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TNAP, an essential player in membrane lipid rafts of neuronal cells

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Abstract

The tissue non-specific alkaline phosphatase (TNAP) is a glycosyl-phosphatidylinositol (GPI) anchored glycoprotein which exists under different forms and is expressed in different tissues. As the other members of the ecto-phosphatase family, TNAP is targeted to membrane lipid rafts. Such micro domains enriched in particular lipids, are involved in cell sorting, are in close contact with the cellular cytoskeleton and play the role of signaling platform. In addition to its location in functional domains, the extracellular orientation of TNAP and the fact this glycoprotein can be shed from plasma membranes, contribute to its different phosphatase activities by acting as a phosphomonoesterase on various soluble substrates (inorganic pyrophosphate -PPi-, pyridoxal phosphate -PLP-, phosphoethanolamine -PEA-), as an ectonucleotidase on nucleotide-phosphate and presumably as a phosphatase able to dephosphorylate phosphoproteins and phospholipids associated to cells or to extra cellular matrix. More and more data accumulate on an involvement of the brain TNAP both in physiological and pathological situations. This review will summarize what is known and expected from the TNAP localization in lipid rafts with a particular emphasis on the role of a neuronal microenvironment on its potential function in the central nervous system.

Keywords: TNAP activity, neuronal function, extra cellular matrix, protein network, microenvironment.

9.1 - The tissue non specific alkaline phosphatase, a GPI-anchored glycoprotein present under different forms in different cellular spaces

9.1.1 -The different forms of TNAP: The tissue non specific alkaline phosphatase (TNAP) belongs to a large family of GPI-anchored ecto-phosphatases whose main function is that of a phospho-monoesterase with an optimal *in vitro* enzymatic activity at alkaline pH (Halling Linder et al. 2012). In contrast to the tissue specific intestinal and placental alkaline phosphatases, TNAP is found in different organs (Hoshi et al. 1997), and exhibits variable amount of expression. For instance, a higher amount of TNAP is expressed in the bone than in the liver and the kidney. Moreover, studies on the regulation of TNAP expression during embryonic life and development (Fonta et al. 2004; Narisawa et al. 1994), are in support of its role during differentiation of the nervous system (Delic and Zimmermann 2011). Depending on promoter regulation and the usage of the first non coding exon, the bone or the liver transcript will be found, leading to a first level of variability in TNAP expression depending on cell specific transcriptional factors (Brun-Heath et al. 2011).

TNAP is a membrane protein maturing in the secretory pathway. It is anchored in the outer leaflet of the plasma membrane through a glycosyl-phosphatidylinositol moiety attached at its C terminus and, as such, localized in raft micro domains. In addition, TNAP is found as a soluble protein in the serum and is also present in extracellular spaces where it exerts functions either associated to matrix vesicles during bone mineralization (Ciancaglini et al. 2006; Wuthier and Lipscomb 2011) or to mucosal surfactant as a defense to pathogens in the lung (Koyama et al. 2002). Another level of diversity is linked to post-translational modifications. TNAP contains 5 potential N-glycosylation sites and a C-terminal GPI moiety. Not only the number of N-glycans relating to the variable occupancy of asparagin sites in the ER (Jones et al. 2005), but also the N-

glycan (Stanley 2011) and the GPI (Maeda and Kinoshita 2011) structures maturing during trafficking in the Golgi apparatus, can vary according to the cellular type. Therefore, different glycoforms of TNAP having the same amino-acid composition are expected and various forms of TNAP have indeed been described by HPLC and electrophoresis analyses (Halling Linder et al. 2013). In this respect, the TNAP N-glycans of the bone isoform contain sialic acid while the liver one does not, and the TNAP function could vary accordingly (Halling Linder et al. 2009).

9.1.2- Implication of a GPI anchoring: The glycosyl-phosphatidylinositol (GPI) core present at the C terminus of different proteins can insert into plasma membrane leaflets. This lipid moiety not only allows the protein attachment to the cell membrane but is also directly involved in cell signaling and trafficking. In the absence of a cytoplasmic domain, intracellular signaling cascade downstream a GPI-protein must be initiated through interaction of the ectodomain of such anchored protein with extracellular domains of transmembrane proteins or of the GPI region with intramembranous scaffold proteins such as caveolin present in specialized lipid rafts (Mouillet-Richard et al. 2000). Indeed, the GPI, by its capacity to interact with cholesterol, is responsible for the localization of such anchored proteins into membrane lipid rafts and replacement of a GPI-anchor by a transmembrane sequence affects raft localization and regulates uptake of receptors. Conversely, the structure of the GPI may affect lipid rafts and membrane composition (Chatterjee and Mayor 2001). Moreover, the complete extracellular orientation of GPI anchored glycoproteins makes them able to interact with extracellular matrix (ECM) and soluble compounds present in the extracellular space.

