



Review

The urokinase receptor: Focused cell surface proteolysis, cell adhesion and signaling

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ABSTRACT

Plasma membrane urokinase-type plasminogen activator (uPA)-receptor (uPAR) is a GPI-anchored protein that binds with high-affinity and activates the serine protease uPA, thus regulating proteolytic activity at the cell surface. In addition, uPAR is a signaling receptor that often does not require its protease ligand or its proteolytic function.

uPAR is highly expressed during tissue reorganization, inflammation, and in virtually all human cancers. Since its discovery, in vitro and in vivo models, as well as retrospective clinical studies have shown that over-expression of components of the uPA/uPAR-system correlates with increased proliferation, migration, and invasion affecting the malignant phenotype of cancer. uPAR regulates the cells–extracellular matrix interactions promoting its degradation and turnover through the plasminogen activation cascade.

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1. Introduction

1.1. Identification of uPAR

The urokinase-type plasminogen activator (uPA) receptor (uPAR), was identified, isolated and cloned as the plasma membrane high-affinity binding-site of the serine protease uPA [1–4].

1.2. Protein synthesis and structure of uPAR

The human uPAR cDNA encodes a polypeptide of 335 amino acids including a N-terminal 22-residue secretion signal peptide and a C-terminal segment (30 amino acids) removable with the attachment of a glycosyl phosphatidylinositol (GPI)-anchor [5]. The mature protein (283 residues) is highly glycosylated and composed of three similarly sized (about 90 residues each) homologous domains (here referred to as DI, DII and DIII) and belonging to the Ly-6/uPAR protein domain family [6]. The biochemical and structural aspects of uPAR have been extensively investigated and reviewed in detail [7,8] and are summarized in Fig. 1.

1.3. uPAR expression

Although uPAR is expressed constitutively in many cell lines, the uPAR gene is inducible, for example in T cells [9], keratinocytes [10–12] and colon [13]. Typical inducers in culture are phorbol esters, growth factors and integrin-mediated signals. The transcription factors that have been shown to bind to the uPAR promoter and to regulate its expression are AP1, PEA3/Ets, Sp1 and Ap2 [14–17]. The expression of uPAR is also regulated at the post-transcriptional level [18–20], possibly through the action of mRNA binding proteins [21] and maybe also by micro RNA's [22].

In the healthy organism, uPAR is moderately expressed in various tissues including lungs, kidneys, spleen, vessels, uterus, bladder, thymus, heart, liver and testis. Strong uPAR expression is observed in organs undergoing extensive tissue remodeling, such as trophoblast cells and migrating, but not resting, keratinocytes at the edge of wounds [11]. In these tissues, macrophages, neutrophils, endothelial cells as well as keratinocytes seem to be the predominant uPAR-expressing cell types. In blood, the expression of uPAR is strongly increased upon activation of neutrophils [23], monocytes [24], T cells [25]. uPAR is also expressed by hematopoietic stem/progenitor cells [26].

A wide variety of human and mouse cancers and most transformed cells overexpress uPAR [27,28]. It is striking that uPAR expression is increased in many pathological conditions, in particular cancer, inflammation and infections [29]. While in most cases this is probably due to activation of transcription factors, for

