The Anaphase Promoting Complex/Cyclosome (APC/C): a versatile E3 ubiquitin ligase

Natalie L. Curtis[1], and Victor M. Bolanos-Garcia[1]*

[1]Faculty of Health and Life Sciences, Department of Biological and Medical Sciences; Oxford Brookes University; Oxford, England, OX3 0BP.

*Author for correspondence (vbolanos-garcia@brookes.ac.uk)

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Abbreviations

Amyloid beta (Aβ), Adenovirus (Ad), Alzheimer's Disease (AD), Adriamycin (ADM), Acute Myeloid Leukemia (AML), Anaphase Promoting Complex/Cyclosome (APC/C), Adult T-cell leukaemia/lymphoma (ATL), Adenosine Triphosphate (ATP), Ataxia telangiectasia and Rad3-related protein **(**ATR), BRCA1-associated RING domain protein 1 (Bard1), Brain-Specific Kinase 2 (BRSK2), Budding uninhibited by benzimidazoles 1 (Bub1), Budding uninhibited by benzimidazoles 3 (Bub3), Budding uninhibited by benzimidazoles related 1 (BubR1), Chicken Anemia virus (CAV), Chicken Anemia Virus-Apoptin (CAV-Apoptin), Cyclic AMP response element binding protein (CBP), Cell division cycle 5 (Cdc5), Cell division cycle 6 protein (Cdc6), Cell Division Cycle 7 (Cdc7), Cell division cycle protein 20 (Cdc20), Cell division cycle protein 23 (Cdc23), Cell division cycle protein 26 (Cdc26), Cell division cycle protein 27 (Cdc27), Cell division control protein 42 (Cdc42), Cell Division Cycle 55 (Cdc55), Cadherin 1 (Cdh1), Cyclin-dependent kinase (Cdk), Cyclin-dependent kinase 1 (Cdk1), Cyclin-dependent kinase 4 (Cdk4), Cyclin-dependent kinase 5 (Cdk5), Chromatin licensing and DNA replication factor 1 (Cdt1), Centromere-associated protein E (CENP-E), Centromere-associated protein F (CENP-F), Checkpoint kinase 1 (Chk1), Chromosome instability and karyogamy protein 1 (Cik1), Casein kinase 1 (CK1), Casein kinase 1 delta (CK1δ), Cytoskeleton-associated protein 2 (CKAP2), Conditional Knockout (cKO), Cyclin-dependent kinases regulatory subunit 1 (Cks1), Central nervous system (CNS), Coimmunoprecipitation (co-IP), Chromosomal passenger complex (CPC), Cryogenic electron microscopy (cryo-EM), Destruction box (D-box), Damaged DNA binding protein 1 (DDB1), Cisplatin (DDP), Deoxyribonucleic acid (DNA), Deoxyribonucleotide triphosphate (dNTPS), Deoxythymidine triphosphate (dTTP) early (E), Adenovirus early region 1A (E1A), E2F Transcription Factor 1 (E2F1), E2F Transcription Factor 3 (E2F3), Early Region 4 Open Reading Frame 4 (E4orf4), Excitatory amino acid (EAA), Epithelial Cell Transforming 2 (Ect2), Euchromatic Histone Lysine Methyltransferase 2 (EHMT2), Electron microscopy (EM) Early mitotic inhibitor (Emi1), Endoplasmic Reticulum (ER), Eyes Absent 1 (EYA1), FANCD2-associated nuclease 1 (FAN1), Filaments in between nuclei protein 1 (Fin1), Forkhead box M1 (FoxM1), Fizzy-related protein homolog (Fzr), Gap 1 (G1), Gap 2 (G2), Glucose-6-phosphate dehydrogenase (G6PD), Granule cell progenitors (GCPs), Glucagon-like peptide (GLP), Glucagon-like peptide-1 (GLP1), Glutaminase 1 (GLS1), Glutamate receptor 1 (GluR1), glioblastoma stem-like cells (GSCs), Glutathione (GSH), Glutathione disulfide (GSSG), Human Adenovirus (HAd), Hepatitis B Virus (HBV), Hepatocellular carcinoma (HCC), Human cytomegalovirus (HCMV), Homologous to the E6-AP Carboxyl Terminus (HECT), Human Gyrovirus-Apoptin (HGyv-Apoptin), Hyaluronan-mediated motility

receptor (Hmmr), Human papillomaviruses (HPV), Histone synthetic lethal 1 (Hsl1) from yeast, Human T-cell lymphotropic virus type 1 (HTLV-1), Hepatoma Up-Regulated Protein (HURP), Inhibitor of Differentiation 1 (Id1), Inhibitor of Differentiation 2 (Id2), Intermediate early (IE), Intermediate protein 72 (IE72), Intermediate protein 86 (IE86), IQ motif containing GTPase-activating protein 1 (IQGAP), Isoleucine Arginine (IR) motif/tail, Janus kinase (JAK), c-Jun N-terminal kinase (JNK), Kilodalton (kDa), Kinesin family member 18A (Kif18A), Kinetochore scaffold 1 (Knl1), late (L), large T antigen (LT), Long-term potentiation (LTP), Mitosis (M), Mitotic arrest deficient 1 (Mad1), Mitotic arrest deficient 2 (Mad2), Mitogen-activated protein kinase (MAPK), Mitotic Checkpoint Complex (MCC), Microcephalin-1 (MCPH1), mediator of DNA damage 1 protein (MDC1), mouse embryonic fibroblasts (MEFs), Male Germ Cell RacGTPase Activating Protein (MgcRacGAP), Modulator of Apoptosis Protein 1 (MOAP-1), Monopolar spindle 1 (Mps1), messenger RNA (mRNA), Nicotinamide adenine dinucleotide phosphate (NADPH), NIMA-related kinase 2A (Nek2A), Neurogenic differentiation factor 2 (NeuroD2), Neurofibrillary tangles (NFT), Ninein-like protein (Nlp), Nuclear Interaction Partner of Alk kinase (NIPA), *N*-methyl-D-aspartate receptors (NMDARs), Neural progenitor cells (NPCs), Nonstructural protein 5A (NS5A), Nucleolar spindle–associated protein (NuSAP), Orf virus (ORFV), Cyclin dependent kinase inhibitor 1 ($p21^{Cip1}$), Cyclin dependent kinase inhibitor 1B ($p27^{Kip1}$), pancreatic ductal adenocarcinoma (PDAC), Premature Dissociation of Sisters (Pds1), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, isoform 3 (PFKFB3), Polo-like kinase 1 (Plk1), Promyelocytic leukaemia bodies (PML) bodies, Protein phosphatase 2 (PP2a), Pentose-Phosphate Pathway (PPP), Retinoblastoma protein (pRb), pro-tosyl-L-arginine methyl ester (proTAME), Postsynaptic density (PSD), Ras-related C3 botulinum toxin substrate 1 (Rac1), Receptor-Associated Protein 80 (RAP80), Ras association domain containing family 1 isoform a (Rassf1a), RING-box protein 1 (RBX1), RING-box protein 2 (RBX2), Ras homolog gene family member A (RhoA), Really Interesting New Gene (RING), Ribonucleic acid (RNA), RNA interference (RNAi), Rho protein kinase 2 (Rock2), Reactive oxygen species (ROS), Synthesis (S), Spindle assembly checkpoint (SAC), Saccharomyces cerevisiae (*S. cerevisiae*), Skp1-Cullin-1-F-box protein (SCF), Shugoshin 1 (Sgo1), SH3 and multiple ankyrin repeat domains protein (Shank), Small interfering RNA (siRNA), Sineoculis homeobox homolog 1 (Six1), S-phase-kinase-associated protein 1 (Skp1), S-phase kinase-associated protein 2 (Skp2), Ski-related novel protein N (SnoN), Speckle-type POZ protein (SPOP), signal transducer and activator of transcription (STAT), Simian virus 40 (SV40), Transforming acidic coiled-coil protein 3 (TACC3), tosyl-L-arginine methyl ester (TAME), testicular germ cell tumours (TGCTs), Transforming growth factor beta (TGF-β), Thymidine kinase (TK1), Thymidylate kinase (TMPK), TNM Classification of Malignant Tumors (TNM), Tetratricopeptide repeat motifs (TPR), Targeting protein for Xklp2 (Tpx2), Tribbles homolog

3 (TRB3), Transformation/Transcription domain-Associated Protein (TRRAP), Tiny yeast comet 1 (Tyc1), Ubiquitin-conjugating enzyme E2 C (Ube2C), Ubiquitin-conjugating enzyme E2 D2 (Ube2D), Ubiquitin carboxyl-terminal hydrolase 1 (UPS1), Ubiquitin specific processing protease 37 (USP37), Whole-exome sequencing (WES).

Abstract

In the present chapter we discuss the essential roles of the human E3 ubiquitin ligase Anaphase Promoting Complex/Cyclosome (APC/C) in mitosis as well as the emerging evidence of important APC/C roles in cellular processes beyond cell division control such as regulation of genomic integrity and cell differentiation of the nervous system. We consider the potential incipient role of APC/C dysregulation in the pathophysiology of the neurological disorder Alzheimer's disease (AD). We also discuss how certain Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) viruses take control of the host's cell division regulatory system through harnessing APC/C ubiquitin ligase activity and hypothesise the plausible molecular mechanisms underpinning virus manipulation of the APC/C. We also examine how defects in the function of this multisubunit protein assembly drive abnormal cell proliferation and lastly argue the potential of APC/C as a promising therapeutic target for the development of innovative therapies for the treatment of chronic malignancies such as cancer.

1. Introduction

Protein degradation through ubiquitination is mediated by the sequential action of activating (E1), conjugating (E2), and ligase (E3) enzymes to render a poly-ubiquitinated protein substrate that is subsequently recognised and degraded by the proteasome (Nakayama and Nakayama 2006; Nalepa *et al*. 2006; Pickart 2001). In the human genome, a small number of ubiquitin E1 and E2 enzymes and hundreds of different E3 ubiquitin ligases have been identified. The much larger number of the latter class of enzymes reflects their role of recruiting specific substrate proteins for targeted ubiquitination, thus conferring substrate selectivity to the Ubiquitin/Proteasome System (UPS) (Nagy and Dikic 2010; Deshaies and Joazeiro 2009; Petroski and Deshaies 2005).

Two major classes of E3 ligases occur in eukaryotes: the Homologous to the E6-AP Carboxyl Terminus (HECT) domain and the Really Interesting New Gene (RING) domain (Deshaies and Joazeiro 2009; Bedford *et al*. 2011). HECT E3s establish a transient, covalent linkage with ubiquitin at a conserved cysteine residue before the ubiquitin molecule is transferred to its substrate. In contrast, RING E3s mediate the transfer of the ubiquitin molecule from the E2 enzyme to a substrate in a process that does not require its physical interaction with the substrate (Metzger *et al.* 2012; Bedford *et al.* 2011; Skaar and Pagano 2009; Rotin and Kumar 2009).

The RING ubiquitin E3 ligases are the largest family of E3 ubiquitin ligases and associated to the regulation of cell division (Nakayama and Nakayama 2006; Nagy and Dikic 2010; Deshaies and Joazeiro 2009). APC/C is one of two multi-subunit RING finger ubiquitin ligase families. The other is the Skp1-Cullin-1-F-box protein (SCF) family, which consists of the scaffold protein Cullin-1, a RING-finger protein RING-box protein 1 (RBX1) or RING-box protein 2 (RBX2) (also known as ROC1/2), the adaptor S-phase-kinase-associated protein 1 (Skp1), and a F-box protein that recruits specific proteins substrates for SCF-mediated degradation (Cardozo and Pagano 2004; Jin *et al.* 2004; Ang and Wade Harper 2005). The

two families of RING finger ubiquitin ligases play pivotal roles in the regulation of the cell cycle as they mediate the proteasome-dependent degradation of cell cycle regulators such as Cyclins and Cyclin-dependent kinase (Cdk) inhibitors (Skaar and Pagano 2009; Harper *et al.* 2002).

Similar to the architecture of the SCF complex, the APC/C assembly contains one Cullin-like subunit (APC2), a RING finger protein (APC11), and an interchangeable substrate recognition subunit (Lipkowitz and Weissman 2011). However, APC/C exhibits a unique molecular architecture as it is composed of 14 to 16 different subunits, depending on the species (Peters 2006; Pines 2011). In the human, the known 14 protein subunits define a holoenzyme complex with a combined molecular mass of approximately 1.5 mega Daltons, consisting of APC1/Tsg24, APC2, APC3/Cell division cycle protein 27 (Cdc27), APC4, APC5, APC6/Cell division cycle protein 6 (Cdc6), APC7, APC8/Cell division cycle protein 23 (Cdc23), APC10/Doc1, APC11, APC13/Swm1, APC15/Mnd2, APC16 and Cell division cycle protein 26 (Cdc26) as well as the co-activator subunit Cell division cycle protein 20 (Cdc20) or Cadherin 1 (Cdh1) (Barford 2011; Foe and Toczyski 2011; McLean *et al*. 2011).

2. Structural Architecture

Recent advances in cryogenic electron microscopy (cryo-EM) methods have revealed important mechanistic details of APC/C formation and regulation and showed that APC/C consists of three sub-complexes: a scaffolding subcomplex, a catalytic and substrate recognition sub-complex, and an arm defined by multiple tetratricopeptide repeat motifs (TPR) (Barford 2015; Chang *et al.* 2015; Chang *et al.* 2014; Alfieri *et al.* 2016; Yamaguchi *et al.* 2016; Zhang *et al*. 2016; Foe and Toczyski 2011; Schreiber *et al.* 2011; Thornton *et al.* 2006; Vodermaier *et al*. 2003). The structural architecture of the APC/C is summarised in **Figure 1 and Figure 2**. The scaffolding sub-complex is composed of APC1, APC4 and APC5; the catalytic sub-complex of the APC2, APC11 (the RING finger protein) and APC10/Doc1 subunits; and the TPR arm, which is defined by the APC3/Cdc27, APC6/Cdc16

and APC8/Cdc23 subunits and delineates a binding surface for the coactivators Cdc20 or Cdh1 and for the subunits Cdc26, APC13/Swm1, and APC16. The APC/C core subunit APC10/Doc1 contributes to substrate recruitment via binding to the Destruction-boxes (D-boxes) of protein substrates (da Fonseca *et al.* 2011; Buschhorn *et al.* 2011; Frye *et al.* 2013). Mechanistic details of APC/C assembly and whether there are additional components of this complex in the cell are key aspects of cell cycle control that remain to be established. The Mitotic Checkpoint Complex (MCC) composed of Budding uninhibited by benzimidazoles related 1 (BubR1), together with Budding uninhibited by benzimidazoles 3 (Bub3), Mitotic arrest deficient 2 (Mad2) and Cdc20, exists both in a free state and stably bound to the APC/C, and its binding to the APC/C is essential to mount an effective Spindle Assembly Checkpoint (SAC) response (Hein and Nilsson 2014; Alfieri *et al.* 2016). The MCC binds and inhibits Cdc20-bound APC/C (APC/C-Cdc20), forming a large complex called APC/C-Cdc20-MCC that contains two copies of Cdc20.

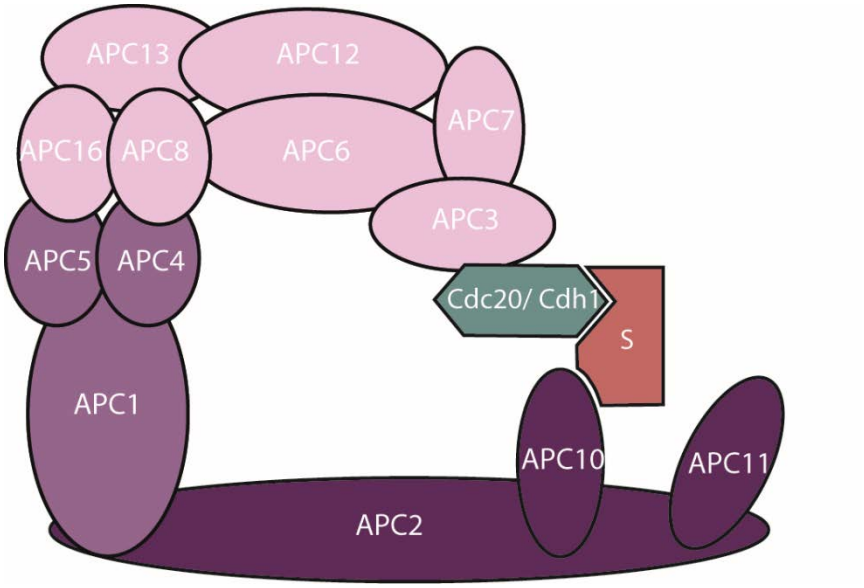

Figure 1. Schematic structure of the APC/C. The APC/C consists of three distinct subcomplexes; a catalytic subcomplex, scaffolding complex and a specificity arm defined by multiple tetratricopeptide repeat motifs (TPR). For clarity the subunits that assemble each subcomplex have been colour-coded, subunits presented are not drawn to scale and not all subunits are depicted. The catalytic sub complex is composed of APC2 and APC11 which contain the Cullin and RING domain respectively and APC10/Doc1 which is the subunit that identifies and recruits specific substrates. The scaffolding subcomplex contains APC1, APC4 and APC5 and holds the catalytic subcomplex and the specificity arm together. The specificity TPR arm is defined by APC3/Cdc27, APC6/Cdc16 and APC8/Cdc23 and delineates a binding surface for APC/C coactivators Cdc20 and Cdh1. These coactivators direct the recruitment of substrates to the complex.

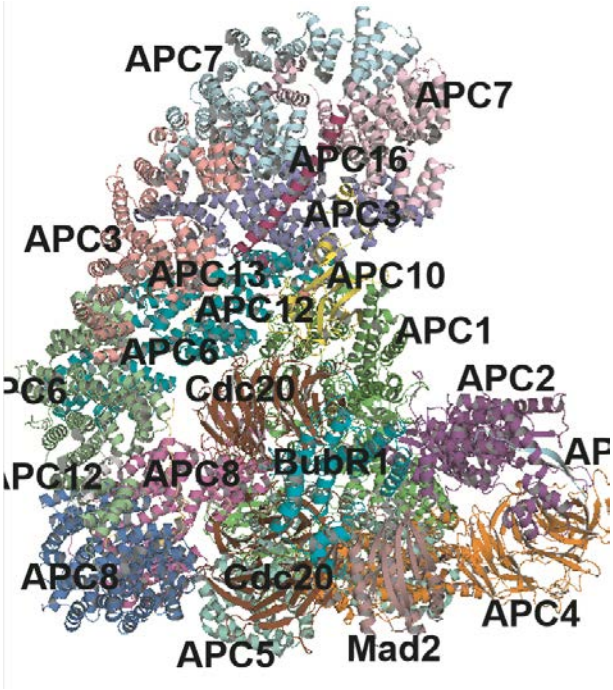

Figure 2. Structural architecture of the APC/C assembly bound to the Mitotic Checkpoint Complex (MCC) as defined by cryo-EM methods (pdb ID 5KHU). The labels indicate the position and copy number of APC/C subunits, coactivators and MCC proteins.

Cryo-EM data of APC/C structure in complex with MCC has revealed new clues about APC/C regulation and showed that the MCC interacts with an APC/C region located in close proximity to the site where a Cdc20 subunit was previously docked (Yamaguchi *et al*. 2016; Alfieri *et al.* 2016). The APC/C-Cdc20-MCC structure shows that BubR1 extends around the two Cdc20 subunits to occupy all degron-binding sites. A consequence of this mode of interaction is that binding of the MCC to APC/C-Cdc20 enables it to act as an allosteric regulator that blocks the access of APC/C substrates. Although it has been established that the WD40 protein Bub3 contributes to the termination of the SAC signal (Alfieri *et al.* 2016; Vanoosthuyse *et al.* 2009), the precise role of this MCC component in the regulation of APC/C function remains obscure. In the cryo-EM structures, the lack of well-defined electron

density around the anticipated Bub3 location of the APC/C-MCC assembly suggests that Bub3 remains largely flexible upon MCC binding to the APC/C. Whether that is the case and how such mode of interaction contributes to fine tune APC/C functions warrants further investigations. Furthermore, the overall similarity of electron microscopy (EM) maps for APC/C-Cdc20-MCC complexes with or without Bub3 raises the questions if MCC subcomplexes lacking Bub3 may be sufficient for the Ube2C-mediated ubiquitination of Cdc20 (Alfieri *et al.* 2016; Yamaguchi *et al.* 2016; Chang. *et al.* 2015).

Unlike the major role of APC/C-Cdc20 in promoting the metaphase to anaphase transition, APC/C-Cdh1 plays a key role in governing cell cycle progression through the Gap 1 (G1) phase by sustaining low Cdk activity, which is achieved by mediating the degradation of mitotic Cyclins (Irniger and Nasmyth 1997), Cdc25A (Donzelli *et al.* 2002), Cyclin-dependent kinases regulatory subunit 1 (Cks1) (Bashir *et al*. 2004) and S-phase kinase-associated protein 2 (Skp2) (Bashir *et al.* 2004; Wei *et al.* 2004). Activation of APC/C-Cdh1 promotes the degradation of Aurora A (Littlepage and Ruderman 2002), Aurora B (Stewart and Fang 2005; Nguyen *et al.* 2005), Cdc20 (Huang *et al.* 2001; Hyun *et al*. 2013), Polo-like kinase 1 (Plk1) (Lindon and Pines, 2004), and Targeting protein for Xklp2 (Tpx2) (Stewart and Fang 2005), leading to mitotic exit.

The APC/C is the ultimate effector of the SAC. In simple terms, the SAC can be defined as an evolutionary conserved mechanism of regulation of higher organisms that prolongs mitosis until all chromosomes have established proper attachment to spindle microtubules. SAC signalling involves the physical interaction of certain central protein components with the kinetochore and microtubules. The kinetochore is a proteinaceous framework that assembles onto centromeric DNA to organise SAC activity, chromosome attachment, and cell cycle progression from metaphase to anaphase (revised in Derive *et al*. 2015; Kapanidou *et al.* 2015; Sacristan and Kops 2015).

In addition to Cdc20, the central SAC components Mitotic arrest deficient 1 (Mad1) and the proteins kinases Budding uninhibited by benzimidazoles 1 (Bub1) and Monopolar spindle 1 (Mps1) enhance the rate of MCC formation and amplify the SAC signals (Hardwick *et al.*

1996; Abrieu *et al.* 2001; Chung and Chen 2002; De Antoni *et al*. 2005; Morrow *et al*. 2005). These core components of the SAC are highly conserved across species and include the Serine/Threonine kinases Bub1 and BubR1, and the dual specificity Serine/Threonine/Tyrosine kinase Mps1. Bub1 kinase is required for the kinetochore localisation of BubR1, Mad2, and the centromere-associated proteins CENP-E and CENP-F (Ciossani *et al.* 2018; Sivakumar and Gorbsky 2015) as well as for the establishment and maintenance of productive attachment to spindle microtubules (Meghini *et al.* 2016; Braunstein *et al*. 2007; Burton and Solomon 2007). Bub1 kinase activity is also necessary for the centromere recruitment of the chromosomal passenger complex (CPC) and Shugoshin 1 (Sgo1) (Williams *et al.* 2017; Baron *et al.* 2016). At least in mitosis, BubR1 functions as a pseudokinase that plays important roles in chromosome segregation but also has reported to play important functions in DNA repair, ciliogenesis and neuron differentiation (revised in Bolanos-Garcia and Blundell 2011). BubR1 together with Bub3, Mad2 and Cdc20, forms part of the MCC, which is assembled in response to improper chromosome attachment to the mitotic spindle to inhibit the APC/C. Mps1 kinase activity is crucial for the conformational activation of Mad2 that is required for Mad2 binding to Cdc20 (Zich and Hardwick 2010), and for the kinetochore recruitment of Mad1 and Mad2 in mitotic cells (Tipton *et al.* 2013; Hewitt *et al.* 2010). In addition to the MCC, the protein Early mitotic inhibitor (Emi1) is an important inhibitor of the APC/C. Both, the MCC and Emi1 inhibitors regulate coactivator binding, substrate recognition, and activity/binding of the E2 enzymes.

