The Light Chain of Factor VIII Comprises a Binding Site for Low Density Lipoprotein Receptor-related Protein*

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In the present study, the interaction between the endocytic receptor low density lipoprotein receptor-related protein (LRP) and coagulation factor VIII (FVIII) was investigated. Using purified components, FVIII was found to bind to LRP in a reversible and dose-dependent manner ($K_d \simeq 60$ nM). The interaction appeared to be specific because the LRP antagonist receptor-associated protein readily inhibited binding of FVIII to LRP ($IC_{50} \simeq 1$ nM). In addition, a 12-fold molar excess of the physiological carrier of FVIII, i.e. von Willebrand factor (vWF), reduced the binding of FVIII to LRP by over 90%. Cellular degradation of $^{125}$I-labeled FVIII by LRP-expressing cells ($\sim 8$ fmol/10$^5$ cells after a 4.5-h incubation) was reduced by approximately 70% in the presence of receptor-associated protein. LRP-directed antibodies inhibited degradation to a similar extent, indicating that LRP is able to bind FVIII at the cell surface and to mediate its transport to the intracellular degradation pathway. Degradation of FVIII was completely inhibited by vWF. Because vWF binding by FVIII involves its light chain, LRP binding to this subunit was studied. In ligand blotting experiments, binding of FVIII light chain to LRP could be visualized. More detailed analysis revealed that FVIII light chain interacts with LRP with moderate affinity ($k_{on} \simeq 5 \times 10^{10}$ M$^{-1}$ s$^{-1}$; $k_{off} \simeq 2.5 \times 10^{-3}$ s$^{-1}$; $K_d \simeq 50$ nM). Furthermore, experiments using recombinant FVIII C2 domain showed that this domain contributes to the interaction with LRP. In contrast, no association of FVIII heavy chain to LRP could be detected under the same experimental conditions. Collectively, our data demonstrate that in vitro LRP is able to bind FVIII at the cell surface and to mediate its transport to the intracellular degradation pathway. FVIII-LRP interaction involves the FVIII light chain, and FVIII-vWF complex formation plays a regulatory role in LRP binding. Our findings may explain the beneficial effect of vWF on the in vivo survival of FVIII.

Low density lipoprotein receptor-related protein (LRP), also known as $\alpha_2$-macroglobulin receptor, is a member of the low density lipoprotein receptor family of endocytic receptors (for a review, see Refs. 1 and 2). It consists of a heavy chain and a light chain, which are associated in a noncovalent manner. The 85-kDa light chain comprises the transmembrane and cytoplasmic domains, whereas the ligand binding regions are located within the 515-kDa heavy chain (3). LRP is abundantly present in various tissues such as the liver, placenta, lung, and brain (4) and is expressed in an array of cell types: parenchymal cells, neurons and astrocytes, Leydig cells, smooth muscle cells, monocytes, and fibroblasts (4). Commonly used cell lines such as monkey kidney COS cells and Chinese hamster ovary (CHO) cells also express LRP (5, 6). The function of LRP is to mediate the binding and transport of ligands from the cell surface to the endosomal degradation pathway (1, 2). Binding and internalization of ligands is antagonized by a 39-kDa chaperone protein designated receptor-associated protein (RAP) (7, 8). Currently, a wide spectrum of structurally and functionally unrelated ligands involved in a variety of processes such as lipoprotein metabolism, cell growth and migration, and neuronal regeneration (1, 2) has been identified. Furthermore, LRP seems to be linked to the process of blood coagulation. This is apparent from the observations that LRP recognizes thrombin/antithrombin and factor Xa/$\alpha_2$-macroglobulin complexes and the Kunitz-type inhibitor tissue factor pathway inhibitor (9–11). In addition, LRP contributes to down-regulation of tissue factor expression at the surface of monocytes (12).

