



**HAL**  
open science

## **Review: Annexin-A5 and cell membrane repair**

A Bouter, R Carmeille, C Gounou, F Bouvet, S A Degrelle, D Evain-Brion, A R  
Brisson

► **To cite this version:**

A Bouter, R Carmeille, C Gounou, F Bouvet, S A Degrelle, et al.. Review: Annexin-A5 and cell membrane repair. *Placenta*, 2015, 36, pp.S43 - S49. <10.1016/j.placenta.2015.01.193>. <hal-04884860>

**HAL Id: hal-04884860**

**<https://hal.science/hal-04884860v1>**

Submitted on 13 Jan 2025

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



Contents lists available at ScienceDirect

## Placenta

journal homepage: [www.elsevier.com/locate/placenta](http://www.elsevier.com/locate/placenta)

## Review: Annexin-A5 and cell membrane repair



A. Bouter<sup>a, \*</sup>, R. Carmelle<sup>a</sup>, C. Gounou<sup>a</sup>, F. Bouvet<sup>a</sup>, S.A. Degrelle<sup>b, c, d</sup>,  
D. Evain-Brion<sup>b, c, d</sup>, A.R. Brisson<sup>a</sup>

<sup>a</sup> Molecular Imaging and NanoBioTechnology, UMR-5248-CBMN CNRS-University of Bordeaux-IPB, Allée Geoffroy Saint-Hilaire, F-33600 Pessac, France

<sup>b</sup> Fondation PremUP, Paris F-75006, France

<sup>c</sup> INSERM, U1139, Paris F-75006, France

<sup>d</sup> Université Paris Descartes, UMR-S1139 Sorbonne Paris Cité, Paris F-75006, France

## ARTICLE INFO

## Article history:

Accepted 26 January 2015

## Keywords:

Membrane repair  
Annexin-A5  
Placenta  
Trophoblast

## ABSTRACT

Annexins are soluble proteins that bind to biological membranes containing negatively charged phospholipids, principally phosphatidylserine, in a  $\text{Ca}^{2+}$ -dependent manner. Annexin-A5 (AnxA5), the smallest member of the annexin family, presents unique properties of membrane binding and self-assembly into ordered two-dimensional (2D) arrays on membrane surfaces. We have previously reported that AnxA5 plays a central role in the machinery of membrane repair by enabling rapid resealing of plasma membrane disruption in murine perivascular cells. AnxA5 promotes membrane repair via the formation of a protective 2D bandage at membrane damaged site. Here, we review current knowledge on cell membrane repair and present recent findings on the role of AnxA5 in membrane resealing of human trophoblasts.

© 2015 Published by IFPA and Elsevier Ltd.

## 1. Annexin-A5

Annexins are soluble proteins present in eukaryotes and absent in yeasts and prokaryotes [1]. Vertebrates have in common twelve annexins, named annexin A1–A13 (AnxA1–AnxA13, the number 12 being non-assigned). Annexins share the property of binding to membranes exposing negatively charged phospholipids in a  $\text{Ca}^{2+}$ -dependent manner [1,2]. They are formed by a four-fold repeat (eight-fold for AnxA6) of a 70 amino-acid domain, highly conserved in sequence, and by a variable amino-terminal ( $N_t$ ) domain which contains phosphorylation sites and binding sites for various molecular partners and is assumed to be responsible for the functional specificity of annexins [2]. Following the determination of AnxA5 structure by X-ray crystallography [3], atomic models have been obtained for many other annexins [4]. Annexins present a common membrane-binding core formed by the cyclic arrangement of the four conserved 70 amino-acid-long domains (Fig. 1). The membrane-binding core has the shape of a slightly curved rhomboid with a convex membrane-binding face where  $\text{Ca}^{2+}$ -binding loops are exposed, and a concave face from which protrudes the  $N_t$  domain.

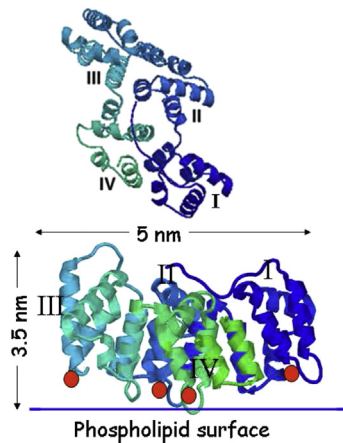
Membrane binding is mediated via four to eight  $\text{Ca}^{2+}$ -binding sites located on the convex face. Annexin binding presents little specificity for anionic lipid head-groups, yet the  $\text{Ca}^{2+}$  concentration required for binding varies considerably between annexins [5,6]. Most *in vitro* membrane binding studies on annexins have been performed with membrane systems containing phosphatidylserine (PS), which is the major negatively charged phospholipid in eukaryotic cell membranes [7]. Binding of annexins to membrane surfaces is very tight and depends on the content in negatively charged phospholipids and the  $\text{Ca}^{2+}$  concentration [8–10]. Equilibrium binding constants of the order of 10 fM have been measured for AnxA5 and membrane vesicles containing 25% PS at 1 mM  $\text{Ca}^{2+}$  [9].

At the functional level, annexins have been shown to participate in numerous membrane-related processes, including exo- and endo-cytosis, vesicle trafficking, regulation of blood coagulation and inflammation, membrane aggregation and fusion, or the regulation of membrane dynamics and organization [2,11]. Strikingly, although annexins do not possess a secretory signal peptide, several of them have been found both intracellularly and extracellularly. Actually, the best defined functions of annexins concern extracellular AnxA1, AnxA2 and AnxA5, which possess anti-inflammatory [12], pro-fibrinolytic [13] and anti-thrombotic [14,15] activities, respectively.

A remarkable property of several members of the annexin family –including AnxA5, AnxB12 (from hydra), AnxA4, AnxA6 and

\* Corresponding author. UMR-5248-CBMN, Bat. B14, Allée Geoffroy Saint-Hilaire, F-33600 Pessac, France. Tel.: +33 (0)5 40006860; fax: +33 (0)5 40002200.

