Published in final edited form as:

Biochim Biophys Acta. 2012 January ; 1821(1): 213–221. doi:10.1016/j.bbali.2011.08.002.

Emerging Roles for Retinoids in Regeneration and Differentiation in Normal and Disease States

Dr. Lorraine J. Gudas

Pharmacology Department, Weill Cornell Medical College of Cornell University, 1300 York Avenue, New York, NY, 10065, USA, Phone: (212)-746-6250, Fax: (212)-746-8858, ljgudas@med.cornell.edu

Abstract

The vitamin A (retinol) metabolite, all-trans retinoic acid (RA), is a signaling molecule that plays key roles in the development of the body plan and induces the differentiation of many types of cells. In this review the physiological and pathophysiological roles of retinoids (retinol and related metabolites) in mature animals are discussed. Both in the developing embryo and in the adult, RA signaling via combinatorial Hox gene expression is important for cell positional memory. The genes that require RA for the maturation/differentiation of T cells are only beginning to be catalogued, but it is clear that retinoids play a major role in expression of key genes in the immune system. An exciting, recent publication in regeneration research shows that ALDH1a2 (RALDH2), which is the rate-limiting enzyme in the production of RA from retinaldehyde, is **highly** induced shortly after amputation in the regenerating heart, adult fin, and larval fin in zebrafish. Thus, local generation of RA presumably plays a key role in fin formation during both embryogenesis and in fin regeneration. HIV transgenic mice and human patients with HIV-associated kidney disease exhibit a profound reduction in the level of RAR β protein in the glomeruli, and HIV transgenic mice show reduced retinol dehydrogenase levels, concomitant with a greater than 3-fold reduction in endogenous RA levels in the glomeruli. Levels of endogenous retinoids (those synthesized from retinol within cells) are altered in many different diseases in the lung, kidney, and central nervous system, contributing to pathophysiology.

Keywords

dendritic cell; emphysema; epigenetics; intestine; regeneration; stem cell

Retinoids are stored in cells and active retinoids are generated locally to regulate gene expression

The vitamin A (retinol) metabolite, all-trans retinoic acid (RA), is a signaling molecule that plays key roles in the development of the body plan and induces the differentiation of many types of cells [1-3]. This review will focus on recent discoveries about the roles of retinoids in specific tissues. Although much remains to be learned, a basic outline of RA's molecular mechanisms of action has been established (Fig. 1). Retinol is obtained from our diet – we can't synthesize retinol in our cells. Retinol is taken up into some cells in the body via a

© 2011 Elsevier B.V. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

specific cell surface receptor for sRBP (RBP4) called Stra6 (Fig. 1) [4, 5]. Transcriptionally *inactive* metabolites of retinol (e.g. retinyl esters) are stored within the body in different cell types, including the liver and lung, and retinol metabolites *active* in regulating transcription (e.g., retinoic acid) are generated from these inactive retinol metabolites at precise times and in specific cell types [3] (Fig. 1). Transcriptionally active retinoids, such as RA, bind to the retinoic acid receptors α , β , and γ , that form heterodimers with retinoid X receptors α , β , and γ ; all six receptors are members of the nuclear receptor family of proteins (Fig. 1). These transcription factors mediate the majority of actions of RA and other transcriptionally active RA metabolites. However, the mechanisms that control the levels and activities of the enzymes that produce metabolites such as RA from retinol are not fully understood [6-11]. Importantly, levels of endogenous retinoids (those synthesized from retinol within our cells) are altered in many different diseases, contributing to pathophysiology (see below). In many cell types RA promotes cell differentiation and a concomitant reduction in cell proliferation; however, there also are examples of cell types that require RA for cell proliferation. Examples of both of these actions of RA are presented in this review.

Retinoid receptors act as “classical” transcription factors, but they can also promote epigenetic changes, i.e. long-term modifications of chromatin that don’t involve mutations or changes in the actual DNA sequence, during cell differentiation [12-15]. Alterations in the epigenome mediated by RA signaling can lead to cell differentiation and these alterations are an important aspect of the use of retinoids for cancer treatment [1, 16]. RARs themselves can also be modified by amino acid modifying enzymes [17]. A current challenge in this field is to understand how variations in this basic RA-associated transcriptional regulatory pathway serve to control both normal and abnormal development and the functions of various types of cells in the adult organism. This review focuses primarily on recent research that has established a role for endogenous RA in the maturation and function of several differentiated cell types, also noting the potential use of retinoids as pharmacological agents. In each case I will try to be cognizant of five key questions:

1. How is the local generation of RA achieved and does altered endogenous retinoid metabolism contribute to disease?
2. What are the retinoid target genes (both primary and secondary) in each cell type?
3. Do retinoids elicit epigenetic changes that contribute to functions in responsive cells?
4. Do alterations in transcription pathways contribute to the development of disease?
5. Can retinoids be pharmacologically useful in diseases that are specific to each tissue or cell type?

Differentiation of Foxp3⁺ Regulatory T Cells in the Intestine Requires the Metabolic Conversion of Retinol to Retinoic Acid: the Role of Mucosal Dendritic Cells

Foxp3⁺ regulatory T cells (T_{regs}) are essential for the establishment and maintenance of immune tolerance. Many T_{regs} develop their regulatory activity in the thymus, but Foxp3⁺ T_{regs} (CD4(+) CD25(+) Foxp3⁺ T cells) can also differentiate from naive precursor/progenitor cells in the periphery. Over the past few years a role for endogenous RA in the regulation of the differentiation of these naive precursor cells has begun to be delineated. Recent work has demonstrated that stimulation of the Forkhead transcription factor Foxp3⁺ T_{reg} cell maturation and responses by CD103⁺ DCs requires both TGF β and endogenous RA [18, 19]. Expression of the Forkhead transcription factor FoxP3 is essential for the production of T_{reg} cells [20], and transforming growth factor β (TGF β) promotes T_{reg} development [18]. *In vivo*, CD103⁺ mesenteric lymph node dendritic cells (DCs) can induce the development of Foxp3⁺ T_{reg} cells. Thus, RA is an essential cofactor in T_{reg} cell

differentiation from naïve precursor cells [18, 21]. As might be expected, vitamin A deficient mice show greatly reduced mucosal DC activity and fail to induce Foxp3⁺ T cells [22]. Thus, pharmacological doses of active retinoids may be useful therapeutic agents in Crohn's disease or other inflammatory intestinal disorders (see below). In fact, anti-TNF α (tumor necrosis factor α) therapy for inflammatory bowel disease also increases both the number and activity of Foxp3⁺ T_{reg} cells [23].

If RA is essential for the production of T_{regs}, how is it generated in appropriate amounts? Endogenous RA synthesis from retinol is regulated, at least in part, by Toll-like receptor (TLR) signaling in DCs, which increases the level of aldehyde dehydrogenase family 1, subfamily A2 (ALDH1a2, also called RALDH2 in DCs (Fig. 1)). This enzyme produces endogenous RA from the substrate retinaldehyde, thereby both stimulating the production of Foxp3⁺ T_{reg} cells and inhibiting T_H-17/T_H-1 and IL-23 activated autoimmune responses [22]. Interestingly, addition of prostaglandin E2 (PGE2) during DC differentiation inhibits ALDH1a2 expression and the differentiation of mouse and human DCs, blocking their induction of CCR9 expression upon T cell priming. Conversely, blocking PGE2 signaling *increases* the number of DCs, and this causes the systemic generation of RA-producing DCs and the subsequent priming of CCR9⁺ T cells in non-intestinal sites, e.g. the spleen [24].

The Wnt- β -catenin pathway in intestinal dendritic cells also regulates the anti-inflammatory response in part by stimulating T_{reg} cell induction/differentiation through an increase in expression of ALDH1a2, which produces RA [25]. Consistent with these data, β -catenin deficiency in dendritic cells results in a much more severe inflammatory response in a murine model of inflammatory bowel disease, suggesting that β -catenin signaling in dendritic cells promotes a tolerogenic state at least in part by increasing the levels of the endogenous, anti-inflammatory factors RA, IL-10, and TGF β [25].

Inflammation can alter the differentiation of naïve CD4⁺ T-cells and result in a decrease in the tolerogenic properties of mesenteric lymph node CD103⁺ DCs. The decrease in tolerogenic properties of these DCs is associated with lower expression of TGF β 2 and ALDH1a2; for example, CD103⁺ DCs isolated from mice with colitis exhibit reduced ability to induce Foxp3⁺ T_{reg} cells [26]. Similarly, while under normal physiologic conditions mesenteric lymph node DCs direct the differentiation of naïve CD4⁺ T cells to Foxp3⁺ T_{reg} cells, in the presence of the cytokine interleukin-15 (IL-15) these DCs showed reduced ability to generate differentiated Foxp3⁺ T_{reg} cells [27]. This was shown in a mouse model of celiac disease [27].

