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Review

Tyrosine phosphatases as key regulators of StAR induction and cholesterol transport: SHP2 as a potential tyrosine phosphatase involved in steroid synthesis

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ABSTRACT

The phospho-dephosphorylation of intermediate proteins is a key event in the regulation of steroid biosynthesis. In this regard, it is well accepted that steroidogenic hormones act through the activation of serine/threonine (Ser/Thr) protein kinases. Although many cellular processes can be regulated by a crosstalk between different kinases and phosphatases, the relationship of Ser/Thr phosphorylation and tyrosine (Tyr)-dephosphorylation is a recently explored field in the regulation of steroid synthesis. Indeed in steroidogenic cells, one of the targets of hormone-induced Ser/Thr phosphorylation is a protein tyrosine phosphatase. Whereas protein tyrosine phosphatases were initially regarded as household enzymes with constitutive activity, dephosphorylating all the substrates they encountered, evidence is now accumulating that protein tyrosine phosphatases are tightly regulated by various mechanisms.

Here, we will describe the role of protein tyrosine phosphatases in the regulation of steroid biosynthesis, relating them to steroidogenic acute regulatory protein, arachidonic acid metabolism and mitochondrial rearrangement.

Contents

1. Introduction

Protein phosphorylation is an integral component of signal transduction pathways within eukaryotic cells and is regulated by the fine interplay of protein kinases and phosphatases. Numerous cellular responses are regulated by reversible phosphorylation of serine (Ser), threonine (Thr) and tyrosine (Tyr) residues.

Tyrosine phosphorylation is a fundamental mechanism for numerous important aspects of eukaryote physiology, as well as for human health and disease. Compared to protein phosphorylation in general, phosphorylation of tyrosine residues is a mechanism extensively present only in multicellular eukaryotes (Hunter, 1987).

Although tyrosine phosphorylation accounts for less than 1% of the phosphoproteome, it plays a disproportionately large role in disease as nearly 50% of the 90 human tyrosine kinases are implicated in cancer (Alonso et al., 2004).

Tyrosine phosphorylation is used for communication between and within cells, it participates in the regulation of shape and motil-

Abbreviations: StAR, steroidogenic acute regulatory protein; AA, arachidonic acid; Acsl4, acyl-CoA synthetase 4; Acot2, acyl-CoA thioesterase 2; PTP, protein tyrosine phosphatase; ACTH, adrenocorticotropin; LH, luteinizing hormone; cAMP, cyclic adenosine monophosphate; PKA, cAMP dependent protein kinase; AII, angiotensin II; Tyr, tyrosine; Ser, serine; Thr, threonine; PAO, phenyl arsine oxyde; BPA, benzyl phosphonic acid; P450scc, cholesterol side-chain cleavage cytochrome P450 enzyme.

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ity of cells, decisions to proliferate versus differentiate, cellular process like regulation of gene transcription, mRNA processing, and transport of molecules in or out of the cells. Tyrosine phosphorylation also plays an important role in the coordination of these processes among neighboring cells, in embryogenesis, organ development, tissue homeostasis, and the immune system (Hertog et al., 2008; Solan and Lampe, 2009; Souza et al., 2009; Tonks and Neel, 1996). Abnormalities in tyrosine phosphorylation are implicated in the pathogenesis of numerous inherited or acquired human diseases from cancer to immune deficiencies (Alonso et al., 2004).

Although it is generally agreed that tyrosine phosphorylation is regulated by the equal and balanced action of protein tyrosine kinases and protein tyrosine phosphatases, proportionately much more research has focused on protein kinases. Recent findings have now led to the emerging recognition that protein tyrosine phosphatases (PTPs) play specific and active dominant roles in setting the levels of tyrosine phosphorylation in cells and in the regulation of many physiological processes (Hertog et al., 2008).

In this review we will discuss the role of PTPs in the regulation of steroid synthesis.

2. Regulation of PTP activity by steroidogenic hormones

Whereas PTPs were initially regarded as household enzymes with constitutive activity, dephosphorylating all the substrates they encountered, evidence in favor of tight regulation of PTP activity by various mechanisms is now accumulating. Like protein phosphorylation, dephosphorylation by PTPs is required in a cell-compartment-specific manner. Protein–protein interaction domains and compartment-specific targeting domains in PTPs serve to achieve the required PTP localization from the cell surface to the nucleus (Hertog et al., 2008).

