REVIEW

Dynamic reciprocity: the role of annexin A2 in tissue integrity

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Abstract Interactions between cells and the extracellular matrix are integral to tissue development, remodelling and pathogenesis. This is underlined by bi-directional flow of information signalling, referred to as dynamic reciprocity. Annexin A2 is a complex and multifunctional protein that belongs to a large family of Ca^{2+}-dependent anionic phospholipid and membrane-binding proteins. It has been implicated in diverse cellular processes at the nuclear, cytoplasmic and extracellular compartments including Ca^{2+}-dependent regulation of endocytosis and exocytosis, focal adhesion dynamics, transcription and translation, cell proliferation, oxidative stress and apoptosis. Most of these functions are mediated by the annexin A2-S100A10 heterotetramer (AIIt) via its ability to simultaneously interact with cytoskeletal, membrane and extracellular matrix components, thereby mediating regulatory effects of extracellular matrix adhesion on cell behaviour and vice versa. While Src kinase-mediated phosphorylation of filamentous actin-bound AIIt results in membrane-cytoskeletal remodelling events which control cell polarity, cell morphology and cell migration, AIIt at the cell surface can bind to a number of extracellular matrix proteins and catalyse the activation of serine and cysteine proteases which are important in facilitating tissue remodelling during tissue repair, neoangiogenesis and pathological situations. This review will focus on the role of annexin A2 in regulating tissue integrity through intercellular and cell-extracellular matrix interaction. Annexin A2 is differentially expressed in various tissue types as well as in many pathologies, particularly in several types of cancer. These together suggest that annexin A2 acts as a central player during dynamic reciprocity in tissue homeostasis.

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Abbreviations

AIIt	(annexin A2 - S100A10 heterotetramer)
DR	(Dynamic Reciprocity)
ECM	(Extracellular Matrix)
EMT	(Epithelial to Mesenchymal Transition)
F-actin	(filamentous actin)
PS	(phosphatidylserine)
PtdIns (4,5)P2	(phosphatidylinositol-(4,5)-bisphosphate)

Introduction

The organisation of cells into tissues requires continuous communication between cells and their extracellular microenvironment which is largely made up of fibrillar extracellular matrix (ECM), capillaries, fibroblasts, immune and inflammatory cells (Bissell et al., 2005). These interactions are bidirectional, they are both biomechanical and biochemical in nature and are termed dynamic reciprocity (DR). DR is a well-established concept in cell biology describing the mechanisms of intercellular and cell-ECM interactions (Xu et al., 2009). Within this concept the ECM is not only considered as an inert scaffold that structurally supports cells and tissues but rather as a signalling entity (Nelson and Bissell, 2006).

DR signals are transduced to and from the nucleus, cytoplasm and extracellular microenvironment through membrane-barriers including the nuclear membrane and the plasma membrane (Xu et al., 2009). Information flow between the cytoplasm and the extracellular microenvironment is usually relayed by transmembrane proteins and receptors that function as signal mediators—the best characterised example being integrins which can bind simultaneously to ECM components

and the cytoskeleton (Chiquet, 1999). DR signals encompass adhesion molecules, hormones, cytokines, growth factors or ECM components derived from neighbouring cells to cell surface receptors (Xu et al., 2009). While biochemical signals can alter gene expression by triggering intracellular signalling cascades, intercellular and cell-ECM biomechanical interactions control tensional forces which are conducted through the cytoskeleton to the cell nucleus (Chiquet, 1999). These forces control the shape of cells and the cell nucleus and have been shown to regulate chromatin organisation and hence gene expression (Iyer et al., 2012). The extracellular microenvironment is, therefore, crucial for regulating cell behaviour—it enables cells which contain identical genetic information to behave in a tissue-specific and synchronized manner (Bissell et al., 2005).

The continuous nature of DR implicates this communication process as essential not only to tissue homeostasis in established or differentiated tissues, but also during tissue development and wound repair. Dysregulation of DR and lack of control of normal cellular regulation can result in pathogenesis (Xu et al., 2009).

