Non-genomic loss of PTEN function in cancer

Non-genomic loss of PTEN function in cancer: not in my genes

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Abstract

Loss of function of the PTEN tumour suppressor contributes to the development of many cancers. However, in contrast to classical models of tumour suppression, partial loss of PTEN function appears to be frequently observed in the clinic. Additionally, studies of both humans and mice with reductions in *PTEN* gene dosage indicate that even partial loss of PTEN function is sufficient to promote some cancer types, particularly in the breast. PTEN expression appears to be tightly controlled both transcriptionally and post-transcriptionally, with several recent studies implicating oncogenic microRNAs in PTEN suppression. Additionally, the lipid phosphatase activity of PTEN can be post-translationally regulated via inhibitory phosphorylation, ubiquitination or oxidation. Here we discuss these multiple mechanisms of PTEN regulation. We also put into context recent proposals that changes in this regulation might drive tumour development and address the accompanying evidence for their clinical significance.

Introduction

Cancer is believed to develop because some cells within an organism become aberrant and hyperproliferative through the accumulation of genetic and epigenetic changes. The genes most frequently modified in cancer are frequently categorised into two groups: *oncogenes*, in which a gain of function drives tumour formation and *tumour suppressors*, in which a loss of function promotes tumour development. The lipid phosphatase, PTEN, is a tumour suppressor originally identified by two research groups in 1997 [1, 2]. Mutations of the *PTEN* gene occur at some significant frequency in almost all human tumour types and mutation of at least one allele occurs in a third or more of breast, colon, prostate and lung tumours [3, 4]. Accordingly, in a recent cancer genomics study aiming to distinguish between deletions driven by chromosomal instability and those driven by phenotypic selection, *PTEN* was proposed to be the tumour suppressor locus in the human genome with the greatest selection for loss [5].

Biochemically, PTEN is a phosphatase that dephosphorylates phosphatidylinositol 3,4,5-trisphosphate (PIP_3), the lipid product of the class I phosphoinositide 3-kinases (PI3K) [6]. This discovery was rapidly followed by a wealth of genetic and cell-based evidence showing that PTEN is a ubiquitous inhibitor of PI3K-dependent signalling [7]. PI3K/PTEN signalling (and the PIP_3 lipid they control) influence cellular behaviour through a large and diverse set of cellular PIP_3-binding proteins, the best characterised of which are the AKT

protein kinases [8-10]. In this way, PI3K and PTEN orchestrate cellular responses to growth factors, cytokines, integrins and other intercellular mediators and contribute to the growth, motility, survival and metabolic responses of many cell types. PTEN also has robust protein phosphatase activity *in vitro* and has been proposed to play a tumour suppressor role in the nucleus, independently of plasma membrane-localized PIP_3 [11-14]. However, the significance of these other mechanisms of action is currently unclear and space constraints do not allow us to consider them further here. The loss of PTEN function in tumours should also be viewed in the context of the broader PI3K pathway, in which an alternative route of PIP_3 metabolism is provided by the family of phosphoinositide 5-phosphatases, in particular the SHIP enzymes, which convert PIP_3 to the alternate signal $PI(3,4)P_2$ (Figure 1).

Partial loss of PTEN function

For many years the ‘gold standard’ of evidence for functional dysregulation in human tumours has been genetic mutation data, in large part due to apparent clarity provided by the identification of novel sequence variants and the usually robust nature of DNA sequence data. Accordingly, it has been clear for many years that the great majority of PTEN mutations identified in tumours inhibit the function of the enzyme [15, 16]. However, improvements in other technologies in tumour pathology, including immunohistochemistry and microarray analysis of both gene expression and copy number has meant that it is now possible to

analyse multiple tumour characteristics with greater confidence. This broadening of tumour pathology, and in particular the increased confidence in PTEN immunohistochemical data [17, 18] has greatly augmented our understanding of the mechanisms by which PTEN function is lost in tumours. A picture has emerged that biallelic mutation of PTEN occurs at highest rates in endometrial carcinoma and glioblastoma, but only in 15% or less at other tumour sites, such as prostate, breast, colon and lung. However, in all of these tumour types, mutation of one copy of *PTEN* and loss of protein expression is far more common [4, 16, 18-20].

A wealth of data tells us that several PTEN-regulated processes can be controlled by small changes in the expression level of the phosphatase and the phenotypic consequences of inheriting one wild-type and one mutant allele of *PTEN* are significant in both humans and mice [4, 21-24]. For example Cowden syndrome patients who inherit a PTEN mutation are reported to have a greatly increased lifetime risk of breast (25-50%), thyroid (10%) and endometrial (5-10%) cancers relative to the broader population [21], with some estimates of lifetime cancer risk being as high as 89% [25]. Such patients also show a number of developmental abnormalities, including several forms of benign growths, in particular those in the skin, mucous membranes, breast and thyroid but also macrocephaly [21].

Among the most remarkable examples of the dose-dependence of PTEN function are studies of a series of *Pten* transgenic mice that combine wild-type, null and hypomorphic alleles to express levels of Pten ranging from 0, 25%, 50%, and 75% to 100% of wild-type levels either throughout the animal or selectively in the prostate [23, 26]. Both lifetime tumour burden and cellular P-Akt closely follow the expression level of Pten and relative to wild-type mice, both are significantly increased by even a modest drop to 75% of normal expression, especially in the breast. This is in stark contrast to some other enzymes. For example, Pdk1 transgenic mice (expressing only 10% of the normal dose of this kinase) are viable and fertile. They show normal activation of the best characterised substrates of Pdk1, whereas Pdk1 null animals die at E9.5 [27].