E-mail address: [a.bouter@cbmn.u-bordeaux.fr](mailto:a.bouter@cbmn.u-bordeaux.fr) (A. Bouter).



**Fig. 1.** Structure of soluble AnxA5. (Top) 3D structure of an AnxA5 molecule observed from the concave side, along a direction perpendicular to the membrane plane. AnxA5, the smallest annexin, presents four  $\alpha$ -helical domains, numbered I to IV [3]. (Bottom) Side view of an AnxA5 molecule. Four  $\text{Ca}^{2+}$  ions are represented, one per  $\alpha$ -helical domain (red dot). The approximate position of the membrane surface is indicated (blue line). These figures were created with PyMOL (<http://pymol.org>) from PDB code 1AVR.

the  $(\text{AnxA2-p11})_2$  tetramer complex-is to self-organize into two dimensional (2D) arrays at the level of biological membranes [16–19]. This property has been mostly investigated in the case of AnxA5, which is the smallest annexin and consists only of the conserved membrane-binding core of annexins. Extensive studies by electron microscopy [16–19] and atomic force microscopy [10,20,21] have revealed the successive steps involved in the formation of AnxA5 2D crystals on a membrane surface (Fig. 2). When soluble AnxA5 molecules encounter a membrane containing negatively charged phospholipids in the presence of  $\text{Ca}^{2+}$ , monomeric AnxA5 molecules bind to the membrane surface, membrane-bound AnxA5 assemble rapidly into trimers [22] which self-organize into 2D ordered arrays, even at low surface density [10]. Two main types of crystals are formed by AnxA5 depending on the PS content and the  $\text{Ca}^{2+}$  concentration. At low and medium PS

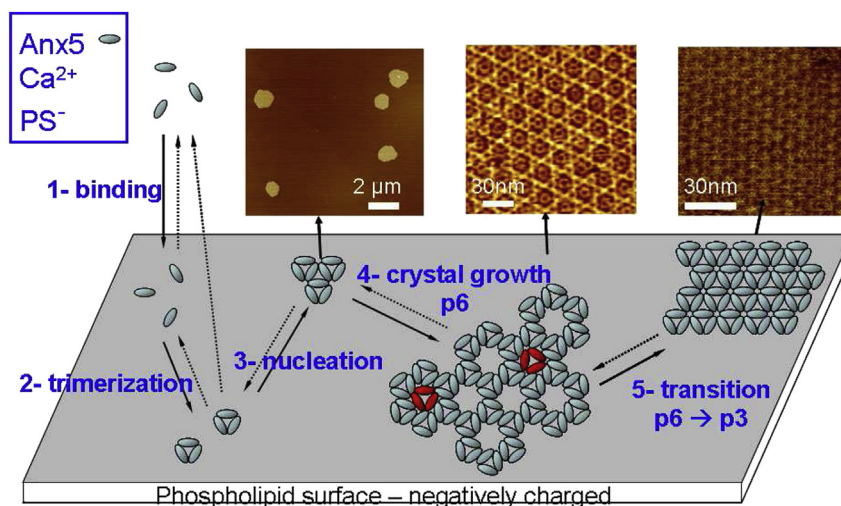
content (5–20%), open hexagonal arrays with p6 symmetry form and are stable, while crystals with p3 symmetry predominate at high PS-content ( $\geq 40\%$ ) and high  $\text{Ca}^{2+}$  concentration [10,23,24].

Given the central roles played by self-assembly processes in the construction of supra-molecular structures essential for cell's life, e.g. cell membranes, microtubules or virus capsids to name a few, the unique tendency of AnxA5 to self-assemble into 2D arrays on membranes in the presence of mM  $\text{Ca}^{2+}$  opened the hypothesis that these 2D arrays could support a cellular function. This has led ultimately to the discovery of the role of AnxA5 2D arrays in cell membrane repair, as described below.

## 2. Cell membrane repair

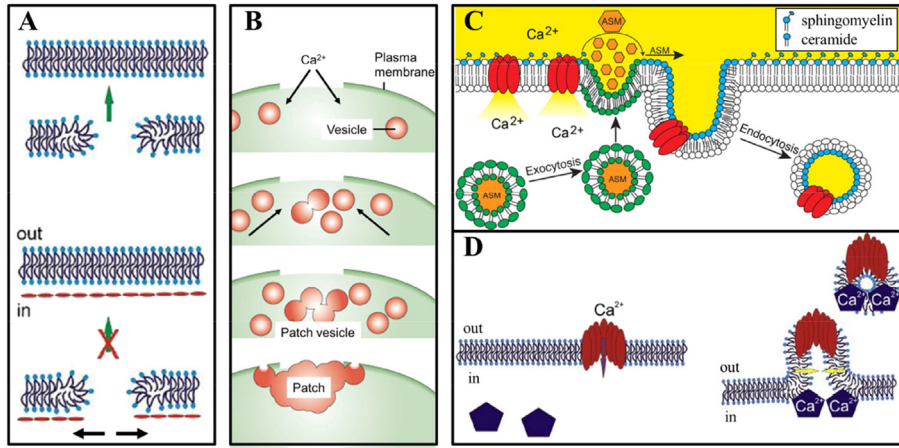
Cells from many tissues, such as skeletal or cardiac muscle, skin, gut epithelium or vascular endothelium, are exposed to mechanical stress in physiological conditions. This results often in the formation of tears in cell membrane [25]. Membrane rupture is followed by a rapid increase in the intracellular  $\text{Ca}^{2+}$  concentration, which is in the  $\mu\text{M}$  range in cell resting state while the extracellular  $\text{Ca}^{2+}$  concentration is in the mM range. The rise in intracellular  $\text{Ca}^{2+}$  concentration activates a repair machinery that ensures a rapid resealing and repair of membrane ruptures [26]. Failure in membrane resealing may lead to cell death and contribute to the development of degenerative diseases, such as muscular dystrophies [27]. According to current models of cell membrane repair [25,28], membrane resealing is an active process that requires extracellular  $\text{Ca}^{2+}$ , intracellular vesicles and a step of  $\text{Ca}^{2+}$ -dependent exocytosis [26]. Although small disruptions may be resealed spontaneously in synthetic liposomes, the presence of a cortical cytoskeleton in cells creates considerable membrane tension which prevents passive resealing (Fig. 3A).