Annexin A2—structure and localisation

Annexins are a multifunctional family of Ca^{2+}-regulated membrane phospholipid-binding proteins which are highly conserved in the majority of unicellular and multicellular organisms. The annexin family consists of more than 160 members present in more than 65 different eukaryotic species (Gerke and Moss, 2002). Annexins were named after the Greek word *annex*, which means to bridge or attach, describing the intrinsic property of annexins to link biological structures. Indeed, the high evolutionary conservation of the annexin family indicates that these proteins are integral to cellular function (Gerke and Moss, 2002).

Annexin A2 is an important member of the annexin family that has been associated with many pathologies including autoimmune (Bastian, 1997), haemorrhagic syndromes (Menell et al., 1999) and most conspicuously with cancer (Liu et al., 2003; Lokman et al., 2011; Shiozawa et al., 2008; Zhong et al., 2009). Annexin A2 is a 36 kDa protein made up of an N-terminal domain and the annexin family-conserved endonexin fold in the C-terminal domain (Barton et al., 1991). The endonexin fold consists of four 70-amino acid repeats which form alpha-helices and binds Ca^{2+} ions (Barton et al., 1991). The C-terminus interacts with filamentous actin (F-actin) (Filipenko and Waisman, 2001) as well as membrane anionic phospholipids (Glenney, 1985), with a preference for phosphatidylinositol-(4,5)-bisphosphate (PtdIns(4,5)P2) in a cholesterol-dependent manner (Rescher et al., 2004). Both these interactions are regulated by intracellular Ca^{2+}-levels as well as posttranslational modification of the annexin A2 N-terminus (Glenney, 1985).

Annexin A2 can occur as a monomer or in heterotetrameric form (Gerke and Weber, 1985). The heterotetramer—also referred to as AIIt—consists of two monomeric annexin A2 molecules bridged by a central dimer made up of the cytosolic S100A10 protein from the EF-hand superfamily of Ca^{2+}-regulated proteins (recently reviewed by Bharadwaj et al. (Bharadwaj et al., 2013)). AIIt functions to stabilize and prevent ubiquitin-mediated proteosomal degradation of complexed S100A10, which plays an important role in proprotease activation at the cell surface (He et al., 2008; Puisieux et al., 1996). Further, the symmetrical structure of the AIIt complex allows each complexed annexin A2 protein to interact with membrane phospholipids, cytoskeletal F-actin and ECM components. It is therefore likely that the simultaneous interaction between any two of these components gives AIIt its signal transducing ability (Gerke et al., 2005; Gerke and Moss, 2002) and implicates it as a bridging protein in a number of important cell processes that involve membrane-cytoskeletal dynamics including vesicular trafficking (Roth et al., 1993; Valapala and Vishwanatha, 2011; Wang et al., 2007), signal transduction (Babbin et al., 2007), cell polarity (Grieve et al., 2012; Rankin et al., 2013), cellular transformation (Krishnan et al., 2004; Zheng et al., 2011), cell motility (Rankin et al., 2013; Zhai et al. 2011) and cellular stress responses (Genetos et al., 2010; Madureira et al., 2012); comprehensively reviewed with focus on the protein's heterotetrameric structure and function by Bharadwaj et al. (Bharadwaj et al., 2013). Monomeric annexin A2, on the other hand, has a low affinity for membrane phospholipids and is therefore unlikely to be associated with membrane-cytoskeletal dynamics (Gokhale et al., 2005). It is primarily found in the cytosol and nucleus, where it is has been shown to interact with mRNA and DNA (Hollas et al., 2006). Functionality of annexin A2 can therefore be divided into three spatial domains separated by membranes namely the extracellular microenvironment, the cytoplasm and the nucleus.

As the abovementioned processes play important roles in tissue homeostasis and DR (Schultz et al., 2011; Xu et al., 2009), this review will discuss the role of annexin A2 in the various cellular compartments in the broad context of DR.