The maturation/differentiation of naïve T cells to Foxp3⁺ T_{reg} cells involves binding of the RA-bound retinoic acid receptor:retinoid X receptor (RAR:RXR) heterodimer protein complex to a DNA binding site in enhancer 1 of the Foxp3 gene, and this binding increases the transcriptional activation of the Foxp3 gene in response to RA [28]. Major epigenetic changes also occur at the Foxp3 gene during the differentiation of CD4⁺ T cells to Foxp3⁺ T_{reg} cells [29], and it is reasonable to speculate that RA may play a role in establishing these epigenetic changes.

In a practical application, the differentiation of human naïve CD4⁺ cells to immunosuppressive Foxp3⁺ T_{reg} cells was accelerated when the naïve CD4⁺ cells were treated with RA in addition to IL-2 and TGF β ; the authors suggest that this differentiation strategy could be used to treat human autoimmune diseases and to prevent allograft rejection [30]. Another potential method for reducing the severity of autoimmune diseases is by preventing the differentiation of proinflammatory IL-17⁺ “T_H17” cells. This can be accomplished by treatment of naïve T cells with digoxin, a cardiac glycoside [31]. Interestingly, digoxin is a specific inhibitor of the transcriptional activity of the nuclear

receptor ROR γ t. Digoxin inhibited murine T_H17 cell differentiation without enhancing or inhibiting the differentiation of other T cell lineages; moreover, digoxin delayed the onset and reduced the severity of autoimmune disease in mice [31, 32].

Retinoid response elements in immune genes

Like the Foxp3 gene (see above), the murine CCR9 gene has a RA response element half-site in its 5'-flanking region to which the RAR/RXR heterodimer complex binds, and the presence of this site is critical for RA-induced promoter activity. In addition, the transcription factor *nuclear factor activator of T cells isoform 2* (NFATc2) directly binds to RAR α and RXR α , and NFATc2 enhances the binding of RAR α to the RA response element half-site in the CCR9 promoter in murine naïve CD4⁺ T-cells [33].

RA and the differentiation of Dendritic Cells (DCs)

Another aspect of the actions of dendritic cells became clearer when Feng et al [22] demonstrated that a subset of cells *in the bone marrow* express the retinoic acid (RA)-synthesizing enzyme ALDH1a2 and are able to provide RA to DC precursors in the bone marrow. These bone marrow-derived DCs then differentiate further, resulting in both increased expression of CCR9 and ALDH1a2 and an increase in mucosal DC markers and actions; these mucosal DC activities include induction of Foxp3⁺ regulatory T cells, IgA-secreting B cells (see below), and gut-homing molecules (Fig. 2). RA also stimulates the synthesis of TGF- β in bone marrow derived DCs [18].

While a role for dendritic cells in the regulation of the differentiation of naïve precursor cells to T_{reg} cells is now established, the signals that stimulate ALDH1a2 transcription and activity in these dendritic cells must still be better defined. Recently, RA-mediated signaling in gut-associated DCs was shown to require MyD88, an adapter protein essential for downstream signaling of all toll-like receptors except TLR3 [34, 35]. Presumably, other signaling pathways that interact with RA in dendritic cells remain to be discovered. The source of retinol, and the levels retinol and retinyl esters in DCs must also be identified. One source of retinol is bile, which can imprint intestinal CD103⁺ DCs to generate T_{reg} cells [36]. The mechanism that delivers RA from DCs to naïve precursor cells must also be determined.

The genes that depend on RA for the maturation of T-cells are only beginning to be catalogued, but it is clear that retinoids play a role in the expression of key genes in the immune system. It is uncertain to what extent RA signaling cooperates with other signaling proteins in the generation of epigenetic changes that control the fate of immune cells and their progeny. These will be fertile areas of research, especially if retinoid agonists and antagonists can be used to modify immune responsiveness to ameliorate autoimmune disorders or to increase the responsiveness of the immune system to infection or tumor cells.

Inflammation, Retinoids, and ABC Transporters

Endogenous retinoids are essential for the maturation and function of many cells of the immune system. RA, produced by DCs, plays a major role in the regulation of the differentiation of CD4⁺ naïve T-cells into Foxp3⁺ T_{reg} cells (Fig. 2). Open questions include how RA is transported out of DCs and into T-cells; how RA is synthesized at the proper level to allow a balanced immune response; and how the RA signal is turned off in these cells. In this context the retinaldehyde ABC family transporter protein ABCA4, which is specifically expressed in retinal rod photoreceptors on the internal disc membranes, may provide an example. It transports retinaldehyde between the luminal and cytosolic faces of the disc membrane [37-39]. While this transport occurs in the context of the specialized functions of the eye, related transporters may play a role in RA transport in immune cells.

Expression of the ATP-binding cassette transporter A1 (ABCA1), involved in efflux of several types of small molecules, is induced by members of the nuclear receptor family of transcription factors, including the retinoic acid receptors (RARs) [40]. Further analysis of the ABCA1 proximal promoter identified binding sites for both RAR α and the transcription factor NFAT [41]. Lipopolysaccharide (LPS) pretreatment reduced both the nuclear level of RAR α and ABCA1 expression [41]. (LPS is the major ligand for the Toll-like receptor 4 (TLR4) and addition of LPS is used to generate an inflammatory response). RA-induced responses of the genes involved in RA metabolism, CYP26A1 and CYP26B1, were also greatly reduced in the livers of rats with LPS-induced inflammation, again indicating that inflammation leads to aberrant RA metabolism and loss of RA homeostasis [42].

Endogenous RA Regulates the Differentiation of Memory B Cells

The synthesis of RA from retinol is also necessary for regulating the transcription factor NFATc1 expression and for the differentiation and maintenance of the natural memory B cell compartment [43]. B1 cells are a subtype of B cells that generates the majority of the natural serum IgM and the gut IgA antibodies; thus, B1 cells constitute a key component of early immune responses to pathogens. A vitamin A-deficient diet greatly reduces NFATc1 expression in B1 cells, and, concomitantly, substantially reduces B1 cell numbers. As a result, vitamin A-deficient mice show a lower level of serum IgM and are unable to mount T cell-independent antibody responses to bacterial antigens [43, 44]. Again, understanding the molecular signals that mediate the differentiation of stem cells to B1 cells will provide useful information for pharmacological manipulation of T-cell independent antibody responses.

Tissue Regeneration: Intriguing Roles for Retinoids and a Key Role for ALDH1a2

Mammals can regenerate their tissues/organs to only a very limited extent, with the exception of the liver. However, some other vertebrate organisms can readily regenerate many different body parts. Because tissue regeneration and tissue repair show major mechanistic overlap in terms of genomic responses and both processes involve cell differentiation, it is not surprising that retinoids, both endogenously derived and externally added, have some fascinating effects on regeneration. Since the pioneering research of Maden [45] demonstrating striking effects of exogenous retinol on amphibian limb regeneration, the field has moved forward and retinoid effects have been shown in numerous vertebrate and protochordate animals.

Rats deficient in retinol can regenerate their livers so retinol does not appear to be essential for such regeneration [46]. However, both exogenous NIK-333, an acyclic synthetic retinoid, and exogenously added RA accelerated liver regeneration after a 70% partial hepatectomy [47].

Zebrafish can regenerate several body parts. One of the most exciting recent reports in regeneration area showed that ALDH1a2 (RALDH2), which is the rate-limiting enzyme in the production of RA from retinaldehyde (Fig. 1), was **highly** induced shortly after amputation in the regenerating heart, adult fin, and larval fin in zebrafish [48]. Thus, local generation of RA presumably plays a key role in fin formation during both embryogenesis and in fin regeneration. Fin regeneration begins with the formation of a wound epithelium, followed by blastema formation (epimorphic regeneration); subsequently, distal to proximal cell proliferation takes place [49, 50]. Cell differentiation then occurs so that a fully functional structure results. Induction of ALDH1a2 is required to establish the wound epithelium and the blastema, early steps in regeneration; expression of the ALDH1a2 gene is regulated in part by Wnt and FGF/ERK signaling during this regeneration [48]. These data suggest that the production of endogenous RA from retinol (via retinaldehyde) during the

regeneration process is essential for regeneration to occur in the zebrafish fin. Consistent with these ideas is the fact that $RAR\gamma$ is expressed in regenerating zebrafish caudal fins [51].

Msx genes, members of the homeobox family of genes (see below), are also induced during zebrafish fin regeneration [48], and, similarly, during more limited mouse digit regrowth/repair *Msx* mRNA levels greatly increased, but *ALDH1a2* mRNA levels did not [52]. It is plausible that the inability of mammals to induce *ALDH1a2* after tissue amputation is one reason why mammals have a more limited ability to regenerate body parts.

