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Direct interaction between ER membrane-bound PTP1B and its plasma membrane-anchored targets

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Abstract

The ubiquitously expressed protein tyrosine phosphatase PTP1B is involved in the regulation of numerous cellular signaling pathways. PTP1B is anchored to the ER membrane while many of its substrates are localized to the plasma membrane. This spatial separation raises the question how PTP1B can interact with its targets. In our study we demonstrate direct interaction of PTP1B with the Ser/Thr kinase PKCδ, the non-receptor tyrosine kinase Src and the insulin receptor which all are key enzymes in cellular signaling cascades. Protein complex formation was visualized *in vivo* using Bimolecular Fluorescence Complementation (BiFC). We demonstrate that complex formation of PTP1B with plasma membrane-anchored proteins is possible without detachment of PTP1B from the ER. Our data indicate that the dynamic ER membrane network is in constant contact to the plasma membrane. Local attachments of the two membrane systems enable a direct communication of ER- and plasma membrane-anchored proteins. The reported formation of membrane junctions is an important step towards the understanding of signal transmissions between the ER and the plasma membrane.

Keywords: Bimolecular Fluorescence Complementation; Insulin receptor; PKC; PTP1B; Src

1. Introduction

It is generally accepted that PTP1B plays an important role in regulation of cell function [1–8], however little is known about the regulation of PTP1B activity itself. Human PTP1B consists of 435 amino acids. With its C-terminus it is anchored to the endoplasmic reticulum (ER), and its catalytic domain is orientated towards the cytosol [9]. There is evidence that reversible oxidation of PTP1B by stimulus-dependent production of reactive oxygen species can inhibit PTP1B function [9–11]. Furthermore, PTP1B activity might be modulated by protein phosphorylations by PKC, Akt and other Ser/Thr protein kinases [12,13]. However, any change in PTP1B activity is ineffective as long as the spatial separation of ER-bound PTP1B and its targets in the plasma membrane persists. There are three models which could explain the encounter of PTP1B with its plasma membrane-anchored targets: (i) PTP1B is released from the ER by proteolytic removal of its ER membrane anchor [14,15], (ii) the targets are delivered from the plasma membrane to the ER by vesicle trafficking [16] or (iii) the local attachment of ER membrane to the plasma membrane could permit a direct interaction of proteins within the contact area [17]. In particular the third model is intriguing because it could explain many of the postulated ER-plasma membrane interactions including the constant down-regulation of insulin receptor activity in resting cells [18,19] or the activation of store-operated calcium influx after intracellular calcium store-depletion [20–22].

To demonstrate physical interaction of PTP1B with various target proteins we used the method of Bimolecular Fluorescence Complementation (BiFC) [23,24]. The BiFC method is based on the complementation among two non-fluorescent fragments of YFP (YN: amino acids 1–154, YC: amino acids 155–238) when they are brought together by complex formation of proteins fused to each fragment. The method allows the selective visualization of protein complexes within living cells without any interference by fluorescence signals from non-bound proteins. As interacting partners of PTP1B we selected PKCδ [25], a cytosolic protein that translocates to the plasma membrane in the presence of PMA, the post-translationally membrane-attached

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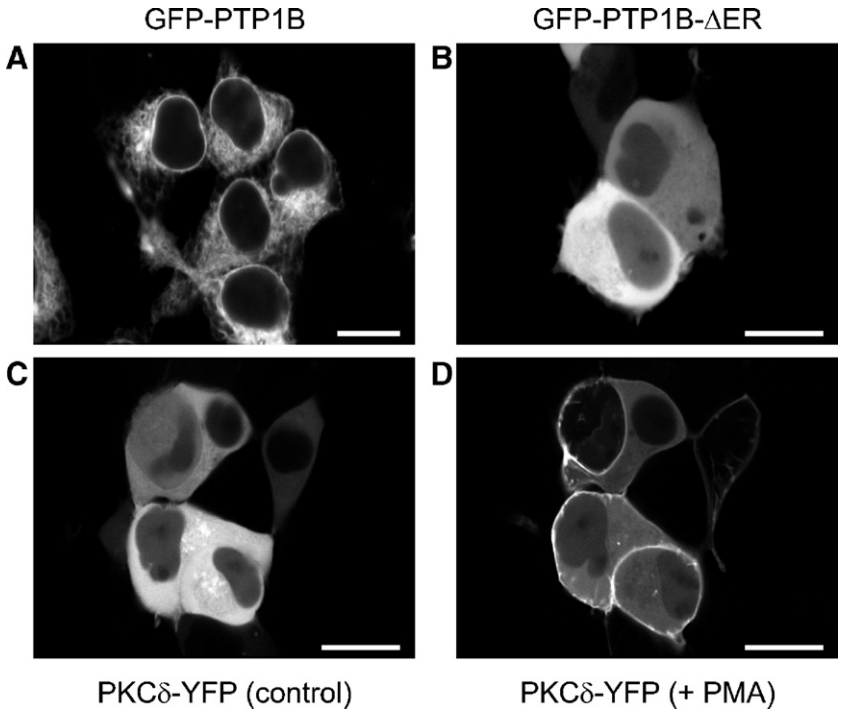