This finding that small experimentally generated changes in PTEN expression can lead to significant increases in cancer risk strongly indicates that cellular mechanisms that cause small reductions in PTEN catalytic activity might also have significant clinical importance. Consistent with this concept, clinical immunohistochemical and expression array data from many tumour types show that reductions in PTEN expression are frequently observed in tumours that retain at least one wild-type copy of the PTEN gene. Because most tumours retain some expression of active PTEN protein, it is an exciting development that evidence for the post translational inhibition of PTEN function in tumours has recently emerged [28, 29]. In addition, several proteins have now been identified as regulators of PTEN function that are themselves mutated, over-expressed or

lost in tumours and appear to mediate effects on tumour development through changes in PTEN. These proteins appear to regulate PTEN through effects on PTEN phosphorylation [30, 31], ubiquitination [32], oxidation [33] and via direct inhibition of PTEN's catalytic activity [34, 35] and will be discussed in more detail below (see also Table 1 and Figure 2).

PTEN function: constitutive activity and post-translational inhibition?

The *PTEN* gene encodes a single 403 amino acid protein (Figure 2). Genetic deletion or RNAi-mediated knock-down of PTEN from many (but not all) unstimulated cultured cells or tissues leads to robust increases in PIP_3-dependent AKT phosphorylation and (where measured) cellular PIP_3 levels themselves [36-38]. This strongly argues that PTEN plays a continuous physiological role in suppressing PIP_3 levels in many cell types and is consistent with the constitutive activity of PTEN purified from multiple sources [6, 39, 40] and the lack of evidence for stimulus driven PTEN activation. However, several mechanisms of acute stimulus-driven PTEN inhibition, (mediated via mechanisms including oxidation, phosphorylation and ubiquitination) have been described. These data imply that several regulatory cellular responses co-ordinate control of PIP_3-dependent signalling by simultaneous activation of PI3K and inhibition of PTEN. Also, it is known that many diverse cellular proteins (probably more than 100) are either regulated directly by PIP_3 binding or by AKT phosphorylation. Thus, loss of constitutive PTEN function would be expected to

affect a very diverse set of proteins and the cellular responses they control, each potentially with distinct dose relationships for PIP_3 activation. Therefore, we would argue that incremental functional and pathological consequences of PTEN loss across a wide range of expression levels seem more consistent with these established models of signalling than a classical tumour suppressor model in which only complete loss of function drives tumour formation. This hypothesis also seems consistent with the occurrence of multiple PI3K pathway mutations in the same tumours [41].

PTEN ubiquitination controls its expression, localisation and activity

As described above, at least some important processes controlled by PTEN, such as tumour suppression in the breast and the regulation of total cellular $PtdInsP_3$ levels appear to be responsive to small changes in the expression level of the phosphatase [4, 26]. Cellular PTEN concentrations are controlled by regulation of both its synthesis and degradation. We will discuss the regulation of PTEN stability here and mechanisms controlling PTEN synthesis below. It was shown some years ago that the addition of ubiquitin chains to PTEN (polyubiquitination) can target PTEN for destruction, through the classical ubiquitin proteasome pathway (reviewed by Wang and Jiang [29]). However, it is now clear that the post-translational regulation of many proteins by the ligation of ubiquitin (or ubiquitin-related polypeptides) exhibits significant diversity in the number of ubiquitin units, the ubiquitin lysine residues participating in chain

formation, and the functional consequences on the target protein. In accordance with this, it appears that whereas polyubiquitination of PTEN can target PTEN for destruction [42, 43], monoubiquitination can drive nuclear import [14] and either modification appears to directly inhibit catalytic activity [44].

The first identified ubiquitin E3 ligase with activity against PTEN was the HECT domain E3 ligase, NEDD4, which was purified using a biochemical PTEN ubiquitination assay [32]. Xuejun Jiang's research group showed that NEDD4 can ubiquitinate PTEN efficiently *in vitro* and interacts with the phosphatase in cells. They also showed that increasing or reducing cellular NEDD4 expression leads to reduced or increased PTEN expression respectively and corresponding PTEN dependent changes in cellular transformation and tumorigenicity in xenografted tumour models. Finally they identified an inverse correlation between NEDD4 expression and levels of PTEN protein, but not mRNA, in a group of patients with bladder cancer [32].

However, the identity of the dominant E3 ubiquitin ligases responsible for PTEN ubiquitination is controversial because analysis using two lines of mice lacking NEDD4 found no changes in the expression and localisation of PTEN or in AKT activity in embryonic fibroblasts or heart tissue [45]. The RING domain E3 ligase, XIAP has also been proposed to mediate PTEN ubiquitination because knockdown of XIAP expression appears to cause reduced AKT phosphorylation and PTEN ubiquitination and increased PTEN levels [46]. However, further

independent support for the significance of NEDD4, or perhaps a NEDD4 related ligase, in the regulation of PTEN has been provided by work with retinal ganglion cells and with astrocytes in which over-expression of a dominant negative NEDD4 protein or NEDD4 siRNA respectively increased PTEN expression [47, 48]. Similarly, studies of the NDFIP proteins show that these proteins can act as regulators of the NEDD4/Itch family of E3 ligases and also control the ubiquitination and expression of PTEN [49]. However, the expression of either dominant negative NEDD4 proteins or NDFIP proteins would be expected to affect other related members of the NEDD4/ITCH ligase family [48, 49]. It therefore seems probable that several E3 ligases for PTEN may exist.

These studies have provided some insight into how PTEN can be regulated by ubiquitination, but currently there is little information about the functions and consequences of PTEN ubiquitination in physiology and pathology. Increased PTEN polyubiquitination appears to be responsible for the reduced PTEN expression observed in cells exposed to stresses such as zinc or hyperosmotic stress [44, 50, 51] and in some tumours [32, 47]. However, the observed reversibility of PTEN ubiquitination [52] and evidence for direct control of both activity [44] and localisation [14] of PTEN suggest that exciting new findings might emerge regarding the acute control over PTEN function.

