Lipoproteins and their receptors in embryonic development: more than cholesterol clearance

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Previously, the relevance of lipoproteins and their receptors has mainly been discussed in terms of cholesterol clearance in the adult organism. Now, findings from nematodes to fruit flies to mammals all point towards novel and unexpected roles for lipoprotein metabolism in the control of key regulatory pathways in the developing embryo, including signaling through steroid hormones and throughout the hedgehog and Wnt signaling pathways. Here, we discuss the emerging view of how lipoproteins and their receptors regulate embryogenesis.

Introduction

All lipids transported in the plasma or extracellular fluid of multicellular organisms are solubilized by specialized transport particles, of which the most common are lipoproteins (see Box 1 for details). Lipoproteins traffic lipids such as cholesterol from the tissue of origin to target sites, where the lipid cargo is delivered via lipoprotein receptor-mediated uptake (Havel and Kane, 2001). So far, the biological significance of lipoproteins has mainly been discussed in terms of cholesterol homeostasis in the adult organism. Here, lipoproteins regulate the supply of the sterol required for many cellular activities, including membrane formation, the synthesis of steroid hormones and the post-translational modification of proteins (for example, the activation of hedgehog) (Havel and Kane, 2001) (see Box 2 for details). Our focus on adult lipoprotein metabolism has chiefly been guided by its importance as a risk factor for cardiovascular disease. Disturbances in lipoprotein metabolism in patients may result in dramatic increases in plasma cholesterol levels (hypercholesterolemia) and, consequently, in atherosclerosis and premature death from coronary artery disease (Goldstein et al., 2001).

Early on, evidence implied that lipoproteins have an equally important yet poorly understood function in embryonic development; evidence that has come from humans and experimental animal models with metabolic malformation syndromes, in which the pharmacological or genetic inactivation of key factors in cholesterol and lipoprotein metabolism causes developmental anomalies (reviewed by Kelley, 2000). These syndromes include inborn errors in cholesterol biosynthesis, such as Smith-Lemli-Opitz syndrome (SLOS), in which mutations in the gene encoding 7-dehydrocholesterol reductase (DHCR7) cause cleft palate and holoprosencephaly (Fitzky et al., 1998); mevalonic aciduria, a defect of mevalonate kinase, which causes facial dysmorphology and skeletal dysplasia (Hoffmann et al., 1993); as well as desmosterolosis, a malfunction of desmosterol reductase, which is associated with macrocephaly, ambiguous genitalia and short limbs (FitzPatrick et al., 1998). Also, the targeted inactivation



Lipoproteins are spherical macromolecules of 10-1200 nm diameter. They are composed of a core of neutral lipids that include mostly cholesterol ester and triglycerides but also fat-soluble vitamins. The core of the particle is surrounded by an amphipathic shell of polar phospholipids and cholesterol. The density of lipoproteins is inversely related to their size and reflects the unique composition of the lipid core. Accordingly, they are categorized into six classes, including high-density (HDL), low-density (LDL) and very low-density lipoproteins (VLDL). Embedded in the shell of lipoproteins are specialized apoproteins, such as apolipoproteins in vertebrates (e.g. APOB and APOE), apolipophorines in insects, as well as vitellogenins in all egg-laying species. The apoproteins play decisive roles in the cell biology of lipoproteins. Some are required for the assembly and secretion of the particles from donor tissues, a process that takes place in the rough endoplasmic reticulum (ER) and involves the activity of lipid transfer proteins such as the vertebrate microsomal triglyceride transfer protein (MTP) (see figure). MTP lipidates the nascent polypeptide chain of APOB that is co-translationally transported into the lumen of the ER. A primordial particle is formed that further increases in lipid content to transform into a mature lipoprotein, which is secreted via the secretory pathway of the cell. Other functions of apoproteins involve the interaction with enzymes (e.g. lipases) that modify the lipid content of circulating lipoproteins. Finally, some apoproteins interact with lipoprotein receptors to deliver the lipid cargo to target cells. Receptor interaction results in the endocytic uptake and lysosomal catabolism of the particles, as with LDL. Alternatively, lipids can be extracted from the lipoproteins that are attached to their receptors on the cell surface, as with vertebrate HDL and insect lipophorins.

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Box 2. Main functions of lipoproteins

The main role of lipoproteins is to transport structural and nutritional lipids throughout the organism. This function is conserved throughout evolution. Thus, lipoproteins are formed in tissues that absorb lipids from the diet or that serve as a major lipid storage pool in the organism (the fat body in insects, the liver in vertebrates). Following release into circulatory fluids, lipoproteins deliver their cargo to target tissues that require these lipids. Lipoprotein-bound triglycerides serve as an energy source, whereas cholesterol is a component of cell membranes and is required for steroid hormone and bile acid synthesis. In addition, lipoproteins also have more specialized functions in lipid homeostasis. Vitellogenins are lipoproteins that are formed in all egg-laying species. They deliver yolk lipid to the oocyte prior to egg deposition. High-density lipoproteins (HDL) are particles secreted from peripheral tissues that shuttle excessive lipids back to the liver for deposition. In humans, this so-called 'reverse cholesterol transport' counteracts the accumulation of cholesterol in vessel walls and the formation of atherosclerotic plaques.

of genes in lipoprotein metabolism, such as those encoding apolipoprotein B (APOB) (Farese et al., 1995), microsomal triglyceride transfer protein (Raabe et al., 1998) and the lipoprotein receptor LRP2 (Willnow et al., 1996), cause developmental abnormalities in mice, in particular brain formation defects. Not surprisingly, the favored hypothesis to explain these defects has been guided by our view of adult lipoprotein metabolism, in as much as insufficient levels of cholesterol in embryonic tissues were considered to be at the heart of the problem.

