Title: Cyclophilin function in Cancer; lessons from virus replication

Authors: Paul T.M. Lavin and Margaret M. McGee*

UCD School of Biomolecular & Biomedical Science, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland

*corresponding author: margaret.mcgee@ucd.ie

Tel: + 353 1 7166771
Fax: + 353 1 2837211

Abstract
Cyclophilins belong to a group of proteins that possess peptidyl prolyl isomerase activity and catalyse the cis-trans conversion of proline peptide bonds. Cyclophilin members play important roles in protein folding and as molecular chaperones, in addition to a well-established role as host factors required for completion of the virus life cycle. Members of the cyclophilin family are overexpressed in a range of human malignancies including hepatocellular cancer, pancreatic cancer, non-small cell lung cancer, gastric cancer, colorectal cancer and glioblastoma multiforme, however, their precise role in tumourigenesis remains unclear. In recent years, mounting evidence supports a role for prolyl isomerisation during mammalian cell division; a process with striking similarity to plasma membrane remodelling during virus replication. Here we will summarise our current understanding of the role of cyclophilins in cancer. We will review the function of cyclophilins during mammalian cell division and during HIV-1 infection, and highlight common processes involving members of the ESCRT and Rab GTPase families.

Introduction
The interconversion of protein backbone between cis and trans, catalysed by peptidyl prolyl isomerases (PPIases), is an important event that alters protein structure and activity and regulates a wide spectrum of cellular functions in normal physiological processes. For example, isomerisation mediates the spatiotemporal control of fundamental processes including cell cycle progression, cell proliferation and cell death [1–4]. In recent years, mounting evidence implicates deregulated isomerase activity in a range of age-related pathologies including neurodegeneration, cardiovascular disease and cancer [5]. As a result, isomerases have gained significant interest as therapeutic targets in the treatment of these diseases.

The PPIase enzyme family is comprised of three structurally distinct groups; cyclophilins (Cyps), FK-506 binding proteins (FKBPs) and parvulins [1]. Although members differ in substrate specificity, all PPIases catalyse the cis-trans conversion of X-proline peptide bonds.
X-proline isomerisation is a slow rate limiting step in many reactions. PPIases accelerate the process by stabilisation of the cis-trans transition state [1] and as such they can assist in protein folding [6,7] and transport [5]. Furthermore, they can function as molecular chaperones independently of their PPIase activity [8]. The parvulin Pin1 differs from the cyclophilin and FKBP families in that it specifically catalyses the isomerisation of proline peptide bonds that are immediately preceded by phosphorylated serine or threonine [9].

The cyclophilin family is highly conserved through evolution and is comprised of 8 cyclophilin and 10 cyclophilin-like members in humans (Table 1) that are localised in different cellular compartments including the cytosol, endoplasmic reticulum, mitochondria and nucleus [10,11]. CypA, the first member to be discovered [12], is a 165 amino acid protein containing a cyclophilin-type domain between amino acids 2-163. Structurally, CypA forms a right-handed β-barrel, comprised of eight antiparallel β-strands, which is flanked by an α-helix at each end. The cyclophilin domain, which is shared with all other cyclophilin members (Figure 1), forms a hydrophobic pocket that is the binding site for proline-containing peptides as well as the enzymatic site of the enzyme.

The FK506-binding proteins (FKBPs) are comprised of 13 human FKBPs which localise to the cytoplasm (FKBP12, 12.6, 25, 36, 38, 51, and 52), the endoplasmic reticulum (FKBP19, 22, 23, 60, and 65) and the mitochondria (FKBP13) [10]. The parvulin family is composed of two members, Pin1 and Par14. Pin1 is conserved in many organisms, however Par 14 is only found in higher eukaryotes [10].

Cyclophilins and FKBPs are also designated as Immunophilins because they are the intracellular target of the chemically unrelated immunosuppressive drugs, cyclosporine A (CsA) and FK506, that are used in the suppression of graft rejection following organ transplantation [13]. CypA was first found to bind and form a ternary complex with CsA [12] and it was demonstrated that CypA/- mice are resistant to CsA-mediated immunosuppression [14]. Subsequently, several other family members were found to bind CsA, including CypB [15], CypC [16] and CypD [17]. The dissociation constants for CsA binding to CypA, CypB, CypC, and CypD are 36.8 nM [18], 9.8 nM [18], 90.8 nM [18] and 3 nM [19] respectively. CsA and FK506 bind and inhibit the PPIase activity of cyclophilins and FKBPs respectively; however, the immunosuppressive action of each drug does not occur by PPIase inhibition. Instead, immunosuppression is achieved by a gain-of-function mechanism whereby the immunophilin-drug complex associates with and inhibits calcineurin, a protein phosphatase that dephosphorylates nuclear factor for activation of T-cells (NF-AT) [20]. Inhibition of NF-AT dephosphorylation prevents its nuclear translocation and the stimulation of cytokines that are required for T-cell proliferation [20].

Although cyclophilins have been associated with diverse cellular functions including protein folding [6,21] and trafficking [22,23], their precise function in normal cells remains unclear. Only a relatively small number of substrates have been identified to date, which may be explained by the spatiotemporal nature of isomerase-substrate interaction. CypA substrates...
include the homo-oligomeric α7 neuronal nicotinic receptor [24] and transferrin [25] which require CypA for correct protein folding. CypA binds to the cell surface receptor, CD147, and regulates its transport to the plasma membrane [26]. Consistent with this, CsA significantly reduced the surface expression of CD147. CypA also controls T-cell activation by prolyl isomerisation of interleukin-2 tyrosine kinase (Itk) which inhibits its catalytic activity [27].

