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Preview

Nanofibrils to Track Phosphatase Activity on Live Cells

Caroline Fonta[1,*]

In this issue of *Chem*, Xu and colleagues have developed a new method of detecting ectophosphatase activity in cells maintained in culture, allowing the spatio-temporal pattern of alkaline phosphatase activity to be followed at the single-cell level.

Alkaline phosphatases (ALPs) contribute to a variety of physiological and pathological processes. In humans, three genes encoding tissue-specific ALPs are clustered on chromosome 2. The distribution of these three isoenzymes is restricted to the placenta (PLAP), intestine (IAP), and germ cells (GCAP), also known as ALPP, ALPI, and ALPP2, respectively (used by Xu and colleagues[1]). One additional gene (*ALPL*) located on chromosome 1 produces TNAP (also known as ALPL, used by Xu and colleagues[1]), the tissue-nonspecific alkaline phosphatase that is present in various tissues such as bone, liver, lung, brain, and blood vessels. This isoenzyme shows a widespread distribution and, moreover, has been found in all mammalian genera examined. ALPs can also be found in bacteria, yeast, and phytoplankton.[2]

ALPs share an ectoplasmic location and phosphomonoesterase activity. Despite their vast distribution and multiple functions, the substrates and metabolic mechanisms in which these isoenzymes are involved have not been exhaustively documented. To date, the functions of ALPL in bone mineralization have been defined through investigations on hypophosphatasia, a rare inborn disease caused by mutations in *ALPL*. A bone-targeted recombinant human ALPL has recently been designed to treat the most severe forms of the disease.[3] ALPP, ALPP2, and ALPI are known to be associated with tumors and malignancy. Therefore, localization and kinetics of ALP activity in normal and pathological tissues and cells continue to be an important endeavor.

A variety of assays and methodologies have been developed to detect ALP expression and activity in various types of samples. Besides the specificity of the method, their use is determined by the type, size, and treatment of the sample and the detection limit. Moreover, interpretation of the data has to face the complexity of the ALP family. For example, the membrane-bound ALPL is also secreted either as a soluble protein shed from the membrane by phospholipases or as a protein associated with vesicles. Promoter regulation of the first non-coding exon of *ALPL* leads to two different transcripts each expressed preferentially in bone or liver tissue. In addition, post-translational modifications produce different ALPL glycoforms (Figure 1). Such variability suggests that ALPL function could vary accordingly. Consequently, experiments aimed at measuring ALP activity need to be designed with tight control of these parameters.

Past and recent literature has reported numerous techniques for measuring ALP activity in fluids or tissues. Concerning solid samples such as cells and tissues, in situ detection of ALPs is generally based on histochemical and immunohistochemical techniques. These methods enable qualitative and semiquantitative analyses for both ALP activity and detection. In addition, in situ hybridization is the most reliable procedure for analyzing mRNA expression of a particular ALP isoenzyme. These methods are generally carried out on fixed cells or tissues. The principle of detecting ALP activity consists of using a phosphorylated substrate whose products released by ALP activity react with chromogens. This final reaction can be observed in optic (light or fluorescent) or transmission electron microscopy. The enzymatic activity can also be directly detected with the use of substrates yielding either fluorescent or chemiluminescent products, permitting the detection of low levels of ALP activity. These methods generally show some limitations in terms of specificity, autofluorescence, photostability, diffusion, or compatibility with other co-stainings. More recent techniques have aimed to overcome these drawbacks,[5,6] and the method proposed by the Xu group[1] has promising perspectives in this context. The authors designed an artificial substrate, a phosphorylated D-peptide coupled to a fluorophore that becomes visible after self-assembly of the peptide. Interestingly, this substrate is kept outside the cell. Thus, when phosphate is removed by ectophosphatases, self-assembly of this peptide substrate is triggered, which generates an extracellular fluorescent signal. The pericellular fluorescent nanofibrils appear visible after about 1 hr of incubation and continue

