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Review

Phosphoinositide 3-kinase beta: when a kinase is more than a kinase

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ABSTRACT

Phosphoinositide 3-kinases regulate numerous intracellular signals and essential biological processes; defects in their expression or regulation are implicated in diverse pathologies. For these reasons, research efforts have focused on generating inhibitors of the catalytic activity of these proteins. Recent evidence nonetheless indicates important non-catalytic properties for these kinases. These kinase-independent roles include a scaffold function in the organization of complexes with other proteins or with DNA, which suggests that pharmacological inhibition of phosphoinositide 3-kinase activity will not always result in loss of function.

KEYWORDS: phosphoinositide 3-kinase beta, DNA repair, scaffold functions, RFC, PIKK

ABBREVIATIONS

DSB, double-strand breaks; GPCR, G protein-coupled receptors; NHEJ, non-homologous end joining; PI, phosphatidylinositol; PIKK, phosphatidylinositol kinase-related kinase; PI3K, phosphoinositide 3-kinase; RFC, replication factor C

INTRODUCTION

Phosphoinositide 3-kinases (PI3K) are a family of enzymes able to transfer phosphate to the 3' position of the inositol ring of membrane phosphoinositides. The members of this family are grouped into different classes based on structural conservation and substrate specificity. Class I enzymes have received the most attention as their lipid products, phosphatidylinositol (PI)(3,4)P_2 and PI(3,4,5)P_3, have critical functions in signal transduction. Resting differentiated cells have low levels of these products, which increase following stimulation of receptors for growth factors, cytokines or chemokines. In cell membranes, PI(3,4)P_2 and PI(3,4,5)P_3 head groups associate with the lipid-binding domains in downstream enzymes, initiating a cascade of events necessary to trigger cell survival, migration and division [1-4]. Recent studies, nonetheless, question the classical view of PI3K as enzymes that exclusively generate 3-poly-phosphoinositides, and show that some additional critical functions of these kinases are independent of their enzymatic activity.

Class I catalytic functions

All PI3K transfer phosphate to the 3' position of the inositol ring of phosphoinositides. They are divided into four classes based on structural similarities and substrate selectivity (Figure 1). Class I PI3K use PI(4,5)P_2 to produce PI(3,4,5)P_3 and through 5′-dephosphorylation, PI(3,4)P_2; members of this class are heterodimers composed of a conserved p110 catalytic subunit and a regulatory subunit. There are four p110 catalytic isoforms: p110α, β, γ, and δ. Except for p110γ, also termed class I_B, which binds to p101 and p84/87 regulatory subunits and is activated mainly by G protein-coupled receptors (GPCR), the remaining

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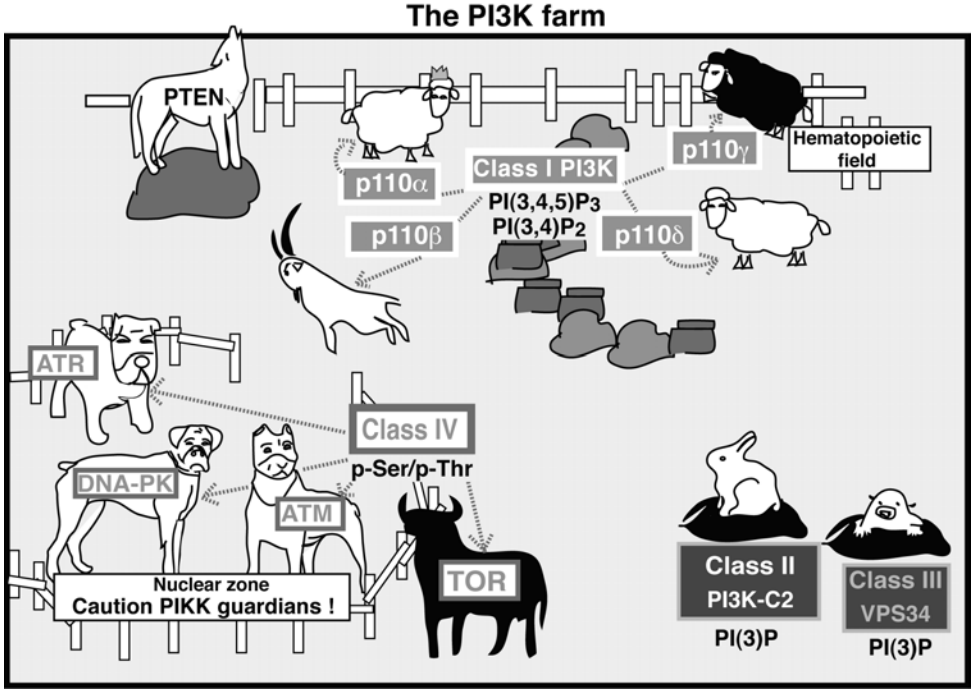