In recent years, deregulated cyclophilin-mediated PPIase activity has been implicated in tumour proliferation, invasion and metastasis [28–30] and is associated with acquired chemoresistance [31–34]. Despite this, the precise signals that are regulated by each cyclophilin in tumour cells remain poorly understood. Improved understanding of cyclophilin function in cancer cells is critical to reveal strategies for therapeutic intervention. Recently, it was shown that CypA plays a role in the division of leukaemia and lymphoma cells, where it facilitates the completion of cytokinesis and the generation of two new daughter cells [35], however the precise mechanism involved is not yet established. In contrast, cyclophilin proteins have an established role as critical host factors required for viral infection [36–39]. During infection, virions target and hijack host proteins involved in topologically similar processes to facilitate assembly and release of progeny from the host cell. This review is a summary of our current understanding of the role of cyclophilins in cancer, with a focus on understanding their role in the regulation of cytokinesis and genome stability. Although much detail remains to be revealed, the review highlights interesting parallels that exist between the role of CypA in the analogous processes of cytokinesis and viral budding that may provide new insight into cyclophilin function in cancer.

**Cyclophilin Expression in Normal and Cancer Cells**

Members of the Cyclophilin family have been implicated in a range of cancers including lung, breast, liver, and prostate [40]. CypA was identified as a novel hepatocellular carcinoma marker that was overexpressed in patient-derived tissue in comparison to normal and cirrhotic liver tissue [41]. It was also found that CypA is significantly overexpressed in pancreatic cancer cell lines and in human pancreatic adenocarcinoma tissue when compared to their normal counterparts, and addition of exogenous CypA significantly stimulated cancer cell proliferation [2]. Analysis of matched normal and lung cancer tissue showed overexpression of CypA in the cancer tissue [42]. Furthermore, siRNA mediated suppression of CypA in non-small cell lung tumour xenografts resulted in reduced cell growth [28]. In addition, CypA was found to be overexpressed in metastatic melanoma [43] and gastric adenocarcinoma [44] when compared to their normal counterparts. CypA was also found to be overexpressed in esophageal cancer cell lines [45] and in clinical endometrial carcinoma specimens [46]. Reduction of CypA levels in the endometrial carcinoma cells using RNAi technology significantly suppressed of cell growth and induced apoptosis [46].

CypB is implicated in the proliferation and survival of breast, liver, brain, and myeloma cancer. siRNA mediated repression of CypB expression in ductal breast epithelial tumour
cells decreased cell growth, proliferation and motility [47]. CypB interacts with the transcription factor Stat3 in HepG2 liver cells which mediates the interleukin-6 family of cytokines [48]. Inhibition of CypB in Stat3-dependent human myeloma cell lines resulted in apoptosis, suggesting that CypB acts as a pro-survival protein in these cells [48]. Furthermore, CypB is overexpressed in malignant glioma tissue and suppression of CypB resulted in reduced cell growth and survival in vitro and in vivo [49].

CypC was identified as a novel gene marker for detecting circulating tumour cells in patients with ovarian cancer, and increased CypC expression in circulating tumour cells after chemotherapy is associated with poor patient survival [50]. CypD is also significantly upregulated in ovarian cancer [51], breast cancer [51], uterus cancer [51] and prostate cancer [52]. Cyp33 is significantly upregulated in glioblastoma compared to non-neoplastic brain tissue [53]. PPIL1 is overexpressed in patient-derived colon cancer tissue, and siRNA mediated suppression of PPIL1 in the human colon cell line SNUC4 suppressed cell growth [54]. PPIL3 mRNA is overexpressed in human glioma tissues [55]. Gene mutations of RANBP2 have also been identified in colorectal cancer tissue [56] while a RANBP2-ALK fusion gene has been detected in acute myelomonocytic leukaemia [57] and inflammatory myofibroblastic tumour [58]. Furthermore, RANBP2 is upregulated in multiple myeloma [59]. In contrast, NKTR was found to be down regulated in cancer-associated prostatic fibroblast tissue when compared to matched normal fibroblast tissue [60].

**Cyclophilins and Chemoresistance**

Cyclophilin family members are associated with cancer chemoresistance. Overexpression of CypA is associated with resistance of prostate cancer cells to cisplatin-induced cell death and it is proposed that CypA suppresses cisplatin-induced ROS production and the loss of the mitochondrial membrane potential [34]. Consistent with that, loss of CypA expression increased mitochondrial membrane depolarization and reduced survival following H2O2 treatment [34]. Furthermore, there is evidence that CypA is transcriptionally regulated by the hypoxia inducible factor (HIF)-1α under hypoxic conditions [34][61] and it is suggested that up-regulated CypA desensitises cells to hypoxia-induced cell death [34].

CypA is down regulated in melphalan-resistant MCF7 breast cancer cells when compared to non-resistant cells [62], and it is proposed that downregulation of CypA allows evasion of apoptosis by inhibition of apoptosis-inducing factor (AIF) [63]. Furthermore, CypA-overexpressing endothelial liver cells display resistance to doxorubicin and vincristine, which was accompanied by up-regulation of cytokines such as interleukins and chemokines [64]. CypA is also overexpressed in paclitaxel resistant endometrial cancer cells, HEC-1-B/TAX and AN3CA/TAX. Knockdown of CypA with siRNA significantly inhibited cell proliferation and invasion when exposed to paclitaxel, which was accompanied by reduced phosphorylation of Akt and the MAPK ERK1/2, p38 and JNK suggesting that overexpression of CypA allows for enhanced MAPK activity [45].
Other cyclophilins have also been implicated in chemoresistance. Under hypoxic conditions HIF-1α upregulates CypB in human hepatocellular carcinoma and CypB knockdown significantly reduced cell survival when subjected to hypoxia, cisplatin, or H₂O₂ treatment [32]. This suggests that CypB may play a similar role to CypA [34] in cisplatin resistance by protecting the cells against ROS induced stress. Furthermore, overexpression of another family member, RANBP2, was detected in cisplatin resistant ovarian carcinoma [65].