Coagulation factor VIII (FVIII) is the precursor of its activated derivative, which stimulates factor IXa-mediated activation of factor X (for recent reviews, see Refs. 13 and 14). The fact that deficiency or dysfunction of FVIII is associated with severe bleeding tendencies demonstrates that this cofactor is indispensable for appropriate hemostasis. FVIII comprises a domain structure (A1-A2-B-A3-C1-C2) (15) and circulates in plasma predominantly as a heterodimeric protein consisting of a metal ion-linked light and heavy chain (16, 17). The heavy chain (90–220 kDa) contains the A1-A2-B domains and is heterogeneous as a result of limited proteolysis within the B domain. The light chain (80 kDa) consists of the A3-C1-C2 domains. The amino- and carboxyl-terminal ends of FVIII light chain together comprise the binding site for von Willebrand factor (vWF) (18, 19), the physiological carrier protein of FVIII (20). The FVIII precursor is converted into its activated derivative upon limited proteolysis by thrombin (21, 22). Activated FVIII consists of the A2 domain, which is noncovalently associated with the metal-ion linked A1/A3-C1-C2 dimer, whereas modified Eagle's medium:F12; FVIII, factor VIII; GST, glutathione $S$-transferase; RAP, receptor-associated protein; vWF, von Willebrand factor; SPR, surface plasmon resonance.

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1 The abbreviations used are: LRP, low density lipoprotein receptor-related protein; CHO, Chinese hamster ovary; DMEM:F12, Dulbecco’s modified Eagle’s medium:F12; FVIII, factor VIII; GST, glutathione $S$-transferase; RAP, receptor-associated protein; vWF, von Willebrand factor; SPR, surface plasmon resonance.
the B domain and the amino-terminal part of the A3 domain have been removed (23). Due to the release of the amino-terminal part, high affinity binding to vWF is lost (18). vWF prevents the FVIII precursor from binding to components of the factor X-activating complex (14, 24, 25). Furthermore, the half-life of FVIII is considerably reduced in the absence of vWF (26, 27), indicating that vWF prevents FVIII from premature clearance. However, the mechanism by which FVIII is removed from the circulation has remained unidentified.

In the present study, we investigated the possibility that FVIII is recognized by the multifunctional receptor LRP. To this end, the interaction between LRP and FVIII or its constituent subunits has been addressed using purified components. In addition, cellular degradation of FVIII has been studied. It is demonstrated that LRP recognizes FVIII as a ligand, and that binding involves the light chain of FVIII. Furthermore, both LRP-mediated binding and degradation of FVIII are downregulated by vWF. Our data are in support of a mechanism in which LRP contributes to binding and internalization of FVIII.

EXPERIMENTAL PROCEDURES

Materials—Glutathione-Sephase 4B and protein A-Sepharose CL-4B were from Amersham Pharmacia Biotech. Microtitre plates were from Dynatech (Ploegingen, Germany). Cell culture plates, fetal calf serum, penicillin, and streptomycin were from Life Technologies, Inc. Dulbecco’s modified Eagle’s medium:F12 (DMEM:F12) medium was from BioWittaker (Verviers, Belgium). The BIAcoreTM2000 biosensor system and reagents (amino-coupling kit and CM5 sensor chips) were from Biacore AB (Uppsala, Sweden).

Proteins—FVIII light chain, thrombin-cleaved FVIII light chain, and FVIII heavy chain were prepared as described previously (25, 28). The integrity of the isolated subunits was assessed in reconstitution experiments as described previously (28). As expected, isolated subunits could effectively be reassembled into biologically active heterodimers according to a two-site model, using equations described previously (33). Data fitting to a one-site model proved inappropriate, as judged from residual plots and statistical parameters (data not shown).