GPI-anchored proteins can be shed from the membrane by phosphatidylinositol-phospholipases (PIPLC, PIPLD) that specifically recognize GPI structures, then releasing the protein moiety from its anchor. Soluble TNAP is indeed detected in the serum. In contrast, proteins with their

GPI (Kooyman et al. 1998) have been demonstrated to be able to integrate the plasma membrane of other cells, a mechanism known as the “painting” of membranes. This illustrates the possibility for such GPI anchored proteins to transfer between cells as well as their affinity for lipids. *In vitro* experiments indicate that such exogenously added proteins may integrate different micro domains (Legler et al. 2005) of new membranes without loss of their activity as shown for different GPI anchored proteins (Bate and Williams 2011; Premkumar et al. 2001). However, most of these observations have been obtained experimentally and with artificial membranes, and whether TNAP secreted in the circulation is released by phospholipases or may carry its GPI is not often documented.

As already noticed, the GPI moiety can be differentially modified according to tissues and cell types expressing the anchored protein. Importance of this structure in the case of TNAP has been highlighted by the fact that mutations affecting different steps in the synthesis of the glycolipid anchor are involved in human diseases of hyperphosphatasia. Details on GPI synthesis are exposed in the chapter 16.

During biosynthesis, maturation of lipid rafts takes place in the secretory pathway where GPI anchor proteins are sorted from other proteins and packaged into ER-derived vesicles (Kinoshita et al. 2013). Besides raft association, the GPI lipid moiety confers specific membrane trafficking properties to the attached protein, such as apical targeting in polarized cells and axonal and dendritic localization in neuronal cells (Chatterjee and Mayor 2001). Mechanisms of GPI anchored protein endocytosis are less defined and could depend on the cell type and the protein. While different pathways have been described, endocytosis of GPI-proteins is suspected to be important in the regulation of cellular signaling (Lakhan et al. 2009). TNAP has also been found in secreted vesicles during mineralization. These vesicles are formed either intracellularly from

multi vesicular body released as exosomes or directly at the plasma membrane as is the case with the matrix vesicles forming by interaction with components of the ECM, including binding of membrane anchored TNAP with matrix collagens II and X (Wuthier and Lipscomb 2011). Briefly, secreted exosomes are formed within the multi vesicular bodies (MVB) arising from the conjunction of vesicular transport between the endocytic and the secretory pathway. The first step is the formation of endocytic vesicles and the sequestration of early endosomes targeted to MVB where they are incorporated as intraluminal vesicles by invagination of the MVB membrane. MVB are either degraded by fusion with the lysosome or released in the extracellular space as exosomes upon fusion with the plasma membrane. Extracellular vesicles (EVs) are also secreted from neuronal cells and have recently been shown to be important for cell to cell communication operating both during physiological or pathological process in the central nervous system. For instance, in normal situation EVs are involved in the maintenance of axonal integrity during myelination by allowing a bidirectional communication between neurons and oligodendrocytes. They are also suggested to have a role in interneural exchange of information within functional brain networks. EVs which are present in high amount in some brain disorders such as neurodegenerative diseases have the capability to propagate the pathology by allowing cell transmission of toxic proteins as described for the prion protein or the amyloid β in Alzheimer disease (Rajendran et al. 2014). EVs harbor different composition according to their cell origin, and different signatures according to the neurodegenerative disorder. Therefore, it would be of interest to look whether TNAP could be present at the surface of such EVs.

9.1.3- TNAP in membrane lipid rafts: Since TNAP, as a GPI anchored protein, is enriched in membrane lipid rafts (MLR), its function must also relate to some features of these specialized micro domains. Although the definition of lipid rafts is still into debate, their

existence as functional signaling platforms is fully recognized (Lindner and Naim 2009). These micro domains, which are enriched in cholesterol and sphingolipids, form lipid ordered patches at the plasma membrane surrounded by disordered lipid phase containing phospholipids and unsaturated fatty acids. This confers differences in the lateral diffusion of membrane proteins in raft and non raft domains and in their biochemical properties, such as the solubility in non ionic detergents, one of the characteristics used to define rafts (Silvius 2005). The specific lipid and protein composition of rafts is in support of the involvement of membrane micro domains in different cellular processes including cell sorting, endocytosis and signaling (Head et al. 2014).

