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Cytoskeleton

Cell Movement, Cytokinesis and Organelles Organization

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Editors

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CYTOSKELETON: CELL MOVEMENT, CYTOKINESIS AND ORGANELLES ORGANIZATION

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Preface

In this book, the authors review the effects of uPAR on the actin, intermediate filaments and microtubules that constitute the cell cytoskeleton, and the mechanisms used by uPAR to modulate intracellular signalling and temporally fine tune cytoskeletal dynamics. The effects of cytoskeletal components of uPAR expression and cellular distribution are examined as well. In addition, Duchenne Muscular Dystrophy (DMD) is an inherited disorder characterized by progressive muscular degeneration and cognitive impairment due to mutations in the DMD gene. Elucidation of the molecular basis for this illness has determined that cytoskeleton and the product of the DMD gene called dystrophin work in a coordinated way to maintain fibre muscle integrity. The authors describe the scientific path that allows elucidation of the molecular basis of DMD. Furthermore, cell motility is central to many aspects of cell, tissue, and organ function in both health and disease. Some of the more popular published experimental approaches to study cell motility are discussed. Also examined are the IQGAP family of proteins, which are now known to act at the interface between cellular signalling pathways and the actin cytoskeleton.

Chapter I - Since the cloning of the urokinase receptor (uPAR) in 1985, challenging data on the structure and the roles of this receptor have been reported. Originally described as a protease receptor that binds the urokinase-type plasminogen activator (uPA) and thus a central element of the plasminogen activation cascade, uPAR is now also recognized as an anchorage (by binding to the extracellular matrix protein, vitronectin) and a signalling receptor. uPAR is a glycosylphosphatidylinositol (GPI)-anchored protein that does not possess an intracellular domain, but uPAR is nonetheless a full signalling receptor with major functions in cell adhesion, migration, and proliferation. This receptor successfully achieves these functions through its lateral relationships with a wide array of other membrane receptors such as seven-transmembrane domain receptors, integrins and growth-factor receptors. The regulation of these fundamental biological functions implies that uPAR also finely controls the state of organization of the cell cytoskeleton. Indeed, either cell spreading, motility or cytokinesis cannot be correctly executed without the appropriate cytoskeleton reorganization. In this chapter, the authors review the effects of uPAR on the actin, intermediate filaments and microtubules that constitute the cell cytoskeleton, and the mechanisms used by uPAR to modulate intracellular signalling and temporally fine tune

cytoskeletal dynamics. In addition, the authors also discuss the effects of cytoskeletal components on uPAR expression and cellular distribution.

Chapter II - According to present knowledge, systemic realization of genetic activity in the dynamic spatial organization of the genome in the nucleus provides such a level of plasticity of complex biological systems that allows them to adequately respond to environmental stimuli or signals during the development, modulate and shift the balance of contacting components and dimensions of their interactions, resulting in structural rearrangements. The chromosome positions within the nucleus determine both normal development and progression of genomic diseases, i.e., changes according to the environmental requirements, current needs of the organism, and its individual experience. At the same time, the striking output of the evolution of higher organisms, largely ignored to date, is that only 1.2% of the mammalian genome encodes proteins and the vast majority of the expressed information is in RNA. There are hundreds of thousands of non-coding (nc) RNAs, as well as many other yet-to-be-discovered small regulatory RNAs . A new paradigm envisions the interactions between these two worlds, the one of protein and the other of RNA, as providing a dynamic link between the transcriptome and the environment and, therefore, the progressive maturation and functional plasticity of the nervous system in health and disease. Also, a wide repertoire of ncRNAs plays an important role in chromatin organization, gene expression, and disease etiology via a signal cascade of actin remodeling (LIMK1, cofilin, actin). The activity of the protein kinase LIMK1 that controls spine development, local dendritic translation at postsynaptic sites and ionotropic glutamate receptor trafficking is regulated by a brain-specific miRNA miR-134. This miRNA is localized to the synapto-dendritic compartment of rat hippocampal neurons and negatively regulates the size of dendritic spines--postsynaptic sites of excitatory synaptic transmission. Moreover, LIMK1 hemizygosity is considered to cause cognitive defects in a genome disorder Williams syndrome. Drosophila is a helpful model organism to determine the sequence of events in this system of hierarchical relationships. Drosophila LIMK1 gene (*agnostic*) with a specific chromosome architecture around the gene capable of generating miRNAs, recapitulates many features both of Williams syndrome and of neurodegenerative disorders. Mutants in the gene have increased expression of LIMK1 and cofilin, modified chromosome packaging and homologous and nonhomologous pairing, implemented in different rates of unequal recombination. Also, they display congofilic inclusions both in the adult brain and larval tissues presumably leading to severe defects in learning and memory during courtship conditioning.

