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REVIEW ARTICLE

Regulation of the synthesis, processing and translocation of lipoprotein lipase

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INTRODUCTION

Lipoprotein lipase (LPL) has its physiological site of action at the luminal surface of capillary endothelial cells where the enzyme hydrolyses the triacylglycerol component of circulating lipoprotein particles (Fig. 1), chylomicrons and very low density lipoproteins, to provide free fatty acids and 2-monoacylglycerol for tissue utilization [1–4]. LPL, therefore, plays a primary role in triacylglycerol metabolism and accordingly is widely distributed in the following tissues: adipose tissue (white/brown), heart, mammary gland (lactating), skeletal muscle (red), adrenal, ovary, thoracic aorta, spleen, small intestine, testes, lung, kidney, brain (hippocampus) and liver (neonatal). Free fatty acids supplied by the LPL reaction in white adipose tissue will be re-esterified for the storage of energy as triacylglycerols, whereas fatty acids will be oxidized to provide an energy source in the heart and to regulate thermogenesis in brown adipose tissue. In some tissues such as brain, a physiological role for LPL is not yet completely clear. In recent years, the description of the tissue distribution of LPL originally based on determinations of catalytic activity [4] has been complemented by measurements of LPL mRNA by Northern blotting and *in situ* hybridization [5–14]. In general, adipose tissue, heart and lactating mammary gland have the highest transcriptional and catalytic activity for LPL. Relative LPL catalytic activity and mRNA content in some guinea pig tissues is shown in Table 1. A similar pattern for the distribution of LPL mRNA was observed in rat tissues [12,13]. However, considerable species-dependent variations in tissue LPL have been reported. For example, substantial LPL mRNA was measured in mouse kidney [11], whereas little or no LPL mRNA could be detected in rat and guinea pig kidney [7,12,13]. The endothelium-bound enzyme (Fig. 1) is often referred to as "functional LPL" since displacement of the enzyme by perfusion with heparin [4,15] or inhibition of enzyme activity by antibodies to LPL [16] resulted in marked reduction in the ability of perfused hearts, for example, to degrade lipoproteins.

LPL is subject to developmental regulation [13,14]. Although LPL is usually described as an extra-hepatic enzyme, significant LPL activity can be measured in neonatal livers [17]. The capacity for hepatic synthesis of LPL is markedly reduced in adult liver [18]; therefore, LPL activity in adult livers (Table 1) is mainly due to hepatic uptake of the enzyme from the circulation [8]. The predominant cellular location of LPL is not always known in a multi-cellular tissue, and can be influenced by the stage of development. LPL mRNA was low in adult rat brains [13] compared to neonatal rat brains where the strongest hybridization signal was localized to the hippocampus [10]. In the neonatal heart, the majority of LPL activity is present in mesenchymal cells [19], whereas the cardiac myocyte represents the predominant source of LPL in the adult heart [7,20].

LPL activity can be altered in a tissue-specific manner, which is physiologically important because it directs fatty acid utilization according to the metabolic demands of individual tissues so that the degradation of triacylglycerol-rich lipoproteins can be targeted to specific sites. For example, fasting results in a reduction in LPL activity in adipose tissue but an increase in cardiac tissue [21–24]; as a result, fatty acids are diverted away from storage in adipose tissue to meet the metabolic demands of the heart under conditions of caloric deprivation. Cold exposure produces a selective stimulation of LPL in brown adipose tissue [25,26]. Another example is the dramatic increase in mammary gland LPL activity with a corresponding decrease in adipose tissue LPL activity during lactation [4,27] so that circulating triacylglycerols are preferentially utilized for milk synthesis. Thus, LPL has a pivotal role in total body lipid metabolism. The regulation of LPL is also important in some clinical disorders of lipid metabolism such as obesity where fat cell LPL activity is markedly elevated [2,28].

The most unique characteristic of LPL is the stimulation of enzyme activity by apolipoprotein CII [1,29]. Thus, circulating

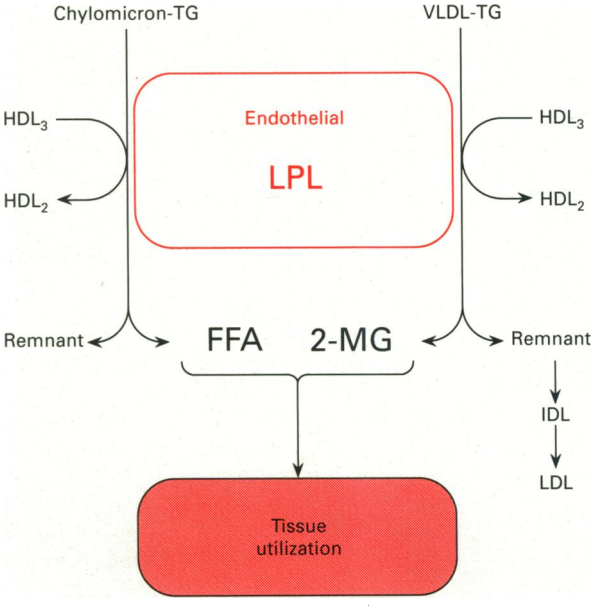

Fig. 1. Metabolism of circulating triacylglycerol (TG)-rich lipoproteins by endothelial lipoprotein lipase, resulting in the formation of free fatty acids (FFA) and 2-monoacylglycerol (2-MG) for tissue utilization

Abbreviations: VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein.

Abbreviations used: CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; endo H, endo-β-*N*-acetylglucosaminidase H; LPL, lipoprotein lipase; PI-PLC, phosphatidylinositol-specific phospholipase C.

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Table 1. LPL in guinea pig tissues: relative catalytic activity, mRNA content and rates of synthesis

Results are from experiments with tissues from adult guinea pigs [6,8,9,43,66]. Values for LPL catalytic activity and relative rates of protein synthesis ([^{35}S]methionine incorporation into immunoprecipitable protein) are expressed as a percentage of adipose tissue (set as 100%). The relative abundance of LPL mRNA was estimated from Northern blots. N.D., not detectable.

Tissue	LPL activity (%)	LPL mRNA	LPL synthesis (%)
Adipose tissue	100	+++	100
Heart	86	+++	56
Lung	25		6
Liver	15	N.D.	N.D.
Spleen	7	++	
Ovary	7	+	
Adrenal	5	+	
Kidney	2	N.D.	

triacylglycerol-rich lipoprotein particles provide both the substrate and a specific activator for the LPL reaction (Fig. 1). The importance of apolipoprotein CII as a co-factor for LPL is illustrated graphically by the extreme hypertriglyceridaemia observed in patients with a genetic deficiency of this apoprotein [30]. The CII-activation of LPL can be modulated by other apoproteins in circulating lipoprotein particles. In the laying hen, VLDL particles contain large amounts of apoliprotein VLDL-II. Schneider *et al.* [31] demonstrated that apolipoprotein VLDL-II inhibited CII-stimulated LPL activity in chicken follicular granulosa cells, and proposed that this inhibition ensured the efficient delivery of triacylglycerols to the oocyte for subsequent use as an energy source by the embryo.

Studies on the regulation of LPL have been facilitated by the use of several model systems. LPL is secreted into milk from the lactating mammary gland [7]. The physiological function of milk LPL is unclear, but the presence of LPL in bovine milk [32] has greatly facilitated the characterization of the biochemical properties of the enzyme by providing a convenient source for purification. Cultured cells and cell lines have also provided useful model systems to examine the synthesis, processing and secretion of LPL. There is a marked increase in LPL activity when 3T3-L1 fibroblasts [33] differentiate into adipocytes [34,35]. Cultured 3T3-L1 adipocytes, along with Ob17 [36] and 3T3-F442A adipose cells [37] have been used extensively for investigations of LPL synthesis and secretion.