Extracellular annexin A2

The role of Annexin A2 in ECM degradation

The extracellular matrix is composed of a network of fibrous proteins, glycoproteins and proteoglycans which form a scaffold to which cells adhere (Xu et al., 2009). While all tissues contain ECM, the composition, dimension and elasticity of the ECM differs between tissue types depending on tissue function (Midwood et al., 2004). ECM integrity is important in

maintaining tissue-specific function, therefore, loss of ECM integrity during development or due to injury or disease results in the release of ECM-bound signalling molecules and changes in the tensional forces exerted on the cell (Bissell et al., 2005). Signalling molecules and biomechanical perturbations trigger intracellular signalling cascades that change gene expression which facilitate either ECM-degradation or synthesis of ECM components (Bissell et al., 1982).

Cell surface AIIt is a well-known facilitator of ECM degradation through its ability to simultaneously interact with the cytoskeletal, membrane and extracellular matrix components, thereby providing a structural link between ECM components and proteolytic enzymes (Dassah et al., 2009; Kwon et al., 2002; Mai et al., 2000), reviewed by Roda et al. (Roda et al., 2003). AIIt is not a classically secreted protein complex; it normally resides in the cytoplasm but translocates to the cell surface in response to cell stress- or injury-induced increases in cytoplasmic Ca^{2+} levels (Deora et al., 2004). ECM degradation is a vital part of the wound repair process, and the S100A10 dimer component of AIIt is capable of binding the proprotease plasminogen and mediate its activation into plasmin by the plasminogen activators tPA and uPA, hence initiating downstream proteolytic cascades (Brownstein et al., 2004; Dassah et al., 2009; Fogg et al., 2002; Kassam et al., 1998; MacLeod et al., 2003; Mai et al., 2000). Indeed, it is now widely accepted that the cell-surface plasmin-regulatory functions are solely attributed to the S100A10 subunits of AIIt. In vascular endothelium, for example, high levels of cell surface annexin A2-bound S100A10 (AIIt) regulate haemostasis by activating plasminogen, which degrades fibrin-rich blood clots (Surette et al., 2011). Moreover, highly motile cells, such as macrophages and the majority of metastatic cancer cells, also utilize this S100A10-mediated protease activation localised at the cell surface in the context of AIIt in order to facilitate migration through protease-initiated ECM degradation (Brownstein et al., 2004; Choi et al., 2003; Lokman et al., 2011; O'Connell et al., 2010; Phipps et al., 2011; Shiozawa et al., 2008; Zhang et al., 2004; Zhong et al., 2009).

Annexin A2-mediated cell-cell adhesion

The symmetrical AIIt molecule can potentially interact with neighbouring phospholipid membranes and therefore may serve as a bridge between two adjacent cells. Indeed, cell-surface AIIt has been shown to mediate cell homing and heterotypic cell-cell adhesion of hematopoetic stem cells to osteoblasts and vascular endothelial cells in the bone marrow microenvironment (Jung et al., 2007). AIIt-mediated cell homing and cell-cell adhesion was also observed between breast cancer cells and the microvascular endothelium (Myrvang et al., 2013). Interestingly, cell-cell adhesion only occurred between cell types expressing high levels of cell

surface AIIt, and siRNA knockdown or treatment with an annexin A2 antibody inhibited cell homing and adhesion while annexin A2 overexpression strengthened cell-cell interactions (Jung et al., 2007). In addition, annexin A2 may have a central role during metastasis, since many metastatic cancer cells have elevated levels of cell surface AIIt which may facilitate ECM degradation, particularly via its S100A10-mediated plasminogen activation as mentioned above, cell homing and heterotypic cell-cell adhesion (Myrvang et al., 2013).