Vitamin A and the Heart: Regulation of Cell Differentiation

After injury to the zebrafish heart cardiomyocytes proliferate at the wound site and the heart regenerates. *ALDH1a2* induction by both the endocardium, an endothelial cell layer that lines the inside of the cardiac chambers, and the epicardium, a mesothelial layer that surrounds the cardiac chambers, is required for heart regeneration after trauma or tissue injury in zebrafish. Furthermore, the induction of *ALDH1a2* generates local RA [53-55] (Fig. 3). LPS injection can also induce *ALDH1a2* in the zebrafish endocardium, suggesting that injury may cause inflammatory cytokines to induce *ALDH1a2* in tissues of animals that can regenerate, whereas *ALDH1a2* is not induced in animals such as mice in which heart regeneration does not take place [52, 53]. Another view is that rapid induction of *ALDH1a2* in the heart endocardium after zebrafish heart amputation is part of an anti-inflammatory response to prevent scar formation [53]. Interestingly, in epicardial cells *ALDH1a2* is a direct transcriptional target gene of the *Wt1* (Wilms' tumor 1) protein [56], a zinc-finger transcription factor that plays a major role in organ development.

Vitamin A and Organ Regeneration

During kidney regeneration many genes involved in kidney organogenesis are reactivated. In zebrafish, inhibition of histone deacetylase (HDAC) activity in combination with RA expanded the renal progenitor cell population. These results suggest that HDAC, RA, and the renal progenitor cells are mechanistically in the same signaling pathway [57]. Moreover, in the kidney *ALDH1a2* was dramatically upregulated in podocytes in puromycin aminonucleoside-induced nephrosis (pan-nephrosis), suggesting that local endogenous RA production assists in the repair of podocytes [58].

In the protochordate ascidian *Polyandrocarpa misakiensis*, after the posterior half of the body is amputated the anterior half has no esophagus, stomach, or intestine. Nevertheless, within one week these organs are regenerated. This regeneration process in this protochordate is also dependent on endogenous RA synthesis [59]. In *Polyandrocarpa*, endogenous RA synthesis during regeneration leads to transdifferentiation of atrial epithelium into the gut, forming a new gut [59].

There are many reports of enhancement of central nervous system (CNS) regeneration/repair by the provision of exogenous retinoids. For example, 9-*cis*RA was recently shown to enhance CNS remyelination and $RXR\gamma$ was induced during the CNS remyelination process in rats [60]. Inflammatory cytokine release from astrocytes was reduced by RA [61]. In a model of spinal cord injury, treatment of rats with the synthetic retinoid AM80 resulted in greater recovery of spinal cord injury related motor function, and the authors demonstrated that the ability of AM80 to promote regeneration/repair was via promotion of differentiation of neural stem cells by increasing *TrkB* expression [62]. Stable $RAR\beta_2$ expression in dorsal root ganglia neurons *in vitro* enabled their axons to regenerate across the inhibitory dorsal root entry zone and project into the gray matter of the spinal cord in an *in vivo* model. The regenerated neurons increased neuronal activity in the spinal cord, and rats with enhanced $RAR\beta_2$ expression showed major improvement in sensorimotor tasks [63]. Expression of

exogenous RAR β enhanced axonal regeneration in adult sensory neurons in rats [64]. One mechanism by which RAR β :RA acts to enhance axonal regeneration after CNS injury is via inhibition of myelin activated Nogo receptor (NgR) signaling [65]. Furthermore, an RAR β agonist induced axonal outgrowth of descending corticospinal fibers and promoted recovery of the injured adult spinal cord [66]. Interestingly, RAR β can be activated by direct protein deacetylation via the activity of the NAD-dependent deacetylase, SIRT1, in the brain [67]. In conclusion, the actions of RARs and ALDH1a2 during larval and adult fin and heart regeneration drive both cell proliferation and cell differentiation. Furthermore, RA has major effects on both kidney and CNS regeneration/repair processes. Whether ALDH1a2 is induced in the CNS during these regeneration processes has not been addressed.

RA Stimulates the Differentiation of Podocytes and Reduces HIV-Associated Nephropathy

Retinol deficiency during development is associated with renal abnormalities and malformations [68]. During kidney development stromal cells require retinol-mediated signals to control Ret expression in the ureteric bud [68]. RA is also involved in patterning early nephron progenitor cells in zebrafish nephrogenesis [69].

Human immunodeficiency (HIV) disease is associated with a nephropathy in which the podocytes, epithelial cells in the Bowman's capsule in the kidneys that wrap around the capillaries of the glomerulus, proliferate abnormally and dedifferentiate [70]. The dedifferentiation of podocytes associated with HIV infection also is reflected in the loss of differentiation markers such as nephrin and Wilm's tumor 1 [70]. Nef-activated Src-STAT3 and MAPK 1 and 2 pathways are induced in HIV-infected podocytes, leading to enhanced proliferation and dedifferentiation [71]. RA reduces podocyte proliferation and stimulates podocyte differentiation, apparently via an increase in cyclic AMP [72].

HIV transgenic mice and human patients with HIV-associated kidney disease exhibit a profound reduction in the level of RAR β protein in the glomeruli, and HIV transgenic mice show reduced retinol dehydrogenase 1 and 9 levels, concomitant with a greater than 3-fold reduction in endogenous RA levels in the glomeruli [73]. Thus, there is a major impairment in endogenous RA production from retinol in the glomeruli in this HIV mouse model. AM580, a specific RAR α agonist, reduces proteinuria, reduces glomerulosclerosis, and restores podocyte differentiation in this HIV transgenic mouse model [73]. This work suggests that HIV infection leads to a reduction in the synthesis of RA from retinol in podocytes, subsequently causing podocyte dedifferentiation and loss of proper podocyte function. RA also has shown significant, beneficial actions in experimental models of glomerulonephritis, lupus nephritis, and diabetic nephropathy [74-76]. For instance, pharmacological doses of RA suppressed inflammatory changes in the diabetic rat kidney, and proteinuria was also decreased by RA in this experimental model [74]. Thus, retinoids play key roles in the kidney and retinoid based therapies are emerging as useful therapies for kidney disorders.

Retinoic Acid and Retinoic Acid Receptors Play a Major Role in the Alveoli of the Lung

The lung primordium forms from the primitive foregut during development and this process requires endogenously generated retinoic acid [77], but retinoids also play key roles in the formation and continued functioning of lung alveoli. Alveoli contain three major epithelial cell types. Type I epithelial cells carry out gas exchange, while type II epithelial cells make surfactant and can regenerate alveolar epithelium following injury. Type III epithelial cells

may function as chemoreceptors. There is evidence that Type I cells are derived from type II cells.

The lung is a major tissue for storage of retinol as retinyl esters [78, 79]. Exogenous RA can stimulate retinol uptake and storage in the lung; for example, when neonatal rats were treated with retinol combined with retinoic acid (RA; 9-cis-RA; Am580, an analog of RA) lung retinyl esters increased approximately 5-7 times more than after an equal amount of retinol alone [80]. Thus, retinoids are stored in the lung and active retinoids regulate the level of precursor storage.

Furthermore, the three RARs play distinct roles at different times during alveoli development. For instance, RAR β functions to *inhibit* alveoli formation in the perinatal period, but not at later times in development, whereas RAR α is needed for the proper number of alveoli to develop *after* the perinatal period in mice. RAR γ null mice show a lower number of alveoli compared to Wt mice and RAR γ expression is required during the first four weeks after birth for proper numbers of lung alveoli to form. RAR β null mice also exhibit a decrease in respiratory function compared to Wt mice [81-86].

Retinoids must play a role in the functioning of the mature lung because they are proving to be pharmacologically useful in treating certain lung diseases. For example, chronic obstructive pulmonary disease (COPD) is a chronic, inflammatory lung disease associated with progressive airflow obstruction. The main risk factor worldwide for COPD is smoking tobacco. Emphysema is a chronic respiratory disease, a severe form of COPD in which the air sacs (alveoli) of the lungs are permanently damaged, causing a decrease in the number of alveoli and in lung function. In a rat elastase emphysema model, RA reverses the emphysema phenotype [87, 88]. These results generated much excitement, though the same results were not seen in adult mice [89], in a cigarette smoking mouse model of emphysema [90], or in another elastase model [91]. In a dexamethasone model in which dexamethasone treatment of newborn mice permanently disrupts alveolar development, RA treatment at later times in development was able to restore normal alveolar structures [92].

These rodent studies led to a clinical trial in humans, the FORTE trial [93], which demonstrated a slight benefit of retinoid treatment. The REPAIR trial is currently underway in patients with emphysema secondary to alpha-1-antitrypsin deficiency (AATD). These patients are being used because they resemble patients with smoke-induced emphysema and are being treated with palovarotene, an RAR γ agonist [94, 95].

Because we don't fully understand which cells in the alveoli have stem/progenitor cell properties, it is difficult to comprehend the results of the retinoid treatments in the various emphysema models and clinical trials. Some cells with stem/progenitor properties have been isolated from the lung [96-98], but detailed lineage tracing experiments are still required. Retinoids may have different effects on cells in a diseased lung or a diseased lung may have different stem cell populations.

