Bone Morphogenetic Protein-1 (BMP-1) Cleaves Human Proapolipoprotein A1 and Regulates Its Activation for Lipid Binding†

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ABSTRACT: Apolipoprotein A1 (apo A1), the major protein of high-density lipoprotein, is secreted as a proprotein and then cleaved by an uncharacterized metalloproteinase. Here this enzyme is identified as C-terminal procollagen endoproteinase/bone morphogenetic protein-1 (BMP-1). Studies with recombinant BMP-1, BMP-1 antibody, and BMP-1 siRNA establish this proteinase as the major or only apo A1-converting activity secreted by human liver-derived (HepG2) cells and CHO cells stably expressing human apo A1. BMP-1 stimulates the conversion of newly secreted proapo A1 to its phospholipid- (PL-) binding form. In this way it promotes formation of functional HDL and reverse cholesterol transport, while inhibiting filtration and clearance of uncleaved proprotein. α_2-Macroglobulin, a protease inhibitor secreted as part of the innate immune response, inhibits BMP-1 activity and blocks the maturation of proapo A1. The decrease in circulating apo A1 levels that is characteristic of the response to inflammation and infection may be mediated, at least in part, via BMP-1 by this novel mechanism.

Apolipoprotein A1 (apo A1)[1] is the major protein of serum high-density lipoprotein (HDL). It is the only protein of prebeta-migrating HDL (prebeta$_1$-HDL), a lipid-poor HDL that is a major early acceptor of peripheral cellular free cholesterol (FC) (*1*). Apo A1 forms part of the reverse cholesterol transport (RCT) pathway that limits FC accumulation in peripheral tissues (*2*). It is secreted as a 249 amino acid (aa) proprotein, mainly from hepatic and intestinal cells. Cleavage N-terminal to two acidic aa (RHFWQQ↓DEPP) in plasma and extracellular fluids by an unidentified metalloproteinase generates the mature 243 aa polypeptide (*3*−*5*). This normally makes up >95% of circulating apo A1 (*6*). Deletion of the sequence coding for the prohexapeptide of human preproapo A1 from cDNA transfected into BHK cells was associated with a decrease in the secretion rate of apo A1 (*7*). Otherwise, a function for proapo A1 cleavage has not been identified.

We recently found that a major part of apo A1 synthesized by human liver- and intestine-derived cells was secreted in the form of a stable lipid-free monomer. It had a Stokes radius (SR) of 2.6 nm. ATP-binding cassette transporter A1 (ABCA1) at the cell surface promoted conversion of this 2.6 nm form of apo A1 to a more loosely folded (3.6 nm SR) conformation, which had gained the capacity to bind phospholipid (PL) (*8*). These data show that synthesis of prebeta$_1$-HDL involves a reaction sequence that includes consecutive steps of apo A1 refolding and lipid binding.

The properties of 3.6 nm lipid-poor apo A1 were found to be very similar to those of plasma prebeta$_1$-HDL, which has a PL content of 2 mol of PL/mol of apo A1 and an apparent diameter of ∼7 nm by nondenaturing PAGE (*8*).

In human Tangier disease, ABCA1 activity is congenitally deficient. Circulating apo A1 levels are very low in spite of normal synthesis rates (*9*, *10*). Though much of Tangier apo A1 circulates as the proprotein, the rate of proapo A1 cleavage in Tangier plasma is within normal limits (*11*). This finding suggests that proapo A1 conversion might be an intermediate step in the formation of biologically active prebeta$_1$-HDL. In the present research, the apo A1 proprotein convertase is identified and its role in the maturation of prebeta$_1$-HDL defined.

