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Review

Phosphoinositide signaling in sperm development

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ABSTRACT

Phosphatidylinositol phosphates (PIPs)[1] are membrane lipids with crucial roles during cell morphogenesis, including the establishment of cytoskeletal organization, membrane trafficking, cell polarity, cell-cycle control and signaling. Recent studies in mice (*Mus musculus*), fruit flies (*Drosophila melanogaster*) and other organisms have defined germ cell intrinsic requirements for these lipids and their regulatory enzymes in multiple aspects of sperm development. In particular, PIP levels are crucial in germline stem cell maintenance, spermatogonial proliferation and survival, spermatocyte cytokinesis, spermatid polarization, sperm tail formation, nuclear shaping, and production of mature, motile sperm. Here, we briefly review the stages of spermatogenesis and discuss the roles of PIPs and their regulatory enzymes in male germ cell development.

Contents

1. Introduction

1.1. Stages of spermatogenesis

Spermatogenesis, the process that gives rise to fertile sperm, occurs in a similar manner in many animal species, including model organisms such as mice (*Mus musculus*) and fruit flies (*Drosophila melanogaster*) (reviewed in [1–4]). Male germ cell development starts with specification of primordial germ cells (PGCs), which migrate and associate with somatic cells that form the gonad. Within the gonad, PGCs localize to regions that are competent to serve as stem cell (SC) niches, where they develop into spermatogonial SCs (SSCs) or germline SCs (GSCs) in mice or flies, respectively. SCs divide to form new SCs and spermatogonial daughter cells. The daughter cells proliferate, undergoing sequential rounds of mitosis and incomplete cytokinesis to form syncytial

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[1] Bru, Brunelleschi (Drosophila TRAPPII subunit); DAG, diacylglycerol; Fwd, Four wheel drive (Drosophila PI4KIIIβ); GDNF, glial cell-derived neurotrophic factor; Gio, Giotto (Drosophila PITP); GOLPH3, Golgi phosphoprotein 3; GSC, germline stem cell; γTuRC, γ-tubulin ring complex; IGF, insulin-related growth factor; IGFR, IGF receptor; ILP, insulin-like peptide; InR, insulin receptor; IP_3, inositol trisphosphate; IP6K, inositol polyphosphate 6-kinase; PGC, primordial germ cell; PI, phosphatidylinositol; PI3P, PI 3-phosphate; PI4P, PI 4-phosphate; PI5P, PI 5-phosphate; $PI(3,4)P_2$, PI 3,4-bisphosphate; $PI(3,5)P_2$, PI 3,5-bisphosphate; $PI(4,5)P_2$, PI 4,5-bisphosphate; $PI(3,4,5)P_3$, PI 3,4,5-trisphosphate; PI3K, PI 3-kinase; PI4K, PI 4-kinase; PIP, PI phosphate; PIP2, $PI(4,5)P_2$; PIP3, $PI(3,4,5)P_3$; PIP5K, PIP 5-kinase; PLC, phospholipase C; Pten, phosphatase and tensin homolog (PIP_3 phosphatase); RA, retinoic acid; RAR, retinoic acid receptor; Sau, Sauron (Drosophila GOLPH3); SC, stem cell; SCF, stem cell factor; Sktl, Skittles (Drosophila PIP 5-kinase); SSC, spermatogonial stem cell; TRAPPII, trafficking transport and protein particle II.

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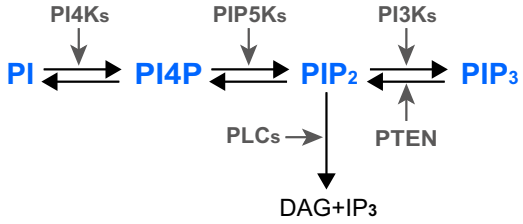

Fig. 1. Phosphoinositide pathway showing catalytic conversion of phosphatidylinositol (PI) to PI 4-phosphate (PI4P), which is further phosphorylated to form PI 4,5-bisphosphate (PIP_2) and PI 3,4,5-trisphosphate (PIP_3), by PI 4-kinases (PI4Ks), PIP 5-kinases (PIP5Ks), and PI 3-kinases (PI3Ks), respectively. PIP_2 can be hydrolyzed to yield second messengers diacylglycerol (DAG) and inositol trisphosphate (IP_3) by phospholipase C enzymes (PLCs). The phosphatase and tensin homolog (PTEN) dephosphorylates PIP_3, converting it back to PIP_2.

groups of spermatogonia. These germ cell syncytia are called cysts in Drosophila. Following a series of synchronous mitotic amplification divisions, spermatogonia differentiate into spermatocytes, which then undergo meiosis. As in the earlier mitotic divisions, meiotic cytokinesis is incomplete, resulting in syncytia of interconnected haploid spermatids. Post-meiotic spermatids undergo an extensive period of differentiation, or spermiogenesis, in which they form sperm-specific organelles, including the sperm head, acrosome, basal body, specialized mitochondria and flagellum. At the end of spermiogenesis, mature sperm are separated from each other by removal of the remnants of incomplete cytokinesis, released from the gonad, and stored prior to use in fertilization. These processes are well understood at a morphological level in many organisms. However, the cellular mechanisms that control various stages of male germ cell development remain active areas of investigation.

One common theme emerging from recent studies is the role of phosphatidylinositol phosphate (PIP) signaling in male fertility. PIPs are bioactive lipid molecules that play roles in multiple cell types during different stages of sperm development. For example, PIPs play a crucial role in regulating dynamic remodelling of cell adhesions in Sertoli cells, which are epithelial cells that encapsulate mammalian male germ cells and support their development [5–8] (reviewed in [9]). PIPs are required for migration of PGCs to populate the developing gonad in nematodes and zebrafish [10,11]. In addition, PIPs are needed for key aspects of sperm function, including activation, motility, capacitation and fertilization (reviewed in [12–15]). PIPs have cell-intrinsic roles in male germ cell development and differentiation in animals (the subject of this review).