The phospho-dephosphorylation of intermediate proteins is a key event in the regulation of steroid biosynthesis (Olson et al., 1993; Podesta, 1979; Podesta et al., 1979a,b; Rae et al., 1979; Sala et al., 1979). Steroids are synthesized in specialized steroidogenic cells of the adrenal gland, ovary, testis, placenta and brain and are essential for maintaining normal body homeostasis and reproductive capacity. The biosynthesis of all steroid hormones begins at the mitochondria with the conversion of cholesterol into pregnenolone by the cholesterol side-chain cleavage cytochrome P-450 enzyme (P450scc) (Crivello and Jefcoate, 1980; Privalle et al., 1983). The transport of cholesterol to the inner mitochondrial membrane and the availability of cholesterol for P450scc constitute the rate-limiting step of steroidogenesis (Crivello and Jefcoate, 1980; Privalle et al., 1983). This step is regulated through the hormonal activation of different signal transduction mechanisms, all of which conduct to the activation of different protein kinases (Podesta et al., 1979a,b; Spat and Hunyady, 2004). Thus, the process of protein phospho-dephosphorylation is a common and crucial event in the mechanism of hormone action upon regulation of steroid synthesis. In this regard, it is well accepted that steroidogenic hormones act through the activation of serine/threonine (Ser/Thr) protein kinases. Adrenocorticotropin (ACTH) and lutropin (LH)/chorionic gonadotropin (CG) signal transduction pathways, in adrenal and Leydig cells respectively, include cyclic adenosine monophosphate (cAMP)-, and cAMP-dependent protein kinase (PKA)-dependent phosphorylation events (Dufau et al., 1977; Podesta et al., 1978; Schimmer et al., 1977), whereas Angiotensin II (AII) and K^+ in the adrenal zona glomerulosa promote phosphorylation events that are not dependent on cAMP and the consequent activation of PKA (Spat and Hunyady, 2004).

Although many cellular processes can be regulated by a crosstalk between different kinases and phosphatases, the relationship between Ser/Thr phosphorylation and Tyr-dephosphorylation is

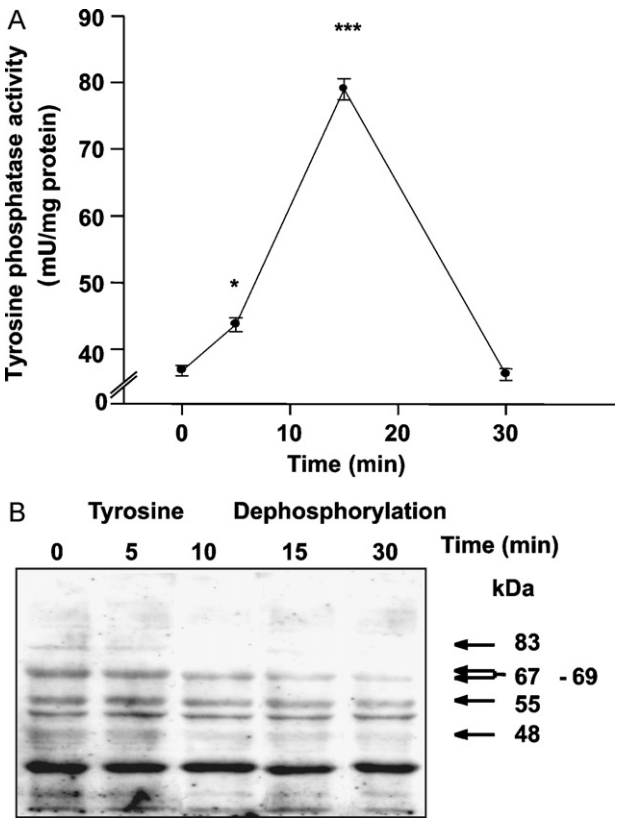

Fig. 1. Protein tyrosine phosphatase in rat adrenal gland. Panel A: PTP activity measured (as [^{32}P]Tyr-RCM-lysozyme dephosphorylation) in the cytosol of adrenal zona fasciculata obtained from control, or ACTH-treated rats for the indicated times. This is a representative experiment, independently performed three times. Data are expressed as mean ± SEM of triplicate experiments. $^{***}P < 0.001$; $^{*}P < 0.05$ control vs. each time (ANOVA followed by Tukey test). Panel B: Cytosolic proteins of adrenal zona fasciculata of ACTH-treated rats were analyzed by Western blot using anti-phosphotyrosine antibody. Numbers on the right indicate molecular masses of tyrosine dephosphorylated proteins in response to ACTH. This is a representative experiment, independently performed twice.
(Reprinted from Paz et al. (1999) with permission of John Wiley and Sons.)

a recently explored field in the regulation of steroid synthesis. Indeed, in steroidogenic cells one of the targets of hormone-induced Ser/Thr phosphorylation is a protein tyrosine phosphatase. We have previously described that *in vivo* treatment with ACTH results in an increase in total PTP activity in adrenal zona fasciculata (Fig. 1). The stimulation has a rapid onset (5 min), reaches a maximum (two-fold) after 15 min of corticotropin administration and returns to basal levels after 30 min (Paz et al., 1999). The increase in PTPs activity is accompanied with a decrease in phosphotyrosine proteins. *In vivo* corticotropin treatment decreased the phosphotyrosine signal in several bands when the proteins were analyzed by Western blot using an anti-phosphotyrosine antibody (Fig. 1). In addition, other reports are consistent with PKA induced shape changes in adrenocortical cells being mediated through the activation of tyrosine phosphatase activity and the dephosphorylation of paxillin (Han and Rubin, 1996; Whitehouse et al., 2002). All together, those results support the view that the morphological and functional responses to activation of the PKA-signalling pathway in steroidogenic cells are intimately linked and mediated by PTP activity.

3. Role of PTPs in steroid synthesis

The question of how the increase in PTP activity relates to the hormonal control of steroid synthesis was the subject of several