EXPERIMENTAL PROCEDURES

Cell Culture. CHO cells stably transfected with the human apo A1 gene under control of the metallothein promoter (CHO-A1 cells) were grown until nearly confluent in 3.5 cm plastic wells containing DMEM/Ham's F-12 medium (1/1 v/v) with 10% fetal bovine serum (FBS) (*12*). To induce apo A1 synthesis, the cells were transferred to the same medium containing 30 μM $ZnSO_4$ and 30 μM $FeSO_4$. After 24 h this was removed, and the cells were washed (×2) with phosphate-buffered saline, pH 7.4 (PBS). Serum-free medium (0.3 mL) with Zn^{2+} and Fe^{2+} and 1 mg mL^{-1} high MW dextran (T-400; Pharmacia/NEN, Parsipanny, NJ) was added. Further incubation was carried out for 8−24 h. Wild-type CHO (CHO-WT) cells, that express no detectable apo A1,

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[1] Abbreviations: ABCA1, ATP-binding cassette transporter A1; aa, amino acid; apo A1, apolipoprotein A1; BMP-1, bone morphogenetic protein-1; CUB, complement C1r,C1s/Uegf/Bmp1; EGF, epidermal growth factor; FC, free cholesterol; HDL, high-density lipoprotein; PL, phospholipid(s); PMSF, phenylmethanesulfonyl fluoride; SR, Stokes radius.

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were grown under the same conditions, as previously reported (*8*).

Human liver-derived HepG2 cells, which secrete apo A1 constitutively (*13*), were grown to near confluence in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS. The cells were washed in PBS (×3) and cultured in serum-free DMEM/dextran for 4−6 h.

In some experiments CHO-A1 or HepG2 cells were preincubated (24 h) with [^{3}H]choline (Perkin-Elmer, Boston, MA; 10 μCi mL^{-1} medium) to label cell PL (*8*). The cells were washed to remove unincorporated isotope. Secreted proteins were collected into serum-free medium for 12−24 h (CHO-A1 cells) or 4−6 h (HepG2 cells), prior to analysis.

A peptide (FWQQDEPP) encompassing the endopeptidase cleavage site of proapo A1 (*3*) and an unrelated peptide with a similar acidic site (GMVGDDPY) were synthesized by Genscript, Edison, NJ. A mutant peptide (FWQQSSPP) lacking the proapo A1 acidic cleavage site was also prepared. Each was added at a final concentration of 2.5 mM to the medium from apo A1-secreting cells. The effects of these peptides on apo A1 propeptide cleavage, the conversion of 2.6 to 3.6 nm secreted apo A1, and PL binding were determined as described below.

Immunoassays. A peptide consisting of a repeat of the apo A1 prosequence with an added C-terminal cysteine residue (RHFWQQRHFWQQC) (*14*) was synthesized by Biosynthesis, Lewisville, TX, linked covalently to keyhole limpet hemocyanin, and injected into rabbits. The antiserum obtained was affinity-purified using agarose-immobilized antigen. The purified globulin (propeptide antibody) was used to assay newly secreted human A1 in the culture media of CHO-A1 or HepG2 cells. A similar assay was described by Barkia et al. (*15*). Polyclonal antibody to human apo A1 was raised in goats (*8*). Other monoclonal antibodies and recombinant protein were from R & D Systems, Minneapolis, MN. Protease inhibitors (1,10-phenanthroline, PMSF, actinonin, amastin, α_2-macroglobulin) were from Sigma, St. Louis, MO.

Solid-phase immunoassay of human bone morphogenetic protein-1 (BMP-1) in HepG2 cell medium was carried out on PVDF sheets using a Minifold-1 blot system (Whatman, Sanford, ME), anti-BMP-1 antibody (no. 1927), and recombinant BMP-1 (both from R & D Systems, Minneapolis, MN).

Electrophoresis. Nondenaturing PAGE was carried out in preformed 10−20% (w/v) gradient gels (Bio-Rad, Hercules, CA) (*8*). Briefly, cell culture medium was brought to 15.5% w/v sucrose, 0.03% Na_2-EDTA, and 0.1% bromophenol blue (pH 8.25). Electrophoresis was for 20 h at 40 V and 4 °C. Electrotransfer to nitrocellulose membranes (0.2 μm; Whatman, Florham Park, NJ) was followed by Western blotting with the propeptide or apo A1 antibodies. Signals from reactive bands were quantified by computerized scanning densitometry. PL radioactivity in proteins recovered after electrophoresis from [^{3}H]choline-labeled cells was assayed by liquid scintillation spectrometry (*8*). SDS−PAGE was carried out in 12% w/v gels, also from Bio-Rad, and processed in the same way.