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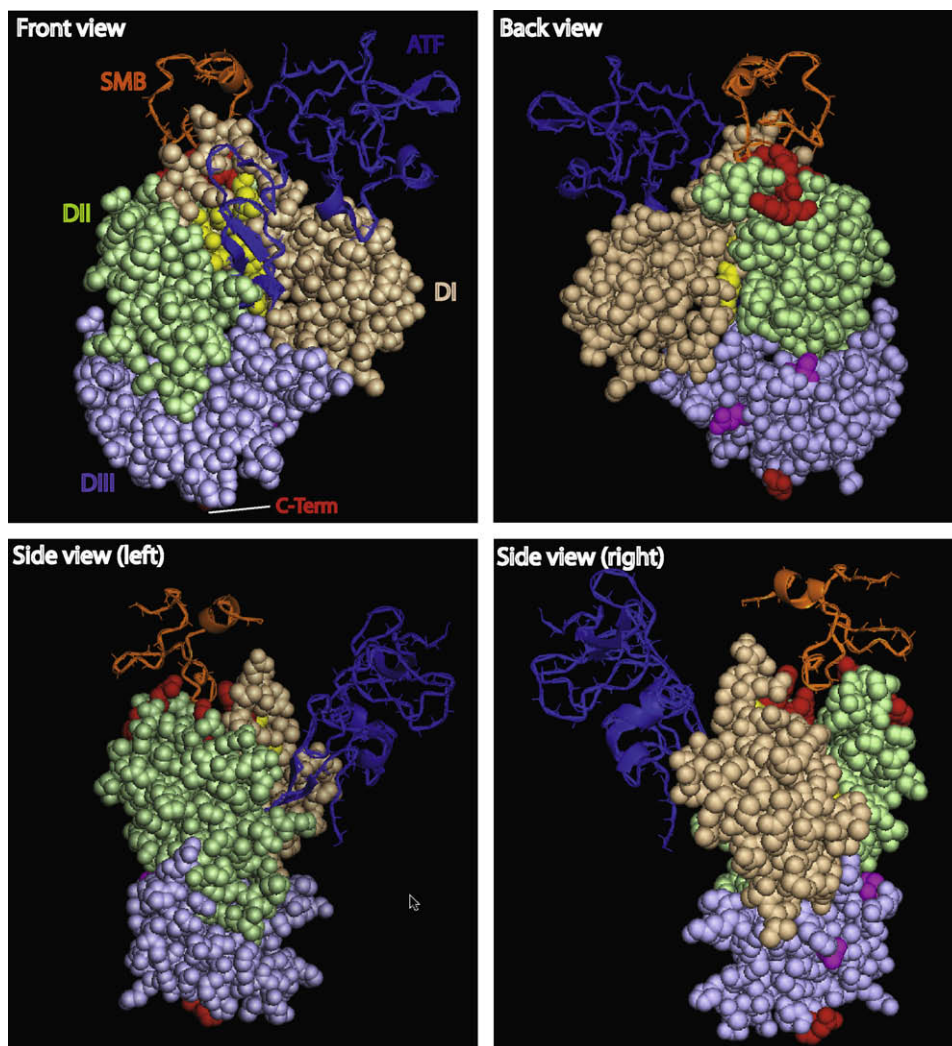


Fig. 1. Topology of the ternary complex between uPAR, uPA and vitronectin. The crystal structure of uPAR (atoms shown as spheres) with residues belonging to domains DI, DII and DIII color-coded wheat, pale-green and pale-blue, respectively. The amino-terminal fragment of uPA (ATF) and the somatomedin B domain of vitronectin (SMB) are shown as ribbons and colored blue and orange. Selected residues in uPAR important for VN binding (W32, R58, I63, R91 and Y92) and uPA-binding (L31, R53, L55, Y57, T64 and L66) are colored red and yellow, respectively. Two residues implicated in the interaction between uPAR and integrins (S245 and D262) are shown in purple. The structure has been oriented so that the C-terminal residue in the uPAR structures points downward and the SMB domain upwards (i.e. towards the ECM). Note that the interaction sites for ATF and SMB are entirely non-overlapping and that there is no molecular contact between these two polypeptides. The images were constructed using the coordinates deposited in the Protein Data Bank (PDB) with the code number 3BT2 and the MacPyMOL software (<http://www.pymol.sourceforge.net>).

example Sp1 [30], it should be noted that in at least two types of cancer, ductal pancreatic cancer and breast carcinomas, the uPAR gene is frequently amplified [31,32].