One example that illustrates the shortcomings of this concept is holoprosencephaly (HPE), a midline defect that is characterized by the fusion of the forebrain hemispheres. HPE is the most common developmental forebrain anomaly in humans, affecting as many as 1 in 250 pregnancies (Wallis and Muenke, 2000). In mice, craniofacial abnormalities consistent with HPE are caused by mutations in the gene that encodes *Dhcr7* (as in human SLOS) (Fitzky et al., 2001; Wassif et al., 2001), in sonic hedgehog (Shh) (Chiang et al., 1996) and also in Lrp2 (Willnow et al., 1996). Accordingly, the inadequate supply of cholesterol from endogenous biosynthesis or from maternal sources absorbed via the yolk sac was believed to be responsible for hampering the formation of cell membranes or the activation of SHH, a regulator of forebrain patterning. However, this hypothesis has been challenged by a number of observations. For example, cholesterol levels are normal in approximately 10% of all SLOS patients (Kelley and Hennekam, 2000). Also, cholesterol-mediated activation of SHH is not impaired in murine models of the disease (Cooper et al., 2003). Finally, sustained expression of LRP2 in the murine yolk sac does not rescue Lrp2-null mice from forebrain malformations (Spoelgen et al., 2005).

This left the puzzling question of what the true contribution of lipoprotein metabolism to embryonic development might be. The surprising answer came with the findings of recent studies that pointed towards lipoproteins and their receptors having unexpected roles as mediators of embryonic signaling pathways.

This review discusses the emerging view that lipoproteins actively participate in signaling pathways by regulating the distribution and local delivery of key regulators of cellular differentiation processes, such as sterols and lipid-linked morphogens. These findings provide a new paradigm in developmental biology that is certain to revise the current perception of lipoproteins as mere cargo transporters of membrane cholesterol.

Lipoprotein receptors are essential for embryonic development

Major advances in our understanding of embryonic lipoprotein metabolism have come from animal models in which dysfunctional members of the LDL receptor gene family have given rise to developmental defects. The LDL receptor gene family represents the main class of endocytic lipoprotein receptors, which are expressed in many tissues in organisms as distantly related as nematodes and mammals (Fig. 1). The LDL receptor is the archetypal member of the family and has structures that are representative of a receptor involved in cellular cholesterol uptake (Fig. 2A). The significance of this receptor for systemic cholesterol homeostasis is underscored by the pathological features of patients who suffer from familial hypercholesterolemia (FH). FH is caused by heritable LDL receptor gene defects and results in the inability of individuals to clear cholesterol-rich LDL from the blood stream, leading to excessive levels of circulating cholesterol (Goldstein et al., 2001). These features are shared by models of LDL receptor deficiency in mice and rabbits (Table 1).

The LDL receptor could be dispensable for embryogenesis, as judged by the normal development of organisms that lack this receptor function. Thus, it came as a surprise when loss of expression of other family members had profound consequences for developmental processes in both laboratory animals (Table 1) and humans (Table 2). The interpretation of these results has been complicated by the fact that, in contrast to the LDL receptor, other receptors not only bind apolipoproteins but also a multitude of different macromolecules, including proteases, protease inhibitors, vitamin carriers and signaling molecules (reviewed by Beffert et al., 2004; Nykjaer and Willnow, 2002). In some instances, linking developmental defects to impaired lipoprotein metabolism was obvious, as with mutations in genes that encode vitellogenin receptors. Loss of receptor activity in C. elegans rme-2 mutants (Grant and Hirsh, 1999), in Drosophila yolkless (yl) (Schonbaum et al., 1995) and in the chicken restricted-ovulator strain (Bujo et al., 1995) prevents the deposition of yolk (vitellogenesis), so causing female sterility. By contrast, the abnormal layering of neurons in the cortex and cerebellum of mouse models with very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (APOER2; also known as LRP8 - Mouse Genome Informatics) deficiencies has unambiguously been linked to a defect in the binding and transmembrane signaling of reelin, a secreted factor that provides guidance cues to migrating neurons in the developing brain (Bock and Herz, 2003; Hiesberger et al., 1999; Trommsdorff et al., 1999). There is no evidence to suggest that lipids are involved in the latter phenomenon. However, there are yet other members of the gene family that impact on embryogenesis, as judged from the developmental abnormalities seen in genetargeted mice (Table 1). In these cases, regulatory roles for lipoproteins and their lipid cargo remained a hypothesis to be explored. For example, disruption of the Lrp1 gene causes lethality of affected embryos around mid-gestation (Herz et al., 1993), defects that are possibly linked to impaired formation of the liver (Roebroek et al., 2006). Mice deficient for Lrp6 suffer from axial truncation and abnormal head and limb structures (Pinson et al., 2000), whereas $Lrp4^{-/-}$ mice have more-specific defects in late embryogenesis that affect limb formation (Johnson et al., 2005). Finally, the development of the forebrain and reproductive organs is impaired in Lrp2-deficient mouse embryos, phenotypes that are partially recapitulated in models of impaired cholesterol biosynthesis.



