR in the context of a heterodimer with either TR or VDR.

Current evidence suggests that the receptor through which HR acts in the skin is the vitamin D receptor (VDR). HR interacts with VDR in multiple biochemical assays, including GST pulldown and coimmunoprecipitation [Hsieh et al., 2003]. Interaction of HR with VDR is weaker than interaction with TR and may require other proteins (C.C.T., unpublished observations). The region of VDR interaction with HR overlaps the sites responsible for interaction with TR and ROR (Figure 1); the specific amino acids required for interaction of HR with VDR have not been determined. In contrast to its role with TR, HR

represses VDR activity both in the absence and presence of vitamin D [Hsieh et al., 2003]. HR can inhibit expression from both synthetic and naturally occurring VDR-responsive promoters, and in cultured keratinocytes, a cell type in which HR and VDR are normally expressed ([Hsieh et al., 2003] and G.M.J. Beaudoin and C.C.T., unpublished observations). *Hr* and VDR mRNAs colocalize in a subset of cells in the skin, indicating that the biochemically defined interaction of HR and VDR can occur *in vivo* [Hsieh et al., 2003]. Both mice and humans with mutations in VDR have a hair loss phenotype which resembles that of mice and humans with mutations in *Hr* [Li et al., 1997b; Yoshizawa et al., 1997]. Notably, although mutations in VDR cause hair loss, vitamin D deficiency does not result in hair loss either in mice or humans [Sakai et al., 2001]. This observation indicated that transcriptional activation by vitamin D-bound VDR is not essential for hair cycle regulation, and suggested that repression could be important for this function. Recent work supporting the role of repression by VDR in hair cycling has come from studies showing that mutant VDRs which do not activate transcription in response to vitamin D can rescue the hair loss phenotype of VDR null mice [Skorija et al., 2005]. The mutant VDRs retain the ability to bind HR and repress basal transcription, suggesting that repression by unliganded VDR via HR is essential for VDR function in the skin [Skorija et al., 2005].

Thus, multiple studies have shown that HR functions as a corepressor for nuclear receptors. By analogy with other corepressors, HR may possess additional functional properties. For example, it is not yet known whether HR can bind to antagonist-bound steroid receptors, and/or act as corepressors for other transcription factors [Jepsen and Rosenfeld, 2002; Privalsky, 2004]. The data summarized above is from rat HR, but the human HR protein also functions as a corepressor [Wang et al., 2007]. Thus, the function of *Hr* is conserved between mammalian species.

Hr expression

Hr mRNA is most abundantly expressed in the skin and brain. Within the skin, *Hr* mRNA is localized to specific cells of the epidermis and hair follicles. Early in postnatal development, *Hr* mRNA is detected in epidermis, then expression is downregulated as development proceeds. In hair follicles, *Hr* mRNA is present throughout the hair cycle (Figure 2, described below) [Panteleyev et al., 2000]. Surprisingly, analysis of HR protein expression showed that follicles actively growing hair do not contain detectable HR protein [Beaudoin et al., 2005]. The discordance between *Hr* mRNA expression and protein expression points to posttranscriptional or posttranslational control of HR protein expression and/or stability. Indeed, recent work has shown a novel mechanism of translational regulation residing in a conserved upstream open reading frame (ORF), supporting the idea that regulation of HR protein expression is critical for its role in regulating hair growth [Wen et al., 2009]. The expression and function of HR

protein in the skin has been studied in detail and is described below.