Chapter III - Duchenne Muscular Dystrophy (DMD) is an inherited disorder characterized by progressive muscular degeneration and cognitive impairment due to mutations in the DMD gene. This disease seriously affects the quality of life of DMD patients causing death due to cardiac and respiratory complications before 30 years of age. Elucidation of the molecular basis for this illness has determined that cytoskeleton and the product of the DMD gene called dystrophin works in a coordinated way to maintain fibre muscle integrity. It is known that cytoskeleton via its association with the integrin complexes modulates migration of stem cells during embryogenesis to give rise to diverse tissues and organs. This association is also important in performing basic neurological processes such as synaptogenesis. Recent findings have shown that the DMD gene is also manifested in the

central nervous system where it has different functions from that seen in muscle. Among the most clearly defined tasks of DMD gene in neurons is its involvement in modulating the formation of beta 1-integrin complexes. This chapter describes the scientific path that allowed elucidation of the molecular basis for Duchenne Muscular Dystrophy. The up dated knowledge to explain the role of the DMD gene in neurological functions is also described together with the advances in the design of therapeutic strategies focused on restoration of the DMD gene function in patients.

Chapter IV - Cell motility is central to many aspects of cell, tissue, and organ function in both health and disease. Examples include the morphogenetic events of embryogenesis, the functioning of the immune system, wound healing, angiogenesis, and the metastatic spread of cancer. Consequently, numerous methods have been developed to study cell motility. One rapidly growing area of interest in the cell motility field involves the interactions between tumor cells, surrounding stromal cells, and mesenchymal stem cells (MesSCs), which can be recruited to tumor sites. Important motility-related questions include how MesSCs are able to home to tumor sites, and, once present at a tumor site, how they affect the metastatic potential of the tumor cells. This chapter discusses and compares some of the more popular published experimental approaches to study cell motility, with an emphasis on assays that are suitable for studies of MesSC-tumor cell interactions *in vitro*. Both two-dimensional and three-dimensional cell motility assays are described, along with the specific strengths and weaknesses of each assay for interrogating specific subcomponents of the motility process. The authors also describe a novel murine breast cancer-MesSC model system that is well suited for *in vitro*, as well as *in vivo* studies of the motility events associated with tumor cell-MesSC interactions.

Chapter V - Animal centrosome an organelle consists of centrioles and associated structures surrounded by pericentriolar material, is the major microtubule organizing center in interphase cell and spindle pole component in mitosis and indispensable organelle for cilia and flagella formation.

In diploid cells, the centrosome usually includes not more then two centrioles and since the number of centrosomes in the cell is determined by the number of centrioles, cells have developed effective mechanisms to control centriole formation and to tightly coordinate this process with DNA replication. Duplication of this organelle and chromosomes redoubling during DNA replication are two principal events of cell cycle in the course of cell division progression. The principal question is what starts earlier, the duplication of centrioles or DNA replication? During last five years, some proteins, which participate in the process of procentriole formation, were found, but temporal sequences of cell cycle events are not still fully investigated. Traditionally the time of centriole replication beginning denote like G_1/S or even S, although precise analysis was not ever undertaken.