The purpose of this Review will be to evaluate the considerable progress made in the last 3 years in the cell biology of LPL, particularly the synthesis and processing of LPL and translocation of the enzyme to its physiological site of action at the surface of vascular endothelial cells. Particular attention will be directed to the regulation of LPL in adipose tissue and heart.

SYNTHESIS, PROCESSING AND RELEASE OF LPL FROM PARENCHYMAL CELLS AND TRANSLOCATION TO THE ENDOTHELIUM

Although the physiological site of action for LPL is at the luminal surface of blood vessels where the endothelium-bound enzyme can interact with circulating lipoproteins (Fig. 1), vascular endothelial cells do not synthesize LPL. Camps *et al.* [7] were able to observe LPL protein by immunohistochemistry at the endothelial surface of blood vessels in adipose tissue, heart and diaphragm of guinea pigs but were unable to detect LPL mRNA by *in situ* hybridization over endothelial cells in any of these tissues. Positive hybridization for LPL mRNA was detected over the major cell types in these tissues (adipocytes, myocytes), indicating that the endothelial LPL protein originated in parenchymal cells. Thus, it appears that the metabolic requirements of the parenchymal cell governs the production of LPL. On the other hand, LPL mRNA was localized in macrophages, a minority cell type, in lung and spleen [8], and both epithelial cells [7] and interstitial cells [27] may be a source of LPL in mammary gland. Blanchette-Mackie *et al.* [38] have immunolocalized LPL in mouse hearts to the sarcoplasmic reticulum, Golgi sacs, and transport vesicles in cardiac myocytes (78% of total LPL), the extracellular space (3–6%) and the capillary endothelium (18%). Thus, LPL in heart is synthesized in cardiac myocytes [7] and then translocated to functional binding sites on the surface of vascular endothelial cells. However, LPL mRNA has been localized to cardiac interstitial cells in cholera toxin-treated rat hearts [39], indicating that LPL synthesis can occur in both mesenchymal cells and cardiac myocytes under some circumstances. The various steps involved in the synthesis and processing of LPL in parenchymal cells will be examined next, along with mechanisms associated with the translocation of LPL to the endothelium (Fig. 2).

Isolation of the cDNA for LPL and transcription of the LPL gene

The complete amino acid sequence of LPL has been deduced from the cloning and sequencing of cDNAs encoding human [40,41], bovine [42], guinea pig [43], mouse [11,13] and chicken [44] LPL. The human clone encodes for a protein of 475 amino acids, including a hydrophobic leader sequence of 27 amino acids; the mature protein contains 448 amino acids with a molecular mass of 50394 daltons [40]. The amino acid sequence of LPL from mouse, cow and guinea pig, predicted from their cDNAs, share about 90% identity to human LPL. Chicken LPL contains 15–17 additional C-terminal residues and shows 73–77% similarity to mammalian LPL's.

The human LPL gene has been mapped to the p22 (short arm) region of chromosome 8 [45] and genomic clones have been isolated and characterized [41,46]. The gene spans approx. 30 kb and contains ten exons and nine introns. Six functional sites have been putatively identified within the LPL monomer [46,47]: (i) the 27-amino-acid signal peptide which is cleaved from the protein; (ii) a catalytic site; (iii) a site for interaction with the CII cofactor; (iv) an interfacial binding site; (v) a polyanion binding site for interaction with heparan sulphate proteoglycans at the vascular endothelium; and (vi) a site for noncovalent subunit-subunit interactions. There is correspondence between these functional sites and exon units [46,48]. Exon 1 codes for the 5′-untranslated region and the signal peptide, exon 2 encodes an N-linked glycosylation site (consensus sequence Asn-Xaa-Ser(Thr) where X is any amino acid but proline), exon 4 codes for the interfacial lipid binding region, exon 5 encodes the active site (consensus sequence Gly-Xaa-Ser-Xaa-Gly), exon 6 encodes the putative heparin-binding domain, exon 8 codes for a domain containing another N-linked glycosylation site, and exon 10 encodes the 3′ untranslated region. The domain structure of LPL has recently been reviewed [29]. A requirement for the predicted active site serine (Ser-132) has been confirmed by site-directed mutagenesis [49]. Mutations in the LPL gene that produce hypertriglyceridaemia (type I hyperlipoproteinaemia) due to familial LPL deficiency have been identified recently. For example, single mis-sense mutations have been localized to exon 4 at codon 142 [50] and to exon 5 at codons 176 [51], 188 [52,53], and 194 [54], where a single amino acid substitution in LPL resulted in catalytically inactive enzyme protein.

Although a single gene encodes for LPL, the expression of

LPL is tissue specific, suggesting that transcription of the LPL gene is regulated by tissue-specific *cis*-acting elements [12]. While the mechanism of tissue specific regulation is not understood, four transcription initiation sites, two promoter elements and several enhancer motifs have been identified in the 5′ upstream region of the LPL gene [46]. Transfection of plasmids containing deletion mutants of the 5′-LPL promoter region linked to a reporter gene into 3T3-L1 adipocytes has revealed the presence of both negative and positive *cis*-acting regulatory elements [55].

LPL belongs to a gene family which includes hepatic lipase and pancreatic lipase, but not lecithin cholesterol acyl transferase, lingual lipase or hormone-sensitive lipase [11,56,57]. *Drosophila* yolk proteins 1, 2 and 3 (vitellogenins) exhibit sequence similarity to LPL but lack lipase activity [58]. Kirchgessner *et al.* [41] originally proposed that LPL, hepatic lipase, pancreatic lipase and the yolk proteins 1,2 and 3 evolved from a common ancestral gene; the evolutionary relationships between members of this lipase gene superfamily have recently been reviewed by Hide *et al.* [59].

In humans, there are two mRNA species of 3350 and 3750 bp that arise from alternative sites of 3′ terminal polyadenylation [40]. Multiple mRNA species have also been observed in all other species with the exception of rat [11,13]. The significance of multiple mRNA species is presently unclear.

Translation of LPL mRNA: synthesis of enzyme protein

The synthesis of LPL in the rough endoplasmic reticulum (Fig. 2) can be measured by the incorporation of a radiolabelled amino acid into immunoprecipitable LPL, identified after SDS/polyacrylamide-gel electrophoresis and autoradiography as a protein band with a subunit mass of approx. 55 kDa in experiments with adipocytes [35,61–63], CHO cells [64] and perfused guinea pig hearts [65]. Similar results were obtained from pulse incubations of tissue pieces from guinea pig adipose tissue, mammary gland, lung, diaphragm and heart [66]. The highest relative rates of LPL synthesis [66] were observed in adipose tissue, heart and lactating mammary gland (Table 1), consistent with measurements of catalytic activity and LPL mRNA [7,43]. LPL is a glycoprotein, containing 8–12% carbohydrate [32,63]. Therefore, the molecular size of immunoprecipitable LPL (55 kDa) is consistent with the subunit mass of approx. 51 kDa deduced from the cDNAs for LPL. Ultracentrifugation at physiological pH and ionic strength [32] and radiation inactivation [67] have indicated that active LPL exists as a homodimer.