Competition Experiments—Purified LRP (125 fmol/well) was adsorbed onto microtiter wells in 50 mM NaHCO3 (pH 9.5) in a volume of 50 μl for 16 h at 4 °C. LRP-coated and noncoated wells were blocked with 1% (w/v) gelatin in 150 mM NaCl, 2 mM CaCl2, 0.01% (v/v) Tween 20, and 20 mM Heps (pH 7.4) in a volume of 100 μl for 30 min at 37 °C. After washing, FVIII (40 nM) was added in the same buffer to LRP-coated and noncoated wells in the presence of 0.1 μM GST-RAP in a volume of 50 μl and incubated for 3 h at 37 °C. Bound FVIII was quantified by incubating for 15 min at 37 °C with peroxidase-labeled antibody CLB-CAg 117.

Ligand Degradation Experiments—Cellular degradation of FVIII was examined essentially as described elsewhere (34). CHO cells (ATCC CCL-61) were grown to 80–95% confluence in 24-well plates in DMEM:F12 medium supplemented with 10% (v/v) fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Before incubation, cells were washed extensively with DMEM:F12 medium. 125I-labeled FVIII (20 nM) was then added in a volume of 200 μl in DMEM:F12 medium containing 1% (w/v) bovine albumin and 5 mM CaCl2. In some experiments, 125I-labeled FVIII was added in the presence of vWF (500 nM), GST-RAP (1 μM), or protein A-Sepharose purified polyclonal antibodies directed against LRP (0.9 mg/ml). After a 35 min incubation at 37 °C, cells were washed three times with 500 μl of DMEM:F12 to remove nonbound material. Subsequently, incubation was allowed to proceed for another 4.5 h in a volume of 7.5 ml of the same buffer containing 5 mM CaCl2 for 18 h at room temperature. After washing the blots, bound FVIII light chain was detected using the peroxidase-labeled anti-FVIII light chain-directed antibody CLB-CAg 69. Binding was visualized using 3,3-diaminobenzidine tablets (Ken-En-Tec, Copenhagen, Denmark).

RESULTS

FVIII Binding to Immobilized LRP The interaction between LRP and FVIII was first investigated by SPR analysis using purified components. As shown in Fig. 1, an increase in response was observed as FVIII (80 nM) was passed over immobilized LRP (8 and 25 fmol/mm2), demonstrating that FVIII associates with LRP. Binding appeared to be dose-dependent in that the highest response was detected in case of the highest density of LRP (Fig. 1). Upon replacement of FVIII solution with buffer, the response started to decline gradually, indicating that FVIII dissociates from immobilized LRP. The interaction between LRP and FVIII was studied in more detail by assessment of the association and dissociation rate constants k on and k off. The results are summarized in Table I. Binding of FVIII heterodimer to LRP could be described using a two-site model. The affinity constants (Kd) derived from k on and k off values are 59 and 65 nM, respectively. These data indicate that FVIII is able to bind to LRP with moderate affinity in a revers-
It appeared that 125I-labeled FVIII was rapidly degraded by the CHO cells, which express LRP constitutively (6). In this regard, degradation of FVIII was examined in competition experiments using the FVIII carrier protein vWF and the LRP antagonist RAP. With regard to RAP, its effect was tested in an immunosorbent assay by incubating immobilized LRP (125 fmol/well) with mixtures of FVIII (40 nM) and various concentrations of GST-RAP (0–1 μM). Residual FVIII binding was subsequently determined using an anti-FVIII-directed antibody. The amount of FVIII bound decreased dose-dependently in the presence of vWF (10–500 nM) (Fig. 2A). The interaction between FVIII and LRP was further analyzed in competition experiments using the FVIII carrier protein vWF and the LRP antagonist RAP (125 fmol/well) in a volume of 50 μl in 150 mM NaCl, 2 mM CaCl2, 0.01% (w/v) gelatin, and 20 mM Hepes (pH 7.4) at a flow of 5 μl/min for 2 min at 25 °C. Ligand solution was subsequently replaced with buffer to initiate dissociation. Response is indicated in Resonance Units (RU) and is corrected for nonspecific binding, which was less than 5% relative to binding to LRP-coated channels.