Retinoid metabolism is altered in several lung disorders. The enzymes involved in the generation and metabolism of retinoids become abnormal in humans even before smokers show evidence of clinical disease, suggesting that retinoid signaling is aberrant in very early smokers, before emphysema develops [99, 100]. Strikingly, exposure to cigarette smoke or to the carcinogen benzo(a)pyrene alone induces retinol (vitamin A) depletion in the lungs of rats, which is associated with the development of emphysema [101]. Moreover, the severity of the emphysema was inversely correlated with the level of retinol in this rat model; and, retinol deficiency itself, even without carcinogen, can cause emphysematous changes in rat lungs [102]. Thus, retinol deficiency appears in an early stage of COPD disease, and adequate retinol levels might be essential to the physiological renewal of the lung which is

disrupted in smoking. If retinoid metabolism and/or signaling is abnormal early in the development of emphysema, this could complicate treatment of late stage disease with pharmacological doses of retinoids. Ultimately, a better understanding of the normal stem cells in lung alveoli will assist us in designing more effective treatments using retinoids as pharmacological doses for COPD.

Hox Genes are Key Mediators of Cell Memory in the Adult during Tissue Differentiation/Regeneration

The examples above demonstrate the actions of endogenous and exogenous retinoids in adult animals in influencing the process of cell differentiation, but what are the molecular signaling pathways controlled by the RARs during the differentiation of adult stem cells? Hox (homeobox) genes encode transcription factors that regulate major aspects of differentiation in adult tissues, and the expression of these genes is known to regulate positional identity in the developing embryo [103]. Most of the Hox genes are located in linear clusters (Hox A, B, C, D clusters) of genes on a chromosome and Hox genes are expressed in nested anterior-posterior and proximal-distal patterns colinear with their genomic position from 3' to 5' of each of the four major Hox gene clusters. Hox genes are transcriptionally activated by RA in stem cells in a complex, sequential process that involves epigenetic mechanisms such as specific histone acetylation and methylation [13, 104-111].

Noncoding (nc) RNAs also play an important role in the transcriptional regulation of Hox gene expression. For instance, a 2.2 kilobase ncRNA residing in the HOXC locus, called HOTAIR, represses transcription in trans across 40 kilobases of the HOXD locus. HOTAIR interacts with the inhibitory Polycomb Repressive Complex 2 (PRC2) proteins and is required for PRC2 occupancy and histone H3 lysine-27 trimethylation of the HOXD locus [112, 113]. Recently, an intergenic transcriptional activity located between the human HOXA1 and HOXA2 genes was shown to exhibit myeloid-specific expression and increased expression during granulocytic differentiation. The novel gene, termed HOTAIRM1 (HOX antisense intergenic RNA myeloid 1), is transcribed antisense to the HOXA genes, originates from the same CpG island that embeds the start site of HOXA1 at the 3' end of the Hoxa chromosomal cluster, and does not encode a protein. Knockdown of HOTAIRM1 led to a decrease in RA-induced differentiation of myeloid cells in culture [114]. HOTTIP, a long intergenic noncoding RNA (lincRNA) transcribed from the 5' tip of the HOXA locus, coordinates the activation of several 5' HOXA genes *in vivo*. The DNA element at the 5' end of the Hoxa chromosomal Hox gene cluster near HoxA13, *HOTTIP* for 'HOXA transcript at the distal tip', exhibits bivalent H3K4me3 and H3K27me3 marks, and a histone modification pattern associated with poised regulatory sequences. *HOTTIP* encodes a 3,764-nt, spliced and polyadenylated lincRNA that initiates ~330 bases upstream of *HOXA13*. Only the strand antisense to the *HOXA* genes is transcribed. HOTTIP RNA binds the protein WDR5 directly and targets WDR5/MLL complexes across HOXA, causing histone H3 lysine 4 trimethylation (H3K4me3) and subsequent gene transcription. For HOTTIP RNA activation of its target genes induced proximity is required. Thus, lincRNAs appear to organize numerous chromatin domains in order to coordinate long-range gene activation by transmitting information from higher order chromosomal structures to epigenetic changes [115]. That knockdown of HOTAIRM1 results in a reduction in RA-induced myeloid cell differentiation shows that the lincRNAs must interact with the RAR associated signaling pathway, but how this occurs is not clear at present.

There is increasing evidence that maintenance of the appropriate HOX transcriptional program in adult cells, such as mesenchymal stem cells and fibroblasts, may provide positional memory to pattern epithelia not only during embryogenesis and regeneration, but also in adults [103, 116-119]. For example, both embryonic origin and Hox gene expression

status distinguish neural crest-derived from mesoderm-derived skeletal progenitor cells, and both of these characteristics, embryonic origin and Hox gene expression, influence the process of adult bone regeneration [116, 118] (Fig. 4). In addition, the retinoic acid receptor γ (RAR γ) plays a major regulatory role in the inhibition of heterotypic bone ossification, ectopic bone formation within soft tissues after surgery or trauma, by controlling aspects of the differentiation of mesenchymal stem cells [120]. Thus, there is evidence that retinoic acid signaling is involved in maintaining cell positional memory via Hox gene expression patterns.

Hox gene expression is also regulated during lung alveolarization [121], and in zebrafish fin regeneration [122]. Importantly, a Hoxa1-Pbx1/2-Meis2 protein complex binds a regulatory element in the ALDH1a2 gene, regulating RA synthesis during early embryonic axial patterning [123]. In the developing hindbrain RA and Hox protein-regulated enhancers mediate RAR β expression, and RAR β is a direct transcriptional target gene of Hoxb4 [124].

Hoxb1, Hoxa1, and Hoxa3, targets of RA signaling [13, 106-111, 125, 126], are expressed in separate sub-domains in the second heart field, a progenitor cell population from which much of the heart, including the atria, right ventricle, and outflow tract, is derived [127]. Endogenous RA and proper combinatorial Hox gene expression patterns are required for the achievement of the correct three-dimensional location of these Hox-expressing progenitor cells [127].

Thus, both in the developing embryo and in the adult, RA signaling via combinatorial Hox gene expression is important for cell positional memory. It is not clear if alterations of this positional memory contribute to disease progression or if it can be changed by pharmacological approaches. The regulation of ALDH1a2 transcription is complex and different signaling pathways appear to be involved in various cell types. Because this pathway is central to the generation of transcriptionally active retinoids from inactive precursors, the manner in which ALDH1a2 is regulated in different cell types should be very informative. Other challenges for researchers in this area include the elucidation of the epigenetic changes that maintain cell type and regional-specific gene expression programs, including Hox gene expression patterns, in the adult, and the delineation of mechanisms by which RA signals intercellularly. These studies will guide the therapeutic uses of retinoids in virtually every organ system.

Acknowledgments

I would like to thank Ms. Tamara Weissman and Ms. Lissett Checo for editorial assistance, Dr. John Wagner and Ms. Megan Ricard for critically reading this manuscript, and NIH grants NIAAA R01 AA018332, NIDCR R01 DE010389, and NCI R01 CA043796 for support.

References

1. Gudas LJ, Wagner JA. Retinoids regulate stem cell differentiation. *J Cell Physiol.* 2011; 226:322–330. [PubMed: 20836077]
2. Means AL, Gudas LJ. The roles of retinoids in vertebrate development. *Annu Rev Biochem.* 1995; 64:201–233. [PubMed: 7574480]
3. Mongan NP, Gudas LJ. Diverse actions of retinoid receptors in cancer prevention and treatment. *Differentiation.* 2007; 75:853–870. [PubMed: 17634071]
4. Chassaing N, Golzio C, Odent S, Lequeux L, Vigouroux A, Martinovic-Bouriel J, Tiziano FD, Masini L, Piro F, Maragliano G, Delezoide AL, Attie-Bitach T, Manouvrier-Hanu S, Etchevers HC, Calvas P. Phenotypic spectrum of STRA6 mutations: from Matthew-Wood syndrome to non-lethal anophthalmia. *Hum Mutat.* 2009; 30:E673–681. [PubMed: 19309693]