3. APC/C regulation is intricate

In vertebrates, full APC/C substrate ubiquitination activity is modulated by two E2 class of ubiquitin ligases: UbcH10 (also named Ubiquitin-conjugating enzyme E2 C (Ube2C)) and UbcH5 (also known as Ubiquitin-conjugating enzyme E2 D (Ube2D)) (Xie *et al.* 2014; Aristarkhov *et al.* 1996) as well as the binding of the APC/C coactivators Cdh1 and Cdc20.

By using UbcH10 and the Lys11-specific 'elongating' E2 enzyme Ube2S, APC/C build Lys11-linked ubiquitin chains as a degradation signal (Komander and Rape 2012; Rape 2010; Wickliffe *et al.* 2011; Ye and Rape 2009). The APC/C binds to substrates that contain D-box [RXXL] and KEN box motifs (Glotzer *et al*. 1991; Pfleger and Kirschner 2000). These motifs define a class of degrons that play an important role in the regulation of protein degradation. The APC/C coactivators Cdc20 and Cdh1 are characterised by having a structure largely organised as a WD40 domain and the presence of C-box and Isoleucine Arginine (IR)-motifs, all of which mediate direct binding to the APC/C (Chang *et al.* 2015; Izawa and Pines 2012; Schwab *et al*. 2001; Passmore *et al.* 2005; Vodermaier *et al*. 2003; Visintin *et al.* 1997). Besides the D-box and KEN box motifs, Cdc20 and Cdh1 bind to proteins that harbour the ABBA motif (for Acm1, Bub1, BubR1, and Cyclin A) which is also known as the Phe box (Di Fiore *et al.* 2016; Di Fiore *et al*. 2015; Diaz-Martinez *et al.* 2015). The region that defines the longitudinal axis of the WD40 repeat mediates binding of KEN box, D-box and ABBA motif-containing substrates (Chao *et al*. 2012; He *et al.* 2013; Passmore *et al.* 2005; Carroll and Morgan 2005; Matyskiela and Morgan 2009; Buschhorn *et al.* 2011; da Fonseca *et al*. 2011). The *bona fide* APC/C substrates reported to date are summarised in **Table 1**.

Table 1. Bona fide APC/C substrates reported to date

Protein substrate	**Function(s)**	**APC/C co-activator**	**Reference(s)**
Acm1 from budding yeast	Regulation of APC/C through Cdh1 inhibition	Cdh1/Cdc20	Enquist-Newman *et al*. 2008
Anillin	Completion of cytokinesis	Cdh1	Zhao and Fang 2005
Aurora A kinase	Regulation of cell cycle progression	Cdh1	Littlepage and Ruderman 2002

Aurora B kinase	Regulation of spindle assembly, chromosome alignment and segregation	Cdh1	Stewart and Fang 2005; Nguyen *et al*. 2005
BRCA1-associated RING domain protein 1 (Bard1)	Regulation of spindle pole formation	Cdh1/Cdc20	Song and Rape 2010
Brain-Specific Kinase 2 (BRSK2)	Regulation of BRSK2 centrosome localisation during mitosis	Cdh1	Li *et al*. 2012
Budding uninhibited by benzimidazoles 1 (Bub1) kinase	Regulation of chromosome segregation and spindle checkpoint signalling	Cdh1	Qi and Yu 2007
Cell division cycle 5 (Cdc5) Polo-like kinase	Regulation of Cdc14 localisation	Cdh1	Visintin *et al*. 2008
Cell division cycle 6 (Cdc6)	Control of DNA replication initiation	Cdh1	Petersen *et al*. 2000
Cell Division Cycle 7 (Cdc7) kinase	Activator of S-phase kinase (ASK; also known as Dbf4)	Cdh1	Yamada *et al*. 2013
Cell Division Cycle 20 (Cdc20)	APC/C coactivator; recruitment of APC/C substrates for proteasome-dependent degradation	Cdh1	Huang *et al.* 2001; Hyun *et al*. 2013
Cdc25A phosphatase	Regulation of Cyclin-dependent kinase 1 (Cdk1) activity in mitosis	Cdh1	Donzelli *et al*. 2002
Chromatin licensing and DNA replication factor 1 (Cdt1)	Initiation of DNA replication in association with Cdc6	Cdh1	Sugimoto *et al*. 2008
Centromere protein F (CENP-F)	Control of chromosome segregation and kinetochore formation in mitosis	Cdc20	Gurden *et al*. 2010
Chromosome instability and karyogamy protein 1 (Cik1) from budding yeast	Regulation of the mitotic spindle and microtubule functions	Cdh1	Benanti *et al*. 2009

Cytoskeleton-associated protein 2 (CKAP2), also known as TMAP	Roles in mitotic spindles assembly and maintenance	Cdh1	Seki and Fang 2007; Hong *et al*. 2007
Cyclin-Dependent Kinases Regulatory Subunit 1 (Cks1)	Regulation of G1/S transition through interaction with S-phase kinase-associated protein 2 (Skp2)	Cdh1	Bashir *et al*. 2004
Claspin	Activation of Checkpoint kinase 1 (Chk1) and regulation of DNA damage repair	Cdh1	Gao *et al*. 2009; Bassermann *et al*. 2008
Clb2	Control of degradation of the mitotic entry regulator Swe1	Cdh1	Simpson-Lavy *et al*. 2009
Conductin	Inhibitor of the Wnt signalling pathway	Cdc20	Bassermann *et al*. 2008
Cyclin A	Control of S phase and G2/M transition	Cdc20/Cdh1	den Elzen and Pines 2001; Geley *et al*. 2001
Cyclin B	Activation of Cdk1 and regulation of the G2/M transition	Cdc20/Cdh1	Clute and Pines 1999
E2F Transcription Factor 1 (E2F1)	Regulation of G1/S transition and apoptosis	Cdc20/Cdh1	Budhavarapu *et al*. 2012
E2F Transcription Factor 3 (E2F3)	Roles in cell cycle exit and neuronal differentiation	Cdh1	Ping *et al*. 2012
Epithelial Cell Transforming 2 (Ect2)	Regulation of Ras homolog gene family member A (RhoA) in mitosis	Cdh1	Liot *et al*. 2011
Euchromatic Histone Lysine Methyltransferase 2 (EHMT2)	Regulation of histone H3K9 methylation and senescence	Cdh1	Takahashi *et al*. 2012
Eyes Absent 1 (EYA1)	Control of cell proliferation and M-to-G1 transition	Cdh1	Sun *et al*. 2013
FANCD2-associated	Confers cellular resistance against DNA inter-strand	Cdh1	Lai *et al*. 2012

nuclease 1 (FAN1)	crosslinking agents		
Filaments in between nuclei protein 1 (Fin1) from budding yeast	Stability of the mitotic spindle	Cdh1	Woodbury and Morgan 2007
Forkhead box M1 (FoxM1) transcription factor	Regulation of S and M phase progression	Cdh1	Park *et al*. 2008
Geminin	Inhibition of DNA replication	Cdh1	McGarry and Kirschner 1998
Glucagon-like peptide-1 (GLP1)	Regulation of histone H3K9 methylation and senescence	Cdh1	Takahashi *et al*. 2012
Glutaminase 1 (GLS1)	Glutaminolysis	Cdh1	Colombo *et al*. 2011
Glutamate receptor 1 (GluR1)	Regulation of homeostatic plasticity	Cdh1	Fu *et al*. 2011
HEC1	Regulation of kinetochore microtubule dynamics and mitotic exit	Cdh1	Li *et al*. 2011
Hyaluronan-mediated motility receptor (Hmmr)	Required for spindle pole localisation of Targeting protein for Xklp2 (Tpx2)	Cdh1/Cdc20	Song and Rape 2010
Histone synthetic lethal 1 (Hsl1) from yeast	Regulation of Mitogen-activated protein kinase (MAPK) pathway signalling	Cdh1	Simpson-Lavy *et al*. 2009
Hepatoma Up-Regulated Protein (HURP)	Microtubules crosslinking	Cdh1/Cdc20	Song and Rape 2010
Inhibitor of Differentiation 1 (Id1)	Inhibition of dendrite growth	Cdc20	Kim *et al*. 2009
Inhibitor of Differentiation 2 (Id2)	Stimulation of axon growth	Cdh1	Lasorella *et al*. 2006
IQ motif containing GTPase-activating protein 1 (IQGAP) from yeast	Regulation of cytokinesis	Cdh1	Ko *et al*. 2007
c-Jun N-terminal kinase (JNK)	Regulation of cell survival, cell differentiation, and mitotic exit	Cdh1	Gutierrez *et al*. 2010

Kid from frog	Regulation of spindle formation and of chromosome movements in mitosis and meiosis	Cdh1	Feine *et al*. 2007
Kinesin family member 18A (Kif18A)	Control of chromosome congression	Cdc20	Sedgwick *et al*. 2013
Liprin-α	Regulation of synaptic size	Cdh1	Teng and Tang 2005; van Roessel *et al.* 2004
Mcl-1	Negative regulation of apoptosis	Cdc20	Harley *et al*. 2010
Microcephalin-1 (MCPH1)	Regulation of chromosome condensation and Cdk1 activation	Cdh1	Meyer et al. 2019
Mes1 from fission yeast	Dual effector of the APC/C (eg, substrate and inhibitor)	Cdc20	Kimata *et al*. 2008
Male Germ Cell RacGTPase Activating Protein (MgcRacGAP)	Control of cytokinesis and cell cycle progression; regulation of RhoA, Ras-related C3 botulinum toxin substrate 1 (Rac1) and Cell division control protein 42 (Cdc42)	Cdh1	Nishimura *et al*. 2013
Modulator of Apoptosis Protein 1 (MOAP-1)	Modulator of apoptosis and DNA damage repair	Cdh1	Huang *et al*. 2012
Monopolar spindle 1 (Mps1) kinase	Regulation of the spindle assembly checkpoint and chromosome-microtubule attachments	Cdh1/ Cdc20	Cui *et al*. 2010
NEDL2 ubiquitin ligase	Control of p73 and Ataxia telangiectasia and Rad3-related protein **(**ATR) kinase levels	Cdh1	Lu *et al*. 2013
NIMA-related kinase 2A (Nek2A)	Regulation of centrosome separation and spindle formation	Cdc20	Hayes *et al*. 2006; Hames *et al*. 2001

Neurogenic differentiation factor 2 (NeuroD2)	Negative regulation of presynaptic differentiation	Cdc20	Yang *et al*. 2009
Ninein-like protein (Nlp)	Regulation of centrosome maturation	Cdh1/Cdc20	Wang and Zhan 2007
Nuclear Interaction Partner of Alk kinase (NIPA)	Regulation of nuclear Cyclin B1 degradation	Cdh1	Klitzing *et al*. 2011
Nrm1 from yeast	Transcriptional activation of MBF and Mcm1 target genes	Cdh1	Ostapenko and Solomon 2011
Nucleolar spindle–associated protein (NuSAP)	Control of microtubules dynamics	Cdh1/Cdc20	Song and Rape 2010
p190	Regulation of cell mobility	Cdh1	Naoe *et al*. 2010
Cyclin-dependent kinase inhibitor 1 ($p21^{Cip1}$)	Inhibition of cyclin-dependent kinase activity	Cdc20	Amador *et al*. 2007
6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase-3 (PFKFB3)	Regulation of glycolysis	Cdh1	Herrero-Mendez *et al*. 2009
PHF8 demethylase	Regulation of histone H3 and H4 expression	Cdc20	Lim *et al*. 2013
Polo-like kinase 1 (Plk1)	Control of mitotic spindle formation	Cdh1	Lindon and Pines 2004
PR-Set7 methyltransferase	Control of cell cycle progression	Cdh1	Herrero-Mendez *et al*. 2009; Wu *et al*. 2010
Rad17	Activation of DNA damage checkpoint mechanisms	Cdh1	Zhang *et al*. 2010
Receptor-Associated Protein 80 (RAP80)	Recruitment of BRCA1 to DNA damage sites	Cdh1/Cdc20	Cho *et al*. 2012
RCS1	Regulation of the metaphase-to-anaphase transition	Cdh1	Zhao *et al*. 2008
REV1 polymerase	Control of stalled DNA replication	Cdc20	Chun *et al*. 2013
Securin	Catalytic activity inhibition of the protease Separase	Cdc20/Cdh1	Michaelis *et al*. 1997; Uhlmann *et al*. 1999

Shugoshin 1 (Sgo1)	Prevention of premature sister-chromatid separation	Cdh1	Karamysheva *et al*. 2009; Christensen *et al*. 2007
Sineoculis homeobox homolog 1 (Six1)	Regulation of cell development	Cdh1	Christensen *et al*. 2007
S-phase kinase-associated protein 2 (Skp2)	Promotes degradation of Cdk inhibitors Cyclin dependent kinase inhibitor 1B ($p27^{Kip1}$) and $p21^{Cip1}$	Cdh1	Wei *et al*. 2004; Bashir *et al*. 2004
Ski-related novel protein N (SnoN)	Inhibition of Transforming growth factor beta (TGF-β) signalling and stimulation of axon growth	Cdh1	Stegmuller *et al*. 2006
Sp100 scaffold protein	Control of transcription regulation and apoptosis	Cdc20	Wang *et al*. 2011
Transforming acidic coiled-coil protein 3 (TACC3)	Regulation of mitotic spindle assembly and chromosome segregation	Cdh1	Jeng *et al*. 2009
Thymidine kinase 1 (TK1)	Control of genome stability	Cdh1	Ke *et al*. 2005; Ke *et al*. 2007
Thymidylate kinase (TMPK)	Regulation of Deoxythymidine triphosphate (dTTP) production and genome stability	Cdh1	Ke *et al*. 2005
Tribbles homolog 3 (TRB3)	Regulation of Endoplasmic Reticulum (ER) stress-induced cell death	Cdh1	Ohoka *et al*. 2010
Transformation/Transcription domain-Associated Protein (TRRAP)	Condensation of chromatin and control of chromosome segregation	Cdh1/Cdc20	Ichim *et al*. 2014
UbcH10 E2 ubiquitin ligase	Essential factor of the APC/C	Cdh1	Rape and Kirschner 2004
Ubiquitin carboxyl-terminal hydrolase 1 (UPS1)	Regulation of DNA repair and genomic stability	Cdh1	Cotto-Rios *et al*. 2011
Yhp1 from yeast	Transcriptional activation of MBF and Mcm1 target	Cdh1	Ostapenko and Solomon 2011

	genes		

APC/C E3 ubiquitin ligase activity is tightly regulated by multiple mechanisms including acetylation, phosphorylation, the binding of inhibitors, subcellular localisation, and destabilisation of its activators and/or component subunits acetylation (Glotzer *et al.* 1991; Touati *et al.* 2018; Hein *et al*. 2017; Craney *et al.* 2016; Höckner *et al.* 2016). Tight regulation of cell cycle progression by the APC/C E3 ligase requires its physical and sequential interaction with its two co-activators Cdh1 and Cdc20, which form an APC/C-Cdh1 or an APC/C-Cdc20 complex, respectively. Essentially, the APC/C-Cdh1 complex is primarily active during the end of mitotic exit and early G1 phase of the cell cycle whereas the APC/C-Cdc20 complex regulates the transition from metaphase to anaphase and mitotic exit.

Towards the Gap 2 (G2) phase, Cdc20 is phosphorylated by Cyclin-dependent kinase 1 (Cdk1) and other mitotic kinases, resulting in the activation of the APC/C-Cdc20 complex (Hein *et al.* 2017; Höckner *et al.* 2016; Kramer *et al.* 2000; Kraft *et al.* 2003). However, spindle misalignment or improper kinetochore attachments to sister chromatids trigger SAC signalling, resulting in the inhibition of APC/C E3 ubiquitin ligase activity through sequestering Cdc20 from the APC/C core complex by the MCC (Yu 2002; Yu 2007; Bharadwaj and Yu 2004; Musacchio and Salmon 2007; Musacchio 2011; Lara-Gonzalez *et al.* 2012).

Extensive phosphorylation of the APC/C occurs during mitosis, where more than 50 phosphorylation sites have been identified (Touati *et al.* 2018; Craney *et al.* 2016). Cdk, Bub1, and Mitogen-activated protein kinase (MAPK) kinases inhibit APC/C-Cdc20 E3 ligase activity during the spindle checkpoint (Chung and Chen 2003; Tang *et al.* 2004; Yudkovsky *et al.* 2000) whereas APC/C activation of the ubiquitin chain-forming E2 subunit of Ube2S is impaired when Cdc20 is phosphorylated by protein phosphatase PP2AB56 (Lee *et al.* 2017). Furthermore, depletion of Kinetochore scaffold 1 (Knl1), a large kinetochore protein (2000 amino acid residues in the human) that acts as an organising centre and recruits BubR1 to

the kinetochore, prevents PP2AB56 and Ube2S binding to Cdc20 (Craney *et al.* 2016). These features suggest that the regulated phosphorylation/dephosphorylation of Cdc20 acts as a molecular rheostat that contributes to modulate APC/C ubiquitin E3-ligase activity through a finely controlled assembly and disassembly of APC/C complexes.

The D-box motifs of the Ras association domain containing family 1 isoform a (Rassf1a) mediate the binding of this protein to the APC/C-Cdc20 complex, resulting in the inhibition of APC/C ubiquitin ligase activity during mitotic entry (Chow *et al.* 2012; Song and Rape 2010). Similar to the case of MCC-mediated inhibition, Rassf1a only suppresses APC/C-Cdc20 activity. In contrast, the F-box protein Emi1 (also called FBXO5) inhibits the APC/C-Cdc20 complex in Synthesis (S) and G2 phases (Reimann *et al.* 2001) and the APC/C-Cdh1 complex during the transition from G1 to S phase (Miller *et al.* 2006; Hsu *et al.* 2002). Unlike Emi1, the Emi1 homolog Emi2 inhibits APC/C to regulate meiotic cell division in a process that probably involves the Emi2 RL motif and additional residues (Liu *et al.* 2006; Tung *et al.* 2005).

An additional layer of regulation of APC/C-Cdc20 activity in response to the status of the mitotic spindle involves fine-tune cycles of Cdc20 synthesis and degradation and the formation of the APC/C-Cdc20-MCC complex (Izawa and Pines 2015; London and Biggins 2014). Indeed, the ubiquitination of the Cdc20 residues Lys485 and Lys490 trigger extensive conformational transitions that enable the adoption of an active, open state that docks the E2 subunit of Ube2C thus fine-tuning APC/C ubiquitination activity that ultimately ensures a tight control of mitosis progression. The fact that Cyclin B and Securin are quickly degraded once the last sister chromatid is properly attached to the kinetochore supports the view that SAC signalling and mitotic control are connected (Hagting *et al.* 2002; Clute and Pines 1999).

The picture emerging from these studies is that of one sophisticated regulatory mechanism involving extensive cooperative interactions that act in a concerted fashion to ensure proper chromosome segregation and cell cycle progression. This manner, depending on the precise requirements by the cell at a given point in time, involves interactions that control APC/C ubiquitin E3-ligase activity that can act either as an inhibitor or as an activator of the complex

in order to maintain a stable genome. The fact that defects in APC/C regulation results in genome stability and aneuploidy provides evidence of the evolutionary advantage of this intricate mode of cell cycle regulation. The fact that most of the germline knockout mice targeting the APC/C pathway are embryonic lethal, confirms further the essential role of APC/C in the control of the eukaryotic cell cycle.

4. Cell-cycle independent functions of APC/C E3 ligase

APC/C has cell-cycle-independent functions in the cell, including regulation of genomic integrity and cell differentiation of the nervous system (Puram and Bonni 2011; Hu *et al.* 2011; Wasch *et al.* 2010; Manchado *et al.* 2010). Protein regulators of DNA damage repair and genomic stability such as Claspin (Gao *et al.* 2009; Bassermann *et al.* 2008), Ubiquitin carboxyl-terminal hydrolase 1 (UPS1) (Cotto-Rios *et al.* 2011), and Rad17 (Zhang *et al.* 2010) as well as the proteins G9a and Glucagon-like peptide (GLP) (Takahashi *et al.* 2012) have been reported to be *bona fide* Cdh1 substrates. However, a deeper understanding of the physiological role of the APC/C-Cdh1 complex in these cellular processes warrants further investigations. If confirmed, the interactions will expand further the repertoire of APC/C functions to include important roles in apoptosis and senescence.

In the last two decades, the association of the APC/C with numerous crucial functions in the central nervous system (CNS), including axon guidance, synaptic development and plasticity, neurogenesis, neuronal morphogenesis and neuron differentiation, survival and metabolism has been ascertained. The APC/C-Cdh1 complex plays a role in the differentiation and function of the nervous system, particularly in the control of axon growth and patterning during normal brain development (Konishi *et al*. 2004). Such regulatory role in neuronal development is mediated by the targeting of the axon growth-promoting factors Inhibitor of Differentiation 2 (Id2) and Ski-related novel protein N (SnoN) by the APC/C-Cdh1 complex, leading to their subsequent degradation by the proteasome (Stegmuller *et al.* 2006; Lasorella *et al*. 2006). The APC/C-Cdc20 complex seems to also play important roles in

neuronal development as it regulates dendrite morphogenesis and presynaptic differentiation, in a process that involves degradation of the transcription factors NeuroD2 (Yang *et al*. 2009) and Inhibitor of Differentiation 1 (Id1) (Qian *et al.* 2010).

Further studies showed that synaptic plasticity, synaptic size and the bioenergetic and antioxidant status of neurons are controlled by APC/C-Cdh1-dependant degradation of Glutamate receptor 1 (GluR1) (Fu *et al*. 2011) and Liprin-α (Teng and Tang 2005; van Roessel *et al.* 2004). Despite these advances, whether APC/C deficiency is associated with neurological and psychiatric disorders such as learning and memory impairment remains largely obscure.

