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Review: Placental syncytiotrophoblast membranes – domains, subdomains and microdomains

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ABSTRACT

Human placental syncytiotrophoblast (STB) is an epithelium responsible for materno-fetal exchange. Ions play multiple roles in STB, as in other transport epithelia. We have been interested in the character and functional expression of ion channels in STB membrane fractions. Characterization of ion channels and their relationship with different domains, subdomains and microdomains of STB membranes is important to explain the intracellular mechanisms operating in the placental barrier. The aim of this paper is to summarize our work on this subject. We isolated and purified basal membrane (BM) and two fractions from the apical membrane, a classical fraction (MVM) and a light fraction (LMVM). They were used either for reconstitution into giant liposomes or for transplantation into Xenopus oocyte membranes followed by electrophysiological recordings to characterize chloride and cationic channels in STB from term human placenta. In addition, Western blot analysis, using ion channel antibodies, was performed on purified apical and basal membrane fractions. We also reported the presence of two functional microdomains (lipid rafts) in LMVM and MVM, using detergent resistant membranes (DRMs) and cholesterol-sensitive depletion. Moreover we found evidence of cytoskeletal participation in lipid rafts of different composition. Our results contribute to knowledge of the ion channels present in STB membranes and their participation in the physiology of this epithelium in normal and pathological pregnancies.

1. Introduction

Fetal growth and development are dependent on the transport functions of the placenta. Human syncytiotrophoblast (STB) separates maternal and fetal blood and may be viewed as an epithelium segregating two fluid compartments. It is responsible for many of the functions performed by the placenta, including transport of oxygen, nutrients and waste products, hormone production and immune tolerance [1]. This syncytium forms the outer surface of the placental villous tree. Its apical plasma membrane faces the maternal intervillous space and its basal plasma membrane faces the endothelium of the fetal capillaries. Owing to its syncytial nature, it constitutes the major barrier to maternal–fetal exchange of water, nutrients and electrolytes [2,3]. Transport of solutes from maternal to fetal circulation must involve movement across both plasma membranes. Many proteins participate in this function, including transporters, exchangers and ion channels [3]. In STB, as in other epithelia, ion channels are essential for a variety of physiological processes, including cell volume regulation, solute transport, maintenance of membrane potential and signal transduction.

Epithelial cells polarize during differentiation, leading to the formation of two distinct plasma membrane domains, the apical and basal domains with distinct protein and lipid compositions (Fig. 1). The organization and maintenance of ion channels within a specific plasma membrane domain plays an important role in determining its physiological function. Maintenance of ion channels within a particular plasma membrane domain is, in part, mediated through interactions with cytoskeletal proteins. In addition, interactions between epithelial ion channels and cytoskeletal proteins play a role in the regulation of channel activity and in their intracellular trafficking [4].

Ion channels do not work alone. Their interaction with membrane lipids can be highly specific and is often essential to their functional and structural integrity [5]. In particular, many studies have demonstrated that membrane cholesterol is a major regulator of ion channels (see review [6]). Cellular membranes were once considered to exist as a homogeneous “liquid crystalline phase”. However, the lateral organization of the bilayer is heterogeneous with selective association of lipids and proteins in discrete microdomains of the membrane: the lipid raft [7]. These domains were initially defined operationally as detergent resistant membranes (DRMs) since they remained insoluble in non-ionic detergents. The DRMs were found to be relatively abundant in