A contact with the actin and tubulin components of the cytoskeleton is required to maintain, beside cell integrity, the organization and distribution of micro domains at the plasma membrane. Interaction of actin filament with MLR allows their correct repartition during polarization of epithelial, endothelial and neuronal cells, in particular during growth cone formation important for axonal guidance. Such stabilization and coalescence of membrane subdomains lead to the formation of regions of high protein density and specific composition involved in adherence and cell signaling (Head et al. 2014).

Composition and dynamics of MLR will also depend on the cell type and ECM microenvironment. The numerous cellular functions associated to MLR depend on a fine interplay with the cytoskeleton but also with the ECM, both being important to cellular communication and regulatory processes. However, much has still to be learnt about the way subcellular assembly in particular in neurons occurs and regulates functional properties. GPI anchored proteins, such as TNAP, may contribute by providing scaffold properties to this organization.

9.2- TNAP, different forms, different functions.

9.2.1- The tissue specificity. Little is known about the regulation and the role of the different TNAP isoforms. However, since the enzymes responsible for post-translational modifications in the glycosylation pathway may vary between organs and even between tissues of an organ, such modifications could contribute to variation in the potential of the extracellular domain of TNAP to interact with different substrates. The role of the glycosylation on TNAP activity has been suggested (Halling Linder et al. 2009). In addition, the trafficking, degradation and function of TNAP have been shown to be affected by different mutations (Chapter 2) interfering with either the GPI or N-glycosylation pathways and responsible for genetic disorders of hyperphosphatasia (Chapter 16) or hypophosphatasia (Ishida et al. 2003; Ito et al. 2002; Sultana et al. 2013), respectively.

The tissue in which TNAP is expressed may also influence its function by the fact that encountered substrates may differ whatever the enzyme being circulating or associated to raft micro domains. Therefore, TNAP exhibits specific or shared activities in different organs (see Chapter 8), which are impacted by the different ligands that can be dephosphorylated by TNAP. Beside circulating phosphomonoester substrates, such as PPi important in bone mineralization, and PLP (a derivative of vitamin B6) or nucleotide-phosphate involved in neurotransmitter metabolism, some phosphoproteins and phospholipids have also been described to be TNAP substrates therefore, enlarging the potential roles of this phosphatase. Dephosphorylation of such substrates may be involved in functions as different as (i) defense against pathogen by detoxification of LPS endotoxin or by counteracting inflammatory processes in the lung, intestine or kidney (Goldberg et al. 2008; Kapojos et al. 2003; Koyama et al. 2002), (ii) regulation of membrane ion channel such as CFTR in cystic fibrosis (Becq et al. 1994) or in secretory activity

of the liver biliary epithelium (Alvaro et al. 2000), (iii) transport activities as is the case of insulin in brain micro vessels or drug and detoxification (Calhau et al. 2002a; Calhau et al. 2002b; Martel et al. 1996), and (iv) yet unknown functions involving interaction with other plasma membrane proteins (Scheibe et al. 2000) or matrix protein, such as laminin important for neuronal differentiation (Ermonval et al. 2009a). As reviewed by Diaz-Hernandez (Chapter 18), TNAP also acts on extracellular phosphorylated tau with implication in Alzheimer' disease (Diaz-Hernandez et al. 2010).

Importantly, although TNAP substrates are circulating in blood vessels and TNAP is expressed in different organs, TNAP exhibits functions that are tissue specific. This is supported by the observation of an effect of vitamin B6 on neurological dysfunction (seizure) but not on mineralization or other aspects of TNAP deficiency (Narisawa et al. 2001). In line with this, human newborns with perinatal hypophosphatasia did not exhibit epileptic symptoms anymore when treated with pyridoxin (Yamamoto et al. 2004) but still died rapidly from further complications independent of PLP specificity (Nunes et al. 2002). Moreover, while TNAP allows bone mineralization by usage of its P_{Pi} substrate, it is only in abnormal situation that ectopic calcification can be observed. It is the case of vascular calcification (Lomashvili et al. 2014), kidneys' stones (Moochhala et al. 2008), breast cancer micro calcification (Cox et al. 2012) or after traumatic brain injury (Toffoli et al. 2008). A case of vessel mineralization has also been documented in the central nervous system of cervids suffering of the chronic wasting disease (CWD) caused by prion protein aggregation (Hamir et al. 2008). This raises the question of a possible implication of TNAP in the course of this pathological CWD disease and in link with the prion protein, the agent of the disease.

