scriptional derepression and the cell cycle most likely reflects the opportunity for synthesis of new DNA that is less methylated and more accessible than that of the parent strand (*6*). After differentiation, epigenetic changes and allelic expression patterns persist for multiple cell divisions, showing that they are both stable and inheritable. Thus, T_H1 and T_H2 lymphocytes exhibit memory of both specificity and function, reflecting the stability of both types of differentiation. The mechanisms of this memory, however, are different. Stability of clonal specificity results from genetic recombination, whereas stability of function is accomplished by epigenetic modification.

Why should cytokine genes be expressed from only one allele? The clearest examples of monoallelic expression of autosomal genes are found in two organ systems for which cellular specificity and population diversity are essential: the immune system (antigen receptors on T, B, and natural killer cells) and the nervous system (olfactory receptors on olfactory epithelial cells) (*7*). Monoallelic expression (or allelic exclusion) ensures that most individual cells express only one member of a family of receptors encoded by highly homologous genes, resulting in each cell having only one of many possible specificities. The functional significance of monoallelic expression of cytokine genes is less obvious and may simply reflect the rate limitations of chromatin remodeling at these loci.

The question of instructive versus selective differentiation has been addressed in other hematopoietic cell lineages. Both instruction and selection have been reported for various lineage-restricted growth and differentiation factors (*8*). For example, macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor appear to instruct different fates in bipotential granulocyte-macrophage progenitors. In contrast, erythropoietin supports expansion of cells committed to the erythroid lineage (selection) but is not required for progenitor cells to make that commitment. Perhaps the example that is most relevant to T_H differentiation is the analogous functional differentiation of B cells involving isotype switch recombination of immunoglobulin heavy chain genes. Cytokines (including IL-4 and IFN-γ) can dramatically alter the switching between immunoglobulin isotypes in differentiating B cells (*9*). Antibody isotype switching requires transcriptional activation of heavy chain genes, and IL-4 and IFN-γ regulate switching by inducing transcription of specific heavy chain genes. The result of cytokine action on differentiation is, thus, instructive, not selective. This instruction takes the form of changes in the probability of different outcomes within the intrinsic constraints of a stochastic process. For example, the probability that B cells will switch to IgE ranges from <0.0001 to >0.01 in the absence or presence, respectively, of IL-4.

The regulation of T_H1 and T_H2 differentiation by differentiative inducer cytokines such as IL-12 or IL-4 may not be adequately described as either strictly instructive or strictly selective. The rate-limiting nature of chromatin remodeling of cytokine gene loci introduces an element of probability into the process, much as it does for antibody isotype switching for B cells. Cytokine inducers of T_H1 or T_H2 differentiation could alter the odds of stable chromatin remodeling of specific cytokine gene loci. Extremes of T_H1 or T_H2 differentiation may be achieved either by subsequent selection or by large changes in probability (see the figure). To decide among these models, it may prove necessary to produce the equivalent of an embryologist's fate map, accounting for the birth, differentiation, and death of all descendants of an individual T cell stimulated under highly controlled conditions.

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PERSPECTIVES: BIOCHEMISTRY

Seeking Ligands for Lonely Orphan Receptors

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Cells are exposed to a plethora of chemicals—metabolic intermediates, hormones, and compounds in the environment. One way in which cells adapt to these physiological and toxicological challenges is through nuclear receptors, which bind to these molecules, move to the nucleus, and initiate changes in gene transcription. Currently we know of about 70 different nuclear receptors, but only half of these have ligands that have been identified (*1*). The remaining receptors with unknown ligands are called orphan nuclear receptors. Identifying ligands for these receptors is a huge challenge but is one that the pharmaceutical industry is eager to take on. Drugs that mimic such ligands could be of particular value in the treatment of diseases that are caused by defects in the biochemical pathways in which these nuclear receptors are involved.

Two reports on pages 1362 and 1365 of this issue from the groups of Willson and Mangelsdorf (*2, 3*) and one in this month's *Molecular Cell* (*4*) now show that bile acids, important regulators of cholesterol homeostasis, are the physiological ligands of the farnesoid X receptor (FXR), an orphan member of the nuclear receptor family. This finding implicates FXR in the regulation of one of the key biochemical pathways in the body.