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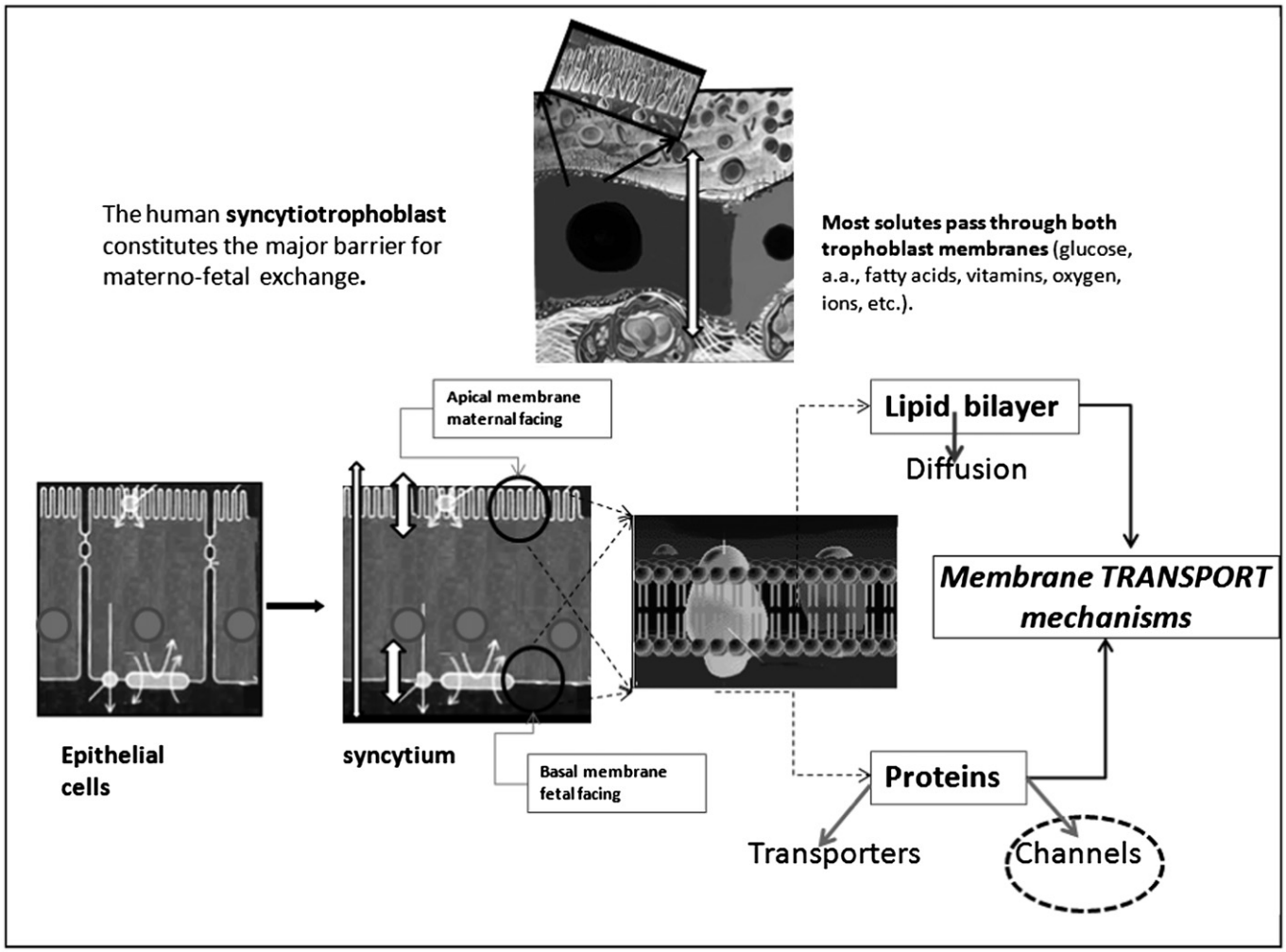

Fig. 1. The syncytiotrophoblast (STB) extends over the surfaces of all villous trees. "Unlike other epithelia, the syncytiotrophoblast is not composed of individual cells but represents a continuous, uninterrupted, multinucleated surface layer without separating cell borders" [1]. Long arrows represent the transplacental exchange between maternal and fetal blood. The small arrows represent transport through plasma membranes of STB.

cholesterol, sphingomyelin, and glycolipids such as GM1 ganglioside. At the Keystone Symposium on Lipid Rafts and Cell Function, lipid rafts were defined as, "small and highly dynamic membrane microdomains (10–20 nm) that are enriched in cholesterol and sphingolipids that compartmentalize cellular processes" [8]. This kind of arrangement creates membrane regions with distinct properties and structural composition.

Segregation of proteins into domains, subdomains and microdomains is closely linked to protein function. Therefore it is relevant to study the relationship of ion channels with subdomains and microdomains in the normal placenta and ask whether alterations in their segregation can be related to pathology as in other cells and tissues [9]. Our interest has been to study the functional characteristics of ion channels, their expression and their relationship with the domains, subdomains and microdomains of STB membranes.