Isoelectric focusing (IEF) electrophoresis was carried out in 5% w/v acrylamide minigels containing 5% glycerol and 2% ampholine (pH range 3.5−7.5) together with protein standards (Bio-Rad, Richmond, CA). Electrophoresis was for 1 h (100 V), then for 1 h (250 V), and then for 30 min (500 V). The gels were electrotransferred to PVDF membranes in 0.7% acetic acid at 100 V (1 h). Membranes were blotted with goat apo A1 antibody as described above.

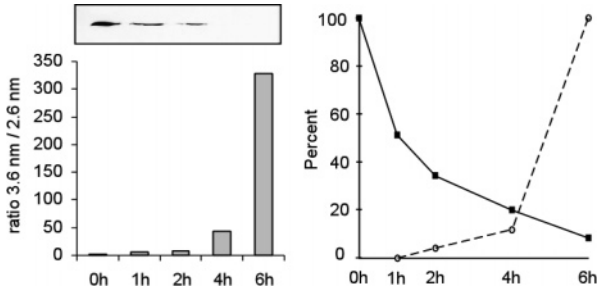

FIGURE 1: Medium from HepG2 cells (6 h) was transferred to [^{3}H]-choline-labeled CHO-WT cells and incubated at 37 °C. Proapo A1 cleavage (upper left) was assayed with propeptide antibody after SDS−PAGE as described in Experimental Procedures. The ratio of apo A1 in the 3.6 nm (product) and 2.6 nm (precursor) fractions in the same experiments (lower left) was determined by densitometric scanning after native PAGE by electrotransfer and Western blotting with polyclonal apo A1 antibody. Right: Comparison of the rates of propeptide cleavage and apo A1 conversion, relative to zero time (100%). Key: squares, percent of proapo A1 remaining; circles, percent of total apo A1 in the 3.6 nm fraction. Data are of duplicate assays, reproducible ± 5%, and representative of four experiments.

siRNA Transfection. BMP-1 knockdown was carried out in HepG2 cells (2×10^5 cells per well in 12-well plates) in the presence of 30 nmol of human BMP-1 silencer validated siRNA (Ambion 105352) and 10 μL per well of siPORT *NeoFx* transfection agent. Other wells contained either 30 nmol of GAPDH siRNA or negative control (−1) siRNA (Ambion). All cells were grown at 37 °C with a medium change 24 h post-transfection. After a total of 48 h the cells were washed (×3) with PBS, and then apo A1 was collected in 200 μL of serum-free medium for 24 h. IEF electrophoresis, electrotransfer, and Western blotting with apo A1 antibody were carried out as described above.

RESULTS

Properties of Apo A1 Convertase. Medium was collected from HepG2 cells for 4 h, conditions when the 2.6 nm species was the major form of apo A1 (*8*). When these media were transferred to the surface of washed CHO-WT cells, the rapid disappearance of propeptide was associated with a conversion of precursor apo A1 (2.6 nm) to product (3.6 nm) that was noticeably delayed (Figure 1) probably owing to a slow rate of conversion of apo A1 bound to the cell surface (*16*). The rate of propeptide cleavage in HepG2 medium was the same when CHO-WT cells were absent (data not shown) even though, under these conditions, 3.6 nm apo A1 is not formed (*8*).

Further analysis of the relationship between proapo A1 and its product, the mature apo A1 polypeptide, was made by isoelectric focusing, taking advantage of the additional positive charge represented by the arginine residue in the propeptide (*5*, *6*). HepG2 cells were incubated in serum-free medium at 37 °C. After 1 h, when >95% of apo A1 was still in the 2.6 nm form, 93.6 ± 3.0% ($n = 3$) of apo A1 was present as proapo A1. After 6 h incubation, when 72% of apo A1 was in the 3.6 nm form, 66.5 ± 8.4% ($n = 4$) of apo A1 was in the mature polypeptide band (Figure 2).

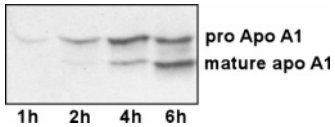

FIGURE 2: IEF electrophoresis of apo A1 in medium from HepG2 cells. The data show its proapo A1 content as a function of incubation time at 37 °C. Analysis was carried out as described under Experimental Procedures. The data are representative of three experiments.