Fig. 1. The LDL receptor family. The structural organization of members of the LDL receptor family. Their extracellular domains contain clusters of complement-type repeats that are the site of ligand binding, and also β -propellers, which are essential for the pH-dependent release of ligands in endosomes. The cytoplasmic tails harbor recognition sites for adaptor proteins that are involved in protein trafficking and signal transduction. All ectodomains share significant sequence similarity in line with the ability of the receptors to bind apolipoproteins. By contrast, the cytoplasmic domains are unique, indicating distinct cellular fates for ligands internalized by individual receptors. Receptors on the left are considered to be core members of the protein family as their extracellular domains are built from a unifying module of amino-terminal complement-type repeats, followed by a carboxyl-terminal cluster of β -propellers and epidermal growth factor-type repeats. This module can exist in single (e.g. LDLR) or multiple (e.g. LRP2) copies in the receptors (e.g. SORLA). APOER2, apolipoprotein E receptor 2; Ce, *C. elegans*; LDLR, low-density lipoprotein receptor; LRP, LDL receptor-related protein; MEGF7, multiple epidermal growth factor-type repeat containing protein 7; RME-2, receptor-mediated endocytosis-2; SORLA, sortilin-related receptor with A-type repeats; VLDLR, very low-density lipoprotein receptor.

In the following, we discuss studies that have uncovered the molecular mechanisms that underlie the developmental phenotypes in mutants of LDL receptor gene family members, and that indeed confirm central roles for the embryonic lipid transport machinery in developmental processes.

Lipoprotein receptors deliver precursors of intracellular steroid hormones

Nematodes express several receptors of the LDL receptor gene family, including RME-2 and *C. elegans* LRP-1 (Ce-LRP-1). RME-2 is a typical vitellogenin receptor that is expressed in oocytes and that mediates the endocytic uptake of yolk, a lipoprotein particle that consists of vitellogenins complexed with intestine-derived lipids. Loss of receptor activity in *rme-2* mutants causes impaired vitellogenesis and reduced embryonic survival (Grant and Hirsh, 1999). These findings are consistent with a role for lipoprotein metabolism in the supplementation of the fertilized oocyte with lipids prior to egg deposition, a function conserved throughout evolution (Box 2, Table 1). Perhaps more rewarding in terms of

conceptual advances has been the analysis of a second lipoprotein receptor in C. elegans, Ce-LRP-1, the homolog of LRP2 (megalin) in mammals (Yochem and Greenwald, 1993). Ce-LRP-1 is strongly expressed on the apical surface of the hypodermis, which comes into contact with the worm's environment. Recessive mutations in ce-lrp-*1* produce a complex phenotype, involving arrested larval growth and the inability to shed the old cuticle during molting (Yochem et al., 1999). Although the exact mechanism that underlies these phenotypes remains obscure, two additional observations are remarkable. The larval growth arrest phenotype in *ce-lrp-1*-deficient larvae resembles features observed in dauer (enduring), a specialized diapause stage of L3 larvae that is induced by hostile conditions, such as starvation or high population density. Furthermore, ce-lrp-1-deficient phenotypes could be reproduced in the wild-type larvae by cholesterol depletion, implicating defects in cholesterol homeostasis in the mutant phenotype. Because nematodes are auxotrophic for cholesterol, which they take up from the environment through the hypodermis, these findings indicate an obvious role of Ce-LRP-1 in supplementing nematodes with the



Fig. 2. Lipoprotein receptors mediate uptake of lipoprotein-bound cholesterol. (**A**) Vertebrate lipoproteins are taken up by the LDL receptor via binding of apolipoproteins B or E present in the shell of the lipoprotein particle. Following endocytosis, the receptor discharges the lipoprotein particle in endosomes before recycling back to the cell surface. Internalized apolipoproteins are degraded in lysosomes, while cholesterol enters the cellular membrane pool via the endoplasmic reticulum (ER) or is converted into steroid hormones in mitochondria or is stored as cholesterol esters in cytoplasmic lipid droplets. The exit of cholesterol from lysosomes requires the activity of Niemann-Pick type C1 (NPC1), an integral membrane protein that acts as transporter for sterols. (**B**) *C. elegans* LRP-1 (Ce-LRP-1) also mediates the endocytic uptake of cholesterol-rich lipoproteins. Following release from endocytic compartments, cholesterol is converted into the steroid hormone gamravali, which blocks nuclear hormone receptor DAF-12-dependent induction of larval growth arrest. PM, plasma membrane.