9.2.2- The impact of membrane on TNAP activity. In view of the various forms of TNAP, and the fact it can be associated to cells or be secreted as a soluble or vesicle associated protein, it is important to know whether the secreted and the GPI forms of TNAP exhibit the same activity. While a local effect can be expected from the GPI anchored protein, the secreted TNAP may have a more global effect.

An influence of plasma membranes on enzymatic activity has indeed been demonstrated for GPI anchored enzymes. The 5'ectonucleotidase (Lehto and Sharom 1998), the dipeptidase (Brewis et al. 1994) and the TNAP (Kothekar et al. 2014), exhibit higher specific activities after release from the membrane by phospholipase C cleavage of the GPI anchor. However, it does not seem to be always the case. In particular, in the upper mentioned studies, the group of Brewis using similar pig kidney membrane model as the group of Kothekar, did not find any impact of membranes on TNAP activity (Brewis et al. 1994). Besides, artificial membrane reconstitutions have shown an impact of the lipid composition and membrane curvature on alkaline phosphatase activity (Sesana et al. 2008).

As expected from the role of matrix vesicles formed at the site of bone mineralization the orientation of TNAP present at their surfaces is the same as at the plasma membrane since both are released by PIPLC treatment. Two types of vesicles either secreted in the medium (medium vesicles, MeV), or forming at the membrane of chondrocytes (matrix vesicles, MV), have been described and both expressed high level of TNAP. However, only the MV type is able to induce mineralization (Wuthier and Lipscomb 2011). This emphasizes the importance of the membrane micro environment for the function of TNAP.

9.3- The case of the neuronal TNAP: a modulator role of the neuronal TNAP depending on its microenvironment ?

9.3.1- Lipid rafts and neuronal functions: Membrane lipid rafts (MLR) constitute signaling platforms linking extracellular compounds from milieu or matrix to intracellular compartments through membrane proteins acting as receptors (Head et al. 2014). Evidence of a role of these micro domains in different types of neurotransmitter signaling has been obtained (Allen et al. 2007), including : (i) ionotropic and G-protein coupled neurotransmitter receptors such as serotonin (Bjork et al. 2010), (ii) downstream effectors such as ions channels, phosphodiesterase, adenylyl cyclase, phospholipases (Head et al. 2006), (iii) neurotransmitter transporters (Cuddy et al. 2014). It has also been established that MLR are important in synaptic transmission and plasticity (Newpher and Ehlers 2009; Sebastiao et al. 2013) and in axonal growth and guidance (Guirland and Zheng 2007). In addition, lipid micro domains are in close contact with the cytoskeleton, what allows to maintain and regulate precise localization of proteins at the synapse and in dendritic spine (Chen and Sabatini 2012). Many of these studies were concluded by combining different approaches that included (i) localization of actors of neurotransmission, (ii) use of agonists and antagonists and (iii) the effect of the perturbation of raft organization on neurotransmission by drugs interfering with their lipidic constituents, in particular, the cholesterol (main lipid in MLR). Another domain of interest is the node of Ranvier rich in GPI anchored proteins, revealing the role of lipid microenvironment in the regulation of trafficking and association of axo-glial complexes constituting axonal nodal subdomains (Labasque and Faivre-Sarrailh 2010). This is in support with the fact that TNAP activity has been demonstrated to localize in the white matter at myelin-free axonal region, namely at the node of Ranvier, and at the level of synaptic contact (Fonta et al. 2005).

Importance of the extracellular matrix (ECM) in such neuronal activities is now well recognized (Dityatev et al. 2010). Components of the ECM are secreted by different cell types and in the case of the central nervous system (CNS), they build perineural nets of specific compositions associated to glial cells, neuronal cells or oligodendrocytes. The ECM, which fills extracellular spaces in the CNS, has the particularity to contain little collagen and fibronectin as compared to other tissues. By contrast, it contains a high level of carbohydrates carried by glycosaminoglycans, either linked to proteins such as the chondroitin and heparan proteoglycans family that can be sulfated (CSPG and HSPG), or free, as is the hyaluronic acid. It also contains diverse glycoproteins such as laminin. These molecules interact with each other to form a net but also with cell surface receptors thereby providing some scaffolds that promote clustering of cell surface proteins as required for some signaling function in lipid rafts. Diverse molecules of the ECM have been shown to be involved in proliferation, migration and cell differentiation. The perineural nets in the CNS enwrap dendrites protruding from cell bodies and synaptic contacts as well as nodes of Ranvier, therefore participating in neuronal functions important for synaptic plasticity in adult brain, which include regulation of long term potentiation by interaction of ECM molecules with different types of receptors (Dityatev and Schachner 2003). Ion channels, neurotransmitter receptors and other compounds present in lipid rafts may be differentially regulated by ECM molecules produced by different neuronal cell types to further activate tyrosine kinase and downstream signaling cascades coupled to the raft through the cytoskeleton. In this context, a glycotranscriptome study revealed differential expression of genes involved in glycosylation pathways while comparing serotonergic and noradrenergic derived neuronal cells. In particular, 9 among the 44 transcripts differentially expressed in these bioaminergic neurons (out of the 375 glycosylation relating transcripts tested) relate to glycosaminoglycan metabolism associated to some ECM molecules (Ermonval et al. 2009b). The ECM will