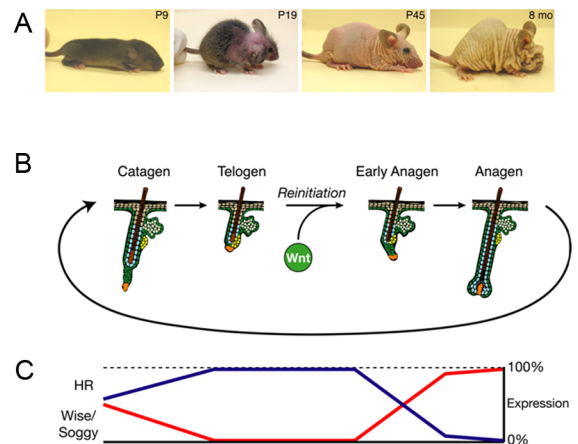


Figure 2. Model for HR action in hair regrowth. A) Onset of phenotype in *Hr* knockout mice. Note that initial hair growth is normal, but after the first hair cycle (around P17) the hair is shed and does not regrow. *Hr*-rhino and knockout mice get progressively more wrinkled with age. P9, postnatal day 9; P19, postnatal day 19; P45, postnatal day 45; 8 mo, 8 months. B) Schematic representation of hair cycle. Wnt signaling initiates the transition from rest (telogen) to growth (anagen). C) Summary of HR protein expression through the hair cycle in relation to Wise and Soggy mRNA expression. Regression (catagen) is characterized by the upregulation of HR protein and concurrent downregulation of Wise/Soggy mRNA. HR protein expression during rest (telogen) is predicted to repress Wise/Soggy mRNA expression, allowing Wnt activation. Subsequent to the reinitiation of hair growth (anagen), HR protein is downregulated while Wise/Soggy mRNA increases, resulting in a transient decrease in Wnt signaling required for proper hair growth. In *Hr* mutants, uncontrolled expression of Wise/Soggy and possibly other Wnt inhibitors prevents hair regrowth by suppressing Wnt signaling. Figure adapted from (Thompson et al., 2006).

In the brain, *Hr* mRNA and protein are broadly expressed, including in cerebellum, somatosensory cortex, hippocampus, dentate gyrus, retina and inner ear [Cachon-Gonzalez et al., 1999; Potter et al., 2002; Thompson, 1996]. In neonatal rodent brain, *Hr* mRNA expression is induced by TH, which is significant because of the well-established role of TH in brain development [Thompson, 1996; Thompson and Bottcher, 1997; Thompson and Potter, 2000]. Expression of *Hr* is also regulated by TH in other tissues, although notably not in skin or in adult brain [Thompson, 1996].

Hr mRNA is expressed at low levels in several other tissues. *Hr* mRNA was detected in a wide range of tissues as early as embryonic day 12.5 in mice [Cachon-Gonzalez et al., 1999]. Tissues with relatively high levels of *Hr* expression include oral, nasal, bladder and rectal epithelia, which are of interest based on the proposed function of HR protein in skin epithelial cells [Beaudoin et al., 2005]. *Hr* mRNA was also detected in embryonic cartilage and in embryonic and adult retina ([Cachon-Gonzalez et al., 1999]; J. M. Sisk and C.C.T., unpublished observations).

Notably, expression is detected in a number of tissues in which thus far no phenotype has been observed in *Hr* mutants. This may reflect redundancy with other corepressors, or regulation of protein expression. For

example, the onset of the skin phenotype is correlated with detection of HR protein. Some phenotypic abnormalities have been observed. In the brain, abnormalities in neuronal morphology, inner ear and retinal cytoarchitecture have been observed [Cachon-Gonzalez et al., 1999; Garcia-Atares et al., 1998]. Alterations in the cerebellar cortex include changes in the size and shape of Purkinje cells, which may be related to HR interaction with ROR α , as both ROR α and TH play roles in Purkinje cell development and function [Dussault et al., 1998; Garcia-Atares et al., 1998; Hamilton et al., 1996; Matysiak-Scholze and Nehls, 1997; Steinmayr et al., 1998]. While the majority of patients with mutations in *Hr* have not been assessed for neural function, there are two cases of patients homozygous for a mutation in *Hr* that suffer from mental retardation [Aita et al., 2000; del Castillo et al., 1974]. In colon, *Hr^{fl}* mice show hyperproliferation of the colon epithelium and an increased number of villi [Cachon-Gonzalez et al., 1999]. This reflects aspects of the skin phenotype and may be typical of other tissues with epithelial components in which HR is expressed.

Cellular mechanism and cellular biology

The HR protein is nuclear [Potter et al., 2002; Thompson and Bottcher, 1997]. In some cells, HR is found in subnuclear structures that contain both HDACs and corepressors known as matrix-associated deacetylase (MAD) bodies [Downes et al., 2000; Potter et al., 2001]. HR colocalizes with HDACs and other corepressors, including SMRT, HDAC3 and HDAC 5 [Potter et al., 2001]. Biochemical studies have revealed the presence of N-CoR and SMRT in large multiprotein complexes that include HDACs, TBL1, TBLR1, as well as other proteins (reviewed in [Privalsky, 2004]). Although similar biochemical studies have not been performed with HR, based on functional conservation, HR may be part of multiprotein complexes that include other corepressors.

The HR protein is phosphorylated *in vivo*, initially shown by immunoprecipitation of metabolically labeled cell extracts (G. B. Potter and C. C. T., unpublished observations). Proteomic profiling of phosphoproteins in human skin fibroblasts has identified two sites of phosphorylation in human HR (S416, S425) [Yang et al., 2006]. It is not known if phosphorylation is important for corepressor function as has been shown for SMRT [Hong and Privalsky, 2000].