Cellular Degradation of FVIII—To address the contribution of LRP to the transport of FVIII to the endosomal degradation pathway, cellular degradation of FVIII was examined in experiments using CHO cells, which express LRP constitutively (6). It appeared that 125I-labeled FVIII was rapidly degraded by CHO cells (~8 fmol/10^5 cells after 4.5 h), and degradation did not occur in the presence of vWF (Fig. 3). The addition of GST-RAP (1 μM) inhibited degradation of 125I-labeled FVIII by approximately 65% (Fig. 3). Moreover, a similar extent of inhibition was observed using polyclonal antibodies directed against LRP (Fig. 3). This demonstrates that LRP contributes to the cellular uptake and subsequent degradation of FVIII.

Effect of GST-RAP and vWF on the FVIII-LRP Interaction—The interaction between FVIII and LRP was further analyzed in competition experiments using the FVIII carrier protein vWF and the LRP antagonist RAP. With regard to RAP, its effect was tested in an immunosorbent assay by incubating immobilized LRP (125 fmol/well) with mixtures of FVIII (40 nM) and various concentrations of GST-RAP (0–1 μM). Residual FVIII binding was subsequently determined using an anti-FVIII-directed antibody. The amount of FVIII bound decreased dose-dependently in the presence of vWF (10–500 nM) (Fig. 2A). The interaction between FVIII and LRP was further analyzed in competition experiments using the FVIII carrier protein vWF and the LRP antagonist RAP (125 fmol/well) in a volume of 50 μl in 150 mM NaCl, 2 mM CaCl2, 0.01% (w/v) gelatin, and 20 mM Hepes (pH 7.4) at a flow of 5 μl/min for 2 min at 25 °C. Ligand solution was subsequently replaced with buffer to initiate dissociation. Response is indicated in Resonance Units (RU) and is corrected for nonspecific binding, which was less than 5% relative to binding to LRP-coated channels.

Effect of Thrombin Cleavage on FVIII Light Chain Binding to LRP—Because both vWF and LRP bind to FVIII light chain (18, Fig. 4), we tested the possibility that LRP and vWF share similar sites within this part of the FVIII molecule. FVIII comprises two sites that are involved in vWF binding, one of which is located between residues 1649 and 1689 at the amino-terminal part of the light chain (18, 36). Thrombin-cleaved FVIII light chain, which lacks this particular sequence, was therefore compared with intact light chain for binding to LRP by SPR analysis to reveal the kinetic parameters k_on and k_off on and off. Thus, these data strongly suggest that FVIII light chain comprises a site that is recognized by LRP.

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tained for both intact and thrombin-cleaved FVIII light chain, indicating that these proteins are similar in their interaction with LRP. Apparently, binding to LRP is independent of the amino-terminal part of FVIII light chain.

**Interaction between LRP and the FVIII C2 Domain—**Apart from its amino-terminal part, the carboxyl-terminal C2 domain of FVIII light chain also comprises a vWF binding site (19). Because vWF binding is inhibited by the C2 domain-directed antibody ESH4, the effect of this antibody on LRP binding was tested. As shown in Fig. 5A, ESH4 interfered with FVIII light chain binding to LRP. Inhibition appeared to be specific because ESH4 was unable to affect binding of tissue-type plasminogen activator/plasminogen activator inhibitor-1 complexes to LRP (data not shown). Moreover, other FVIII light chain directed antibodies (CLB-CAG A and CLB-CAG 69) were unable to interfere with LRP binding (data not shown). Thus, these data suggest that the FVIII C2 domain contributes to the interaction with LRP. This was further investigated using recombinant C2 domain. However, even at high concentrations of this 17.5-kDa fragment (500 nM), only a modest association with LRP was observed (Fig. 5B, line I). The conformation of the C2 domain may be affected by residues elsewhere in FVIII light chain or by the C2 domain-directed antibody ESH8 (37). Binding of the C2 domain to LRP was therefore addressed in the presence of this antibody. When complexes of C2 domain and antibody ESH8 were applied to LRP, a pronounced, dose-dependent increase in response could be observed (Fig. 5B, lines II–IV), indicating that ESH8 promotes binding of the isolated C2 domain to LRP. Binding was dependent on exposure of the LRP binding site in the C2 domain because C2 domain-ESH8 complexes or C2 domain alone did not associate to LRP in the presence of antibody ESH4 (Fig. 5B, lines V and VI). Collectively, these data strongly suggest that a LRP binding site is present within the C2 domain of FVIII light chain.

