Review

How viruses affect the cell cycle through manipulation of the APC/C

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Viruses frequently exploit host cell cycle machineries for their own benefit, often by targeting 'master switches' of cell cycle regulation. By doing so, they achieve maximum effect from minimal input. One such master switch is the anaphase promoting complex or cyclosome (APC/C), a multicomponent ubiquitin ligase and a dominant regulator of the cell cycle. A growing number of viruses have been shown to target the APC/C. Although differing strategies are employed, viral manipulation of the APC/C seems to serve a common purpose, namely, to create an environment supportive of viral replication. Here, the molecular mechanisms employed by these viruses are summarized and discussed.

Viral targeting of the cell cycle

As obligate intracellular parasites, viruses require an infected cell to provide an environment conducive to their replication including resources enabling extensive viral genome synthesis. Often this involves some viral manipulation of cell cycle regulation. The cell cycle can be divided into four phases (Figure 1). The orderly progression through these phases is driven by oscillating levels of cyclin-dependent kinase (Cdk) activity, which in turn reflect the accumulation and abrupt proteolysis of cyclins, the activating subunits of Cdks. The degradation of cyclins and many other proteins involved in cell cycle transitions occurs via the ubiquitin–proteasome pathway [1]. The formation of polyubiquitin chains that tag a protein for proteasomal degradation arises from the sequential activity of three enzymes (Figure 2), with substrate specificity being determined by the last enzyme in the sequence, the ubiquitin ligases. The APC/C is a multicomponent ubiquitin ligase that has attracted much interest due to its crucial roles in many aspects of cell biology, but especially in cell cycle regulation [2]. A growing number of viruses have been shown to target the APC/C for their own replication benefit.

Regulation of the APC/C

The APC/C is active from mid-mitosis through to late G1 phase and thereby regulates both cell division and the timing of re-entry into S phase by targeting key cell cycle regulators such as cyclin B, cyclin A, and E2F1 for proteasome degradation in an orderly coordinated fashion (Figure 2) [2,3]. Human APC/C, with a molecular mass of 1.5 MDa, is composed of at least 12 subunits with the catalytic core formed by APC11, a RING-H2 protein, and APC2, a cullin-like protein (Figure 2). Substrate recruitment is mediated at least in part by either of two adaptor proteins, Cdh1 and Cdc20 [4]. Subsequent ubiquitination of substrates is facilitated by the recruitment of ubiquitin-charged ubiquitin conjugating enzymes (E2) through the APC11/2 module [5,6].

APC/C^{Cdc20} is active from early metaphase to telophase facilitating mitotic exit. In human cells, although APC/C^{Cdc20} is formed in the G2 phase, the APC/C activity is suppressed by the APC/C inhibitors such as Emi1, RASSF1A, Rae1–Nup98, and the spindle assembly checkpoint (SAC) until the SAC is satisfied (Figure 3a) [7–11]. Emi1, a pseudosubstrate of the APC/C, binds and inhibits the APC/C activity by blocking the interaction of Cdc20 and Cdh1 with the APC/C [9]. RASSF1A suppresses the APC/C activity by binding to Cdc20, thus blocking its interaction with the APC/C (Figure 3a) [8,12]. Inhibition of APC/C^{Cdh1} is also achieved by the Rae1–Nup98 complex, which binds APC/C^{Cdh1} and inhibits its activity through an unknown mechanism [10]. The crucial SAC, activated by a lack of interactions between microtubules and kinetochores, further inhibits APC/C^{Cdc20} by forming the mitotic checkpoint complex (MCC) (Figure 3a), a potent APC/C inhibitory complex that 'locks' APC/C^{Cdc20} in an inactive state [13]. Collectively, these inhibitors suppress the APC/C's activity, allowing crucial proteins to accumulate and ensuring mitotic progression. Once the SAC is satisfied, APC/C^{Cdc20} is permitted to direct the degradation of mitotic proteins such as cyclin B, facilitating mitotic exit.