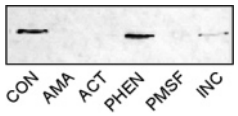

FIGURE 3: Effects of inhibitors on the cleavage of propeptide from proapo A1. Medium was removed from CHO-A1 cells after 16 h. Aliquots were kept on ice (CON) or incubated for 16 h at 37 °C without additive (INC) or in the presence of amastatin (AMA, 100 μM), actinonin (ACT, 100 μM), 1,10-phenanthroline (PHEN, 1 mM), or PMSF (1 mM). After SDS−PAGE and electrotransfer, proapo A1 was determined by Western blotting with propeptide antibody.

Table 1: Substrates of Astacin Family Protease BMP-1[a]

BMP-1	
procollagen α1	YYRA ↓ DDAN
laminin 5	CYSG ↓ DENP
prolysyl oxidase	GMVG ↓ DDPY
probiglycan	FMMN ↓ DEEA
myostatin	DVQR ↓ DDSS
dental matrix protein-1	GMQS ↓ DDPE
endorepellin	SGGN ↓ DAPG

[a] The sequences shown, derived from GenBank, are for human proteins. The specificity of BMP-1 for these bonds is consistent with the merops.sanger protease database (http://merops.sanger.ac.uk).

Consistent with previous data (*3*, *4*) propeptide cleavage was completely blocked in CHO-A1 medium by 1,10-phenanthroline, an inhibitor of metalloproteinases (Figure 3). Similar results were obtained using medium from HepG2 cells (data not shown). There was no inhibition by PMSF, which blocks serine proteinases. Only a few metalloproteinases cleave N-terminal to acidic residues (D or E). These include several Zn^{+}-dependent astacin family proteases (*17*). Tolloid proteases and some meprins generate products with N-terminal D/E sequences. Though many are membrane-associated, procollagen C-endoproteinase/bone morphogenetic protein-1 (BMP-1), an alternative splice product of the mammalian tolloid (Tld) gene, is soluble and secreted by fibroblasts, hepatocytes, and other cells (*18*, *19*). BMP-1 plays an important role in connective tissue formation by processing precursors of procollagens (*20*). Meprins cleave the protein precursors of many hormonal peptides, but these enzymes are expressed mainly in renal and intestinal cells. Their activities are actinonin-sensitive (*21*). Actinonin did not inhibit proapo A1 conversion, nor did amastin, an inhibitor of N-terminal proteinases (*22*) (Figure 3). The properties of proapo A1 converting enzyme thus resembled those of a soluble tolloid protease.

BMP-1 cleaves several substrates at sites that resemble that of proapo A1 (Table 1). It mediates C-terminal cleavage of procollagen and also activates prolysyl oxidase to promote the cross-linking of collagen microfibrils. Prolysyl oxidase conversion by BMP-1 is inhibited by GMVGDDPY (peptide 1) (Figure 4), which contains its endoproteinase site (*23*).

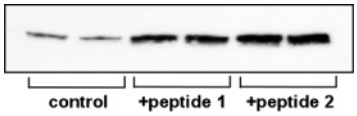

FIGURE 4: Effects of cleavage site peptides of prolysyl oxidase (GMVGDDPY, peptide 1) and proapo A1 (FWQQDEPP, peptide 2) (2.5 mM final concentration in each case) on proapo A1 levels in medium from CHO-A1 cells (24 h incubation) determined by Western blotting following SDS−PAGE.

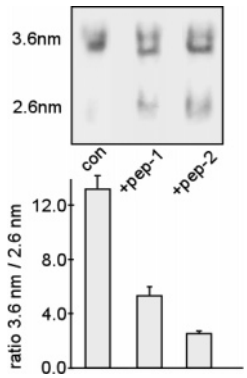

FIGURE 5: Effects of competitive peptides on apo A1 conversion. Upper: 2.6 and 3.6 nm species of newly secreted apo A1 from CHO-A1 cells in the presence and absence of the peptides described in the legend to Figure 3, determined after native PAGE with polyclonal apo A1 antibody. Lower: The same data expressed as the ratio of 3.6 nm/2.6 nm particles. The data are means ± 1 SD of three to four experiments.