In present study, the ultrastructural analysis of centrosomes from cells that were previously in one's lifetime observed after mitosis was used in combination with autoradiography of the same cells. PE cells were individually monitored after mitosis and procentriole appearance was detected by electron microscopy as soon as 5–6 h after mitosis. This period was 1–2 h shorter than minimal duration of G_1-phase in PE cell line. Ultrastructural serial sections analysis of centrosomes in the cells with known "cell cycle age" in combination with ^{3}H-thimidine autoradiography study of the same cells directly

confirmed that centrioles duplication started earlier than cells entered in S-phase of cell cycle, i.e., preceded the DNA replication. The data were obtained showed that centriole duplication started before the beginning of DNA replication.

Chapter VI - The first members of the IQGAP family of proteins were characterised over 15 years ago. It is now known that these molecules act at the interface between cellular signalling pathways and the actin cytoskeleton. They bind to a diverse range of signalling molecules – including those involved in calcium, GTPase, kinase and growth factor signalling. One intriguing interaction is that between mammalian IQGAP1 and the myosin essential light chain isoform, Mlc1sa. Although this has been demonstrated *in vitro*, its *in vivo* role is not known. Indeed, it would be tempting to dismiss it as an experimental artefact, except for the existence of a parallel interaction in the budding yeast, *Saccharomyces cerevisae*. In this organism, the IQGAP-like protein (Iqg1p) interacts with a myosin essential light chain (Mlc1p). This interaction is critical for the correct execution of cytokinesis. IQGAP-like proteins also play key roles in cytokinesis in other fungi. Recent work implicating mammalian IQGAP1 in cytokinesis may help explain the role of the interaction in higher eukarytotes.

Chapter VII - Polarity is a fundamental cell property essential for differentiation, proliferation and morphogenesis in unicellular and multicellular organisms. It is well known that polarized distribution of F-actin is important in providing the driving force for directional migration in mammalian leukocytes and *Dictyostelium* cells. Phosphoinositide (PI) signaling, including phosphatidylinositol kinases and phospholipases, is also critical for the formation of cell polarity in these cells. A monospore from the marine red alga *Porphyra yezoensis* is well known as a migrating plant cell and thus is a unique and useful material for investigating polarity determination in plant cells. As in leukocytes and *Dictyostelium* cells, monospore migration requires asymmetrical distribution of F-actin, whose establishment is regulated by the phosphatidylinositol 3-kinase and phospholipase C, whereas phospholipase D is involved in the maintenance of F-actin distribution. These findings indicate that the regulation of F-actin asymmetry by PI signaling cascades is evolutionarily conserved in terms of the establishment of cell polarity in migrating eukaryotic cells.

Chapter VIII - The possibility that an ensemble of neural-membrane lipids could regulate the duration of the ion-channel "open" conformation may have direct implications for local gating of the action potential (AP). The control of channel dynamics is equivalent to controlling the transmembrane ion conductances responsible for neural depolarization and hyperpolarization. Accordingly, if a membrane region in an axon were to contain (for example) clusters of Na^+ channels with relatively longer open states, such a structure would be conducive to a local depolarization (spike) and the continuing propagation of the neural impulse. By contrast, if the Na^+ channels had brief open states, the structure would be conducive to impulse propagation failure. Modulation of the membrane state through other channel types would of course also be possible. A good example is the A-current delayed-rectifier K^+ channel (K_A), currently the object of intensive research because of its possible role in conduction failure. The K_A channel is gated by membrane hyperpolarization, which it increases and prolongs by rapidly conducting potassium ions out of the cytosol. A prolonged open time for this channel could hypothetically strengthen the delayed-rectifier effect and significantly increase the probability of conduction block.