N-linked glycosylation of newly synthesized LPL

The glycosylation of LPL initially involves the transfer *en bloc* of a lipid-linked oligosaccharide [Glc_3-$Man_9(GlcNAc)_2$] to specific arginine residues of the nascent polypeptide co-translationally (Fig. 3). The N-linked carbohydrate moiety of LPL then undergoes a series of modifications as the enzyme is transported through a series of distinct intracellular compartments, each with a specific complement of glycoprotein processing enzymes [68,69]. The three terminal glucose residues are removed first by glucosidases I and II in the endoplasmic reticulum, followed by removal of one mannose by an α-mannosidase (Fig. 3), to give a high-mannose structure [Man_8-$(GlcNAc)_2$–protein] which is then transferred to the Golgi complex (Fig. 3).

The Golgi complex consists of a sorting and receiving centre called the *cis*-Golgi network, a processing centre (Golgi stacks), and a sorting and exiting centre called the *trans*-Golgi network (Fig. 2). The exact location of Golgi processing enzymes is not established unequivocally and may vary in different cell types [70,71]. Also, the number of topologically distinct compartments within the Golgi stack is not clear; however for the purposes of this review, three compartments (*cis*, medial and *trans*; Fig. 2) will be considered [70]. As a glycoprotein moves through the Golgi processing stacks by vesicular transport from the *cis*- to the *trans*-cisternae [72], the oligosaccharide is further modified by processing enzymes (Fig. 3). Three mannoses are removed by the action of mannosidase I in the *cis*-Golgi. After the addition of GlcNAc in the medial-Golgi, two additional mannose residues are removed by mannosidase II. The resulting structure [GlcNAc-$Man_3(GlcNAc)_2$–protein] is then modified by a series of transferase reactions in the *trans*-Golgi to add additional GlcNAc, galactose and sialic acid residues to give a variety of complex oligosaccharide chains.

Pulse-chase experiments ([^{35}S]methionine incorporation into immunoprecipitable LPL) have shown that newly synthesized LPL in the endoplasmic reticulum contained high-mannose oligosaccharide chains sensitive to endo-β-*N*-acetylglucosaminidase H (endo H), which were subsequently processed to complex (endo H-resistant) forms in the Golgi [24,63,73,74]. The conversion of a glycoprotein from an endo H-sensitive to an endo H-resistant form is a marker for the passage of molecules from the endoplasmic reticulum to the medial/*trans*-Golgi [75]. Mature LPL protein contained two complex oligosaccharide chains in mouse and rat adipocytes [24,63,74]: a precursor–product relationship between high-mannose and complex forms was observed. Both intracellular forms (55–58 kDa) were deglycosylated to a 51 kDa species by glycopeptidase F [63,76]. Mature LPL from guinea pig [73] and chicken adipocytes [77] contained three oligosaccharide chains, two complex and one high-mannose. Therefore, LPL can exist in several glycoforms.

N-linked glycosylation of LPL is required for catalytic activity. Tunicamycin is an antibiotic that inhibits the synthesis of the dolichol-linked oligosaccharide (Fig. 3) so that N-linked glycosylation is blocked [78]. Tunicamycin decreased LPL catalytic activity in adipocytes [35,74,79,80], cardiac mesenchymal cells [81] and macrophages [82]. Incubation of cells with tunicamycin characteristically resulted in the synthesis of a smaller form of LPL (48–52 kDa), due to the absence of N-linked oligosaccharides [35,63,76,79,81], that was retained in the endoplasmic reticulum [74]. Similar results were obtained when N-linked glycosylation of LPL was inhibited in cultured rat adipocytes by glucose deprivation [79]. Semenkovich *et al.* [83] have reported that site-directed mutagenesis of human LPL at Asn-43 (an N-linked glycosylation site) and subsequent transfection into COS M-6 cells resulted in the synthesis of catalytically inactive LPL. However, these studies have not established if addition of the oligosaccharide is sufficient for LPL activity, or if oligosaccharide processing is necessary. Treatment of cultured Ob17 adipocytes with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) to block the energy-dependent transfer of proteins from the endoplasmic reticulum to the Golgi [78] resulted in the accumulation of catalytically inactive LPL in the endoplasmic reticulum [84]. Monensin blocks the passage of glycoproteins from medial to *trans*-Golgi cisternae (Fig. 3; [78]). Treatment of adipocytes [74,80,84] and macrophages [82] with monensin resulted in the retention of active LPL in the Golgi. Therefore, it was concluded that activation of LPL by glycosylation was associated with processing of oligosaccharide chains in *cis*/medial Golgi [84,85]. In contrast, Ben-Zeev *et al.* [86] have concluded that the acquisition of catalytic activity by LPL occurred in the endoplasmic reticulum as a result of the trimming of glucose residues in oligosaccharide chains by endoplasmic reticulum glucosidases, based on several experimental approaches. COS cells transfected with an LPL cDNA construct containing a specific tetrapeptide (KDEL) at the C-terminus that results in recycling and retention in the endoplasmic reticulum