PTEN oxidation

PTEN is a member of the Protein Tyrosine Phosphatase family, which shares a reactive catalytic site cysteine nucleophile and the accompanying potential to be regulated by active site oxidation [53, 54]. Initial studies showed that a fraction of the cellular PTEN protein becomes oxidised in response to the endogenous generation of reactive oxygen species (ROS) stimulated by several ligands, including growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and insulin. This correlates with a ROS-dependent activation of downstream AKT phosphorylation [40, 55-57]. More recently, PTEN oxidation has been implicated in the development of T cell acute lymphoblastic leukemias (T-ALL) that display constitutively high levels of ROS [28]. Evidence also supports PTEN oxidation as a key mechanism responsible for the multi-organ tumorigenesis observed in mice lacking the hydrogen peroxide metabolising enzyme, Prdx1 [33]. These findings implicate ROS (specifically hydrogen peroxide) in the oxidation of PTEN, which supports the proposed role for H_2O_2 in mediating the oxidation of phosphatases during receptor tyrosine kinase signal transduction [58]. However, the observation of multiple oxidised forms of PTEN *in vitro* and evidence for the modification of PTEN both through S-nitrosylation and in response to arachidonic acid signalling suggest that multiple mechanisms of PTEN redox regulation exist [53, 59-62].

PTEN phosphorylation

PTEN is constitutively phosphorylated to high stoichiometry upon a cluster of C-terminal serine and threonine residues (Ser380/Thr382/Thr383/Ser385) that maintain the phosphatase in a stable ‘closed’ conformation with reduced plasma membrane localisation and lipid phosphatase activity [16, 63, 64]. It seems likely that this partially reflects a cytosolic pool of the phosphatase, as in contrast, it appears that PTEN incorporated into stable high molecular weight complexes is poorly phosphorylated on these sites [65, 66]. The phosphorylation of these sites appears to be mediated by CK2 [43]. PTEN is also phosphorylated with lower stoichiometry at Thr366 and Ser370, phosphorylation at the latter site by CK2 appears to promote subsequent phosphorylation at Thr366 by GSK3 [67, 68]. Tyrosine phosphorylation of PTEN has also been reported on several occasions, implicating several potentially phosphorylated residues, but these sites currently remain to be confirmed using high quality mass spectrometry or well validated phosphorylation site-specific antibodies.

Two proteins have been proposed to act as tumour suppressors through the promotion of PTEN phosphorylation and stabilisation: PICT1 (also known as GLTSCR2) and RAK (also known as FRK). Both were identified initially as direct PTEN binding proteins that were able to promote PTEN phosphorylation [31, 69] In the case of the RAK tyrosine kinase, experiments individually mutating several tyrosine residues of PTEN suggests this might be through direct phosphorylation of the exposed PTEN Tyr336 (see previous paragraph), whereas knockdown of PICT1 expression was shown to promote C-terminal dephosphorylation of PTEN

Ser380, although the mechanism is unclear [31, 69]. The evidence is strong that knocking down the expression of these proteins is able to transform cells in culture and, in the case of RAK, confers tumorigenicity on xenograted breast tumour cells *in vivo*. It was also shown that these effects only occur in cells expressing PTEN [30, 31]. Finally, there is evidence that the function of these proteins is lost in human tumours. *RAK/FRK* is located at chromosome 6q21, a region of frequent loss in many tumour types including breast [70, 71]. The evidence for selective focal loss of *PICT1/GLTSCR2* is perhaps even stronger. Studies have identified reduced expression of PICT1 in neuroblastoma, which correlates with reduced PTEN protein, yet retained PTEN mRNA. In glioblastoma *PICT1* deletion, mutation and reduced expression have been described, and finally, reduced expression of PICT1 has been shown in expression profiling of ovarian tumours [30, 72, 73].

P-Rex2 and SIPL1: direct oncogenic inhibitors of PTEN activity?

Two recent studies have presented exciting results concerning two potential PTEN regulators, P-Rex2 and SIPL1, identified through proteomic and functional over-expression library screens respectively [34, 35]. Both proteins were shown to bind PTEN directly, could directly inhibit phosphatase activity *in vitro* against PIP_3, and in cells, the manipulation of expression of P-Rex2 or SIPL1 could affect AKT phosphorylation in a PTEN-dependent manner [34, 35]. RNAi-mediated knockdown or over-expression of P-Rex2 and SIPL1 were also able to control

xenograft tumour formation *in vivo* [34, 35]. However, in the case of SIPL1, in xenografted HeLa cell experiments, knockdown of PTEN enhanced tumour development as expected, but knockdown of SIPL1 strongly reduced tumour growth even in cells in which PTEN expression had been demonstrably knocked down, suggesting that SIPL1 could promote tumour formation by PTEN-independent pathways [35]. Finally, analysis of clinical samples identified a correlation between the expression of PTEN with P-Rex2 or SIPL1 expression in groups of breast and cervical cancers respectively [34, 35]. Furthermore, mutations in P-Rex2 have been identified in several tumour types at a frequency of around 3% [34].

Epigenetic and transcriptional regulation of PTEN

In addition to gene mutations/deletions, protein post-translational modifications and protein-protein interactions affecting its activity and stability, PTEN undergoes a complex regulation by multiple epigenetic and transcriptional mechanisms (Figure 3). This additional level of regulation appears to play a crucial role in various cancer or metabolic disorders, in which PTEN expression is altered with no apparent muations or deletions of the gene.

Epigenetic mechanisms

PTEN promoter hypermethylation might represent an alternative mechanism by which the tumour suppressive activity of PTEN is lost. Hypermethylation of CpG islands in the PTEN promoter was reported in several type of cancers, including prostate cancer, colorectal cancers, breast cancer, hepatocellular carcinoma, gastric carcinoma, melanoma, lung cancer, endometrial carcinoma, glioma and haematological malignancies [74-83]. Although silencing of tumour suppressor genes by hypermethylation of their promoters is a hallmark in many cancers, this mechanism should be considered with caution regarding PTEN. Indeed, PTEN promoter hypermethylation does not always strictly correlate with the loss of PTEN protein expression, and it is still unclear if hypermethylation observed in many studies occurs on the promoter of PTEN, or its pseudogene [84].