1.4. uPAR function

1.4.1. Proteolytic functions

Coherent with uPA being a protease, uPAR is involved in the regulation of extracellular proteolysis because it promotes cell-surface activation of plasminogen, generating plasmin [33]. Connected to its role in extracellular proteolysis, uPAR also mediates the internalization of inactive complexes between uPA and the inhibitory serpins PAI-1 and PN-1 [34,35] in cooperation with members of the low-density lipoprotein receptor family [36]. This leads to the degradation of the uPA:inhibitor complexes in the lysosome and the subsequent recycling of uPAR to the cell surface [36]. This allows the generation and regeneration (after uPAR recycling) of active cell surface-bound plasmin and hence the spatial focusing of extracellular proteolysis. For this reason uPAR was

immediately proposed as an important regulator of the invasive properties of cancer cells [37].

1.4.2. Non-proteolytic uPAR functions

In addition to extracellular proteolysis, many biological activities of the receptor occur independently of the protease activity of uPA and/or are activated by over-expression of the receptor even in the absence of uPA. These functions are largely related to the regulation of the interactions between the cells and the surrounding extracellular matrix (ECM).

uPAR interacts functionally with matrix vitronectin (VN), adhesion receptors of the integrin family and G protein-coupled receptors. uPAR and integrins cooperate in migration of monocytes [38], fibrosarcoma HT1080 [39], melanoma [40], MCF-7 breast cancer [41], fibroblasts [42] and many other cells. Moreover, both anti-uPAR and anti-integrins antibodies inhibit cell migration induced by uPA [42]. Finally, inhibitors of G protein-coupled receptors, such as pertussis toxin, also inhibit uPA-induced migration [43]. This activity may be at least in part due to the cleavage between do-

mains I and II of uPAR, which generates an SRSRY amino-terminus. This peptide has chemotactic, pertussis-toxin sensitive, activity, induces ERK1/2 phosphorylation and might be expected to be a ligand of a G protein-coupled receptor. Indeed, it has been shown that the family of formyl peptide G proteins-coupled receptors (FPR and FPRL) is involved in mediating uPA-induced migration and is highly sensitive to SRSRY-peptides [44]. Likewise, the DIIDIII-fragment of uPAR is a potent chemoattractant for several different cell lines [43,45], most likely via p56/59hck and ERK1/2 phosphorylation. Inhibitors of tyrosine kinases or of heterotrimeric G proteins block the chemotactic response to DIIDIII and the induction of phosphorylation of p56/59hck. Indeed, DIIDIII has been reported to interact directly with, and to signal through the FPRL1 chemokine receptor inducing p56/59hck phosphorylation [44]. Likewise, FPR receptors appear to respond in chemotaxis to DIIDIII-derived peptides in human hematopoietic stem cells [46].

Over-expression of uPAR promotes cell spreading, migration and invasion in fibroblasts and several different tumor cell lines, and is mediated by the extracellular matrix protein VN [47–49]. This activity is triggered by a direct interaction between uPAR and matrix VN [48], requires integrin dependent signaling and results in p130Cas-Crk and DOCK180 dependent Rac activation [47,49]. It has been concluded that the interaction between uPAR and VN may be necessary and sufficient for uPAR to modulate cell shape changes and signaling. Indeed, all alanine substitutions which affect this biological activity of the receptor also display reduced VN binding [48]. Furthermore, a chimeric membrane-anchored PAI-1 molecule mimics uPAR function recapitulating VN-adhesion and uPAR signaling activity, even though these two proteins display no structural homology [48].

As regulator of proliferation, uPAR over-expression constitutively activates the EGFR pathway in many human cancer cell lines [50]. This correlates well with the over-expression of uPAR in many human cancers [51]. In these cell lines, uPAR over-expression activates EGF Receptor in the absence of EGF and induces an unbalance between p38 and p42/44. The balance between pro-apoptotic p38MAPK and the proliferation activating ERK1/2 is shifted in favor of the ERK1/2, and results in constitutive cycling. On the contrary, down-regulation of uPAR reduces the malignancy of cancer cell lines and induces a state of dormancy [52,53]. These data agree with the phenotype of the uPAR Ko mouse keratinocytes in which the EGFR cannot be activated by EGF, resulting in deficient proliferation [54]. However, the overall role of uPAR in proliferation must be more complex and may be cell-type specific, since, unlike keratinocytes, uPAR Ko embryo fibroblasts proliferate faster than wt and display a stronger tumorigenic activity upon transformation with Ras and Myc [55].