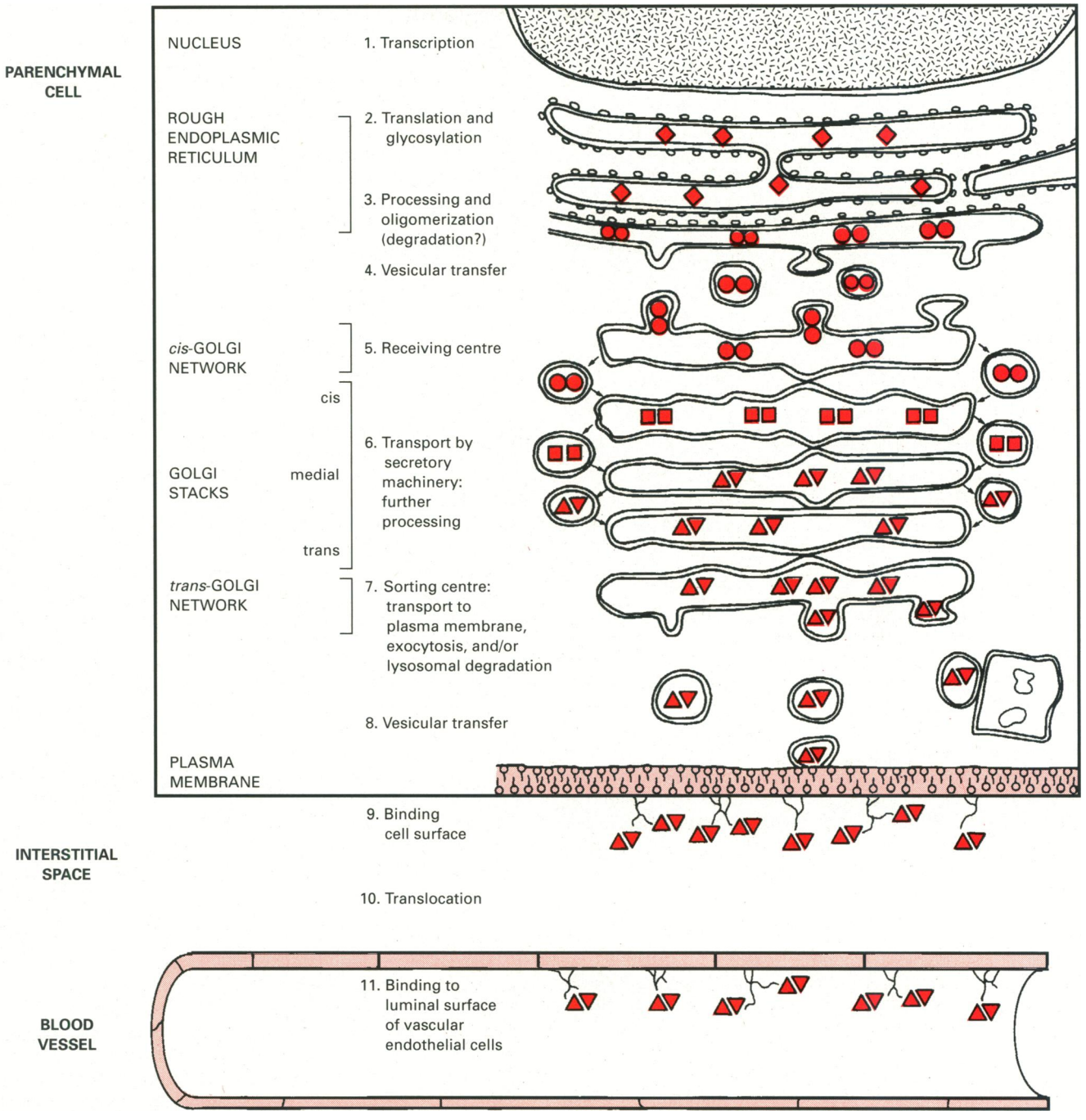

Fig. 2. Synthesis, processing and translocation of lipoprotein lipase

After transcription of the LPL gene in the nucleus of a parenchymal cell in a tissue, LPL will be synthesized (translation) and N-glycosylated in the rough endoplasmic reticulum (enzyme protein represented by ◇). Trimming of terminal glucose residues in the oligosaccharide chains attached to the LPL protein results in the acquisition of catalytic activity in the endoplasmic reticulum that is probably secondary to oligomerization (dimerization ○○). Some LPL protein may be degraded at this stage. Active LPL is then transferred to the Golgi complex and undergoes further processing (□□) to yield complex oligosaccharide chains (▽△). The fully processed LPL is sorted in the *trans*-Golgi network for delivery either to lysosomes for degradation or to the parenchymal cell surface where the enzyme is bound to heparan sulphate proteoglycans. Finally, the enzyme is translocated by unknown mechanisms to functional heparan sulphate proteoglycan binding sites on the luminal surface of the capillary endothelium where the hydrolysis of triacylglycerol-rich lipoproteins takes place.

(Fig. 3) exhibited a marked increase in intracellular LPL activity; oligosaccharide chains in the KDEL-LPL were endo H-sensitive, establishing the localization of the active enzyme in the endoplasmic reticulum. Ben-Zeev *et al.* [86] also demonstrated that LPL was catalytically active with high-mannose (endo H-sensitive) oligosaccharide chains when the temperature-dependent vesicular transfer of glycoproteins from the endoplasmic reticulum to Golgi (Fig. 3) was inhibited by incubation of cultured CHO cells at 16°C. In addition, specific glycoprotein processing enzyme inhibitors [68,69,78] were used to determine the requirement for individual processing steps in the activation of LPL by glycosylation. *N*-Methyldeoxynojirimycin and castanospermine inhibit glucosidases in the endoplasmic reticulum, whereas Golgi mannosidases I and II can be inhibited

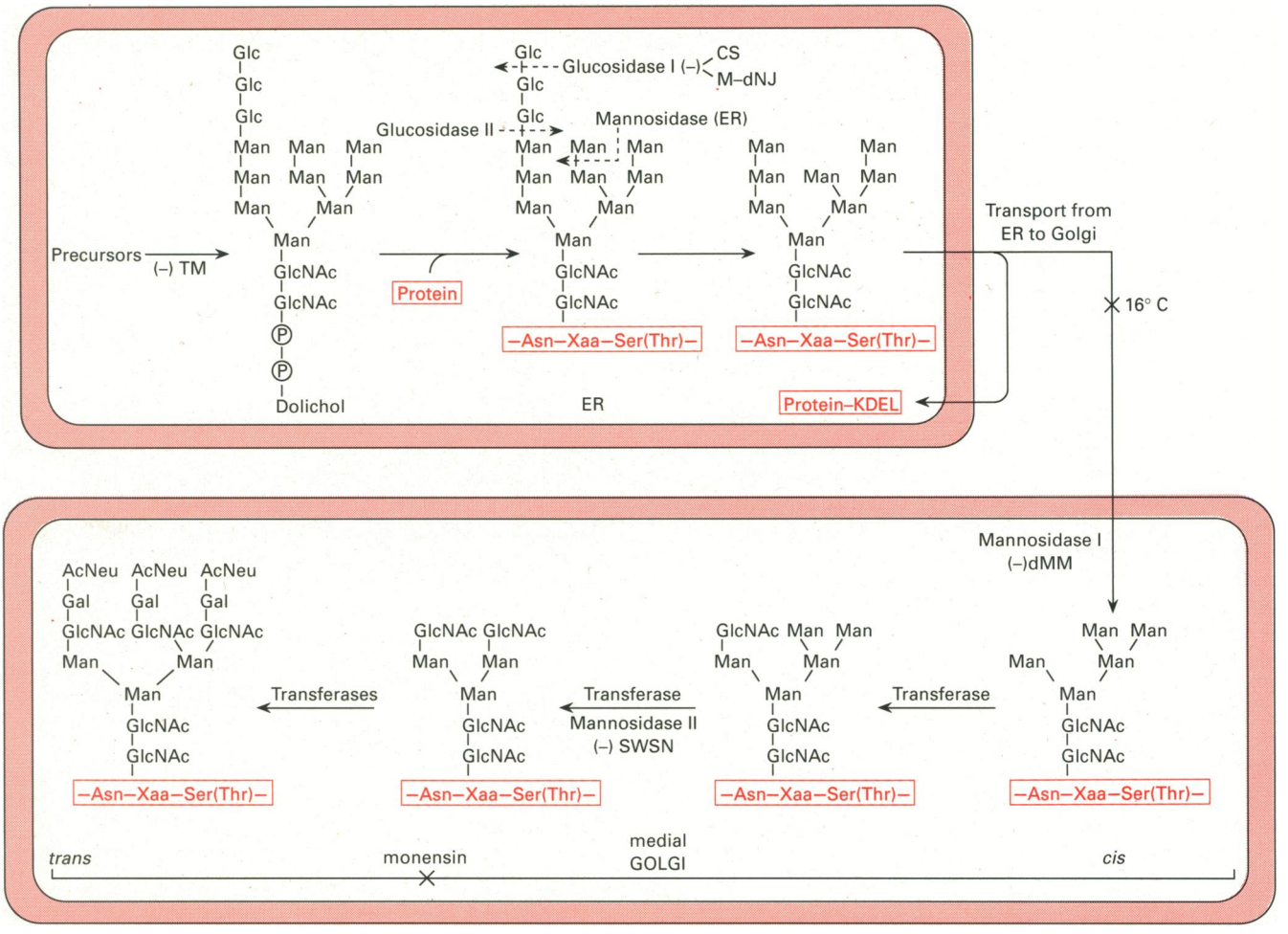

Fig. 3. Reactions involved in the synthesis and processing of N-linked glycoproteins in the endoplasmic reticulum (ER) and Golgi

The sites where inhibitors of the biosynthesis of the oligosaccharide chain and of specific glycoprotein processing enzymes act are indicated (–), along with the location where the transport of glycoproteins can be blocked (×). Abbreviations: Tm, tunicamycin; CS, castanospermine; M-dNJ, *N*-methyldeoxynojirimycin; dMM, deoxymannojirimycin; SWSN, swainsonine.