When this peptide was added to the incubation medium of CHO-A1 cells, proapo A1 remaining was increased 3.6 ± 0.3-fold above control levels. In the presence of FWQQDEPP (peptide 2), which included the apo A1 cleavage site, proapo A1 remaining was 4.6 ± 0.3-fold higher. Control peptide (FWQQSSPP) was without effect.

Under the same experimental conditions, peptide 1 (GMVGDDPY) increased the proportion of newly secreted apo A1 remaining in the 2.6 nm fraction after 24 h from 7 ± 5% to 37 ± 7% of total apo A1 (Figure 5, upper panel). Peptide FWQQDEPP increased it to 39 ± 3% under the same conditions. As a result, the mass of apo A1 in the 3.6 nm fraction (relative to that in 2.6 nm apo A1) significantly decreased (Figure 5, lower panel). These data showed that a BMP-1 peptide inhibitor also mediated a reduction in proapo A1 processing. Together, the data in Figures 3 and 4 confirmed a relation between apo A1 propeptide cleavage and the maturation of newly secreted apo A1 into its lipid-binding (3.6 nm) form.

Identification of BMP-1 as Proapo A1 Convertase. The secretion of BMP-1 into the medium of HepG2 cells was confirmed by solid-phase immunoassay. After 40 h this contained 12.6 ± 3.2 ng mL^{-1} ($n = 3$). To determine if BMP-1 cleaved proapo A1, HepG2 cell medium was incubated at 37 °C without addition, or in the presence of BMP-1 antibody, or with added rBMP-1. The effect was assayed in IEF gels (Figure 6, left). Propeptide cleavage was accelerated by rBMP-1 and reduced by antibody. Antibody to BMP-1 inhibited, while rBMP-1 accelerated, the conversion of 2.6 nm apo A1 to its 3.6 nm product (Figure 6, middle). Finally, BMP-1 siRNA increased by 6.8 ± 0.1-fold the level of proapo A1 in HepG2 cell medium (Figure 6, right). An unrelated (GAPDH) siRNA was without effect. Taken together, these studies strongly suggest that BMP-1

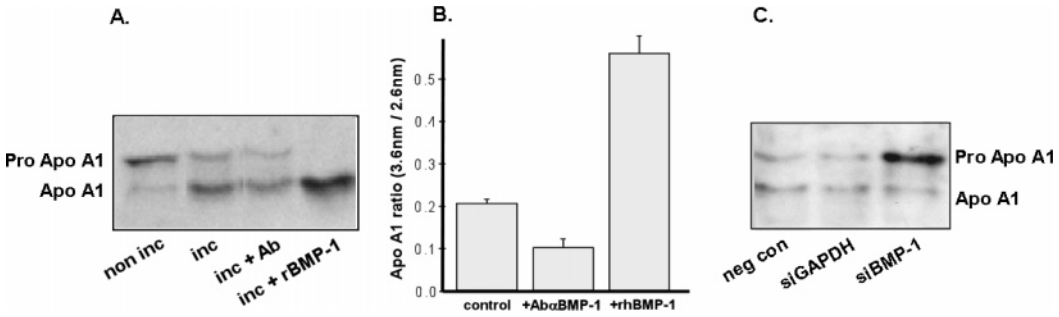

FIGURE 6: Effects of BMP-1 on proapo A1 cleavage. Left: Effects of rBMP-1 or BMP-1 antibody on proapo A1 cleavage in medium from HepG2 cells (1 h). Medium was analyzed without further incubation, or incubation without additives, or in the presence of BMP-1 antibody (5 μg) or of rBMP-1 (25 ng/mL). Samples were analyzed by IEF as described in the legend to Figure 2. Middle: Effect of the same conditions on the proportions of 3.6 and 2.6 nm apo A1 fractions determined by native PAGE. Right: Effect of BMP-1 siRNA on propeptide conversion in HepG2 cell medium. Sham transfection [con(−)], transfection with GAPDH siRNA [con(+)], or transfection with BMP-1 siRNA (BMP-1) was carried out as described in Experimental Procedures. After 24 h, medium was collected and assayed by IEF.