5. An incipient role of the APC/C in Alzheimer's Disease?

There is accumulating evidence of a direct involvement of APC/C-Cdh1 in numerous Alzheimer's Disease (AD) pathophysiological hallmarks with substantial indication that deregulation of APC/C substrates has significant implication in AD. For example, brains of individuals suffering from AD exemplify an accumulation of APC/C substrates, implying APC/C deregulation. Furthermore, it has recently been depicted that the APC/C is inactivated in AD (reviewed in Fuchsberger *et al.* 2017). Key AD pathological hallmarks include senile plaque development comprising the toxic peptide Amyloid beta (Aβ) and production of neurofibrillary tangles (NFT) by hyperphosphorylation of the microtubule associated protein tau, with subsequent aggregate formation. These pathophysiological hallmarks have been associated with numerous processes exemplified in AD including ectopic cell cycle re-entry, oxidative stress, excitotoxicity, impaired long-term potentiation (LTP), and deregulation of numerous signalling pathways, collectively resulting in the subsequent development of neurodegeneration in AD. It is postulated that these disease-related processes possibly correlate to deregulation of the APC/C and its substrates (**summarised in Figure 3**) (Fuchsberger *et al*. 2017).

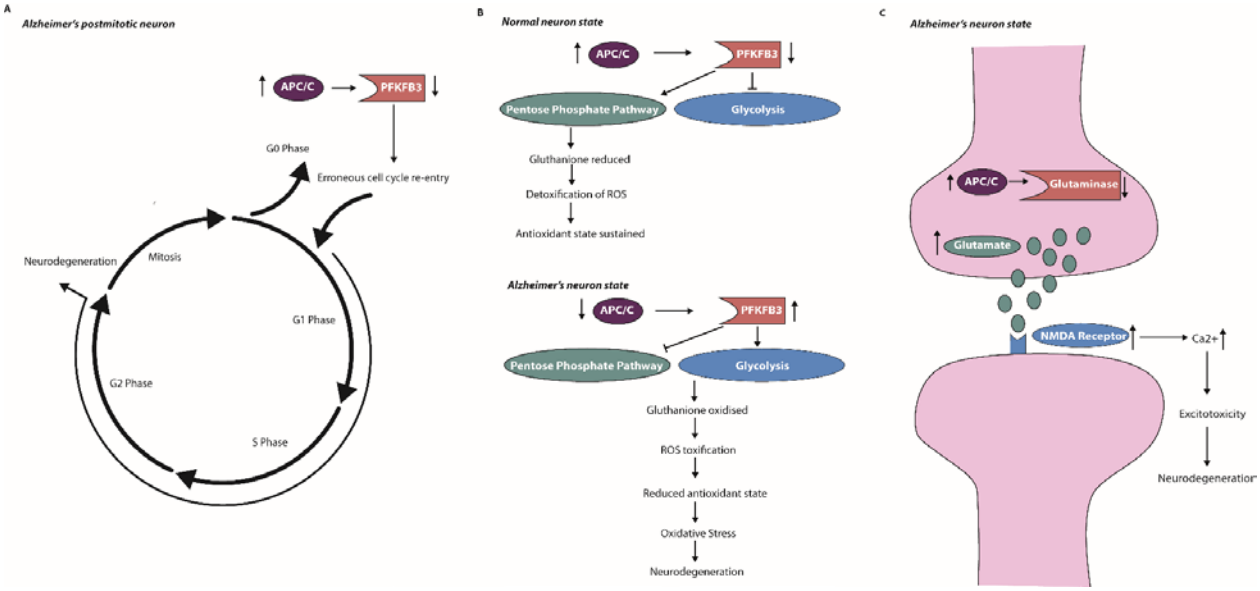

Figure 3. Association of APC/C dysregulation in Alzheimer's disease. (A) Inactivation of APC/C-Cdh1 causes an upregulation in APC/C substrate Cyclin B1 that allows an erroneous re-entry in the cell cycle which leads to subsequent neurodegeneration. (B) In normal state neuron, APC/C-Cdh1 activity is high and APC/C substrate 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, isoform 3 (PFKFB3) is low. Glycolysis is switched off and the pentose phosphate pathway is used which in turn reduces glutathione that subsequently detoxifies Reactive Oxygen Species (ROS) and maintains the antioxidant state. In an Alzheimer's neuron, APC/C-Cdh1 activity is low and APC/C substrate PFKFB3 is high. The pentose phosphate pathway is switched off and glycolysis is used which in turn oxidises glutathione and leads to a reduced antioxidant state and subsequent neuronal death. (C) APC/C-Cdh1 downregulation causes an increase in APC/C substrate glutaminase a critical precursor for synthesis of the neurotransmitter glutamate. The resulting high levels of the latter amino acid overstimulates the N-methyl-D-aspartate (NMDA) receptor, which subsequently leads to an increase in Ca2+ thus causing excitotoxicity and neuronal death.

APC/C association with the ectopic cycle in AD

Differentiated cells including neurons which are post-mitotic cells, remain in the G0 phase in a quiescence state, due to an active downregulation of cell cycle-related proteins. In neurons, the cell cycle protein Cyclin B1 and the glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 are actively downregulated by the APC/C-Cdh1 (Almeida 2012). This downregulation is fundamental in averting the erroneous re-entry of post-mitotic neurons into the G1 and S phase of the cell cycle and for preserving neurons reduced antioxidant status. However, it has been exemplified that in response to CNS insults, neurons can retain the ability to re-enter the cell cycle under pathological conditions (Almeida 2012). Moreover, it has been depicted that APC/C activity is not only crucial for regulating cell cycle progression in proliferating cells but also required for neuronal survival. APC/C-Cdh1 is active in postmitotic neurons, regulating axonal growth and patterning in the developing brain (Konishi *et al*. 2004).

The mounting evidence illustrating the accumulation of Cyclin B1 in neurons of brains from individuals suffering AD, demonstrates this APC/C substrate plays an important role in AD pathological hallmarks of neuronal loss and apoptosis. Indeed, Mosch and collaborators have reported an elevated content of DNA and Cyclin B1 in AD neurons, indicating reactivation of their cycle and progression towards the S phase (Mosch *et al*. 2007). It has been shown that the APC/C activator Cdh1 was predominantly expressed *in vivo* throughout terminal differentiation of cortical neurons. In contrast, expression of the APC/C activator Cdc20 was severely decreased during neuron differentiation, strongly indicating that Cdh1 is the sole APC/C activator active in postmitotic, terminally differentiated neurons (Almeida *et al.* 2005). In cortical neurons where Cdh1 was depleted, Cyclin B1 accumulation and subsequent neuronal degeneration ensued, strongly indicating that the increased Cyclin B1 levels are implicated in apoptotic neuronal death (Almeida *et al.* 2005). Furthermore, Cyclin B1 depletion in Cdh1-depleted cortical neurons significantly increased cell survival, signifying Cdh1 promotes active Cyclin B1 degradation to facilitate postmitotic neuron survival,

impeding neurons from entering an erroneous S phase with subsequent apoptotic neuron degeneration.

Collectively these findings depict APC/C activator Cdh1 is an essential survival protein for postmitotic neurons and its inactivation and deregulation stipulate a potential mechanism for the erroneous reactivation of Cyclin B1 that has been reported to precede ectopic cell cycle and subsequent neuronal death in AD (Almeida *et al.* 2005).

Under excitotoxic conditions *N*-methyl-D-aspartate receptors (NMDARs) overactivation occurs in AD and stimulates Cyclin-dependent kinase 5 (Cdk5) activation, which in turn induces triple phosphorylation of Cdh1 residues Ser-40, Thr-121 and Ser-163 with subsequent nuclear export of Cdh1 and APC/C-Cdh1 inactivation. Consequently, nuclear localisation and accumulation of Cyclin B1 in cortical neurons ensues, with successive induction of neuronal apoptotic death (Maestre *et al.* 2008). Intriguingly, NMDAR residue Ser-1232 is phosphorylated by Cdk5 mediating NMDAR synaptic transmission (Li *et al.* 2001). It is possible that overactivation of NMDAR, which arises in prolonged release of neurotransmitter glutamate that occurs in AD, potentially instigates a Cdk5-NMDAR activation feedback loop that contributes to neurodegeneration response propagation. If so, inactivation of APC/C-Cdh1 stimulated by Cdk5 with subsequent elevated Cyclin B1 levels potentially provides a novel pathway in neurodegeneration ensuing NMDAR stimulation (Maestre *et al.* 2008). Furthermore, endogenous glutamatergic stimulation of rat cortical primary neurons induces Cdk5-dependent-mediated phosphorylation of Cdh1 with subsequent Cdh1 accumulation in the cytoplasm and disassociation from the APC3 core protein with consequential APC/C-Cdh1 inactivation (Veas-Pérez de Tudela *et al.* 2015). This inactivation of the APC/C-Cdh1 causes depletion of $p27^{Kip1}$ and switches on a Cyclin D1-Cyclin-dependent kinase 4 (Cdk4)-retinoblastoma protein (pRb) pathway inducing an erroneous S-phase entry of post-mitotic neurons and subsequent neuronal death. These findings indicate APC/C-Cdh1 actively suppresses ectopic cell cycle and neuronal death, emphasising its fundamental neuroprotection function (Veas-Pérez de Tudela *et al*. 2015).

In addition, it has been exemplified that localisation of Cdk5 not its total amount is a key

determinant in neuron postmitotic state. This is because when primary neurons were stimulated to re-enter the cell cycle a loss of nuclear Cdk5 was observed. This feature is found in neurons from individuals suffering AD and AD mouse models with Cdk5 redistributed to the cytoplasm (Zhang *et al.* 2008). Consequently, cell cycle suppression is alleviated with subsequent erroneous and lethal cell cycle proceeding, potentially resulting in further neuronal stress (Zhang *et al.* 2008). A similar principle applies during normal cell cycle. When a neuron enters S-phase Cdk5 is re-localised to the cytoplasm, where it is unstable and ubiquitinated by APC/C-Cdh1 with subsequent degradation by the proteasome. The Cdk5 ubiquitination site seems to overlap with the binding region implicated in Cdk5 physical interaction with the protein p35. When p35 levels are elevated Cdk5 ubiquitination is impaired, resulting in its attenuated degradation by the proteasome (Zhang *et al.* 2012). It is postulated that an unsuccessful normal clearance pathway would exacerbate elevated Cdk5 kinase activity outcome, and in conjunction with alleviated cell cycle suppression, indicates that Cdk5 participates in ectopic cell cycle of AD through numerous mechanisms. The timing of Cdk5 ubiquitination in early S-phase is out of phase with APC/C-Cdh1 activity, which is ordinarily inactivated prior to entry into S-phase. This signifies a previously unidentified APC/C-Cdh1 activity in G1/S phase. It is possible that APC/C-Cdh1 subunits change and consequently alters substrate specificity favouring Cdk5 over other APC/C substrates. Alternatively, APC/C is primarily located in the cytoplasm in neurons, however its localisation may be altered (Zhang *et al*. 2012).

Collectively these findings provide strong indications that inactivation and downregulation of APC/C-Cdh1 is implicated in the AD ectopic cell cycle. Further findings have corroborated a role of APC/C-Cdh1 in S-phase entry. Ubiquitin ligase complex SCF regulates S-phase entry by inducing Cdk inhibitors $p21^{Cip1}$ and $p27^{Kip1}$ degradation. In G1 phase, SCF complex subunits Skp2 and Cks1 are unstable and their degradation is facilitated by APC/C-Cdh1. It has been exemplified that APC/C-Cdh1 silencing in HeLa cells at G1 phase stabilises the SCF complex with subsequent increased $p21^{Cip1}$ and $p27^{Kip1}$ degradation and an abundance of cells at S-phase. Collectively, it is exemplified in more ways than one, that APC/C-Cdh1

inactivation potentially causes erroneous re-entry at S-phase (Aulia and Tang 2006).

APC/C involvement in oxidative stress in AD

There is mounting evidence illustrating that during AD the brain tissue is subjected to oxidative stress (protein, lipid and DNA oxidation and glycoxidation), which is denoted by an imbalance in homeostasis due to the escalated radical production of reactive oxygen species (ROS) or reduced antioxidative defence. (Gella and Durany 2009; Huang *et al.* 2016). A significant attribute of the antioxidant defence mechanism is the low molecular weight reducing equivalent glutathione (GSH), a small molecule that is accountable for the maintenance of an appropriate redox potential in the cell. Glutathione most significant function is the donation of electrons to ROS with the purpose to scavenge the ROS.

The concentration of intracellular GSH declines in aged mammalian brain regions including the hippocampus (Sasaki *et al.* 2001; Sandhu and Kaur 2002; Calabrese *et al.* 2004; Zhu *et al.* 2006), consequently creating a state in which ROS production rate surpasses the capability of the antioxidant defence mechanism, inducing a condition that favours oxidative stress defence (Gella and Durany 2009). Furthermore, ROS can react with proteins, lipids, DNA and other molecules potentially modifying their structure and function and subsequently affects organs and tissues. Importantly, the brain is particularly susceptible to ROS due to its constitution of easily oxidisable lipids and activity, which results in a high oxygen consumption rate (Huang *et al.* 2016).

Oxidative stress partakes in the induction of Aβ deposition, hyperphosphorylation of the protein tau and consequently synaptic and neuronal loss, indicating that oxidative stress is a critical contributor to AD pathophysiology (Gella and Durany 2009). The damage caused by ROS mediates alterations in numerous signalling pathways in neurons that are associated with oxidative stress and neurodegeneration. Among these, APC/C-Cdh1 downregulation stimulates an alteration in the oxidant and antioxidant homeostasis of neurons (Fuchsberger *et al.* 2017). In comparison to other brain cells including astrocytes, neurons have the highest oxygen consumption rate. However, the glycolysis rate, the metabolic process

accountable for producing the majority of energy needs in human cells, is relatively low (Almeida *et al.* 2004). Glucose-derived glucose-6-phosphate is the crucial metabolite linking glycolysis to the pentose-phosphate pathway (PPP), an alternative pathway to glycolysis for glucose oxidation. Utilising the PPP, neurons metabolise glucose. PPP is the key metabolic pathway accountable for regenerating Nicotinamide adenine dinucleotide phosphate (NADPH), a vital reducing cofactor for many oxidoreductases (Almeida 2012). A central PPP regulatory enzyme is glucose-6-phosphate dehydrogenase (G6PD) that promptly reacts to the surge in demand for NADPH essential for sustaining the neurons antioxidant state. This antioxidant state is crucial in preserving glutathione in its reduced form (GSH), which consequentially is critical for detoxification of ROS. The PPP supplies the majority of reducing equivalents in the form of NADPH, which are required for reduction of glutathione disulfide (GSSG) to GSH, a reaction catalysed by glutathione reductase (Wamelink *et al.* 2008). 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, isoform 3 (PFKFB3) is a central enzyme in regulating and activating glycolysis. Its activation is fundamental for the prompt activation of glycolysis and subsequent energy production from glucose as well as protection from apoptosis. Its virtual depletion in neurons provides clues about neurons severe sensitivity to energy diminution and neurodegeneration (Almeida *et al.* 2004).

It has been depicted that PFKFB3 contains a ^{142}Lys-Glu-Asn (KEN) box motif that is continually targeted for ubiquitination by the APC/C-Cdh1 and subsequent proteasomal degradation (Herrero-Mendez *et al.* 2009). In neurons APC/C-Cdh1 activity is high and subsequently PFKFB3 abundance is low. In contrast, astrocytes exhibit high PFKFB3 concentration and very low Cdh1 levels, with a concomitant low APC/C-Cdh1 activity (Herrero-Mendez *et al.* 2009). Moreover, APC/C-Cdh1 activity dictates the regulation of glucose consumption rate through both glycolysis and the PPP. PFKFB3 upregulation through either Cdh1 inhibition or PFKFB3 overexpression instigated glycolysis activation concurrently with a decrease in the PPP. As PPP is critical for glutathione reduction at the expense of NADPH for neuroprotection, this shift in glucose consumption towards glycolysis through the expense of a decrease in PPP, increased the oxidation of

glutathione and production of ROS, promoting a state favouring oxidative stress and subsequent neuronal death. These observations depict APC/C-Cdh1 as fundamental for regulating the glycolytic and PPP pathways to generate an antioxidant state for neuroprotection at the expense of glucose utilisation for bioenergetics purposes (Herrero-Mendez *et al.* 2009), which are met by other sources (Herrero-Mendez *et al*. 2009).

Furthermore, it has been demonstrated in cortical neurons that NMDAR activation through APC/C-Cdh1 inhibition induced stabilisation of PFKFB3, instigating a metabolic alteration of increased glycolysis activity and reduced PPP activity with subsequent oxidative stress and neuronal apoptosis (Rodriguez-Rodriguez *et al.* 2013). Furthermore, overactivation of NMDA receptor represses APC/C-Cdh1 activity through a Ca^{2+}-Cdk5-mediated signalling pathway, which causes phosphorylation of Cdh1 and the subsequent inactivation of the APC/C-Cdh1. This inactivation of the APC/C-Cdh1 complex not only stabilises PFKFB3, causing increased glycolysis and reduced PPP action, but also stimulates the accumulation of Cyclin B1, illustrating that the NMDAR-APC/C-Cdh1-PFKFB3 axis triggers oxidative stress and subsequent neuronal death by excitotoxicity as well as neuronal death by ectopic cell cycle (Zhou *et al.* 2016).

Stabilisation of PFKFB3 and accumulation of Cyclin B1 link APC/C-Cdh1 inactivation/downregulation with neuronal death through oxidative stress and ectopic cell cycle. Future studies are imperative to further comprehend and ascertain whether Cdh1 deficiency or APC/C-Cdh1 inactivation exert a pivotal regulatory role in the mechanisms that trigger neurodegeneration.

APC/C connection with excitotoxicity in AD

Excitotoxicity is characterised as consequential cell death from excitatory amino acid (EAA) toxic actions. In the mammalian CNS, the key EAA neurotransmitter is glutamate, thus ordinarily neuronal excitotoxicity alludes to neuronal injury and death ensuing prolonged exposure to synaptic release of glutamate and the successive deregulated Ca^{2+} homeostasis (Dong *et al*. 2009; Arundine and Tymianski 2003). Glutamate activates postsynaptic

ionotropic EAA receptor NMDAR, which opens its associated ion channel enabling Ca^{2+} and Na^{+} ion influx. While normal functioning of a cell requires physiological elevations of intracellular Ca^{2+}, the surplus of Ca^{2+} ions can overwhelm Ca^{2+} regulatory mechanisms and is exceptionally neurotoxic, inducing protein, membrane lipids and nuclei acid degradation, oxidative stress, altered synaptic plasticity and eventual subsequent neuronal necrosis and apoptosis (Arundine and Tymianski 2003; Dong *et al*. 2009).

Compelling evidence indicates that in AD soluble Aβ peptide oligomers provoke synaptic dysfunction and loss. This Aβ-mediated synaptic toxicity is dependent on NMDAR overstimulation which instigates increase cytoplasmic Ca^{2+} (Tu *et al*. 2014). Although the molecular mechanisms remain not fully understood, it has been alluded that Aβ induces these events by causing an aberrant increase in extrasynaptic glutamate abundance through impeding uptake of glutamate or stimulating release of glutamate from glial cells (Tu *et al.* 2014).

In a primary culture of neurons, Aβ caused Cdh1 proteasomal-dependent reduction which subsequently stabilised APC/C-Cdh1 substrate glutaminase, a critical neuronal glutamate production source. Consequently, glutamate levels increased and further intraneuronal Ca^{2+} dysregulation ensued (Fuchsberger *et al.* 2016). Furthermore, glutamate also reduced Cdh1 levels and caused glutaminase accumulation, indicating potential of positive feedback loop of Cdh1 inactivation. Inhibition of glutaminase averted glutamate excitotoxicity and subsequent apoptosis in Aβ treated neurons. In addition, *in vivo* findings utilising microinjection of either Aβ or glutamate in the CA1 region of the rat hippocampus, exemplified re-localisation of Cdh1 from the nucleus to the cytoplasm and increased glutaminase levels. Furthermore, elevated levels of glutaminase were depicted in APP/PS1 mice compared to wild-type control mice. These findings collectively indicate that elevated glutaminase and subsequent glutamate generation contribute to AD excitotoxicity (Fuchsberger *et al.* 2016).

It has been exemplified that both glutamate- and glutaminase-immunoreactive pyramidal neurons in the hippocampus and cerebral cortex illustrate morphological abnormalities with

irregular shortened and disorganised dendritic fields in AD individuals compared to age-matched controls. In conjunction with these observed degenerative changes both glutamate- and glutaminase-immunoreactive pyramidal neurons depicted NFT (Kowall and Beal 1991). Collectively, the mounting evidence of glutaminase accumulation and the finding of Aβ-induced Cdh1 reduction stabilises glutaminase, provides a probable vindication for the observations in AD.

Furthermore, under excitotoxic conditions NMDAR hyperactivation inhibits APC/C-Cdh1 function through a Ca^{2+}-Cdk5-mediated signalling pathway. This inactivation results in PFKFB3 stabilisation, increased glycolysis and reduced PPP action and Cyclin B1 accumulation. These findings of APC/C-Cdh1 inactivation/downregulation during excitotoxicity proposes a link between Ca^{2+} dynamics and glucose metabolism regulation (Zhou *et al.* 2016). The intracellular Ca^{2+} elevation results in mitochondrial Ca^{2+} overload accountable for production of ROS and release of cytochrome c, both critically contributing to glutamate-induced excitotoxicity (Luetjens *et al.* 2000). Oxidative stress related to excitotoxicity also causes mitochondrial fragmentation and this imbalance in mitochondrial dynamics can induce overactivation of NMDAR, further promoting excitotoxicity (Nguyen *et al*. 2011). Thus, it can be depicted that a metabolic imbalance ensuing activation of NMDAR is significantly implicated in excitotoxicity (Rodriguez-Rodriguez *et al.* 2013).

It is evident that all these intracellular events converge at the ubiquitous pathways of necrosis and apoptosis. While oxidative stress has been associated with glutamate and Aβ-mediated neurotoxicity, ectopic cell cycle and perturbations in systems involving glutamate are potentially implicated in chronic AD neurodegeneration pathogenesis. Aβ-mediated neurotoxicity and neurodegeneration have been related with NMDAR overactivation, with subsequent excitotoxicity ensuing that potentially augments the localised neuronal susceptibility in a manner consistent with the pathogenesis of AD (Dong *et al*. 2009). Furthermore, Aβ induces surplus ROS production, dependent on NMDAR activation (De Felice *et al.* 2007). This indicates that NMDAR activity dysregulation and oxidative stress potentially devise dual deleterious roles in AD (Dong *et al*. 2009). Furthermore, the evidence

of Aβ-induced APC/C-Cdh1 degradation and subsequent glutaminase accumulation is postulated as a further molecular link between the AD pathogenic factor Aβ and slow excitotoxicity in which APC/C-Cdh1 inactivation/downregulation appears to be the crucial intermediate mechanism.