sterol from the environment (Yochem et al., 1999). But what conceptual advances in our understanding of cholesterol metabolism and its role in larval development have emerged from these studies? The surprising twist came from studies by Kurzchalia and colleagues, who uncovered why nematodes need cholesterol (Matyash et al., 2004). They demonstrated that reproductive growth in nematodes requires an intracellular steroid hormone, tentatively termed gamravali, that inhibits the activity of the nuclear hormone receptor DAF-12 (Fig. 2B). In the presence of gamravali, the DAF-12-dependent program to undergo diapause is blocked and normal larval growth ensues. Although not proven formally, the resemblance between ce-lrp-1 mutants and sterol-depleted larvae strongly suggests a model in which the precursor cholesterol is specifically delivered to target cells via the lipoprotein receptor Ce-LRP-1, enabling the formation of intracellular steroid hormones that regulate dauer larvae formation and molting (Fig. 2B) (Entchev and Kurzchalia, 2005; Matyash et al., 2004). Support for this model comes from studies by Grigorenko et al., who demonstrated that loss of the polytopic endoprotease Ce-IMP-2, which is related to mammalian presenilins, also results in larval growth arrest and molting phenotypes. Ce-IMP-2 is proposed to perform regulated intramembranous cleavage of Ce-LRP-1, liberating the intracellular domain of the receptor in response to ligand binding (Grigorenko et al., 2004). However, whether and how possible signals through the receptor tail regulate steroid hormone signaling is still a matter of debate.

In mammals, the role of lipoprotein receptors in the delivery of cholesterol to steroidogenic tissues (such as the adrenal gland) for steroid hormone synthesis has long been appreciated. However, these pathways act systemically; newly synthesized hormones are released in the circulatory system to act at distant sites in the organism. Rather than acting in a paracrine fashion, this novel steroid-uptake pathway uncovered in *C. elegans* acts cell autonomously as it specifically delivers the precursor to the target cells for the formation of essential intracellular hormones. Similar concepts have been discussed in vertebrates, in which neurosteroids (steroid hormones, such as pregnenolone, dehydroepiandrosterone and progesterone, that are produced in neurons) are proposed to protect the brain from neurodegenerative processes (Lee and McEwen, 2001; Wojtal et al., 2006).

Lipoprotein receptors deliver androgens and estrogens

A role for lipoprotein receptors in the cell-type-specific delivery of steroid hormone signals during development has also been confirmed in mice.

Lrp2 is highly expressed in a number of absorptive epithelia, including yolk sac, neural tube, kidney and reproductive organs of the mammalian embryo. In addition to other abnormalities (Table 1), Lrp2-deficient mice suffer from defects in the maturation of reproductive organs, including maldescent of the testis in males (cryptorchidism) and impaired opening of the vaginal cavity in females (Hammes et al., 2005). These features are reminiscent of rodents treated with anti-androgens and antiestrogens during the embryonic or postnatal period of life, respectively (Ashby et al., 2002; Spencer et al., 1991), implicating impaired sex steroid signaling in the phenotypes of Lrp2 mutant mice. A clue to understanding the role of LRP2 in the embryonic

Table 1. Loss-of-function models of the LDL receptor family

Receptor	Expression	Organism	Phenotype	References	
LDL receptor	Vertebrates	Rabbit (Watanabe heritable hyperlipidemic, WHHL)	Hypercholesterolemia	(Tanzawa et al., 1980)	
		Mouse (targeted gene disruption)	Hypercholesterolemia	(Ishibashi et al., 1993)	
VLDL receptor (vitellogenin receptor)	Vertebrates	Mouse (targeted gene disruption)	Dysplastic cerebellum, reduced adipose tissue mass	(Trommsdorff et al., 1999)	
		Chicken (restricted-ovulator)	Impaired vitellogenesis, female sterility	(Bujo et al., 1995)	
Yolkless	Insects	Drosophila (yolkless)	Impaired vitellogenesis, female sterility	(Schonbaum et al., 1995)	
RME-2	Nematodes	C. elegans (rme-2 null)	Impaired yolk deposition, reduced embryonic viability	(Grant and Hirsh, 1999)	
LRP8 (APOER2)	Vertebrates	Mouse (targeted gene disruption)	Dysplastic hippocampus and cerebellum	(Trommsdorff et al., 1999)	
LRP4 (MEGF7)	Vertebrates	Mouse (targeted gene disruption)	Impaired limb formation, polysyndactyly, neuromuscular junction defects	(Johnson et al., 2005; Simon- Chazottes et al., 2006; Weatherbee et al., 2006)	
		Cattle (mulefoot disease)	Syndactyly	(Drogemuller et al., 2007; Duchesne et al., 2006; Johnson et al., 2006)	
LRP5	Vertebrates and insects	Mouse (targeted gene disruption)	Low bone mass, hypercholesterolemia, impaired insulin secretion	(Fujino et al., 2003; Kato et al., 2002)	
LRP6 (Arrow)	Vertebrates and insects	Mouse (targeted gene disruption)	Abnormal body axis	(Pinson et al., 2000)	
		Xenopus (null mutant)	Impaired dorsal axis and neural crest formation	(Tamai et al., 2000)	
		Drosophila (arrow null)	Inhibition of Wingless-dependent patterning	(Wehrli et al., 2000)	
LRP1	Vertebrates	Mouse (targeted gene disruption)	Embryonic lethality	(Herz et al., 1992; Roebroek et al., 2006)	
LRP1B	Vertebrates	Mouse (targeted gene disruption)	Unknown	(Marschang et al., 2004)	
LRP2 (megalin; Ce-LRP-1)	Vertebrates and invertebrates	Mouse (targeted gene disruption)	Holoprosencephaly, impaired maturation of reproductive organs, renal dysfunction	(Willnow et al., 1996; Nykjaer et al., 1999; Hammes et al., 2005)	
		Rat (induced autoimmune disease)	Glomerular nephritis (Heymann nephritis)	(Raychowdhury et al., 1989)	
		C. elegans (ce-lrp-1 null)	Molting defect, larval growth arrest	(Yochem et al., 1999)	
SORLA (LR11; SORL1)	Vertebrates and invertebrates	Mouse (targeted gene disruption)	Alzheimer's disease	(Andersen et al., 2005)	
Highlighted receptors are those for which loss-of-function is associated with developmental defects.					