2. Domains and subdomains in human syncytiotrophoblast

The apical and basolateral plasma membranes of epithelial cells differ strongly in lipid and protein composition. Human STB is a multinucleated epithelium formed by the fusion of precursor cytotrophoblast cells producing a syncytium that separates maternal and fetal blood. To control solute flow in one defined direction, STB maintains a polarized organization with two distinct plasma membrane domains (Fig. 1). Analysis of transplacental transport implies the understanding of transport through both the fetal-facing basal membrane and the maternal-facing microvillous membrane. Thus development of a cellular model of syncytiotrophoblast ion transport requires characterization of ion channels present in the apical and basal membranes. The isolation and purification of apical and basal membranes have provided us with the material for investigation of specific placental transport mechanisms, including ion channels. Human placental microvillous membrane vesicles were prepared as described in Jimenez et al. [10], a method that allows simultaneous isolation of apical and basal membranes from the same placenta from term pregnancy. This method is a modification of that described by Illsley et al. [11] incorporating some steps from our previous protocol for obtaining apical membrane and a step to isolate plasma membrane free of mitochondrial membranes. Interestingly using differential sucrose density migration, we were able to isolate two fractions from the apical (maternal-facing) membrane: the classical microvillous membrane (MVM), used by us and other authors to study transport mechanisms, and a light microvillous membrane (LMVM) [10] (Fig. 2). The purity of isolated membranes was evaluated routinely by enzymatic assays, binding studies and immunoblotting for marker proteins. Alkaline phosphatase is a marker enzyme