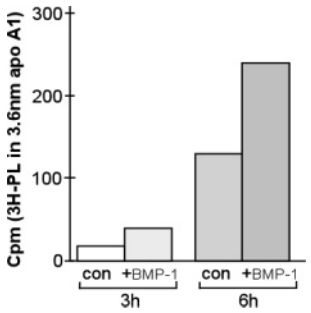

FIGURE 7: Effects of rBMP-1 on PL binding to 3.6 nm apo A1. Medium from CHO-A1 cells was collected (4 h) and then transferred to [^{3}H]choline-labeled CHO-WT cells without addition (control) or in the presence of rBMP-1 (50 ng). Duplicate samples were taken for analysis after 3 and 6 h. ^{3}H-PL label recovered in the 3.6 nm apo A1 band is shown. No label was detected in 2.6 nm apo A1. Duplicate assays were reproducible ± 5%. Data shown is representative of three experiments.

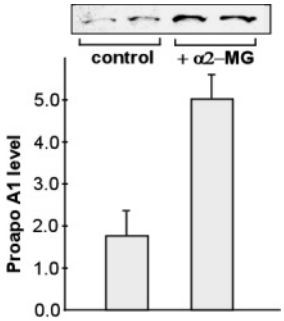

FIGURE 8: Effects of α_2-macroglobulin on proapo A1 conversion. Medium from HepG2 cells was collected after 4 h and then incubated for 16 h at 37 °C in the presence or absence of protease inhibitor (0.2 mg mL^{-1}). Levels of proapo A1 were determined by SDS−PAGE and Western blotting. After incubation in the presence of α_2-macroglobulin, proapo A1 levels were 106 ± 10% (n = 3) of those in nonincubated medium, indicating inhibition of propeptide cleavage to be complete under the conditions described.

was the major or only activity responsible for the cleavage activity observed and confirmed the link seen earlier between apo A1 propeptide cleavage and the conversion of newly secreted apo A1 to its PL-binding form.

Role of BMP-1 in Prebeta-HDL Formation. Medium from CHO-A1 cells was incubated with CHO-WT cells that had been labeled with [^{3}H]choline. ^{3}H-PL was recovered in the 3.6 nm fraction, but none was found in 2.6 nm apo A1, consistent with our previous report (*8*). When rBMP-1 was added (Figure 7), the recovery of PL in 3.6 nm apo A1 was increased 1.7 ± 0.2-fold. These data support the hypothesis that propeptide cleavage can accelerate the maturation of the PL-binding form of newly secreted apo A1. The PL content in 3.6 nm apo A1, determined from PL-specific radioactivity, was 1.9 ± 0.2 mol/mol of apo A1, consistent with previous reports (*8*, *24*).

α_2-Macroglobulin and the Processing of Apo A1. α_2-Macroglobulin is a tetrameric protease inhibitor recently shown to inhibit BMP-1 activity in collagen processing (*25*). Plasma levels of this inhibitor, whose secretion is stimulated by inflammation or infection, reach >1.5 mg mL^{-1} (*26*). To determine if α_2-macroglobulin regulated cleavage of apo A1 propeptide, it was added to the medium of HepG2 cells and the effect on proapo A1 conversion determined (Figure 8). These data showed that macroglobulin strongly inhibited proapo A1 cleavage when present at levels relevant to those in plasma during the inflammatory response (*27*).

DISCUSSION

Protein convertases catalyze the cleavage of many newly secreted proproteins, usually at the carboxyl terminus of a consensus sequence rich in positively charged amino acids (R/K-x-x-R/K-R↓) (*28*). Though one (NARC-1) cleaves C-terminal to a glutamine residue, it is PMSF-sensitive and not a metalloproteinase. The protease that cleaves proapo A1 has quite different properties and has not been previously identified.