Figure 1. The PI3K farm. Classical and atypical forms of the PI3K classes. Whereas class II and class III enzymes produce PI(3)P, generally in the endosomal compartment, class I isoforms (white) graze on the cell surface, where they generate $PI(3,4)P_2$ and $PI(3,4,5)P_3$, which mediate cell survival, division and migration. $PI(3,4)P_2$ and $PI(3,4,5)P_3$ levels are reduced by the action of the phosphatase PTEN. Among class I isoforms, p110α and p110β are ubiquitous, whereas p110δ and p110γ are more abundant in hematopoietic tissue. In addition, p110γ (the black sheep) is found at low levels in the heart, where it has a critical non-catalytic function in the control of cardiac contraction. The p110β isoform also produces phosphoinositides, but mimics class IV Ser/Thr kinases (also termed PIKK) in their functional role in preserving DNA integrity. Class IV/PIKK are also heterogeneous, most PIKK members are Ser/Thr kinases; while some members such as DNA-PK, ATR and ATM safeguard DNA integrity, TOR watches for growth factor and energy availability. The critical function of p110β in the control of genomic integrity appears to be independent of its kinase activity, but requires its scaffold ability to form complexes with proteins that bind and act on DNA.

class I enzymes bind to and are stabilized by p85 regulatory subunits. The p85 are adaptor molecules with several protein-protein interaction domains including SH2, SH3, Pro-rich and BcR homology domains. p85 bind to p110 via an inter-SH2 region; the SH2 domains are necessary for the activation of p110 by Tyr kinases (TyrK). Regulatory subunits also control p110 intracellular localization and activation, which is also affected by Ras [1-4].

Class I PI3K exhibit distinct expression patterns; p110δ and p110γ are expressed at highest levels in hematopoietic tissues and their deletion or inactivation impairs the immune response [5-7]. p110γ also regulates cardiac function, as discussed below. p110α and p110β are ubiquitous and their expression is essential for embryonic development [3]. p110α is activated by growth factor receptor stimulation and it controls cell cycle entry and cell growth [8-10]. p110β also responds to growth factors and GPCR [11] and presents activity peaks during cell cycle progression that always follow those of p110α [12]; p110β regulates DNA homeostasis. The $PI(3,4)P_2$ and $PI(3,4,5)P_3$ products of class I enzymes activate downstream targets such as Akt (also called PKB), Rho GTPases and mTOR, which are able to drive cell survival, division and migration [13]. Given their ability to modulate critical cell responses, alterations in class I enzyme levels or activity have been linked to various diseases and are promising targets for therapeutic intervention in

cancer and inflammatory diseases [4]. The most frequent alteration in the PI3K pathway in cancer is inactivation of the phosphatase and Tensin homologue (PTEN), which dephosphorylates the 3' position of the inositol ring of PI, showing that the pro-tumorigenic action of class I PI3K is the modulation of $PI(3,4)P_2$ and $PI(3,4,5)P_3$ levels [2, 4].