This chapter examines the possible role of neural membrane microdomains in regulating the propagation of the action potential. These data are particularly important because they appear consistent with the concept that "local switches" regulate AP propagation (Scott, 1995). The chapter begins with an overview of the experimental evidence for AP conduction failure. It then examines the two major alternative models: one emphasizing the role of impedance mismatch due to neuron branching geometry; the other emphasizing the role of prolonged hyperpolarization due to the A-current potassium channel (K_A). A model emphasizing the possible interaction of a microdomain-cytoskeleton system with neuron branching geometry will then be presented (Wallace, 2004).. Because of the large number of studies bearing on the subject, the K_A channel will be used as the basic example, although the possible contributions of other channel types will be briefly discussed. The chapter concludes with a discussion of how microdomain regulation of AP propagation may explain a number of neuron features that strikingly depart from cable properties.

Chapter IX - Actin is the main component of the microfilament system in all eukaryotic cells and is essential for most intra- and inter-cellular movement including muscle contraction, cell movement, cytokinesis, cytoplasmic organisation and intracellular transport. The polymerisation and depolymerisation of actin filaments in nonmuscle cells is highly regulated and the reorganisation of the actin cytoskeleton can occur within seconds after chemotactic stimulation. There are many proteins which are involved in the regulation of the actin cytoskeleton. These include receptors which receive chemotactic stimuli, G proteins, second messengers, signalling molecules, kinases, phosphatases and transcription factors. These proteins are varied and numerous and are involved in multiple pathways. Despite the large number of proteins, there are not enough to coordinate the various responses of the cytoskeleton. An additional level of regulation is conferred by scaffolding proteins.

Due to the presence of numerous protein interaction domains, scaffolding proteins can tether various proteins to a certain location within the cell to facilitate the rapid transfer of signals from one protein to the next. This colocalisation of the components of a particular pathway also helps to prevent unwanted crosstalk with components of other pathways. Tethering receptors, kinases, phosphatases and cytoskeletal components to a particular location within a cell helps ensure efficient relaying and feedback inhibition of signals to enable rapid activation and inactivation of responses. Scaffolding proteins are also thought to stabilise the otherwise weak interactions between particular proteins in a cascade and to catalyse the activation of the pathway components. There are numerous scaffolding proteins involved in the regulation of the cytoskeleton and this chapter has focussed on examples from several groups of scaffolding proteins including the MAPK scaffolds, the AKAPs, scaffolds of the post synaptic density and actin binding scaffolding proteins.

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Chapter I

Follow the Leader: When the Urokinase Receptor Coordinates Cell Adhesion, Motility and Proliferation with Cytoskeleton Organization

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Abstract

Since the cloning of the urokinase receptor (uPAR) in 1985, challenging data on the structure and the roles of this receptor have been reported. Originally described as a protease receptor that binds the urokinase-type plasminogen activator (uPA) and thus a central element of the plasminogen activation cascade, uPAR is now also recognized as an anchorage (by binding to the extracellular matrix protein, vitronectin) and a signalling receptor. uPAR is a glycosylphosphatidylinositol (GPI)-anchored protein that does not possess an intracellular domain, but uPAR is nonetheless a full signalling receptor with major functions in cell adhesion, migration, and proliferation. This receptor successfully achieves these functions through its lateral relationships with a wide array of other membrane receptors such as seven-transmembrane domain receptors, integrins and growth-factor receptors. The regulation of these fundamental biological functions implies that uPAR also finely controls the state of organization of the cell cytoskeleton. Indeed, either cell spreading, motility or cytokinesis cannot be correctly executed without the appropriate cytoskeleton reorganization. In this chapter, we review the effects of uPAR

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on the actin, intermediate filaments and microtubules that constitute the cell cytoskeleton, and the mechanisms used by uPAR to modulate intracellular signalling and temporally fine tune cytoskeletal dynamics. In addition, we also discuss the effects of cytoskeletal components on uPAR expression and cellular distribution.

Introduction

Glycosylphosphatidylinositol (GPI)-anchored proteins form a unique class of proteins that are bound to the outside leaflet of the plasma membrane. This family of receptors includes functionally diverse proteins such as enzymes, adhesion molecules, surface antigens, others active proteins and receptors [for reviews see Paulick and Bertozzi, 2008; Orlean and Menon, 2007].