Collectively these studies confirm that upregulation of cyclophilin protein is associated with a range of cancers (Table 2), and supports a role for cyclophilins in tumourigenesis and acquired chemoresistance. Thus, cyclophilins may represent valuable biomarkers for chemoresistance and potential therapeutic targets to sensitise cancer cells to chemotherapy. In support of this, treatment of cisplatin-resistant hepatocellular carcinomas with the cyclophilin inhibitors, CsA or sanglifehrin A (SFA), in combination with cisplatin, synergistically enhanced apoptosis [33]. It is only in recent years that the role of cyclophilin proteins, and other PPIases, in cancer has begun to be explored. To date, reports suggest that PPIases are associated with an increase in cell proliferation, migration and invasion [2,23,30]

The Mammalian Cell Cycle

The cell cycle involves the co-ordinated division of a cell into two genetically identical daughter cells. Progression through the cell cycle is regulated by cyclin-dependant kinases (Cdks); a family of serine/threonine protein kinase that require binding to cyclin regulatory subunits for biological activity. Thus, the synthesis and degradation of the cyclin proteins during the cell cycle ultimately drives cell cycle progression [66]. Transition through the cell cycle is also controlled by checkpoints that are switch-like transitions that regulate S phase entry, mitotic entry and mitotic exit and are active at the G1/S boundary, G2/M boundary and at the metaphase/anaphase boundary respectively. Progression through the cell cycle can be halted at these checkpoints if conditions for successful cell division are not met [67].

Mitosis (M) phase of the cell cycle is divided into prophase, metaphase, anaphase, telophase and cytokinesis (Figure 2B). During prophase the nuclear membrane breaks down, and duplicated chromosomes, which are comprised of two sister chromatids, condense while the centrosomes nucleate the mitotic spindles which are constructed from microtubules [68]. During metaphase the spindle microtubules attach to the sister chromatids via their kinetochores, and they align at the centre of the mitotic spindle. During anaphase the sister chromatids are separated and move to opposite poles of the mitotic spindle. Separation of sister chromatids during anaphase should take place only when all chromosomes are attached to the bipolar mitotic spindle via their kinetochores. Separation is promoted by the anaphase-promoting complex (APC) [68], an E3 ubiquitin ligase that targets the degradation of securin, which is an inhibitor of the protease separase. Degradation of securin liberates active separase, which is free to degrade the cohesion proteins that hold sister chromatids together [68]. APC also targets cyclin B for degradation, which culminates in inactivation of
Cdk1 and marks mitotic exit [66]. During telophase, the two sets of daughter chromosomes arrive at the spindle poles, where they decondense and a new nuclear envelope is formed. Finally, cytokinesis involves the separation of the cytoplasm, organelles and DNA of a dividing cell into two new daughter cells [69].

**Cytokinesis and Abscission**

The first event of cytokinesis is the formation of the central spindle, a narrow zone of bundled overlapping non-kinetochore microtubules at the midzone between separating chromosomes. Central spindle formation requires the microtubule binding and bundling protein PRC1, and the motor protein KIF4 that blocks microtubule growth thereby promoting normal midzone architecture [68]. Activation of PRC1 causes microtubule bundling to form the midzone which serves as a platform for the localisation of other critical components of the spindle, including centralspindlin and Chromosomal Passenger Complex (CPC) [70] (Figure 3). Centralspindlin is a heterotetramer comprised of two molecules of MKLP1 and two molecules of CYK4 which contains a GTPase-activating protein (GAP) domain [71]. The CPC is composed of Aurora B and three non-enzymatic proteins required for Aurora B regulation, INCENP, Survivin, and Borelin [72]. Aurora B phosphorylates MKLP1, promoting the recruitment of centralspindlin to the midzone, which tethers the central spindle to the plasma membrane [73]. PRC1 and MKLP2 recruit Plk1 to the central spindle where it phosphorylates the centralspindlin subunit CYK4, generating a binding site for Ect2, a guanine nucleotide exchange factor for Rho GTPases [68]. Ect2 binds and is directed towards the plasma membrane around the centre of the dividing cell. Ect2 activates RhoA by conversion of RhoA-GDP into RhoA-GTP, which promotes contractile ring assembly at the equatorial membrane [74]. As the actomyosin ring is constricted, the central spindles at the midzone become densely packed to form the midbody, located at the centre of the intercellular bridge. The midbody acts as a protein scaffold for components required for the final stage of cytokinesis known as abscission. The midbody recruitment of Centrosome protein of 55 kDa (Cep55) is essential for abscission. During early mitosis Erk2/Cdk1 phosphorylates Cep55 at Ser425 and Ser428 which results in Cep55 disassociation from the spindle poles [75]. Phosphorylation of Cep55 at Ser425 and Ser428 allows for the recruitment and phosphorylation by Plk1 on S436 within a C-terminal region containing the binding site for MKLP1 [75–77]. While there is general agreement that Plk1 phosphorylation controls Cep55 localisation during cytokinesis, Plk1 phosphorylation is reported to act as a positive and negative regulator of Cep55 function [75–77]. One model proposes that Plk1 phosphorylation is required for Cep55 localisation and function at the midbody [75], whereas the other model proposes that Plk1 prevents Cep55 from interacting with the central spindle and midbody protein MKLP1 in anaphase. The progressive degradation of Plk1 during mitotic exit exposes the MKLP1 binding site which allows Cep55 recruitment to the midbody [77].