Class II and III

The class II enzymes, termed PI3K-C2α, β, and γ in mammals, are less well-defined. Class II PI3K phosphorylate PI, PI(4)P and $PI(4,5)P_2$ *in vitro*, nonetheless, *in vivo* they only produce PI(3)P, a product also generated by class III PI3K [14] (Figure 1). PI3K-C2 have large catalytic subunits (>170 kDa), an extended N-terminal domain and a C-terminal region with a PX and a C2 domain, but they lack associated regulatory subunits. Homologues of PI3K-C2 are found in *Drosophila melanogaster* and *Caenorhabditis elegans*, but not in yeast. Class II enzymes are found predominantly in low-density microsomes and the cell nucleus. PI3K-C2α is expressed in epithelium, vascular endothelium and smooth muscle. PI3K-C2β is also widely expressed with highest levels in thymus and placenta, and C2γ is expressed mainly in the liver [14]. The activation mechanism of class II PI3K is poorly defined; class II PI3K is only moderately activated by TyrK and GPCR [15].

The precise function of class II-produced PI(3)P remains unclear. PI(3)P is enriched in intracellular membranes, including early endosomes, multivesicular bodies/late endosomes, phagosomes, and the Golgi apparatus [16, 17]; it permits endosomal recruitment of proteins with PX o FyVe domains, such as FAB1 (FyVe finger-containing PI 5-kinase), GTPase-activating proteins, NADPH subunits, and several proteins involved in membrane transport. PI3K-C2 are specifically associated with processes of endocytosis, exocytosis [18] and autophagy [19]. In flies, the only class II homologue (Pi3k68D) is necessary for endosomal sorting, probably through regulation of PI(3)P levels [20, 21]. The knockout mouse of PI3K-C2α is embryonic lethal, and its deletion in endothelium indicates an essential function in angiogenesis and vascular barrier function [22, 23]. PI3K-C2α appears to have a selective role in the regulation of PI(3)P levels in endothelial cell endosomes, since its depletion, but not that of PI3K-C2β or of class III PI3K, severely impairs vesicle trafficking and vascular endothelial (VE)-cadherin junction assembly [22]. PI3K-C2α is also selective in mediating sphingosine1p-induced endothelial cell migration [23]. In contrast, the PI3K-C2β isoform regulates cell migration in epidermoid carcinoma cells and uterine cervical tumor cells [24-26], which shows that class II selective functions are cell type-dependent.

The class III PI3K, Vps34, was identified in yeast. Its only substrate is PI, and it is probably responsible for the production of most PI(3)P at endosomal membranes. Vps34 forms a heterodimer with the vesicular protein-sorting protein Vps15p; Vps34/Vps15p are conserved from simple eukaryotes to plants and mammals. Vps15p is myristoylated in the N terminus and has an inactive kinase domain, as well as HEAT domains (Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1) and WD (Trp-Asp) repeats; the latter mediates association of Vps15p with Rab5, a GTPase that regulates vesicle transport [14, 27]. Vps34 is not activated by TyrK growth factor receptors, but is induced by glucose, amino acids and some GPCR. Vps34 activation is also regulated in multimolecular complexes. For instance, Vps34 associates with beclin 1, ATG14L (autophagy related-14) and uVRAG (ultraviolet radiation resistance-associated gene); incorporation of Bax-interacting factor 1 to this complex after nutrient deprivation stimulates Vps34 [28, 29]. Vps34 is involved in the regulation of vesicle traffic/protein sorting, in polarized endocytic recycling and in autophagy induction [17, 19, 28, 30-33].

Class II and class III PI3K produce the same lipid and might share effector proteins, although their distinct activation mechanisms, tissue distribution and intracellular localization appear to determine their functional specificity. The relevance of fine-tuned regulation of cell PI(3)P levels was recently highlighted by reports showing the involvement, in human disease, of enzymes that regulate this lipid. Human neuropathies and myopathies are linked to germ line mutations in the PI(3)P phosphatase myotubularin or the FAB1 PI(3)P 5-kinase [33]; Vps34 is also implicated in schizophrenia, and Vps34, PI3K-C2α and PI3K-C2β

have been linked to survival and growth of cancer cells [reviewed in 16].