contribute then to shape synaptic plasticity by imposing diffusion constraints for neuronal factors (neurotransmitters, trophic factors) and to regulate different neuronal functions in a spatio-temporal manner.

9.3.2- Role of the neuronal TNAP: TNAP is expressed in different cell types (endothelial, neuronal) in the brain, and in different areas of the central nervous system (CNS) (see Chapter 5). The different known activities of TNAP, its various isoforms and locations in intracellular and extracellular spaces in the CNS as well as its spatio-temporal regulation during neuronal development, identified this ectophosphatase with an important role in the CNS. Studies on hypophosphatasia deficiencies and associated neuronal complications have added information supporting this view. However, understanding the contribution of TNAP in neuronal functioning is at an early stage, and further investigations are required to learn more about it.

Different studies have brought some clues on the way neuronal TNAP could exert activities in the brain. The difficulties are in integrating all the data obtained in relation with the different specificities and regulations of TNAP. Questions then arise of how these activities may be influenced by the local environment of the TNAP and, conversely, does TNAP influence its partners in close vicinity in lipid rafts. As already mentioned and without going into details given in other chapters, the phosphatase activities of TNAP, mainly linked to its phosphomonoesterase and nucleotidase functions, are contributing to neuronal homeostasis. Regulation of the PLP substrate, a cofactor of many enzymes among which decarboxylases are key enzymes involved in the metabolism of different monoamine neurotransmitters (serotonin, norepinephrine, GABA), is essential for bioaminergic neuron activities. Another important role of TNAP, relates to the regulation of the adenosine signaling through purinergic receptors that can be obtained locally by sequential dephosphorylation of extracellular ATP, ADP, AMP, to adenosine (Matsuoka and Ohkubo 2004). However, TNAP can be found in the blood circulation and adenosine could be generated in the brain microvasculature (Pettengill et al. 2013) as well as the pyridoxal since the

PLP can not cross the blood brain barrier and / or enter cells and must be dephosphorylated first (detailed in Chapter 11). It is thus not an easy task to establish whether these regulatory cofactors that act locally are produced in the near surrounding of their receptors. Interestingly, TNAP is a metalloenzyme dependent on zinc for its stability and acquisition of its functionality raising the question of the requirement of zinc transporter for activation of TNAP (Fukunaka et al. 2011). It is noticeable that in the CNS, metals and in particular Zn have to be tightly regulated. Considering only the enzymatic activities of TNAP and its differential expression already raised important questions such as : (i) what are the substrates found around the TNAP in extracellular space, in particular at the synaptic cleft, (ii) are the receptors of the products of TNAP in close vicinity with the enzyme to ensure local effects, (iii) can TNAP act on membrane compounds of surrounding cells or on matrix phosphoproteins, or at distance through the generation of circulating dephosphorylated products.

9.3.3- Role of microenvironment on TNAP function, an hypothesis on its role in the central nervous system: Other neuronal functions could relate to the ability of TNAP to dephosphorylate membrane or matrix phosphoproteins and to its participation to protein complexes of high density in lipid rafts known to be important in neuronal signaling. Therefore, it can be expected to see an impact of micro domain composition in differentiated cells on the functional outcomes of TNAP in relation with soluble substrates, membrane receptors or matrix proteins. In this context, TNAP has been shown to be expressed both in serotonergic and noradrenergic neurons upon neuronal differentiation of the neuroectodermic 1C11 cell line. TNAP expression in lipid rafts of bioaminergic neurons correlates with the detection of its specific activity whose inhibition impacts the metabolism of the corresponding neurotransmitter (Ermonval et al. 2009a). Also, TNAP colocalizes with the cellular prion protein, PrP^C, in micro domains of bioaminergic 1C11 derivatives. PrP^C was previously shown to participate in a signaling platform with the caveolin and fyn (Mouillet-Richard et al. 2000), modulating the