A recent discovery was that the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, which are known for their role in membrane scission during viral budding and
the formation of multivesicular bodies, also play a key role in abscission [68,78,79](Figure 4). The protein family, which is comprised of 4 complexes (0, I, II, III) and VPS4, are involved in membrane remodelling during cytokinesis and abscission [68,80]. Cep55 is the key protein directing ESCRT recruitment. It recruits the ESCRT-I subunit Tsg101, and ALIX to the midbody which allows for recruitment of ESCRT-III complex to the flanking midbody ring [79]. ESCRT-III accumulates at the abscission site and promotes membrane severing by the formation of constricting helical oligomers and the loss of the midbody microtubules at the intercellular bridge [68]. This is coupled with ESCRT-III recruitment of the ATPase spastin which displays microtubule severing activity and is believed to be responsible for the final scission and separation of the daughter cells (Figure 4) [68]. ESCRT-III also recruits the disassembly factor AAA-ATPase VPS4 which releases the ESCRT-III from the membrane, allowing it to bind elsewhere [68]. Additional membrane-trafficking proteins localise to the intercellular bridge and play a role in the final stages of cytokinesis including dynamin, soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins (SNAREs), Rab proteins and exocyst components [80,81]. However, while many of the proteins that are important for abscission have been identified, their precise mechanism is still unclear.

Cytokinesis failure and Cancer

The majority of cancer cells contain large-scale alterations in chromosome structure and number [83-86]. Loss or gain of whole chromosomes, known as aneuploidy, has long been associated with cancer, however, there has been much debate surrounding how aneuploid cells arise and whether they are a cause or consequence of cancer [83–86]. The cumulative evidence to date suggests that aneuploidy promotes tumourigenesis [83 –86]. In that context, a long standing hypothesis is that failure of cell division generates unstable tetraploid cells that represent the first step to aneuploidy and tumourigenesis [84]. In support of this, mutation of adenomatous polyposis coli (APC) in colorectal cancer inhibits spindle anchoring during anaphase and results in cytokinesis failure with the generation of tetraploid genomes in vivo [87]. Transient inhibition of cytokinesis generates genetically unstable tetraploid cells that promote tumourigenesis in p53−/− mouse mammary epithelial cells [88]. Furthermore, tetraploidy and chromosome instability are early events detected in human cervical cancer tissue [89]. Mouse ovarian surface epithelial cells derived from an intermediate tetraploid cell, generated through chromosome mis-segregation during mitosis, exhibited aneuploidy and were tumourigenic in vivo [85]. Overexpression of Aurora B in murine epithelial cells give rise to tetraploid cells that formed tumours when injected into mice [90]. Genomic analysis of the tumours revealed widespread genomic reorganisation including trisomies, amplifications, and deletions [90]. Impaired integrin traffic, which is critical for cell adhesion, induces aneuploidy via a near-tetraploid intermediate and results in oncogenic transformation in vitro and in vivo [91]. In addition, loss of the Breast Cancer Susceptibility Gene, BRCA2 [92] or the LATS1 tumour suppressor [93] is accompanied by cytokinesis defects, suggesting a role for these tumour suppressors in cytokinesis. Aneuploid cells that arise from tetraploidy are most likely generated due to multiple centrosomes and multipolar mitoses, which can lead to gain or loss of whole
chromosomes and to chromosome breaks. Collectively, these studies support the 100 year old hypothesis proposed by Theodor Boveri [94] that abnormal mitosis promotes genome instability and cancer.

The mitotic checkpoint is the major regulator of chromosome segregation during mitosis. Mitotic checkpoint components including Mad1, Mad2, Bub1, BubR1, Bub3 and CENP-E are recruited to kinetochores on chromosomes and they act to inhibit anaphase onset and mitotic exit by inhibiting the APC [95,96]. Defects in mitotic checkpoint genes results in numerical aneuploidy. In recent years a number of animal models that exhibit aneuploidy and/or chromosome instability due to mutation in mitotic checkpoint genes have been tested for their tumour forming ability. Results indicate that Mad+/− and Cdc20+/AAA mice as well as those that overexpress Bub1 or Mad2 promote tumourigenesis. On the otherhand, mutations that suppress tumourigenesis have also been reported including securin −/−, while others had no effect on tumour incidence including inactivated Bub1 or Bub3−/−, while mutations such as Bub1+/−, BubR1 +/− and Mad2+/− either promote or suppress tumourigenesis depending on the context. Thus, overall, the tumour promoting ability of aneuploidy and chromosome instability is dependent on the context and the specific genes involved (for review see [97]). Interestingly, data from mouse models and patients indicates that low and intermediate rates of chromosome instability can promote tumours, whereas, high rates of chromosome instability is incompatible with viability leading to cell death and tumour suppression, and correlates with improved patient outcomes [97]. Thus, a better understanding of the role of aneuploidy and genome instability in tumourigenesis is important in order to exploit the process therapeutically.

The role of Cyclophilin and Cyclophilin-like proteins in the Cell Cycle

ESS1, a homolog of Pin1, was first identified in *Saccharomyces cerevisiae* as a novel protein required for growth [98]. Studies in yeast found that loss of ESS1 led to defective cytokinesis revealing a role in cell division. Moreover, the CypA homolog, CYP1, was sufficient to rescue ESS1 loss in yeast, and CYP1 becomes overexpressed in stable ESS1 null yeast [99,100]. Thus, it was proposed that ESS1 regulates the expression of genes required for cell growth [101]. ESS1 binds directly to the Sin3-Rpd3 histone deacetylase complex and the Set3C histone deacetylase complex and modulates their deacetylase activity [100,102]. CYP1 also interacts and regulates Sin3-Rpd3 histone in yeast [103]. These findings suggest that ESS1 and CYP1 act within the same pathway to control transcription of cell cycle genes in yeast.