5. Sun H, Kawaguchi R. The membrane receptor for plasma retinol-binding protein, a new type of cell-surface receptor. *Int Rev Cell Mol Biol.* 2011; 288:1–41. [PubMed: 21482409]
6. Cunningham TJ, Chatzi C, Sandell LL, Trainor PA, Duester G. Rdh10 mutants deficient in limb field retinoic acid signaling exhibit normal limb patterning but display interdigital webbing. *Dev Dyn.* 2011; 240:1142–1150. [PubMed: 21360789]
7. D'Ambrosio DN, Clugston RD, Blaner WS. Vitamin A Metabolism: An Update. *Nutrients.* 2011; 3:63–103. [PubMed: 21350678]
8. Kumar S, Sandell LL, Trainor PA, Koentgen F, Duester G. Alcohol and aldehyde dehydrogenases: Retinoid metabolic effects in mouse knockout models. *Biochim Biophys Acta.* 2011
9. Lee SA, Belyaeva OV, Wu L, Kedishvili NY. Retinol Dehydrogenase 10 but Not Retinol/Sterol Dehydrogenase(s) Regulates the Expression of Retinoic Acid-responsive Genes in Human Transgenic Skin Raft Culture. *J Biol Chem.* 2011; 286:13550–13560. [PubMed: 21345790]
10. Linke T, Dawson H, Harrison EH. Isolation and characterization of a microsomal acid retinyl ester hydrolase. *J Biol Chem.* 2005; 280:23287–23294. [PubMed: 15767260]
11. Wang C, Kane MA, Napoli JL. Multiple retinol and retinal dehydrogenases catalyze all-trans-retinoic acid biosynthesis in astrocytes. *J Biol Chem.* 2011; 286:6542–6553. [PubMed: 21138835]
12. Hudlebusch HR, Skotte J, Santoni-Rugiu E, Zimling ZG, Lees MJ, Simon R, Sauter G, Rota R, De Ioris MA, Quarto M, Johansen JV, Jorgensen M, Rechnitzer C, Maroun LL, Schroder H, Petersen BL, Helin K. MMSET is highly expressed and associated with aggressiveness in neuroblastoma. *Cancer Res.* 2011; 71:4226–4235. [PubMed: 21527557]
13. Kashyap V, Gudas LJ, Brenet F, Funk P, Viale A, Scandura JM. Epigenomic reorganization of the clustered Hox genes in embryonic stem cells induced by retinoic acid. *J Biol Chem.* 2011; 286:3250–3260. [PubMed: 21087926]
14. Martens JH, Brinkman AB, Simmer F, Francoijs KJ, Nebbioso A, Ferrara F, Altucci L, Stunnenberg HG. PML-RAR α /RXR Alters the Epigenetic Landscape in Acute Promyelocytic Leukemia. *Cancer Cell.* 2010; 17:173–185. [PubMed: 20159609]
15. Subramanyam D, Belair CD, Barry-Holson KQ, Lin H, Kogan SC, Passegue E, Billelo R. PML-RAR α and Dnmt3a1 cooperate in vivo to promote acute promyelocytic leukemia. *Cancer Res.* 2010; 70:8792–8801. [PubMed: 20861188]
16. Tang XH, Gudas LJ. Retinoids, retinoic acid receptors, and cancer. *Annu Rev Pathol.* 2011; 6:345–364. [PubMed: 21073338]
17. Huq MD, Tsai NP, Khan SA, Wei LN. Lysine trimethylation of retinoic acid receptor- α : a novel means to regulate receptor function. *Mol Cell Proteomics.* 2007; 6:677–688. [PubMed: 17205979]
18. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103⁺ DCs induces Foxp3⁺ regulatory T cells via a TGF- β and retinoic acid-dependent mechanism. *J Exp Med.* 2007; 204:1757–1764. [PubMed: 17620361]
19. Hoechst B, Gamrekashvili J, Manns MP, Greten TF, Korangy F. Plasticity of human Th17 cells and iTregs is orchestrated by different subsets of myeloid cells. *Blood.* 2011; 117:6532–6541. [PubMed: 21493801]
20. Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nat Immunol.* 2007; 8:277–284. [PubMed: 17220892]
21. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, Cheroutre H. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science.* 2007; 317:256–260. [PubMed: 17569825]
22. Feng T, Cong Y, Qin H, Benveniste EN, Elson CO. Generation of mucosal dendritic cells from bone marrow reveals a critical role of retinoic acid. *J Immunol.* 2010; 185:5915–5925. Figure 2 Copyright 2010. The American Association of Immunologists, Inc. [PubMed: 20944006]
23. Boschetti G, Nancey S, Sardi F, Roblin X, Flourie B, Kaiserlian D. Therapy with anti-TNF α antibody enhances number and function of Foxp3(+) regulatory T cells in inflammatory bowel diseases. *Inflamm Bowel Dis.* 2011; 17:160–170. [PubMed: 20848510]

24. Stock A, Booth S, Cerundolo V. Prostaglandin E2 suppresses the differentiation of retinoic acid-producing dendritic cells in mice and humans. *J Exp Med.* 2011; 208:761–773. [PubMed: 21444662]
25. Manicassamy S, Reizis B, Ravindran R, Nakaya H, Salazar-Gonzalez RM, Wang YC, Pulendran B. Activation of beta-catenin in dendritic cells regulates immunity versus tolerance in the intestine. *Science.* 2010; 329:849–853. [PubMed: 20705860]
26. Laffont S, Siddiqui KR, Powrie F. Intestinal inflammation abrogates the tolerogenic properties of MLN CD103+ dendritic cells. *Eur J Immunol.* 2010; 40:1877–1883. [PubMed: 20432234]
27. Depaolo RW, Abadie V, Tang F, Fehlner-Peach H, Hall JA, Wang W, Marietta EV, Kasarda DD, Waldmann TA, Murray JA, Semrad C, Kupfer SS, Belkaid Y, Guandalini S, Jabri B. Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. *Nature.* 2011; 471:220–224. [PubMed: 21307853]
28. Xu L, Kitani A, Stuelten C, McGrady G, Fuss I, Strober W. Positive and negative transcriptional regulation of the Foxp3 gene is mediated by access and binding of the Smad3 protein to enhancer I. *Immunity.* 2010; 33:313–325. [PubMed: 20870174]
29. Toker A, Huehn J. To be or not to be a reg cell: lineage decisions controlled by epigenetic mechanisms. *Sci Signal.* 2011; 4:pe4. [PubMed: 21285410]
30. Lu L, Zhou X, Wang J, Zheng SG, Horwitz DA. Characterization of protective human CD4CD25 FOXP3 regulatory T cells generated with IL-2, TGF-beta and retinoic acid. *PLoS One.* 2010; 5:e15150. [PubMed: 21179414]
31. Huh JR, Leung MW, Huang P, Ryan DA, Krout MR, Malapaka RR, Chow J, Manel N, Ciofani M, Kim SV, Cuesta A, Santori FR, Lafaille JJ, Xu HE, Gin DY, Rastinejad F, Littman DR. Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORgamma activity. *Nature.* 2011; 472:486–490. [PubMed: 21441909]
32. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor RORgamma directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell.* 2006; 126:1121–1133. [PubMed: 16990136]
33. Ohoka Y, Yokota A, Takeuchi H, Maeda N, Iwata M. Retinoic acid-induced CCR9 expression requires transient TCR stimulation and cooperativity between NFATc2 and the retinoic acid receptor/retinoid X receptor complex. *J Immunol.* 2011; 186:733–744. [PubMed: 21148038]
34. Villablanca EJ, Wang S, de Calisto J, Gomes DC, Kane MA, Napoli JL, Blaner WS, Kagechika H, Blomhoff R, Roseblatt M, Bono MR, von Andrian UH, Mora JR. MyD88 and Retinoic Acid Signaling Pathways Interact to Modulate Gastrointestinal Activities of Dendritic Cells. *Gastroenterology.* 2011; 141:176–185. [PubMed: 21596042]
35. Wang S, Villablanca EJ, De Calisto J, Gomes DC, Nguyen DD, Mizoguchi E, Kagan JC, Reinecker HC, Hacohen N, Nagler C, Xavier RJ, Rossi-Bergmann B, Chen YB, Blomhoff R, Snapper SB, Mora JR. MyD88-Dependent TLR1/2 Signals Educate Dendritic Cells with Gut-Specific Imprinting Properties. *J Immunol.* 2011; 187:141–150. [PubMed: 21646294]
36. Jaensson-Gyllenback E, Kotarsky K, Zapata F, Persson EK, Gundersen TE, Blomhoff R, Agace WW. Bile retinoids imprint intestinal CD103(+) dendritic cells with the ability to generate gut-tropic T cells. *Mucosal Immunol.* 2011; 4:438–447. [PubMed: 21289617]
37. Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, Gerrard B, Baird L, Stauffer D, Peiffer A, Rattner A, Smallwood P, Li Y, Anderson KL, Lewis RA, Nathans J, Leppert M, Dean M, Lupski JR. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet.* 1997; 15:236–246. [PubMed: 9054934]
38. Radu RA, Hu J, Yuan Q, Welch DL, Makshanoff J, Lloyd M, McMullen S, Travis GH, Bok D. Complement system dysregulation and inflammation in the retinal pigment epithelium of a mouse model for Stargardt macular degeneration. *J Biol Chem.* 2011; 286:18593–18601. [PubMed: 21464132]
39. Sun H, Molday RS, Nathans J. Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. *J Biol Chem.* 1999; 274:8269–8281. [PubMed: 10075733]