agonist response of serotonin (5-HT) receptors (Mouillet-Richard et al. 2007). The complex network of interactions between actors of neurotransmission in lipid rafts is also illustrated by the fact that the G-protein coupled serotonin receptors and the serotonin transporter are both localizing in lipid rafts (Sebastiao et al. 2013). Moreover, 5-HT receptor activation allows neurite outgrowth and neuronal survival by triggering downstream signaling cascades (Fricker et al. 2005). It has to be added that the 5-HT neurotransmitter exerts its function through 14 different subtypes of 5-HT receptors and dysregulated metabolism is implicated in anxiety and depression disorders (Bjork et al. 2010). Other studies have pointed to the role of TNAP enzymatic activity in neuronal differentiation (Delic and Zimmermann 2011) and neuritogenesis by promoting axonal growth of hippocampal neurons (Diez-Zaera et al. 2011). In this latter study, a coordinated action of TNAP and purinergic receptors colocalizing at the level of the axonal growth cone was required for axonal growth to occur.

While interaction of TNAP with matrix collagen during mineralization has been largely investigated, it is only recently that the neuronal TNAP has been shown to be able to dephosphorylate the phospho-laminin. In addition, the inactivation of TNAP by levamisol stabilizes the phosphorylated form of the laminin and influences its binding to PrP^C (Ermonval et al. 2009a). The laminin by interacting with different membrane proteins such as integrins, PrP^C, the laminin receptor, have been ascribed roles in differentiation, survival and migration of neurons but also in neuronal plasticity (Nishimune et al. 2004; Sperling et al. 2012).

Altogether these studies revealed the complexity of the interplay between different partners required for neuronal functioning and positioned TNAP as an essential player in the multi-molecular complexes constituting lipid rafts, whose composition may depend on the cellular context and environment and lead to diverse functions. All these compounds and their ligands are

tightly regulated and imbalanced conditions may lead to dysfunction of the whole system, as in the case of seizures associated to severe hypophosphatasia. A role of TNAP on extracellular missorted phosphorylated Tau (Diaz-Hernandez et al. 2010; Zempel and Mandelkow 2014) a protein involved in a neurodegenerative disorder (the Alzheimer disease, AD) has also recently been reported (Kellett et al. 2011; Vardy et al. 2012). A link has also been made between AD and lipid rafts by the group of Hooper, adding to the complexity of interactions between partners in micro domains. Indeed, their studies show that amyloid β oligomers bind to PrP^C and activate fyn kinase only when PrP^C is localized in lipid rafts. They also documented a role of PrP^C in zinc uptake besides its role in homeostasis of other metals such as Cu²⁺. Interestingly, both a diminution of Zn transport and an alteration of raft composition were occurring with aging, one of the identified risk factor in AD (Watt et al. 2014). These different studies illustrate how clusters in MLR of protein complexes of different compositions may have different outcomes.

It is clear that TNAP may have different functional impacts on different neuronal cells according to its micro environment in the CNS. An hypothesis could be that this enzyme exerts a modulator role on its partners within lipid raft complexes, whose composition may vary as well as those of the extracellular matrix and the secreted compounds able to serve as substrate or receptor ligands. The determination of the composition of protein complexes associated with TNAP in the rafts, in different situations, should provide a better understanding of its neuronal functions.

9.4 - Conclusion

Although increasing evidence has been obtained for a direct role of TNAP during neuronal development, in blood brain barrier function and for neurotransmission, the physio-pathological involvement of TNAP in the central nervous system is far from being fully understood. The

differential role that this ubiquitous ecto-phosphatase harbors in various organs, points to the importance of TNAP regulation occurring at different levels of expression (transcriptional, post translational, compartment targeting) and depending on its environment in the rafts and available ligands, altogether impacting its activity and function. In addition, TNAP being part of dynamic complexes in the rafts, it may also exert a modulator role on its partners in micro domains contributing to neuronal activity. This is also true for other molecules, as shown for the PrP^C, present in multi-molecular complexes among micro domains whose composition varies in different neuronal cell types. Interplays between different compounds, as shown in fig.1, may then converge towards signaling cascades important in neuronal functions such as cell adhesion and survival, neurite outgrowth and neuronal homeostasis.