Three main mechanisms of membrane repair have been proposed, which include the steps of membrane resealing and lesion removal. The classical model from McNeil and collaborators in the late 1990s proposed that the increase of intracellular  $\text{Ca}^{2+}$  concentration triggered the fusion of intracellular vesicles and the formation of a lipid patch enabling the membrane to reseal via an exocytosis-like process (Fig. 3B) [29,30]. McNeil and coworkers

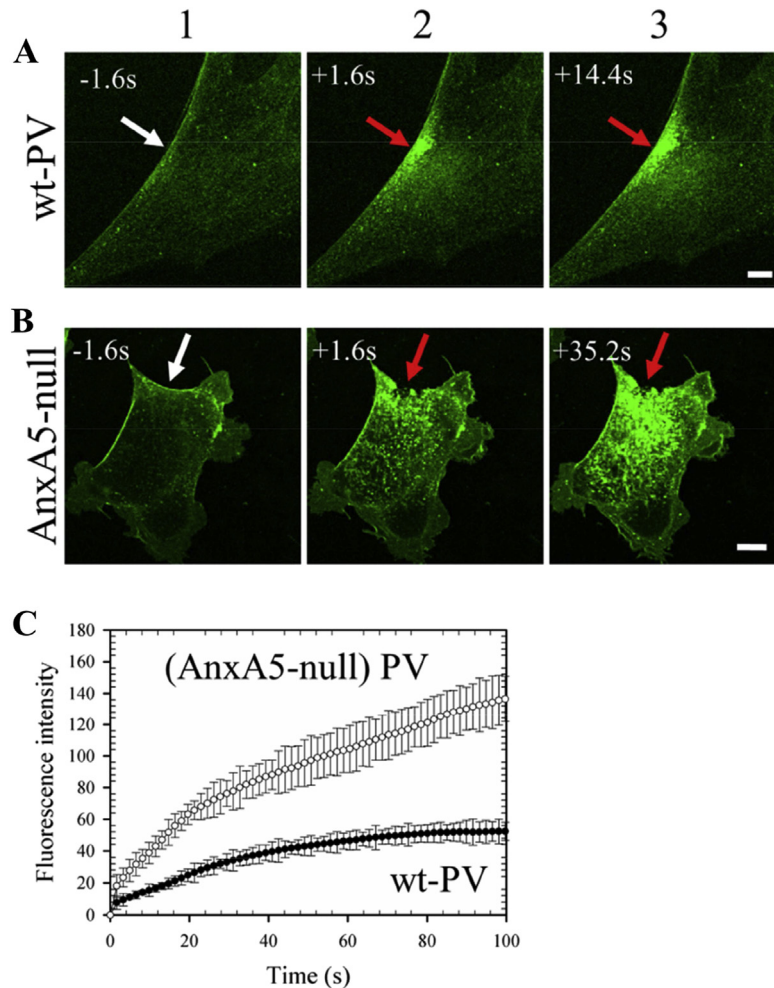


**Fig. 2.** 2D self-assembly of AnxA5 on a negatively charged phospholipid surface. Three successive steps take place when soluble AnxA5 molecules encounter a membrane containing negatively charged phospholipids, mainly phosphatidylserine (PS), in the presence of  $\text{Ca}^{2+}$ : 1) monomeric AnxA5 molecules bind to the membrane in a  $\text{Ca}^{2+}$ -dependent manner; 2) membrane-bound AnxA5 molecules form trimers; 3) trimers self-assemble into 2D ordered arrays, of p6 or p3 symmetry. This model is mainly based on electron microscopy and atomic force microscopy studies. The three upper panels display the nucleation, the crystal growth and the p6/p3 transition steps, as observed in atomic force microscopy.

Adapted from Ref. [10].



**Fig. 3.** Models of cell membrane repair. (A) In synthetic liposomes small disruptions is resealed spontaneously (upper panel). In contrast, considerable membrane tension due to the cortical cytoskeleton prevents passive resealing in nucleated cell (lower panel). Adapted from Ref. [40]. (B) The lipid patch hypothesis.  $Ca^{2+}$  influx triggers fusion of intracellular vesicles, which form a lipid patch. Through an exocytic-like process, the patch reseals the disrupted site. Adapted from Ref. [28]. (C) Lesion removal through ceramide-driven invagination. Bacterial toxins insert into cell plasma membranes and form pores (red) leading to the entry of  $Ca^{2+}$  which activates lysosome exocytosis and secretion of acid sphingomyelinase (ASM). The hydrolysis of sphingomyelin head groups induces ceramide-driven invagination and lesion removal via an endocytic process. Adapted from Ref. [34] (©2010 Tam et al. Journal of Cell Biology. 189:1027–1038. doi:10.1083/jcb.201003053). (D) Lesion removal by membrane shedding.  $Ca^{2+}$  influx through a toxin-induced pore activates annexins (blue diamonds) translocation to the plasma membrane. Annexins induce membrane folding which leads to the formation of an outward curvature and the release of microparticles into the extracellular milieu. Adapted from Ref. [65].