The urokinase receptor (uPAR) belongs to the latter sub-family of GPI-anchored proteins. uPAR is a multi-ligand and multi-functional receptor that regulates pericellular proteolysis and protease activation, gene expression, cell proliferation, adhesion and migration. These roles are reflected at the molecular level by the functioning of uPAR which is known as a protease (that binds urokinase), anchorage (that binds vitronectin), and as a signalling receptor. The latter is certainly the most surprising characteristic because uPAR being a GPI-anchored protein has no intracellular domain. However, this receptor successfully mediates signals to the cell through its lateral relationships with a wide array of other receptors namely seven-transmembrane domain receptors such as FPRL1; endocytic receptors such as LRP-1 (low density lipoprotein receptor-related protein-1), VLDL-R (Very Low-Density Lipoprotein Receptor), the mannose 6-phosphate/IGF-II receptor (CD222, CIMPR), or uPARAP (Endo180); caveolin; the gp130 cytokine receptor; tyrosine kinase receptors such as the EGF receptor (EGFR), the PDGF receptor (PDGFR) or the IGF-1 receptor (IGF-1R); and integrins [for reviews see Blasi and Carmeliet, 2002; Degryse, 2003, 2008; Ragno, 2006].

The partners of uPAR are structurally different and exert various molecular functions. This diversity certainly explains how uPAR manages basic but essential physiological processes for instance cell adhesion, migration and proliferation. At the first glance, these processes are divergent and have rather contrasting goals i.e. immobility vs. motion vs. growth respectively. However, the least common denominator of all these processes is the cell cytoskeleton. Without a tight and timely control of the cytoskeleton organization, all these fundamental processes would fail or would not even start. It is remarkable that uPAR succeeds in regulating the specific organization of the cytoskeleton during such divergent cellular processes.

In this chapter, we focus on the relationship between uPAR and the cell cytoskeleton summarizing our current knowledge. We also discuss the influence of the cytoskeleton on uPAR.

Structure of a Leader

The Extracellular Domains of uPAR

In humans, the gene of uPAR is located on chromosome 19 (19q13.1-q13.2), which is then transcribed into a 1.4 kb mRNA and later translated into a 335-residue precursor. At the N-terminal, the removal of a signal peptide of 22 amino acids leads to a 313-residue single polypeptide chain presenting five potential glycosylation sites. uPAR is modified post-translationally at the C-terminal by the cleavage of a 30-residue GPI anchor sequence signal and the attachment of the GPI anchor. Full-length uPAR consists of a 283-residue single-chain protein. The molecular weight of the non-glycosylated polypeptide is approximately 35,000 while the one of the glycoprotein is about 55,000-60,000 [Nielsen et al., 1988; Roldan et al., 1990; Blasi and Carmeliet, 2002].

uPAR is a cysteine-rich molecule, and disulphide bonds give a structure comprised of 3 homologous domains. Domain I is located at the N-terminus while the GPI anchor added post-translationally is placed at the C-terminus of domain III [for reviews on uPAR see Blasi and Camerliet, 2002; Degryse, 2003, 2008; Ragno, 2006]. Domain II contains the D2A sequence (residues $_{130}$IQEGEEGRPKDDR$_{142}$ of human uPAR) that was the first identified region of uPAR involved in uPAR-integrin interactions [Degryse et al., 2005]. The minimum active sequence composed by the four residues GEEG was also reported [Degryse et al., 2005]. D2A-derived synthetic peptide binds at least to integrins $\alpha v\beta 3$ and $\alpha 5\beta 1$ inducing integrin- but not uPAR-dependent signalling thereby stimulating cell migration. Moreover, our most recent experimental data demonstrated that D2A is also mitogenic and represents the first identified mitogenic sequence of uPAR [Eden et al., in preparation]. So far, the D2A sequence is the only region of uPAR that has been described to have intrinsic signalling activities inducing cell migration and growth [Degryse et al., 2005]. Changing the two glutamic acids into two alanines in the D2A motif generated two inhibitors of cell migration, the D2A-Ala and GAAG peptides [Degryse et al., 2005]. Interestingly, D2A-Ala and GAAG are also inhibitors of cell proliferation and tumour growth in vivo [Eden et al., in preparation]. Other sites of uPAR-integrin interactions have been identified in the domain III of uPAR [Chaurasia et al., 2006; Wei et al., 2007]. The synthetic peptide corresponding to sequence $_{240}$GCATASMCQ$_{248}$ of human uPAR disrupts suPAR-$\alpha 5\beta 1$ integrin complex [Chaurasia et al., 2006]. In addition, mutants of uPAR bearing single point mutation S245A or H249A, and D262A fail to associate to integrins $\alpha 5\beta 1$ and $\alpha 3\beta 1$ respectively [Chaurasia, 2006; Wei, 2007].