The most important primary bile acids in humans are cholic acid (the most abundant) and chenodeoxycholic acid. Bile acids are oxidation products of cholesterol with the enzyme cholesterol 7α-hydroxylase as the rate-limiting step in their synthesis. Cholic acid and chenodeoxycholic acid differ only in that cholic acid has a hydroxyl group at the 12α position and requires an extra enzyme, 12α-hydroxylase, for synthesis. Bile acids have two important functions in the gut: to facilitate solubilization and disposal of cholesterol (see the figure) and to facilitate absorption of dietary fat and fat-soluble vitamins. They are synthesized from cholesterol by two distinct pathways. The first is the classical "neutral" pathway in which cholesterol 7α-hydroxylase catalyzes the first and rate-limiting step (*5*). In the second (and more recently discovered) "acidic" pathway (*6*), oxysterol 7α-hydroxylase replaces cholesterol 7α-hydroxylase as the primary synthetic enzyme (*5*). The acidic pathway begins with the oxidation of a cholesterol side chain to form 27-hydroxy cholesterol. Although the neutral pathway usually predominates, the acidic pathway is important, for example, in babies with a mutation in the oxysterol 7α-hydroxylase gene (*7*). The three reports now

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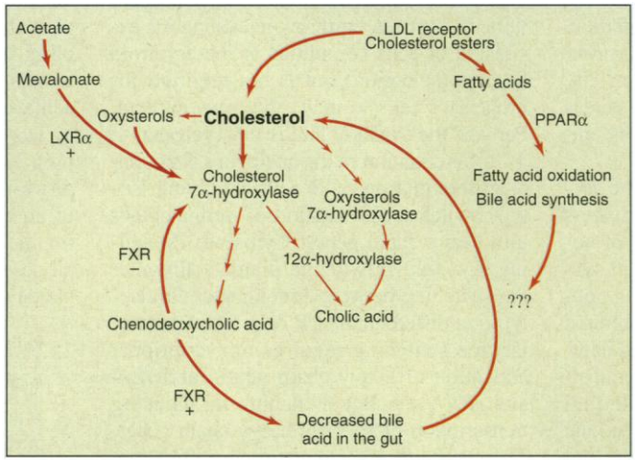
demonstrate that chenodeoxycholic acid but not cholic acid binds to FXR (*2–4*). Moreover, when bound to bile acids, FXR down-regulates the transcription of cholesterol 7α-hydroxylase and activates the gene encoding a candidate bile acid transporter protein, which transports bile acids from the gut to the liver (*3*). The result is a decrease in the amount of bile acid in the gut. Hence, through binding to FXR, bile acids can regulate their own synthesis and transport.

One of the most notable findings is that chenodeoxycholic acid, not cholic acid, is the principal ligand of FXR. Chenodeoxycholic acid is therefore a crucial regulator of cholesterol 7α-hydroxylase expression and of cholesterol homeostasis (its regulatory effect on cholesterol levels has been known for decades) (*8, 9*). The enzyme that catalyzes the synthesis of cholic acid, 12α-hydroxylase, is thus an important branch point enzyme and potential feedback mechanism in bile acid biosynthesis because it regulates the ratio of cholic acid to chenodeoxycholic acid.

The affinity (K_d) of FXR binding to bile acids is in the micromolar range, about three orders of magnitude higher than the nanomolar K_d values for steroid hormone receptors bound to their ligands. Micromolar K_d values have been observed for other orphan nuclear receptors: the peroxisomal proliferator activated receptor (PPAR), which binds fatty acids, and the liver X receptor (LXRα), which binds oxysterols. These high K_d values are consistent with the relatively high tissue concentrations of the lipid ligands of these receptors. Clearly, nuclear receptors should no longer be regarded exclusively as high-affinity receptors, because FXR, PPAR, and LXRα all bind their ligands with relatively low affinity.