2. Regulation of uPAR activity

2.1. Regulation of uPAR activity by receptor shedding and cleavage

Two types of post-translational modifications are believed to regulate uPAR location and activity globally and irreversibly: uPAR “shedding” and uPAR “cleavage”. These events affect uPAR activity as a whole as they completely, and irreversibly, change the location and/or destroy or activate a given activity of the receptor. Whereas uPAR shedding releases the entire protein moiety from the cell surface generating soluble uPAR (suPAR), uPAR cleavage causes the release of the N-terminal domain (DI) from the rest of the receptor (DIIDIII). So, uPAR shedding solubilizes uPAR reducing the number of receptors on the cell surface, while uPAR cleavage removes the essential DI, inactivating the binding to most ligands. These two modifications may occur individually or together on a single uPAR

molecule generating (at least) 4 distinct forms of uPAR in addition to the native receptor: suPAR, GPI-anchored DIIDIII, soluble DIIDIII (sDIIDIII) and the free DI fragment. Each of these forms of uPAR has different biological activity and has been found both in vitro and in vivo [56,57].

uPAR shedding occurs either by the action of a phospholipase such as phosphatidylinositol-specific phospholipase D (GPI-PLD) [58], or by proteolytic cleavage of the polypeptide chain close to the GPI-anchor. Several proteases, including plasmin, tissue kallikrein 4 and bacterial metalloproteinases, are able to cleave synthetic peptides derived from the juxtamembrane region of uPAR [59–61]. All cell surface activities of uPAR (i.e. plasmin generation, internalization of the uPA:serpin complexes, cell adhesion to VN, regulation of integrin-function, etc.) are reduced by uPAR shedding. Furthermore, released soluble uPAR and uPAR fragments can be biologically active and may function in a remote paracrine way. Soluble uPAR displays intact uPA binding and may act as an uPA-scavenger. Moreover, suPAR can interfere with cellular uPAR functions, for example with integrins, inhibiting the activity of cell surface uPAR [62]. Other forms of soluble uPAR, like the DIIDIII-fragment, display potent chemotactic activities [43,63].

The second type of uPAR hydrolysis, referred to as uPAR “cleavage”, is a proteolytic event in the linker region connecting domains I and II of uPAR resulting in the generation of two uPAR fragments known as DI and DIIDIII. The cleavage releases the DI fragment from the cells, but the DIIDIII-fragment may either remain associated with the cell membrane or be released from the cell surface by receptor shedding as described above. The linker region connecting DI and DIIDIII in uPAR is prone to hydrolysis by a variety of proteases including uPA, plasmin, neutrophil elastase, and by a number of different matrix metalloproteinases (MMPs) [64–66]. While cleavage of purified soluble uPAR by uPA is an inefficient process that does not require the high-affinity interaction between the two molecules [64], cleavage of cell surface uPAR by uPA is accelerated and requires binding of uPA to uPAR [67]. Two explanations for the accelerated cleavage of cell surface uPAR by uPA have been proposed. First, it has been suggested that the exposure of the linker region connecting DI and DIIDIII is different in soluble and GPI-anchored uPAR [66,68]. Second, dimerization and/or clustering of uPAR in specific lipid membrane domains known as lipid rafts may position the catalytic domain of bound uPA in a favorable position for the cleavage of flanking receptor molecules [69]. Independently of the responsible enzyme and mechanism, cleavage strongly affects the biological activity of uPAR. On one hand, the physical separation of the DI and DIIDIII-fragments practically abolishes uPA and VN binding, the lateral association with integrins and consequently the biological activity of uPAR in both extracellular proteolysis and cell signaling via cell adhesion. On the other hand, uPAR cleavage generates fragments endowed with strong, *bona-fide* chemokine-like activities in a variety of cell systems.