Linker regions are present in between domain I and II, and domain II and III. The DI-DII linker region is highly sensitive to proteolytic cleavage and harbors the first identified chemotactically active sequence SRSRY (human sequence) that is involved in the interactions with seven-transmembrane domain receptors such as FPRL1 [Resnati et al., 1996, 2002; Fazioli et al., 1997; Degryse et al., 1999; de Paulis et al., 2004; Gargiulo et al., 2005; Seleri et al., 2005]. The SRSRY motif is not always exposed on the uPAR surface. By binding to uPAR, urokinase (uPA) induces a crucial change of conformation that results in the exposition of the SRSRY chemotactic epitope. This conformational change permits uPAR-seven-transmembrane domain receptor interaction by metamorphosing uPAR into a

ligand of FPRL1 which in its turn mediates signalling and promotes cell migration (Figure 1A).

uPAR exists under different forms. Soluble uPAR (suPAR) is generated by the degradation of the GPI anchor. Moreover, high levels of the soluble forms of uPAR are markers of cancers and correlate with poor clinical prognosis [Stephens et al., 1999; Mustjoki et al., 1999; Sier et al., 1999]. Proteolytic cleavage in the DI-DII linker region of both membrane-bound uPAR and suPAR by a variety of proteases including uPA produces DI fragment and DIIDIII-uPAR [Ploug and Ellis, 1994]. Interestingly, DIIDIII-uPAR is chemotactically active mimicking the effects of uPA. This fact suggests that the proteolytic cleavage has led to the exposition of the SRSRY motif and that DIIDIII-uPAR can bind to FPRL1 [Fazioli et al., 1997]. However, the proteolytic cleavage can occur at other sites in the DI-DII linker region generating two kinds of DIIDIII-uPAR possessing or not the intact SRSRY sequence. Therefore, only full-length uPAR and suPAR, SRSRY-DIIDIII-uPAR and SRSRY-DIIDIII-suPAR can stimulate cell migration. Furthermore, all forms of DIIDIII-uPAR cannot bind to uPA or vitronectin (VN). In contrast, the two-chain kinin-free high molecular weight kininogen (HKa) is capable of binding domains II and III of both DIIDIII-uPAR and full-length uPAR in a Zn^{2+}-dependent manner [Colman et al., 1997; Chavakis et al., 2000]. HKa and VN are competitive binding partners providing uPAR with interesting anti-adhesive and adhesive properties.

The three-dimensional structure of uPAR has been resolved recently [Llinas et al., 2005; Huang et al., 2005; Barinka et al., 2006; Huai et al., 2006]. The three external domains form a globular-like structure in the form of a "croissant" creating a central pocket where uPA the main ligand of uPAR can bind. The crystal data are coherent with previous biochemical studies showing that all three domains are involved in the binding of uPA but with domain I being predominant. The crystal structure also revealed that the whole external part of uPAR constitutes a very large surface useful for the binding of the other soluble ligands (Table 1) and lateral partners of uPAR (Table 2).

The Lipid Anchor of uPAR

The GPI anchor is certainly the most original and important characteristic of uPAR. Due to this lipid anchor, uPAR is entirely located on the outer side of the plasma membrane. uPAR has neither transmembrane nor intracellular domains.