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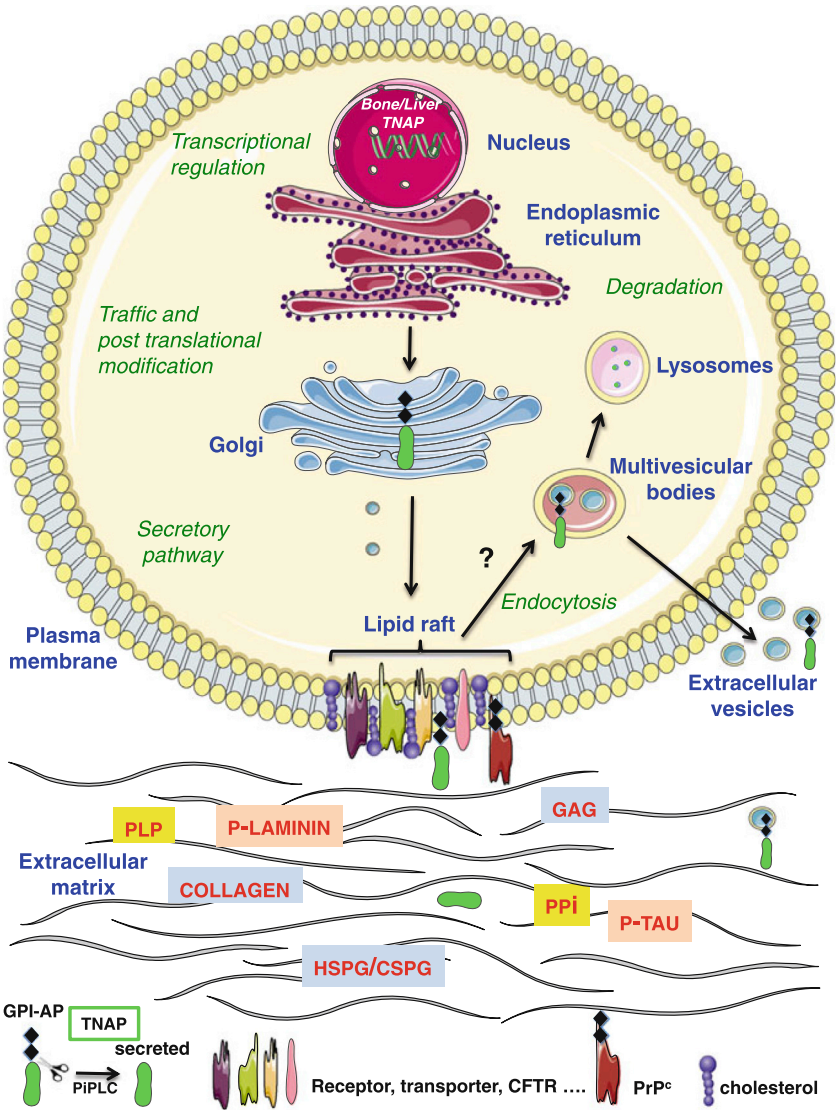

Figure 1. TNAP, or ALPL, Environments in Intra- and Extracellular Compartments

Reprinted from Ermonval et al.[4] with permission of Springer. Copyright 2015 Springer Science+Business Media. The original legend is as follows: Fig. 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 (phosphatidylinositolphospholipase C) release or endocytosis. Endosomes can then reach multivesicular bodies (MVBs the sequestration of early endosomes targeted) from which TNAP could be either sent to degradation or be released on extracellular vesicles (EVs). 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

to accumulate with increasing incubation time for a few hours. The process then slows down. The nanofibrils can be washed out, suggesting that further experiments could probe phosphatase activity on the same cellular preparation. The method has been tested on different cell types—cancer cells and "normal" cells (e.g., stromal cells and osteoblasts)—known to express different ALPs, especially ALPL and ALPP, at various levels.

The enzyme-instructed self-assembly (EISA) method presented by Xu and colleagues is designed for living cell lines. This novelty opens up new exciting perspectives and raises further questions. For example, could the method be used for analyzing ALPL activity in neuronal cells so we can better understand how ALPL functions in the nervous system, about which very little is known? Interestingly, the method can discriminate between two types of cells tested in a co-culture and characterized by different levels of ALP activity. Could the EISA method be extended to tissue sections, and could it be used in neurophysiological and pharmacological in vitro experiments on brain slices? This would require that the substrate easily penetrate the intercellular space to reach the cells in their natural context. In addition, it is unclear how nanofibril accumulation over a long period of time would modify the cell environment and consequently ALP activity. Nanofibrils may block the enzyme catalytic sites. Moreover, as another member of the ALP family, ALPL is integrated into lipid rafts of the cell membrane through a glycosylphosphatidylinositol (GPI) anchor. Such molecular assemblies can act as platforms enabling signal transfer between the extra- and intracellular milieus. The interactions between the enzyme ectodomain and the extracellular matrix and soluble compounds present in the extracellular space can initiate intracellular signaling cascades. Moreover, membrane lipid rafts are associated with cytoskeletal