2.2. The uPAR interactome and its regulation

As a GPI-anchored receptor molecule, the signaling activity of uPAR relies on its interaction with other proteins. Since its discovery about twenty years ago, a wide variety of uPAR interactors have been reported in the literature. Based on the level of evidence available, these interactors may be divided into two groups. The first group is formed by the serine protease uPA and the extracellular matrix protein VN, which may be considered the “core” uPAR ligands for which extensive, independent and coherent biological, biochemical and structural evidence is available. Recently, it has been proposed that this “ménage à trois” between uPAR, uPA and VN may be sufficient to explain most, or all, of the pleiotropic cellular effect of uPAR [48,70].

The second group of interactors encompasses a long series of proteins for which the directness of the interactions as well as their structural basis is poorly understood. Nevertheless, much evidence has accumulated that the physical and functional interaction of uPAR with this group of proteins is an essential part of the biology of uPAR (reviewed in [29,71,72]). In brief, uPAR functionally interacts with a variety of receptor tyrosine kinases, like EGFR and PDGFR [50,54,73,74]; a series of integrins (reviewed in [75]); caveolin [76]; and receptors of the low-density lipoprotein receptor family including the LDL receptor-related protein (LRP) and LRP1B [36,77,78]. Certain forms of uPAR (i.e. the soluble DII/III-fragment) interact with the G-protein-coupled receptors FPR, FPRL1 and FPRL2 [44,79]. In addition, uPAR has been shown to associate with the cation-independent Mannose 6-phosphate/insulin-like growth factor-II receptor (CIMPR/IGF-II receptor) that has been implicated in the targeting of uPAR to lysosomes [80]. Finally, by chemical cross-linking uPAR has also been shown to associate with the collagen receptor uPARAP/Endo180 [81] in a process that requires the contemporary binding of pro-uPA.

As a consequence of the above listed interactions, uPAR activates various intracellular signaling molecules such as the tyrosine kinase Src, the serine kinase Raf, focal adhesion kinase (FAK), p130Cas and extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK), among others. Activation of these proteins results in profound changes in cell proliferation, adhesion and migration.

2.2.1. The uPA/uPAR interaction

2.2.1.1. Structural basis for the interaction between uPA and uPAR. The high-affinity binding (K_d in the low nanomolar range) of uPA to cells [1,2] led to the identification, purification and cloning of uPAR [3,4]. This interaction has been extensively studied at the biochemical level on purified proteins (reviewed in [7]). Crystal structures of uPAR in complex with an antagonistic peptide or with the receptor-binding part of uPA (the amino-terminal fragment, ATF) have been solved [82–84]. The two proteins interact through the N-terminal growth-factor-like domain (GFD) of uPA [85] and a large hydrophobic binding pocket involving residues from all three domains of uPAR (recently reviewed in [8]). The extended nature of the uPA/uPAR-interface renders the affinity of the interaction relatively insensitive to single amino-acid substitutions in uPAR, but highly dependent upon the intact three-domain structure of the receptor [86,87]. The structure of uPAR and its interaction with uPA is presented in Fig. 1.

2.2.1.2. Regulation of the uPA/uPAR interaction. As a receptor for uPA, uPAR may be considered “constitutively active” as high-affinity binding occur without the need for any additional co-factors. The interaction however requires the intact three-domain structure of uPAR explaining why cleavage of uPAR in the linker region connecting DI and DII is an irreversible inhibitory event. Cleavage of uPAR by uPA might act as a negative-feedback mechanism in extracellular proteolysis, although the actual occurrence and relevance of this feedback still has to be determined. In addition to uPAR cleavage, the affinity of the interaction with uPA is moderately dependent upon expression levels and on the type and degree of uPAR glycosylation [88,89]. The existence of intact cell surface uPAR incapable of uPA binding [90] has been reported, suggesting that poorly understood “cryptic” forms of the receptor may also exist.