development of reproductive organs has come from studies on the function of the receptor in the adult kidney (Nykjaer et al., 1999). These studies demonstrated that LRP2 mediates the retrieval of the steroid 25-hydroxyvitamin D₃ from the primary urine into cells of the renal proximal tubules, the major cell type in the body that is responsible for the conversion of this inactive precursor into the biologically active steroid hormone 1, 25dihydroxyvitamin D₃. Intriguingly, cellular uptake of 25hydroxyvitamin D₃ does not proceed by the non-specific diffusion of the free steroid through the plasma membrane as postulated in the free hormone hypothesis (Mendel, 1989). Instead, this uptake involves receptor-mediated endocytosis of 25-hydroxyvitamin D₃ bound to its plasma carrier, the vitamin D binding protein (DBP; also known as GC globulin - Mouse Genome Informatics) (Nykjaer et al., 1999). Similarly, LRP2 was also found to be required for the cell-type-specific uptake of androgens and estrogens complexed with the sex hormone binding globulin (SHBG) (Hammes et al., 2005) (Fig. 3). The latter observation suggested a model in which tissues in the reproductive tract of

 $Lrp2^{-/-}$ embryos fail to specifically acquire carrier-bound sex steroids, resulting in impaired action of androgens and estrogens, despite normal circulating levels of these hormones.

Developmental processes that regulate testicular descent in male embryos provide a molecular explanation for LRP2 action. During late gestation, the male gonad migrates from the lower kidney pole into the inguinal region (close to the bladder neck), completing the first step towards ultimate descent into the scrotum. This initial movement of the male gonad is critically dependent on the differential fate of two tissues of the genital mesentry that attach to this organ: the growth of the gubernaculum and the regression of the cranial suspensory ligament (CSL) (Heyns and Hutson, 1995). The gubernaculum attaches to the caudal end of the gonad and tracts it towards the bladder neck. By contrast, the CSL attaches to the cranial pole of the testes, tethering them to the dorsal abdominal wall. Regression of the CSL in males is essential to enable descent of the testes and depends on exposure of its primordium to fetal testicular androgens (van der Schoot and Elger, 1992). Lrp2 mutant male

Receptor	Mutation	Disease	References
LDL receptor	Loss-of-function (familial, autosomal dominant)	Familial hypercholesterolemia (impaired clearance of LDL)	(Goldstein et al., 2001)
VLDL receptor	Loss-of-function (familial, autosomal recessive)	Autosomal recessive cerebellar hypoplasia (ataxia, mental retardation)	(Boycott et al., 2005)
LRP5	Loss-of-function (familial, autosomal recessive)	Osteoporosis-pseudoglioma syndrome (reduced bone mass)	(Gong et al., 2001)
	Gain-of-function (familial, autosomal dominant)	High-bone-mass trait (increased osteogenic activity)	(Little et al., 2002)
LRP6	Missense mutation (familial, autosomal dominant)	Autosomal dominant early coronary artery disease (hyperlipidemia, hypertension, diabetes)	(Mani et al., 2007)
LRP1B	Loss-of-function (sporadic)	Esophageal squamous cell carcinoma, non-small- cell lung cancer	(Liu et al., 2000; Sonoda et al., 2004)
LRP2	Loss-of-function (autosomal recessive)	Donnai-Barrow syndrome (proteinuria, brain malformations, diaphragmatic hernia)	(Kantarci et al., 2007)
SORLA	Polymorphisms (sporadic)	Alzheimer's disease	(Andersen et al., 2005; Lee et al., 2007; Rogaeva et al., 2007)

Table 2. Human diseases of the LDL receptor family

embryos fail to induce regression of the CSL, a defect known to cause cryptorchidism (van der Schoot and Elger, 1992; Zimmermann et al., 1999). In wild-type mice, expression of *Lrp2* is seen in the epithelium of the mesonephric tubules in close proximity to the primordium of the CSL, indicating the



Fig. 3. Lipoprotein receptors mediate uptake of steroid

hormones bound to carrier proteins. In the circulation, androgens are solubilized by the sex hormone binding globulin (SHBG). Cell-type-specific uptake of SHBG-bound androgens involves the recognition of SHBG by LRP2 (megalin), followed by receptor-mediated endocytosis. In a process that parallels endocytic uptake of cholesterol in lipoproteins (see Fig. 2A), the protein moiety (here SHBG) is degraded in lysosomes, while the steroid enters the cytoplasm to exert its action (the induction of nuclear androgen receptors). Similar hormone-uptake pathways have been documented for SHBG-coupled estrogens and vitamin D binding protein-bound 25-hydroxyvitamin D₃. PM, plasma membrane.

involvement of the receptor in the spatially and temporarily restricted uptake of androgens into target tissues that are responsible for inducing CSL regression (Fig. 3).