AIIt-mediated cell-cell adhesion may also play a role in phagocytosis. It has been suggested that macrophage cell surface AIIt facilitates phagocytosis of apoptotic lymphocytes expressing the acidic phospholipid and apoptotic marker phosphatidylserine (PS). For example, it has been shown that treatment of lymphocytes or macrophages with a monoclonal annexin A2 antibody inhibited macrophage-mediated phagocytosis of PS-presenting lymphocytes (Fan et al., 2004). Interestingly, it was found that AIIt, with its ability to laterally organise lipids and membrane constituents in the plasma membrane via lipid rafts (see next section), sequesters PS and generates PS-rich domains in artificial membranes (Menke et al., 2005). AIIt-mediated domain clustering of PS at the cell surface could present a concentrated signal for phagocytic cells to recognise apoptotic lymphocytes. As both PS-presenting lymphocytes and macrophages have high levels of cell surface AIIt, AIIt may mediate cell-cell adhesion between apoptotic lymphocytes and macrophages, their close proximity encouraging phagocytosis (Fan et al., 2004; Myrvang et al., 2013). It must be noted, however, that not all phagocytic cells express high levels of cell surface AIIt, suggesting that there may be multiple mechanisms driving cell recognition and subsequent phagocytosis (Fan et al., 2004).

Intracellular annexin A2

While both monomeric and heterotetrameric annexin A2 is found in the cytoplasm, the latter has been shown to have a far greater affinity for membrane phospholipids. Using surface plasmon resonance analysis on giant unilamellar vesicles, Gokhale et al. (2005) found AIIt to have at least a 10x greater affinity for PtdIns(4,5)P2-containing vesicles compared to the monomer (Gokhale et al., 2005). Further, as a consequence of low phosphoinositide affinity, monomeric annexin A2 would not be able to compete with other PtdIns(4,5)P2-binding proteins for membrane PtdIns(4,5)P2 unless the interaction was augmented by binding to other membrane constituents. The authors therefore concluded that heterotetrameric and not monomeric annexin A2 is associated with membrane PtdIns(4,5)P2 interactions. While many of the papers referenced in this review do not distinguish between the monomeric and

heterotetrameric form of annexin A2, the study conducted by Gokhale et al. (2005) demonstrated that AIIt is more likely to be associated with membrane dynamics. As such, this review will refer to AIIt for all annexin A2 functions associated with membrane dynamics.

At physiological Ca^{2+} concentrations, the heterotetrameric AIIt is associated with PtdIns(4,5)P2-rich membranes, where it interacts with and bundles actin filaments (Gerke and Moss, 2002). Research suggests that AIIt predominantly co-localises with F-actin at regions of active membrane-cytoskeleton remodelling including cell-cell junction formation (Hansen et al., 2002), vesicular transport (Morel and Gruenberg, 2009; Valapala and Vishwanatha, 2011), cell polarization (Hayes et al., 2006) and cell migration (Babbin et al., 2007). AIIt's involvement in the aforementioned processes is likely due to its role as a lipid raft organiser whereby it acts to recruit Rho GTPases - key regulators of membrane-cytoskeletal dynamics (reviewed by Heasman and Ridley (Heasman and Ridley, 2008)).

The role of annexin A2 in membrane organisation and trafficking

Lipid rafts are minute (approximately 10 nm) and short-lived free-floating membrane regions enriched in sphingolipids and cholesterol, which selectively incorporate and organise membrane components and associated proteins (reviewed by Simons and Sampaio (Simons and Sampaio, 2011)). Interaction between the actin cytoskeleton and cell membrane components, via adaptor or bridging proteins such as AIIt, can initiate lipid raft clustering into newly-formed lipid microdomains and stabilize them (Chichili and Rodgers, 2007). Lipid microdomains serve as cell signalling platforms involved in intracellular signalling cascades (Simons and Sampaio, 2011), are required for vesicle budding (Simons and Sampaio, 2011), and cause cell polarity as a result of asymmetrical distribution of membrane components (Cao et al., 2012). Using artificial lipid membranes, Gokhale et al. demonstrated that AIIt, initiates lateral aggregation of PtdIns(4,5)P2 into distinct microdomains (Gokhale et al., 2005). In addition, as a membrane-cytoskeletal adaptor protein, AIIt affects F-actin-mediated lipid raft clustering and stabilisation of lipid microdomains (Chichili and Rodgers, 2007; Hayes et al., 2006). As a result, lipid rafts and AIIt have been shown to be integral to membrane trafficking events such as endocytosis and exocytosis (Drucker et al., 2013; Valapala and Vishwanatha, 2011). The membrane-cytoskeletal remodelling activities of AIIt are regulated by post-translational modifications, the most significant being phosphorylation of annexin A2 at Tyr-23 which occurs under stress-induced elevations of intracellular Ca^{2+} (Zheng et al., 2011) such as heat stress (Deora et al., 2004), hyperosmotic shock (Hayes et al., 2004) and hypoxia (Genetos et al., 2010). Annexin A2 phosphorylation at Tyr-23 is often brought about by insulin receptor kinase (Rescher et al., 2008) and the Src