2.2.2. The uPAR/VN interaction

2.2.2.1. Structural basis for the uPAR/VN interaction. The discovery of VN as a ligand for uPAR came from the observation that the adhesion of stimulated monocytes to serum-coated surfaces is enhanced by ligand-occupancy of uPAR [91,92]. Fractionation of

serum identified VN as the component responsible for the increased adhesion [92], and several lines of evidence confirmed uPAR to be the responsible membrane receptor [93]. In contrast to integrin binding, the interaction of uPAR with VN does not require divalent cations and does not involve the RGD-motif present in this extracellular matrix protein.

The X-ray structure of the ternary complex between uPAR, ATF and the somatomedin B (SMB) domain of VN has been determined [94] and is in good accordance with the major findings of two independent and complete, alanine scans of uPAR [48,95]. Although initial experimentation pointed towards an interaction between regions within DII/III of uPAR [93,96] and the heparin binding domain of VN [97] there is now compelling evidence that the interaction is entirely mediated by a composite epitope exposed on the DI/DII interface of uPAR and the N-terminal somatomedin B domain of VN (reviewed in [8]). Although more than 30 different alanine substitutions noticeably impair uPAR-mediated cell binding to VN only a handful of these do so also in the presence of uPA [48]. Two of these residues, W32 and R91, are located in the uPAR:SMB interface of the crystal structure [94] (see Fig. 1) and their substitution with alanine results in particularly low VN binding [48,95]. The W32A and R91A mutations both display normal uPA binding affinity [48,87] and thus represent excellent candidate mutations for use in structure function analyses aimed at understanding the physiological importance of the uPAR/VN interaction. Both the W32A and R91A uPAR mutants do however display some residual VN binding [48,95] and care should be taken in using these mutants to document the existence of VN independent uPAR functions [98].

Importantly, the SMB domain of VN also harbors an overlapping high-affinity binding site for PAI-1 and is located adjacent to the RGD motif mediating integrin binding [99]. Indeed, several alanine substitutions in the SMB domain of VN impair not only uPAR [87,100] but also PAI-1 binding to the same domain, rendering them of little use in structure–function studies in biological systems where PAI-1 may be present. The identification of mutations in the SMB domain that selectively impair uPAR and/or PAI-1 binding would greatly facilitate future studies aimed at addressing the relative importance of these two interactions in the biology of uPAR.

2.2.2.2. Regulation of the uPAR/VN interaction. As for uPA, the binding of VN to uPAR requires the intact three-domain structure of the receptor [101,102]. This is explained by the fact that the binding epitope for VN in uPAR involves residues in both DI and DII [94]. The binding of soluble recombinant uPAR to immobilized VN is a high-affinity interaction (K_d in the low nanomolar range) and is strongly dependent upon concomitant uPA binding [93,95,103]. On the contrary the binding of VN to immobilized uPAR is rather low affinity (1 μ M range) and only moderately affected by uPA binding [95].

The high-affinity interaction between uPAR and VN has been suggested to require uPAR dimerization and/or oligomerization [69,104,105]. Although binding experiments using purified proteins strongly suggest that uPA regulates VN binding by controlling uPAR dimerization [104] the data are not entirely conclusive. First, while the model used to explain the uPA dose-dependence of uPAR binding to VN predicts that dimerization is a high-affinity reaction, complexes containing dimeric uPAR cannot readily be detected by gel filtration [104]. Second, the model, as well as the experimental evidence, indicate that the high-affinity VN binding complex between uPAR and uPA has a 2:1 stoichiometry [104] and not the 1:1 ratio observed in the uPAR:ATF:SMB crystal structure [94].

In contrast to uPA, VN binding to uPAR is thus a highly regulated and complex process. In its native state uPAR displays no or little

VN binding. However, uPA binding, receptor oligomerization and partitioning to discrete membrane domains trigger VN binding.

2.2.3. The interaction between uPAR and integrins

2.2.3.1. Identification of the uPAR–integrin interaction. The interaction of uPAR with integrins was originally demonstrated by the co-immunoprecipitation of uPAR and integrins in cell extracts [106]. The isolation of an uPAR-binding peptide from a phage library [107] that disrupted both co-immunoprecipitation with integrins and VN-adhesion, provided functional significance to the interaction [108].