Lipoproteins transport lipid-linked morphogens

As well as trafficking signaling sterols, lipoproteins have recently also been shown to bear an unexpected protein cargo: lipid-linked morphogens of the hedgehog (Hh) and Wnt families (Panáková et al., 2005). The association of both Hh and the Wnt family protein Wingless (Wg) with lipoproteins has important consequences for imaginal disc development in Drosophila. The Drosophila lipoprotein Lipophorin is made in the fat body, a tissue that has functions similar to those of the vertebrate liver and adipose tissue. The apolipoprotein moiety of these particles, Apolipophorin (also known as Retinoid- and fatty-acid binding protein - Flybase), is a member of a large conserved family of lipid binding proteins that include APOB, the protein that represents the structural scaffolds of most lipoprotein particles in vertebrates (Shelness and Ledford, 2005) (see Box 1). These particles circulate through the larval hemolymph to the developing imaginal tissues, where they become associated with both Wg and Hh by an active process that is not yet fully understood. Lowering the systemic level of Lipophorin particles reduces the long-range, but not the short-range, signaling capacity of these morphogens and alters their trafficking behavior (Panáková et al., 2005).

How does lipoprotein association affect the function of morphogens? One obvious possibility is that they act as vehicles for the spread of lipid-linked morphogens, whose membrane affinity might otherwise prevent their long-range dispersal. This model implies that endocytic receptors or other surface binding sites for lipoproteins might influence the spread or downregulation of lipoprotein/morphogen signals. It seems unlikely that the LDL receptor itself influences the spread of morphogens because LDL receptor-deficient organisms develop normally, without alterations in Hh or Wg signaling. However, the possibility remains that other LRPs influence morphogen trafficking. The Drosophila LRP5/6 homolog Arrow is essential for Wg signaling (reviewed by He et al., 2004). Although LRP5/6 is only distantly related to other LRPs (see Fig. 1 for details), evidence exists that it might actually bind to lipoproteins in mice (Kim et al., 1998; Magoori et al., 2003). Although Arrow is not required for Wg internalization, it does appear to influence the rate of Wg degradation after endocytosis (Marois et al., 2006).



Fig. 4. Roles for lipoprotein association in morphogen signaling. (**A**) Heteromeric receptor complex formation between Frizzled and LRPs (e.g. LRP1, Arrow/LRP5/6) via simultaneous binding of Wnt ligands and apoproteins in lipoprotein particle may affect canonical Wnt signaling pathways. (**B**) Binding of Hh-modified lipoproteins to Patched and to LRPs may control Hh signaling directly or via cellular uptake of sterols with regulatory function. Extrac., extracellular; Intrac., intracellular; PM, plasma membrane. TCF, T cell factor.

Lipoprotein receptors LRP1, LRP2 and LRP4 also have important roles in development, but whether they affect the spread of morphogens has not been specifically examined. However, LRP2 appears to be able to internalize SHH in cultured rat yolk sac cells (McCarthy et al., 2002). Finally, heparan sulfate proteoglycans can also bind and internalize lipoproteins independently of LRPs (MacArthur et al., 2007; Wilsie et al., 2006) and clearly have important and ubiquitous functions in regulating the spread of Wnt and Hh family proteins (Blair, 2005; Hacker et al., 2005; Nybakken and Perrimon, 2002b). It will be interesting to examine whether they do so by interacting with lipoproteins.

But lipoprotein association might have other advantages beyond morphogen mobilization. Signaling in the context of a lipoprotein particle, rather than as a simple morphogen molecule, might allow additional regulatory possibilities. For example, the presence of multiple copies of Wg or Hh on the same lipoprotein particle generates a multivalent ligand complex that might be able to promote homomeric clustering of their cognate receptors. Of greater interest still, the apolipoprotein moiety itself, or other lipoproteinassociated factors, might bind to additional receptors on the cell surface, leading to formation of heteromeric receptor complexes (Fig. 4). The clustering of morphogen receptors with other receptor classes would expand the repertoire of signals that could be generated by an isolated morphogen. For example, Arrow/LRP5/6 must be clustered with Frizzled to transmit the Wg signal (reviewed by He et al., 2004). Although speculative, clustering might be affected by simultaneous binding of Frizzled to Wnt and Arrow to apolipoproteins in the morphogen-modified lipoprotein. Similarly, LRP1 also has been suggested to form competing complexes with Frizzled in cultured cells, inhibiting Wnt signaling (Zilberberg et al., 2004). This observation supports the intriguing possibility that multiple interactions between Wg, Frizzled, LRPs and lipoproteins could regulate Wg signaling pathways during development (Fig. 4A).