40. Costet P, Lalanne F, Gerbod-Giannone MC, Molina JR, Fu X, Lund EG, Gudas LJ, Tall AR. Retinoic acid receptor-mediated induction of ABCA1 in macrophages. *Mol Cell Biol.* 2003; 23:7756–7766. [PubMed: 14560020]
41. Maitra U, Parks JS, Li L. An innate immunity signaling process suppresses macrophage ABCA1 expression through IRAK-1-mediated downregulation of retinoic acid receptor alpha and NFATc2. *Mol Cell Biol.* 2009; 29:5989–5997. [PubMed: 19752193]
42. Zolfaghari R, Cifelli CJ, Lieu SO, Chen Q, Li NQ, Ross AC. Lipopolysaccharide opposes the induction of CYP26A1 and CYP26B1 gene expression by retinoic acid in the rat liver in vivo. *Am J Physiol Gastrointest Liver Physiol.* 2007; 292:G1029–1036. [PubMed: 17185629]
43. Maruya M, Suzuki K, Fujimoto H, Miyajima M, Kanagawa O, Wakayama T, Fagarasan S. Vitamin A-dependent transcriptional activation of the nuclear factor of activated T cells c1 (NFATc1) is critical for the development and survival of B1 cells. *Proc Natl Acad Sci U S A.* 2011; 108:722–727. [PubMed: 21187378]
44. Ross AC, Chen Q, Ma Y. Vitamin A and retinoic acid in the regulation of B-cell development and antibody production. *Vitam Horm.* 2011; 86:103–126. [PubMed: 21419269]
45. Maden M. Vitamin A and pattern formation in the regenerating limb. *Nature.* 1982; 295:672–675. [PubMed: 7057925]
46. Hu Z, Fujio K, Marsden ER, Thorgeirsson SS, Evarts RP. Hepatic regeneration in vitamin A-deficient rats: changes in the expression of transforming growth factor alpha/epidermal growth factor receptor and retinoic acid receptors alpha and beta. *Cell Growth Differ.* 1994; 5:503–508. [PubMed: 7519440]
47. Kimura M, Watanabe M, Ishibashi N, Yanagida S, Ogihara M. Acyclic retinoid NIK-333 accelerates liver regeneration and lowers serum transaminase activities in 70% partially hepatectomized rats, in vivo. *Eur J Pharmacol.* 2010; 643:267–273. [PubMed: 20619257]
48. Mathew LK, Sengupta S, Franzosa JA, Perry J, La Du J, Andreasen EA, Tanguay RL. Comparative expression profiling reveals an essential role for raldh2 in epimorphic regeneration. *J Biol Chem.* 2009; 284:33642–33653. [PubMed: 19801676]
49. Gibert Y, Gajewski A, Meyer A, Begemann G. Induction and prepatterning of the zebrafish pectoral fin bud requires axial retinoic acid signaling. *Development.* 2006; 133:2649–2659. [PubMed: 16774994]
50. Kawakami A, Fukazawa T, Takeda H. Early fin primordia of zebrafish larvae regenerate by a similar growth control mechanism with adult regeneration. *Dev Dyn.* 2004; 231:693–699. [PubMed: 15499559]
51. White JA, Boffa MB, Jones B, Petkovich M. A zebrafish retinoic acid receptor expressed in the regenerating caudal fin. *Development.* 1994; 120:1861–1872. [PubMed: 7924993]
52. Wang G, Badylak SF, Heber-Katz E, Braunhut SJ, Gudas LJ. The effects of DNA methyltransferase inhibitors and histone deacetylase inhibitors on digit regeneration in mice. *Regen Med.* 2010; 5:201–220. [PubMed: 20210581]
53. Kikuchi K, Holdway JE, Major RJ, Blum N, Dahn RD, Begemann G, Poss KD. Retinoic acid production by endocardium and epicardium is an injury response essential for zebrafish heart regeneration. *Dev Cell.* 2011; 20:397–404. [PubMed: 21397850]
54. Lepilina A, Coon AN, Kikuchi K, Holdway JE, Roberts RW, Burns CG, Poss KD. A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. *Cell.* 2006; 127:607–619. [PubMed: 17081981]
55. Stuckmann I, Evans S, Lassar AB. Erythropoietin and retinoic acid, secreted from the epicardium, are required for cardiac myocyte proliferation. *Dev Biol.* 2003; 255:334–349. [PubMed: 12648494]
56. Guadix JA, Ruiz-Villalba A, Lettice L, Velecela V, Munoz-Chapuli R, Hastie ND, Perez-Pomares JM, Martinez-Estrada OM. Wt1 controls retinoic acid signalling in embryonic epicardium through transcriptional activation of Raldh2. *Development.* 2011; 138:1093–1097. [PubMed: 21343363]
57. de Groh ED, Swanhart LM, Cosentino CC, Jackson RL, Dai W, Kitchens CA, Day BW, Smithgall TE, Hukriede NA. Inhibition of histone deacetylase expands the renal progenitor cell population. *J Am Soc Nephrol.* 2010; 21:794–802. [PubMed: 20378823]

58. Suzuki A, Ito T, Imai E, Yamato M, Iwatani H, Kawachi H, Hori M. Retinoids regulate the repairing process of the podocytes in puromycin aminonucleoside-induced nephrotic rats. *J Am Soc Nephrol.* 2003; 14:981–991. [PubMed: 12660332]
59. Kaneko N, Katsuyama Y, Kawamura K, Fujiwara S. Regeneration of the gut requires retinoic acid in the budding ascidian *Polyandrocarpa misakiensis*. *Dev Growth Differ.* 2010; 52:457–468. [PubMed: 20507359]
60. Huang JK, Jarjour AA, Nait Oumesmar B, Kerninon C, Williams A, Krezel W, Kagechika H, Bauer J, Zhao C, Evercooren AB, Chambon P, Ffrench-Constant C, Franklin RJ. Retinoid X receptor gamma signaling accelerates CNS remyelination. *Nat Neurosci.* 2011; 14:45–53. [PubMed: 21131950]
61. van Neerven S, Nemes A, Imholz P, Regen T, Denecke B, Johann S, Beyer C, Hanisch UK, Mey J. Inflammatory cytokine release of astrocytes in vitro is reduced by all-trans retinoic acid. *J Neuroimmunol.* 2010; 229:169–179. [PubMed: 20826012]
62. Takenaga M, Ohta Y, Tokura Y, Hamaguchi A, Shudo K, Okano H, Igarashi R. The effect of Am-80, a synthetic retinoid, on spinal cord injury-induced motor dysfunction in rats. *Biol Pharm Bull.* 2009; 32:225–231. [PubMed: 19182380]
63. Wong LF, Yip PK, Battaglia A, Grist J, Corcoran J, Maden M, Azzouz M, Kingsman SM, Kingsman AJ, Mazarakis ND, McMahon SB. Retinoic acid receptor beta2 promotes functional regeneration of sensory axons in the spinal cord. *Nat Neurosci.* 2006; 9:243–250. [PubMed: 16388307]
64. Yip PK, Wong LF, Pattinson D, Battaglia A, Grist J, Bradbury EJ, Maden M, McMahon SB, Mazarakis ND. Lentiviral vector expressing retinoic acid receptor beta2 promotes recovery of function after corticospinal tract injury in the adult rat spinal cord. *Hum Mol Genet.* 2006; 15:3107–3118. [PubMed: 16984961]
65. Puttagunta R, Schmandke A, Floriddia E, Gaub P, Fomin N, Ghyselinck NB, Di Giovanni S. RA-RAR- β counteracts myelin-dependent inhibition of neurite outgrowth via Lingo-1 repression. *J Cell Biol.* 2011; 193:1147–1156. [PubMed: 21690307]
66. Agudo M, Yip P, Davies M, Bradbury E, Doherty P, McMahon S, Maden M, Corcoran JP. A retinoic acid receptor beta agonist (CD2019) overcomes inhibition of axonal outgrowth via phosphoinositide 3-kinase signalling in the injured adult spinal cord. *Neurobiol Dis.* 2010; 37:147–155. [PubMed: 19800972]
67. Donmez G, Wang D, Cohen DE, Guarente L. SIRT1 suppresses beta-amyloid production by activating the alpha-secretase gene ADAM10. *Cell.* 2010; 142:320–332. [PubMed: 20655472]
68. Batourina E, Gim S, Bello N, Shy M, Clagett-Dame M, Srinivas S, Costantini F, Mendelsohn C. Vitamin A controls epithelial/mesenchymal interactions through Ret expression. *Nat Genet.* 2001; 27:74–78. [PubMed: 11138002]
69. Wingert RA, Davidson AJ. Zebrafish nephrogenesis involves dynamic spatiotemporal expression changes in renal progenitors and essential signals from retinoic acid and *irx3b*. *Dev Dyn.* 2011; 240:2011–2027. [PubMed: 21761484]
70. Barisoni L, Bruggeman LA, Mundel P, D'Agati VD, Klotman PE. HIV-1 induces renal epithelial dedifferentiation in a transgenic model of HIV-associated nephropathy. *Kidney Int.* 2000; 58:173–181. [PubMed: 10886562]
71. He JC, Husain M, Sunamoto M, D'Agati VD, Klotman ME, Iyengar R, Klotman PE. Nef stimulates proliferation of glomerular podocytes through activation of Src-dependent Stat3 and MAPK1,2 pathways. *J Clin Invest.* 2004; 114:643–651. [PubMed: 15343382]
72. He JC, Lu TC, Fleet M, Sunamoto M, Husain M, Fang W, Neves S, Chen Y, Shankland S, Iyengar R, Klotman PE. Retinoic acid inhibits HIV-1-induced podocyte proliferation through the cAMP pathway. *J Am Soc Nephrol.* 2007; 18:93–102. [PubMed: 17182884]
73. Ratnam KK, Feng X, Chuang PY, Verma V, Lu TC, Wang J, Jin Y, Farias EF, Napoli JL, Chen N, Kaufman L, Takano T, D'Agati VD, Klotman PE, He JC. Role of the retinoic acid receptor-alpha in HIV-associated nephropathy. *Kidney Int.* 2011; 79:624–634. [PubMed: 21150871]
74. Han SY, So GA, Jee YH, Han KH, Kang YS, Kim HK, Kang SW, Han DS, Han JY, Cha DR. Effect of retinoic acid in experimental diabetic nephropathy. *Immunol Cell Biol.* 2004; 82:568–576. [PubMed: 15550114]