**DISCUSSION**

The surface of cells comprises numerous receptors that contribute to the binding and internalization of plasma proteins. Among these receptors is LRP, a multifunctional, endocytic receptor that is involved in the transport of a wide spectrum of ligands from the cell surface to the endosomal degradation pathway. In the present study, evidence is provided that LRP recognizes coagulation procofactor FVIII as a ligand. First, in a system using purified components, FVIII proved to bind to LRP in a manner that is reversible and dose-dependent (Fig. 1). Furthermore, binding could be inhibited efficiently in the presence of the FVIII carrier protein vWF or the LRP antagonist RAP (Fig. 2). Finally, both RAP- and anti-LRP-directed antibodies interfered with cellular degradation of 125I-labeled FVIII (Fig. 3). It is of interest to note that inhibition of LRP does not fully prevent FVIII degradation. Whether or not residual degradation involves a receptor-mediated process is currently under investigation. Irrespective thereof, our data indicate that the transport of FVIII from the cellular surface to the intracellular degradation pathway involves LRP. Because FVIII is structurally and functionally unrelated to the ligands of LRP that have been described thus far, it therefore provides a novel member of the already extensive range of established ligands for LRP.

The affinity by which FVIII heterodimer binds to LRP was found to be approximately 60 nM (Table I). This is in the same range as reported for some of the other LRP ligands, such as hepatic lipase (52 nM) (38), β-amyloid precursor protein (80 nM) (39), two-chain urokinase (60 nM) (40), and plasminogen activator inhibitor-1 (35 nM) (33). The data obtained for FVIII binding to LRP are in agreement with a two-site binding model (Table I), indicating that multiple regions contribute to the interaction with LRP. FVIII light chain proved similar to the FVIII heterodimer in that LRP binding involves two classes of binding sites (referred to in Table I as 1 and 2). Furthermore, both proteins display similar affinity with regard to the class 1 binding site (Table I). Therefore, it seems conceivable that FVIII light chain serves an important role in the interaction between FVIII and LRP. It is noteworthy that the class 1 association and dissociation rate constants for FVIII heterodimer differ from that for FVIII light chain by 25-fold (Table I), suggesting that exposure of the LRP binding site in FVIII light chain is distinct from that in the FVIII heterodimer. The same may be true for the class 2 binding site because its

**TABLE I**

Kinetic parameters for the binding of FVIII or its derivatives to LRP

| Ligand                        | $k_{\text{on}}$ | $k_{\text{off}}$ | $K_d$  |
|-------------------------------|-----------------|------------------|--------|
| association rate constants (1) | 7.1 (±1.1) $\times 10^2$ | 1.2 (±0.1) $\times 10^6$ | 59 ± 10 |
| Light chain                   | 9.8 (±0.2) $\times 10^3$ | 1.5 (±0.4) $\times 10^5$ | 65 ± 17 |
| Thrombin-cleaved light chain  | 2.5 (±0.2) $\times 10^3$ | 4.8 (±1.5) $\times 10^4$ | 52 ± 17 |
| Thrombin-cleaved light chain  | 4.3 (±0.2) $\times 10^2$ | 3.3 (±0.3) $\times 10^4$ | 130 ± 13 |
| Thrombin-cleaved light chain  | 3.3 (±0.1) $\times 10^3$ | 7.6 (±2.2) $\times 10^4$ | 43 ± 13 |
| Thrombin-cleaved light chain  | 5.3 (±0.4) $\times 10^2$ | 2.8 (±0.5) $\times 10^4$ | 189 ± 15 |
affinity for LRP is 2–3-fold lower in FVIII light chain than in FVIII heterodimer. One explanation for this observation may be that the exposure of LRP binding sites within FVIII light chain depends on the presence of FVIII heavy chain. A similar mechanism has been reported previously for the interaction between FVIII light chain and vWF. The affinity for vWF increases 10-fold when FVIII light chain is associated with FVIII heavy chain (19).