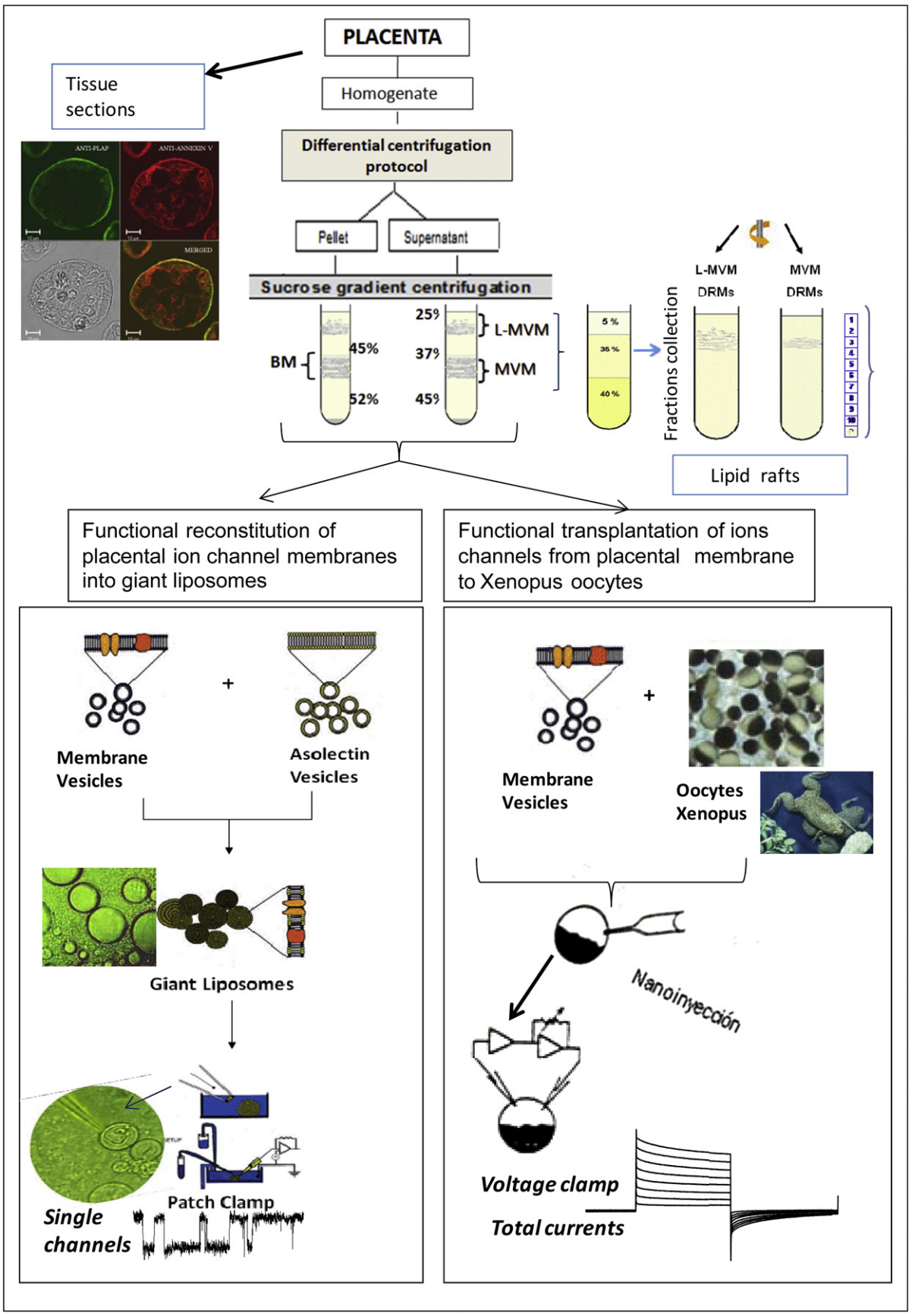

Fig. 2. Purified syncytiotrophoblast membranes were obtained through a double protocol of apical and basal membrane isolation, which includes differential centrifugations, basal membrane precipitation with $MgCl_2$ and band separation through sucrose step gradients [10]. Purified membranes were used for reconstitution into giant liposomes [20] or incorporation into Xenopus oocyte membranes [29], followed by electrophysiological recordings. In addition, microdomains were extracted from MVM and LMVM using 1% Triton X-100 and sucrose flotation. Afterwards, we collected the samples and probed them by Western and dot blot for different rafts and non-rafts markers. Enzymatic assays, Western blots using specific markers and ion channel antibodies were performed on purified apical and basal membrane fractions, and lipid rafts to support our functional findings.

for apical epithelial membranes like STB. Adenylate cyclase and β-adrenergic receptor, both located in the basal membrane but not in the microvillous surface [10,11], have been historically used for the STB basal membrane. The purity and cross-contamination of the purified membranes were comparable to those of other preparations reported for single or paired apical and basal membrane purification.

2.1. Subdomains LMVM and MVM

We have described the characteristics of two fractions (LMVM and MVM) from the apical STB domain. We determined that both fractions are enriched in the apical membrane markers. However, they have specific characteristics that suggest a possible association with microvillous membrane subdomains [12]. The strategy was to determine alkaline phosphatase (PLAP) activity, cholesterol/protein ratio and to identify proteins from the specialized cytoskeleton (Fig. 3). Alkaline phosphatase activity in LMVM and MVM was enriched ∼26 and ∼21-fold, respectively, compared to the initial tissue homogenate and Western blot analysis also demonstrated the presence of alkaline phosphatase in both LMVM and MVM fractions, suggesting that both fractions came from the apical domain of STB. The cholesterol content of the two apical fractions was such that the cholesterol/protein ratio of LMVM was 1.7-fold higher than the MVM ratio. Ezrin, β-actin and cytokeratin 7 (CK-7), which are localized in different parts of the cytoskeletal apical domain [13–16], were analyzed for LMVM and MVM. The results showed that ezrin and β-actin proteins, putatively associated with the microvillous finger-like projection region, were significantly more concentrated in the MVM than in the LMVM fraction. CK-7, a component of the intermediate filaments in whole trophoblast epithelia, was more abundant in LMVM than in MVM [12]. In summary, our results suggest that LMVM corresponds to the apical subdomain forming the bottom part of the microvilli (the base of these finger-like projections), which interacts with the intermediate filaments of the trophoblast cytoskeleton. MVM could then correspond to the microvillous finger-like region of the apical subdomain of STB. Fig. 3 shows a possible model of MVM and LMVM localization in the microvilli of STB.