Class IV/PIKK

Class IV is a structurally unique family of protein Ser/Thr kinases whose catalytic domains are similar to those of mammalian and yeast PI3K [34, 35] (Figure 1). As these enzymes do not produce 3-poly-phosphoinositides, the family was renamed PIKK (phosphatidylinositol kinase-related kinase). Six PIKK have been described in eukaryotes: ATM (ataxia telangiectasia mutated), ATR (ATM- and Rad3-related), SMG-1 (suppressor with morphogenetic effect on genitalia-1), DNA-PK (DNA-dependent protein kinase), TOR (target of rapamycin, also called FRAP) and TRRAP (transformation/transcription domain-associated protein) [36]. PIKK are large proteins (270-470 kDa) that share structural elements such as α-helical repeats in the N terminus, a conserved catalytic domain, and the FAT domain (FRAP, ATM, TRAP) [37]. Of the six reported PIKK, ATM, ATR, TRRAP and TOR are found from *Saccharomyces cerevisiae* to mammals, whereas DNA-PK and SMG-1 arose during metazoan evolution [36, 38].

Several PIKK are involved in DNA repair processes (DNA-PK, ATM and ATR); TOR is an energy and growth factor availability sensor [39, 40], SMG-1 participates in an mRNA quality control mechanism [reviewed in 41, 42] and TRRAP is an essential component of histone acetyltransferase complexes [reviewed in 43]. TOR also differs from PIKK in its substrate specificity; its best-studied substrate, 4EBP1, is phosphorylated at two Thr residues (Thr36 and Thr45) within duplicated STTPGG sequences, whereas DNA-PK, ATM, ATR and SMG-1 are Ser/Thr-Gln-directed kinases [44-48].

DNA damage is a relatively frequent event. Genotoxic agents such as UV light or γ-irradiation and processes such as DNA replication can introduce errors in DNA, which must be repaired to preserve genomic integrity [49, 50]. Cells have developed a number of repair mechanisms, including base excision and nucleotide excision repair, and repair of double-strand breaks (DSB), the most harmful form of damage [49-51]. DNA repair involves recognition of damaged DNA by sensor proteins, recruitment of checkpoint modulators that slow down cell cycle progression, and enrollment of enzymes able to repair the lesion.

There are two related mechanisms for DSB repair: non-homologous end joining (NHEJ) and homologous recombination [50]. DSB repair sites are marked by the formation of large protein aggregates, termed foci, at sites of replication stress, single strand DNA, or DSB. The first stage of the repair process is detection of DNA damage; Mre11/Rad50/Nbs1 is considered the earliest DSB sensor and is shared by NHEJ and homologous recombination [50-53]. In this complex, Rad50 binds directly to DSB ends and stabilizes Mre11, which trims "bad" ends (hairpins, etc) to allow binding of other proteins such as DNA-PK [50, 53]. DNA-PK is essential for the checkpoint activation step, as it induces the p53-dependent $p21^{Cip}$ expression that restrains cell cycle progression [36, 38]. The function of the third complex component, Nbs1 (mutated in the human chromosomal instability disorder), is less well-understood; Nbs1 binds ATM to DSB, thereby enhancing the checkpoint response, since ATM phosphorylates and activates p53 and Chk2 [50, 54]. The NHEJ process (the most common mechanism in G1 phase and in V(D)J recombination) is completed by ligation of the two DNA ends via ligase 4 associated to XRCCl [49, 50].