PTEN transcription is also directly or indirectly regulated by histone acetylation/deacetylation. In particular, a recent study demonstrated that SALL4, a transcription factor required for the maintenance of pluripotency and self-renewal of embryonic stem cells, binds to the PTEN promoter. Binding of SALL4 represses PTEN transcription by recruiting Mi-2/NuRD, an epigenetic repressor complex containing a chromatin remodelling ATPase and a histone deacetylase [85, 86]. Histone deacetylase inhibitors, such as trichostatin A, were also shown to upregulate PTEN transcription in hepatoma and fibroblastic cells. However, in this case PTEN transcriptional upregulation might be indirect and related to activation of the transcription factor Egr1 [87, 88].

Transcriptional regulation

Experimental evidence and sequence analyses of the PTEN core promoter have identified several potential binding sites on regulatory sequences of the PTEN gene for transcription factors (Figure 3), which might positively or negatively regulate PTEN transcription [89-91]. Among factors positively regulating PTEN expression at the transcriptional level, early growth response factor 1 (Egr-1) was shown to trigger PTEN upregulation in UV-irradiated cells and in mammary glands stimulated by IGF2 [92, 93]. Consistent with these findings, PTEN overexpression in the gastric mucosa of aging rats was correlated with Egr-1 upregulation [94]. C-promotor binding factor 1 (CBF-1) is another transcription factor, that can exert dual control on PTEN transcription. Indeed, CBF-1 in complex with other proteins acts as a transcriptional repressor, but when Notch-1 is activated, it binds to CBF-1 and modifies the repressor complex protein composition thus converting CBF-1 into a transcriptional activator for PTEN [95]. In this regard, Notch-1 signalling is impaired in prostate adenocarcinoma suggesting that the transcriptional repressive activity of CBF-1 may contribute to the loss of PTEN expression with prostate tumorigenesis [96].

Binding sites on the PTEN promoter for peroxisome proliferator-activated receptor (PPAR)γ have also been reported [89]; however whether PPARγ promotes or represses PTEN mRNA transcription is unclear and seems to differ depending of the cell type analyzed. Indeed, in cancer and epithelial cells PPARγ

upregulated PTEN transcription [89, 97-99] whereas in muscle and adipocytes cells it had inverse effects [100], suggesting that cell specific co-activators/corepressors might be involved in these regulatory mechanisms. In both cases however, PPARγ-dependent modulation of PTEN expression appeared beneficial either by exerting a tumour suppressor and anti-inflammatory effects (PTEN upregulation in cancer/epithelial cells) [89, 97-99] or by increasing insulin sensitivity (PTEN downregulation in insulin-sensitive muscle or adipose cells) [100]. Of note, PPARβ/δ repressed PTEN transcription in lung carcinoma cells [101] but through mechanisms involving nuclear factor (NF)κB (see below). In the context of metabolic diseases, the adipokine resistin was equally shown to upregulate PTEN expression by inducing Atf2 activation and binding to the PTEN promoter [102]. Investigations of PTEN regulation by p53 led to the discovery of a complex and surprising relationship between the two tumour suppressors. Indeed although p53 appears to stimulate transcription of PTEN [103], high p53 expression triggers PTEN protein proteosomal degradation [104]. PTEN interaction with p53 seems to be required for p53 functions and to stabilize p53 by preventing Mdm2-mediated degradation [103, 105, 106]. This reciprocal regulation at multiple levels provides an interesting example of a tumour suppressor network, in which a single genetic hit or dysregulation of one tumour suppressor may impact on the expression of others.

Other factors have been equally shown to negatively regulate PTEN transcription through various mechanisms. Snail1, a transcriptional repressor

triggering epithelial-to-mesenchymal transition, and Id-1, an oncogene inactivating several tumour supressors and promoting cell growth, bind to the PTEN promoter and inhibit its transcription by preventing binding of p53 to the PTEN promoter [107, 108]. Two other transcription factors, Bmi-1 and c-Jun, which are dysregulated in various cancers, also bind to the PTEN promoter and inhibit its transcription but through still poorly known mechanisms [109, 110]. Finally, NFκB appears also to inhibit PTEN transcription through direct or indirect mechanisms and a reciprocal regulation of PTEN and NFκB expression/activation has been observed in cancer cells [111, 112]. At least two putative binding sites for NFκB are present on the PTEN promoter and direct binding of NFκB to these sites was reported to repress PTEN transcription in MEF cells [113]. However, NFκB appears to control PTEN transcription also through indirect mechanisms. For example, NFκB represses PTEN transcription in NIH 3T3 cells by sequestering CBP/p300, a PTEN transcriptional activator [111], whereas NFκB activation in hepatocytes triggers PTEN downregulation by stimulating miR-21 biosynthesis and PTEN mRNA degradation [91, 114].

microRNAs

A further degree of complexity in the regulation of PTEN mRNA transcription and translation is provided by the action of several microRNAs (miRNAs), which have been demonstrated to interact with the 3' untranslated region of the PTEN mRNA. miRNAs are short single-stranded RNA molecules of approximately 19-