It can be envisioned that TNAP activities may vary according to raft dynamics and extracellular composition at the level of the synapse or the perineural net constituting a particular matrix. Neuronal homeostasis also relates to neurotransmitter concentration, whose equilibrium may change according to the neuronal state, the brain area and whether pathological situation occurs such as the one met in some neurodegenerative diseases when extracellular matrix is affected. The abundance of extracellular phosphorylated tau protein in Alzheimer disease is an example. In this context, the new opening field of neuronal vesicles released in the extracellular space and their role in neuronal communication as well as in neurodegenerative diseases may have an impact on the outcome of TNAP activity.

Importance of the microenvironment leads to think it will be of great interest to look at the TNAP as part of networks, whose function may depend on its different forms, cellular localization and therefore, accessibility to its different substrates. It will be determinant to know what are the different partners of TNAP, specially in lipid rafts, to understand the potential role this ecto

enzyme may have in the modulation of neuronal functions. The establishment of the link of TNAP with different key players of signaling neuronal networks (Chapter 10) but also other networks of interaction with the ECM, the cytoskeleton and intracellular trafficking processes, as well as with other systems such as the immune system where for instance adenosine also play a key role as regulator of immune functions (Pettengill et al. 2013), will allow to model the impact of specific TNAP substrates either in physiological or pathological situations. At this point, it is crucial to understand what is the resilience of the system and therefore, to identify which of the network links can be modified without perturbation and which ones are essential and will affect the whole system and lead to disorders in case of homeostatic imbalance. In this respect, the case of epilepsy that may involve TNAP corresponds to a loss of homeostasis triggering synchronization of neuronal network leading the system to become out of control. This supports a modulator role of TNAP associated to the fine tuning of neuronal homeostasis in the context of connected network.

Legend to figure

Figure 1: Schematic representation of the different environments of TNAP found during its trafficking in intra- and extra-cellular compartments. The different levels of regulation that affect TNAP expression in a cell type specific manner appear in green letters. This includes the transcriptional level involving factors that interact with the bone and liver promoters, the different post-translational modifications impacting TNAP glycoform composition all along the secretory pathway and the expression level and stability of TNAP in its final destination *i.e.* the membrane lipid rafts. TNAP in lipid rafts can indeed be submitted to regulation following PiPLC (phosphatidylinositol-phospholipase C) release or endocytosis. Endosomes can then reach multivesicular bodies (MVB) from which TNAP could be either sent to degradation or be released on extracellular vesicles (EV). The different cell compartments are named in blue letters. The extracellular orientation of the GPI anchored protein (GPI-AP) at the plasma membrane or at the surface of micro vesicles, as well as of the secreted form of TNAP, allows this enzyme to be in contact with its various substrates or ligands (shown in red letters) circulating in the extracellular space such as the phosphomonoester substrates (inorganic pyrophosphate -PPi-, pyridoxal phosphate -PLP-) or compounds of the extracellular matrix or perineural net (collagen, laminin, GAG and proteoglycans...) involved in different cellular functions. In addition, its clustering in specialized microdomains supports its participation in different signalling functions depending on the microenvironment constituted by its close partners. In the case of neuronal cells, the proximity of TNAP with receptors, transporters or other membrane channels present in lipid rafts and themselves depending on regulatory processes could contribute to neuronal homeostasis as part of neuronal networks.

The abbreviations in the figure are as follows: GAG for glycosaminoglycan, HSPG/CSPG respectively for heparan sulfate proteoglycans and chondroitin sulfate proteoglycans, CFTR for cystic fibrosis transmembrane receptor and PrP^C for the cellular prion protein.