In order to characterize our purified membranes from a functional point of view, we used them either in reconstitution of giant liposomes or for incorporation into Xenopus oocyte membranes for electrophysiological recording (Fig. 2). We have characterized calcium and chloride currents in the basal membrane [17,18] and several types of channels in the apical membrane (Fig. 4). Thus, in the classical MVM, we have described a maxi chloride channel and its regulation [19–24], a non-specific cation channel [25] and the epithelial sodium channel ENaC [26]. In the light microvillous membrane (LMVM) we have described potassium channels [27–29]. Our electrophysiological studies evidenced different frequencies in the occurrence of the electrical activity observed for the maxi chloride channel and potassium channels in MVM and LMVM, suggesting a possible asymmetrical distribution between these domains. Since we have not performed *in extenso* functional studies for the different types of channels in all membrane fractions, however, we cannot rule out their presence in one fraction or the other.

3. Microdomains: two distinguishable lipid raft subsets from purified MVM and LMVM

We studied differential expression of microdomain lipid rafts in both purified apical membrane fractions (LMVM and MVM) from STB and explored their possible associations with the cytoskeleton.

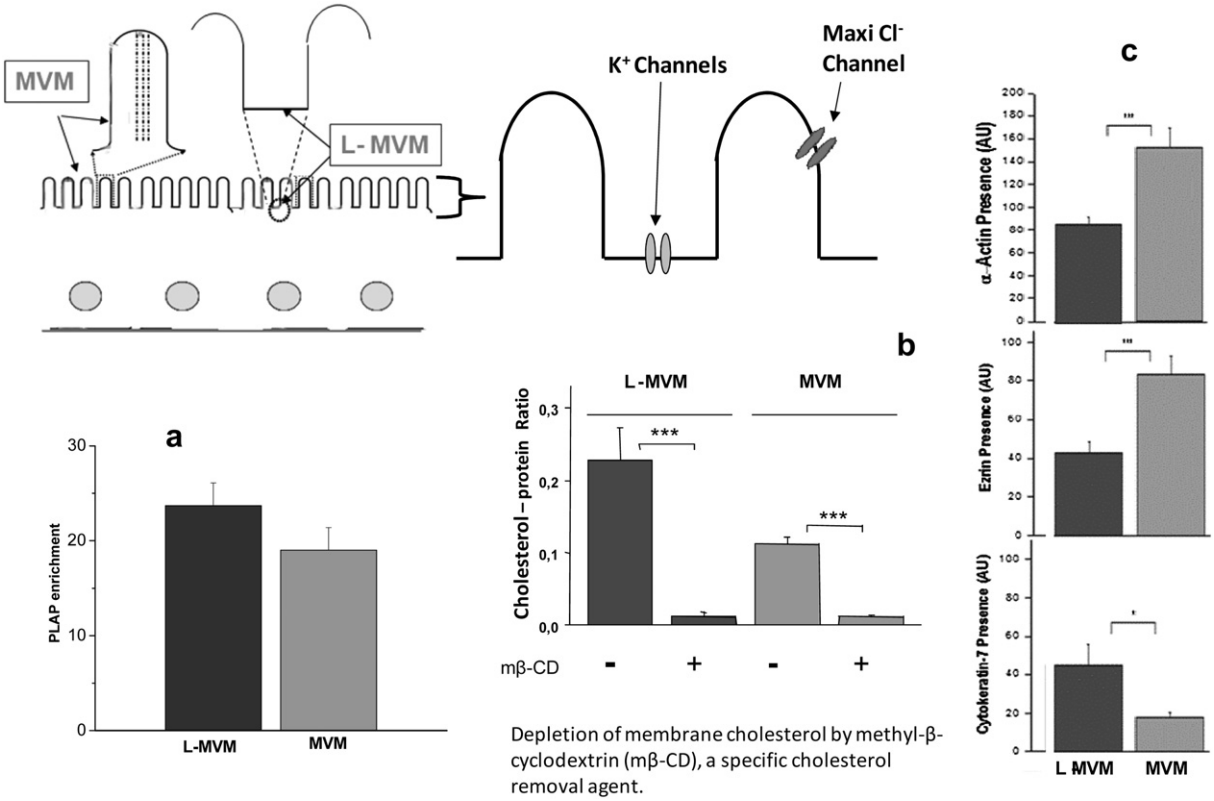

Fig. 3. The two fractions from the apical membrane (MVM, LMVM) have: (a) similar enrichment of placental alkaline phosphatase (PLAP), an epithelial apical membrane marker, (20–25-fold relative to the initial tissue homogenate). Data are courtesy of C. Vallejos; (b) different cholesterol content; (c) differential expression of cytoskeletal proteins (CK-7, ezrin and β-actin).