A positive identification of the LRP binding site on the FVIII light chain was achieved using the monoclonal antibody ESH4 (Fig. 5), which has previously been described to be directed against the FVIII C2 domain (42). This suggests that the C2 domain contributes to the interaction with LRP. Indeed, purified recombinant C2 domain displayed modest binding to LRP (Fig. 5B). Surprisingly, the binding of C2 domain to LRP was markedly increased in the presence of antibody ESH8 (Fig. 5B), in a manner that was more pronounced than that observed for other antibodies (data not shown). It has been well established that antibody ESH8 is able to change the conformation of the C2 domain in thrombin-cleaved FVIII light chain, resulting in altered affinities for vWF and phospholipids (37, 43). It seems conceivable therefore that this antibody provokes a similar event in the isolated C2 domain, which then results in a more optimal exposure of the LRP binding site. The presence of a binding site for LRP within the C2 domain may explain the inhibitory effect of vWF (Fig. 2B), which is known to bind to the C2 domain of FVIII (19). However, whether vWF and LRP share a common binding site within the C2 domain or whether vWF interferes with binding by sterical hindrance remains to be determined.

In plasma, FVIII is in a dynamic equilibrium with vWF in which approximately 95% of the FVIII molecules have been calculated to be in complex with vWF (44). In complex with vWF, cellular uptake and degradation of FVIII is almost fully

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**Fig. 4. Binding of FVIII subunits to LRP.** A, binding of FVIII heavy (I) or light chain (II) to immobilized LRP (25 fmol/mm²) was examined as described in the legend of Fig. 1. The sensorgrams obtained using 100 nM of each subunit are shown. B, purified LRP (1 µg) was subjected to a 5% SDS-polyacrylamide gel electrophoresis gel under nonreducing conditions and transferred to nitrocellulose. Filters were then preincubated with 1% (w/v) non-fat milk powder in 100 mM NaCl, 0.01% (v/v) Tween 20, and 20 mM Hepes (pH 7.4) before incubation with FVIII light chain (50 nM) in the absence (lane I) or presence (lane II) of GST-RAP (125 nM) in the same buffer containing 5 mM CaCl₂ for 16 h at room temperature. Bound FVIII light chain was detected using the peroxidase-labeled anti-FVIII light chain-directed antibody CLB-CAg 69 and 3,3-diaminobenzidine. The mobility of molecular mass markers is indicated at the left.

**Fig. 5. Binding of FVIII or its C2 domain to LRP in the presence of anti-FVIII C2 domain antibodies.** A, immobilized LRP (16 fmol/mm²) was incubated with FVIII light chain (150 nM) in the presence or absence of antibody ESH4 as described in the legend of Fig. 1. The maximal response (RU), corrected for nonspecific binding (less than 5%), is shown at the indicated antibody concentrations. B, LRP, immobilized at a CM5 sensor chip at 16 fmol/mm² was incubated with the C2 domain (500 nM; line I) or with complexes of the C2 domain with antibody ESH8. Complexes consisted of 100 nM C2 domain (line II), 200 nM C2 domain (line III), or 400 nM C2 domain (line IV) with 500 nM ESH8. Similarly, complexes of C2 domain (400 nM) with ESH4 (500 nM) and complexes of the C2 domain (400 nM) with both antibodies (500 nM each) were incubated with LRP (lines V and VI, respectively). Complexes were allowed to form for 45 min before SPR analysis.
suppressed (Fig. 3), indicating that the contribution of LRP to the cellular uptake of FVIII-vWF complexes in vivo is limited. However, in the absence of vWF, FVIII is degraded efficiently in a process that involves LRP (Fig. 3). In this view, our findings may provide an explanation for the beneficial effect of vWF that has been reported with regard to the expression of recombinant FVIII in CHO cells (17, 45). vWF may well contribute to the intravascular clearance of FVIII, but they also have a considerably reduced half-life of FVIII by LRP due to the absence of vWF.