**Fig. 4.** AnxA5 promotes membrane repair in perivascular cells. Responses of wild-type (A) and AnxA5-null (B) murine perivascular (PV) cells after laser irradiation in the presence of 2 mM  $Ca^{2+}$ . (A) Image series showing the response of a wild-type (wt) PV cell. The cell presents an increase of cytoplasmic fluorescence intensity limited to an area closed to the wounding site. Bar = 10  $\mu$ m. (B) Response of an AnxA5-null PV cell. The cell presents a macroscopic membrane rupture at the irradiated area and a large increase of cytoplasmic fluorescence intensity. Bar = 10  $\mu$ m. In all frames, the area of membrane irradiation is marked with a white arrow before irradiation and a red arrow after irradiation. Image frames 1 and 2 were recorded 1.6 s before and 1.6 s after irradiation, respectively, as indicated. (C) Kinetics of FM1-43 fluorescence intensity increase for wt-PV cells (filled circles) and AnxA5-null PV cells (empty circles) after membrane damage. For wt-PV cells, the fluorescence intensity reaches a plateau after about 90 s, indicating cell membrane has resealed stopping further entrance of FM1-43 molecules. For AnxA5-null PV cells, the fluorescence intensity increases continuously, indicating the absence of membrane resealing. Adapted from Ref. [49].

hypothesized that several fusion pores formed between the edges of the patch and the plasma membrane surrounding the disruption site, similarly to the “vertex” fusion process observed when two yeast vacuoles fuse [31]. Complete connection between the patch and the plasma membrane may be created by lateral expansion of the fusion pores. While membranes of the patch, together with the residual wounded plasma membrane, are mostly released into the extracellular milieu, the lower membrane portion of the patch may restore cell membrane at the wounded site [28]. Most early investigations that led to the ‘lipid patch’ hypothesis did not use specific markers ensuring the identification of injury-induced intracellular vesicles [29,30], preventing the determination of their endo- or exo-cytic origin. Lysosomes were the first intracellular vesicles proposed to participate in membrane resealing [30]. They were thought to contribute to the lipid patch formation until the detection of lysosomal marker Lamp1 revealed a punctate distribution surrounding the disrupted site, inconsistent with the localization of lysosomes in the patch [32]. Actually, the entry of  $\text{Ca}^{2+}$  into wounded cells triggers not only lysosome exocytosis but also a form of endocytosis [33], which is induced by the secretion of lysosomal acid sphingomyelinase [34]. Exogenously-added acid sphingomyelinase enables plasma membrane repair even in the absence of  $\text{Ca}^{2+}$  [35]. Based on this, a new model has emerged according to which  $\text{Ca}^{2+}$  entry through a membrane disruption activates the exocytosis of lysosomes, which secrete acid sphingomyelinase in the extracellular milieu. Subsequently, hydrolysis of the phosphorylcholine head groups of sphingomyelin trigger ceramide-driven membrane invagination giving rise to the lesion removal by endocytosis (Fig. 3C) [33,34,36].

In addition, the processes of membrane resealing and lesion removal can also be achieved by microvesiculation shedding (Fig. 3D) [37–39]. These processes may involve the ESCRT machinery [39] or neutral sphingomyelinase that triggers ceramide production in the inner leaflet of plasma membrane giving rise to an outward curvature (Fig. 3D) [40].

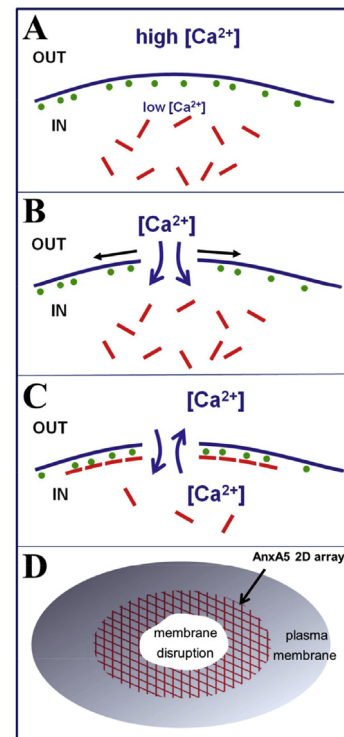
The implementation of one or a combination of membrane repair mechanisms depends probably on the size and the type of injury. It is noteworthy that the emerging models based on the lysosomal enzyme-induced endocytosis rely mostly on the observation of toxin-treated cells, while most studies that led to the ‘lipid patch’ hypothesis have been conducted on cells submitted to mechanical tears. Major differences are expected with regards to molecular consequences induced by these two types of injury, which may explain the various operating processes of membrane repair.

Several proteins of the membrane repair machinery have now been identified. Dysferlin has been shown to participate in the membrane fusion step of the repair process [41,42]. Mutations of the dysferlin gene are responsible for muscular dystrophies [41,43]. Several dysferlin-binding proteins have also been identified, including MG53, AHNAK, calveolin-3, calpain-3, as well as several annexins [42,44,45]. In mouse primary muscle cells, AnxA1 and AnxA2 have been proposed to trigger the recruitment and fusion of intracellular vesicles during the lipid patch formation and promote its attachment to the plasma membrane, by interacting with dysferlin [42]. On the other hand, in dysferlinopathic mice that present a defect in membrane repair, it has been shown that AnxA1–AnxA2 do not interact with dysferlin. In the zebrafish skeletal muscle, membrane repair has been shown to involve the controlled and sequential assembly of a highly ordered scaffold comprising dysferlin, AnxA6, AnxA2 and AnxA1 [46]. The membrane repair machinery is therefore becoming unraveled, although the specific role of each component and their interplay remain largely to be elucidated. Unlike dysferlin, which has been found to facilitate the secretion of acid sphingomyelinase [47], most proteins of the repair

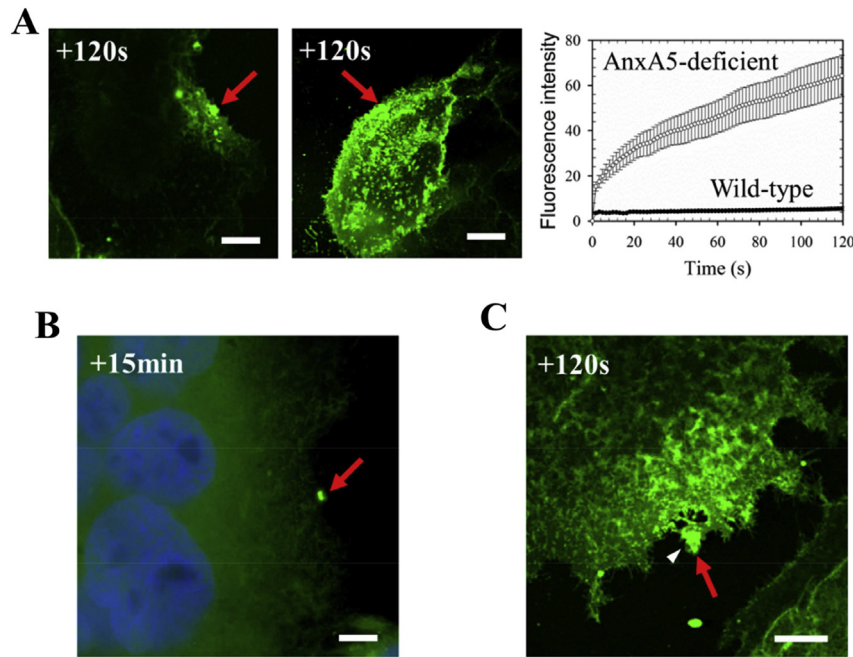
machinery were analyzed solely according to the lipid patch model. Future investigations might consider their participation in membrane repair in the light of the emerging models.