by deoxymannojirimycin and swainsonine, respectively (Fig. 3). Incubation of CHO cells with glucosidase inhibitors resulted in a reduction in LPL activity with no change in LPL mass, so that enzyme specific activity was markedly diminished [86]. Masuno *et al.* [87] have observed similar results with 3T3-L1 adipocytes where an 18 h incubation with castanospermine reduced intracellular LPL activity by 80%. In contrast to these results with glucosidase inhibitors [86,87], treatment of CHO cells [86] and adipocytes [73,74] with deoxymannojirimycin resulted in an endo H-sensitive form of LPL that was catalytically active. LPL was also active in brown adipocytes incubated with swainsonine [74]. LPL in guinea pig adipocytes can be isolated in a totally high-mannose form (all three chains) which was catalytically active [73]. Therefore, the acquisition of catalytic activity by LPL is not associated with processing in the Golgi, but instead requires the trimming of glucose residues in the oligosaccharide chains of LPL by the action of glucosidases in the endoplasmic reticulum [86]. Catalytically active LPL is a homodimer [32,67] and most proteins are oligomerized in the endoplasmic reticulum [88]. Therefore, glucose trimming by glucosidases may induce dimerization and activation of LPL; as a result, LPL retained in the endoplasmic reticulum after incubation of CHO cells at 16°C or addition of the KDEL sequence in COS cells was catalytically active [86]. Vannier *et al.* [84] originally concluded that LPL activation required processing in the Golgi because of their observation that CCCP treatment resulted in the retention of inactive LPL in the endoplasmic reticulum. However, incubation of thyroid slices with CCCP decreased the formation of dolichol-linked oligosaccharides and thus reduced protein glycosylation [89]; therefore the inactive LPL in the endoplasmic reticulum of CCCP-treated Ob17 cells may not have been glycosylated [84].

The combined lipase-deficient (*cld/cld*) mouse has a single, recessive, autosomal mutation within the T/t complex of chromosome 17 that causes a deficiency of both LPL and hepatic lipase activities [90,91]. Although homozygotes develop normally *in utero*, they display massive hypertriglyceridaemia if allowed to suckle and die within 3 days. *cld/cld* mice expressed normal mRNA levels for LPL, and increased amounts of high-mannose LPL that was catalytically inactive and retained within the endoplasmic reticulum; as a result, LPL specific activity was reduced to less than 5% of control in a variety of tissues from *cld/cld* mice [76,91,92]. The presence of inactive LPL mass in the endoplasmic reticulum of *cld* tissues is similar to the effect of the glucosidase inhibitors in CHO cells [86]. Exactly how the *cld/cld* mutation causes LPL retention within the endoplasmic reticulum and reduced catalytic activity is not known. The gene disrupted by the *cld* mutation must have a specific function within the lipase processing pathway since other secretory N-linked glycoproteins such as adipsin were processed normally in *cld* adipocytes [92]. Glycosylation is often required for correct folding

and/or packaging of proteins. Improperly folded proteins, in general, are slow to leave the endoplasmic reticulum and are bound to a soluble binding protein (BiP), that associates with protein aggregates [88,93]. A human patient with type I hyperlipoproteinaemia has also been characterized as having an inactive high-mannose LPL retained in the endoplasmic reticulum in adipose tissue [94].

Turnover of LPL: intracellular degradation

Glycoproteins processed by the Golgi are sorted in the *trans*-Golgi network (Fig. 2) for: (i) delivery to lysosomes; (ii) incorporation into secretory vesicles; and (iii) delivery to the plasma membrane. Inhibition of protein synthesis in adipocytes with cycloheximide resulted in a rapid reduction in cellular LPL activity, with a $t_{\frac{1}{2}}$ of about 40 min [84,95]. Pulse-chase experiments have also revealed a rapid turnover of LPL in adipocytes [35,60,62,63]; only a small fraction of the radiolabelled LPL that disappeared from cells was recovered in the medium, indicating extensive intracellular degradation. Approximately 80% of newly-synthesized LPL was degraded in adipocytes incubated under basal conditions [62,63]. The intracellular site for the degradation of LPL may be lysosomes because leupeptin reduced rates of LPL turnover [63], although Semb and Olivecrona [95] suggested some LPL degradation may occur in the endoplasmic reticulum. The degradation of newly-synthesized proteins in the endoplasmic reticulum can be substantial, particularly if secretion is inhibited [96]. Therefore the very rapid degradation of inactive LPL in tunicamycin-treated adipocytes [63] likely occurred in the endoplasmic reticulum. Recently, Liu and Olivecrona [65] have performed pulse-chase experiments on LPL in perfused guinea pig hearts. After a 90 min chase, 65% of radiolabelled LPL was still present in the heart, indicating that the turnover of myocardial LPL was slower than in adipocytes.

Secretion of LPL and translocation to the endothelium

Protein secretion from cells occurs via two general mechanisms, termed constitutive and regulated [72,88,97]. "Constitutive" refers to a process in which proteins are secreted as they are synthesized, without any intracellular accumulation. Rates of constitutive protein secretion may be altered pre-translationally. Secretion is "regulated" if newly synthesized proteins are stored in secretory vesicles until the cell receives a stimulus from an appropriate secretagogue, upon which large amounts of protein are secreted at a rate much higher than the protein synthetic rate. Constitutive and regulated secretory proteins are processed by a common secretory pathway that diverges at the level of the *trans*-Golgi network (Fig. 2). All cell types exhibit constitutive secretion while some cells exhibit both constitutive and regulated secretion.

LPL has been localized to secretory vesicles in cardiac myocytes [38] and adipocytes [98]. Very low rates of constitutive (spontaneous) LPL release into the medium have been observed with Ob17 [85] and 3T3-L1 adipocytes [35], cardiac myocytes [99,100] and cardiac mesenchymal cells [19]. Moderate rates of spontaneous LPL secretion have been reported for guinea pig adipocytes [95] and CHO cells [64] and very high rates have been observed with preadipocytes [80] and 3T3-F442A adipocytes [101]. Measurement of constitutive (spontaneous) LPL release from cells will likely include both exocytosis of newly-synthesized LPL (secretion) as well as the displacement of pre-formed enzyme bound to the cell surface.

Heparin stimulates the release of LPL activity into the incubation medium of a variety of cells [3], due to displacement of the enzyme from cellular heparan sulphate proteoglycan binding sites that are similar to binding sites for "functional LPL" at the capillary endothelium (see below). Thus, the heparin-induced release of LPL initially reflects the displacement of pre-formed enzyme that had been secreted previously and bound to the cell surface, in contrast to the spontaneous release of LPL which includes a secretory component of newly synthesized enzyme. Inactive LPL in tunicamycin-treated cells [35,63,74,79,81], *cld* adipocytes [76] and CHO cells incubated with glucosidase inhibitors [86] was not secreted. Golgi processing is not required for secretion since high-mannose (catalytically active) forms of LPL in adipocytes were released spontaneously [73] and in response to heparin [74]. Heparin did not influence rates of LPL synthesis but markedly reduced the intracellular degradation of LPL in adipocytes [62,63,95], presumably because the heparin-induced release of LPL into the medium diverted newly-synthesized LPL away from intracellular sites of degradation.

Heparan sulphate proteoglycans are heterogeneous macromolecules consisting of a glycosaminoglycan (heparan sulphate), covalently linked to a core protein via a link trisaccharide [102,103]. Glycosaminoglycans are long, unbranched polysaccharide chains of repeating disaccharide units (amino sugar and a uronic acid). Glycosaminoglycans are highly negatively charged due to the presence of sulphate and carboxyl groups on the sugar residues [103]. Since glycosaminoglycans are polyanions, they interact with positively-charged molecules, including lipoprotein lipase and hepatic lipase. Heparan sulphate proteoglycans are present in the extracellular matrix (glycocalyx), intercalated within the plasma membrane and in intracellular secretory organelles. Glycosaminoglycan chains enter both regulated and constitutive secretory pathways, co-packaged with other secretory proteins [104].