APC/C contribution to Long-Term Potentiation impairment in AD

Long-term potentiation (LTP) is a form of activity-dependent plasticity that triggers continual synaptic transmission augmentation (Bliss and Cooke 2011). APC/C-Cdh1 has been implicated in cortical plasticity, learning and memory (Li *et al.* 2008). A study analysed *cdh1*-knockout mice, in which a gene-trap construct was inserted into intron 5 of *cdh1*. Cdh1 protein was absent in homozygous *cdh1*-knockout mice and an approximately 50% decrease was observed in heterozygous *cdh1*-knockout mice (Li *et al.* 2008). In homozygous *cdh1*-knockout mice, the absence of Cdh1 caused early lethality. In heterozygous *cdh1*-knockout mice compared to wild-type, no gross alteration in morphology of hippocampus or other brain regions was observed, however impairment in the late-phase LTP induced by multiple spaced trains of high-frequency stimulation was demonstrated. Furthermore, in hippocampal slices from heterozygous *cdh1*-knockout mice late-phase LTP declined back to baseline levels within 3 hours after the final train of high-frequency stimulation compared to the robust late-phase LTP observed in wild-type mice. These results propose that APC/C-Cdh1 potentially plays a vital role in late-phase LTP induction likely through mediating the ubiquitination and subsequent degradation of unidentified proteins that negatively regulate late-phase LTP, corroborating the concept that both protein synthesis and degradation are crucial for the long-lasting form of synaptic plasticity expression and maintenance (Li *et al.* 2008; Fonseca *et al.* 2006).

In addition, it has been shown that Cdh1 is essential for amygdala-dependent memory. In *cdh1* conditional knockout (cKO) mice contextual and cued fear memory, which both require a functioning amygdala, were defective. Furthermore late-phase LTP impairment was observed in amygdala slices (Pick *et al.* 2012). In conjunction with these observations, in

amygdala *cdh1* cKO mice a Cdh1 expression reduction was noted. In addition, an elevated expression of the synaptic scaffolding protein SH3 and multiple ankyrin repeat domains protein (Shank), and a NMDAR subunit, NR2A (both of which are postsynaptic density (PSD) proteins), ensued LTP-inducing stimulation in *cdh1* cKO mice amygdala slices. These findings indicate and are consistent with the concept that ordinarily APC/C-Cdh1 is implicated in amygdala synaptic plasticity and memory and regulating in the PSD the appropriate protein complexes following neuronal activity (Pick *et al.* 2012).

Collectively, these findings propose that either at various development stages or in different cell types, Cdh1 has distinctive responsibilities in different regions of the brain. It is probable that Cdh1 activity divergent effects in the hippocampus and amygdala are attributable to the various Cdh1 substrates diverse expression in these brain regions (Pick *et al.* 2012).

Furthermore, heterozygous *cdh1*-knockout mice compared to wild-type mice performed poorly in the hippocampus-dependent process of contextual fear conditioning, suggesting *cdh1* haploinsufficiency instigates hippocampus-dependent memory impairments and that the ubiquitination and subsequent degradation of APC/C protein substrates mediated by APC/C-Cdh1 is vital in learning and memory (Li *et al.* 2008). In addition, it has been demonstrated in a Morris water maze test that cKO of APC/C subunit APC2 in excitatory adult forebrain neurons in mice decreases capability in spatial memory formation and abrogates the capability of extinguishing contextual fear conditioning, indicating a hippocampal functional defect (Kuczera *et al.* 2010).

The stability of the dendrite destabiliser Rho protein kinase 2 (Rock2) is regulated by APC/C-Cdh1. In *cdh1* cKO mice, loss of APC/C-Cdh1 function in adult neurons causes Rock2 accumulation and increased activity with subsequent destabilisation of cortex and hippocampus dendrites, in conjunction with cognitive impairment and neurodegeneration in mice. Elimination of these pathological events ensued Rock2 inhibition. These findings indicate APC/C-Cdh1-mediated degradation of Rock2 regulates the integrity of the dendritic structural and functional network depicting Cdh1 as a crucial molecular factor in AD pathogenesis (Bobo-Jiménez *et al*. 2017).

Although these findings indicate that the APC/C potentially participates in hippocampus-dependent memory and learning, its implication in synapse function and plasticity remains to be fully investigated. In particular, the underlying molecular mechanisms of APC/C contributions in learning and memory remain elusive (Puram and Bonni 2011).

APC/C implications in neurogenesis impairment in AD

The two hallmark features of neurogenesis are proliferation and differentiation. Although causal links have not been ascertained, a variety of critical molecules implicated in the pathogenesis of AD seem to exert positive or negative regulation of new neurons generation (Mu and Gage 2011).

Studies conducted in Cdh1 knockout mouse model, have depicted that Cdh1 elimination shortens G1 phase duration and extends S-phase duration in Neural progenitor cells (NPCs), significantly delaying NPCs cell cycle exit, which promotes replicative stress that consequently initiates NPCs p53-mediated apoptotic death with an overall reduction in newly differentiated cells (Delgado-Esteban *et al.* 2013). In addition, *in vitro* investigations into neuronal differentiation suggested the rapid degradation of APC/C-Cdh1 substrate Skp2 with subsequent stabilisation of Cdk inhibitor $p27^{Kip1}$ that ultimately leads to cell cycle exit. Both differentiation of a rat pheochromocytoma cell line PC12 and human neuroblastoma cell line SH-SY5Y to neuronal phenotypes required elimination of Skp2 and stabilisation of $p27^{Kip1}$. This proposes that at least for cell differentiation *in vitro*, the APC/C-Cdh1-Skp2- $p27^{Kip1}$ axis define a conserved signalling pathway (Delgado-Esteban *et al*. 2013). Furthermore, Cdh1 phosphorylation status is crucial for neuronal differentiation and survival *in vitro*. In culture NPCs, phosphorylated and non-phosphorylated Cdh1 delayed or accelerated cell cycle exit and neurite extension, respectively. As non-phosphorylated Cdh1 does not impede APC/C-Cdh1 activity, APC/C substrate Cyclin B1 degradation is promoted, with subsequent Cdk1 activity inhibition supporting the neuronal phenotype. Besides the negative effect on the progress of neuronal differentiation, phosphorylated Cdh1 also causes NPCs apoptotic death (Delgado-Esteban *et al*. 2013).

Collectively, these findings indicate that functional APC/C-Cdh1 activity is a necessity for both neurogenesis *in vivo* and cortical neuronal differentiation *in vitro*, coupling the NPCs cell exit with neuronal differentiation (Delgado-Esteban *et al*. 2013).

Furthermore, utilising mouse cerebellar granule cell progenitors (GCPs) as a model of brain neurogenesis, the role of casein kinase 1 delta (CK1δ) in the CNS progenitor cell expansion was exemplified. Conditional loss of CK1δ in GCPS or small interfering RNA (siRNA) knockdown of CK1δ in wild-type GCPs demonstrated diminished GCPs proliferation. In addition, administration of a CK1δ specific inhibitor also illustrated GCPs proliferation reduction *in vitro* and *ex vivo*. In contrast overexpression of CK1δ resulted in increased proliferation of GCPs (Penas *et al.* 2015).

Moreover, CK1δ is an APC/C-Cdh1 substrate and conditional Cdh1 deletion in GCPS caused elevated CK1δ levels. CK1δ is the sole isoform of Casein kinase 1 (CK1) that is targeted by APC/C-Cdh1 for degradation in the developing cerebellum. The mechanism in which APC/C-Cdh1 acquires specificity for CK1δ with regards to GCPs proliferation remains elusive. CK1δ is the only CK1 isoform that comprises a N-terminal D-box motif, all other CK1 isoforms contain D-boxes that could potentially facilitate turnover by the APC/C-Cdh1, however would necessitate upstream signalling pathway activation for recognition by APC/C-Cdh1. Further investigations ascertaining GCP-specific interactors or substrates should help to elucidate the mechanism in which APC/C-Cdh1 regulates CK1δ levels during cell proliferation (Penas *et al.* 2015).

Collectively, these findings indicate that CK1δ controls neurogenesis of GCPs and that APC/C-Cdh1 mediated degradation of CK1δ regulates GCPs proliferation (Penas *et al.* 2015). Cdh1 is essential for appropriate neurogenesis, which consequently has crucial implications in the regulation of mammalian brain size, thus establishing Cdh1 as an important player in the molecular pathogenesis of neurological disorders (Delgado-Esteban *et al.* 2013).

6. Manipulation of the APC/C by Viruses

APC/C manipulation by viruses can severely affect cell cycle control and other physiological attributes of animal cells including the human. These qualities in conjunction with many viruses during infection controlling the host cell cycle including oncoviruses, denote the APC/C as an attractive viral target for establishing an intracellular environment favouring viral replication (Mo *et al.* 2012). There is mounting evidence that numerous viruses target the APC/C. Two excellent reviews examining how viruses control the host cell cycle through manipulating the APC/C listed 7 viruses and identified several viral proteins that target the APC/C (summarised in **Table 2 and Figure 4**) (Mo *et al.* 2012; Fehr and Yu 2013). As discussed below, viruses utilise a diverse collection of molecular mechanisms, accentuating the capability of modulating APC/C function through convergent evolution. Importantly, such manipulation retains the mutual trait of enabling viruses to maintain their replication (Fehr and Yu 2013).

Table 2. Proposed mechanisms of APC/C manipulation by viruses.

Virus	Viral protein expressed	Mode of APC/C manipulation	Postulated mechanism of APC/C manipulation	Oncovirus	Reference(s)
Human T cell lymphotropic virus type 1 (HTLV-1)	Tax	Direct	Directly interacts with APC/C-Cdh1 and APC/C-Cdc20. Mechanism of action remains elusive although altering APC/C phosphorylation state is a probable cause.	Yes	Liu *et al*. 2004; Liu *et al.* 2003
Hepatitis B virus (HBV)	X	Direct	Binds BubR1 potentially disrupting MCC inhibition towards APC/C-Cdc20 by promoting Cdc20 association and premature activation of APC/C. Binds DDB1, however mechanism of action remains elusive. Impairs APC/C-Cdh1 proteolytic machinery.	Yes	Kim *et al*. 2008; Martin-Lluesma *et al.* 2008; Pandey and Kumar 2012).
Orf virus (ORFV)	PACR	Direct	Imitates APC11 interacting with APC2 and appears to be integrated into APC/C in place of APC11. Lacks ubiquitin ligase activity potentially inhibiting E2-E3 interaction crucial for APC/C function. May cause redirection of APC/C activity rather than simple inhibition.	No	Mo *et al.* 2009; Mo *et al.* 2010
Human papillomavirus	E2	Direct	Directly interacts with Cdh1 and Cdc20.	Yes	Bellanger *et al.* 2005;

(HPV)			Instigates accumulation of Cyclin B and induces redistribution of Cdh1 to insoluble cytoplasmic aggregates, inhibiting APC/C by refusing it access to Cdh1. Binds BubR1 and Mad2. As stabilises Cyclin B implies potential MAD2/BUBR1/Cdc20/E2 complex formation, preventing SAC inactivation and MCC disassembly subsequently inhibiting APC/C.		Tan *et al*. 2015
HPV	E6/E7	Direct	Elevated Cdc20 and UbcH10 levels cause impulsive APC/C activation and mitotic exit. E7 causes transcriptional upregulation and stabilisation of APC/C inhibitor Emi1. Emi1 upregulation may inhibit APC/C substrates degradation and cause prometaphase delay.	Yes	Patel and McCance 2010; Yu and Munger 2013
CAV	Apoptin	Direct	Interacts with APC1 initiating APC/C disruption with APC/C subunits degradation and APC/C substrate stabilisation. Interaction with APC1 combined with increased Cyclin B1 causes APC/C inactivation sufficient for apoptosis induction. Relocalises MDC1 and APC3 to PML bodies with subsequent degradation and consequential APC/C	No	Teodoro 2004; Coster *et al.* 2007; Kucharski *et al.* 2011 Mo *et al.* 2012; Lee *et al.* 2009; Wiebusch and Hagemeier 2010

			disassociation and inhibition. APC/C and MDC1 disruption during infection possibly impedes APC/C-Cdh1 which if active would perturb viral replication.		
Ad	E1A	Direct	APC5/APC7 and E1A compete with one another in binding CBP/p300. E1A regulates APC/C-CBP/p300 complexes ability by disrupting the interaction consequently impeding their functions. Inhibition of CBP/p300–APC/C-dependent transcriptional activation of genes involved in G1 arrest may ensue and utilisation of CBP/p300 complexes in absence of APC/C promotes S-phase entry and cellular DNA synthesis. How E1A affects APC/C activity remains elusive.	No	Turnell *et al.* 2005; Mo *et al.* 2012
Ad	E4orf4	Direct	E4orf4 binds PP2A. E4orf4-PP2A complex directly interacts with APC/C altering APC/C activity. Both APC/C-Cdc20 inhibition and activation could involve different potential PP2A targets within APC/C and within its modulators. Binding site and mechanism of action remains elusive. E4orf4 and its effectors Ynd1p and Cdc55 potentially functionally interact with Cdc20 and	No	Mui *et al.* 2010; Kornitzer *et al.* 2001; Kleinberger 2015; Maoz *et al.* 2005

			Cdh1.		
HCMV	pUL97	Direct	Induces phosphorylation of Cdh1 impeding Cdh1 interacting with APC/C and APC/C-Cdh1 activity.	Potential association	Tran *et al.* 2010; Wiebusch *et al.* 2005
HCMV	pUL21a	Direct	Interacting partners identified as APC3, APC7 and APC8. Essential and sufficient for inducing APC4 and APC5 degradation with subsequent APC/C disassembly. Which APC/C subunit pUL21a directly binds and precise mechanism inducing APC4 and APC5 degradation remains elusive. Possibly binds neighbouring subunit of APC4 and APC5 or recruits protein degradation enzyme targeting subunits for degradation.	Potential association	Fehr *et al.* 2012
SV40	LT	Indirect	Interacts with Bub1 causing a compromised SAC potentially by disrupting MCC integrity which instigates APC/C deregulation.	Yes	Cotsiki *et al.* 2004; Hein *et al.* 2008
HCV	NS5A	Indirect	Cellular factors targeted and mechanism of action remains elusive. Decreased degradation of APC/C substrates Cyclin B and Securin denote elevated Cyclin B and Securin levels perturb the normal timing of the mitotic cell cycle.	Yes	Baek *et al.* 2006

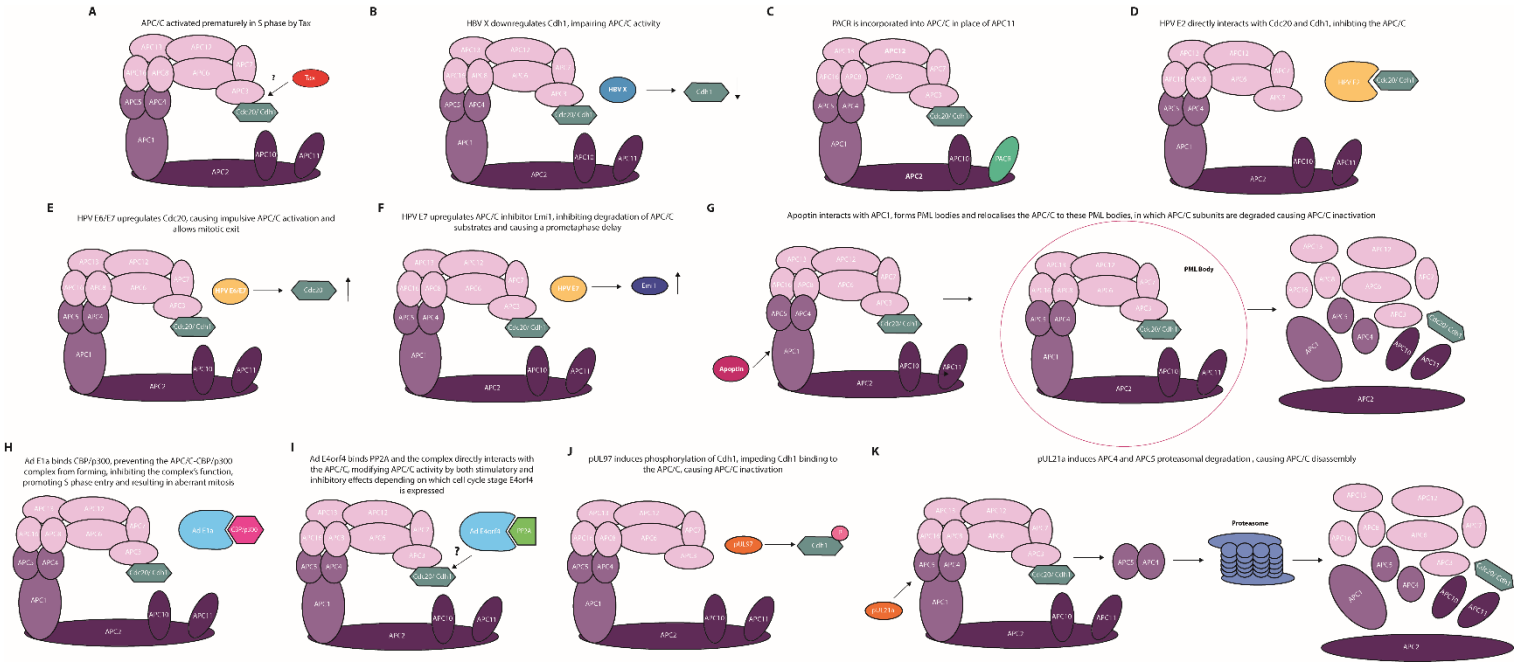

Figure 4. Proposed mechanisms of human APC/C manipulation by viruses. (A) Human T cell lymphotropic virus type 1 (HTLV-1) Tax interacts with APC/C-Cdh1 and APC/C-Cdc20, prematurely activating the APC/C during S phase. Despite these advances the mechanism of action remains elusive. (B) Hepatitis B virus (HBV) HBVX downregulates Cdh1, impairing APC/C-Cdh1 proteolytic machinery and correlates with an unscheduled entry into S phase. (C) Orf virus (ORFV) poxviral anaphase promoting complex/cyclosome regulator (PACR) inhibits the APC/C by acting as a non-functional mimic of APC11 and is incorporated into the APC/C in place of APC11. (D) Human papillomavirus (HPV) E2 directly interacts with Cdh1 and Cdc20 and redistributes Cdh1 to insoluble cytoplasmic aggregates, inhibiting the APC/C through denying the complex access to its coactivator. (E) Human papillomavirus (HPV) E6 and E7 causes elevated Cdc20 levels resulting in impulsive APC/C activation. (F) Human papillomavirus (HPV) E7 upregulates APC/C inhibitor Emi1, impeding APC/C function and subsequently APC/C substrate degradation, resulting in a prometaphase delay. (G) Chicken Anemia Virus (CAV) Apoptin interacts with APC1 and relocalises the APC/C to promyelocytic leukaemia (PML) bodies causing degradation of APC/C subunits and

subsequent APC/C inactivation. (H) Adenovirus (Ad) E1A competes with APC5/APC7 in binding CBP/p300, therefore disrupts the formation of the APC/C-CBP/p300 complex and consequently impedes the complex's functions promoting S-phase entry and aberrant mitoses. (I) Adenovirus (Ad) E4orf4 can have both stimulatory and inhibitory effects on the APC/C in a PP2A-dependent manner, however the binding site and mechanism of action remains elusive. (J) Human cytomegalovirus (HCMV) pUL97 induces phosphorylation of Cdh1, impeding Cdh1 interacting with the APC/C resulting in APC/C inactivation. (K) Human cytomegalovirus (HCMV) pUL21a induces APC4 and APC5 proteasomal degradation, resulting in APC/C disassembly and subsequent APC/C inactivation.

Human T-cell lymphotropic virus type 1 Tax:

The retrovirus Human T-cell lymphotropic virus type 1 (HTLV-1), is the etiological agent of the aggressive leukaemia adult T-cell leukaemia/lymphoma (ATL) and neurological disorder HTLV-1-associated myelopathy/tropical spastic paraparesis. The viral transactivator/oncoprotein HTLV-1 Tax, facilitates potent viral transcription activation and targets mechanisms of cell control including cell cycle progression to sustain viral replication and stimulate infected T-cell proliferation (Boxus and Willems 2009).

Tax-expressing cells manipulate the APC/C through provoking a delay in cell cycle phases S/G2/M by directly interacting and prematurely activating APC/C-Cdc20 before mitosis onset. This correlates with decreased levels of APC/C substrates Cyclin A, Cyclin B1, Securin and Skp2 (Liu *et al*. 2004; Liu *et al.* 2003). Tax directly interacts with APC/C-Cdh1 and APC/C-Cdc20 complexes, denoting Tax likely binds a core APC/C subunit, however the action mechanism remains elusive, although altering APC/C phosphorylation state is a probable cause (Liu *et al*. 2004). More recently an inverse correlation between expression of Tax and numerous Cdc20 substrates abundance including Cyclin B and Bim has been exemplified (Wan *et al.* 2014). Defective mitosis induced by Tax resulting in chromosome instability and cytokinesis failure that leads to severe aneuploidy as reflected in pathological

findings of micro- and multinucleated cells, postulates a molecular explanation for recurrent defective chromosomes in infected T-cells and highly aneuploid attribute of ATL cells (Liu *et al.* 2003; Liang *et al*. 2002). Collectively, it is depicted that premature APC/C activation by Tax, has a probable significant contribution in ATL development.

Hepatitis B Virus HBV X

Hepatitis B Virus (HBV) is a primary etiological factor for hepatocellular carcinoma (HCC) development. It has been conveyed that HBV X protein binds to BubR1. Both the HBV X binding domain and Cdc20 binding domain of BubR1 overlap, thus HBV X binding to BubR1 potentially disrupts BubR1 capability of binding Cdc20 and its inhibitory activity towards the APC/C-Cdc20 complex. Consequently, Cdc20 is released from the MCC and can prematurely activate the APC/C before spindle pole alignment, thus potential chromosome missegregation can ensue. This provides a prospective insight into viral pathogen-induced defective mitosis (Kim *et al*. 2008). However, further investigations have not been able to confirm HBV X disrupts the SAC and MCC but instead it has been depicted that HBV X binds the E3 ubiquitin ligase damaged DNA binding protein 1 (DDB1) and this potentially contributes to the induction of chromosome instability. The molecular mechanisms and effects mediated from this interaction remains elusive (Martin-Lluesma *et al.* 2008).

More recently it has been depicted HBV X impairs APC/C-Cdh1 proteolytic machinery (Pandey and Kumar 2012). Cell division cycle 6 (Cdc6) is a key component in DNA replication. During cell cycle progression Cdc6 is tightly controlled and a target of APC/C-Cdh1 for proteolysis in quiescence and G1 phase restricting cell proliferation and preventing replication reinitiation (Petersen *et al*. 2000). It has been demonstrated that in the presence of HBV X, Cdh1 is down-regulated with elevated Cdc6 levels ensuing, accentuating the importance of APC/C-Cdh1 regulation in cell cycle control and the establishment of this system in HBV X-induced oncogenic phenotype (Pandey and Kumar 2012). This impairment of APC/C-Cdh1-dependent protein degradation pathway instigating intracellular stability of Cdc6 correlates with an unscheduled entry of cells into S-phase (Pandey and Kumar 2012).