When NHEJ fails, the cells proceed to homologous recombination (the most common mechanism in G2 phase and in collapsed replication forks in S phase). Homologous recombination also begins with Mre11/Rad 50/Nbs1 sensor complex binding to ATM; subsequent resection of 5´ ends permits generation of single-stranded DNA, and recruitment of replication protein A (RPA), ATR, Rad17 and the Rad9/Hus1/Rad1 complex [49-51]. ATR is activated by single strand DNA breaks and operates in conjunction with its partner protein, ATRIP (ATR-interacting partner) [55]. ATM and ATR show partial redundancy; it is not clear what determines their function in different settings, although γ-irradiation activates mainly ATM and UV light activates ATR. ATR generally participates in repair processes that involve single-stranded DNA; therefore, resection of DSB ends is critical for ATR involvement in DNA repair [51]. Both ATR-regulated Chk1 and ATM-regulated Chk2 can phosphorylate

Cdc25A (which controls S phase entry) and Cdc25C (the mitosis-promoting phosphatase) [56, 57]. The last stage of DSB resolution downstream of ATR or ATR involves the action of Rad51 recombinase.

Several participants in the DNA repair response are related to the family of molecular clamps, proteins that form ring-like structures (generally in trimers) around chromatin. The first molecular clamp identified was proliferating cell nuclear antigen (PCNA), which tethers DNA polymerase (Pol)δ to the replication fork [58, 59]. Clamp loading onto chromatin is a complex process carried out by the replication factor C complex (RFC). Variations in this complex regulate different processes; all RFC variants share RFC2-5 subunits, but have a unique RFC-1-like subunit [59]. Rad17 is an RFC-1 subunit that when associated with RFC2-5 subunits, loads the Rad1-Rad9-Hus1 complex onto damaged DNA [60].

Whereas DNA-PK, ATM and ATR kinases regulate DNA repair, they also have central roles in the formation of macromolecular signaling complexes. Gel filtration analyses indicate that both ATM and ATR form part of very high molecular weight protein complexes in mammalian cells [61]. In addition to regulation of DNA damage responses, ATM controls vesicle transport in cytoplasm, insulin signaling and synaptic function. ATR-regulated Chk1 also governs DNA synthesis, and DNA-PK has substrates not involved in DNA repair, including the class I PI3K effector Akt [36]. The clearest example of a pure scaffold function for PIKK is that of TRRAP, which has no kinase activity but regulates the formation of histone acetyltransferase complexes [38, 43].

Class I PI3K kinase-independent functions: p110γ in heart function

Recent findings on the class I PI3K family suggest that some of its members, in particular p110γ and p110β, have kinase-independent functions. Some protein kinases also have non-catalytic functions; epidermal growth factor receptor (EGFR) preserves cell viability in a kinase-independent manner *via* its interaction with p53 upregulated modulator of apoptosis (PUMA) or with the sodium/glucose cotransporter 1 [62, 63]. Src controls focal adhesion kinase (FAK) phosphorylation in both a catalytic and a non-catalytic fashion in colon cancer cells [64], as does ERK as a transcriptional regulator of INF-γ controlled gene expression [65].

PI3K has recently been involved in the control of cardiac function. Whereas p110α regulates cardiac growth and physiological hypertrophy, p110γ regulates cardiac contractility [66]. Contractility is modulated by β-adrenergic receptors (AR), cAMP and protein kinase A (PKA). Catecholamine binding to β-AR promotes adenylyl cyclase activity, raising cAMP levels [67]. p110γ-deficient mice show no structural abnormalities of the heart, although they have marked enhancement in basal contractility due to increased levels of myocardial cAMP [66, 68]. In contrast, mice that express a kinase-dead p110γ show normal cAMP levels and unaltered contractile function [68]. p110γ regulation of myocardial contractility is thus largely unrelated to its kinase activity. Indeed, p110γ is responsible for the assembly of a signaling complex that includes the p84/p87 regulatory subunit, phosphodiesterase 3B, and its potential activator PKA [68, 69]. In this complex, p110γ binds directly to the RIIα regulatory subunit of PKA and promotes PKA-mediated activation of phosphodiesterase 3B and subsequent reduction of cAMP levels. Disassembly of this complex in p110γ-deficient mice leads to a major reduction in phosphodiesterase activity, abnormal cardiac cAMP levels and contractility defects; in contrast, inactive p110γ can form the complex and the mice show no contractility defects [68, 69]. p110γ-deficient mice also have increased infarct size and severely impaired cardiac function in an ischemia model, whereas mice with inactive p110γ resemble controls [70]. These findings indicate a critical kinase-independent role for p110γ in cardiovascular function [reviewed in 71-73]. p110γ also controls platelet aggregation in a kinase-independent manner [74]. Evidence suggests that also a truncated form of p110δ that lacks the kinase domain is able to regulate cell growth and proliferation in HEK-293 cells and embryonic fibroblasts [75].