Heparin displaces LPL bound to cellular heparan sulphate proteoglycan binding sites [3], forming soluble heparin-LPL complexes in the medium. The presence of LPL on the cell surface of adipocytes has been demonstrated directly by immunodetection [105]. The observation that a substantial (40%) fraction of cellular LPL activity in 3T3-L1 adipocytes was released into the medium by a 10 min incubation with heparin at 4°C [35] is also consistent with LPL being bound to heparan sulphate proteoglycans on the cell surface. However, Vannier *et al.* [101] recently suggested that LPL was bound to heparan sulphate proteoglycans within intracellular secretory vesicles so that the enzyme was maintained in an inactive, condensed (cryptic) state. Thus, LPL activity in homogenates of adipocytes and myocytes had to be unmasked by dilution to prevent an underestimation of intracellular LPL activity [106,107]. Consequently, Vannier and Ailhaud [63] proposed two pathways for LPL secretion from 3T3-F442A cells: a constitutive pathway and a regulated pathway where exocytosis of LPL from intracellular secretory vesicles was accelerated by heparin by an unknown mechanism.

In contrast, Cisar *et al.* [108] have provided evidence that LPL is bound to heparan sulphate proteoglycans on the external surface of plasma membranes. Exogenous radioiodinated LPL was bound to the surface of cultured adipocytes. Pretreatment of cells with heparinase or heparitinase reduced high-affinity LPL binding, establishing that LPL was bound to cell surface heparan sulphate proteoglycans [108]. The degree of sulphation of heparan sulphate proteoglycans on the cell surface of adipocytes has recently been demonstrated to affect the binding of LPL [109]. Cisar *et al.* [108] proposed a model of LPL secretion where newly synthesized enzyme is transported to the cell surface where it binds to heparan sulphate proteoglycan receptors; LPL is then either released into the medium or internalized via the receptor and either degraded or recycled back to the cell surface. The observation that exogenous triacylglycerols can be degraded by LPL on the cell surface of intact cardiac mesenchymal cells [19] and cardiac myocytes [110,111] indicates that at least a portion of cellular LPL is bound to the cell surface in a functional state (Fig. 2).

Table 2. Release of LPL activity into the medium of cells by PI-PLC

Cell preparation [reference]	PI-PLC-releasable LPL activity (% of heparin-releasable activity)
3T3-L1 adipocytes [115]	6–10%
Cardiac mesenchymal cells [116]	30–50%
Cardiac myocytes [117]	60–70%
Macrophages [116]	0%

Covalent linkage of proteins to glycosyl phosphatidylinositol molecules is an important mechanism for anchoring a wide variety of proteins to the cell surface [112–114]. The C-terminal α-carbonyl residue of anchored proteins is covalently attached to ethanolamine via an amide bond; the ethanolamine is bound via a phosphodiester linkage to a glycan structure [$(Man)_3$-GlcNac] that is glycosidically linked to the inositol ring of phosphatidylinositol molecules in the lipid bilayer of the cell membrane.

Identification of glycosyl phosphatidylinositol-anchored proteins typically has relied on the release of proteins into the medium after incubation of cells with a bacterial phosphatidylinositol-specific phospholipase C (PI-PLC). Incubation of 3T3-L1 adipocytes [115], cardiac mesenchymal cells [116] and cardiac myocytes [117] with PI-PLC released LPL into the medium. The amount of LPL activity released from different cells by PI-PLC was extremely variable when expressed as a percentage of heparin-releasable LPL activity (Table 2); incubation of macrophages with PI-PLC [116] did not release LPL into the incubation medium. It was concluded from investigations with cardiac myocytes and mesenchymal cells that LPL was not directly linked to the cell surface by a glycosyl phosphatidylinositol membrane anchor [116,117]. Instead, LPL is bound ionically to heparan sulphate proteoglycan molecules, some of which are covalently attached to the cell membrane via a glycosyl phosphatidylinositol membrane anchor (Fig. 4, model A). The proportion of LPL bound to heparan sulphate proteoglycan molecules that are glycosyl phosphatidylinositol-linked and thus releasable by PI-PLC relative to the pool of enzyme bound to non-anchored proteoglycans (Fig. 4, model B) must, therefore, vary markedly between cells (Table 2). Heparin would displace LPL from both pools of heparan sulphate proteoglycans (Fig. 4). The mechanism(s) by which LPL is released *in vivo* from parenchymal cell surface binding site is (are) presently unknown. A constitutively active or hormonally-regulated PI-PLC could contribute to constitutive and regulated rates of LPL release.

Very little information is available concerning the translocation of LPL from sites of synthesis in parenchymal cells across the interstitial spaces to the luminal surface of capillary endothelial cells (Fig. 2). Blanchette-Mackie *et al.* [38] proposed that LPL is transferred along bridges of heparan sulphate proteoglycan molecules that connect the cell surfaces of cardiac myocytes and capillary endothelial cells in the heart. A saturable heparan sulphate proteoglycan-dependent transport system for LPL from the basal to apical surface of cultured aortic endothelial cells has been characterized by Saxena *et al.* [118].

LPL at the vascular endothelium

LPL bound to functional sites on the luminal surface of vascular endothelial cells is released into the circulation, along with hepatic lipase, by the administration *in vivo* of heparin (plasma post-heparin lipolytic activity). Cultured endothelial cells from pulmonary artery [119] or aorta [120,121], with no endogenous LPL activity, bound exogenous bovine milk LPL [120,122]. Binding of radioiodinated LPL to the vascular endothelium in perfused rat hearts has also been detected [123]. The binding of exogenous LPL to cultured endothelial cells was reduced by preincubation with heparinase [119], suggesting that LPL was bound to heparan sulphate proteoglycans on the cell surface. PI-PLC did not reduce the binding of exogenous LPL to cultured aortic endothelial cells [124], therefore LPL must not be bound to a pool of glycosyl phosphatidylinositol-anchored heparan sulphate proteoglycans; similar results were obtained with macrophages (Table 2). The endothelial cell LPL receptor has recently been identified as a 220 kDa proteoglycan [124].

After prolonged incubation of cultured endothelial cells with exogenous LPL, a portion of radioiodinated LPL initially bound to the cell surface was not released by a subsequent heparin incubation [122], suggesting that the enzyme was internalized. LPL internalization was markedly reduced by pretreatment of endothelial cells with heparinase, as observed previously for adipocytes [108]. However, in contrast to adipocytes [108], internalized LPL in aortic endothelial cells was re-cycled back to the cell surface without intracellular degradation [122].

Since vascular endothelial cells do not degrade LPL [122,123], the quantity of functional, endothelium-bound LPL will be determined by the balance between translocation of the enzyme to the endothelium [38,118] from sites of synthesis (Fig. 2), and the release of endothelial LPL into the circulation [123]. Precise regulation of functional, endothelium-bound LPL activity is required to control carefully triacylglycerol catabolism and the supply of fatty acids to tissue cells (Fig. 1). Exogenous LPL bound to cultured endothelial cells was released into the medium by fatty acids [121,125]. Displacement of functional LPL from endothelial binding sites by free fatty acids, the product of the LPL reaction, would prevent the over-supply of fatty acids to tissue parenchymal cells under conditions of excessive lipolysis [121] and complement other regulatory mechanisms whereby free fatty acids inhibit endothelial LPL activity, such as product inhibition and a reduction in CII activation [126]. Peterson *et al.* [127] observed a strong correlation between plasma free fatty acid concentrations and plasma LPL activity in humans. However, fatty acids did not release LPL from either cardiac myocytes or perfused rat hearts [110]; this discrepancy with the results from experiments with cultured aortic endothelial cells [121,125] may be a reflection of differences in cell surface binding sites for LPL, such as the presence or absence of glycosyl phosphatidylinositol membrane anchors for heparan sulphate proteoglycans on the cell surface or different characteristics of the proteoglycan receptor such as polymer length and the degree of N-sulphation [109].