To date, HR does not have a defined enzymatic activity although the presence of a conserved JmjC domain and preliminary results (C.C.T. and J.M. Sisk, unpublished observations) indicate that HR may have histone demethylase activity. As a corepressor, HR does not directly regulate target gene expression but does so via multiple nuclear receptors. Genes that are misregulated in the *Hr* null mouse model have been identified [Beaudoin et al., 2005; Zarach et al., 2004]. Further investigation is required to determine whether any or all of these genes are directly regulated by HR:nuclear receptor complexes.

Systemic biology and physiology

Based on the phenotype of *Hr* mutant mice and humans, *Hr* has an important role in the skin. The skin phenotype in all known murine *Hr* alleles is initially similar (Figure 2A). Animals appear normal for about the first two weeks after birth, after which they begin to lose their hair and eventually all hair is lost. In *hairless* (*Hr^{hr}*) mutant mice, after the initial hair loss the skin remains relatively smooth. In *rhino* (*Hr^{rh}*) mutant mice, once hair is lost the skin subsequently becomes wrinkled [Mann, 1971]. *Hairless* “knockout” mice (*Hr^{-/-}*) made by targeted deletion have a more severe wrinkling phenotype [Zarach et al., 2004]. The severity of the phenotype is correlated with the severity of the deficit in *Hr* mRNA expression [Thompson and Beaudoin, 2006; Zarach et al., 2004].

Mammalian skin is comprised of three distinct structures, the overlying epidermis, hair follicles and sebaceous glands [Stenn and Paus, 2001]. All of these structures are affected in *Hr* mutant skin. The first phenotypic change detected in *Hr* mutant skin is the widening of the hair follicle near the epidermis [Mann, 1971; Zarach et al., 2004], forming as a consequence of aberrant cell proliferation and differentiation [Zarach et al., 2004]. The next alteration observed is that the lower part of the hair follicle disintegrates, and follicles do not reform as they would in wild type mice [Mann, 1971; Montagna et al., 1952; Orwin et al., 1967; Panteleyev et al., 1999]. Loss of hair follicles is complete, as markers expressed in regenerating hair follicles are not present [Panteleyev et al., 1998b; Zarach et al., 2004]. Cysts develop in the dermis [Mann, 1971; Montagna et al., 1952; Zarach et al., 2004], which increase in size with age, due in part to proliferation of the cells lining the cyst [Zarach et al., 2004]. The cysts have properties of sebaceous glands, as they contain lipids and express *Scd1*, but do not express adipocyte-specific markers [Bernerd et al., 1996; Mann, 1971; Zarach et al., 2004]. The cysts in *Hr^{-/-}* skin contribute to the overall thickness of the skin, and the wrinkling phenotype.

How does a single gene/protein affect all of these processes in the skin? Although the epidermis, sebaceous glands, and hair follicles are distinct structures with characteristic properties, all are maintained by the same population of epithelial stem cells. The undifferentiated progenitors of all three components originate as stem cells found in a region of the follicle outer root sheath (ORS) known as the bulge [Alonso and Fuchs, 2003; Tumber et al., 2004]. The current model for stem cell-mediated maintenance of the skin is that the bulge stem cells generate progenitor cells, and that production and maintenance of all three components occurs through the differentiation of progenitor cells into the mature cells that comprise each structure [Alonso and Fuchs, 2003].

Based on the increase in epidermal and sebaceous cells and decrease in hair cells in *Hr^{-/-}* skin, my lab proposed a model in which the role of HR is to regulate epithelial stem cell differentiation, and that disruption of timing in *Hr* mutants leads to changes in cell fate favoring

epidermis and sebaceous cells at the expense of the hair follicle [Zarach et al., 2004].

To test this model, we generated and characterized a gain of function mutant mouse in which the *Hr* cDNA was expressed in K14 positive cells (K14-rHr). Based on the analysis of K14-rHr skin, the model generally holds true ([Beaudoin et al., 2005] and unpublished observations). In K14-rHr epidermis, there is an increased number of undifferentiated and a reduced number of terminally differentiated keratinocytes, indicating a delay in epidermal differentiation. The hair of the K14-rHr transgenic mice is shorter than normal due to reduced production of each lineage of the hair bulb, and the hair cycle is accelerated. Sebaceous glands were not initially detected in K14-rHr skin, indicating delayed sebaceous gland differentiation. Thus, the K14-rHr transgenic phenotype is consistent with the model that HR promotes hair cell fate while suppressing progression into cells that populate the epidermis and sebaceous glands.