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REFERENCES

1. Strickland, D. K., Kounnas, M. Z., and Argraves, W. S. (1995) FASEB J. 9, 890–896
2. Neels, J. G., Horn, I. R., van den Berg, B. M. M., Pannekoek, H., and van Zonneveld, A.-J. (1998) Fibrinolysis Proteolysis 12, 219–240
3. Herz, J., Hamann, U., Rogne, S., Myklebost, O., Gausepohl, H., and Stanley, K. K. (1988) EMBO J. 7, 4119–4127
4. Moestrup, S. K., Gliemann, J., and Pallesen, G. (1992) Cell Tissue Res. 269, 375–382
5. Orth, K., Madison, E. L., Gething, M. J., Sambrook, J. F., and Herz, J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7422–7426
6. Fitzgerald, D. J., Fryling, C. M., Zdanowsky, A., Saelinger, C. B., Kounnas, M., Winkles, J. A., Strickland, D., and Leppa, S. (1995) J. Cell Biol. 125, 1533–1541
7. Herz, J., Goldstein, J. L., Strickland, D. K., Ha, Y. K., and Brown, M. S. (1991) J. Biol. Chem. 266, 21232–21238
8. Bu, G., and Schwartz, A. L. (1998) Trends Cell Biol. 8, 272–276
9. Kounnas, M. Z., Church, F. C., Argraves, W. S., and Strickland, D. K. (1996) J. Biol. Chem. 271, 6523–6529
10. Narita, M., Rudolph, A. E., Milestein, J. P., and Schwartz, A. L. (1990) Blood 91, 555–560
11. Warshawsky, I., Broe, G. J., Jr., and Schwartz, A. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6664–6668
12. Hanik, A., Setiadi, H., Bu, G., McEver, R. P., and Morrissey, J. H. (1999) J. Biol. Chem. 274, 4962–4969
13. Kaufman, R. J. (1998) Thromb. Haemostasis 79, 1068–1079
14. Lenting, P. J., van Mourik, J. A., and Mertens K. (1998) Blood 92, 3983–3996
15. Vehar, G. A., Keyt, B., Eaton, D., Rodriguez, H., O’Brien, D. P., Roblat, F., Oppermann, H., Keck, R., Wood, W. I., Harkin, R. N., Tuddenham, E. G. D., Lawn, R. M., and Capon, D. J. (1984) Nature 312, 337–342
16. Roblat, F., O’Brien, D. P., O’Brien, F. J., Goodall, A. H., and Tuddenham, E. G. D. (1986) Biochemistry 24, 4294–4300
17. Kaufman, R. J., Wadley, L. C., and Dorner, A. J. (1988) J. Biol. Chem. 263, 6352–6362
18. Lollar, P., Hill-Eubanks, D. C., and Parker, C. G. (1988) J. Biol. Chem. 263, 10451–10455
19. Saenko, E. L., and Scandella, D. (1997) J. Biol. Chem. 272, 18007–18014
20. Sadler, J. E. (1998) Annu. Rev. Biochem. 67, 395–424
21. Lollar, P., Knutson, G. J., and Fass, D. N. (1985) Biochemistry 24, 8656–8606
22. Eaton, D., Rodriguez, H., and Vehar, G. A. (1986) Biochemistry 25, 505–512
23. Fay, P. J., Haidaris, P. J., and Smudzin, T. M. (1991) J. Biol. Chem. 266, 8957–8962