Furthermore, HBV X down-regulating APC/C-Cdh1 results in the intracellular stability of deubiquitinase Ubiquitin specific processing protease 37 (USP37) (Saxena and Kumar 2014). In the presence of HBV X, Cyclin A levels throughout the cell cycle are stabilised and maintained, this upregulation in Cyclin A/Cdk2 activity and downregulation of Cdh1 under the HBV X microenvironment potentially creates an environment favouring USP37 upregulation (Saxena and Kumar 2014). Further investigation is imperative for ascertaining to what extent HBV X regulation of the APC/C plays a role in HBV-mediated tumorigenesis.

Orf Virus PACR:

Orf virus (ORFV) is a large double-stranded DNA parapoxvirus that causes an exanthemous disease primarily in sheep and goats and infects differentiated epidermal cells in humans. It has been ascertained that the ORFV gene ORFV014 encodes for a RING-H2 protein called poxviral anaphase promoting complex/cyclosome regulator (PACR) with substantial sequence homology to APC/C core subunit APC11 (Fleming *et al*. 2015; Mo *et al*. 2009).

PACR only exists in the parapoxvirus family. PACR expression causes dysregulated cell cycle with fewer cells in G1 phase, more in S, and an accumulation of cells in G2/M as well as APC/C substrate accumulation collectively in a manner consistent with defective APC/C function (Mo *et al*. 2009). PACR imitates APC11 in its capability via its N-terminal to interact directly with APC/C core subunit APC2 and appears to be integrated into the APC/C in place of APC11 (Mo *et al.* 2009; Mo *et al.* 2010). However, the catalytic residues present in E3 ligases are absent in PACR, resulting in a non-functional enzymatic domain in PACR that lacks ubiquitin ligase activity. This feature correlates with sequence differences in the region between the 6th and 8th Cys/His as evidenced from an APC11 and PACR enzymatic domain swap that converses their ubiquitin ligase activity (Mo *et al.* 2009). The lacking ubiquitin ligase activity correlates with the absence of residues fundamental for E2-E3 interaction, consequently PACR may inhibit the E2-E3 interaction. This in turn suggests that the integration of PACR into the APC/C in place of APC11 impedes ubiquitin-charged conjugating enzyme E2 recruitment, which is crucial for APC/C function (Mo *et al*. 2009).

Overexpression of PACR demonstrates G2/Mitosis (M) phase arrest with an increase in APC/C substrates, thus confirming that PACR impedes the APC/C by imitating a non-functional APC11 (Mo *et al*. 2009).

It has been demonstrated that ORFV infection may stimulate differentiated epidermal skin cells to enter a S-phase-like state providing cellular factors to sustain viral replication, a consequence of APC/C function disrupted by PACR (Mo *et al.* 2009). ORFV does not encode its own thymidine kinase (TK) and ribonucleotide reductase (RR) (Mo *et al.* 2010), two enzymes vital for nucleotide metabolism providing nucleotides for DNA replication and the cellular version are APC/C substrates (Ke and Chang, 2004; Chabes *et al.* 2003). This depicts the ORFV exploits APC/C inhibition as an alternative method for acquiring specific enzymatic activity to sustain viral replication (Mo *et al.* 2009; Mo *et al.* 2010).

Interestingly, PACR appears to integrate into an otherwise intact APC/C with APC/C subunits APC2, APC3 and APC4 coprecipitating with PACR, indicating that a full complex is assembled with PACR in place of APC11 (Mo *et al.* 2010). This may result in the redirection of APC/C activity as opposed to simple inhibition. Consequently, PACR is referred to as an APC regulator rather than presuming it is an APC/C inhibitor. Whether PACR is capable of such subtle APC/C activity manipulation is the substantial focus of on-going investigations (Mo *et al.* 2010).

<u>Human Papillomavirus E2:</u>

Human papillomaviruses (HPVs) are small DNA viruses infecting human epithelial skin cells or mucosal sites specifically the cervix, stimulating hyperproliferative lesions resulting in cervical cancer. Different strains are categorised into two types; high risk comprise viruses with malignant properties and related to cervical cancer and low risk contain viruses accountable for benign growths. Viral manipulation of the host cell to endow an environment favouring replication is essential for viral genome replication and high-level amplification in differentiated, non-dividing cells that depends almost entirely on host factors.

The HPV E2 protein as a viral transcription and replication factor has well ascertained roles.

It has been depicted that E2 from high risk not low risk HPV strains directly interacts with Cdh1 and Cdc20 disrupting APC/C function, stimulating a mitotic block (a G2/M arrest) and often subsequently metaphase-specific apoptosis (Bellanger *et al.* 2005). HPV E2-expressing cells that abscond the mitotic block exemplify aberrant long metaphases and subsequently polyploidy, missegregated chromosomes and amplification of centrosomes, concurrently instigating severe genomic instability (Bellanger *et al.* 2005).

High risk HPV E2 binds Cdh1 and Cdc20 as proficiently as Emi1, a cellular APC/C inhibitor. It was depicted that HPV E2 co-localises with Cdh1 in insoluble intracellular structures, alluding that HPV E2 inhibits the APC/C by refusing it access to Cdh1 (Bellanger *et al.* 2005). Interestingly, in contrast to Emi1, in an *in vitro* degradation assay HPV E2 failed to inhibit APC/C activity, signifying that HPV E2 specific mode of action may differ to Emi1.

Furthermore, it has been ascertained that HPV E2 instigates accumulation of APC/C substrate Cyclin B and induces redistribution of Cdh1 to insoluble cytoplasmic aggregates (Bellanger *et al.* 2005). More recently, it has been depicted that in addition to Cdc20, HPV E2 binds the MCC proteins BubR1 and Mad2 and that these interactions correlate with mitotic block, DNA breaks and aneuploidy. As HPV E2 is known to stabilise APC/C substrates including Cyclin B, this implies that interaction of HPV E2 with MCC proteins potentially enables the formation of Mad2-BubR1-Cdc20-HPV E2 complex, which prevents SAC inactivation and the disassembly of the MCC at the end of metaphase, subsequently obstructing SAC inactivation, with APC/C inhibition ensuing and cells arrested in mitosis (Tan *et al*. 2015). This advocates that APC/C inhibition is one potential mechanism for HPV to stimulate tumours. However, it is yet to be ascertained whether HPV E2-induced genomic instability directly ensues APC/C inhibition or can be attributed to other functions of this multifunctional protein.

As HPV E2 is essential for viral DNA it is postulated that regulation of the APC/C may also be imperative for viral genome replication, exemplified by increased HPV E2 levels resulting in increased viral episomal copy number (Penrose and McBride 2000). The generation of a mutant E2 protein with its capability of APC/C inhibition specifically abrogated would be

useful to dissect more precisely the mechanism of APC/C manipulation by HPV and how harnessing the control of the APC/C is exploited to promote replication and tumorigenesis.

In addition, it has been demonstrated that HPV E6 and HPV E7 gene expression facilitates circumventing the G2 DNA damage and SAC despite DNA damage presence. Elevated Cyclin B, Cdc20 and UbcH10 levels were observed in HPV E6 and HPV E7 expressing cells (Patel and McCance 2010). It is plausible that elevated Cyclin B permits evading G2 DNA damage checkpoint and enables M phase entry. It is also probable that high Cdc20 and UbcH10 levels results in impulsive APC/C activation and ensuing SAC inactivation, with Cyclin B degraded allowing mitotic exit.

Furthermore, it has been demonstrated that HPV16 E7 oncoprotein manipulates the APC/C. HPV16 E7 interferes with degradation of APC/C substrates with transcriptional upregulation and stabilisation of APC/C inhibitor Emi1 in HPV16 E7-expressing mitotic cells (Yu and Munger 2013). The subsequent abnormally high levels of Emi1 may consequently inhibit degradation of APC/C substrates and cause the prometaphase delay displayed in HPV16 E7-expressing cells with Emi1 depletion partially reversing inhibition of APC/C substrate degradation. The detection of HPV16 E7 targeting Emi1, suggests APC/C inhibition potentially provides a critical role in HPV E7-induced mitotic abnormalities (Yu and Munger 2013). The correlation between genomic instability and HPV E6 and HPV E7 expression in malignant lesions, in which the HPV E2 open reading frame is disrupted, is a probable consequence of uncontrolled APC/C activation (Patel and McCance 2010; Fehr and Yu 2013). If so, HPV is the first virus revealed to supress and activate the APC/C at different disease progression stages.

Chicken Anemia Virus Apoptin:

Chicken anemia virus (CAV) is a small single-stranded virus targeting bone marrow hematopoietic cells and thymus T cell precursors, instigating severe thymocytes and erythroblastoid cells depletion in the bone marrow through apoptosis, inducing severe anemia and immunodeficiency in young chickens (Noteborn 2004; Los *et al.* 2009).

CAV expresses three proteins, one being a 14 kiloDalton (kDa) non-structural protein referred to as VP3 or Apoptin owing to its capability of selectively stimulating a severe G2/M arrest and ensuing apoptosis in tumour cells rendering this protein a desirable aspirant for novel cancer therapies (Noteborn 2004; Teodoro 2004). Apoptin selective killing correlates with its subcellular localisation; cytoplasmic and nuclear in non-transformed and transformed cells respectively (Danen-van Oorschot *et al*. 2003; Teodoro 2004).

The APC/C has been identified a nuclear Apoptin target with Apoptin interacting with APC/C subunit APC1 in transformed cells exemplified in coimmunoprecipitation (co-IP) mass spectrometry analysis and immunoblot analysis (Teodoro 2004; Kucharski *et al.* 2016). The APC/C binding domain has been mapped to Apoptin C-terminal domain and is both required and adequate for apoptosis induction. The association of Apoptin with APC1 in transformed cells initiates APC/C disruption with subsequent degradation of some APC/C subunits and APC/C substrate stabilisation, consequently instigating a G2/M phase arrest and inducing apoptosis in a p53-independent fashion (Teodoro 2004). Apoptin induces the formation of promyelocytic leukaemia (PML) nuclear bodies and relocalises the APC/C to these PML bodies in which APC/C subunits including APC3 are degraded with consequential APC/C inhibition. This APC/C inhibition is probably due to Apoptin cell-type specific localisation and activity and its capability to shuttle between the nucleus and cytoplasm (Heilman *et al.* 2006). This eludes that apoptosis is likely influenced by Apoptin capability in controlling the APC/C. This premise is supported by studies where siRNA knockdown of APC1 was carried out to stimulate G2/M arrest and apoptosis, which closely resembled the effect of Apoptin-mediated APC/C inhibition (Teodoro 2004). Further investigations are required to determine how Apoptin provokes APC/C disassociation and if this necessitates APC/C redistribution to PML bodies.

More recently, it has been depicted Human Gyrovirus-Apoptin (HGyv-Apoptin) a homolog of Chicken Anemia Virus-Apoptin (CAV-Apoptin), has a similar mechanism of action in manipulating the cell cycle as CAV-Apoptin through inhibiting APC/C function. Like CAV-Apoptin, HGyv-Apoptin induces G2/M arrest and abnormal spindle in cancer cells. HGyv-

Apoptin appears to disrupt the cell cycle most likely by inhibiting APC/C function, as HGyv-Apoptin expressing cells maintain high Cyclin B1 expression, blocking mitotic exit and cell cycle completion selectively in cancer cells, while having no effect on cell cycle progression in normal cells (Chaabane *et al.* 2017).

Furthermore, the mediator of DNA damage 1 protein (MDC1), a fundamental protein in the DNA damage response and a protein that associates with the APC/C subunit Cdc27, colocalises in PML bodies with Cdc27 when Apoptin is expressed resulting in the degradation of MDC1 (Coster *et al.* 2007; Kucharski *et al.* 2011). As APC/C-Cdh1 activity is upregulated in response to DNA damage and is essential for sustaining the subsequent G2 arrest, APC/C and MDC1 disruption during CAV infection possibly impedes APC/C-Cdh1, which if were active would perturb viral replication (Mo *et al.* 2012; Kucharski *et al.* 2011; Lee *et al.* 2009; Wiebusch and Hagemeier 2010).

Intriguingly, it has been shown that while Apoptin-null CAV is defective in DNA synthesis, an Apoptin point mutant has the capacity of viral DNA synthesis but failed to support virus particle production (Prasetyo *et al*. 2009). Thus, further investigations are required to define the potential role of APC/C regulation in CAV replication.

Adenovirus E1A:

Adenovirus (Ad) are double-stranded DNA viruses, which cause diverse clinical illnesses such as upper respiratory infections, conjunctivitis, tonsillitis, and gastroenteritis in mammalian species. Interestingly, although it is not known to have oncogenic potential in humans, human Ad (HAd) can stimulate transformation of cells in tissue culture (Fehr and Yu 2013).

The HAd early region 1A (E1A) gene is accountable for viral gene transcription activation with extensive influences on host cell gene expression and renowned for negatively regulating many cellular transformation pathways including pRB, cyclic AMP response element binding protein (CBP) and p300 (Mo *et al.* 2012; DeCaprio 2009). The discovery of E1 protein capability in binding and inhibiting pRB was the first demonstration of a direct

interaction between an oncogene and tumour suppressor (Whyte *et al*. 1988).

CBP/p300 are large lysine acetyltransferases acting as transcriptional coactivators through chromatin modification, controlling numerous transcriptional pathways including p53 and E2F (Goodman and Smolik 2000). It has been established that APC/C subunits APC5 and APC7 interact with CBP/p300 *in vivo*, correlating the APC/C with transcriptional activity of CBP/p300 (Turnell and Mymryk 2006; Turnell *et al.* 2005). The interaction between APC5/APC7 and CBP/p300 contributes significantly to their function with APC5/APC7 expression stimulating CBP/p300 activity and CBP knockdown impeding APC/C ubiquitin ligase activity (Turnell *et al.* 2005). In addition, APC5/APC7-CBP/p300 complex exemplifies ubiquitin ligase activity towards APC/C substrates and it has been demonstrated that both APC5/APC7 enhance p53-dependent transactivation of a $p21^{Cip1/Waf1}$ promoter-tethered reporter and E2F-1–DP-1-dependent transactivation through directly interacting with the respective promoters in a CBP/p300-dependent manner (Turnell *et al.* 2005). Intriguingly, APC5/APC7 contain within their primary sequences *bona fide* CBP/p300 protein-protein interaction domains illustrating homology to E1A, with APC5/APC7 and E1A competing *in vitro* with one another in binding CBP/p300. Importantly, wild-type APC5/APC7 overexpression competitively inhibited the binding of E1A to CBP/p300 and consequently impeded E1A transforming ability, while APC5/APC7 mutants did not supress E1A-induced transformation. Furthermore, APC5/APC7 knockdown enhanced the transformation ability of a transformation-defective E1A mutant unable to bind CBP/p300 (Turnell *et al.* 2005)

Collectively, these findings propose E1A regulates APC/C-CBP/p300 complexes ability by disrupting the interaction, consequently impeding their functions. Subsequently, inhibition of APC/C-CBP/p300-dependent transcriptional activation of genes involved in G1 arrest (e.g. $p21^{Cip1/Waf1}$) may ensue, and the utilisation of CBP/p300 complexes in the absence of APC/C to induce gene supporting progression into S-phase, together promotes S-phase entry and cellular DNA synthesis, with APC/C deregulation ultimately resulting in aberrant mitoses (Turnell *et al.* 2005; Mo *et al.* 2012). However, how E1A affects APC/C activity is yet to be directly examined, thus the effects of APC5/APC7 overexpression and knockdown may

be indirect. Biochemical data is fundamental in ascertaining if E1A specifically disrupts APC/C-CBP/p300 complex activity.

Adenovirus E4orf4:

Another protein HAd encodes is Early Region 4 Open Reading Frame 4 (E4orf4), which can stimulate a G2/M phase cell cycle block and acts as effective as an Apoptin in its ability to induce apoptosis of transformed cells (Noteborn 2009; Mui *et al.* 2010). These effects are dependent on E4orf4 binding to protein phosphatase 2A (PP2A). The E4orf4-PP2A complex is also associated with manipulating APC/C activation in which the complex directly interacts with APC/C to alter its activity (Mui *et al.* 2010; Kornitzer *et al.* 2001).

It has been demonstrated in *Saccharomyces cerevisiae* (*S. cerevisiae*) and in mammalian cells that E4orf4 exerts its effects principally through directly binding to the Cell Division Cycle 55 (Cdc55) regulatory subunit of PP2A. Expression of E4orf4 induces G2/M cell accumulation with elevated Clb2-Cdc28/Cdk1 activity, alluding E4orf4 disrupts cell cycle timing and regulates mitotic kinase activity (Mui *et al*. 2010). In yeast, it has been indicated the cell cycle arrest is irreversible (Kornitzer *et al*. 2001). Overexpression of Clb2 stimulates high Cdk1 kinase activity and severely reduces cell viability, denoting unregulated Clb2-Cdc28/Cdk1 activity is lethal for cells and is a potential mechanism for the Cdc55-dependent toxicity of E4orf4 (Mui *et al*. 2010). Cdk1 is known to phosphorylate APC/C subunits and participate in APC/C-Cdc20 activation (Ciosk *et al.* 1998; Cohen-Fix *et al*. 1996). Premature Dissociation of Sisters (Pds1)/Securin is a substrate of APC/C-Cdc20 that is ubiquitinated and degraded resulting in sister chromatid segregation necessary at anaphase. During S phase, Pds1 is usually stable, impeding Esp1 protease activity against Scc1. Interestingly, it has been depicted that E4orf4 provokes premature degradation of Pds1 in S-phase arrested cells and consequently seems to induce the premature release of Esp1 with subsequent inappropriate loss of Scc1. It seems plausible that reduced Pds1 and Scc1 levels are caused by the premature APC/C-Cdc20 activation that is observed in E4orf4-expressing cells, which is a probable consequence of Cdc55-dependent increase in

Clb2-Cdc28/Cdk1 activity and supports the hypothesis that increased mitotic kinase activity stimulated by E4orf4 expression is a potential crucial mechanism for inducing apoptosis. APC/C-Cdh1 activity was not altered under these conditions (Mui *et al.* 2010). Ase1 and Pds1 are substrates of APC/C-Cdh1 and APC/C-Cdc20 respectively. In contrast with studies undertaken by others indicating premature APC/C-Cdc20 activation, stabilisation of Ase1 and increased Pds1 levels were demonstrated in the presence of E4orf4, indicating the latter protein inhibits both APC/C complexes (Kornitzer *et al*. 2001). A possible explanation for both APC/C-Cdc20 inhibition and activation could involve the existence of different potential PP2A targets within the APC/C and within its modulators that are potentially targeted differentially by E4orf4-P22A complex in S-phase and mitosis. For example, one such target could be APC/C inhibitor Emi1, which is itself inhibited by Cdk1 phosphorylation. Although in S-phase Emi1 is not usually phosphorylated, a Cdc55-dependent E4orf4-induced Cdk1 activation potentially could cause phosphorylation of Emi1 with subsequent premature APC/C activation. Alternatively, during mitosis Cdc55-dependent APC/C inhibition may be more prominent, while Emi1 is degraded at mitotic entry (Kleinberger 2015).

While APC/C inhibition may account at least in part for the E4orf4-induced G2/M arrest, how premature APC/C-Cdc20 activation in S-phase assists in this process remains elusive as are the binding site and mechanism that E4orf4 utilises for regulating the APC/C (Kleinberger 2015; Kornitzer *et al*. 2001). Thus, future studies ascertaining a better understanding of the interaction between E4orf4 and the cell cycle are imperative.

A classical genetics approach identifying yeast genes that are required for E4orf4 mediated toxicity in *S. cerevisiae* revealed Ynd1p, a Golgi NTPDase, functionally interacted with Cdc55 and contributed in an additive manner to E4orf4-induced toxicity (Maoz *et al.* 2005). As full resistance to E4orf4 was illustrated in a double mutant lacking both Ynd1p and Cdc55, it appears Ynd1p and Cdc55 potentially are the only direct effectors of E4orf4. Biochemical studies revealed that Ynd1p associated with Cdc55 and this interaction was disrupted when E4orf4 was expressed. However, it is improbable that removal of Ynd1p or Cdc55 from a complex containing their common target is the only effect that instigates

E4orf4 toxicity since E4orf4 expression is not equivalent to Ynd1p or Cdc55 deletion. Instead, overexpression of one protein and deletion of the other is necessary to cause toxicity in yeast. A pathway in which a joint target for PP2A and Ynd1p may be functional has been suggested (Maoz *et al*. 2005). As previously illustrated mutants defective in APC/C activating subunits Cdc20 and Cdh1/Hct1 are supersensitive to E4orf4-induced toxicity, with Cdc20 mutant cells supersensitive to Cdc55 overexpression (Kleinberger 2015). It has also been depicted Cdh1/Hct1 mutant cells are more sensitive to Ynd1p and Cdc55 overexpression than wild-type cells and Cdc20 mutant cells are supersensitive to Ynd1p overexpression. Thus, it is postulated that E4orf4, as well as E4orf4 effectors Ynd1p and Cdc55, functionally interact with Cdc20 and Cdh1/Hct1 (Maoz *et al.* 2005).

Expression of E4orf4 in both yeast and mammalian cells modifies the activity of the APC/C, with effects both stimulatory and inhibitory, depending on which cell cycle stage the protein is expressed (Mui *et al.* 2010; Kornitzer *et al.* 2001). Furthermore, it is yet to be ascertained that regulation of the APC/C by E4orf4 occurs during Ad infection. In cultured human cancer or primary cells, E4orf4 is non-essential for viral replication; the influence E4orf4 has on Ad biology remains elusive (Miron *et al.* 2008). Thus, future studies ascertaining whether E4orf4 regulates the APC/C during infection, elucidating its significance on virus replication, and identifying E4orf4 mechanism of action are imperative.

Human Cytomegalovirus pUL97:

Human cytomegalovirus (HCMV) is a large double-stranded DNA that infects a broad range of cell types and establishes a persistent and latent infection in human hosts (Bhattacharjee *et al.* 2012). HCMV is a ubiquitous virus asymptomatically infecting the majority of the human population (Ranganathan *et al.* 2011). While immunocompetent individuals maintain a reservoir of HCMV asymptomatically, it is a frequent source of severe infectious complications in immunocompromised individuals and a leading viral cause of birth defects (Bhattacharjee *et al.* 2012; Ranganathan *et al.* 2011). Furthermore, mounting evidence proposes a possible link of HCMV with certain human cancers (Ranganathan *et al.* 2011;

(Soroceanu and Cobbs 2011). Viral infection with HCMV-encoded proteins can produce the hallmarks of cancer including genomic instability (Fortunato *et al.* 2000), inflammation (Britt 2008), angiogenesis (Caposio *et al.* 2011), cell migration (Vomaske *et al.* 2010), proliferation (Hume and Kalejta 2009), inactivation of the immune response (Powers *et al.* 2008) and apoptotic pathways (Brune 2011) and in conjunction with HCMV specifically detected at low levels in a variety of human cancers, denotes HCMV an intriguing human cancer virus candidate (Ranganathan *et al*. 2011).