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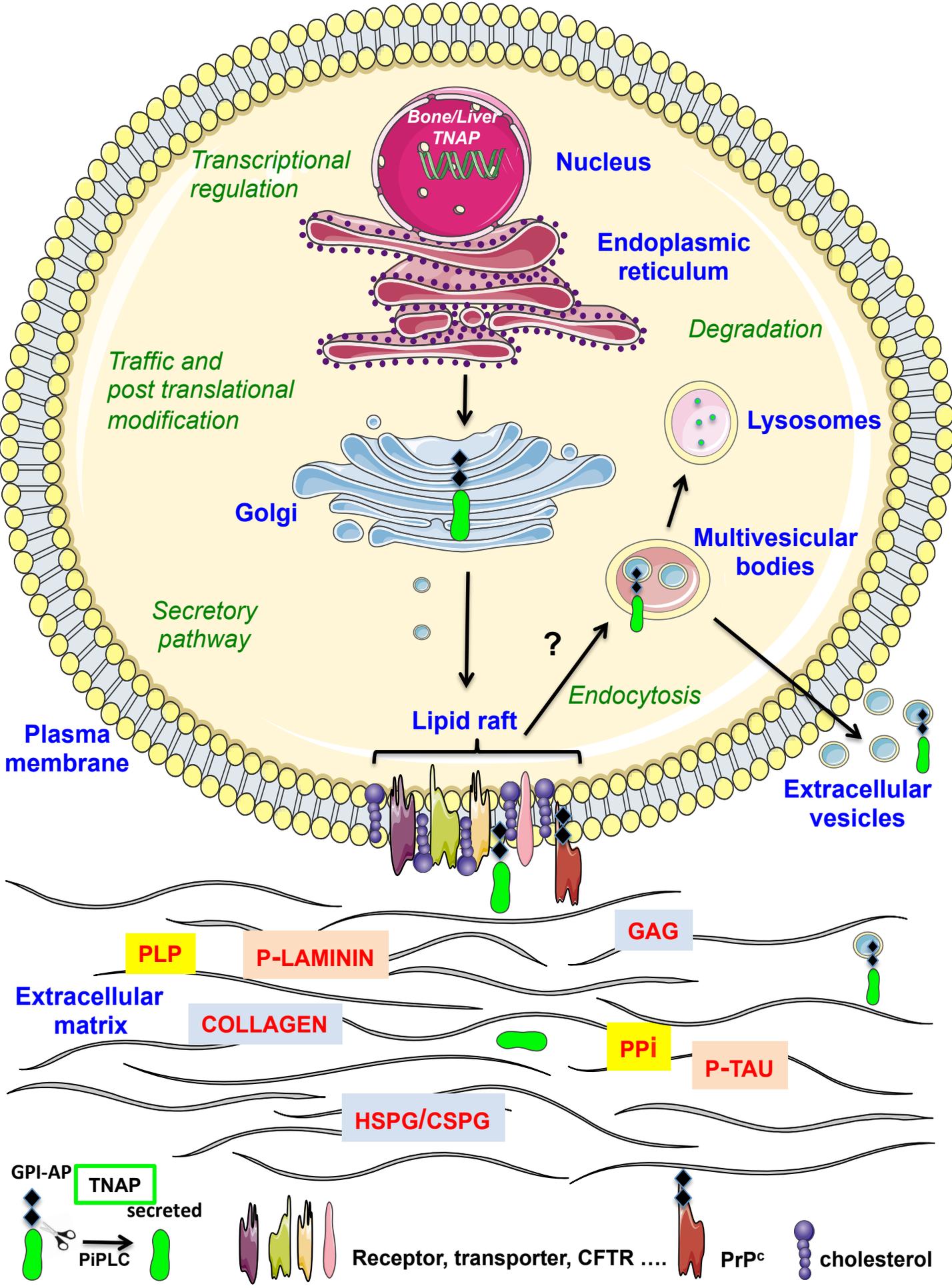
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Legend to figures

Figure 1: Schematic representation of the different environments of TNAP found during its trafficking in intra- and extra-cellular compartments. The different levels of regulation that affect TNAP expression in a cell type specific manner appear in green letters. This includes the transcriptional level involving factors that interact with the bone and liver promoters, the different post-translational modifications impacting TNAP glycoform composition all along the secretory pathway and the expression level and stability of TNAP in its final destination *i.e.* the membrane lipid rafts. TNAP in lipid rafts can indeed be submitted to regulation following PiPLC release or endocytosis. Endosomes can then reach multivesicular bodies (MVB) from which TNAP could be either sent to degradation or be released on extracellular vesicles (EV). The different cell compartments are named in blue letters.

The extracellular orientation of the GPI anchored protein (GPI-AP) at the plasma membrane or at the surface of micro vesicles, as well as of the secreted form of TNAP, allows this enzyme to be in contact with its various substrates or ligands (shown in red letters) circulating in the extracellular space such as the phosphomonoester substrates (inorganic pyrophosphate, P_i, pyridoxal phosphate, PLP..) or compounds of the extracellular matrix or perineural net (collagen, laminin, GAG and proteoglycans...) involved in different cellular functions. In addition, its clustering in specialized microdomains supports its participation in different signalling functions depending on the microenvironment constituted by its close partners. In the case of neuronal cells, the proximity of TNAP with receptors, transporters or other membrane channels present in lipid rafts and themselves depending on regulatory processes could contribute to neuronal homeostasis as part of neuronal networks.

The abbreviations in the figure are as follows : GAG for glycosaminoglycan, HSPG/CSPG respectively for heparan sulfate proteoglycans and chondroitin sulfate proteoglycans, CFTR for cystic fibrosis transmembrane receptor and PrP^C for the cellular prion protein.