Proapo A1 Convertase Is a Tolloid Proteinase, Bone Morphogenetic Protein-1 (BMP-1). The propeptide site of apo A1 is consistent with cleavage sequences of other BMP-1 substrates: aspartic acid in the P+1 position, a hydrophobic amino acid in positions P3 and/or P4, and small residues at P−1 and P−2. These features are also consistent with the effects of mutation in the propeptide cleavage site of human apo A1 expressed in *Escherichia coli* (*29*). The response of proapo A1 cleavage to inhibitors matched those of a tolloid protease, not a classic protein convertase, N-terminal peptidase, or meprin. A peptide encompassing the cleavage site of the known BMP-1 substrate prolysyl oxidase inhibited proapo A1 maturation. Recombinant hBMP-1 accelerated the conversion of proapo A1 to apo A1. Monoclonal antibody to BMP-1 inhibited convertase activity in cell culture media. Finally, siRNA directed against BMP-1 markedly inhibited the formation of mature apo A1. Together, these data strongly

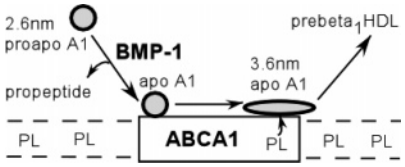

FIGURE 9: A model of prebeta$_1$-HDL genesis from newly secreted (2.6 nm) apo A1, suggesting the roles of BMP-1 and ABCA1.

suggest that BMP-1 is the major or only significant activity cleaving proapo A1 in the medium of CHO and HepG2 cells.

Like other tolloid family astacin proteases, BMP-1 is made up of an N-terminal catalytic domain, followed by several EGF-like sequences and CUB motifs. The latter are ~100 aa sequences rich in β-sheet structure, important in substrate recognition by endoproteinases (*30*). Though proapo A1 has not been previously identified as a BMP-1 substrate, the renal tubular protein cubilin, whose sequence includes 27 CUB domains, binds apo A1 as part of a reabsorption/catabolism pathway mediating the turnover of lipid-poor apo A1 by renal tubular epithelium (*31*, *32*). While further research is needed, it seems likely that the CUB domains of BMP-1 may be involved in substrate recognition of the apo A1 proprotein.

Role of BMP-1 in HDL Biogenesis. A major part of apo A1 secreted by human liver- and intestine-derived cells, as well as CHO-A1 cells, is a 2.6 nm globular monomer that lacks the ability to bind either PL or FC (*8*). Facilitation of the formation of lipid-binding HDL promotes RCT (*32*). We showed earlier that conversion of newly secreted apo A1 to its 3.6 nm PL-binding form required the presence of the cell surface lipid transporter ABCA1. Incorporation of competitive peptide FWQQDEPP decreased the rate of conversion of proapo A1 to its mature form. Anti-BMP-1 monoclonal antibody, which inhibited the hydrolysis of proapo A1, caused an accumulation of 2.6 nm apo A1 in the medium of CHO-A1 and HepG2 cells. In contrast, an increase in medium BMP-1 levels accelerated formation of 3.6 nm apo A1. These data establish a link between apo A1 propeptide cleavage and the maturation of newly secreted apo A1 into PL-binding prebeta$_1$-HDL.

BMP-1 cleavage continued in the absence of cell membranes, but conversion of 2.6 nm precursor apo A1 to its 3.6 nm product was completely dependent on the presence of ABCA1 (*8*). This suggests that removal of the propeptide from apo A1 normally precedes the conversion of apo A1 to its lipid-binding form (Figure 9). Consistent with this model, we found BMP-1 to influence subsequent steps of HDL biogenesis: formation of the 3.6 nm protein product and lipidation by PL.

The accelerated clearance of immature lipid-free and lipid-poor apo A1 by the kidney is well established (*33*). This rapid turnover is probably mainly responsible for the very low total apo A1 levels that are characteristic of genetic ABCA1 deficiency (Tangier disease). Apo A1 synthesis rate and proapo A1 converting activity are within the normal range (*10*, *34*) in this syndrome.

During inflammation, α_2-macroglobulin levels increase significantly (*26*) and circulating HDL levels are reduced (*35*, *36*). We found that when this protease inhibitor was added to cell medium at concentrations relevant to those present in the circulation in response to inflammation, proapo A1 processing was almost completely inhibited. This suggests that α_2-macroglobulin could contribute to the regulation of circulating HDL levels, by inhibiting the processing and lipidation of newly secreted apo A1 via BMP-1 and enhancing its renal clearance. It remains to be determined if BMP-1 and/or α_2-macroglobulin levels contribute to differences in HDL levels that depend on age, gender, and other factors in vivo. In summary, BMP-1-mediated cleavage of proapo A1 may play an important role in HDL processing under both physiological and pathophysiological conditions.

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