REGULATION OF LPL ACTIVITY

Fasting

The reciprocal effects of fasting in adipose tissue and heart where LPL activity is reduced and increased, respectively, represents an interesting example of tissue-specific LPL regulation. The reduction in LPL activity in guinea pig fat pads due to fasting was greater than in isolated adipocytes [22,23], therefore the translocation of LPL to functional endothelial sites may have been decreased. The fasting-induced decline in LPL activity was greater than the reduction in LPL synthesis and LPL mRNA, particularly in younger animals [23]. Glucose refeeding produced a rapid increase in guinea pig adipose tissue LPL activity with only a small increase in LPL mRNA [22], and a carbohydrate-rich meal increased human adipose tissue LPL activity with no change in enzyme mass or LPL mRNA [128]. Therefore, although changes in LPL mRNA contribute to the nutritional regulation of adipose tissue LPL activity [23,129], post-translational mechanisms are important. Doolittle *et al.* [24] observed that a

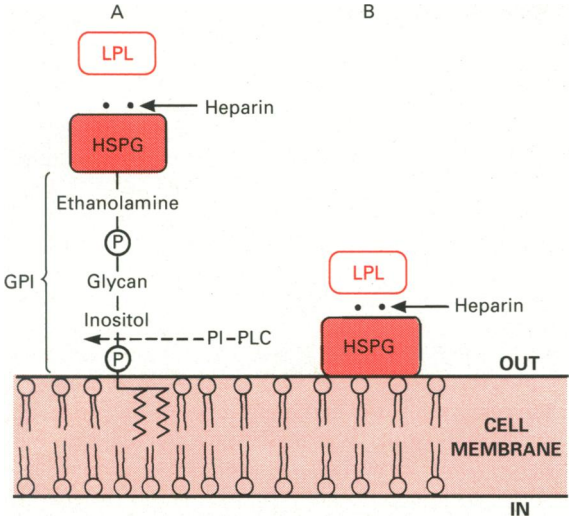

Fig. 4. Models for the attachment of lipoprotein lipase to the cell surface

Abbreviations: HSPG, heparan sulphate proteoglycans; GPI, glycosyl phosphatidylinositol.

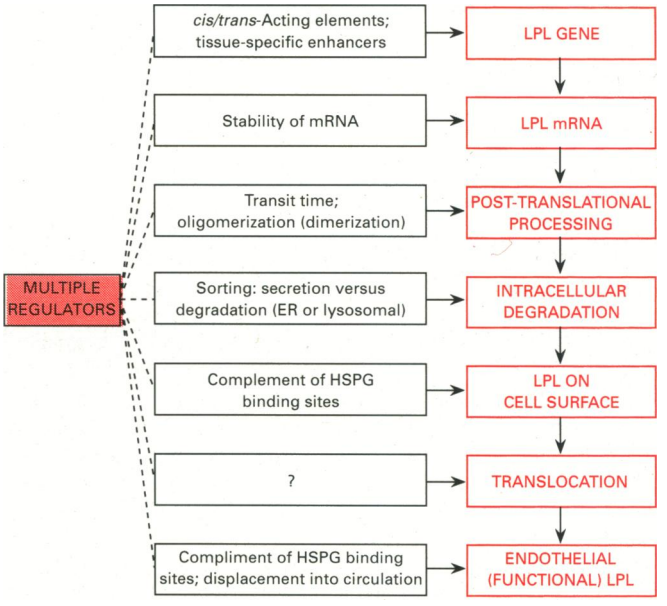

Fig. 5. Possible sites and potential mechanisms for regulation of LPL

Abbreviations: HSPG, heparan sulphate proteoglycans; ER, endoplasmic reticulum.

12 h fast produced a reduction in LPL catalytic activity in rat fat pads with no change in enzyme mass so that LPL specific activity was decreased, which was accompanied by an increase in LPL mRNA and synthesis rates. A reduction in catalytic activity without a change in total LPL mass was the result of the redistribution of LPL from the functional (endothelium-bound) pool to high-mannose (inactive) forms in adipocytes. This redistribution could be caused by diverting LPL from a secretory pathway into an intracellular degradative pathway; increased synthesis of the inactive LPL precursor then would keep the total LPL mass (active and inactive) constant [24].

Fasting rats for 12 h increased both myocardial LPL activity and mass so that LPL specific activity was unchanged; LPL mRNA and relative rates of synthesis were unchanged [24]. Thus, LPL in heart is regulated by post-translational mechanisms that are different from those operating in adipose tissue [24]. On the other hand, Ladu *et al.* [129] observed an increase in both rat heart LPL activity and mRNA after 24 h of fasting. Therefore, heart LPL is subject to nutritional regulation by both pre- and post-translational mechanisms, depending on the duration of the fast. Although LPL activity in guinea pig adipose tissue is more sensitive to nutritional regulation than in mouse or rat adipose tissues [22], fasting produced no significant increase in LPL activity or LPL mRNA in guinea pig heart [22,130].

The regulation of LPL activity in adipose tissue and heart in response to fasting is complex, and a number of important questions remain. What are the specific nutritional signals (hormones, metabolites) that are recognized by adipocytes and cardiac myocytes, and how are these signals transduced into producing a reciprocal response in functional LPL activity? Do the specific cell types responsible for LPL synthesis and processing in these tissues recognize different signals, or are there selective, tissue-specific biochemical mechanisms that can produce reciprocal effects on enzyme activity in response to the same signal?

Insulin and diabetes

The hypertriglyceridaemia associated with insulin-dependent diabetes mellitus is, in part, due to decreased degradation of circulating triacylglycerol-rich lipoproteins [131] because of changes in the apoprotein content of lipoprotein particles [132] and decreased adipose tissue LPL activity [133]. Insulin has well-documented direct effects on LPL in adipose tissue *in vitro*. Insulin increased immunodetectable LPL on the cell surface of adipocytes [106] and enhanced constitutive (spontaneous) rates of LPL release into the medium [34,115,134]. Insulin also increased cellular LPL activity, rates of LPL synthesis and LPL mRNA levels in adipocytes [61,135,136]. Insulin did not stimulate LPL gene transcription in nuclear run-on assays [136], so insulin-induced increases in steady-state LPL mRNA levels must have been due to changes in mRNA stability (post-transcriptional mechanism). On the other hand, insulin treatment of 3T3-L1 adipocytes produced an increase in LPL catalytic activity with no change in LPL mRNA and a decrease in LPL synthetic rate, implicating post-transcriptional and post-translational mechanisms [137]. Therefore, insulin has a direct stimulatory effect on adipose tissue LPL activity, although the precise biochemical mechanism depends on the particular cellular preparation.