1.2. Phosphatidylinositol phosphates

PIPs (also called phosphoinositides) are low-abundance phospholipids found in the cytoplasmic leaflet of cellular membranes. The precursor lipid phosphatidylinositol (PI) consists of two fatty acid chains connected by a diacylglycerol (DAG) backbone and a phosphodiester bond to an inositol head group that is exposed to the cytoplasm. PIP pathway kinases and phosphatases modify the 3, 4 and 5 positions of the inositol ring to generate and interconvert seven species of PIPs: PI3P, PI4P, PI5P, PI(3,4)P_2, PI(3,5)P_2, PI(4,5)P_2 and PI(3,4,5)P_3.

Of these, PI4P, PI(4,5)P_2 and PI(3,4,5)P_3 are the most abundant and form the focus of this review (Fig. 1). PIPs control cell growth and division, membrane and lipid transport, cytoskeletal organization, organelle biogenesis and signal transduction by two general mechanisms: (1) direct binding and recruitment of target proteins, leading to modulation of their activity or function; and (2) hydrolysis to yield second messengers (for example, DAG and inositol trisphosphate [IP_3]), which further promote cell signaling and homeostasis.

Subcellular distribution of PIPs is largely controlled by local recruitment or activation of PIP pathway enzymes. Indeed, enzymes that generate the same PIP species can produce localized pools of PIPs in different places in the cell. For example, the three (in flies) or four (in mammals) PI 4-kinases (PI4Ks) localize to and control PI4P synthesis in distinct organelles, namely the Golgi, *trans*-Golgi network, plasma membrane and endosomes (reviewed in [16]). The PIP 5-kinases (PIP5Ks) and PI 3-kinases (PI3Ks) that synthesize PI(4,5)P_2 and PI(3,4,5)P_3 (henceforth PIP_2 and PIP_3) are found primarily at the plasma membrane, yet also play significant roles in endo-/lysosomal trafficking and in the nucleus [17–19] (reviewed in [20,21]). Recent studies have revealed cell-intrinsic roles for PIPs in spermatogenesis in mice, flies and other animal models. Here, we summarize current knowledge regarding roles of PI4P, PIP_2 and PIP_3 and their regulatory enzymes in sperm development. In particular, we focus on the role of PIP_3 signaling in regulating cell-cycle progression in mitosis and on the roles of PIP_2 and PI4P in controlling cell morphogenesis during meiotic cytokinesis and spermiogenesis.

2. PIP_3 signaling in mitosis

2.1. Primordial germ cells

PI3K signaling through PIP_3 and Akt promotes cell-cycle progression and cell growth (Fig. 2; reviewed in [22]). Cellular levels of PIP_3 are tightly regulated by PI3K and the phosphatase and tensin homolog Pten, which regulate stemness in diverse cell types (reviewed in [22–24]). In early (fetal) mouse germ cells, PIP_3 signaling acts downstream of retinoic acid (RA) as a potent activator of cell proliferation; this pathway must therefore be turned off to promote

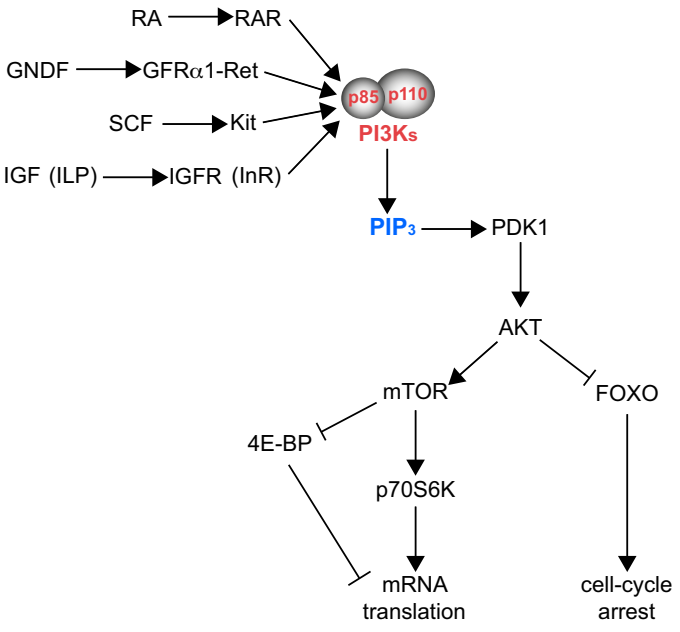

Fig. 2. PI3K signaling in mouse and Drosophila spermatogenesis. During mouse sperm development, PI3K is activated by four ligand–receptor interactions: retinoic acid (RA) and its receptor RAR; glial cell-derived neurotrophic factor (GDNF) and its receptor GFRα/Ret; stem cell factor (SCF) and its receptor Kit; and insulin-like growth factor (IGF) and its receptor IGFR. In Drosophila, PI3K is activated by insulin-like peptides (ILPs) via the insulin receptor (InR). Activated PI3K phosphorylates PI 4,5-bisphosphate (PIP_2) to form PI 3,4,5-triphosphate (PIP_3), which then recruits and activates PDK1. PDK1 phosphorylates and activates AKT. AKT has a dual role in spermatogenesis. On one side, AKT inhibits FOXO transcription factors (which promote cell-cycle arrest), leading to cell proliferation and survival. On the other side, AKT regulates mRNA translation by activating mTOR, which in turn activates p70S6 kinase (p70S6K) and inhibits 4E-BP, leading to protein synthesis.