In many reviews on uPAR the fact that GPI anchoring is a very complicated and metabolically expensive process is too often simply not mentioned [for reviews see Orlean and Menon, 2007; Paulick and Bertozzi, 2008]. GPI anchors are widely present among eukaryotic organisms including protozoa, yeasts, fungi, plants, insects, and mammals. GPI anchoring is extremely important in the embryonic development of mammals as the deficiency of GPI biosynthesis results in fetal lethality [Orlean and Menon, 2007; Paulick and Bertozzi, 2008]. About >20 genes are involved in the synthesis of the anchor that take place in the endoplasmic reticulum. It is only when completed that the whole GPI anchor will be assembled to the protein bearing a GPI anchor sequence signal at the C-terminus. The common structure of the GPI anchor consists of three domains: a phosphoethanolamine linker

(that is bound to the C-terminus of the protein), a conserved glycan core, and a phospholipid tail. The glycan core is the subject of variability meaning that the GPI anchors from diverse GPI-anchored proteins are not identical [Orlean and Menon, 2007; Paulick and Bertozzi, 2008]. In the literature, examples of the influence of the nature of the GPI anchor on the functions of the proteins can be found [Nicholson and Stanners, 2006]. However, so far no correlation has been reported between the structure and the function of the GPI anchors. The exact functions of the complex GPI anchors are still being discussed [Paulick and Bertozzi, 2008].

The same uncertainty can be extended to the functions of the GPI anchor of uPAR. Very little knowledge can be claimed beside the fact that the GPI anchor provides uPAR with a convenient and efficient link to the cell surface. However, a few interesting statements can reasonably be made based on the published literature. First, the GPI anchor seems to influence the conformation of uPAR. It is indeed well known that soluble and GPI-bound uPAR have different conformations [Høyer-Hansen et al., 2001; Andolfo et al., 2002]. A similar change of conformation has also been reported for Thy-1 another GPI-anchored protein [Barboni et al., 1995].

The presence of the GPI anchor may represent a convenient signal to address a protein in particular domains of the plasma membrane, the lipid rafts [Varma and Mayor, 1998; Friedrichson and Kurzchalia, 1998; Nicholson and Stanners, 2006]. This is also true for uPAR that was reported to be present in lipid rafts and caveolae [Okada et al., 1995; Stahl and Mueller, 1995; Koshelnick et al., 1997; Wei et al., 1999; Schwab et al., 2001; Cunningham et al., 2003; Sitrin et al., 2004; Sahores et al., 2008]. In addition, uPA enhances uPAR redistribution into lipid rafts [Sahores et al., 2008]. The effect of uPA suggests that the conformation of the receptor may exert an influence on uPAR localization into lipid rafts [Sahores et al., 2008]. Dimerization of uPAR was also suggested to redirect uPAR into lipid rafts and to be required for VN binding [Sidenius et al., 2002; Cunningham et al., 2003].

The influence of the GPI anchor on uPAR has not been thoroughly studied. In one study, the presence or the absence of the GPI anchor does not appear to interfere with uPAR functioning [Li et al., 1994]. The comparison of uPAR with a chimeric uPAR built using the extracellular domains attached to the transmembrane and intracellular domains of the α chain of the IL-2 receptor, showed that the kinetics of binding, internalization and degradation of the uPA/Plasminogen activator inhibitor-1 (PAI-1) complex were identical [Li et al., 1994]. These data are consistent with the fact that cleaved suPAR (that exposes the SRSRY epitope) can mimic the effects of uPAR [Resnati et al., 1996].

A more recent study employed a reverse strategy. The GPI anchor of uPAR was associated with different external domains [Madsen et al., 2007]. In that report, a chimeric receptor composed of the GPI anchor of uPAR and PAI-1 was compared to uPAR. PAI-1 was chosen because this serpin binds to VN in a similar region [Okumura et al., 2002]. The chimeric receptor mimicked the effects of uPAR on cell morphology suggesting that the external domains of uPAR play a minor role [Madsen et al., 2007].