24. Nesheim, M., Pittman, D. D., Giles, A. R., Fass, D. N., Wang, J. H., Slonskys, D., and Kaufman, R. J. (1991) J. Biol. Chem. 266, 17815–17820
25. Lenting, P. J., Donath, M. J. S. H., van Mourik, J. A., and Mertens, K. (1994) J. Biol. Chem. 269, 7150–7155
26. Over, J., Sixma, J. J., Bouma, B. N., Vlooswijk, R. A. A., and Beeser-Visser, N. H. (1981) J. Clin. Lab. Med. 97, 332–344
27. Mancucci, P. M., Tenson, P. M., Castaman, G., and Rodeghiero, F. (1992) Blood 78, 3130–3137
28. Donath, M. J. S. H., Lenting, P. J., van Mourik, J. A., and Mertens, K. (1995) J. Biol. Chem. 270, 3648–3655
29. Brinkman, H. J., Koster, P., Mertens, K., and van Mourik, J. A. (1997) Biochem. J. 323, 735–740
30. Fijvaandraat, K., Celie, P. H. N., Turenhout, E. A. M., ten Cate, J. W., van Mourik, J. A., Mertens, K., Peters, M., and Voorberg, J. (1998) Blood 91, 2347–2352
31. Moestrup, S. K., and Gliemann, J. (1991) J. Biol. Chem. 266, 14011–14017
32. Bradford, M. M. (1976) Anal. Chem. 72, 248–254
33. Horn, I. R., Moestrup, S. K., van den Berg, B. M. M., Pannekoek, H., Nielsen, M. S., and van Zonneveld, A.-J. (1995) J. Biol. Chem. 270, 11770–11775
34. Conese, M., Olson, D., and Blasi, F. (1994) J. Biol. Chem. 269, 17886–17892
35. Mikhailenko, I., Kounnas, M. Z., and Strickland, D. K. (1995) J. Biol. Chem. 270, 9545–9549
36. Leyster, A., Verbeet, M. P., Brodiniewicz-Proba, T., van Mourik, J. A., and Mertens, K. (1989) Biochem. J. 257, 679–683
37. Saenko, E. L., Shima, M., Gilbert, G. E., and Scandella, D. (1996) J. Biol. Chem. 271, 27424–27431
38. Kounnas, M. Z., Chappell, D. A., Wong, H., Argraves, W. S., and Strickland, D. K. (1995) J. Biol. Chem. 270, 8307–8312
39. Kounnas, M. Z., Murr, R. D., Rebeck, G. W., Bush, A. L., Argraves, W. S., Tanzi, R. E., Hyman, B. T., and Strickland, D. K. (1995) Cell 82, 331–340
40. Kounnas, M. Z., Henkin, J., Argraves, W. S., and Strickland, D. K. (1993) J. Biol. Chem. 268, 21862–21867
41. Yakhyaev, A., Mikhailenko, I., Strickland, D., and Saenko, E. (1997) Blood 90, (suppl.) 31a
42. Scandella, D., Gilbert, G. E., Shima, M., Nakai, H., Eagleson, C., Felch, M., Prescott, R., Rajalakshmi, K. J., Hoyer, L. W., and Saenko, E. (1995) Blood 86, 1811–1819
43. Saenko, E. L., Scandella, D., Yakhyaev, A. V., and Greco, N. J. (1998) J. Biol. Chem. 273, 27918–27926
44. Noe, D. A. (1996) Haemostasis 26, 289–303
45. Wise, R. J., Dorner, A. J., Krane, M., Pittman, D. D., and Kaufman, R. J. (1991) J. Biol. Chem. 266, 21948–21955