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 PrPC for the cellular prion protein.

components, which contribute to the organization and distribution of these micro-domains at the plasma membrane. Proteins located in raft or non-raft domains in the cellular membrane can diffuse laterally, and their allocation takes part in cell signaling and trafficking.[7] Consequently, ALP is part of dynamic molecular complexes in the lipid rafts, whose interplay might converge toward signaling cascades inside the cell[4] (Figure 1). Therefore, the membrane environment is important in the analysis of the spatial and temporal kinetics of ALP activity and the physiological responses that depend on it. Previous work[8] has shown that nets of xenofibrils block the molecular and vesicular exchange between cancer cells and their environment, affecting cell viability, adhesion, and migration. Also, abnormal extracellular fibrils play a role in the pathogenesis of neurodegenerative disorders (Alzheimer disease and Parkinson disease). Thus, reciprocally, spatio-temporal control of the cell environment through manipulation of nanofibril nets would offer new paradigms for understanding the functions of different ALP forms (isoenzymes, isoforms, and membrane-bound and soluble forms) in cellular processes.

The method developed by Xu and colleagues overcomes several requirements for probing ALP activity under spatial and temporal resolution on live cells: a lack of substrate toxicity, background fluorescence, and product diffusion; compatibility between the method and other probing assays; and application to various types of cells. Its advantages also include a one-step detection procedure and the use of classical confocal microscopy. The substrate seems easily available in terms of quantity. This method will be very useful for sorting cell types and screening ALP inhibitors or engineered enzymes in the context of fundamental or applied research.

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1. Zhou, J., Du, X., Berciu, C., He, H., Shi, J., Nicastro, D., and Xu, B. (2016). Chem *1*, this issue, 246–263.

2. Millan, J.L. (2006). Mammalian Alkaline Phosphatases: From Biology to Applications in Medicine and Biotechnology (Wiley-VCH).

3. Whyte, M.P., Greenberg, C.R., Salman, N.J., Bober, M.B., McAlister, W.H., Wenkert, D., Van Sickle, B.J., Simmons, J.H., Edgar, T.S., Bauer, M.L., et al. (2012). N. Engl. J. Med. *366*, 904–913.

4. Ermonval, M., Baychelier, F., and Fonta, C. (2015). Subcell. Biochem. *76*, 167–183.

5. Cox, W.G., and Singer, V.L. (1999). J. Histochem. Cytochem. *47*, 1443–1456.

6. Halbhuber, K.J., Krieg, R., Fischer, P., König, K., Nasse, H., and Dietz, W. (2002). Cell. Mol. Biol. (Noisy-le-grand) *48*, OL343–OL358.

7. Head, B.P., Patel, H.H., and Insel, P.A. (2014). Biochim. Biophys. Acta *1838*, 532–545.

8. Kuang, Y., Shi, J., Li, J., Yuan, D., Alberti, K.A., Xu, Q., and Xu, B. (2014). Angew. Chem. Int. Ed. Engl. *53*, 8104–8107.

Preview

Greater Porosity with Redox Reaction Speeds Up MOF Color Change

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In this issue of *Chem*, Dincă and colleagues show that immobilization of redox-active naphthalene diimide moiety into a mesoporous metal-organic framework structure (MOF-74) allows the rapid and reversible transparent-to-dark electrochromism and has a potential application in smart windows.

As our brand-new Boeing 787 Dreamliner rose into the Tokyo skies en route to Paris, the pilot banked smoothly over the capital, and morning sunlight shot dazzlingly into the cabin. Passengers who had never before flown on a Dreamliner began to search for the pull-down blinds, but they sought in vain, for Boeing's flagship airplane has replaced them with a discrete series of switches. The Dreamliner employs smart windows dependent on the ability to darken or lighten on command through the utilization of electrochromic materials able to change color and transparency through electron injection. Thanks to modern technology, fumbling with window

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