22 nucleotides, which are partially complementary to one or more messenger RNA molecules. Their best characterized function is to downregulate protein expression through translational repression or degradation of target mRNAs [115]. Several miRNAs have been shown to play a role in tumorigenesis or metabolic disorders by downregulating PTEN. These include miR-17-92 in lymphoproliferative disease and autoimmunity [116], miR-19a in leukaemia and Cowden syndrome [117-119], miR-26a in high grade glioma [120], miR22 and the miR106b~25 cluster in prostate tumorigenesis [36], miR155 in hepatic carcinogenesis [121], miR-214 in ovarian cancer [122], miR-216a and miR-217 in diabetic kidney diseases [123] and miR-21 in multiple cancers, inflammation and metabolic diseases (reviewed in [124, 125]). Multiple miRNAs probably contribute simultaneously to the loss of PTEN expression in specific cancers, but this remains to be firmly established. Little is known about molecular mechanisms controlling the expression of miRNAs, except for miR-21, one of the most frequently dysregulated oncogenic microRNA (oncomir) in cancer, for which both transcriptional and post-transcriptional mechanisms have been described [124]. At the transcriptional level, several functional binding sites in the miR-21 promoter have been identified, (i.e. for AP-1, Ets/PU.1, C/EBP-α, NF1, SRF, p53, STAT3 and NFκB) [91, 124]. Interestingly, transforming growth factor (TGF)β (a potent inhibitor of PTEN expression in some cell types), upregulates miR-21 expression at post-transcriptional steps [126], but was also suggested to exert its effect on PTEN expression through the induction of two other miRNAs, miR-216a and miR-217 [123]. Finally, a recent study has proposed a new mechanism

regulating the expression of PTEN, (which might also apply to other tumour suppressors) that involves coding-independent functions of the PTEN pseudogene *PTENP1* (an untranslated copy of the *PTEN* coding sequence). The mRNA of the pseudogene *PTENP1* appears to act as a decoy for PTEN-targeting miRNAs, thus preventing the negative regulatory effects of miRNAs on PTEN expression [127]. The relevance of this mechanism in cancer is further supported by the focal and independent loss of the PTENP1 locus in sporadic colon cancer [127].

Cancer therapeutics targeting PTEN function?

The PI3K signalling pathway appears to contribute to driving the formation of most human tumours and frequently to resistance of these tumours to existing therapies [20, 128]. The pathway is therefore the target of intense drug discovery activity [129, 130]. The emergence of evidence, discussed in this review, that tumour development is promoted by modifiers of PTEN function has raised the possibility of these modifiers as novel drug targets. The most appealing targets would seem to be functional inhibitors of PTEN from druggable enzyme groups such as ubiquitin ligases or kinases that selectively inhibit PTEN function (see Table I), in particular CK2, NEDD4-1 and XIAP. Although selective inhibitors of these three enzymes would not be expected to act solely through effects on PTEN, these effects provide mechanistic support for existing drug development programmes [131, 132].

In targeting the broader PI3K pathway, most effort has gone into developing small molecule inhibitors of the Class I PI3K enzymes themselves as well as downstream components including the AKT, PDK1 and mTOR kinases; several agents are in clinical trials [129, 130]. It is important to note that low cellular PIP_3 is not always a good thing and of concern in these programmes are potential metabolic side-effects caused by inhibiting the PI3K-dependent steps in insulin responsive signal transduction. This focus on targeting the positive mediators of PI3K signalling stems from the belief that screening drug-like small molecules is more likely to succeed in identifying enzyme inhibitors than enzyme activators. However, there have been successes from other areas in the development of enzyme activators [133], and some limited attention has been paid to PTEN itself as a drug target in cancer. Indeed, selective small molecule activators of the alternate PIP_3 5-phosphatase, SHIP, have been described that can inhibit cellular PI3K dependent signalling and induce apoptosis in SHIP-expressing multiple myeloma cells [134]. This proof-of-concept development of a phosphatase activator provides further potential approaches to targeting the PI3K pathway.

Concluding remarks

In summary, novel insight into the mechanisms leading to the loss of PTEN function in tumours should identify novel therapeutic approaches, but also provides a deeper understanding of oncogenic PI3K/PTEN signalling that will

assist the development and eventual use of the many agents in development targeting this pathway. It seems likely that continued intense activity in this broad research field will provide many areas of progress and eventual clinical success stories.

Figure Legends

Figure 1. The core PI 3-kinase signalling pathway. An established model for the synthesis of PIP_3 by the receptor-stimulated action of the class I PI 3-kinase enzymes and the metabolism of PIP_3 by PTEN is presented. An alternative route of metabolism of PIP_3 is provided by the action of the phosphoinositide 5-phosphatase enzymes, such as SHIP, which convert PIP_3 to $PI(3,4)P_2$. PIP_3 and $PI(3,4)P_2$ affect many cellular processes through overlapping groups of selective lipid-binding proteins that include the AKT kinases. The significance of reported mechanisms of receptor driven PTEN inhibition is currently unclear.

Figure 2. Regulation of the PTEN protein. **A.** The PTEN protein is shown, including an expansion of the regulatory C-terminal tail, identifying residues phosphorylated by CK2 and GSK3. Identified sites for ubiquitination of lysine residues 13 and 289 are also represented. The $PI(4,5)P_2$ binding site and adjacent cytoplasmic localisation signal are located at the N-termunus [135, 136]. **B.** A model for the conformational regulation of PTEN is presented, in which C-terminal phosphorylation promotes an electrostatic interaction between this highly acidic C-terminal tail and basic surfaces of the N-terminus, phosphatase and C2 domains (shown in blue). In the open conformation, these basic surfaces of PTEN interact with the acidic inner surface of the plasma membrane. The catalytic core of PTEN, comprising the phosphatase and C2 domains (PHOS/C2) is shown as an oval, with the less structured termini being shown as lines [39].

This model was developed from the original proposal of Vazquez et al [66]. The N-terminal poly-basic region appears to selectively interact with $PI(4,5)P_2$ [137] and also contribute to the nuclear accumulation of PTEN [138]. PIP_3 is shown interacting with the active site.

Figure 3. Control of PTEN expression and activity. The diagram summarizes the various mechanisms by which PTEN expression and activity can be dysregulated in pathological conditions. These include: i) epigenetic inhibitory mechanisms such as methylation of the PTEN promoter and histone acetylation; ii) positive and negative regulation of PTEN mRNA transcription by various transcription factors; iii) PTEN mRNA degradation and translational repression by microRNAs; and iv) post-translational modifications of the protein and interaction with other cellular factors which may affect the activity, localization and stability of the PTEN protein.