### 3. Discovery of the role of AnxA5 in membrane repair

In view of the 2D self-assembly properties of AnxA5, we wondered whether AnxA5 and its 2D arrays could play a role in membrane repair. This question was first addressed at the level of mouse perivascular cells, for which an AnxA5 knock-out cell line had been established [48]. We compared the responses to membrane damage of wild-type cells expressing AnxA5 and cells lacking AnxA5 [49]. A well-established procedure of membrane rupture by laser irradiation was applied, while membrane repair was assessed by measuring the entrance within the cell of FM1-43, a dye that becomes fluorescent upon inserting into intracellular membranes [41]. Wild-type cells were able to repair membrane ruptures spontaneously and rapidly. In contrast, cells lacking AnxA5 exhibited a defect in their machinery of membrane repair (Fig. 4). The addition of exogenous AnxA5 was shown to restore membrane repair ability, while an AnxA5 mutant lacking the property of 2D self-assembly failed to promote membrane resealing. This study established that AnxA5 plays a central role in the membrane repair machinery by forming a 2D bandage at the level of torn membrane edges, thus preventing the expansion of membrane wound and facilitating the final steps of membrane resealing (Fig. 5) [49].



**Fig. 5.** Model of the function of AnxA5 in membrane repair. (A) Intact cell membrane. The extracellular and intracellular milieus differ by their high versus low  $\text{Ca}^{2+}$  concentrations, and the absence versus presence of AnxA5 (red rods) and PS (green spheres), respectively. (B) Local rupture of a cell membrane. Forces resulting from membrane tension tend to expand the tear laterally (black arrows). (C, D) The formation of AnxA5 2D arrays at the torn membrane edges stops the expansion of the wound. Upon membrane rupture, a microenvironment is formed where the intra- and extra-cellular milieus mix, providing optimal conditions for the formation of tight complexes between  $\text{Ca}^{2+}$ , PS and AnxA5. AnxA5 molecules bind to PS molecules exposed at ruptured membrane edges, form trimers and 2D arrays. These AnxA5 2D self-assemblies stabilize the membrane and stop expansion of the rupture. (D) Top view of a membrane disruption with AnxA5 2D arrays surrounding the rupture.



**Fig. 6.** AnxA5 promotes membrane resealing in human trophoblasts. (A) Representative images showing the response of a wild-type BeWo ST (left-hand panel) and an AnxA5-deficient BeWo ST (middle panel) 120 s after 110-mW infrared-laser irradiation in the presence of 1 mM  $\text{Ca}^{2+}$ . Whereas the human choriocarcinoma BeWo cell line remains as mononucleated cells in free-cAMP growth condition, cell differentiation and fusion occur in the presence of 100- $\mu\text{M}$  8-Br-cAMP for 48 h, forming large multinucleated cells (referred as BeWo ST). AnxA5-deficient BeWo ST were obtained by transduction with lentiviral particles containing AnxA5-targeting shRNA [61]. The area of membrane irradiation is marked with a red arrow (also in B, C). Bars = 20  $\mu\text{m}$  (right-hand panel) For wild-type cells (filled circles) the FM1-43 fluorescence intensity reaches a plateau after about 30 s, indicating cell membrane resealing. For AnxA5-deficient cells (empty circles), the fluorescence intensity increases continuously, indicating the absence of membrane resealing. (B) Subcellular localization of endogenous AnxA5 in a damaged wild-type ST (left-hand panel) analyzed by immunocytofluorescence. Cells were irradiated, fixed with paraformaldehyde, immunostained for AnxA5 (green) and counterstained with DAPI (blue). Endogenous AnxA5 binds exclusively to the disrupted site of the injured membrane. Bar = 20  $\mu\text{m}$ . (C) Lesion removal in a wild-type ST after cell membrane injury. The image was recorded 120 s after irradiation in the presence of FM1-43 (green). The white arrowhead points to the removal of a large membrane fragment containing the disrupted site. Bar = 20  $\mu\text{m}$ .

#### 4. AnxA5 promotes membrane resealing in human trophoblasts

Annexins are abundant in the placenta where they represent about 1% of total proteins [50–52]. AnxA5 was discovered in the placenta and named initially PAP-1, for placental anti-coagulant protein-1 [53].

The multinucleated syncytiotrophoblast (ST) layer covering the surface of the placenta and bathing in maternal blood is the site of numerous placental functions and plays a major role throughout pregnancy [54–56]. The ST layer expands through the continuous recruitment by fusion of underlying progenitor cytotrophoblasts (CT), and is therefore regenerated along pregnancy. The ST apical membrane that faces maternal blood is made of abundant but fragile microvilli. These microvilli are subjected to shear stresses from the maternal blood flux within the intervillous space, which result in numerous events of membrane rupture. In addition, the syncytial surface delivers into the maternal blood numerous ST-derived particles named either syncytial knots or sprouts [57]. An exacerbated deportation of trophoblast knots and sprouts into maternal blood is observed in preeclampsia, which is responsible for an inflammatory response of maternal endothelial cells directly involved in the physiopathology of preeclampsia [58,59]. The unexpected presence in ST of dysferlin, a cell membrane repair protein hitherto considered specific to skeletal muscle, suggested the existence of a plasma membrane repair machinery in the placenta [60]. The molecular mechanisms leading to the shedding of ST-derived particles and the required processes of membrane repair in the placenta are yet poorly understood.