2.2.3.2. Structural basis of the uPAR/integrin interaction. The original phage derived uPAR: integrin antagonistic peptide P25 [108] displays some homology to a linear sequence present in the propeller domain of the α_M chain of Mac-1. An integrin peptide (called M25) derived from this sequence was likewise found to bind uPAR and block uPAR: integrin co-immunoprecipitation [62]. The corresponding peptide from the $\alpha 3$ integrin chain (called $\alpha 325$) was also found to bind uPAR and block its interaction with this integrin [109]. Comparisons of the three peptide sequences reveal that even though there is clear homology between P25 and M25, as well as between M25 and $\alpha 325$, there is only *one* residue which is conserved in all three peptides. This is remarkable as all three peptides are reported to bind uPAR and have essentially the same biological activity. Coherently with the predicted importance of the histidine residue common to the three peptides [110], a single alanine substitution (H245A in $\alpha 3$) is sufficient to abolish the biological activity of uPAR in $\alpha 3\beta 1$ -dependent mesenchymal transition [110]. Several studies have also evidenced a strong functional interaction between uPAR and the fibronectin (FN) receptor $\alpha 5\beta 1$ [111,112] as well as with the VN receptors $\alpha v\beta 3$ [49,113] and $\alpha v\beta 5$ [114]. However, both $\alpha 5$ and αV chains lack this critical histidine residue [109], suggesting that uPAR may interact with these integrins in a different way. In support of such alternative interactions, peptides derived from the $\beta 1$ integrin sequence, as well as a specific $\beta 1$ mutant (S227A), impair both the physical and functional association between uPAR and $\alpha 5\beta 1$ [112].

Attempts to identify the regions of uPAR involved in the interaction with integrins have been published [113,115,116] and uPAR mutants with deficient integrin interaction(s) have been reported [115,116]. The uPAR residues implicated in the interaction with integrins are: E134, E135, S245, H249 and D262. The residues identified in these three studies however do not point towards a single coherent binding site in uPAR but rather suggest the existence of multiple and diverse binding sites. In this context it should be noted that a comprehensive study aimed specifically at the unbiased functional identification of the integrin binding site in uPAR failed to detect any such site and also excluded all the previously identified sites [48]. Hence, the wealth of evidence underlying the concept of integrin–uPAR interaction is still in need of a convincing structural basis.

2.2.3.3. Regulation of the uPAR/integrin interaction. Little is known about how uPAR–integrin interactions are regulated. As for VN binding the association between these receptors requires the intact 3-domain structure of uPAR [117] and is promoted by uPA binding [62,112,118]. Binding of ligand to the integrin also seems to favor the interaction [50,112].

2.2.4. The homotypic uPAR interaction

The existence and functional relevance of uPAR dimerization was initially deduced from the peculiar biphasic uPA dose-dependence of suPAR binding to immobilized VN [95,104] which can be accurately explained only if the high-affinity VN binding form of uPAR is a dimer [104]. Indeed, on the surface of living cells uPAR

dimerizes as evidenced by chemical cross linking [69], photon counting histogram (PCH, [105,119] and fluorescence energy transfer (FRET, [105]).

Self-association of uPAR can be demonstrated *in vitro* by co-immunoprecipitation experiments using differentially tagged suPAR molecules. Under these conditions the process is regulated by uPA binding and displays a dose-dependence very similar to that observed for VN binding. In living cells dimeric uPAR is preferentially located in detergent insoluble membrane domains, i.e. lipid rafts, suggesting that membrane partitioning may also regulate dimerization [69]. The cause/consequence connection between uPAR dimerization and lipid raft association is however not clear.