We still do not understand the mechanisms that regulate the expression of oxysterol 7α-hydroxylase (the principal enzyme of the alternative pathway of bile acid synthesis) and 12α-hydroxylase, or the parts played by other orphan receptors in the regulation of cholesterol homeostasis. It is well established that, when bound to its ligand, LXRα can induce the synthesis of cholesterol 7α-hydroxylase, thus opposing the effect of FXR. The ligand for LXRα is not bile acid but oxysterol (*10*), an oxidized metabolite of cholesterol. Mice genetically engineered to be deficient in LXRα appear to be normal, but when they are fed a very high cholesterol diet their livers become full of cholesterol (*10*). As animals deficient in 7α-hydroxylase survive without evident problems, does this mean that oxysterol 7α-hydroxylase can replace cholesterol 7α-hydroxylase under normal dietary conditions? It is noteworthy that LXRα and FXR, which have opposing effects on cholesterol 7α-hydroxylase synthesis, communicate directly with each other (*4*). In the presence of FXR, bile acids repress transcriptional activity of LXRα, but they have no effect in the absence of FXR.



Bilious biochemical pathways. A number of nuclear receptors are involved in the biochemical pathways that regulate cholesterol homeostasis. For example, FXR binds bile acids that are important in the disposal of cholesterol. When bound to bile acids, FXR switches off (–) production of cholesterol 7α-hydroxylase (which is the rate-limiting step in bile acid synthesis) and switches on (+) synthesis of bile acid transporter proteins, leading to a decrease in bile acid in the gut and an increase in cholesterol levels in the blood. Another nuclear receptor, LXRα, which binds oxysterols, induces the synthesis of bile acids by up-regulating (+) cholesterol 7α-hydroxylase. In addition to the classical pathway of bile acid synthesis, there is an alternative pathway in which oxysterol 7α-hydroxylase is the rate-limiting enzyme.

Another player in the story of the regulation of cholesterol and bile acid synthesis is PPARα. When bound to fatty acids, PPARα stimulates the proliferation of peroxisomes and induces synthesis of several enzymes involved in the β-oxidation of fatty acids. PPARα is not only activated by fatty acids but also by lipid-lowering drugs, such as the fibrates, which stimulate proliferation of peroxisomes (*11*). Peroxisomes catalyze the β-oxidation of fatty acids, the synthesis of cholesterol and other isoprenoids, and certain steps in the oxidation of cholesterol to bile acids. Interestingly, FXR was originally identified as a receptor for farnesol, an intermediate in the synthesis of cholesterol and other isoprenoids by peroxisomes (*12*). FXR, LXRα, and PPARα may therefore regulate both fatty acid and cholesterol homeostasis. These two pathways are probably coregulated because the amount of free fatty acids and cholesterol in cells is increased when cholesterol esters (delivered by low density lipoproteins to lysosomes) are hydrolyzed into their component parts (cholesterol and fatty acids). The cell has to take care of both of these potentially toxic molecules at the same time suggesting that the pathways for disposal of cholesterol should be coordinated with those involved in fatty acid disposal.

Receptors for hormones such as glucocorticoids, estrogen, and thyroid hormone also regulate cholesterol homeostasis. Another orphan receptor, the pregnane X receptor (PXR), down-regulates cholesterol 7α-hydroxylase expression and increases bile acid flow (*13*). This promiscuous receptor is activated by many pharmaceutical agents. Its most effective endogenous ligand is corticosterone, and one of its most potent pharmaceutical ligands is the antibiotic rifampicin. Interestingly, the ansamycins, which are derived from rifampicin but lack antibacterial activity, are hypolipidemic agents that effectively lower plasma cholesterol levels, perhaps by binding to PXR.

The 70 different nuclear receptors characterized thus far may be just a small sampling of a much larger family. A recent scan of the *Caenorhabditis elegans* genome for two–zinc finger structures (a characteristic feature of nuclear receptors) reveals the presence of 228 such proteins in this nematode (*14*). It is, of course, not clear that mammalian homologs for all of these nematode nuclear receptors exist. However, it is tempting to speculate that there are many more nuclear receptors and their ligands waiting to be discovered.

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