In view of the role of AnxA5 in membrane repair and the high amount of AnxA5 in human placenta, we hypothesized that AnxA5 may also play a role in membrane repair of human trophoblasts [61]. We showed that wild-type ST reseal  $\mu\text{m}^2$ -size membrane disruptions rapidly, within 30 s, whereas cells lacking AnxA5 fail to reseal membrane ruptures (Fig. 6A). Similar results were obtained for CT [61]. It is unlikely that the fast membrane resealing observed in ST is accomplished through ceramide-driven invagination induced by exocytosis of lysosomal enzymes. For instance, it was observed that secretion of significant amount of acid sphingomyelinase can take up to several minutes [34], which is inconsistent with membrane resealing occurring in the second range. Our results suggest that membrane resealing in human trophoblasts occurs via the formation of a lipid patch.

Wild-type ST express AnxA5, which is homogeneously distributed within the cell, in both the cytoplasm and the nucleus. Few seconds after membrane injury, cytosolic AnxA5—as well as extracellular AnxA5—is recruited to the disruption site, where it is retained several minutes after membrane resealing (Fig. 6B). The intracellular  $\text{Ca}^{2+}$  concentration has been found to drop rapidly after membrane resealing [37,39], at a level that is inconsistent with AnxA5 binding to PS. The presence of AnxA5 at the disrupted site several minutes after membrane resealing suggests therefore that it is trapped in a lipid environment where  $\text{Ca}^{2+}$  remains at mM concentration. Frequently, the lipid material accumulated at the disruption site of ST was found to detach from the cell, either as a large fragment (about 100- $\mu\text{m}^2$ , Fig. 6C) or as membrane debris (about 5- $\mu\text{m}^2$  [61]). Importantly, this process was observed to start after completion of membrane

resealing, which suggests that the release of lipid material corresponds to the removal of the lesion.

## 5. Conclusions

Cell membrane rupture creates a microenvironment where extracellular and intracellular components mix; in particular, the high intracellular concentrations of PS and AnxA5 mix with the high extracellular concentration of  $\text{Ca}^{2+}$ . Upon binding to PS molecules at the wounding site of injured cell, AnxA5 self-assembles into 2D arrays. The formation of AnxA5 2D arrays at a ruptured cell membrane strengthens the membrane [62,63] by counteracting membrane tension due to cytoskeleton attachment. AnxA5 may thus prevent the expansion of the tear and facilitate membrane resealing. By participating in the earliest step of membrane repair, according to a process based only on the  $\text{Ca}^{2+}$  influx and the presence of PS at the plasma membrane, AnxA5 functions likely in a universal and ubiquitous way whatever the subsequent membrane resealing and lesion removal mechanisms.

The human trophoblast, which is the primary barrier separating maternal blood from fetal blood, is a fragile tissue. Various types of damages may affect the ST plasma membrane and result in the exposure of PS-containing membranes to maternal blood. In view of the pro-coagulant properties of PS-exposing membranes, efficient mechanisms of membrane repair must operate in the placenta to prevent onset of blood coagulation, which could be life-threatening for the fetus.

Extracellular—as well as cytosolic—AnxA5 binds to the disrupted site of injured plasma membrane as early as few seconds after membrane wounding and promotes membrane resealing. Rand and collaborators have proposed that extracellular AnxA5 forms a 2D anti-thrombotic shield covering the surface of placental ST in physiological conditions [64]. They proposed also that this protective shield was disrupted by anti-phospholipid antibodies and that the correlated exposure of pro-coagulant phospholipids was responsible for recurrent pregnancy losses observed in patients presenting the anti-phospholipid antibody syndrome [14,64]. It would be relevant to revisit this interesting hypothesis in the context of membrane repair.

The presence of many annexins in the placenta [50–52], together with the participation of several members of the annexin family in membrane repair processes, raise the question of a collective role of these proteins in the protection and repair of membrane injuries in trophoblasts.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgments

This study was supported by ANR (grant 11-BSV1-03501-Placenta5 to A.R.B.) and by AFM-Téléthon (grant AFM-téléthon-17140 to A.B.).