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PTEN regulator	Effect on PTEN function	PTEN dependence	Clinical mutation data	Clinical expression data	Mechanism for PTEN effects	Transformation in culture
P-Rex2	inhibitor	x	x	x	direct inhibition	x
SIPL1	inhibitor	unclear		x	direct inhibition	x
NEDD4-1	inhibitor	x		x	ubiquitination	x
PICT1/GLTSCR2	activator	x	x	x	stabilisation	x
RAK/FRK	activator	x	x		stabilisation	x
Prdx1	activator	x			oxidation protection	x
CK2	inhibitor	unclear		x	phosphorylation	
XIAP	inhibitor	unclear		x	ubiquitination	x

Table I. Evidence for candidate proto-oncogenes and tumour suppressors acting indirectly through regulation of PTEN

Fig 1

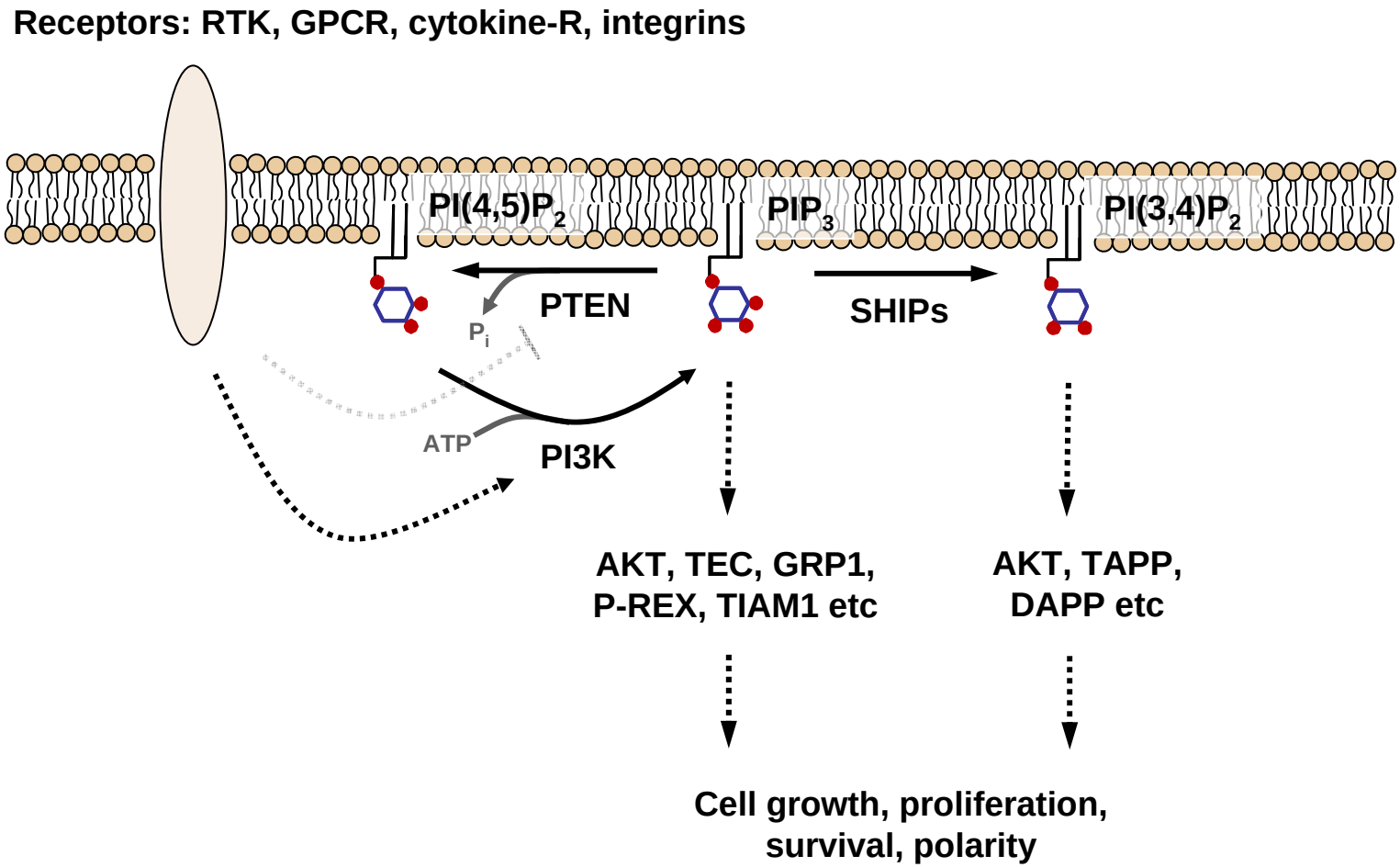

Receptors: RTK, GPCR, cytokine-R, integrins
PI(4,5)P2
PIP3
PI(3,4)P2
PTEN
Pi
SHIPs
ATP
PI3K
AKT, TEC, GRP1,
P-REX, TIAM1 etc
AKT, TAPP,
DAPP etc
Cell growth, proliferation,
survival, polarity