family of tyrosine kinases (Hayes and Moss, 2009) which results in AIIt interaction with Rho GTPases and subsequent upregulation of the Rho/ROCK pathway which triggers cytoskeletal remodelling (Rescher et al., 2008). In addition, annexin A2 Tyr-23 phosphorylation inhibits AIIt-F-actin binding and this, together with the Rho/ROCK signalling, causes extreme membrane-cytoskeletal remodelling through loss of focal adhesions and cell-substratum contacts and ultimately leads to a more motile, mesenchymal cell phenotype (Rescher et al., 2008). Epithelial to mesenchymal (EMT) transition is an essential process during inflammation and wound repair as cells must degrade damaged ECM, proliferate and reorganise themselves to replace damaged tissue (Kovacic et al., 2012). Moreover, EMT is important for cancer cell dissemination. Therefore, AIIt, bridging the membrane and cytoskeleton, together with kinases, can respond to stress signals in order to ultimately restore tissue homeostasis.

Annexin A2 is central in cell polarization processes

In order to best explain the role of AIIt as a membrane-cytoskeletal adaptor protein during dynamic remodelling processes, we will discuss AIIt in the context of polarised epithelial cells. As mentioned before, cell polarity is the spatial segregation of membrane constituents which enables a cell to perform specialised functions (Cao et al., 2012; Simons and Sampaio, 2011). To that effect polarised epithelial cells, which line organ surfaces and cavities, have distinct apical and basolateral surface domains separated by tight junctions. As well as serving as a protective barrier, sheets of epithelial cells regulate the vectorial transport of molecules between compartments. The vesicular transport system not only aids in transport of molecules, but maintains cell polarity through dynamic sorting and translocation of membrane constituents to the apical or basolateral surface (Cao et al., 2012).

Epithelial cells interact with neighbouring cells via adherens junctions and tight junctions (Leslie et al., 2010). During adherens junction formation, transmembrane E-cadherin proteins on opposing cells dimerise, forming a bridge between two cells (Yamada et al., 2005). Tight junctions are derived from adherens junctions and require the fusion of the exoplasmic leaflet of the lipid bilayer on opposing cells (Lee et al., 2004). In addition to facilitating intercellular communication via paracellular transport, tight junctions form a circumferential belt around the cell which mediates cell polarity by separating the apical and basolateral domains (Cao et al., 2012). Independent experiments using calcium chelation to disrupt cell-cell junctions showed that AIIt is required for the reassembly of both adherens junctions by recruiting E-cadherin to nascent adherens junctions (Yamada et al., 2005) as well as for the reassembly of tight junctions (Lee et al., 2004). In addition, AIIt directs active Rho GTPases Rac1 and Cdc42 to nascent adherens junctions where they facilitate

actin remodelling and cell signalling required for cell-cell junction formation (Hall and Nobes, 2000; Hansen et al., 2002). Thus AIIt functions to facilitate cell-cell interaction and epithelial polarity required for epithelial cell function.