## References

- [1] Moss SE, Morgan RO. The annexins. *Genome Biol* 2004;5:219.
- [2] Gerke V, Creutz CE, Moss SE. Annexins: linking  $\text{Ca}^{2+}$  signalling to membrane dynamics. *Nat Rev Mol Cell Biol* 2005;6:449–61.
- [3] Huber R, Römisch J, Paques EP. The crystal and molecular structure of human annexin V, an anticoagulant protein that binds to calcium and membranes. *EMBO J* 1990;9:3867–74.
- [4] Swairjo MA, Seaton BA. Annexin structure and membrane interactions: a molecular perspective. *Annu Rev Biophys Biomol Struct* 1994;23:193–213.
- [5] Blackwood RA, Ernst JD. Characterization of  $\text{Ca}^{2+}$ -dependent phospholipid binding, vesicle aggregation and membrane fusion by annexins. *Biochem J* 1990;266:195–200.
- [6] Andree HA, Reutelingsperger CP, Hauptmann R, Hemker HC, Hermens WT, Willems GM. Binding of vascular anticoagulant alpha (VAC alpha) to planar phospholipid bilayers. *J Biol Chem* 1990;265:4923–8.
- [7] Devaux PF. Protein involvement in transmembrane lipid asymmetry. *Annu Rev Biophys Biomol Struct* 1992;21:417–39.
- [8] Lu Y, Bazzi MD, Nelsestuen GL. Kinetics of annexin V, calcium, and phospholipid association and dissociation. *Biochemistry* 1995;34:10777–85.
- [9] Tait JF, Gibson DF, Smith C. Measurement of the affinity and cooperativity of annexin V-membrane binding under conditions of low membrane occupancy. *Anal Biochem* 2004;329:112–9.
- [10] Richter RP, Him JLK, Tessier B, Tessier C, Brisson AR. On the kinetics of adsorption and two-dimensional self-assembly of annexin A5 on supported lipid bilayers. *Biophys J* 2005;89:3372–85.
- [11] Raynal P, Pollard HB. Annexins: the problem of assessing the biological role for a gene family of multifunctional calcium- and phospholipid-binding proteins. *Biochim Biophys Acta* 1994;1197:63–93.
- [12] D'Acquisto F, Perretti M, Flower RJ. Annexin-A1: a pivotal regulator of the innate and adaptive immune systems. *Br J Pharmacol* 2008;155:152–69.
- [13] Ling Q, Jacovina AT, Deora A, Febbraio M, Simantov R, Silverstein RL, et al. Annexin II regulates fibrin homeostasis and neoangiogenesis in vivo. *J Clin Invest* 2004;113:38–48.
- [14] Rand JH, Wu X-X. Antibody-mediated interference with annexins in the antiphospholipid syndrome. *Thromb Res* 2004;114:383–9.
- [15] Ueki H, Mizushima T, Laoharatchathanan T, Terashima R, Nishimura Y, Rieanrakwong D, et al. Loss of maternal annexin A5 increases the likelihood of placental platelet thrombosis and foetal loss. *Sci Rep* 2012;2:827.
- [16] Mosser G, Ravanat C, Freyssinet JM, Brisson A. Sub-domain structure of lipid-bound annexin-V resolved by electron image analysis. *J Mol Biol* 1991;217:241–5.
- [17] Voges D, Berendes R, Burger A, Demange P, Baumeister W, Huber R. Three-dimensional structure of membrane-bound annexin V. A correlative electron microscopy–X-ray crystallography study. *J Mol Biol* 1994;238:199–213.
- [18] Oling F, Bergsma-Schutter W, Brisson A. Trimers, dimers of trimers, and trimers of trimers are common building blocks of annexin a5 two-dimensional crystals. *J Struct Biol* 2001;133:55–63.
- [19] Govorukhina N, Bergsma-Schutter W, Mazères-Dubut C, Mazères S, Drakopoulou E, Bystrykh L, et al. Self-assembly of annexin A5 on lipid membranes. *Landes Biosciences, Kluwer Academic/Plenum Publishers*; n.d. p. 61–78.
- [20] Reviakine I, Bergsma-Schutter, Brisson. Growth of protein 2-D crystals on supported planar lipid bilayers imaged in situ by AFM. *J Struct Biol* 1998;121:356–61.
- [21] Reviakine I, Bergsma-Schutter W, Mazères-Dubut C, Govorukhina N, Brisson A. Surface topography of the p3 and p6 annexin V crystal forms determined by atomic force microscopy. *J Struct Biol* 2000;131:234–9.
- [22] Langen R, Isas JM, Luecke H, Haigler HT, Hubbell WL. Membrane-mediated assembly of annexins studied by site-directed spin labeling. *J Biol Chem* 1998;273:22453–7.
- [23] Brisson A, Bergsma-Schutter W, Oling F, Lambert O, Reviakine I. Two-dimensional crystallization of proteins on lipid monolayers at the air–water interface and transfer to an electron microscopy grid. *J Cryst Growth* 1999;196:456–70.
- [24] Reviakine I, Bergsma-Schutter W, Morozov AN, Brisson A. Two-dimensional crystallization of annexin a5 on phospholipid bilayers and monolayers: a solid–solid phase transition between crystal forms. *Langmuir* 2001;17:1680–6.
- [25] McNeil PL, Steinhardt RA. Plasma membrane disruption: repair, prevention, adaptation. *Annu Rev Cell Dev Biol* 2003;19:697–731.
- [26] Steinhardt RA, Bi G, Alderton JM. Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. *Science* 1994;263:390–3.
- [27] Glover L, Brown RH. Dysferlin in membrane trafficking and patch repair. *Traffic* 2007;8:785–94.
- [28] McNeil PL, Kirchhausen T. An emergency response team for membrane repair. *Nat Rev Mol Cell Biol* 2005;6:499–505.
- [29] Miyake K, McNeil PL. Vesicle accumulation and exocytosis at sites of plasma membrane disruption. *J Cell Biol* 1995;131:1737–45.
- [30] McNeil PL, Vogel SS, Miyake K, Terasaki M. Patching plasma membrane disruptions with cytoplasmic membrane. *J Cell Sci* 2000;113(Pt 11):1891–902.
- [31] Wang X, Campos B, Kaetzel MA, Dedman JR. Secretion of annexin V from cultured cells requires a signal peptide. *Placenta* 2001;22:837–45. <http://dx.doi.org/10.1053/plac.2001.0724>.
- [32] Reddy A, Caler EV, Andrews NW. Plasma membrane repair is mediated by  $\text{Ca}^{2+}$ -regulated exocytosis of lysosomes. *Cell* 2001;106:157–69.
- [33] Idone V, Tam C, Goss JW, Toomre D, Pypaert M, Andrews NW. Repair of injured plasma membrane by rapid  $\text{Ca}^{2+}$ -dependent endocytosis. *J Cell Biol* 2008;180:905–14.
- [34] Tam C, Idone V, Devlin C, Fernandes MC, Flannery A, He X, et al. Exocytosis of acid sphingomyelinase by wounded cells promotes endocytosis and plasma membrane repair. *J Cell Biol* 2010;189:1027–38.
- [35] Tam C, Flannery AR, Andrews N. Live imaging assay for assessing the roles of  $\text{Ca}^{2+}$  and sphingomyelinase in the repair of pore-forming toxin wounds. *J Vis Exp* 2013:e50531.
- [36] Andrews NW, Almeida PE, Corrotte M. Damage control: cellular mechanisms of plasma membrane repair. *Trends Cell Biol* 2014;24:734–42.