75. Perez de Lema G, Lucio-Cazana FJ, Molina A, Luckow B, Schmid H, de Wit C, Moreno-Manzano V, Banas B, Mampaso F, Schlondorff D. Retinoic acid treatment protects MRL/lpr lupus mice from the development of glomerular disease. *Kidney Int.* 2004; 66:1018–1028. [PubMed: 15327395]
76. Wagner J, Dechow C, Morath C, Lehrke I, Amann K, Waldherr R, Floege J, Ritz E. Retinoic acid reduces glomerular injury in a rat model of glomerular damage. *J Am Soc Nephrol.* 2000; 11:1479–1487. [PubMed: 10906161]
77. Chen F, Cao Y, Qian J, Shao F, Niederreither K, Cardoso WV. A retinoic acid-dependent network in the foregut controls formation of the mouse lung primordium. *J Clin Invest.* 2010; 120:2040–2048. [PubMed: 20484817]
78. Liu L, Gudas LJ. Disruption of the lecithin:retinol acyltransferase gene makes mice more susceptible to vitamin A deficiency. *J Biol Chem.* 2005; 280:40226–40234. [PubMed: 16174770]
79. O'Byrne SM, Wongsiriroj N, Libien J, Vogel S, Goldberg IJ, Baehr W, Palczewski K, Blaner WS. Retinoid absorption and storage is impaired in mice lacking lecithin:retinol acyltransferase (LRAT). *J Biol Chem.* 2005; 280:35647–35657. [PubMed: 16115871]
80. Wu L, Ross AC. Acidic retinoids synergize with vitamin A to enhance retinol uptake and STRA6, LRAT, and CYP26B1 expression in neonatal lung. *J Lipid Res.* 2010; 51:378–387. [PubMed: 19700416]
81. Desai TJ, Chen F, Lu J, Qian J, Niederreither K, Dolle P, Chambon P, Cardoso WV. Distinct roles for retinoic acid receptors alpha and beta in early lung morphogenesis. *Dev Biol.* 2006; 291:12–24. [PubMed: 16427040]
82. Massaro GD, Massaro D, Chambon P. Retinoic acid receptor-alpha regulates pulmonary alveolus formation in mice after, but not during, perinatal period. *Am J Physiol Lung Cell Mol Physiol.* 2003; 284:L431–433. [PubMed: 12533315]
83. Massaro GD, Massaro D, Chan WY, Clerch LB, Ghyselinck N, Chambon P, Chandraratna RA. Retinoic acid receptor-beta: an endogenous inhibitor of the perinatal formation of pulmonary alveoli. *Physiol Genomics.* 2000; 4:51–57. [PubMed: 11074013]
84. McGowan S, Jackson SK, Jenkins-Moore M, Dai HH, Chambon P, Snyder JM. Mice bearing deletions of retinoic acid receptors demonstrate reduced lung elastin and alveolar numbers. *Am J Respir Cell Mol Biol.* 2000; 23:162–167. [PubMed: 10919981]
85. Snyder JM, Jenkins-Moore M, Jackson SK, Goss KL, Dai HH, Bangsund PJ, Giguere V, McGowan SE. Alveolarization in retinoic acid receptor-beta-deficient mice. *Pediatr Res.* 2005; 57:384–391. [PubMed: 15635054]
86. Wongtrakool C, Malpel S, Gorenstein J, Sedita J, Ramirez MI, Underhill TM, Cardoso WV. Down-regulation of retinoic acid receptor alpha signaling is required for sacculization and type I cell formation in the developing lung. *J Biol Chem.* 2003; 278:46911–46918. [PubMed: 12947094]
87. Massaro GD, Massaro D. Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. *Nat Med.* 1997; 3:675–677. [PubMed: 9176496]
88. Tepper J, Pfeiffer J, Aldrich M, Tumas D, Kern J, Hoffman E, McLennan G, Hyde D. Can retinoic acid ameliorate the physiologic and morphologic effects of elastase instillation in the rat? *Chest.* 2000; 117:242S–244S. [PubMed: 10843928]
89. Fujita M, Ye Q, Ouchi H, Nakashima N, Hamada N, Hagimoto N, Kuwano K, Mason RJ, Nakanishi Y. Retinoic acid fails to reverse emphysema in adult mouse models. *Thorax.* 2004; 59:224–230. [PubMed: 14985558]
90. March TH, Bowen LE, Finch GL, Nikula KJ, Wayne BJ, Hobbs CH. Effects of strain and treatment with inhaled aII-trans-retinoic acid on cigarette smoke-induced pulmonary emphysema in mice. *COPD.* 2005; 2:289–302. [PubMed: 17146994]
91. Lucey EC, Goldstein RH, Breuer R, Rexer BN, Ong DE, Snider GL. Retinoic acid does not affect alveolar septation in adult FVB mice with elastase-induced emphysema. *Respiration.* 2003; 70:200–205. [PubMed: 12740518]
92. Stinchcombe SV, Maden M. Retinoic acid induced alveolar regeneration: critical differences in strain sensitivity. *Am J Respir Cell Mol Biol.* 2008; 38:185–191. [PubMed: 17717321]
93. Roth MD, Connett JE, D'Armiento JM, Foronjy RF, Friedman PJ, Goldin JG, Louis TA, Mao JT, Muindi JR, O'Connor GT, Ramsdell JW, Ries AL, Scharf SM, Schluger NW, Sciurba FC, Skeans

- MA, Walter RE, Wendt CH, Wise RA. Feasibility of retinoids for the treatment of emphysema study. *Chest*. 2006; 130:1334–1345. [PubMed: 17099008]
94. Hind M, Stinchcombe S. Palovarotene, a novel retinoic acid receptor gamma agonist for the treatment of emphysema. *Curr Opin Investig Drugs*. 2009; 10:1243–1250.
 95. Stolk J, Cooper BG, Stoel B, Rames A, Rutman O, Soliman S, Stockley R. Retinoid treatment of Emphysema in Patients on the Alpha-1 International Registry. The REPAIR study: study design, methodology and quality control of study assessments. *Ther Adv Respir Dis*. 2010; 4:319–332. [PubMed: 20926506]
 96. Fujino N, Kubo H, Suzuki T, Ota C, Hegab AE, He M, Suzuki S, Yamada M, Kondo T, Kato H, Yamaya M. Isolation of alveolar epithelial type II progenitor cells from adult human lungs. *Lab Invest*. 2011; 91:363–378. [PubMed: 21079581]
 97. Kajstura J, Rota M, Hall SR, Hosoda T, D'Amario D, Sanada F, Zheng H, Ogorek B, Rondon-Clavo C, Ferreira-Martins J, Matsuda A, Arranto C, Goichberg P, Giordano G, Haley KJ, Bardelli S, Rayatzadeh H, Liu X, Quaini F, Liao R, Leri A, Perrella MA, Loscalzo J, Anversa P. Evidence for human lung stem cells. *N Engl J Med*. 2011; 364:1795–1806. [PubMed: 21561345]
 98. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell*. 2005; 121:823–835. [PubMed: 15960971]
 99. Quinn AM, Harvey RG, Penning TM. Oxidation of PAH trans-dihydrodiols by human aldo-keto reductase AKR1B10. *Chem Res Toxicol*. 2008; 21:2207–2215. [PubMed: 18788756]
 100. Wang R, Wang G, Ricard MJ, Ferris B, Strulovici-Barel Y, Salit J, Hackett NR, Gudas LJ, Crystal RG. Smoking-induced upregulation of AKR1B10 expression in the airway epithelium of healthy individuals. *Chest*. 2010; 138:1402–1410. [PubMed: 20705797]
 101. Li T, Molteni A, Latkovich P, Castellani W, Baybutt RC. Vitamin A depletion induced by cigarette smoke is associated with the development of emphysema in rats. *J Nutr*. 2003; 133:2629–2634. [PubMed: 12888649]
 102. Baybutt RC, Hu L, Molteni A. Vitamin A deficiency injures lung and liver parenchyma and impairs function of rat type II pneumocytes. *J Nutr*. 2000; 130:1159–1165. [PubMed: 10801913]
 103. Wang KC, Helms JA, Chang HY. Regeneration, repair and remembering identity: the three Rs of Hox gene expression. *Trends Cell Biol*. 2009; 19:268–275. Figure 4 reprinted from *Trends in Cell Biology* with permission from Elsevier. [PubMed: 19428253]
 104. Fernandez CC, Gudas LJ. The truncated Hoxa1 protein interacts with Hoxa1 and Pbx1 in stem cells. *J Cell Biochem*. 2009; 106:427–443. [PubMed: 19115252]
 105. Ferraiuolo MA, Rousseau M, Miyamoto C, Shenker S, Wang XQ, Nadler M, Blanchette M, Dostie J. The three-dimensional architecture of Hox cluster silencing. *Nucleic Acids Res*. 2010; 38:7472–7484. [PubMed: 20660483]
 106. Gillespie RF, Gudas LJ. Retinoid regulated association of transcriptional co-regulators and the polycomb group protein SUZ12 with the retinoic acid response elements of Hoxa1, RARbeta(2), and Cyp26A1 in F9 embryonal carcinoma cells. *J Mol Biol*. 2007; 372:298–316. [PubMed: 17663992]
 107. Kashyap V, Gudas LJ. Epigenetic regulatory mechanisms distinguish retinoic acid-mediated transcriptional responses in stem cells and fibroblasts. *J Biol Chem*. 2010; 285:14534–14548. [PubMed: 20231276]
 108. Kashyap V, Rezende NC, Scotland KB, Shaffer SM, Persson JL, Gudas LJ, Mongan NP. Regulation of stem cell pluripotency and differentiation involves a mutual regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. *Stem Cells Dev*. 2009; 18:1093–1108. [PubMed: 19480567]
 109. Langston AW, Gudas LJ. Identification of a retinoic acid responsive enhancer 3' of the murine homeobox gene Hox-1.6. *Mech Dev*. 1992; 38:217–227. [PubMed: 1360810]
 110. Langston AW, Thompson JR, Gudas LJ. Retinoic acid-responsive enhancers located 3' of the Hox A and Hox B homeobox gene clusters. Functional analysis. *J Biol Chem*. 1997; 272:2167–2175. [PubMed: 8999919]