The role of annexin A2 in endocytosis

Polarised epithelial cells, as the interface between the internal and external milleu of organs, require mechanisms to facilitate controlled transfer of signals and substances across the membrane (Cao et al., 2012). Vesicular trafficking and cell signalling are intimately connected in a feedback-loop system which functions to regulate both spatial and temporal cellular responses to extracellular stimuli (reviewed by Scita and Di Fiore (Scita and Di Fiore, 2010)). In order to sustain sensitivity to extracellular microenvironment signals, ligand-bound/activated receptors need to be recycled (Scita and Di Fiore, 2010). Endocytosis of ligand-bound receptors facilitates ligand-receptor separation followed by lysosomal degradation or recycling of receptors back to the cell membrane. In this way endocytosis can attenuate ligand-receptor activated cell signalling as well as mediate future signalling by directing recycled receptors to particular membrane regions in a polarised cell via exocytosis (Scita and Di Fiore, 2010). Evidence suggests that AIIt is involved at multiple levels of endocytosis including accumulation of ligand-bound receptors destined for endocytosis by lipid raft clustering (Drucker et al., 2013; Valapala and Vishwanatha, 2011), vesicular budding by forming membrane indentations as a result of line tension in lipid microdomains (Drucker et al., 2013) and vesicular transport (Merrifield et al., 2001; Morel and Gruenberg, 2009; Morel et al., 2009). Further, AIIt, located on early endosomes, nucleates F-actin and is required to initiate actin polymerization-dependent propulsion of endosomes (Merrifield et al., 2001; Morel and Gruenberg, 2009; Morel et al., 2009). It is likely that AIIt facilitates endosome transport by recruiting and/or activating the Rho GTPase Cdc42, which is a known regulator of actin nucleation (Merrifield et al., 2001).

Annexin A2 and its role in endocytosis-mediated cell migration

Cell migration requires rapid disassembly and reassembly of cell-ECM interactions known as focal adhesions (Rankin et al., 2013). Focal adhesions primarily consist of transmembrane integrin proteins that simultaneously interact with the cytoskeleton and ECM components. At the trailing edge of the cell, focal adhesion integrins are endocytosed, sorted and recycled to the leading edge of the cell, where new focal adhesions are formed. In this way dynamic recycling of integrins and thus focal adhesions provides the traction

required for cell motility (Rankin et al., 2013). During wound healing and inflammation, growth factors such as PDGF and VEGF facilitate membrane rearrangement of integrins to actin-rich dorsal circular ruffles where they are internalised via macropinocytosis (Gu et al., 2011). Knockdown of annexin A2 was shown to result in increased focal adhesions and cell immobilisation, suggesting that AIIt is required to promote endosomal recycling of integrins (Rankin et al., 2013).

The role of annexin A2 in exocytosis

A number of studies have suggested that AIIt is required for membrane fusion events. Wang et al. showed that AIIt directly interacts with SNAP-23, a member of the SNARE super family of lipid bilayer fusion proteins. During exocytosis cell membrane SNARE proteins SNAP-23 and syntaxin recognise and form complexes with vesicle-SNARE proteins to facilitate vesicular-plasma membrane fusion. Antibodies against either annexin A2 or SNAP-23 inhibited exocytosis, suggesting that both are required for exocytosis. As exocytosis is favoured under conditions of elevated intracellular Ca^{2+}, AIIt likely senses increased Ca^{2+} levels and interacts with SNAP-23 to facilitate exocytosis. In addition, AIIt may function to organise SNAP-23 into distinct lipid microdomains which can be recognised by vesicle-SNARE proteins to mediate targeted exocytosis (Wang et al., 2007). Interestingly, using fluorescently labelled annexin A2, it was found that exocytosis can result in the transfer of annexin A2 from one cell to another (Valapala and Vishwanatha, 2011). This suggests that annexin A2 may act as a direct intercellular communicator.

Nuclear annexin A2

While the majority of annexin A2 is found in the heterotetrameric form associated with the membrane and the actin cytoskeleton, monomeric annexin A2 is also found in the nucleus (Liu and Vishwanatha, 2007). Here annexin A2 functions as a transporter of particular transcription factors and mRNA, to and from the nucleus, respectively (Hollas et al., 2006). In addition, annexin A2 nuclear translocation has been identified as a protective mechanism against DNA-damage (Madureira et al., 2012).