Insulin-deficient diabetes resulted in a reduced degradation of very-low density lipoproteins by perfused hearts which also exhibited decreased functional (endothelium-bound) LPL activity [138]. Cardiac myocytes isolated from diabetic rat hearts have decreased cellular LPL activity and reduced release of LPL in response to heparin and PI-PLC [117], which could be the cause of the fall in functional LPL activity in the whole heart [138]. Administration of insulin *in vivo* rapidly reversed the effects of diabetes on LPL in cardiac myocytes, and increased the heparin- and PI-PLC-induced release of LPL from control cardiac myocytes [117]. However, incubations of control and diabetic cardiac myocyte preparations with insulin *in vitro* had no effect on either cellular or heparin-releasable LPL activities [139]. Therefore, additional factors active *in vivo*, such as another hormone, may be required for insulin to be effective in an *in vitro* incubation. Alternatively, the decrease in LPL activity in diabetic cardiac myocytes may not be due to insulin deficiency directly, but instead be secondary to one or more of the multiple metabolic factors that are altered in acute models of diabetes. These results clearly indicate that the regulation of LPL in diabetes is subject to tissue-specific mechanisms in adipocytes and cardiac myocytes.

PERSPECTIVE ON FUTURE DIRECTIONS

LPL is a member of a gene family which includes hepatic lipase and pancreatic lipase; all three enzymes evolved from a common ancestral gene [41]. It is likely that the first gene duplication gave a digestive lipase active in the intestine (pancreatic lipase) and a lipase active on circulating lipoproteins in the blood stream that subsequently specialized into LPL and hepatic lipase as a consequence of a second gene duplication [59]. Hepatic lipase can be distinguished from LPL on the basis of its restrictive site of synthesis (liver) and the absence of any cofactor requirement [140]. LPL and hepatic lipase have complementary functions in lipoprotein metabolism. LPL catalyses the hydrolysis of the triacylglycerol component of chylomicrons and very-low-density lipoproteins (Fig. 1). Hepatic lipase degrades triacylglycerols in high-density lipoproteins and the remaining triacylglycerols on the remnant particles produced by the action of LPL [140]. Recently, a new function for LPL has been proposed. LPL enhanced the binding of chylomicron remnants to the low-density lipoprotein receptor-related protein [141], an effect that was not dependent on lipolysis. Therefore, LPL may have a structural role in remnant catabolism in addition to its catalytic function in producing chylomicron remnant particles (Fig. 1).

A great deal of information obtained over the past 3 years regarding the structure and regulation of LPL sets the stage for future progress. It is reasonable to anticipate that considerable emphasis will continue to be placed on structure-function relationships for LPL. The LPL gene encodes for an enzyme with a number of important domains. The requirement of specific amino acids in the primary sequence of LPL for catalytic activity has been determined from site-directed mutagenesis and knowledge of the structural basis for the absence of catalytic activity due to LPL gene mutations [29]. Further progress in assigning specific functions to LPL domains may be achieved through the construction of chimaeric lipases, using domains of LPL and hepatic lipase [142] to establish the specific determinants for CII activation, for example. Finally, knowledge of the three-dimensional structure of LPL from crystal analysis will be a crucial step in establishing structure-function relationships.

LPL is subject to regulation in a variety of physiological (feeding/fasting, cold adaptation, development, lactation, exercise) and pathophysiological (diabetes, obesity, endotoxin infection) situations. It follows that LPL must be regulated by multiple factors interacting at various sites in the synthesis, processing and translocation of LPL, using a number of potential mechanisms (Fig. 5). A major area of focus in the future will be studies into the regulation of LPL gene expression. Transfection of plasmids containing the 5′-LPL promoter linked to a luciferase reporter gene has shown that deletions between -565 and -232 resulted in increased transcription in 3T3-L1 adipocytes but not in Hep G2 cells, establishing the presence of tissue-specific regulatory element(s) in this region [55]. Tissue-specific enhancer sequences that function independently of orientation at considerable distances from the promoter and their regulatory nuclear factors [143], may also regulate LPL gene expression. Transcription of the LPL gene is also regulated by hormones. Cold exposure results in an increase in LPL activity in brown adipose tissue that is due to β-adrenergic stimulation of LPL gene transcription [25,26]. Therefore, a cyclic AMP response element [144] in the LPL promoter will need to be identified in adipocyte transfection studies, along with characterization of tissue-specific transcription factors (proteins) that bind to this region. The mechanism for regulation of transcription factors (e.g., phosphorylation by protein kinase A) will also be an important area of investigation. Steady-state LPL mRNA levels in adipocytes can be increased by insulin [61,136]. Additional work is required to establish if there is an insulin-regulator element in the LPL promoter and to determine the mechanism whereby insulin regulates the stability of LPL mRNA [136]. Recently, enhanced levels of LPL mRNA were observed in macrophage-derived foam cells in atherosclerotic lesions [145]. Therefore, the regulation of LPL expression by cytokines present in the artery wall may play a role in the pathogenesis of atherosclerosis.

LPL is also regulated by tissue-specific post-transcriptional mechanisms during heart development [14] and in response to insulin and fasting in adipose tissue and heart [24,137]. LPL provides a unique example of an enzyme where catalytic activity is dependent upon N-linked glycosylation. The relationship between the trimming of glucose residues in the endoplasmic reticulum and dimerization of LPL needs to be assessed directly. Monomeric (inactive) and dimeric (active) forms of LPL can be monitored by sucrose gradient ultracentrifugation [63] or heparin-Sepharose chromatography [73]. The use of mammalian glycosylation mutants [146] may provide a useful experimental approach to confirm the role of glucosidases in the dimerization and activation of LPL. Elucidating the molecular defect associated with the retention of inactive LPL in the endoplasmic reticulum of cells from *cld/cld* mice will also provide considerable insight into how LPL is regulated. The gene product of the *cld/cld* mouse chromosome 17 could bind LPL (and hepatic lipase) selectively to cause retention of the inactive enzymes. Alternatively, the mutant gene product could be an essential component of the processing and secretory pathways for the lipase enzymes. The sorting of LPL into secretory or degradative pathways in the *trans*-Golgi network may also be regulated by unknown mechanisms.

Factors regulating the synthesis of heparan sulphate proteoglycans and translocation to the cell surface via a secretory pathway [104] could influence the availability of binding sites for LPL. Heparan sulphate proteoglycans are heterogeneous and differences in chemical composition such as the degree of sulphation can affect the binding affinity for LPL [109]. Variations in the complement of proteoglycan binding sites for LPL will almost certainly exist between different cell types (e.g., endothelial and parenchymal cells), with implications regarding the release, recycling and translocation of LPL. Diabetes, for example, could alter the nature of the binding sites for LPL on cardiac myocytes and thus contribute to the observed reduction in LPL release [117,139]. The presence or absence of glycosyl phosphatidylinositol anchors for heparan sulphate proteoglycans in a particular cell will also determine if LPL can be released by either a constitutively-active or regulated PI-PLC. The ability to experimentally distinguish between secretion by exocytosis and release from cell surface binding sites is also an important objective for further research. Finally, much of the future research outlined above will utilize the powerful techniques of cell and molecular biology with appropriate cells or cell lines. An important area of future research that is technically much more difficult because it involves the study of whole organs and tissues is to understand the translocation of LPL from specific cells of origin to its functional site on the luminal surface of vascular endothelial cells. How can the process of translocation be regulated in a reciprocal manner in adipose tissue and heart in response to fasting? What determines the vectorial nature of LPL translocation? The presence of immunoreactive LPL in kidney [7] and liver [8,18] probably is due to uptake of circulating LPL in the blood released from other tissue sites. Is the LPL bound to liver sinusoids functional, or does this uptake represent a degradative pathway requiring reverse transport back to hepatocytes and/or non-parenchymal (Kupffer) cells? Does internalization (endocytosis) and recycling of released LPL back

to the cell surface occur in parenchymal cells *in vivo*? These are exciting challenges for LPL research that will require novel, multi-disciplinary experimental approaches.

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