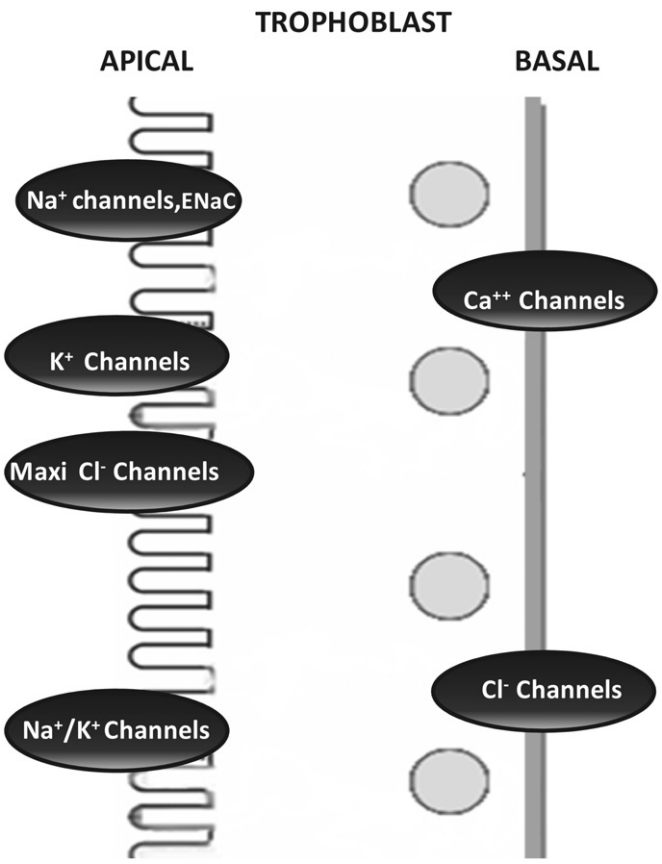

Fig. 4. Summary our contributions to the transplacental transport model. We have investigated the characteristics of anionic and cationic channels present in these membranes: a maxi chloride channel [20–24], a non-specific cation channel [25], and a potassium channel which allow ion permeation in the apical membrane of term human placentas [29]. We have also characterized calcium [18] and chloride currents present in the basal membrane [17].

In both purified fractions we observed features of sphingolipid/cholesterol-enriched membrane microdomains characterized by their resistance to detergent extraction and their ability to float in density gradient centrifugation. However, we obtained two different DRMs as described in Godoy and Riquelme [12] (Fig. 2). Analysis of sucrose density gradient fractions obtained from the DRM protocol was done with three raft markers: PLAP, Anx-2 (a protein associated with cholesterol and cytoskeleton) and GM1 (a glycosphingolipid marker). PLAP and GM1 were higher in LMVM than in MVM lipid rafts. Anx-2 signal appeared as a small peak in the raft fractions of LMVM, and was totally absent in the corresponding fractions from MVM, where the total marker appeared in the non-raft zone. Transferrin receptor (hTf-R), a non-raft marker, was absent from LMVM and MVM raft fractions, indicating that the presence of PLAP, GM1 and Anx-2 in those fractions was not due to contamination from non-raft zones. Observed differences in lipid rafts from apical membranes might be explained by LMVM and MVM deriving from two distinct apical subdomains and the differential cytoskeletal protein expression may be linked to the differential distribution of raft markers between the two apical membrane fractions of STB [12] (Fig. 5A).

In summary, our data support the heterogeneity of STB apical membrane domains, specifically in their protein and lipid composition, substantiating the existence of two purified apical fractions, MVM and LMVM, which may constitute two structurally distinct regions or subdomains in the microvillous membrane. These conclusions are in agreement with a number of studies that suggest heterogeneity in the apical domains and propose that microvilli contain subdomains distinguished by the localization of cytoskeletal protein [30,31].