What about the most striking part of the phenotype, hair loss? Unlike the epidermis and sebaceous gland, the hair follicle is periodically destroyed and regenerated in a cyclic manner (Figure 2B) [Hardy, 1992; Stenn and Paus, 2001]. The hair cycle can be divided into distinct stages: **anagen**, **catagen**, and **telogen**. Active growth of hair occurs during **anagen** [Hardy, 1992; Stenn and Paus, 2001]. Once the hair has grown to an appropriate length, the hair follicle enters **catagen**, a period of regression in which the lower part of the hair bulb is destroyed by apoptosis [Lindner et al., 1997; Stenn and Paus, 2001]. After catagen is completed, the follicle enters a period of rest, **telogen** [Stenn and Paus, 2001]. Hair growth is reinitiated by reentry into anagen and the hair bulb is regenerated [Hardy, 1992; Stenn and Paus, 2001]. Signaling between the cells of the dermal papilla (mesenchymal cells) and bulge (epithelial cells), appears to be critical for re-growth [Jahoda et al., 1984; Oliver and Jahoda, 1988].

The temporal and spatial localization of the HR protein in hair follicles suggested that HR functions in both follicle regression and hair regrowth [Beaudoin et al., 2005]. Within hair follicles, HR protein is detected in the outer root sheath (ORS), which includes the bulge region. HR protein is initially detected as follicles start to regress (catagen), which coincides with the onset of phenotypic alterations in *Hr* mutant mice [Panteleyev et al., 1999; Zarach et al., 2004]. HR expression is maintained through the rest phase (telogen) and during the transition to hair regrowth. Once the hair follicle has regenerated, HR protein is undetectable in follicles actively growing hair (anagen) [Beaudoin et al., 2005]. Evidence that HR plays an active role in reinitiating hair growth came from studies in which the hair re-growth defect in *Hr*^{-/-} mice was rescued by expression of *Hr* in K14-positive cells. Mice that express HR only in K14-positive cells ("transgenic rescue") lose their hair, but regenerate hair follicles and produce normal hair [Beaudoin et al., 2005]. Thus, HR is both necessary and sufficient to reinitiate hair growth.

Transcriptional regulation by HR *in vivo*

Since HR is a corepressor, the defects observed in *Hr* mutant skin presumably arise from altered gene expression, specifically upregulation of gene expression. Putative target genes were identified using microarray analysis to compare gene expression between normal and *Hr*^{-/-} skin at an age prior to observable phenotypic changes (P12). Microarray analysis revealed significant (> 2-fold) upregulation of several genes in *Hr*^{-/-} skin with most of the identified genes playing roles in epidermal differentiation [Zarach et al., 2004]. These genes include filaggrin, loricrin, keratin 10, caspase-14, keratinocyte differentiation associated protein, and calmodulin-4) [Chien et al., 2002; Eckhart et al., 2000; Hwang and Morasso, 2003; Koshizuka et al., 2001; Lippens et al., 2000; Oomizu et al., 2000].

Misregulation of these genes likely underlies the initial epidermal phenotype, as *in situ* hybridization showed that transcriptional changes were localized to the upper part of the hair follicle, the site at which the phenotype is initially manifested [Zarach et al., 2004]. Genes regulated by HR with a role in reactivation of hair growth were identified as genes (identified from the P12 microarray) that were 1) upregulated in *Hr*^{-/-} skin and 2) downregulated (relative to *Hr*^{-/-}) in transgenic rescue skin, at the time hair follicles regenerate (P24) using Northern analysis [Beaudoin et al., 2005]. Genes that met these criteria included Wise (Wnt modulator in surface ectoderm), a protein that modulates Wnt signaling [Itasaki et al., 2003], and Soggy, a protein related to Dickkopfs, which inhibit Wnt signaling [Krupnik et al., 1999; Logan and Nusse, 2004].

The identification of putative inhibitors of Wnt signaling suggested that HR might be regulating this important signaling pathway. Indeed, expression of an endogenous Wnt target gene, *Axin2*, is correlated with the *Hr* genotype; *Axin2* is expressed in wild type and transgenic rescue skin, and is not detected in *Hr* knockout skin [Beaudoin et al., 2005].

