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Exploring phosphatidylinositol 5-phosphate 4-kinase function

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A B S T R A C T

The family of phosphatidylinositol 5-phosphate 4-kinases (PI5P4Ks) is emerging from a comparative backwater in inositide signalling into the mainstream, as is their substrate, phosphatidylinositol 5-phosphate (PI5P). Here we review some of the key questions about the PI5P4Ks, their localisation, interaction, and regulation and also we summarise our current understanding of how PI5P is synthesised and what its cellular functions might be. Finally, some of the evidence for the involvement of PI5P4Ks in pathology is discussed.

Introduction

The phosphatidylinositol 5-phosphate 4-kinases (PI5P4Ks, EC 2.7.1.149) are a family of three in most vertebrates – see (Clarke and Irvine, 2012, 2013). It is generally accepted that the reaction they catalyse *in vivo* is the 4-phosphorylation of PI5P, and because of the much lower abundance of this lipid compared to PI4P (the major precursor of $PI(4,5)P_2$ and substrate of the phosphatidylinositol 4-phosphate 5-kinases, PI4P5Ks), it is also accepted that their most likely function is to remove PI5P and thus control its levels in the cell. If the pool of $PI(4,5)P_2$ synthesised by the PI5P4K route does have a function, it must be a specific and localised one, given that the amount will be much lower than the overall cellular levels of $PI(4,5)P_2$. That concept cannot be ruled out, as, for example, PI5P4Ks can be localised to intracellular membranes (e.g. PI5P4Kγ (Clarke et al., 2008)), where $PI(4,5)P_2$ is present at

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very low levels. Here we review some of the current concepts about the PI5P4K family, how their substrate, PI5P, is synthesised in cells, and what the functions of PI5P4Ks and PI5P may be in cellular physiology and pathology.

Activity, localization and interrelationship of PI5P4Ks

PI5P4Kγ

It is clear that, at least as assayed *in vitro*, the three isoforms of PI5P4Ks have very different PI5P 4-kinase activities (Bultsma et al., 2010; Clarke and Irvine, 2013; Wang et al., 2010), with several orders of magnitude separating the most active (PI5P4Kα) from the least active (PI5P4Kγ). Indeed, the activity of PI5P4Kγ is so low that we have raised the issue of whether it might not function as a PI5P 4-kinase *in vivo* at all (Clarke and Irvine, 2013). But in its vesicular location, exposed perhaps to a local PI5P pool, with millimolar levels of ATP to drive it at a maximum rate, it may have sufficient activity to impact on local PI5P or $PI(4,5)P_2$ levels. Also, we do not yet know for certain how the dimerization of PI5P4Ks with each other (Bultsma et al., 2010; Clarke and Irvine, 2013; Wang et al., 2010) may impact on their activity. When PI5P4Kα is co-immunoprecipitated with overexpressed active or inactive PI5P4Kβ the lipid kinase activity of the complex with active PI5P4Kβ is greater than that of the complex with inactive PI5P4Kβ, and the activity of PI5P4Kβ assayed *in vitro* cannot account for this difference (Bultsma et al., 2010). The implication therefore is that either active PI5P4Kβ is required for full activation of PI5P4Kα, or that PI5P4Kα can activate PI5P4Kβ, and either way, if this applies also to PI5P4Kγ then it may in be more active *in vivo* (as a heterodimer) than *in vitro* assays would suggest.

The idea of PI5P4Kγ as an active PI5P 4-kinase may receive some support from our recent exploitation of a highly specific PI5P4Kγ inhibitor that apparently interacts with the PI5P binding site (J.H.C., M-L.G. et al., unpublished observations). This has clear effects on cell trafficking events in mpkCCD cells, which are mimicked by RNAi knock-down of PI5P4Kγ, and either this is because it is inhibiting the PI5P 4-kinase activity of PI5P4Kγ, or it is displacing the enzyme from its localization in intracellular vesicles and thus altering its ability to target other proteins to these vesicles. In the latter context, as noted above, PI5P4K isoforms can heterodimerise (Bultsma et al., 2010; Clarke and Irvine, 2013; Wang et al., 2010), plus we have produced evidence that PI5P4Kα has the ability to interact with any one of the three PI4P5K isoforms (Hinchliffe et al., 2002), so the concept of PI5P4Kγ being a 'targeting' protein is entirely plausible. The same may be true of PI5P4Kβ (discussed below), and the functional consequences of the PI5P4Ks abilities to heterodimerise remains one of the key unknowns about them.

PI5P4Ks α and β

The interrelationship of the α and β isoforms of PI5P4K is an interesting one that we and others have explored quite extensively, particularly with regard to their localization (and see above for activity considerations). In overexpression experiments, PI5P4Kα tagged at the N- or C-terminus with GFP is largely cytoplasmic when transfected into HeLa cells or cultured rat hippocampal neurons (Ciruela et al., 2000) or DT40 cells (Richardson et al., 2007). The same result was found with Myc-tagged PI5P4Kα in HeLa cells (Bultsma et al., 2010; Bunce et al., 2008) and FLAG-tagged PI5P4Kα in Cos-7 and PAE cells (Hinchliffe et al., 2002). An exception to this pattern is that Boronenkov et al. (Boronenkov et al., 1998) found the FLAG-tagged enzyme to be predominantly nuclear, and sometimes associated with nuclear speckles, in cultured fibroblasts.

Most of the above studies also examined the localization of overexpressed PI5P4Kβ. Ciruela et al. (2000) found it to be predominantly nuclear when N- or C-terminally tagged with GFP in HeLa cells or cultured rat hippocampal neurons. In contrast Bunce et al. (2008) reported 80% of Myc-tagged PI5P4Kβ to be cytoplasmic in HeLa cells, and Bultsma et al. (2010) also found PI5P4Kβ to be predominantly cytoplasmic in HeLa cells, in this case detected by an HA tag. Interestingly, in both of these papers some of the minority of PI5P4Kβ seen in the nucleus was in nuclear speckles (Bultsma et al., 2010; Bunce et al., 2008), and Bunce et al. (2008) demonstrated that this pool of enzyme co-localized with SPOP. Co-localization with nuclear speckles was also reported with FLAG-tagged PI5P4Kβ in cultured fibroblasts but in this case the majority of PI5P4Kβ was nuclear (Boronenkov

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