4. Placental lipid rafts and ion channels

Cell polarity in STB is critical for vectorial transport between maternal and fetal blood. The existence of distinct sets of proteins on the apical and basal membranes enables these membrane domains to function differently. There are multiple differences in cytoskeleton protein distribution and in lipid composition between apical and basal membrane. In epithelial cells in general, cholesterol and sphingomyelin are found in the apical membrane and are the main lipids involved in lipid rafts, whereas phosphatidylcholine and phosphatidylinositol dominate in the basolateral membrane.

The variable ratio of lipids in these membrane domains affects the fluidity of the membrane, the diffusion speed across the membrane and the function of intrinsic membrane proteins [32]. A number of studies have demonstrated that lipid composition affects ion channel function; among them, electrophysiological measurements of voltage-dependent channel activity, testing different lipid bilayer environments, indicate that voltage-dependent K^+ channels are dependent on negatively charged phospholipid molecules [33–35]. Additionally, a variety of ion channels have been shown to be specifically modulated by cholesterol (see review [6]). Usually the sensitivity of membrane proteins to cholesterol is associated with their partitioning to cholesterol-rich membrane domains or lipid rafts [36]. The localization of ion channels in lipids rafts is important for correct electrical signaling in different physiological systems, and ensures that the channels are located close to the signaling molecules that regulate them.

During development of epithelial polarity, the localization, organization and function of cellular organelles and cytoskeletal structures are strictly determined [37–39]. Skeletal proteins have a central role in targeting of different membrane proteins, including ion channels, by forming complexes with transmembrane proteins. The interaction of ion channels with the cytoskeletal protein in epithelial cells not only maintains the polarized expression of ion channels within specific membrane domains, but is also involved in the intracellular trafficking and regulation of channel activity [4]. In particular Montalbetti et al. [40] have demonstrated cytoskeletal regulation of a calcium channel in STB.

It is assumed that ion channels in placental membranes have similar functions to other epithelial channels, and that their activity and consequently their influence on placental function can be substantially modulated by their localization in specialized domains, subdomains and microdomains.

At present our aim is to determine the differential targeting of potassium channel subtypes (TREK, Kir, TASK and Kv), epithelial sodium channel (ENaC) and cytoskeleton proteins (ezrin, actin, CK-7) to either raft or non-raft domains. Our results have shown that, although these proteins are located in both fractions of the apical membranes, some of them are partitioned into cholesterol-rich plasma membrane microdomains or “lipid rafts”. For example, our preliminary results show that the inward rectifying potassium channel Kir 2.1 but not TREK 1 targets to specialized cholesterol-rich lipid raft domains on LMVM and MVM (Fig. 5B).

We have been working on a similar analysis in placental membranes from pathological pregnancies such as intrauterine growth restriction and preeclampsia. Our preliminary results show that lipid raft subsets from pathological MVM and LMVM have a different pattern for markers (PLAP, hTf-R, Anx-2 and GM1) compared with the one observed in apical rafts from normal pregnancies [41], Furthermore, in placentas from pathological pregnancies we did not find differences in expression of