A number of studies have shown that Wnt signaling (like HR function) is critical for hair follicle regeneration [Huelsenken et al., 2001; Lo Celso et al., 2004; Merrill et al., 2001; Niemann et al., 2002; Van Mater et al., 2003]. For example, blocking Wnt signaling by either loss of β -catenin expression or expression of a mutant Lef1 blocks reinitiation of hair growth [Huelsenken et al., 2001; Merrill et al., 2001; Niemann et al., 2002]. Wnt signaling is sufficient for follicle regeneration, as inducing nuclear translocation of β -catenin in a transgenic mouse model induces hair regrowth [Lo Celso et al., 2004; Van Mater et al., 2003]. Transient Wnt signaling appears to be critical, as constitutive expression of a stabilized β -catenin induces skin tumors in mice and humans [Chan et al., 1999; Gat et al., 1998; Lo Celso et al., 2004; Van Mater et al., 2003].

The established role of Wnt signaling in hair regrowth suggested that HR may regulate hair follicle regeneration by repressing the expression of one or more Wnt

inhibitors (Wise, Soggy), thereby allowing Wnt activation. Multiple lines of evidence support this hypothesis. First, Wise inhibits Wnt-induced reporter gene expression, including activation by Wnt10b, one of two Wnts that are proposed regulators of hair regrowth [Beaudoin et al., 2005; Reddy et al., 2001]. Second, expression of HR protein and Wise and Soggy mRNAs is inversely correlated *in vivo*, and HR suppresses expression of the Wise promoter *in vitro* [Beaudoin et al., 2005]. Third, nuclear HR protein colocalizes with expression of a Wnt-responsive reporter gene in the skin of TOP-Gal transgenic mice [Beaudoin et al., 2005; DasGupta and Fuchs, 1999], and no reporter gene expression is detected in the skin of TOP-Gal/*Hr*^{-/-} mice (J. M. Sisk and C.C.T., unpublished observations).

These data support a model for HR action in the skin in which HR regulates hair follicle regeneration by promoting Wnt signaling (Figure 2). The HR protein promotes Wnt/ β -catenin activation, and thus hair growth initiation, by repressing expression of soluble Wnt inhibitors at the proper time in the hair cycle. This model includes a means of inducing transient Wnt signaling, via the decrease in HR protein expression and concomitant increase in Wise expression subsequent to the reinitiation of hair growth. This model also specifies a molecular mechanism to explain the failure of hair follicles to regenerate in *Hr* mutant skin: inhibition of Wnt signaling caused by the persistent expression of Wnt inhibitors.

The function of HR in the epidermis and sebaceous glands may be through Wnt signaling as well, as HR expression in K14-positive cells also prevents the wrinkling phenotype. This suggests that HR expression also rescues the epidermal and sebaceous phenotypes, and is consistent with the idea that HR is regulating epithelial stem cell differentiation via the modulation of Wnt signaling. Unlike reinitiation of hair growth, hair morphogenesis does not seem to be sensitive to loss of *Hr* expression; an alternative factor may regulate Wnt signaling during morphogenesis.

The regulation of Wnt signaling by HR in the hair follicle is likely to be one component of a complex interplay of signaling pathways that regulate hair regrowth. Other pathways that have already been implicated in hair cycling include Sonic hedgehog and BMP signaling [Botchkarev and Sharov, 2004; Callahan and Oro, 2001]. Notably, Wise was independently identified as an inhibitor of BMP signaling (Sostdc1, Ectodin, USAG-1) [Balemans and Van Hul, 2002; Laurikkala et al., 2003; Yanagita et al., 2004] raising the possibility that Wise may also influence BMP signaling in the context of follicle regeneration. In addition, HR may regulate the expression of additional factors that modulate such pathways.

Disease

The clinical importance of *Hr* function was demonstrated when the human ortholog of *Hr* was identified as the gene mutated in two rare human diseases, alopecia universalis and atrichia with papular lesions [Ahmad et al., 1998; Cichon et al., 1998; Sprecher et al., 1998]. It was

subsequently recognized that many cases of alopecia universalis may be misdiagnosed cases of papular atrichia, with the development of papular rash a phenotypic variation [Sprecher et al., 1999a; Sprecher et al., 1999b]. As in mice, humans with these diseases initially have normal hair, but within a few years the hair falls out and in general does not regrow [Ahmad et al., 1998; Cichon et al., 1998; Sprecher et al., 1998]. Both human diseases exhibit histological changes similar to those observed in *Hr* mutant mice [Ahmad et al., 1998; Cichon et al., 1998; Sprecher et al., 1998].