Class I PI3K kinase-independent functions: the case of p110β

p110β is a ubiquitously expressed class I isoform that has an essential function in early embryonic development (days 2-3) [76]. After TyrK and GPCR activation, p110β contributes to cell activation via

the canonical PI3K pathway, by inducing $PI(3,4)P_2/PI(3,4,5)P_3$ formation and subsequent activation of the downstream effector pathway [11, 12]. p110β also has kinase-independent function in the control of DNA homeostasis. Indeed, p110β has a nuclear localization signal that mediates localization in this compartment [77]. p110β expression is necessary for PCNA localization to chromatin, thereby controlling Polδ-regulated elongation of the replicating DNA strands. Whereas p110β inhibition only moderately impairs this process, p110β depletion markedly affects DNA elongation [10]. Although modest, the effect of p110β inhibitors on DNA replication suggests that p110β activity on nuclear membrane phosphoinositides cooperates with the p110β scaffold function for DNA synthesis.

Proteomic analysis identified several p110β-associated proteins including Rad50, Rad9 and Rad17, which mediate DSB repair by homologous recombination. p110β concentrates at DSB foci, and its depletion reduces both ATR and ATM activity and in turn, Chk1 and Chk2 activity. An interesting observation on DBS repair on p110β depleted cells is the defective binding of the Nbs1 sensor protein to the damaged DNA, pointing at p110β as an early mediator of DNA damage sensing. Whereas p110β activity contributed modestly to this function, p110β depletion had a more notable effect; indeed, inactive p110β partially rescued the defective activation of the DSB repair pathway [78].

p110β also regulates chromosome segregation in mitosis. Indeed, p110β depletion promotes chromosome separation even when chromosomes