A number of studies report a role for cyclophilin proteins during progression of the mammalian cell cycle. Fragile histidine triad (FHIT) acts as a tumour suppressor and down regulation is associated with tumour growth [104]. Loss of FHIT expression results in increased CypA levels in mouse lung tissue, mouse kidney cells and in the human lung cancer cells. Treatment of FHIT negative cells with extracellular recombinant CypA led to up regulation of cyclin D1 and activation of Cdk4 permitting transition from G1 to S phase of the cell cycle [104].
CypD has also been implicated in cell cycle progression. Knockdown or deletion of CypD in glioblastoma cells and mouse embryonic fibroblasts enhanced cell proliferation via accelerated entry into S phase [105]. Increased STAT3 transcription and activity were detected in CypD null MEF cells [105]. While the connection between CypD expression and STAT3 is unclear, STAT3 plays a role in G1 to S phase transition by up regulation of cyclin D or inhibition of cell cycle repressors such as p21 [106]. Thus, it is proposed that by its interaction with STAT3, CypD can regulate the G1 to S phase transition. Furthermore, recent work has demonstrated that CypA stimulates phosphorylation of STAT3 and transcription of cyclin D1/surviving [107].

The phosphorylation-dependent PPIase, Pin1, is overexpressed in a large number of tumours [108] and is associated with poor survival [109]. Pin1 plays an important role in the cell cycle by regulating cyclin D1 and influencing the G1 and S phase transition [110] as well as regulating numerous mitotic substrates including NIMA, Wee1, Cdc25, Plk1, CENP-F (for review see [111]). In recent years, several studies have provided a link between PPIase localisation at the centrosome and a role in cytokinesis. Pin1 is localised to the centrosome and undergoes mitosis-specific phosphorylation dependent relocalisation to the midbody [112,113]. The midbody protein Cep55 must be tightly regulated to ensure proper execution of cytokinesis. Pin1 interacts with Cep55 in vitro and in vivo and it is suggested that Pin1 isomerisation of Cep55 at the midbody enhances Plk1 phosphorylation of Cep55 on Ser436, an essential step for the timely execution of cytokinesis [113]. Consistent with that, deregulated Pin1 function, as a result of overexpression or depletion causes cytokinesis defects. For example, overexpression of Pin1 in mouse mammary glands induced malignant mammary tumours that was accompanied by centrosome amplification and aneuploidy [112]. In addition, Pin1 knockout mouse embryonic fibroblasts (MEFs) and HeLa cells display cytokinesis defects [113].

Pin1 also mediates the final separation of daughter cells via interaction with the septin family member, SEPT9 [114]. Specifically, SEPT9 is phosphorylated by Cdk1 at Thr24 which acts as a site for Pin1 isomerisation, and is required for abscission [114]. In addition to SEPT9, a number of septins are implicated in the earlier stages of cytokinesis including SEPT2, SEPT7, and SEPT11 [115] however, the role of isomerisation in the regulation of their activity remains unknown.

CypA localises to the centrosome during interphase in a range of human tumour cells including haematopoietic cells, lung cancer cells and HeLa cervical carcinoma cells [35]. CypA forms part of the spindle poles during early mitosis and relocates to the midzone and midbody during telophase and cytokinesis respectively. Moreover, while CypA PPIase activity is not required for centrosome or midbody localisation, it is required for the timely completion of cytokinesis [35]Bannon et al., demonstrated that expression of wild-type CypA rescued the defect whereas an isomerase defective mutant did not. Furthermore, loss of CypA expression, by homozygous deletion or RNAi, led to supernumerary centrosomes
and a tetraploid genotype indicative of cytokinesis failure. Loss of CypA also significantly reduced clonogenic potential indicating that CypA expression confers a growth advantage to cancer cells [35]. These findings provide a mechanistic link between aberrant expression of CypA and cancer.

More recently it was discovered that RANBP2 localises to the centrosome in mammalian cells [116] and in Drosophila [117]. In addition, RANBP2 was detected at the spindle pole and kinetochore during mitosis in HeLa cells, which was controlled by binding to importin [116]. Downregulation of RANBP2 induces chromosome missegregation and aneuploidy, leading to mitotic catastrophe and cell death [116]. This suggests that RANBP2 plays a crucial role in faithful chromosome segregation, which may provide mechanistic insight into previously reported phenotypes associated with disrupted RANBP2 expression including tumour growth and embryonic lethality in knockout mice [116]. Mice with low levels of RANBP2 were highly sensitive to tumour formation, which is likely due to defective chromosome segregation [118].