111. Martinez-Ceballos E, Gudas LJ. Hoxa1 is required for the retinoic acid-induced differentiation of embryonic stem cells into neurons. *J Neurosci Res.* 2008; 86:2809–2819. [PubMed: 18512762]
112. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature.* 2010; 464:1071–1076. [PubMed: 20393566]
113. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell.* 2007; 129:1311–1323. [PubMed: 17604720]
114. Zhang X, Lian Z, Padden C, Gerstein MB, Rozowsky J, Snyder M, Gingeras TR, Kapranov P, Weissman SM, Newburger PE. A myelopoiesis-associated regulatory intergenic noncoding RNA transcript within the human HOXA cluster. *Blood.* 2009; 113:2526–2534. [PubMed: 19144990]
115. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Protacio A, Flynn RA, Gupta RA, Wysocka J, Lei M, Dekker J, Helms JA, Chang HY. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature.* 2011; 472:120–124. [PubMed: 21423168]
116. Ackema KB, Charite J. Mesenchymal stem cells from different organs are characterized by distinct topographic Hox codes. *Stem Cells Dev.* 2008; 17:979–991. [PubMed: 18533811]
117. Gordon JA, Hassan MQ, Koss M, Montecino M, Selleri L, van Wijnen AJ, Stein JL, Stein GS, Lian JB. Epigenetic Regulation of Early Osteogenesis and Mineralized Tissue Formation by a HOXA10-PBX1-Associated Complex. *Cells Tissues Organs.* 2011
118. Leucht P, Kim JB, Amasha R, James AW, Girod S, Helms JA. Embryonic origin and Hox status determine progenitor cell fate during adult bone regeneration. *Development.* 2008; 135:2845–2854. [PubMed: 18653558]
119. Rinn JL, Wang JK, Allen N, Brugmann SA, Mikels AJ, Liu H, Ridky TW, Stadler HS, Nusse R, Helms JA, Chang HY. A dermal HOX transcriptional program regulates site-specific epidermal fate. *Genes Dev.* 2008; 22:303–307. [PubMed: 18245445]
120. Shimono K, Tung WE, Macolino C, Chi AH, Didizian JH, Mundy C, Chandraratna RA, Mishina Y, Enomoto-Iwamoto M, Pacifici M, Iwamoto M. Potent inhibition of heterotopic ossification by nuclear retinoic acid receptor-gamma agonists. *Nat Med.* 2011; 17:454–460. [PubMed: 21460849]
121. Boucherat O, Franco-Montoya ML, Thibault C, Incitti R, Chailley-Heu B, Delacourt C, Bourbon JR. Gene expression profiling in lung fibroblasts reveals new players in alveolarization. *Physiol Genomics.* 2007; 32:128–141. [PubMed: 17911382]
122. Thummel R, Ju M, Sarras MP Jr, Godwin AR. Both Hoxc13 orthologs are functionally important for zebrafish tail fin regeneration. *Dev Genes Evol.* 2007; 217:413–420. [PubMed: 17437127]
123. Vitobello A, Ferretti E, Lampe X, Vilain N, Ducret S, Ori M, Spetz JF, Selleri L, Rijli FM. Hox and Pbx factors control retinoic acid synthesis during hindbrain segmentation. *Dev Cell.* 2011; 20:469–482. [PubMed: 21497760]
124. Serpente P, Tumpel S, Ghyselinck NB, Niederreither K, Wiedemann LM, Dolle P, Chambon P, Krumlauf R, Gould AP. Direct crossregulation between retinoic acid receptor {beta} and Hox genes during hindbrain segmentation. *Development.* 2005; 132:503–513. [PubMed: 15634700]
125. Huang D, Chen SW, Langston AW, Gudas LJ. A conserved retinoic acid responsive element in the murine Hoxb-1 gene is required for expression in the developing gut. *Development.* 1998; 125:3235–3246. [PubMed: 9671595]
126. LaRosa GJ, Gudas LJ. An early effect of retinoic acid: cloning of an mRNA (Era-1) exhibiting rapid and protein synthesis-independent induction during teratocarcinoma stem cell differentiation. *Proc Natl Acad Sci U S A.* 1988; 85:329–333. [PubMed: 3422432]
127. Bertrand N, Roux M, Ryckebusch L, Niederreither K, Dolle P, Moon A, Capecchi M, Zaffran S. Hox genes define distinct progenitor sub-domains within the second heart field. *Dev Biol.* 2011; 353:266–274. [PubMed: 21385575]

Highlights

- RA signaling via Hox expression is important for cell positional memory in the adult
- ALDH1a2 (RALDH2) is induced after amputation in the regenerating heart and fin in zebrafish
- Levels of endogenous retinoids are altered in many different diseases in adult animals and humans

Uptake and Intracellular Metabolism of Retinol (modified from Mongan and Gudas, 2007).

Figure 1. Major pathways of retinol (vitamin A) metabolism in mammalian cells

Not all of these enzymatic pathways are present in all cells; Stra6 is a receptor for RBP4 (serum retinol-binding protein), and both Stra6 and lecithin:retinol acyltransferase (LRAT) are required for accumulation of retinol within some, but not all cell types. RARE, DNA retinoic acid response element; RAR, retinoic acid receptor; RXR, retinoid X receptor; ADHs, alcohol dehydrogenases; ALDH, aldehyde dehydrogenase; CRBP1, cellular retinol binding protein 1; CRABP1, 2, cellular retinoic acid-binding proteins 1 and 2. Rol, retinol; Ral, retinaldehyde; RA, all-trans retinoic acid (modified from [3]).

FIGURE 2. “Multiple-step model” for RA-mediated mucosal DC generation

One mechanism for T_{reg} production: mucosal DC precursors (pre-DCs) encounter RA, which is metabolized by $ALDH^+$ bone marrow cells from dietary vitamin A, in a bone marrow microenvironment. RA induces pre-DCs to express CCR9 and $ALDH1a2$, and suppresses $SOCS3$ while enhancing $STAT3$ activation. $CCR9^+$ DCs then exit the bone marrow and migrate into the intestine by the chemotactic attraction of CCR9 ligand CCL25. In the intestinal mucosa, commensal TLR (toll-like receptor) ligands and epithelial cell-derived signals further instruct DCs to develop into fully functional “regulatory” mucosal DCs, which are competent to promote the maturation of $Foxp3^+$ Tregs. In contrast, without RA conditioning, pre-DCs develop into “inflammatory” DCs, which produce proinflammatory cytokines in response to TLR7 signals and promote Th1 and Th17 cell differentiation [22].

Figure 3. Model: ALDH1a2 is necessary but not sufficient for regeneration following injury
Depiction of the hypothesis that regeneration occurs only after ALDH1a2 induction.

Figure 4. Hox status and bone regeneration in mouse

Cells expressing Hox genes (for example Hoxa11) are in light blue and those not expressing Hox are indicated in light grey. Hox(+) skeletal stem cells can only heal orthotopic Hox(+) bone injury site (tibia). (a) Hox(+) skeletal stem cells cannot repair a Hox null injury site (mandible). (b) Conversely, Hox null skeletal stem cells will express the ectopic Hox gene when transplanted into a Hox(+) injury site and regenerate the ectopic bone [103].