In addition to a role in Wnt signaling, a possible role for LRPs in Hh signaling has also been suggested based on the recent observation that the Hh-dependent activation of cyclin D transcription in mouse granule neuron precursor cells can be blocked by the receptor-associated protein (RAP; also known as LRPAP1 – Mouse Genome Informatics) – a factor that competitively inhibits ligand binding to LRPs (Vaillant et al., 2007). It will be interesting to determine whether a lipoprotein-Hh association might promote such interactions (Fig. 4B). The above study also showed that the LRP ligand PN-1 (also known as SERPINE2 – Mouse Genome Informatics) specifically interferes with Hh signaling in these cells and causes Hh gain-of-function phenotypes when mutated in mice (Vaillant et al., 2007). One possible explanation for these data is that LRP1 has a positive role in Hh signaling and that PN-1 competes with Hh for binding to this receptor.

Lipoproteins may deliver sterols to regulate the hedgehog pathway

In addition to modulating Wnt and Hh signal transduction across the plasma membrane, endocytic uptake of morphogen-modified lipoproteins may also combine morphogen signaling with cellular delivery of regulatory sterols (Fig. 4B). Recently, studies on the involvement of cholesterol derivatives in Hh regulation have indicated such an exciting possibility. The Hh family controls patterning and proliferation in a wide diversity of tissues and phyla - the basic components of this signaling pathway are depicted in Fig. 5A. Hh signals by binding to and inhibiting Patched (PTC), a twelve-transmembrane-domain protein that is a member of the resistance nodulation division (RND) superfamily of transmembrane transporters (Tseng et al., 1999). In the absence of Hh, PTC is thought to pump a small molecule across the bilayer to repress the activity of Smoothened (SMO) - a seven-passtransmembrane receptor (Chen et al., 2002; Taipale et al., 2002). When repression is relieved, activated SMO induces the transcription of Hh target genes by regulating the stability, nuclear translocation and activation of Cubitus interruptus, a Gli family transcription factor (Fig. 5A) (reviewed by Aza-Blanc and Kornberg, 1999; Kalderon, 2005; Lum and Beachy, 2004; Nybakken and Perrimon, 2002a).

Links between Hh signaling and sterol metabolism have been identified at almost every level of the Hh pathway. Indeed, Hh family proteins are themselves covalently modified by both cholesterol (Porter et al., 1996; Wendler et al., 2006) and palmitate (Pepinsky et al., 1998) in a way that is essential for their function. The Hh receptor, PTC, contains a sterol-sensing domain, and its closest relative within the RND family is Niemann-Pick type C1 (NPC1) – the protein required to mobilize sterols from endocytic compartments (Fig. 2A) (Ikonen and Holtta-Vuori, 2004; Mukherjee and Maxfield, 2004). Hh secretion requires Dispatched, another member of the RND transporter family with a sterol-sensing domain (Burke et al., 1999). Finally, Hh lipid modifications allow association not only with cell membranes, but also with lipoprotein particles - which are, of course, full of sterols (Panáková et al., 2005). Despite this plethora of clues, the exact nature of the relationship between sterols and Hh signaling has remained murky. Recent findings showing how specific sterol derivatives influence Hh pathway activity have begun to provide insight into this relationship.

The plant steroidal alkaloid cyclopamine, derived from *Veratrum californicum*, is a specific Hh pathway antagonist that acts by binding to and inactivating SMO (Chen et al., 2002; Cooper et al., 1998). It has been hypothesized that PTC might pump a structurally

related endogenous molecule across the membrane to repress SMO in vivo. Bijlsma and colleagues now demonstrate that supernatants from PTC-expressing cells contain such a SMO inhibitor. Results from experiments using cells that are mutant for different enzymes in the cholesterol biosynthesis pathway indicate that this inhibitor is likely to be produced from 7-dehydrocholesterol, the precursor of vitamin D_3 (Bijlsma et al., 2006). Interestingly, PTC expression increases the efflux of this class of molecules into the culture medium, where it associates with LDL (Fig. 5C). Bijlsma and colleagues show that vitamin D_3 competes with cyclopamine for binding to SMO-expressing yeast cells and is a potent inhibitor when added to a SMO-transfected mouse fibroblast cell lines. However,



Fig. 5. Sterol derivatives in hedgehog signaling. (**A**) The hedgehog (Hh) signaling pathway. Hh precursors undergo autocatalytic cleavage to generate a biologically active amino-terminal peptide covalently modified by cholesterol (HhN). Following release from cells, HhN acts on Patched to relieve Smoothened (SMO) repression, leading to the activation of the Gli family of transcription factors. (**B**) Oxysterols may positively regulate Hh signaling, either via repression of Patched (solid line) or the activation of SMO through as yet unknown mechanisms (dashed line). (**C**) Vitamin D metabolites negatively regulate SMO. Patched-expressing cells secrete a SMO inhibitor derived from 7-dehydrocholesterol that associates with lipoproteins (solid lines; right panel). In addition to locally synthesized sterols, exogenously derived vitamin D₃ metabolites might also have the potential to affect SMO activity (left panel). These metabolites may enter cells either packaged in lipoprotein particles or complexed with vitamin D binding protein (DBP) via LRPs (dotted lines). Extrac., extracellular; Intrac., intracellular; PM, plasma membrane.