Annexin A2 protects cells from DNA-damage

Madureira et al. found that annexin A2 accumulates in the nucleus in response to DNA-damaging agents. Interestingly, this accumulation was blocked by the antioxidant N-acetyl cysteine indicating that annexin A2 is redox sensitive and its nuclear accumulation occurs in response to oxidative stress. Further, annexin A2 depleted cells were shown to be more

susceptible to DNA damage, suggesting that annexin A2 may protect DNA from genotoxic agents (Madureira et al., 2012). This is of particular importance in epithelial tissues which serve as protective barriers between external and internal organ compartments and are therefore constantly exposed to DNA-damaging compounds.

Annexin A2 regulates transcription and transport of mRNA

Annexin A2 has also been shown to be involved in both transcriptional and translational regulation. Das et al. found that cytoplasmic annexin A2 binds to, and upregulates the activity of phosphorylated transcription factor signal transducer and activator of transcription (STAT6). In response to treatment with the pro-inflammatory cytokine interleukin-4, phosphorylated STAT6 showed annexin A2-dependent migration to the nucleus, where it associates with the DNA-binding complex in association with annexin A2 (Das et al., 2010). Annexin A2 is also involved in facilitating Ca^{2+}-dependent transport, and thereby translation, of certain mRNA transcripts (Fahling et al., 2006; Hollas et al., 2006). This is likely a result of the ability of annexin A2 to simultaneously interact with actin filaments, mRNA and heterogenous nuclear ribonucleoproteins (hnRNP), i.e. short RNA-binding proteins involved in the pre-processing of pre-mRNAs and in mRNA transport (Hollas et al., 2006). In this way annexin A2 facilitates mRNA transport from the nucleus to the endoplasmic reticulum by acting as a bridging protein between the cytoskeleton, mRNA and hnRNPs. The mRNA transcripts that are known to interact with annexin A2 include c-myc (Mickleburgh et al., 2005), the collagen synthesis enzyme collagen prolyl 4 hydroxylase-α (Fahling et al., 2006) and annexin A2 itself (Hollas et al., 2006), the latter being particularly interesting as annexin A2, by regulating its own expression, is likely to enhance annexin A2-mediated DNA-protection as well as membrane-cytoskeletal remodelling required for a number of processes involved in wound repair. One could speculate that the observed overexpression of annexin A2 in many malignancies, particularly in the nucleus, would confer protection against genotoxic agents such as chemotherapy drugs, while increased AIIt would mediate membrane cytoskeletal remodelling - an easy mechanism whereby cancer cells can regulate their own microenviroment and therefore, annexin A2 represent an attractive drug target for treating cancer cells.
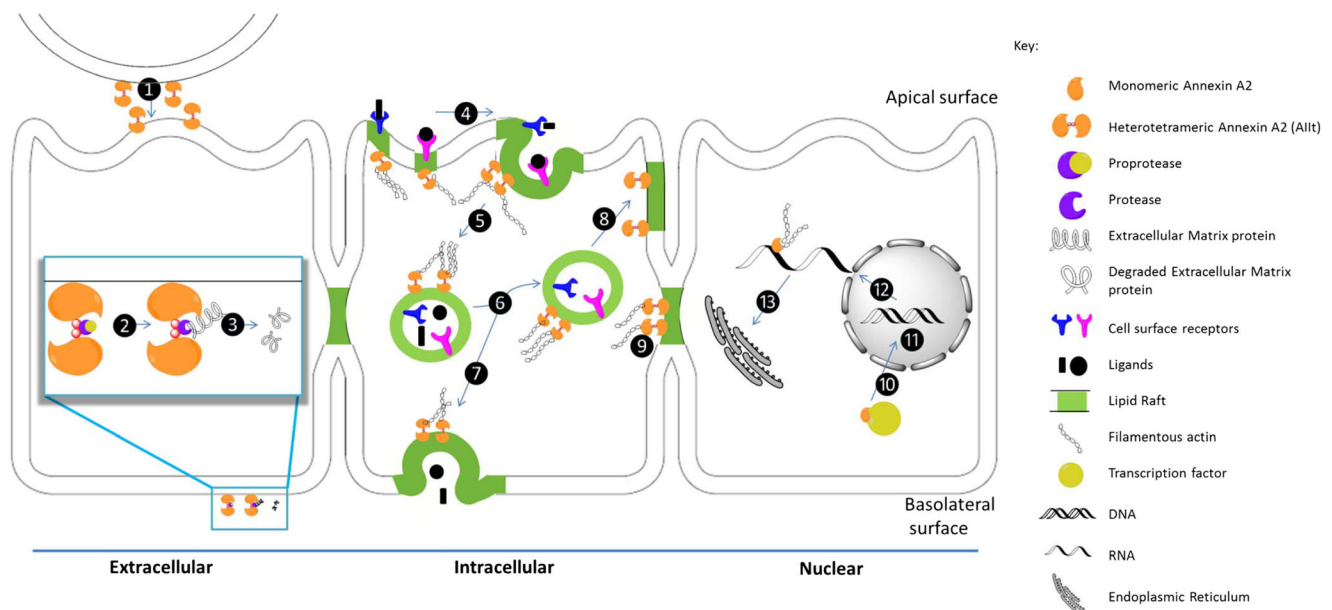