Irrespective of the cell type or strain, there are three classes of gene expression, immediate-early (IE), early (E) and late (L), which are expressed in a well-co-ordinated manner and fundamental for a productive viral infection. E gene expression is dependent on IE gene expression while L gene expression ensues IE and E gene expression (Bhattacharjee *et al*. 2012). HCMV stimulates an intracellular environment favouring viral replication through expressing IE proteins including IE72 and IE86 that disable pRB-family proteins with subsequent E2F-responsive S-phase gene expression (Bain and Sinclair 2007). HCMV also expresses factors and utilises various mechanisms to regulate APC/C function and even disassemble the complex (Tran *et al.* 2010; Tran *et al.* 2007; Wiebusch *et al.* 2005). One mechanism is the activity of a viral protein kinase pUL97 during HCMV infection which induces phosphorylation of Cdh1, a modification known in uninfected cells to impede Cdh1 interacting with the APC/C (Tran *et al.* 2010; Wiebusch *et al.* 2005). It has been established that the APC/C plays a limiting role in the regulation of G0 maintenance and that HCMV rapidly inactivates the G0/G1 APC/C in early infection of quiescent cells with the untimely stabilisation of APC/C substrates consequently ensuing. This APC/C inactivation is instigated by the dissociation of its positive regulator Cdh1. This dissociation is independent of known Cdh1 inhibitors (Wiebusch *et al.* 2005). pUL97 is a viral Cdk functional orthologue, thus has the expected capacity to phosphorylate Cdh1, a natural Cdk target (Hume *et al.* 2008). Compared to wild-type virus infected cells, in pUL97 deletion virus infected cells, Cdh1 was hypophosphorylated and accumulations of APC/C substrates were delayed at early time points. However, by 24 to 36 hours post infection (time at which viral DNA

synthesis occurs) even in pUL97 deletion virus infected cells, APC/C substrates accumulated efficiently alluding that APC/C activation was still occurring (Tran *et al.* 2010). Furthermore, in pUL97 deletion virus infected cells and wild-type virus infected cells, Cdh1 and APC1 association with APC3 had severely reduced 16 hours post infection and was undetectable 24 hours post infection (Tran *et al.* 2010). This depicts that the disassembly of the APC/C occurs independently from Cdh1 phosphorylation and that HCMV must regulate the APC/C through additional mechanisms. APC/C disassembly during infection with localisation of APC/C subunits APC3, APC7, APC8 and APC10 to the cytosol, could account for the subsequent Cdh1 disassociation in pUL97 deletion virus infected cells (Tran *et al.* 2007). Retention of APC1 in the nucleus possibly denotes the necessity for this APC/C subunit in viral replication. Indeed, during mitosis different localisation patterns of APC/C subunits have been detected, implying different APC/C subunits possibly function independently of the APC/C (Acquaviva *et al.* 2004). APC/C disassembly is attributed to the proteasome-dependent degradation of APC4 and APC5 early in infection (6 to 8 hours post infection). The fact that E1 (ubiquitin-activating enzyme) inhibition prevented APC4 and APC5 loss adds support to the notion the degradation of these APC/C subunits is ubiquitin-dependent. This alludes that potentially a cellular ubiquitin ligase is implicated in ubiquitination of the subunits as targets for proteasome degradation, as there are no known HCMV-encoded ubiquitin ligases. Perhaps a viral early protein is obligatory for the degradation of these subunits (Tran *et al.* 2010). Although the primary mechanism of APC/C inactivation appears as the disassembly of the APC/C and targeted APC4 and APC5 degradation, pUL97 inducing Cdh1 phosphorylation possibly endows a small kinetic advantage in inhibiting the APC/C earlier during infection, with complete disassociation of Cdh1 from APC3 occurring before APC1 disassociates from APC3. It is possible that Cdh1 phosphorylation provides another purpose in facilitating viral replication other than mediating APC/C inactivation (Tran *et al.* 2010). However, whether APC/C inhibition by pUL97 solely contributes to HCMV capability to replicate or causes disease remains elusive.

Furthermore, HCMV does not express APC/C substrates thymidine kinase 1 (TK1), and

thymidylate kinase (TMPK) which are essential for DNA metabolism (Wiebusch *et al.* 2005). Through disrupting the APC/C, HCMV may enhance accessibility to these cellular enzymes to acquire vital Deoxyribonucleotide triphosphate (dNTPS) for facilitating its own DNA synthesis. Additionally, through targeting the APC/C, HCMV manipulates quiescent cells into a pseudo-S phase supportive of viral replication, while simultaneously impeding cellular DNA synthesis through expressing other viral factors (Qian *et al.* 2010). Future studies ascertaining these prospects and unravelling the APC substrates potentially vital for viral replication are imperative.

Human Cytomegalovirus Virus pUL21a:

Another factor HCMV encodes that can also regulate the APC/C through inducing APC/C disassembly is pUL21a. pUL21a is a 14.3kDa protein sharing no apparent homology with any known cellular or viral protein, alluding its function is likely to be unique to HCMV biology (Fehr and Yu 2009). pUL21a facilitates efficient viral DNA synthesis that is required for efficient late accumulation of IE transcripts and initiating a productive infection, illustrated by a pUL21a-null virus having a marked defect in viral DNA synthesis (Fehr and Yu 2010).

Interacting partners of pUL21a were identified in a proteomics screen as APC/C subunits APC3, APC7 and APC8, in which pUL21a interacts with the APC/C via pUL21a carboxyl terminus which contains the APC/C binding domain and residues $PR_{109–110}$ which are fundamental for this interaction (Fehr *et al.* 2012). Functional analysis signified pUL21a was essential and sufficient for inducing proteasomal degradation of APC4 and APC5, with subsequent APC/C disassembly. A pUL21a point mutant PR109-110AA ($pUL21a_{PR\text{-}AA}$) virus in which proline-arginine residues were mutated to alanine, incapable of binding to the APC/C was unable to induce degradation of APC4 and APC5, signifying APC4 and APC5 degradation is dependent on pUL21a binding to the APC/C (Fehr *et al.* 2012). pUL21a does not contain a sequence domain that would imply it is an E3 ligase and it is currently unknown which APC/C subunit pUL21a directly binds to. Consequently, the precise mechanism in which pUL21a induces APC4 and APC5 degradation remains elusive. It is plausible that

pUL21a may bind a neighbouring subunit of APC4 and APC5, resulting in APC/C disassembly and subsequent degradation of APC4 and APC5, or possibly pUL21a recruits a protein degradation enzyme such as an E3 ubiquitin ligase to target the subunits for proteasomal degradation (Fehr *et al.* 2012).

More recently, a Cyclin-binding domain (RxL) within pUL21a that confers its ability to bind and target Cyclin A for proteasomal degradation has been discovered (Caffarelli *et al.* 2013). pUL21a inhibition of APC/C would result in increased Cyclin A levels, which in turn would be detrimental to HCMV replication. Cyclin A promotes cellular replication which exhausts vital enzymes and metabolites necessary for viral replication, subsequently HCMV blocking Cyclin A is imperative.

Interestingly, pUL21a is highly unstable undergoing proteasome dependent but ubiquitin independent degradation (Fehr and Yu 2009). It is intriguing to deliberate whether pUL21a directly binds APC4 and APC5 targeting them for ubiquitin independent degradation. Thus, future studies ascertaining which APC/C subunit pUL21a directly binds to, the mechanism by which pUL21a induces degradation of APC4 and APC5 and whether the intrinsic instability of pUL21a contributes to its capability in regulating APC/C are imperative. It is intriguing that both CAV Apoptin and HCMV pUL21a target the APC/C bridge subcomplex, inducing its disassembly to regulate the APC/C. This is consistent with the bridge subcomplex essential function in sustaining stability of the APC/C, alluding different viruses have evolved a conserved strategy converging on this subcomplex as an efficient means in APC/C activity regulation (Fehr and Yu 2013).

It does not appear HCMV directly abolishes the APC/C enzymatic unit, thus ascertaining if the APC/C retains some activity or is modulated to target different substrates during infection is of great significance. A double mutant virus that carried both alanine substitution of proline-arginine residues in pUL21a and a pUL97 deletion exemplified significantly more attenuated viral replication than a pUL97 deletion virus alone, signifying disruption of both pUL21a and pUL97 are synthetically lethal to HCMV replication (Fehr *et al.* 2012). This corroborates the working model that HCMV has evolved a sophisticated strategy by

exploiting two viral factors that it encodes to support viral replication through ensuring successful disruption of the APC/C to incapacitate the APC/C restriction on virus infection. It is plausible that pUL97 and pUL21a have differential roles during infection under different conditions or cell types, serving as a substitute for one another or acting synergistically to fully exploit the virus capability to acquire entire control over the APC/C during infection (Fehr *et al.* 2012). The verity that HCMV utilises various mechanisms to disrupt the APC/C accentuates its critical role in HCMV infection. However, analysing these intertwined viral mechanisms during infection proves overall challenging, due to the existence of other factors targeting the same process (Fehr *et al.* 2012). Future studies unequivocally ascertaining the critical role of APC/C modulation in HCMV replication and mechanistic insight into how APC/C modulation influences HCMV biology are important and a pending assignment in the field.

Furthermore, the mechanism of APC/C inactivation and the relative contributions of pUL21a and pUL97 were described recently (Clark and Spector 2015). In brief, in uninfected cells only pUL21a alone was able to disrupt APC/C function with subsequent APC/C substrate accumulation ensuing. In contrast, pUL97 alone was insufficient to cause APC/C substrate accumulation despite its ability to phosphorylate Cdh1. Despite Cdh1 phosphorylation the APC/C itself remains intact, thus a relatively small amount of unphosphorylated Cdh1 potentially would be adequate for APC/C coactivation. It was also demonstrated that pUL21a was both sufficient and necessary for instigating degradation of APC1 in addition to APC4 and APC5 (Clark and Spector 2015). Knockdown of any of the bridge subcomplex subunits (APC1, APC4, APC5) by siRNA mediated the destruction of the other bridge subcomplex subunits in uninfected cells. In addition, knockdown of the TPR subunit APC8 caused the destruction of all the APC/C bridge subcomplex subunits. This is not completely surprising as the apparent binding of APC1, APC4, APC5 and APC8 is interdependent in *vitro* (Thornton *et al.* 2006) and the solved structure of human APC/C places APC1 beside APC8 (Chang *et al.* 2014). It remains elusive whether the APC/C subunits are degraded by an active process or APC/C subunit degradation is a part of normal APC/C turnover, with

synthesis of new subunits recognised as improperly folded proteins if they do not associate correctly. While this evidence furthers our understanding of HCMV manipulation of cell cycle machinery, ascertaining the functional relevance of APC/C inhibition in HCMV infection *in vivo* and more precisely the mechanisms underpinning APC/C and specific APC/C subunit degradation in the presence of pUL21a warrant future investigations.

Other demonstrations of viruses manipulating the APC/C

Other instances of potential viral manipulation of the APC/C have been reported. Simian virus 40 (SV40) is a small DNA tumour virus, belonging to the polyomavirus family, that produces a productive infection in its natural host, the rhesus macaque, but stimulates oncogenic transformation in nonpermissive hosts such as rodent cells. SV40 large T antigen (LT) manipulates cell cycle regulation through indirectly activating APC/C. LT interacts with Bub1 a component of the potent APC/C inhibitory complex MCC and central SAC regulator. LT expression results in a compromised SAC potentially by disrupting MCC integrity (Cotsiki *et al.* 2004). SAC disruption instigates APC/C deregulation, allowing genomic instability and transformation to ensue, a phenomenon associated with SV40 infection (Hein *et al.* 2008). However how precisely unscheduled APC/C activation by these viruses potentially supports viral replication remains elusive.

Another virus reported to manipulate the APC/C indirectly is Hepatitis C, a virus demonstrated to be a major cause of HCC. It has been depicted that cells expressing Hepatitis C protein Nonstructural protein 5A (NS5A) exhibit increased fractions of mitotic cells, reduced G1 cell populations and decreased degradation of APC/C substrates Cyclin B and Securin, denoting elevated levels of Cyclin B and Securin perturb the normal timing of the mitotic cell cycle. During prolonged mitotic arrest, mitotic checkpoint components have demonstrated inhibiting APC/C averting it from targeting Cyclin B1 and Securin for subsequent degradation prior to the metaphase to anaphase transition. Delay of anaphase onset occurs when mitotic abnormalities arise, triggering apoptosis of the cell to avoid aneuploidy ensuing. However, it has been indicated that NS5A expressing cells by unknown

mechanisms adapt to aneuploidy and return to cell cycle following a prolonged mitotic arrest (Baek *et al.* 2006). Future studies ascertaining the cellular factors targeted by NS5A are imperative.

7. APC/C deregulation drives carcinogenesis

Precise cell cycle control is fundamental in averting oncogenic transformation and is safeguarded by oscillating activities of Cyclins and Cdk. It is well established that the APC/C is instrumental for regulation of these mitotic Cyclins. An intricate network of signals regulates both the APC/C timely activation and inactivation, in which defective regulation often instigates faulty mitotic checkpoint signalling, uncontrolled genome replication and erroneous mitotic exit, mutually causing genomic instability, a prevalent cancer hallmark (Zhou *et al.* 2016). Not only have mutations in APC/C subunits been related to oncogenic transformation but also defective activating and inhibitory mechanisms for APC/C. Thus, attributable to its essential responsibility in mitotic regulation, it is not unexpected that both a direct and indirect association of the APC/C with cancer has been elucidated.

Abnormal expression levels of APC/C subunits are an important contributor to genome instability and cancer (Rahimi *et al*. 2015). A total of 132 missense mutations in APC/C subunits have been reported in cancer, with the majority negatively affecting the structure stability of the subunits (Sansregret *et al.* 2017). These partial APC/C dysfunctions lengthened mitosis, supressed pharmacologically induced chromosome segregation errors and reduced naturally occurring lagging chromosomes in cancer cell lines (Sansregret *et al.* 2017). APC/C components are heterogeneously regulated in breast cancer (Park *et al.* 2005). However, mechanistic details of the process that enables breast cancer cells exhibiting a defective functional APC/C to progress through mitosis remains elusive. In contrast, it has been established that in colon cancer nonsense mutations affect the genes encoding for the APC/C subunits APC4, APC6/Cdc16 and APC8/Cdc23, causing premature

stop codons which in turn result in the expression of truncated proteins of impaired stability (Wang *et al.* 2003). Ectopic expression of the truncation APC8/Cdc23 mutant in colon epithelial cells has confirmed these findings as the APC8/Cdc23 deletion mutant results in a phenotype characterised by an elevated number of cells in G2/M phase after release of G2/M phase arrest with abnormal levels of APC/C substrate Cyclin B1 observed. This indicates that APC8/Cdc23 mutant potentially deregulates cell cycle progression through mitosis, with subsequent delay in mitotic exit through an alteration in the degradation schedule of crucial mitotic regulators (Wang *et al.* 2003).

Significant overexpression of APC/C subunits APC2 and APC7 occur in numerous cancer cell lines and acute myeloid leukaemia (AML) patients, with APC2 upregulation significantly correlating with splenomegaly (Rahimi *et al.* 2015). However, various APC/C expression levels have been observed in different cancer cell lines. The dissimilar APC/C subunit expression level found in different tissues, is a contributing factor to the heterogeneity of APC/C subunit expression in different cancers (Rahimi *et al.* 2015). In lung carcinoma characterised by rapid cell growth, APC7 was produced at high expression levels, while 90% of renal carcinomas, a form of cancer that grow slowly, showed loss of APC7 expression (Park *et al.* 2005). These findings signify that an APC7 expression loss is unique to some carcinomas and APC/C components are heterogeneously regulated across cancer types.

A significant elevation in APC3/Cdc27 expression has been found in gastric cancer (Xin *et al.* 2018). High APC3/Cdc27 expression negatively correlated with 5-year overall survival while significantly positively correlated with lymph node metastasis. In addition, Cdc27 silencing effectively inhibited gastric cancer cell proliferation, invasion and metastasis both *in vivo* and *in vitro* (Xin *et al.* 2018). Collectively, these findings illustrate that Cdc27 upregulation mediates gastric cancer progression and that high Cdc27 expression significantly correlates with tumour size, TNM Classification of Malignant Tumours (TNM) stage and distant metastasis (Xin *et al.* 2018). Cdc27 overexpression has also been noted in breast cancer tissue, where it significantly correlates with disease recurrence whereas

testicular germ cell tumours (TGCTs) are known to harbour recurrent mutations in Cdc27 (Litchfield *et al.* 2015). In the latter case, all Cdc27 mutations identified to date were missense variants characterised by a constantly low frequency of mutant allelic reads, consistent with mutations of Cdc27 only present in a subclone of each tumour sample (Litchfield *et al.* 2015). In addition, whole-exome sequencing (WES) has revealed recurrent mutations of Cdc27 in adrenocortical (Juhlin *et al.* 2015), osteosarcoma (Reimann *et al.* 2014), prostate (Lindberg *et al.* 2013) and lung carcinomas (Ahn *et al.* 2014). Elevated APC3/Cdc27 protein expression level has been illustrated in colorectal cancer and significantly correlated with tumour size, TNM stage, distant metastasis and a poorer survival rate (Qiu *et al.* 2016). Cdc27 overexpression promoted proliferation *in vitro* and *in vivo* with greater tumour growth capacity exhibited while siRNA knockdown of Cdc27 inhibited proliferation with a significant tumour weight and volume reduction (Qiu *et al.* 2016). Upregulated Cdc27 expression has been further correlated with colorectal cancer metastasis (Qiu *et al.* 2017). siRNA knockdown of Cdc27 significantly repressed both migratory and invasive capabilities and sphere-formation capacity of cells while Cdc27 overexpression promoted metastasis and sphere-formation capacity. Furthermore, knockdown of Cdc27 *in vivo* supressed metastasis with a significant reduction in metastatic nodule numbers (Qiu *et al.* 2017).

Abnormally high expression levels of APC11 have been reported in lung cancer (Zhou *et al.* 2018) where high APC11 messenger RNA (mRNA) levels significantly correlated with poorer overall survival for lung adenocarcinoma. siRNA knockdown of APC11 arrested cells in mitosis and significantly reduced cell proliferation, colony formation and migration and invasive capabilities. Conversely, APC11 overexpression promoted cell proliferation and was associated with poorer prognosis. Collectively these findings show that APC11 crucially contributes to the development of lung adenocarcinoma (Zhou *et al.* 2018). APC11 overexpression has also been reported in colorectal cancer cell lines, which significantly

correlated with chromosomal instability, lymphovascular invasion and residual tumours (Drouet *et al.* 2018).

APC/C coactivators association with tumorigenesis

The two APC/C co-activators Cdc20 and Cdh1 have opposing functions in tumorigenesis, with Cdc20 and Cdh1 identified as an oncoprotein and tumour suppressor, respectively. Abnormal Cdc20 expression has been exemplified in diverse cancer types.

Overexpression of Cdc20 in cervical cancer has been reported in high grade squamous intraepithelial lesions and invasive squamous cell carcinoma (Kim *et al.* 2014). In addition, upregulated and downregulated Cdc20 expression was observed in glioblastoma and low-grade gliomas respectively, illustrating Cdc20 as a potential biomarker for glioblastoma (Marucci *et al.* 2018). Overexpression of Cdc20 has been reported in high-risk multiple myeloma patients, which seems to correlate with cellular proliferation and overall poor prognosis (Lub *et al.* 2016).

Cdc20 overexpression also occurs in non-small cell lung and oral squamous cell carcinoma, which results in a significant reduction in overall survival (Kato *et al.* 2012; Moura *et al.* 2014). Moreover, Cdc20 elevated expression significantly correlates with tumour size in non-small-cell lung cancer patients and larger primary tumour size, higher DNA ploidy level and poor prognosis in lung adenocarcinoma patients (Shi *et al.* 2017). Furthermore, gene knockout or knockdown in lung cancer cells causes a decrease in cell proliferation (Yi *et al.* 2018) whereas Cdc20 upregulation in oral squamous cell carcinomas and primary head and neck tumours seems to deregulate the timing of the APC/C in promoting premature anaphase with consequential genome instability and aneuploidy (Mondal *et al.* 2007).

Additionally, Cdc20 is overexpressed in colon cancer cell lines and primary tissues. The elevated Cdc20 expression levels significantly correlated with decreased overall survival and

additional parameters of clinical stage, N and M classification and pathological differentiation (Wu *et al.* 2013). However, further investigation comprising more cases are required to ascertain the clinicopathological significance of elevated Cdc20 expression in colon cancer, particularly in early stages of tumour development.

Significantly elevated Cdc20 expression has been reported in pancreatic tumour tissues and associated with poor differentiation and a reduced 5-year recurrence-free survival rate (Chang *et al.* 2012). Furthermore, knockdown of Cdc20 in pancreatic carcinoma cells enhanced cell cytotoxicity following paclitaxel treatment and increased cell sensitisation to gamma radiation, indicating Cdc20 could provide a promising therapeutic target for treating this devastating disease (Taniguchi *et al.* 2008*)*. More recently, elevated Cdc20 expression exhibited in PDAC tumours, significantly correlated with additional parameters of advanced tumour stage (Dong *et al.* 2019).

Furthermore, screening of Cdc20 expression in 445 breast cancer patients with up to 20 years of follow-up, confirmed Cdc20 overexpression. The elevated Cdc20 levels was associated with aneuploid DNA content and an aggressive course of breast cancer with Cdc20 overexpression in prognostic analyses indicating 2-fold risk of breast cancer death (Karra *et al.* 2014). More recently, siRNA delivery against Cdc20 in triple-negative breast cancer cells inhibited tumour growth, emphasising Cdc20 potential as a valuable therapeutic target for inhibiting tumour growth of highly aggressive metastatic breast cancer cells (Parma *et al.* 2018).

Upregulated Cdc20 expression has been reported in 68.18% of HCC tissues in contrast to adjacent non-malignant tissues. This Cdc20 overexpression positively correlated with tumour differentiation and TNM stage (Li *et al.* 2014). In addition, siRNA knockdown of Cdc20 in HepG2 cells illustrated supressed cell proliferation and induced G2/M phase arrest, (Li *et al.* 2014). More recently, Cdc20 upregulation has been correlated with the additional parameters of poor histological grade, vascular invasion and poorer overall survival

(Menyhart *et al.* 2018). More recently, elevated Cdc20 expression exhibited in hepatocellular carcinoma tumours, significantly correlated with additional parameters of advanced tumour stage (Zhuang *et al.* 2018)

Requirement for APC/C-Cdc20 in sustaining glioblastoma stem-like cells (GSCs) function has been exemplified. Knockdown of Cdc20 through RNA interference (RNAi) considerably diminished GSCs tumourigenicity, while overexpression of Cdc20 augmented GSCs self-renewal capacity and invasiveness, denoting Cdc20 is both necessary and sufficient for GSCs self-renewal, invasion and tumour initiation. Consistent with these findings, treatment with the APC/C-Cdc20 and APC/C-Cdh1 inhibitor pro-tosyl-L-arginine methyl ester (ProTAME) can abrogate the GSCs phenotypes (Mao *et al.* 2015). Upregulation of Cdc20 has been identified in glioblastoma-associated stromal cells and proposed to promote glioblastoma tumorigenesis (Gujar *et al.* 2015).

High Cdc20 expression levels occur in urothelial bladder cancer, with overexpression of Cdc20 associated with high grade, advanced stage, non-papillary growth pattern and distant metastasis. In addition, elevated Cdc20 expression correlates with shorter recurrence-free survival as well as overall survival (Choi *et al.* 2013).