Recently, an autosomal dominant hair loss disorder, Marie Unna hereditary hypotrichosis, was mapped to the *Hr* locus, not to the coding region but to a conserved upstream ORF [Wen et al., 2009]. This elegant work showed that the ORF controlled the level of HR protein expression by a translational mechanism, reinforcing the idea that HR protein levels are critical for modulating hair growth.

As in mouse, there are multiple mutant human *Hr* alleles, which include nonsense and missense mutations. Unlike in mice, the human phenotype is not correlated with the severity of the mutation. While nonsense mutations are presumably loss-of-function, a number of missense mutations have been tested to assess corepressor function (Figure 3).

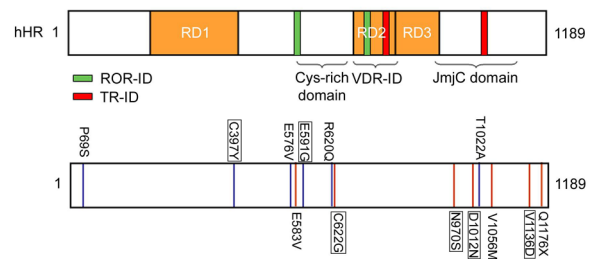


Figure 3. Mutant *Hr* alleles from human patients lack corepressor activity. Naturally-occurring HR mutants tested for corepressor activity. Top, schematic representation of human HR protein (hHR) functional domains. Bottom, positions of mutated residues and altered amino acids. Blue indicates polymorphism, red indicates missense mutation. Mutants were tested for the ability to mediate repression by VDR and TR. Mutations in human HR were tested with VDR, mutants in rat HR (corresponding to the mutations found in human *Hr* alleles) were tested with TR (boxed). All proteins were expressed at equivalent levels except for P69S. All expressed proteins retain interaction with TR and VDR. In general, mutants lack corepressor activity, polymorphisms retain corepressor activity. Missense mutants (red): E583V (Paradisi et al., 2003); C622G/C642G (Aita et al., 2000); N970S/N988S (rat) (Kruse et al., 1999); D1012N/D1030N (rat) (Klein et al., 2002); V1056M (Zlotogorski et al., 2002); V1136D/V1154D (rat) (Cichon et al., 1998); Q1176X (Henn et al., 2002). Polymorphisms (blue): P69S, C397Y/C422Y (rat), A576V, E591G/E611G (rat), R620Q, T1022A (Hillmer et al., 2002).

Surprisingly, mutations do not in general map to established functional domains, although several mutations map to the JmjC domain which has putative histone modifying activity. A set of HR mutants (amino acid changes associated with alopecia/atrichia) and polymorphisms (amino acid changes occurring in HR that are not associated with atrichia/alopecia) were tested for function with VDR [Wang et al., 2007]. This work showed that stably expressed missense mutants (except E583V) lose corepressor activity, while polymorphisms maintain

corepressor activity [Wang et al., 2007]. Some polymorphisms (T1022A) do show partial activity. All mutants retain interaction with VDR, so the lack of corepressor function is not due to loss of receptor interaction. These investigators demonstrated that HR mutants show reduced interaction with HDAC1, suggesting that impaired HDAC interaction contributes to loss of corepressor function.

Supporting this idea, recent work has shown that a human HR isoform (Δ 1072-1126) that arises from alternative splicing maintains interaction with VDR, but lacks both corepressor activity and HDAC1 binding [Malloy et al., 2009]. The deletion in this variant also removes part of the JmjC domain, so disruption of this domain may also contribute to loss of corepressor activity. A subset of HR mutants were tested for function with TR, and showed similar results; all mutants tested maintained interaction with TR but lost corepressor activity [Thompson et al., 2006]. The correlation between corepressor activity and mutant phenotype indicates that disruption of corepressor function underlies congenital hair loss disorders [Thompson et al., 2006; Wang et al., 2007].

Conclusions

The biological role of transcriptional activation by nuclear receptors is well established. Far less is known about the role of repression, but the study of corepressors is helping to elucidate the mechanisms and significance of repression by nuclear receptors. The study of HR over the past decade has contributed to this understanding.

HR corepressor function *in vivo* is essential for maintaining skin function. HR appears to be a member of a distinct class of corepressors that are spatially and temporally restricted, as *Hr* mRNA expression is restricted to specific tissues and/or cell types, and HR protein expression is further restricted temporally. The study of the biochemical and biological roles of HR has revealed its mechanism of action in regulating developmental processes in the skin, which will likely apply to other tissues, as well.

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