- [37] Babiychuk EB, Monastyrskaya K, Potez S, Draeger A. Intracellular Ca(2+) operates a switch between repair and lysis of streptolysin O-perforated cells. *Cell Death Differ* 2009;16:1126–34.
- [38] Babiychuk EB, Monastyrskaya K, Potez S, Draeger A. Blebbing confers resistance against cell lysis. *Cell Death Differ* 2011;18:80–9.
- [39] Jimenez AJ, Maiuri P, Lafaurie-Janvore J, Divoux S, Piel M, Perez F. ESCRT machinery is required for plasma membrane repair. *Science* 2014;343:1247136.
- [40] Draeger A, Monastyrskaya K, Babiychuk EB. Plasma membrane repair and cellular damage control: the annexin survival kit. *Biochem Pharmacol* 2011;81:703–12.
- [41] Bansal D, Miyake K, Vogel SS, Groh S, Chen C-C, Williamson R, et al. Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature* 2003;423:168–72.
- [42] Lennon NJ, Kho A, Bacskai BJ, Perlmutter SL, Hyman BT, Brown RH. Dysferlin interacts with annexins A1 and A2 and mediates sarcolemmal wound-healing. *J Biol Chem* 2003;278:50466–73.
- [43] Liu J, Aoki M, Illa I, Wu C, Fardeau M, Angelini C, et al. Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nat Genet* 1998;20:31–6.
- [44] McNeil AK, Rescher U, Gerke V, McNeil PL. Requirement for annexin A1 in plasma membrane repair. *J Biol Chem* 2006;281:35202–7.
- [45] Cai C, Masumiya H, Weisleder N, Matsuda N, Nishi M, Hwang M, et al. MG53 nucleates assembly of cell membrane repair machinery. *Nat Cell Biol* 2009;11:56–64.
- [46] Roostalu U, Strähle U. In vivo imaging of molecular interactions at damaged sarcolemma. *Dev Cell* 2012;22:515–29.
- [47] Defour A, Van der Meulen JH, Bhat R, Bigot A, Bashir R, Nagaraju K, et al. Dysferlin regulates cell membrane repair by facilitating injury-triggered acid sphingomyelinase secretion. *Cell Death Dis* 2014;5:e1306.
- [48] Brachvogel B, Dikschas J, Moch H, Welzel H, von der Mark K, Hofmann C, et al. Annexin A5 is not essential for skeletal development. *Mol Cell Biol* 2003;23:2907–13.
- [49] Bouter A, Gounou C, Bérat R, Tan S, Gallois B, Granier T, et al. Annexin-A5 assembled into two-dimensional arrays promotes cell membrane repair. *Nat Commun* 2011;2:270.
- [50] Tait JF, Sakata M, McMullen BA, Miao CH, Funakoshi T, Hendrickson LE, et al. Placental anticoagulant proteins: isolation and comparative characterization four members of the lipocortin family. *Biochemistry* 1988;27:6268–76.
- [51] Römisch J, Heimbürger N. Purification and characterization of six annexins from human placenta. *Biol Chem Hoppe-Seyler* 1990;371:383–8.
- [52] Buhl WJ, García MT, Zipfel M, Schiebler W, Gehring U. A series of annexins from human placenta and their characterization by use of an endogenous phospholipase A2. *Eur J Cell Biol* 1991;56:381–90.
- [53] Funakoshi T, Heimark RL, Hendrickson LE, McMullen BA, Fujikawa K. Human placental anticoagulant protein: isolation and characterization. *Biochemistry* 1987;26:5572–8.
- [54] Hay WW. Placental transport of nutrients to the fetus. *Horm Res* 1994;42:215–22.
- [55] Benirschke K, Kaufmann P. *Pathology of the human placenta*. Springer Science & Business Media; 2000.
- [56] Evain-Brion D, Malassine A. Human placenta as an endocrine organ. *Growth Horm IGF Res* 2003;13(Suppl. A):S34–7.
- [57] Burton GJ, Jones CJP. Syncytial knots, sprouts, apoptosis, and trophoblast deportation from the human placenta. *Taiwan J Obstet Gynecol* 2009;48:28–37.
- [58] Knight M, Redman CW, Linton EA, Sargent IL. Shedding of syncytiotrophoblast microvilli into the maternal circulation in pre-eclamptic pregnancies. *Br J Obstet Gynaecol* 1998;105:632–40.
- [59] Johansen M, Redman CW, Wilkins T, Sargent IL. Trophoblast deportation in human pregnancy – its relevance for pre-eclampsia. *Placenta* 1999;20:531–9.
- [60] Vandr  DD, Ackerman WE, Kniss DA, Tewari AK, Mori M, Takizawa T, et al. Dysferlin is expressed in human placenta but does not associate with caveolin. *Biol Reprod* 2007;77:533–42.
- [61] Carmelle R, Degrelle S, Plawinski L, Bouvet F, Gounou C, Evain-Brion D, et al. Annexin-A5 promotes membrane resealing in human trophoblasts. *Biochim Biophys Acta* 2015. <http://dx.doi.org/10.1016/j.bbamcr.2014.12.038>.
- [62] Vénien-Bryan C, Lenne PF, Zakri C, Renault A, Brisson A, Legrand JF, et al. Characterization of the growth of 2D protein crystals on a lipid monolayer by ellipsometry and rigidity measurements coupled to electron microscopy. *Biophys J* 1998;74:2649–57.
- [63] Saurel O, Cézanne L, Milon A, Tocanne JF, Demange P. Influence of annexin V on the structure and dynamics of phosphatidylcholine/phosphatidylserine bilayers: a fluorescence and NMR study. *Biochemistry* 1998;37:1403–10.
- [64] Krikun G, Lockwood CJ, Wu XX, Zhou XD, Guller S, Calandri C, et al. The expression of the placental anticoagulant protein, annexin V, by villous trophoblasts: immunolocalization and in vitro regulation. *Placenta* 1994;15:601–12.
- [65] Draeger A, Schoenauer R, Atanassoff AP, Wolfmeier H, Babiychuk EB. Dealing with damage: plasma membrane repair mechanisms. *Biochimie* 2014;107(Pt A):66–72.