Collectively these reports support a role for prolyl isomerases in the regulation of cell cycle progression and highlight how their deregulation can contribute to tumourigenesis. As aberrant cytokinesis is an early event in tumourigenesis, improved knowledge about proteins that play a role in the regulation of cytokinesis may lead to the development of novel cancer therapeutics. Thus, the identification and characterisation of cyclophilin substrates at the centrosome and midbody will provide new insight into isomerase-mediated events that regulate cytokinesis. CypA is required for viral budding, a process that is topologically similar to cytokinesis. In addition, members of the ESCRT protein family play key roles during these analogous processes and are involved in signalling pathways that are regulated by isomerisation. Similarly, Rab GTPase play important roles during viral budding and cytokinesis and recent evidence supports a role for isomerases in their regulation. The following section will review the role of cyclophilin proteins during viral infection, and highlight common targets and pathways shared with ESCRT proteins and Rab GTPases during viral budding and cytokinesis.
The role of Cyclophilins in the Virus life cycle

CypA is involved in the life cycle of several viruses, including human immunodeficiency virus type 1 (HIV-1), influenza virus, hepatitis C virus (HCV), hepatitis B virus (HBV), vaccinia virus (VV), human cytomegalovirus, human papillomavirus, coronavirus, vesicular stomatitis virus (VSV), and rotavirus [119–128]. A large body of evidence indicates that CypA is an important host factor for successful viral infection and CypA is also incorporated into several enveloped virus particles, such as HIV-1, influenza virus [129–131], however, the function of CypA in virus particles is still unclear.

HIV-1 uncoating

The physiological relevance of cyclophilins in HIV infection was revealed when cells containing a disrupted CypA gene displayed defective HIV replication [129]. The first evidence that cyclophilin proteins function as viral co-factors was reported for CypA in HIV infection [36], and mounting data suggest a multifunctional role for isomerases in the virus life cycle [37,132–136]. An early and critical event in HIV life cycle is uncoating of the viral core that precedes genomic RNA release, reverse transcription, and nuclear import. HIV assembly and disassembly is controlled largely by the Gag-CA protein and changes in the timing of viral uncoating blocks viral infection highlighting the important timing of these events [137]. CypA binds to CA and blocks access to the cellular restriction factor, TRIM5α, which blocks uncoating during the early stage of the HIV life cycle [134,138].

HIV-1 assembly

Assembly of progeny virions and their release from the virus producing host cell, are well co-ordinated processes that are required to complete the virus life cycle. Virion assembly and release are driven by the viral Gag protein and are dependent on host cellular factors. The Gag protein is the major structural protein of the virus capsid and is a 55kDa polyprotein comprising of four subdomains – Matrix (MA), Capsid (CA), Nucleocapsid (NC) and p6, which are interspaced by SP1 and SP2 (Fig 4) [137,139]. It is widely accepted that HIV-1 Gag assembly and budding occur predominantly on the plasma membrane, where the N-terminal myristoylated MA domain mediates membrane anchoring and assembly of Gag. MA membrane binding is coupled with Gag multimerisation at the plasma membrane and is required for particle formation and viral infectivity, however, MA itself is not required for Gag-Gag interactions and particle formation [140–143]. Similarly, p6 recruits components required for viral budding, but does not make Gag-Gag interactions. Critical lateral Gag-Gag interactions that facilitate lattice formation are initiated primarily by CA and SP1, and to a lesser extent NC domains [141].

HIV-1 maturation and budding

The formation of mature virions requires Gag polypeptide processing into its component fragments, together with morphogenesis of a spherical particle into the viral envelop that is lined with MA, and the CA containing conical core harbouring mature dimeric viral NC/RNA complex. Packaging of CypA into HIV-1 virions is essential for efficient replication [119].
Incorporation is mediated through binding to the Gly-89-Pro-90 peptide bond of the N-terminal domain of newly assembled HIV-1 capsid (CA) [144].

p6 is a small protein that does not play a structural role in virion maturation, but it is required for virion incorporation of the viral accessory protein R (Vpr). CypA binds to the N-terminal heptapeptide motif RHFP$_{35}$RIW of Vpr centred at Pro-35 and it catalyses the prolyl cis-trans interconversion of the highly conserved proline residues (Pro-5, -10, -14 and 35) of Vpr [132]. Mutation of Pro-35 disrupts the interaction of Vpr with CypA [136]. It was subsequently found that CypA binds to a non-proline containing 16 residue region of C-terminal Vpr $^{75}$GCRHSRIGVTRQRRAR$^{90}$ with similar affinity as full length Vpr and Arg-80 was identified as a key residue in the C-terminal binding domain [38]. Although the biological significance of the binding of Vpr to CypA remains elusive, several key functions of Vpr are associated with the identified N and C-terminal CypA binding domains. These include G2 cell cycle arrest and apoptosis [38,136,145], suggesting a role for isomerisation in the regulation of these virus-induced processes.

The p6 Gag protein also regulates the final abscission step and budding of nascent virions from the cell membrane by the action of two late assembly (L) domains, PTAP and YPXnL (where X is any amino acid and n=1 to 3 residues) motifs, located within p6 [137,139,141]. The 52 amino acid p6 peptide binds two cellular budding factors; the conserved PTAP motif binds Tsg101 (a component of host ESCRT I complex), whereas the YPXnL motif binds Alix (Apoptosis-linked gene 2 –interacting protein X) [137,139]. The p6 binding to Tsg101 allows recruitment of the core ESCRT-I heterotetramer complex, which facilitates ESCRT-III recruitment. On the other hand, Alix is a Bro domain protein containing a proline rich C-terminal residue that harbours binding sites for interaction partners including the human ESCRT-III protein, CHMP4 [137]. Thus, components of host ESCRT pathway, Tsg101 and Alix, play important roles linking Gag protein to viral budding by recruitment of human ESCRT-III and resolution of the membrane stalk that connects the virion to the host cell. As such, Gag p6 acts as an adaptor protein for the host cell machinery to promote budding. A striking feature of the p6 protein is the high relative content of proline residues located at positions 5, 7, 10, 11, 24, 30, 37, and 49. Recently, CypA was shown to interact with p6 and act as a general catalyst for cis-trans isomerisation of all proline residues in the full length protein [37]. Crucially, the binding motifs of p6 to Tsg101, Alix and Vpr coincide with binding regions and catalytic sites of p6 to CypA [37]. Based on this, it is possible that CypA binding and isomerisation represents an important molecular switch that mediates the interaction of p6 with cellular factors such as Tsg101 at the p6 N-terminal or Alix at the C-terminal.