Although the structural basis for uPAR oligomerization is still unknown, some data suggest that the hydrophobic uPA binding cavity of the receptor may be involved. Indeed, complete saturation of the receptors with uPA actually reduces binding of suPAR to VN [95,104] as well as uPAR–uPAR co-immunoprecipitation [104]. However, the VN:uPAR:uPA high-affinity complex is no longer inhibited by excess uPA, suggesting that the binding cavity on both uPAR molecules in this complex are occupied [104]. In agreement with this possibility, a large number of the residues implicated in uPA-independent uPAR-mediated cell binding to VN (L31, R53, L55, Y57, T64, L66 and E68) have their side chains exposed in the uPA binding cavity of uPAR [48].

3. Dynamics of uPAR membrane localization

3.1. uPAR internalization and recycling

As a cell surface receptor, uPAR is normally located at the external leaflet of the plasma membrane [11]. However, in certain cell types, namely neutrophils, uPAR may be predominantly present in intracellular secretory vesicles and is exposed at the cell surface only upon cell activation [23]. Although predominantly found on the plasma membrane, uPAR localization is regulated in a highly dynamic way by interactions with ligands and other membrane receptors. Binding of uPA:serpin complexes to uPAR results in the formation of quaternary complexes with members of the LRP family [36], which are internalized by clathrin-mediated endocytosis [77]. In this process, the uPA:serpin complexes are degraded in the lysosomes while uPAR recycles back to the plasma membrane [120]. Also in the absence of uPA:serpin complexes the location of uPAR on the cell surface is modulated by at least LRP1b [78] as well as by the cation-independent Mannose 6-phosphate/insulin-like growth factor-II receptor (CIMPR/IGF-II receptor) which may target uPAR to lysosomes [80].

It has recently been found that internalization and recycling of uPAR also takes place constitutively in the absence of ligands, through a pathway that is independent of LRP-1 and clathrin but shares some properties with macropinocytosis. The ligand-independent route does not require uPAR partitioning into lipid rafts, is amiloride-sensitive, independent of the activity of small GTPases RhoA, Rac1 and Cdc42, and does not require PI3K. Constitutively endocytosed uPAR is found in EEA1 positive early/recycling endosomes but does not reach lysosomes in the absence of ligands. Electron microscopy analysis reveals the presence of uPAR in ruffling domains at the cell surface, within macropinosome-like vesicles, and in endosomal compartments [121].

3.2. uPAR membrane partitioning

In the plasma membrane, uPAR partitions in both lipid rafts and more fluid membrane regions [69]. While monomeric uPAR is mainly located in detergent soluble (DS) membrane domains,

dimeric uPAR is preferentially associated with detergent resistant (DRS) membranes or lipid rafts [69]. In detergent resistant membrane (DRM) fractions, uPAR is associated with an environment whose glycosphingolipid composition is different from the average composition of the plasma membrane, as shown by glycosphingolipid analysis of immunoprecipitated uPAR [122]. Moreover, the amount of uPAR found in the DRM changes in the presence of ligands along with the nature of the lipid environment. Indeed, in the absence of ligands the environment is very similar to that of total DRM, enriched in sphingomyelin and glycosphingolipids. However, after treatment of cells with uPA the lipid environment is strongly impoverished of neutral glycosphingolipids [122]. Unlike signaling, however, lipid rafts association is not involved in ligand-dependent or constitutive uPAR internalization.

4. Conclusions

Twenty years of intensive research by many laboratories have underscored the importance of uPAR and its ligands in a variety of biological phenomena. Interestingly the requirement for uPAR is not observed under normal conditions (for example in KO animals in a mouse facility). However, uPAR requirement and function becomes obvious under pathological circumstances, like acute and chronic inflammation, infections, tumorigenesis and induced hematopoietic stem cells mobilization, or under conditions of tissue remodeling or reconstruction. Despite the many investigations over the last 24 years, and despite the solution of uPAR tertiary structure, we are still missing crucial information necessary to understand the molecular basis of its function. Although this is surprising, our feeling is that it reflects its involvement in an hitherto unrecognized general mechanism regulating the coupling of cells to extracellular matrix and influencing cell signaling. The next years will undoubtedly solve some of these mysteries.

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