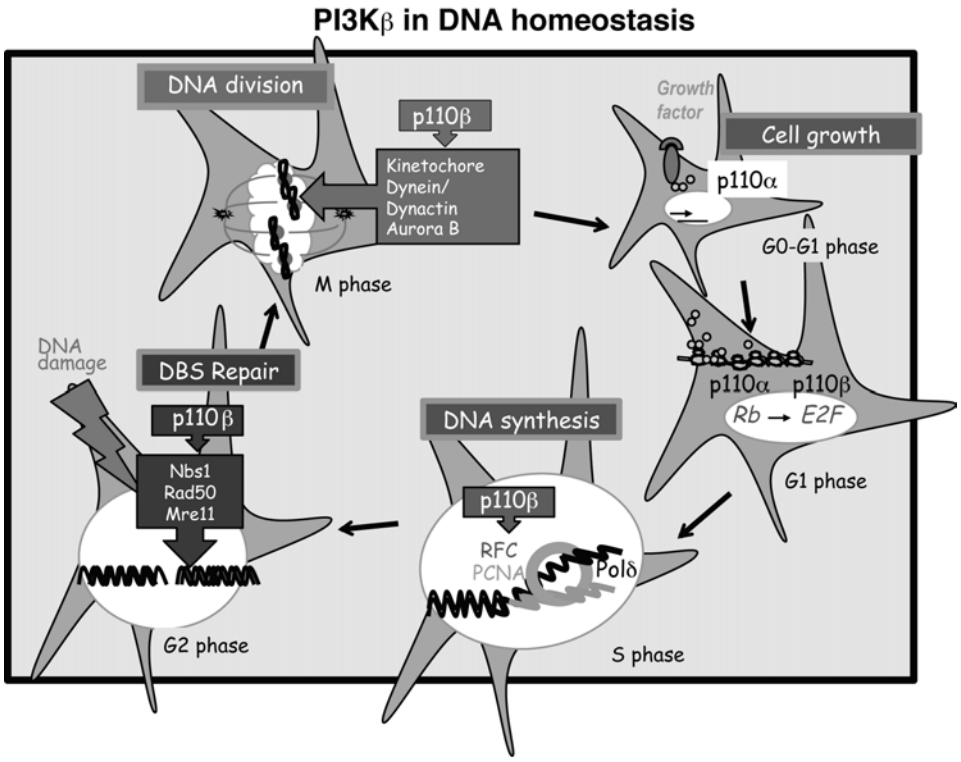

Figure 2. p110β actions in DNA homeostasis. p110β contributes to generation of 3-poly-phosphoinositides after growth factor receptor stimulation, at G0 to G1 transition, but the increase in cell mass is mainly regulated by p110α. In addition, p110β exhibits a scaffold function in the organization of proteins complexes that act on DNA; these include the formation of the RFC complex required for binding to chromatin of PCNA, which tethers DNA polymerase (Pol)δ to the replication fork during S phase. p110β also binds to double strand breaks at the first phases of the DNA repair response, permitting subsequent binding of the MRN sensing complex. During DNA division, p110β regulates activation of Dynein/Dynactin and Aurora B activity in the mitotic kinetochore.

are unaligned, suggesting defective spindle checkpoint action [79]. Accordingly, p110β depletion, but not its inhibition, impairs activation of dynein/dynactin and Aurora B in the kinetochore; the latter responsible for premature inactivation of the spindle checkpoint [79]. As observed with PIKK enzymes, p110β regulates biological processes other than DNA homeostasis, such as EGFR, transferrin receptor recycling and autophagy [80, 81].

These observations show that p110β participates in three important events in genome integrity: PCNA binding to chromatin during replication, Nbs1 binding to DSB, and activation of kinetochore proteins during mitosis (Figure 2). Some of these effects appear to be related to p110β function as a scaffold for RFC. p110β binds directly to RFC-1 and to the RFC-1-like subunits Rad17 and CTF18 [82]. Moreover, p110β depletion leads to defective subcellular localization of RFC-1, impaired RFC complex formation and in turn imperfect binding to chromatin of PCNA, Rad17-RFC and CTF18-RFC molecular clamps [82]. Not all kinase-independent p110β functions depend on RFC; this is the case for its function in Aurora B regulation in mitosis, or for p110β-dependent Nbs1 binding to DSB [78, 79].

CONCLUSION

p110β appears to have two type of functions. In the first of these, it acts as a lipid kinase after receptor stimulation and contributes to the formation of $PI(3,4)P_2$ and $PI(3,4,5)P_3$ in activated cells. p110β kinase activity contributes to cancer, since its activity is necessary for oncogenesis; this is the case in an ERBB2-based model of breast cancer [83] and in prostate cancer development in *pten*-deficient mice [84]. In addition, p110β exhibits an apparently distinct function in the control of DNA homeostasis; for this action p110β utilizes a kinase-independent mechanism and regulates formation (or binding to chromatin) of complexes that act on DNA [10, 78, 82]. p110β non-catalytic function is essential for early embryonic development, as mice with inactive p110β are not early embryonic lethal, as p110β-/- mice [83].

The p110β kinase-independent effects on DNA replication and repair more closely resemble the function of class IV/PIKK proteins than that of class I PI3K enzymes, suggesting that p110β acts as both a class I PI3K enzyme and a class IV/PIKK checkpoint regulator. Independently of its classification as a class I or a class IV enzyme, p110β scaffold function for DNA events shows that p110β is more than a kinase for DNA homeostasis.

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CONFLICT OF INTEREST STATEMENT

None.

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