The role of GTPase during Cytokinesis and Viral budding

The processes of cytokinesis and viral budding also require specific membrane trafficking events to deliver new membrane to the growing furrow and viral bud respectively. The small GTPase superfamily, which is comprised of Ras, Rho/Rac, Rab, Arf and Ran, control diverse events including cell proliferation and differentiation, as well as the regulation of actin
cytoskeleton, membrane trafficking and nuclear transport [146]. In particular, the Rab GTPases represent a large family of over 60 small guanosine triphosphate (GTP)-binding proteins, and localise to distinct membrane compartments where they act as master regulators of vesicular membrane transport on both the endocytic and exocytic, and transcytic pathways [146].

Rab proteins are implicated in the completion of cell division [147]. Completion of cytokinesis in *C. elegans* requires Rab11 dependent membrane trafficking events to deliver new membrane to the furrow and for abscission, and depletion of Rab11 leads to cytokinesis defects including furrow regression and abnormal scission, confirming that endosomes provide membrane for cytokinesis [148]. Consistent with this, Rab11 and its binding partner FIP3 localise to the cleavage furrow during cell division in *C. elegans* and in mammalian cells [149,150].

Two additional GTPases, Arf1 and Arf6, are also implicated in cytokinesis. Arf6 localises to the midbody during telophase and is responsible for the recruitment of Rab11/FIP3 and Rab11/FIP4 complexes [149]. Rab35, which is involved in the early endocytic recycling pathway, plays a crucial role during cytokinesis by mediating the localisation of phosphatidylinositol 4,5, bisphosphate (PIP2) lipid and the septin, SEPT2, to the growing cytokinetic bridge [151]. Thus, the essential role of Rab11 and Rab35, which control distinct endocytic recycling pathways, implies that multiple endocytic routes are essential for cytokinesis. Furthermore, the transport of Rab8 positive vesicles to the midbody during cytokinesis via a dynein-dependent manner implicates the retrograde pathway in the completion of cytokinesis [152]. CypA associates *in vitro* and *in vivo*, via its PPIase domain, with the dynein/dynactin motor protein complex suggesting a role for CypA PPIase activity in cargo binding for retrograde movement along microtubules [153].

Rab21 plays a role in cell division by integrin targeting to the growing cleavage furrow, and loss of Rab21 leads to cytokinesis failure, aneuploidy and tumourigenesis *in vivo* [91]. RABL6A a novel RAB-like protein, plays a role in centrosome regulation and maintenance of chromosome stability in non-transformed cells, key processes that ensure genomic integrity and prevent tumourigenesis [154]. Rab24 is an atypical member of the GTPase family and its function remains largely unknown. It was previously shown that Rab24 and CypA co-localise at the perinucleus in COS cells, implying that they may co-operate to function in a signalling pathway at that position or elsewhere [155]. Importantly, it was recently demonstrated that, like CypA [35], Rab24 is a centrosome protein that is redistributed to the mitotic spindle and midbody during mitotic progression, where is regulates multiple mitotic events including chromosome segregation and cytokinesis [156]. Depletion of Rab24 in COS-7 cells increased chromosome segregation errors and cytokinesis errors implicating Rab24 in the completion of normal cell division [156]. Thus, although there is no evidence to date that Rab function is regulated by isomerisation, it is possible that CypA isomerisation controls the
distribution of Rab24, and other Rabs such as Rab11 and Rab35, to mitotic structures during the cell cycle.

Recent publications show that Rab-controlled trafficking pathways are altered during tumourigenesis. Certain members including Rab25 acts as both an oncogene and a tumour-suppressor gene [157,158]. Accelerated cell migration induced by upregulated Rab11 and Rab25 is associated with increased vesicular transport efficiency in the inner cell compartment and at the plasma membrane [159]. Furthermore, Rab 25 and chloride intracellular channel 3 (CLIC3) drives invasiveness of pancreatic and ovarian cancer by regulating the recycling of α5β1 integrin from late endosomes to the plasma membrane [160]. In contrast, Rab37 suppresses tumour metastasis by exocytosis of the tissue inhibitor of metalloproteinase 1 (TIMP1), thereby inhibiting matrix metalloproteinase 9 (MMP9) [161]. Finally, Rab18 is highly expressed in Hepatitis B virus (HBV)-associated HCC tissue and upregulated Rab18 is mediated by HBV X protein, which promotes hepatoma cell proliferation [162].

Increasing evidence supports a role for Rab proteins in the viral life cycle. For example Rab7A, a major regulator of the late endocytic pathway, is required in the late stages of the HIV-1 replication cycle [163], whereas Rab11-FIP1C and Rab14 direct plasma membrane sorting and particle incorporation of the HIV-1 envelope glycoprotein complex [164]. Furthermore, Rab6 and Vps53 implicate the retrograde pathway in viral entry [165].