Fig. 1 Annexin A2 mediates important cellular functions in the context of dynamic reciprocity at the extracellular, intracellular and nuclear level. Extracellular Annexin A2 (*left panel*): Cell surface AIIt is involved in mediating cell homing and cell-cell adhesion ❶ as well as ECM degradation via the activation of proteases through the S100A10 subunit ❷ and by increasing the catalytic efficiency of proteases by bridging ECM components and proteases ❸. Intracellular Annexin A2 (*middle panel*): Membrane bound AIIt mediates lipid raft clustering into large lipid microdomains which form membrane buds ❹ prior to endocytosis. AIIt orchestrates F-actin polymerisation-dependent propulsion of endocytic vesicles ❺ which mature into multivesicular endosomes where receptors, ligands and extracellular fluid components are sorted ❻ and either sent for lysosomal degradation (not shown), targeted exocytosis of soluble proteins ❼ or targeted receptor recycling ❽. Further, membrane-bound AIIt is involved in the formation of both adherens (not shown) and tight junctions ❾. Nuclear Annexin A2 (*right panel*): Monomeric annexin A2 facilitates nuclear import of transcription factors ❿ and associates with the DNA-binding complex during transcription ⓫. Annexin A2 also facilitates mRNA nuclear export ⓬ and, together with F-actin, transports the mRNA transcript to the endoplasmic reticulum prior to translation ⓭. For illustrating purposes, this is a simplified overview of the localisation and functions of annexin A2 in the various cellular compartments; please refer to the text for details

Conclusion

Annexin A2 is a multifunctional protein which mediates many aspects of intercellular and extracellular microenvironment communication. The defining property of this protein is its ability to act as a bridging protein between various structural elements in and around the cell, whereby it conducts the bidirectional flow of information (Fig. 1). This includes intra- and intercellular plasma membrane interactions, plasma membrane and cytoskeletal interactions, and mRNA and cytoskeletal interactions. Further, annexin A2 function and subcellular location is regulated by extracellular changes which are signalled by fluxes of intracellular Ca^{2+}. Annexin A2 is thus an effector of extracellular microenvironmental signals. The ability of annexin A2 to adapt in response to these changes to alter membrane-membrane and membrane-cytoskeletal dynamics as well as gene transcription and translation implicates this protein as an effector of DR between the cell and its extracellular microenvironment. Therefore, abnormal expression of annexin A2 would corrupt this DR and result in pathogenesis. This is often observed in various types of malignancies where annexin A2 is often abnormally high facilitating survival and dissemination of cancer cells through DNA protection, ECM degradation, cell homing and heterotypic cell-cell adhesion. While annexin A2 has multiple functions which appear to be unrelated at the first glance, when considered in the context of tissue homeostasis it is apparent that all functions of annexin A2 act in synergy to control bidirectional communication between cells as well as cells and the extracellular microenvironment.

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