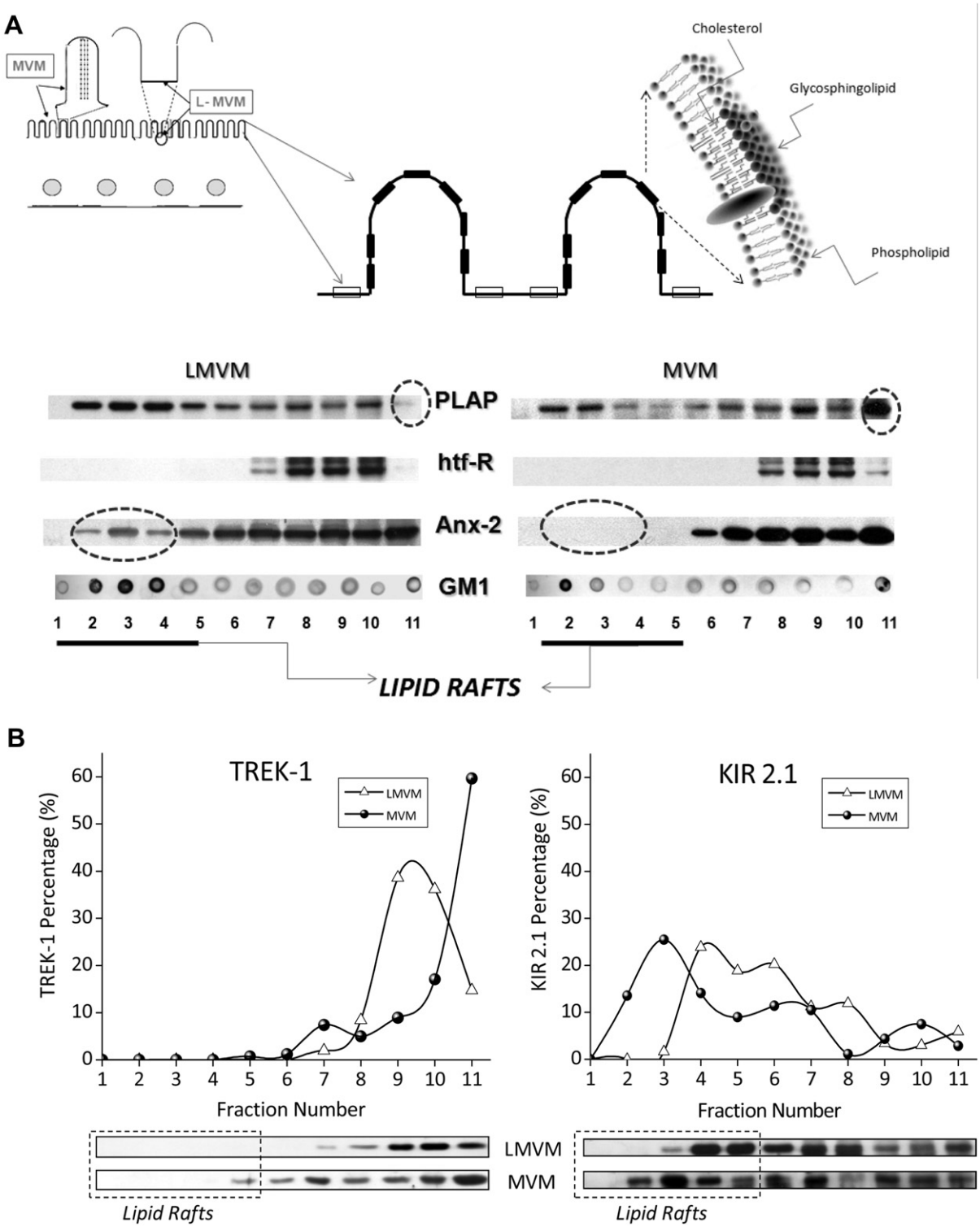

Fig. 5. A) We reported the expression of two distinguishable microdomains (lipid rafts) in purified microvillous membranes (both MVM and LMVM) using detergent resistant membranes and cholesterol-sensitive depletion. B) Distribution of two subtype of K^+ channels between raft and non-raft membrane fractions. These preliminary results show that Kir 2.1 is found in lipid rafts from both placental apical membranes. Data are courtesy of M. Berrios.

cytoskeletal proteins in the apical membrane fractions analogous to those found in apical membranes of normal pregnancy, suggesting that the apical domain, and thus apical rafts, could be less well organized in pathological placentas than in normal placentas [41].

In conclusion, since many functions of the epithelial membrane are based on membrane structure–function correlation, the membrane compartmentalization of the channels and cytoskeleton proteins in the STB may play a role in different intracellular mechanisms in the placental epithelial barrier.

There is a growing number of studies about the relationship between lipid microdomains and ion channels in health and disease [42]. Among them, O'Connell et al. [43], discuss the

localization of ion channels to membrane microdomains within the cardiovascular system and the implications that such localization has for channel trafficking and regulation. A similar type of study should be encouraged for the placenta, because this may be critical to a better understanding of the molecular mechanisms of transport processes in the placental STB from normal and pathological pregnancies.

Conflict of Interest Statement

The authors state they have no conflict of interest.

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