Cdc20 expression is significantly elevated in gastric cancer tumour tissues which positively correlates with tumour size, histological grade, lymph node involvement, TMN stage and poorer overall survival (Ding *et al.* 2014).

In prostate cancer, a correlation between Cdc20 overexpression with high Gleason scores and recurrence after prostatectomy has been revealed (Mao *et al.* 2016). More recently, prostate cancer-derived mutations in Speckle-type POZ protein (SPOP) a protein binding and promoting Cdc20, impedes SPOP ability to bind Cdc20. Consequently, these prostate cancer cells exhibited elevated Cdc20 expression, rendering them more resistant to Cdc20 inhibitor Apcin (Wu *et al.* 2017). Furthermore, both *in vitro* and *in vivo* Cdc20 silencing supressed cell proliferation and enhanced chemosensitivity to docetaxel (Li *et al.* 2016)

whereas Cdc20 overexpression facilitated docetaxel resistance of castration-resistant prostate cancer cell lines, further emphasising Cdc20 potential as a promising therapeutic target (Wu *et al.* 2018).

A system review and meta-analysis illustrated Cdc20 elevated expression correlates with poor survival in majority of solid tumours and indicates Cdc20 is both a novel prognostic marker and therapeutic target. However, further investigations to validate the potential mechanism and influence of Cdc20 as well as its prognostic value in human solid tumour pathogenesis are imperative (Wang *et al.* 2018).

Overall, consistency between findings of associated Cdc20 expression with tumorigenesis and its potential as a valuable prognostic biomarker amongst diverse cancers has been depicted and prospects of Cdc20 as a therapeutic target for cancer considered (Kapanidou *et al.* 2017). Future investigations ascertaining the precise molecular mechanism in which Cdc20-meditated tumorigenesis evolves are imperative.

Cdh1 is essential for downregulating replication and the mitotic spindle checkpoint, with APC^{Cdh1} activity in G1 phase averting the accumulation of essential genes and proteins necessary for another round of DNA replication (de Boer *et al.* 2015).

Cdh1 expression is significantly decreased in diverse cancer types including breast (Fujita *et al*. 2008a) prostate, ovary, liver, brain, (Bassermann *et al.* 2008) and colon (Fujita *et al.* 2008b) tumours with poor patient survival, arguing in favour of Cdh1 function as a tumour suppressor.

Furthermore, APC/Cdh1 has been revealed to target Skp2 for degradation, averting premature Skp2/SCF-meditated destruction of $p27^{Kip1}$, and this subsequently prevents premature entry into S phase, indicating dysregulation of APC/C-Cdh1-dependent proteolysis of substrates is fundamental and likely implicated in tumour initiation (Fujita *et al.* 2008a; Fujita *et al.* 2008b). Significantly reduced Cdh1 and $p27^{Kip1}$ expression and elevated Skp2 expression in breast and colon cancer tissues have been reported (Fujita *et al.* 2008a;

Fujita *et al.* 2008b). In addition, in non-malignant breast and colon cancer tissues, Cdh1 knockdown by RNAi caused increased cellular proliferation and number of cells in S phase with significantly elevated Skp2 levels and consequential attenuation of $p27^{Kip1}$ levels (Fujita *et al.* 2008a; Fujita *et al.* 2008b).

Further studies have identified the potential and significance of the physiological tumour suppressor role of Cdh1. In a xenograft breast cancer mouse model, Cdh1 overexpression significantly reduced implanted tumour size, in contrast to Cdh1 depletion by RNAi which accelerated tumour growth (Fujita *et al.* 2008a). Furthermore, lentiviral-delivered knockdown of Cdh1 by RNAi in primary human fibroblasts and osteosarcoma cells caused aberrant accumulation of numerous APC/C-Cdh1 substrates including Cyclin A, Cyclin B and Aurora A. Subsequent premature and prolonged S phase ensued with significantly delayed mitosis entry, inducing defective chromosome segregation and cytokinesis, collectively stimulating genomic instability (Engelbert *et al.* 2007). Furthermore, depletion of Cdh1 in mouse embryonic fibroblasts (MEFs) caused defects in proliferation and accumulation of aberrations including binucleated and multinucleated cells, chromosomal aberrations such as abnormal numbers and complex translocations, and chromosomal missegregation contributing in aneuploidy (García-Higuera *et al.* 2008).

Furthermore, low expression level of Cdh1 has been associated with poor prognosis in some multiple melanoma patients, with patients demonstrating high Cdh1 expression, exhibiting a significant enrichment of genes related to mature bone marrow plasma cells and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling, in comparison to patients with low Cdh1 expression exemplifying significant enrichment in MYC target genes (Lub *et al.* 2016). Moreover, expression of Cdh1/Fizzy-related protein homolog (Fzr) was reduced in a fully malignant B-cell line as opposed to premalignant cells. Retroviral overexpression of Fzr in B-lymphoma cells caused decreased tumour formation, with tumours that did develop exhibiting diminished or extinguished retroviral Fzr (Wang *et al.* 2000). Furthermore, a high percentage of malignant tumours showed accumulation of Cdh1

in contrast to most benign and some low-grade tumours which exhibit low Cdh1 expression (Lehman *et al.* 2007).

Concomitant with Cdh1 downregulation, several APC/C-Cdh1 substrates including Aurora A, Aurora B, Cdc6, Cdc20, Cyclin B, Rad17 and Tpx2 are most frequently overexpressed in human cancers (Carter *et al.* 2006). Overall, an association between APC/C-Cdh1 downregulation and oncogenesis is evident with reduced APC/C-Cdh1 activity potentially causing the stabilisation and subsequent accumulation of substrates and unscheduled activity that promote the formation of highly proliferative, genetically unstable and poorly differentiated tumours.

APC/C inhibitors contribution to oncogenic transformation

APC/C activity requires meticulous regulation executed by an intricate network of signals including endogenous inhibitory proteins. These endogenous inhibitors have evolutionarily developed to ensure appropriate APC/C ligase activity is accomplished with subsequent accurate progression of the cell cycle and chromosomal stability. Defective inhibitory mechanisms of the APC/C participate in human disease development, particularly cancer (Zhang *et al.* 2014). Fundamental endogenous APC/C inhibitors identified to regulate APC/C activity include Emi1 and SAC core proteins Mad2, BubR1 and Bub3. Deregulation of these inhibitors causes dysregulated APC/C activity and consequential genomic instability indirectly associating the APC/C with cancer. Mad2, BubR1 and Bub3 are three core SAC proteins vital for regulating APC/C activity. These three proteins associate with Cdc20 for the formation of the MCC. This complex monitors chromosome segregation, inhibiting APC/C-Cdc20 activity and averting subsequent degradation of APC/C substrates Cyclin B1 and Securin, consequently delaying metaphase transition to anaphase until all chromosomes have accurately attached to the mitotic spindles. Alternatively, Emi1 crucially functions in

regulating the transition from G1 phase to S phase and progression of mitosis through inhibiting the activity of APC/C-Cdh1 (Zhang *et al.* 2014).

Deregulation of these endogenous APC/C inhibitors has been reported in diverse cancer types with deregulation of Mad2 and Emi1 reported to cause mitotic catastrophe (Hsu *et al.* 2002; Hernando *et al.* 2004). The overexpression of Mad2 has been reported in malignant lymphoma (Alizadeh *et al.* 2000), lung cancer (Garber *et al.* 2001; Heighway *et al.* 2002; Kato *et al.* 2011), hepatocellular carcinoma (Chen *et al.* 2002; Zhang *et al.* 2008), colorectal carcinoma (Rimkus *et al.* 2007), soft tissue sarcoma (Hisaoka *et al.* 2008) and gastric cancer (Wang *et al.* 2009) and correlates to poor prognosis (Hernando *et al.* 2004; Li *et al.* 2003; Tanaka *et al.* 2001; van't Veer *et al.* 2002). In human fibroblasts and cell lines overexpression of Mad2 can induce the stabilisation of APC/C substrates Cyclin B1 and Securin with a subsequent mitotic delay and an aneuploid cell phenotype observed, indicating upregulation in Mad2 potentially exerts an oncogenic function through producing an overactive MCC with subsequent APC/C activity dysregulation and deficit in cell control that initiates genomic instability (Sotillo *et al.* 2007). Furthermore, in transgenic mice Mad2 overexpression causes a diverse range of neoplasias including chromosome breaks, anaphase bridges as well as chromosome gain and losses. These results suggest that Mad2 overexpression may exert oncogenic potentials by generating a hyperactive mitotic checkpoint to override the critical role of the APC/C in the control of cell cycle and genomic stability (Sotillo *et al.* 2007). Furthermore, BubR1 overexpression is also observed in diverse cancers (Liu *et al.* 2009; Lee *et al.* 2009; Pinto *et al.* 2008; Yamamoto *et al.* 2007; Grabsch *et al.* 2003; Seike *et al.* 2002), however the effect of BubR1 overexpression on APC/C substrates stabilisation remains elusive.

Emi1 overexpression has been reported in diverse cancers (Hsu *et al.* 2002; Lehman *et al.* 2007; Gütgemann *et al.* 2008). It has been exemplified that Emi1 through inhibiting the APC/C-Cdh1 stimulates an accumulation of APC/C substrate Cyclin A and subsequent S-phase entry in somatic cells. Furthermore, while Emi1 overexpression accelerates S-phase

and overrides a G block caused by Cdh1 or pRB overexpression, conversely siRNA knockdown of Emi1 impedes accumulation of Cyclin A and subsequent S-phase entry (Hsu *et al.* 2002). Furthermore, it has been exemplified that Emi1 overexpression causes severe mitotic catastrophe including chromosome missegregation and concurrent with these phenotypes a stabilisation of APC/C substrate Cyclin A and Aurora A is observed (Margottin Gouget *et al.* 2003). In addition, in p53-deficient cells, overexpression of Emi1 induces unscheduled cell proliferation, tetraploidy and chromosomal instability. While in p53 wild-type cells this tetraploidy induction by Emi1 overexpression often results in subsequent G1 arrest or apoptotic cell death, in p53-deficient cells, the continual unchecked cellular proliferation in conjunction with severe chromosome missegregation likely causes the aneuploidy phenotype exemplified by diverse cancers (Lehman *et al.* 2006). Overexpression of Emi1 has been reported in HCC and associated with poor survival. *In vitro* studies revealed Emi1 was highly expressed in HCC cell lines with upregulation of Skp2 and APC/C substrates Cyclin A and Cyclin B exemplified while $p27^{Kip1}$ was downregulated. In addition, siRNA knockout of Emi1 inhibited cell proliferation through impeding S phase and mitosis entry and resulted in the prevention of an accumulation of Skp2, Cyclin A and Cyclin B while $p27^{Kip1}$ was upregulated. These findings indicate overexpression of Emi1 has oncogenic potential and participates in HCC cell proliferation through APC/C inhibition with subsequent stabilisation of Skp2, Cyclin A and Cyclin B and consequential degradation of $p27^{Kip1}$ that contributes to HCC pathogenesis (Zhao *et al.* 2013). Furthermore, Emi1 overexpression has been observed in head and neck cancers and correlates with upregulation of APC/C-Cdh1 substrates Cyclin B, Aurora A and Skp2. Head and neck cancers exhibiting high Emi1 expression levels illustrated malignant behaviours including poor differentiation and lymph node metastasis. Additionally, Emi1 knockdown induced downregulation of APC/C substrates Cyclin A, Cyclin B, Aurora A and Skp2, illustrating deregulation of Emi1 contributes to inactivation of APC/C in cancer (Shimizu *et al.* 2013). Moreover, frequent overexpression of Emi1 and APC/C substrates Securin, Plk1, Aurora A and Skp2 in an abundance of diverse cancer types has been reported in malignant tumours in contrast to

benign tumours. Importantly, the APC/C substrates clustered together in numerous tumours with Emi1 elevated expression, indicating a ‘mitotic profile’ in tumours potentially arises from APC/C dysregulation (Lehman *et al.* 2007).

Collectively these findings indicate that deregulation of endogenous APC/C inhibitors can mediate the dysregulation of the APC/C. Overall, whether is it a direct mutation or dysregulation of APC/C subunits, coactivators or inhibitors, APC/C misfunction appears a mutual attribute among diverse cancers.

8. APC/C as an attractive therapeutic target

The indispensable role of APC/C in regulating mitotic progression, accurate chromosomal segregation and mitotic exit as well as its implication in oncogenesis, renders the APC/C an attractive therapeutic target for cancer treatment. Investigations into discovering and developing small molecule inhibitors to suppress cancer growth or induce cancer death have initiated, which could provide a therapeutic window amongst diverse cancers and is crucial for innovating novel prospective cancer therapeutic strategies (Liu *et al.* 2019; Wang *et al.* 2015; Zhou *et al.* 2016). Antimitotic drugs targeting microtubules including vinca alkaloids and taxanes are used to treat diverse cancers (Montero *et al.* 2005). However, the clinical efficacy of these small molecules is limited and attributed to the variable responses of the treated cells (Brito and Rieder 2009; Gascoigne and Taylor 2009). In particular, these drugs cause neurotoxicity likely due to perturbation of neuron microtubules (Jordan and Wilson 2004).

In an attempt to develop antimitotic drugs lacking such side effects, investigations were initiated into targeting specific proteins of the mitotic spindle to activate the SAC and subsequently inactivate the APC/C (Huang *et al*. 2009). In addition, investigations into weakening core proteins of the SAC to impede tumour growth indicates that perturbing the SAC signalling pathway is a promising pool of therapeutic targets for cancer treatment

(reviewed in Kapanidou and Bolanos-Garcia 2014). These findings instigated the exploration and development of small molecule inhibitors that target the mitotic spindle and SAC kinase catalytic sites, some of which have entered clinical trials. Although these molecules are not neurotoxic, the utilisation of inhibitors that target SAC kinases through functioning as Adenosine Triphosphate (ATP)-binding competitors has proved challenging and of limited success. The inhibitors lack of specificity for the intended drug target potentially causes undesirable side effects and drug resistance of cancer cells to rapidly develop, which remain major concerns (Tsai and Nussinov 2013). Cancer cells can resist such killing by premature mitotic exit before apoptosis is initiated due to a weak checkpoint or rapid slippage (Huang *et al*. 2009). Slippage is proposed to occur when APC/C-Cdc20-induced background degradation of Cyclin B1 under an active SAC exceeds a certain threshold before cell apoptosis initiation and consequently prompts the cell to exit from mitosis (Brito and Rieder 2006). To improve clinical efficacy and overcome undesirable side effects, developing inhibitors for more specific targets represents a more appropriate approach for treating diverse cancers.

In recent investigations, it has been exemplified that mitotic exit may serve as an appropriate target owing to its pro-apoptotic outcomes of RNAi knockdown of Cdc20 (Huang *et al*. 2009). Depletion of Cdc20 slowed proteolysis of APC/C substrate Cyclin B1, enabling more time for cell death to be initiated. Such killing of cancer cells was independent of the SAC and could occur by intrinsic apoptosis. Inhibiting mitotic exit causes a permanent mitotic arrest and subsequent mitotic cell death as opposed to other approaches which often cause cell cycle arrest with possible re-entry into a new cell cycle ensuing. These findings depict blocking mitotic exit downstream of the SAC may serve as an improved therapeutic approach for killing apoptosis-resistant, slippage-prone, or SAC defective cancer cells in comparison to antimitotic drugs dependent on targeting the mitotic spindle or the SAC (Huang *et al*. 2009).

Due to the pivotal role of the APC/C in regulating mitotic progression and exit, renders the APC/C an attractive target for development of mitotic exit inhibitors. As the complex determines substrate specificity, potentially more targeted therapies can be designed and

developed. Conversely, the multiplicity and diversity of APC/C substrates can prove challenging for targeted therapy development (Ramanujan and Tiwari 2016). An attractive therapeutic target attribute of APC/C is the modulation of its activity by Cdc20 and Cdh1. The development of inhibitors specifically targeting either of these two APC/C coactivators could reduce the off-target and side effects observed with anti-mitotic agents. Cdc20 is the preferred target as its function and substrates are mainly restricted to mitosis in contrast to Cdh1 which has a more diverse range of functions and substrates (Ramanujan and Tiwari 2016). However, due to Cdh1 implication in maintaining the G0/G1 state and in conjunction with pRB inhibiting transition of G1 to S phase, agonists could be potentially developed to promote these functions. Small compounds stabilising Cdh1 interaction with pRB or preventing its release from pRB could prove valuable in averting proliferation (Ramanujan and Tiwari 2016). The interdependence between Cdh1, SCF, Cdc20 and Emi1 could also possibly be exploited through a combination of antagonists and agonists, signifying future prospects in the design and development of chemotherapeutic agents targeting Cdh1 (Cardozo and Pagano 2007).

Blocking the APC/C through averting Cdc20-dependent mitotic progression with subsequent cell cycle arrest and probable eventual cell death has been the substantial focus in developing inhibitors targeting APC/C. Tosyl-L-arginine methyl ester (TAME) and Apcin are two small size APC/C inhibitors. TAME targets and binds to the APC/C causing a dramatic loss in APC/C activity by perturbing APC/C interaction with its coactivators Cdc20 and Cdh1 (Zeng *et al.* 2010). TAME imitates the IR tail of Cdc20 and Cdh1, thus specifically interferes with the IR tail-dependent interaction between Cdc20 or Cdh1 and APC/C. Functioning as a Cdc20 and Cdh1 inhibitor, TAME disrupts the coactivators recruitment to the complex impeding APC/C activation and subsequent APC/C substrates degradation. Although Cdh1 and Cdc20 bind to common APC/C sites, only APC/C-Cdc20 is activated by phosphorylation (Zeng *et al.* 2010). The phosphorylation-independent activity of Cdh1 is attributable to the increased affinity of Cdh1 for unphosphorylated apo APC/C. This increased affinity is caused by more extensive contacts formed between APC/C and Cdh1 in comparison to APC/C and

Cdc20. This phenomenon also provides an explanation for TAME exemplifying a greater potency towards APC/C-Cdc20 in contrast to APC/C-Cdh1 (Alfieri *et al*. 2017).

The precise mechanism in which TAME impedes the activation of APC/C has been further exemplified (Zeng and King 2012). TAME reduces the binding of Cdc20 to the APC/C through inhibiting free Cdc20 binding the complex and actively promoting the dissociation of pre-bound Cdc20 from the APC through inducing auto-ubiquitination of Cdc20 in *Xenopus* extracts. Both aspects of this mechanism are antagonised by the binding of APC/C substrates including Cyclin B1. This phenomenon potentially verifies why in mitotically arrested *Xenopus* extracts in which APC/C substrates are absent, TAME promotes efficient dissociation of Cdc20 from the APC/C, in contrast to the little effect observed in somatic cells in which APC/C substrates seem to promote Cdc20 binding and suppress Cdc20 auto-ubiquitination (Zeng and King 2012). Nevertheless, TAME inactivates the APC/C and stabilises Cyclin B1 in *Xenopus* extracts by inducing a catalytic defect in the APC/C-Cdc20-Cyclin B1 complex which consequentially slows the initial ubiquitination of unmodified Cyclin B1. As Cyclin B1 becomes ubiquitinated it loses its capability of promoting Cdc20 binding to APC/C in the presence of TAME, the APC/C-Cdc20-substrate-TAME complex remains susceptible to dissociation of Cdc20 as the substrate becomes ubiquitinated, averting the substrate from reaching a threshold of ubiquitination necessary for proteolysis, thus resulting in a persistent mitotic arrest (Zeng and King 2012).

TAME shows low permeability to the cellular membrane, a feature that prompted the search of derivatives of this molecule with better pharmacological properties. These efforts lead to the synthesis of the prodrug pro-TAME, which is processed *in vitro* by esterases to yield the active molecule TAME (Zeng *et al.* 2010). ProTAME exhibits the same inhibitory action as TAME *in vitro*, hindering tumour growth and inducing a mitotic arrest without perturbing the mitotic spindle morphology and function. Intriguingly, the proTAME-induced mitotic arrest is dependent on sustained SAC activity. This is unexpected as treatment with proTAME causes mitotic arrest in cells with normal kinetochore tension development, a condition that ordinarily causes SAC inactivation. The proTAME-induced mitotic arrest in a SAC-dependent

manner could be attributable to proTAME treatment possibly instigating defective interactions between kinetochores and microtubules, producing an abnormally high degree of checkpoint signals in contrast to normal metaphase kinetochores. Alternatively, proTAME potentially could impede inactivation of the SAC, regardless of normal interactions between kinetochore and microtubules. The latter condition is more favoured due to the degree of checkpoint dependence exhibited, far exceeding the degree of kinetochore-microtubule perturbation (Zeng *et al.* 2010). APC/C-dependent ubiquitination or proteolysis is necessary for SAC inactivation. It is postulated and observed that such mutual antagonism between the APC/C and SAC could instigate a positive feedback loop that would augment proTAME inhibitory effects in a SAC dependent manner. This mutual antagonism possibly signifies a system-level behaviour that is controlled by minor abundance alterations in numerous core SAC proteins preceding anaphase (Zeng *et al.* 2010). Furthermore, it has been exemplified that induction of mitotic arrest could be achieved without complete pharmacological inhibition of the APC/C. This was unexpected as a previous investigation utilising RNAi showed that reduction of Cdc20 to very low levels was required for induction of mitotic arrest and in contrast to proTAME-induced mitotic arrest, was not SAC-dependent (Wolthuis *et al*. 2008; Huang *et al*. 2009). Variation in the protein synthesis rates among cells is one potential factor that accounts for the high variability in cellular response to microtubule inhibitors, which could subsequently limit the inhibitors therapeutic effectiveness. In comparison, proTAME-induced mitotic arrest occurs through residual APC/C activity inhibition instead of provoking SAC activation. This mechanism mediating mitotic arrest illustrates reduced dependence on protein synthesis due to the lowered residual APC/C activity rate and consequential lowered protein synthesis required for replenishing APC/C substrates, thus is less prone to mitotic slippage. ProTAME potentially therefore provides enhanced effectiveness in promoting and sustaining mitotic arrest and inducing a greater apoptotic death (Zeng *et al.* 2010).

More recently, the possibility that APC/C activity is necessary for silencing of the SAC has been further investigated (Lara-Gonzalez and Taylor 2012). It was demonstrated that

proTAME does indeed induce a mitotic arrest in a SAC-dependent manner. However, a much simpler explanation has been proposed as to why proTAME-induced mitotic arrest is dependent on the SAC. It has been exemplified that the mitotic arrest induced by treatment with proTAME is attributable to cohesion fatigue induction, a phenotype produced by asynchronous chromatid separation ensuing a prolonged metaphase (Lara-Gonzalez and Taylor 2012). This is a result of the microtubule pulling forces during metaphase eventually overcoming cohesin-based forces holding sister chromatids together (Lara-Gonzalez and Taylor 2012). Subsequent generation of unpaired sisters that are unable to stably attach to microtubules causes reactivation of the SAC (Stevens *et al.* 2011). Thus, proTAME induced mitotic arrest dependent on the SAC is an indirect effect of cohesion fatigue. Treatment that delay cells in metaphase including Cdc20 depletion has illustrated the induction of cohesion fatigue (Huang *et al.* 2009; Stevens *et al.* 2011; Lara-Gonzalez and Taylor 2012). Thus, it is not surprising that cohesion fatigue can be induced by proTAME. It has been depicted that proTAME inhibits APC/C activity, producing a metaphase delay in which if cohesion fatigue occurs, SAC reactivation occurs instigating a prolonged mitotic arrest (Lara-Gonzalez and Taylor 2012). Further investigations identifying and characterising APC/C targets that promote SAC silencing are imperative to ascertain the balance of SAC activating and silencing signals during mitosis, which subsequently can have implications for chemotherapeutic drug development since mitotic exit potentially serves as a valuable therapeutic target for treating cancer.