**Targeting Cyclophilins in Anti-viral and Anti-cancer Therapy**

The pleiotropic involvement of CypA in the lifecycle of various viruses opened the way for the development of broad range antiviral compounds. However, given the growing body of evidence linking deregulated cyclophilin expression with tumour development, metastasis and chemoresistance, it is not surprising that the family has recently gained interest as potential anti-cancer targets. The cyclophilins are already a target for immunosuppression, with Cyclosporine A (CsA), an eleven amino acid cyclic nonribosomal peptide, used to inhibit the immune response in organ transplant patients [13]. The immunosuppressive effect of the CsA-CypA complex, is independent of isomerase inhibition, and occurs by inhibition of the phosphatase calcineurin, which is required for T cell activation [166]. However, off-target effects associated with the CsA scaffold and their high molecular size, has limited their clinical use. In recent years, much effort has focussed on the development of non-immunosuppressant derivatives of CsA with applications as anti-viral agents. Overall, a number of studies report that chemical inhibition of CypA by CsA and non-immunosuppressive CsA analogues can inhibit HIV-1 replication [167–169]. Furthermore, Alisporivir (Debio-025) is a first-in-class non-immunosuppressive cyclophilin inhibitor to enter clinical trial and has shown promise in the inhibition of HIV-1 [167,170] and HCV [171–173].

Emerging data implicating cyclophilin proteins in tumourigenesis provides a rationale to investigate the anti-tumour effect of cyclophilin inhibitors. Early work using the non-
immunosuppressive analogue of CsA, O-acetyl cyclosporin A showed a two-fold increase in sensitivity of lung cancer cells compared to CsA [174] however, little work has been carried out on O-acetyl cyclosporin A since. Interestingly, NIM811 induced apoptotic cell death in human melanoma cells. Moreover, studies in an in vivo murine model of melanoma showed that NIM811 retards tumour progression and significantly decreases tumour volume after intratumoral application [175].

**Conclusion and Future Perspective**

It is intriguing that normal cells harbour relatively high cyclophilin levels [5], yet, knockout studies in mice indicate that CypA is not essential for mammalian cell viability [176]. In contrast, depletion of CypA causes cytokinesis defects and reduces the proliferation of tumour cells, supporting the view that CypA provides a growth advantage to tumour cells. Thus, due to its requirement during cytokinesis of rapidly dividing cancer cells, CypA represents a novel anti-mitotic drug target. Importantly, the findings that CypA is not required for normal cell viability provides a therapeutic window to selectively kill cancer cells and to reverse acquired chemoresistance, without having detrimental effects on normal cells. However, not all cyclophilin proteins are involved in mitosis, yet, cyclophilin inhibitors developed to date block the activity of numerous family members. For instance, NIM811 inhibits CypA, CypC and CypD [177,178]. Therefore, a major challenge is the development of compounds that can inhibit individual family members. Such compounds may have utility in the treatment of cancer and may sensitise resistant cancer subtypes to chemotherapy.

**Conflict of Interest**

There is conflict of interest to declare. The authors wish to Acknowledge Science Foundation Ireland for funding research in this area through 11/RFP/CAN/320.

**Tables and Figures**

**Table 1** – The Cyclophilin and Cyclophilin-Like Family of Proteins

**Table 2** – Cyclophilin and Cyclophilin-Like proteins that are overexpressed (↑) or down regulated (↓) in cancer.
Figure 1 – Conservation of the PPIase domain (yellow) in the Cyclophilin and Cyclophilin-like family

Figure 2 – Overview of the cell cycle (A) The phases of the cell cycle including Gap 1 (G1), Synthesis (S), Gap 2 (G2) and Mitosis (M). (B) The stages of mitosis showing CypA localisation at each stage. The DNA condenses and the nuclear envelope is degraded during prophase. The DNA aligns at the centre of the dividing cell and the spindle attach to the DNA during metaphase. At anaphase the DNA is separated and move towards the spindle poles while the contractile ring starts to form during telophase. During cytokinesis the cytoplasm is divided by the contractile ring which condenses into the midbody. The midbody acts as a platform for the recruitment of proteins required for final abscission event that gives rise to two genetically identical daughter cells.

Figure 3 – Schematic showing the formation of the contractile ring and central spindles during telophase. PRC1 interacts with the motor protein KIF4 which directs KIF4 to the antiparallel MTs, PRC1 bundles the midzone microtubules by cross linking antiparallel microtubules while KIF4 caps the midzone microtubules which limit their growth. Centralspindlin (CS) is recruited to the midbody by the chromosomal passenger complex (CPC) and it tethers the central spindles to the plasma membrane. Plk1 and Pin1 control the translocation of Cep55 from the spindle poles to the centralspindlin complex. Centralspindlin also recruits Ect2 to the plasma membrane where is activates RhoA by conversion from RhoA-GDP to RhoA-GTP. Active RhoA induces contractile ring formation by activating pathways for actin and myosin formation.

Figure 4 – Schematic showing proteins involved in membrane re-modelling during the analogous processes of HIV-1 viral budding and cytokinesis. HIV-1 Gag regulates viral budding by binding the ESCRT-I component, Tsg101, and ALIX to L-domains within p6, which in turn recruits the ESCRT-III complex that is required for resolution of the membrane stalk connecting the virions to the host cell. During cytokinesis Cep55 recruits Tsg101 and ALIX to the midbody where they recruit ESCRT-III. The ESCRT-III complex depolymerises the microtubules and mediates abscission by plasma membrane remodelling along with recycling endosomes that are directed to the abscission site by Rab11 and Arf6 binding. CypA interacts with HIV-1 Gag p6 and acts as a general catalyst for cis-trans proline isomerisation. CypA cis-trans isomerisation is also required at the midbody for the timely completion mammalian cytokinesis.
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**Table 1** – The Cyclophilin and Cyclophilin-Like Family of Proteins
Figure 1 – Conservation of the PPIase domain (yellow) in the Cyclophilin and Cyclophilin-Like family
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Table 2 – Cyclophilin and Cyclophilin-Like proteins that are overexpressed (↑) or down regulated (↓) in cancer.
Figure 4
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