Fig 2A

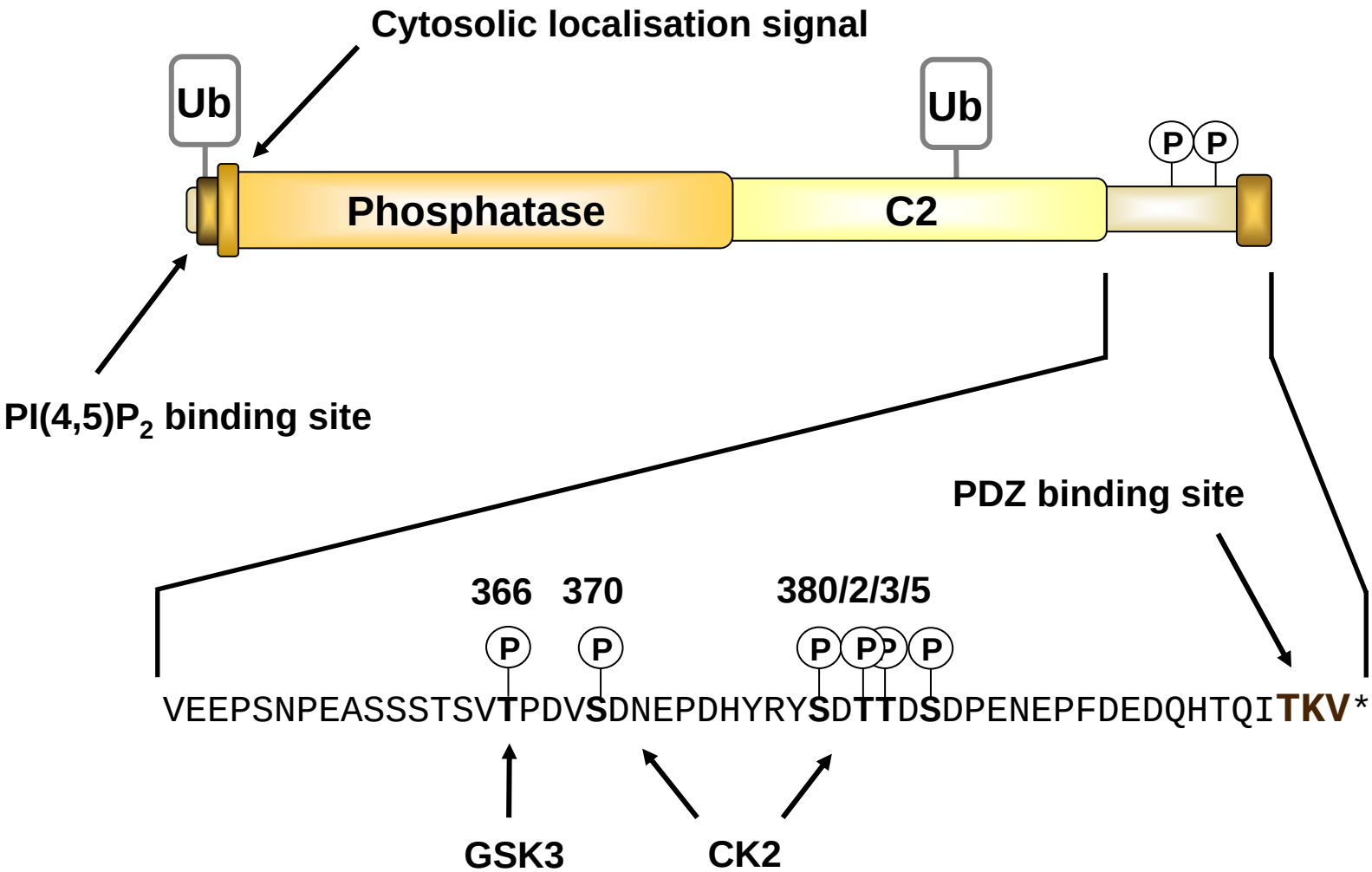

Cytosolic localisation signal
Ub
Ub
P
P
Phosphatase
C2
PI(4,5)P2 binding site
PDZ binding site
366
370
380/2/3/5
P
P
P
P
P
P
VEEPSNPEASSSTSVTPDVSDNEPDHYRYSDTTDSDPENEPFDEDQHTQITKV*
GSK3
CK2