Furthermore, low concentrations of an APC/C inhibitor may be valuable in combination with microtubule inhibitors to sustain mitotic arrest and enhance cell death. The addition of proTAME to HepG2 cells pre-treated with paclitaxel or an Aurora A inhibitor produced a potent antitumor efficacy by causing a further accumulation of cells in mitosis, inducing significant inhibition of mitotic exit and subsequently substantial apoptotic cancer cell death (Giovinazzi *et al.* 2013). In addition, treatment of multiple myeloma cell lines with proTAME results in growth arrest with subsequent apoptosis. When proTAME was then combined with the microtubule inhibitor vincristine, a significant increase in death of multiple myeloma cell

lines and primary cells ensued demonstrating an enhanced anti-myeloma effect. Such findings indicate that APC/C could serve as a promising novel therapeutic target for the treatment of multiple myeloma (Crawford *et al.* 2016). Furthermore, *in vitro* studies revealed that proTAME can enhance the effect of commonly used chemotherapeutic drugs Adriamycin (ADM) and Cisplatin (DDP) in killing osteosarcoma cells, suggesting that the combination of proTAME with chemotherapeutic drugs may constitute an attractive therapeutic approach to treat osteosarcoma (Hu *et al.* 2014).

While Cdh1 exhibits a potential tumour suppressor role, Cdc20 expression positively correlates with cancer and its depletion impedes tumours growth. Hence, the design and development of an inhibitor specifically targeting APC/C-Cdc20 instead of a pan-APC/C inhibitor like pro-TAME should be more selective to kill cancer cells than those overexpressing Cdc20 (Zhang *et al.* 2014). In mitosis, APC/C activation requires the binding of Cdc20 which results in the formation of a complex that recognise substrates containing a D-box). The other small compound inhibitor of the APC/C developed to date is Apcin, which directly binds to Cdc20 and prevents the recognition of D-box containing substrates, consequentially competitively impeding APC/C-dependent ubiquitination of APC/C-Cdc20 substrates (Sackton *et al.* 2014). Apcin binds the pocket defining D-box within Cdc20 WD40 domain, blocking substrate-induced Cdc20 loading onto the APC/C enabling the stabilisation of APC/C substrates containing D-box including Cyclin B1, Cyclin A2 and Securin. However, at low concentrations Apcin is insufficient to inhibit mitotic exit due to substrates outcompeting Apcin binding the pocket, or other mechanisms recruiting substrates to APC/C. Enhanced Apcin efficacy is achieved by combining the compound with TAME (Sackton *et al.* 2014). Furthermore, the combination of these two inhibitors illustrates greater effectiveness in disrupting APC/C activity than when either compound is used alone, demonstrating synergistic effects in inhibiting APC/C dependent proteolysis and mitotic exit when simultaneously perturbing two protein-protein interactions within the APC/C-Cdc20-substrate complex. This synergistic inhibition of mitotic exit does not depend on the SAC, but likely occurs from direct pharmacological inhibition of the APC/C. As dynamic protein

complexes are crucial to ensure homeostasis, these findings depict the possibility of disrupting a protein machine function through simultaneously inhibiting multiple weak protein-protein interactions and potentially provides a novel therapeutic approach for targeting protein complexes that may otherwise prove particularly challenging to target with a single inhibitor (Sackton *et al.* 2014).

More recently, it has been shown that Apcin inhibits cell growth and invasion as well as induces significant apoptosis in osteosarcoma cell lines, indicating Cdc20 possibly serves as a valuable therapeutic target for osteosarcoma treatment (Gao *et al.* 2018). Furthermore, it has been exemplified that myeloma cell lines treated with proTAME caused an accumulation of APC/C substrate Cyclin B1 and increased the number of cells in metaphase. When proTAME was combined with Apcin, an enhanced anti-multiple myeloma effect was observed with greater induction of cell cycle metaphase arrest and subsequent apoptosis, depicting APC/C and Cdc20 could serve as valuable novel therapeutic targets for high-risk multiple myeloma patients (Lub *et al*. 2016).

Investigations into identifying and developing APC/C inhibitors as promising therapeutic agents that target cells that undergo mitotic slippage following treatment with microtubule inhibitors remains ongoing. More recently, it has been illustrated that when in excess, Mad2 binding motif peptides derived from Cdc20; the endogenous 39 amino acid Tiny yeast comet 1 (Tyc1) protein which contains homology to human $p31^{comet}$; and a homologous peptide derived from human $p31^{comet}$ can all function as inhibitors of the APC/C (Schuyler *et al*. 2018). Furthermore, overexpression of these peptides *in vivo* instigates sensitivity to microtubule inhibitors. These peptides bound and inhibited the APC/C perturbing the ability of the coactivators Cdc20 and Cdh1 to bind to the APC/C. When Cdc20 Mad2-binding motif peptides were co-expressed with Tyc1 an increased sensitivity to microtubule inhibitors was observed *in vivo*. Although these APC/C inhibitor peptides are too large and functionally too weak to serve as prospective therapeutic agents, these findings indicate that these peptides can all serve as novel valuable molecular tools to investigate potential mechanisms inhibiting APC/C that induce sensitivity to microtubule inhibitors *in vivo* as a therapeutic strategy

towards the unending aim of targeting cancers treated with microtubule inhibitors that undergo mitotic slippage (Schuyler *et al*. 2018).

Overall, the APC/C serves as a valuable therapeutic target for designing and developing drugs for the treatment of diverse cancers. Through targeting mitotic exit provides an attractive alternative therapeutic strategy in inducing permanent mitotic arrest and subsequent cell death in comparison to other anti-mitotic agents. However, it must be stressed that proTAME is a prodrug that requires activation by esterases to the active impermeable compound TAME, thus upon administration *in vivo,* active esterases in the blood stream will process majority of the proTAME before it reaches target cells and subsequently no uptake of this drug shall occur (Zeng *et al.* 2010). As *in vivo* validation of proTAME is yet to be optimised, the need to design and develop novel more potent APC/C inhibitors that can be validated *in vivo* is imperative for the treatment of diverse cancers that constitute important yet unmet medical needs.

9. Concluding remarks and perspective analysis

Renowned for targeting a diverse array of substrates for ubiquitination and subsequent degradation, the APC/C is an indispensable multifunctional E3 ubiquitin-protein ligase accountable for the regulation of numerous crucial and diverse cellular processes. Attributable to its vital functions, the APC/C is therefore tightly regulated by a network of multiple intricate signals involving extensive cooperative interactions that act in a concerted manner to ensure appropriate APC/C activity. The regulation depends on the required APC/C activity at a given point in time, in which interactions can inhibit or activate the complex.

Although since the discovery of the APC/C its crucial implications in cell cycle regulation has been elucidated and its numerous substrates identified, the remarkable versatility and complexity of functions of this E3 ubiquitin-protein ligase elicits the prospect that additional roles and substrates of this complex are yet to be discovered. The emerging utilisation of

technologies including single molecule analysis and super-resolution microscopy combined with traditional biochemical, cellular and structural biology approaches will enable to uncover novel aspects of this indispensable complex with an unprecedented level of detail. Further investigations as to whether the subunits exhibit different roles independent of the APC/C holoenzyme are imperative. Future work should aim to further our understanding of the structural architecture of the APC/C. In particular, the precise location of the lack in well-defined electron density around the anticipated position of Bub3 in the APC/C-MCC assembly and how its mode of action contributes to fine tune APC/C functions require further investigations. Additionally, recent studies by independent groups have elucidated APC/C functions in the cell that are cell cycle-independent including roles in cell differentiation of the nervous system and DNA damage repair. Diverse protein regulators important for the maintenance of a stable genome and implicated in DNA damage repair are Cdh1 substrates. Future work should aim to deepen our understanding of APC/C-Cdh1 physiological role in these cellular processes and if these interactions are confirmed, they will expand further the APC/C repertoire of functions to incorporate crucial roles in apoptosis and senescence.

The discovery of novel APC/C substrates remains an active and substantial focus for research and is fundamental for advancing our understanding and knowledge of its functions and mode of regulation and their implications in health and disease conditions. Within the last two decades, significant advancements in knowledge have provided novel mechanistic insights into the multiple roles of APC/C assembly in chromosome segregation including functions beyond cell cycle regulation. For example, APC/C-Cdh1 is instrumental in the differentiation and function of the nervous system where it plays vital roles in axon growth regulation and synaptic transmission and plasticity, while APC/C-Cdc20 is implicated in neuronal development, regulating dendrite morphogenesis and presynaptic differentiation.

Perhaps, not surprisingly, dysregulated activity of the APC/C appears to be associated with AD pathophysiology. As the APC/C is an indispensable E3 ligase renowned for its capability of stipulating ubiquitination and subsequent degradation of numerous substrates, in

conjunction with accumulating evidence indicating a role in vital CNS functions, alterations of the APC/C could affect numerous diverse proteins and their functions and ultimately contribute to the development of neurodegenerative diseases. Accumulating evidence depicts an association between downregulation/inactivation of APC/C-Cdh1 and AD pathological hallmarks, with substantial indication that deregulation of APC/C substrates has significant implications in AD with regards to ectopic cell cycle re-entry, oxidative stress, ecotoxicity and impaired LTP. Aβ oligomers and glutamate excitotoxicity both reduce Cdh1 levels, inactivating APC/C-Cdh1 with subsequent APC/C substrate accumulation. Furthermore, it has been exemplified in AD that Cyclin B1, PFKFB3, and glutaminase are elevated due to APC/C-Cdh1 downregulation. It is probable that other APC/C-Cdh1 substrates that are yet to be identified can potentially accumulate in AD due to dysregulation of APC/C activity. Future investigations ascertaining entirely the roles of APC/C-Cdh1 and APC/C-Cdc20 in health and disease are imperative to ascertain whether these complexes can be valuable therapeutic targets for treating AD.

Numerous diverse viruses can affect APC/C functions, suggesting the APC/C is crucially implicated in viral replication and pathogenesis. A common approach amongst some viruses is the disruption of the integrity of the APC/C as exemplified for HCMV, CAV and HPV. Alternatively, the APC/C may be targeted through phosphorylation by HTLV-1 and Ad. ORFV PACR utilises a unique approach incorporating into an otherwise intact APC/C complex through mimicking and competing with APC/C subunit APC11. Although this approach may seem less efficient than targeting substrate recruitment, it elicits the prospect that PACR may redirect APC/C function to fulfil other roles. In the case of oncoviruses, manipulation of the APC/C can lead to premature APC/C-Cdc20 activation which subsequently promotes chromosome instability. For other viruses, APC/C-Cdh1 inhibition stimulates an S-phase like environment while APC/C-Cdc20 inhibition averts the nuclear envelope from reforming, thus enabling nuclear factors to become enriched or accessible for replication of the virus. Intriguingly, viral proteins may be targeted for ubiquitination and

degradation by the APC/C, consequently restricting replication of the virus. Numerous HCMV viral proteins contain the consensus D-box, an APC/C recognition signal regularly observed in APC/C substrates. The precise mechanisms in manipulating the APC/C utilised by diverse viruses illustrate variability, as are the effects exhibited by the numerous viral factors. However, as the functional implications of APC/C manipulation by viruses are gradually unveiled, the emerging picture is of a mutual trait between the expression of viral regulators that are crucial for the virus invasive strategy and the control of APC/C functions, which has implications on viral infection.

The identification of diverse viruses and numerous viral protein regulators in manipulating the APC/C, signifies the potential importance of this cell cycle master regulator in virus infection, with more viral factors likely to be discovered. Currently, mechanistic details of how exactly the reported viruses manipulate the APC/C are yet to be defined. The potential implication of virus-mediated APC/C regulation during virus infection remains largely elusive, complicated further by the fact the majority of the viral APC/C regulators identified to date are known to affect other proteins and consequently, other functions. Undoubtedly, further understanding of the role of APC/C manipulation by viruses is imperative. Future investigations should aim to unveil the precise molecular mechanisms these viruses utilise to modulate the APC/C; how exactly the APC/C is implicated in viral replication and pathogenesis; and what are the specific APC/C subunits and APC/C regulators that are targeted by the viruses.

In instances where APC/C potentially restricts replication of the virus, it will be essential to elucidate if the APC/C targets viral or cellular substrates. It is equally important to establish whether the viruses encode adaptor proteins to drive APC/C ubiquitination towards unknown cellular targets in a manner that specific substrates are targeted for destruction by the proteasome, thus selectively redirecting APC/C activity rather than activating or inhibiting the complex. Furthermore, it would be particularly valuable to ascertain whether viral APC/C modulation significantly effects host cell metabolism, favouring an environment that provides

not only nucleotides for viral replication, but an extensive repertoire of resources and energy required for viral particle synthesis and assembly.

Equally important is the verification of whether a pseudo-S phase state promoted in otherwise quiescent cells is a mutual trait amongst viral APC/C modulation. It would also be of great significance to determine whether other viruses than those already identified, encode factors modulating APC/C ubiquitin ligase activity. The APC/C has been shown to participate in signalling pathways that implicate pRB and p53. However, further studies should aim to unveil whether some of the viruses known to target pRB and p53 pathways do so through APC/C manipulation as well as elucidate if any viral regulators modulate APC/C activity through targeting APC/C inhibitors including Emi1.

In a broader perspective, future investigations into these viral regulators shall be valuable in further comprehending the biology of the APC/C and ascertaining how exactly these viral regulators interact with the APC/C, which will undeniably further our understanding of the structural architecture, assembly and regulation of the complex. It is likely these studies shall have crucial implications for cancer research, with drugs currently being designed and developed to target the APC/C as a potential therapeutic strategy for treating cancer. These viral APC/C regulators may serve as indispensable tools for identifying APC/C attributes that can be utilised as additional therapeutic targets for innovative development of novel drugs. Furthermore, future studies should analyse whether viral APC/C manipulation can sensitise cancer cells to drugs and/or gamma irradiation and proton beam therapy. Apoptin, a small protein from CAV, can selectively kill tumour cells and for this reason it is currently being investigated as a prospective cancer therapeutic strategy. In addition to this viral protein, it would be of great significance to evaluate the potential utilisation and efficacy of these viral factors as novel drug-delivery systems targeting the SAC-kinetochore-microtubule axis. Overall future investigations should collectively aim to advance our understanding of the intricate interplays amongst the APC/C, virus replication, cell cycle and virus-mediated cancer.

The APC/C is the most complex member of the RING finger ubiquitin E3 ligase family. As the APC/C is instrumental in the regulation of multiple vital cellular processes particularly in cell cycle progression control, timely activation and inactivation of the complex is therefore tightly regulated by a network of sophisticated signals. It is not surprising that when the regulation of the APC/C is dysregulated this instigates genomic instability with the APC/C both directly and indirectly associated with cancer.

Accumulating evidence exemplifies dysregulation of the complex either through mutations in core APC/C subunits or the deregulation of both activating and inhibiting APC/C regulators are associated with oncogenic transformation. These deregulations have been extensively investigated particularly for deregulations in both APC/C coactivators which show contrasting effects, with Cdc20 and Cdh1 functioning as an oncoprotein and tumour suppressor respectively. In addition, APC/C-Cdc20 and APC/C-Cdh1 are active at different phases of the cell cycle which in turn potentially differentially affects carcinogenesis. Moreover, it is very probable the different behaviour exemplified by Cdc20 and Cdh1 potentially originates from their tissue-specific expression, with different levels expression in different tissues possibly affecting the cellular function of these two APC/C coactivators. Furthermore, Cdh1 can induce Cdc20 degradation, thus the contrasting carcinogenic effects potentially are a result of excess Cdc20 degradation by Cdh1. While the precise molecular details of how tumorigenesis evolves from APC/C deregulation remain to be fully elucidated, ultimately the direct or indirect deregulation of APC/C activity instigates the formation of highly proliferative, genetically unstable and poorly differentiated tumours, in which APC/C dysregulation potentially can serve as a key prognostic marker and therapeutic target for the treatment of cancer. Accumulating evidence illustrates that mutations in several core APC/C subunits occur in numerous cancer tissues. Future studies should aim to elucidate the causal roles of these subunits in cancer both individually and collectively. Future investigations should also aim to further ascertain the precise molecular mechanisms in which Cdc20-mediated and Cdh1-mediated tumorigenesis evolves.

Currently, the development of new anti-mitotic therapies is one of the most active areas of cancer research. Attributable to its instrumental role in regulating cell cycle progression and the mounting evidence implicating the APC/C in tumorigenesis, renders the complex an attractive therapeutic target for treating cancer. In contrast to other anti-mitotic agents that target microtubules and the spindle assembly checkpoint, targeting mitotic exit provides an attractive innovative novel therapeutic approach in inducing a permanent mitotic arrest and subsequent cell apoptotic death. With other anti-mitotic agents, the clinical efficacy is limited with undesirable effects observed as well as premature mitotic exit and mitotic slippage in cancer cells.

An attractive APC/C therapeutic attribute is its regulation by Cdc20 and Cdh1. Through specifically targeting either of these two coactivators elicits the potential of reducing the off-target and undesirable side effects illustrated with anti-mitotic agents. The inhibitors proTAME and Apcin that target different aspects of APC/C activity, alone illustrate efficient anti-mitotic effects. When these two inhibitors are combined a synergistic inhibition of mitotic exit is observed, depicting the possibility of perturbing a protein machine function through simultaneously inhibiting multiple protein-protein interactions. These two APC/C inhibitors potentially provide an innovative novel therapeutic strategy for targeting protein complexes that may otherwise prove particularly challenging to target with a single inhibitor. However, proTAME requires activation by esterases to render the active impermeable compound TAME. Thus, on administration of proTAME *in vivo* the majority of the drug will be processed before reaching its target cells and consequentially drug uptake will not occur. Future studies should aim to optimise *in vivo* validation of proTAME and regardless as to whether this is achievable, the requirement of novel more potent APC/C inhibitors that can be validated *in vivo* is imperative for the innovate design and development of prospective therapeutic strategies targeting diverse types of cancer.

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Figure Captions

***Figure 1**. Schematic structure of the APC/C. The APC/C consists of three distinct subcomplexes; a catalytic subcomplex, scaffolding complex and a specificity arm defined by multiple tetratricopeptide repeat motifs (TPR). For clarity the subunits that assemble each subcomplex have been colour-coded, subunits presented are not drawn to scale and not all subunits are depicted. The catalytic sub complex is composed of APC2 and APC11 which contain the Cullin and RING domain respectively and APC10/Doc1 which is the subunit that identifies and recruits specific substrates. The scaffolding subcomplex contains APC1, APC4 and APC5 and holds the catalytic subcomplex and the specificity arm together. The specificity TPR arm is defined by APC3/Cdc27, APC6/Cdc16 and APC8/Cdc23 and delineates a binding surface for APC/C coactivators Cdc20 and Cdh1. These coactivators direct the recruitment of substrates to the complex.*

***Figure 2**. Structural architecture of the APC/C assembly bound to the Mitotic Checkpoint Complex (MCC) as defined by cryo-EM methods (pdb ID 5KHU). The labels indicate the position and copy number of APC/C subunits, coactivators and MCC proteins.*

***Figure 3**. Association of APC/C dysregulation in Alzheimer's disease. (A) Inactivation of APC/C-Cdh1 causes an upregulation in APC/C substrate Cyclin B1 that allows an erroneous re-entry in the cell cycle which leads to subsequent neurodegeneration. (B) In normal state neuron, APC/C-Cdh1 activity is high and APC/C substrate 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, isoform 3 (PFKFB3) is low. Glycolysis is switched off and the pentose phosphate pathway is used which in turn reduces glutathione that subsequently detoxifies Reactive Oxygen Species (ROS) and maintains the antioxidant state. In an Alzheimer's neuron, APC/C-Cdh1 activity is low and APC/C substrate PFKFB3 is high. The pentose phosphate pathway is switched off and glycolysis is used which in turn*

oxidises glutathione and leads to a reduced antioxidant state and subsequent neuronal death. (C) APC/C-Cdh1 downregulation causes an increase in APC/C substrate glutaminase a critical precursor for synthesis of the neurotransmitter glutamate. The resulting high levels of the latter amino acid overstimulates the N-methyl-D-aspartate (NMDA) receptor, which subsequently leads to an increase in Ca2+ thus causing excitotoxicity and neuronal death.

Figure 4*. Proposed mechanisms of human APC/C manipulation by viruses. (A) Human T cell lymphotropic virus type 1 (HTLV-1) Tax interacts with APC/C-Cdh1 and APC/C-Cdc20, prematurely activating the APC/C during S phase. Despite these advances the mechanism of action remains elusive. (B) Hepatitis B virus (HBV) HBVX downregulates Cdh1, impairing APC/C-Cdh1 proteolytic machinery and correlates with an unscheduled entry into S phase. (C) Orf virus (ORFV) poxviral anaphase promoting complex/cyclosome regulator (PACR) inhibits the APC/C by acting as a non-functional mimic of APC11 and is incorporated into the APC/C in place of APC11. (D) Human papillomavirus (HPV) E2 directly interacts with Cdh1 and Cdc20 and redistributes Cdh1 to insoluble cytoplasmic aggregates, inhibiting the APC/C through denying the complex access to its coactivator. (E) Human papillomavirus (HPV) E6 and E7 causes elevated Cdc20 levels resulting in impulsive APC/C activation. (F) Human papillomavirus (HPV) E7 upregulates APC/C inhibitor Emi1, impeding APC/C function and subsequently APC/C substrate degradation, resulting in a prometaphase delay. (G) Chicken Anemia Virus (CAV) Apoptin interacts with APC1 and relocalises the APC/C to promyelocytic leukaemia (PML) bodies causing degradation of APC/C subunits and subsequent APC/C inactivation. (H) Adenovirus (Ad) E1A competes with APC5/APC7 in binding CBP/p300, therefore disrupts the formation of the APC/C-CBP/p300 complex and consequently impedes the complex's functions promoting S-phase entry and aberrant mitoses. (I) Adenovirus (Ad) E4orf4 can have both stimulatory and inhibitory effects on the APC/C in a PP2A-dependent manner, however the binding site and mechanism of action remains elusive. (J) Human cytomegalovirus (HCMV) pUL97 induces phosphorylation of Cdh1, impeding Cdh1 interacting with the APC/C resulting in APC/C inactivation. (K) Human*

cytomegalovirus (HCMV) pUL21a induces APC4 and APC5 proteasomal degradation, resulting in APC/C disassembly and subsequent APC/C inactivation.