Fig. 1. Subcellular localization of GFP-PTP1B, GFP-PTP1B-ΔER and PKCδ-YFP in transiently transfected HEK293 cells. GFP-PTP1B is localized to the ER network and the nuclear envelope (A). Removal of the ER membrane anchor resulted in a cytosolic localization of GFP-PTP1B-ΔER (B). In resting cells, PKCδ-YFP is predominantly localized to the cytosol (C). Stimulation of PKCδ-YFP-transfected cells with PMA (100 nM) led to translocation of the bulk of PKCδ-YFP to the plasma membrane (D). The images were taken with a confocal laser scanning microscope. The white bar indicates 10 μm.

non-receptor tyrosine kinase Src [26] which is activated by PTP1B-mediated dephosphorylation, and the insulin receptor which has been shown to form complexes with the catalytic domain of PTP1B [27]. From all proteins we first produced a YFP- or GFP-tagged fusion protein to investigate the detailed subcellular distribution of the respective protein. Then, we replaced the fluorescent protein by the non-fluorescent YN or YC fragment to study *in vivo* protein complex formation with the BiFC method.

Our data demonstrate that a direct and regulated communication between ER-anchored and plasma membrane-anchored proteins is possible. The experiments explain how PTP1B can modify the activity of plasma membrane proteins without being detached from the ER.

2. Material and methods

2.1. Construction of plasmid vectors for YFP and GFP fusion proteins

pEGFP-C1-PTP1B and pEGFP-C1-PTP1B(D181A) were subcloned from human PTP1B-pLexA or human PTP1B(D181A)-pcDNA3 (gift from W. Hofer, University of Konstanz, Germany). The coding region of PTP1B was inserted into pEGFP-C1 (BD Biosciences Clontech) using the *Eco*RI and *Sal*I restriction site. The cytosolic pEGFP-PTP1B-ΔER and pEGFP-PTP1B(D181A)-ΔER were produced by truncation of 28aa from the C-termini. pEGFP-C1-PTP1B and pEGFP-C1-PTP1B(D181A) were cut at the *Xmn*I restriction sites and ligated with the Klenow-fragment blunted *Xba*I site from pEGFP-C1 vector, introducing a stop codon from the vector. pEYFP-N1-PKCδ was subcloned from pcDNA3 vector (gift from M. Schaefer, FU Berlin, Germany). The coding region of human PKCδ was ligated into the *Eco*RI/*Sal*I linearized pEYFP-N1 vector (BD Biosciences Clontech). Src was subcloned from pRK5-cSrc-Kinase (gift from W. Hofer, University of Konstanz, Germany) into the *Eco*RI/*Xma*I restriction sites of pEYFP-N1. pEYFP-N1-insulin-receptor was subcloned from human pRK5-IR (gift from A. Ullrich, MPI für Biochemie, Martinsried, Germany) into the *Nhe*I/*Age*I restriction site of pEYFP-N1. Since only the β-subunit of the IR reveals a cytoplasmic orientation, YFP was fused to the C-terminus of the β-subunit. The stop codon was removed and the *Age*I restriction site was inserted by PCR. All sequences produced by PCR were verified by DNA sequencing.

2.2. Construction of BiFC plasmid vectors

As previously described [24] the DNA sequences coding for the N-terminal (YN) and C-terminal (YC) part of YFP were amplified by PCR from pEYFP-C1 (BD Biosciences Clontech). YN contained the amino acids 1–154 and YC the amino acids 155–238. A *Age*I site at the 5′end and a *Bsr*GI site at the 3′end was introduced with the primers. This allows exchange of the full-length EYFP (amino acids 1–238) in the pEYFP-vector against the YN and YC fragment, respectively. Both constructs were verified by DNA sequencing. BiFC plasmids were constructed by replacing the DNA of the fluorescent protein in pEGFP-C1-PTP1B, pEGFP-C1-PTP1B-ΔER, pEGFP-C1-PTP1B(D181A), pEGFP-C1-PTP1B(D181A)-ΔER, pEYFP-N1-PKCδ, pEYFP-N1-Src or pEYFP-N1-insulin-receptor with the YN or YC fragment using the *Age*I/*Bsr*GI restriction sites. Before fusion proteins were used in BiFC experiments, we always checked that neither the YN nor the YC-fusion protein alone was fluorescent when expressed in HEK293 cells.

2.3. Cell culture, cell transfection and microscopy

HEK293 cells were transfected with 0.5–2 μg DNA per 35 mm Petri dish (transfection reagent Trans-IT-LT1, MoBiTec, Germany) and kept for 18 to 72 h under standard cell culture conditions. GFP-, YFP- and BiFC fluorescence was detected either by conventional fluorescence microscopy (Zeiss Axiovert 135, 40×/1.3 oil Fluar objective, Till Photonics imaging system, excitation with a