whereas the inhibitor produced by their cell line relies on endogenous sterol biosynthesis, in vivo vitamin D_3 can be derived only by UV irradiation of 7-dehydrocholesterol in the skin or by nutritional uptake (Dusso et al., 2005) and would almost certainly have to be taken up from the extracellular fluids into Hh target cells. Endogenous vitamin D_3 that is produced in the skin is transported by DBP through the circulatory system. By contrast, vitamin D_3 absorbed from the diet is delivered by lipoprotein particles (Haddad et al., 1993). Should vitamin D_3 turn out to be the relevant regulator of SMO in vivo, it would suggest possible novel functions for both lipoproteins and their receptors in the regulation of the Hh pathway (Fig. 5C). LRP2, the receptor for uptake of vitamin D_3 metabolites complexed with DBP, might be of particular relevance (Fig. 5C).

Oxygenated forms of cholesterol (oxysterols) are another group of cholesterol derivatives that have been identified as regulators of the Hh pathway. They arise from cholesterol by auto-oxidation or by specific microsomal or mitochondrial oxidation processes. Previously, oxysterols had been shown to regulate cholesterol biosynthetic pathways by interacting with nuclear hormone receptors in the adult organism. Now, oxysterols have also been uncovered as positive regulators of the Hh pathway in mouse medulloblastoma cell lines and in pluripotent mesenchymal cells (Corcoran and Scott, 2006; Dwyer et al., 2007). Medulloblastoma arises frequently in mice heterozygous for patched 1 (Ptch1), and continued proliferation of this tumor depends on activation of the Hh pathway (Berman et al., 2002). It has previously been shown that sterols are required for Hh pathway activation at the level of SMO (Cooper et al., 2003). Corcoran and colleagues confirm that both proliferation and Hh target gene activation in medulloblastoma cells derived from *Ptch1*^{-/-}; *p53*^{-/-} (*Trp53* – Mouse Genome Informatics) mice depend on their ability to synthesize cholesterol. They further show that, when cholesterol synthesis is blocked, target gene activation and proliferation can be restored by the addition of specific oxysterols derived from cholesterol. Their data suggest that the requirement for sterols in SMO activation reflects a signaling function for an oxysterol derivative (Fig. 5B).

Oxysterols have been known for some time to promote the differentiation of bone, rather than adipose tissue, from pluripotent mesenchymal cells (Kha et al., 2004). Dwyer and colleagues now show that oxysterols exert their function in these cells by activating the Hh pathway at or above the level of SMO (Dwyer et al., 2007; Kha et al., 2004). Oxysterols do not further activate the pathway in *Ptch1* homozygous mutant mouse fibroblasts, in which SMO is constitutively active owing to the absence of PTCH1-mediated repression. This fact, along with the failure of oxysterols to compete with cyclopamine for binding to SMO, led these investigators to conclude that oxysterols do not act directly on SMO (Fig. 5B). Instead, they suggest that oxysterols modulate the pump activity of PTC and note that sterol binding to other sterol-sensing domain proteins, such as sterol regulatory element-binding protein cleavage activating proteins (SCAP), has an important regulatory function.

Although it is primarily endogenous synthesis that generates the stimulatory sterol derivative in these systems, it is interesting to consider whether exogenous sources could also contribute in vivo. Hh pathway elements are strictly conserved in *Drosophila*, which, like all insects, do not have the capacity for de novo cholesterol biosynthesis and are dependent on nutritional sources of this sterol (Clayton, 1964; Svoboda, 1999). If the function of oxysterols or vitamin D_3 is conserved in this organism, then lipoproteins and their receptors might have important roles to play in delivering these components. Oxidized lipoproteins might provide an external source of oxysterol.

Conclusion

Starting with the simple concept that lipoproteins provide lipids with structural or nutritional function, our perspective of embryonic lipoprotein metabolism has become significantly more sophisticated, but also much more complex. We now know that lipoproteins act as scaffolds for the assembly of signaling factors and that lipoprotein receptors act as co-receptors in embryonic patterning pathways; cholesterol metabolites represent inducers or repressors of morphogen action in these scenarios.

While an understanding of the molecular concepts of lipid transport and uptake unfolds, there is yet another problem to be tackled concerning the intracellular fate of sterols. What mechanisms determine whether internalized cholesterol is converted into steroid hormones that are destined for secretion, or whether it is turned into intracellular hormones that activate nuclear hormone receptors, or perhaps even converted into regulators of SMO? As in the circulatory fluids, the transport of sterols in the cytoplasm is facilitated by proteins. Thus, a number of transporters have been identified that traffic sterols in and out of organelles and across membrane bilayers. These pumps include members of the ATPbinding cassette (ABC) superfamily as well as NPC1 and related factors (e.g. NPC2, NPC1-like 1). In addition, cytoplasmic carriers that solubilize cholesterol and its metabolites in the aqueous milieu of the cell have been uncovered, which include lipid-transfer proteins (e.g. START-domain-containing proteins) and oxysterolbinding proteins. The elucidation of the mechanisms that link extracellular transport and receptor-mediated uptake of sterols with distinct intracellular trafficking routes (and hence cellular functions) will be one of the future challenges in the field of embryonic lipoprotein metabolism.

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