Fig 2B

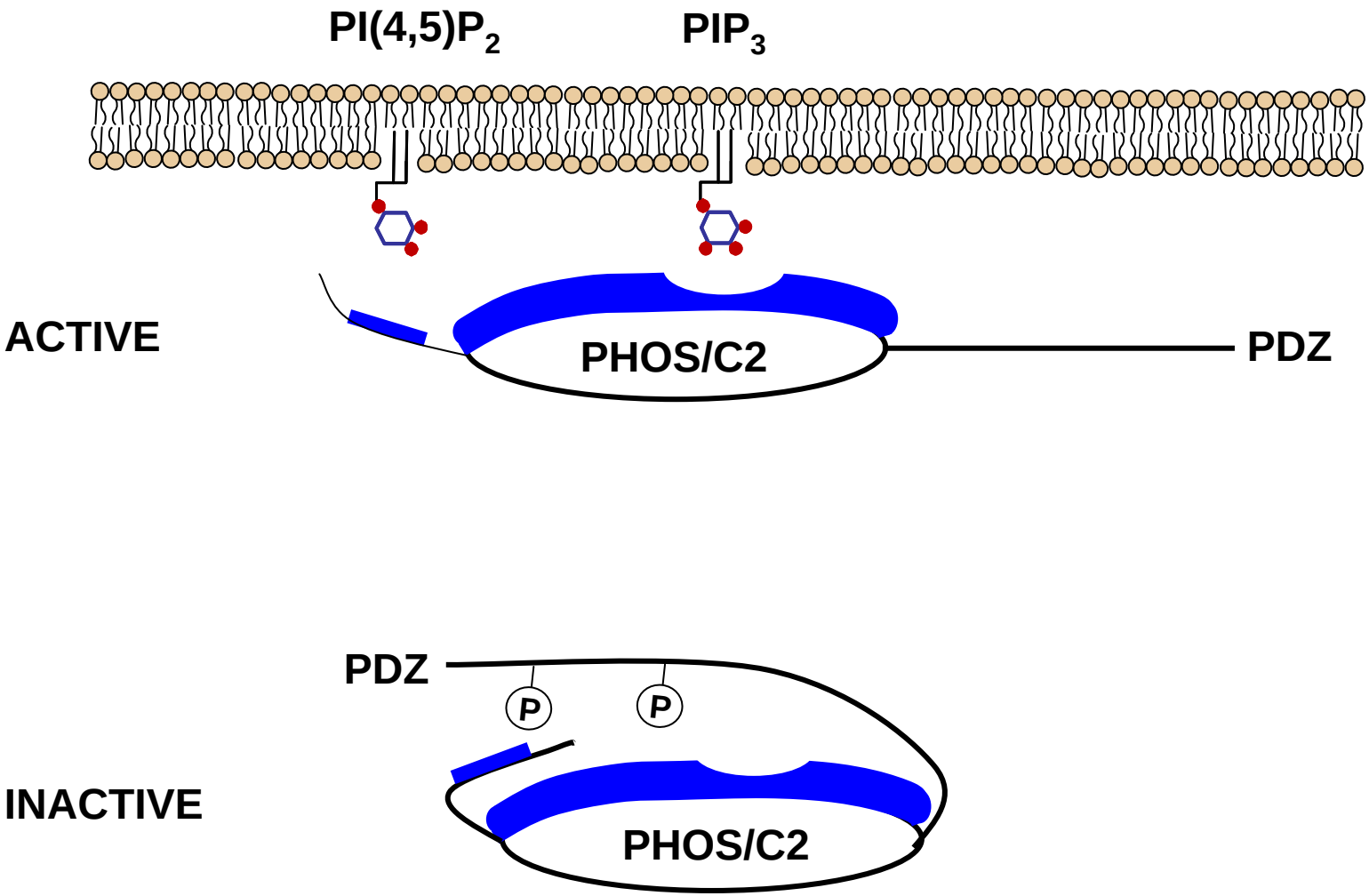
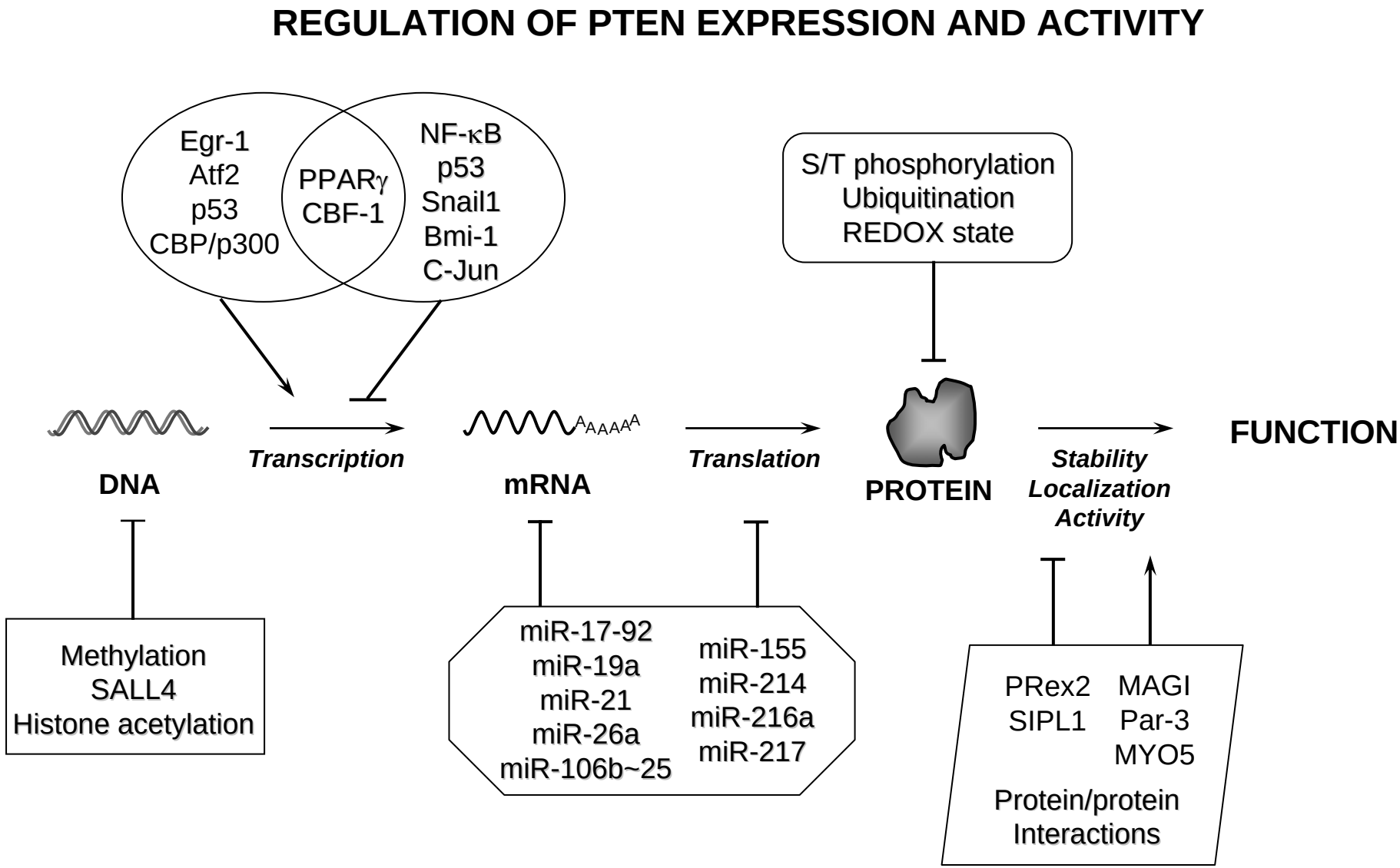


Fig 3


REGULATION OF PTEN EXPRESSION AND ACTIVITY
Egr-1
Atf2
p53
CBP/p300
PPARγ
CBF-1
NF-κB
p53
Snail1
Bmi-1
C-Jun
S/T phosphorylation
Ubiquitination
REDOX state
DNA
Transcription
mRNA
Translation
PROTEIN
Stability
Localization
Activity
FUNCTION
Methylation
SALL4
Histone acetylation
miR-17-92
miR-19a
miR-21
miR-26a
miR-106b~25
miR-155
miR-214
miR-216a
miR-217
PRex2
SIPL1
MAGI
Par-3
MYO5
Protein/protein
Interactions