APC/C^{Cdh1} is formed in late mitosis and maintains G1 phase by sustaining low levels of critical proteins required for S phase entry, such as cyclin A and Skp2 [14,15]. It must be switched off for cells to progress into S phase. Suppression of the APC/C activity at the G1/S phase transition is achieved by a highly coordinated self-regulatory loop [16]. Ubc10, a ubiquitin-conjugating (E2) enzyme responsible for the initial stages of ubiquitin chain formation on the APC/C substrates, and Cdh1 are ubiquitinated and degraded toward the end of G1 (Figure 3b) [17–20]. Meanwhile, Skp2 levels begin to rise and contribute to activation of cyclin A–Cdk through the ubiquitination and degradation of Cdk inhibitors. Consequently, Cdh1 is phosphorylated by Cdk2–cyclin A, resulting in export of Cdh1 to the cytoplasm and consequent APC/C suppression (Figure 3b) [16]. Furthermore, Cdk2–cyclin A enhances transcription of Emi1, a key APC/C inhibitor, which acts as a pseudosubstrate that binds APC^{Cdh1} but is not ubiquitinated.

Synthesis of the deoxynucleotide triphosphates (dNTPs) required for DNA replication is ensured by the stabilization of the APC/C substrates ribonucleotide reductase subunit

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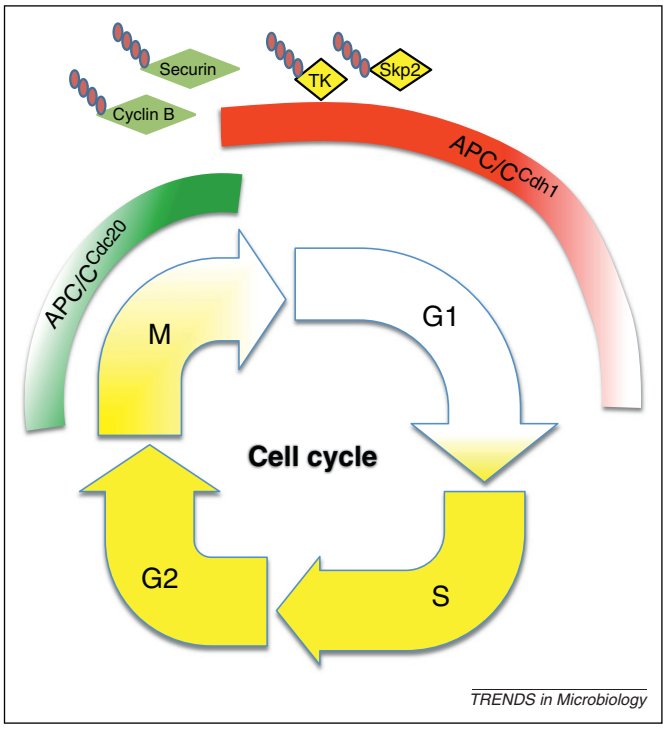

Figure 1. Overview of cell cycle regulation by the anaphase promoting complex or cyclosome (APC/C). The four phases of the cell cycle are shown as yellow arrows. In S phase a cell duplicates its genetic information via DNA replication, and in mitosis (M phase) this genetic information is distributed equally to two daughter cells. The gap phases between these two phases are called G1 and G2. Progression through the cell cycle is driven by oscillating activity of cyclin-cyclin-dependent kinase (Cdks). Cyclin-Cdk activity in each cell cycle phase is represented by a yellow color gradient where the intensity of the color corresponds to enzyme activity. Heightened cyclin-Cdk activity is needed to drive cells into S and M phase whereas low cyclin-Cdk activity is required to drive cells out of M and maintain G1 phase. Two forms of the APC/C exist in cells: APC/C^{Cdc20} and APC/C^{Cdh1}. The timing of activity of the two forms is indicated by green and red bars, respectively, with activity levels corresponding to the intensity of color. APC/C^{Cdc20} is formed in late G2 and its activity is suppressed till the end of M phase. Toward the end of M phase, APC/C^{Cdc20} activity peaks and facilitates M phase exit by ubiquitinating key mitotic proteins such as cyclin B and securin. APC/C^{Cdh1} forms in late M and its activity influences G1 and S phase. High APC/C^{Cdh1} activity is crucial in maintaining G1 phase by targeting proteins such as Skp2 whereas low APC^{Cdh1} activity is needed for S phase entry. APC/C^{Cdh1} activity also influences S phase DNA replication by controlling DNA metabolism through targeting enzymes such as the thymidine kinase (TK).

2 (R2), thymidine kinase 1 (TK), and thymidylate kinase (TMPK) due to the APC/C inhibition at G1/S transition [21–23]. Moreover, the heightened Cdk2–cyclin A activity and stabilization of the pre-replication complex (pre-RC) inhibitor Geminin, a substrate of the APC/C, suppresses further loading of pre-RC onto the origin of DNA replication, ensuring replication of DNA occurs only once per cell cycle [24,25].

In summary, predominant ways of inhibiting the APC/C include phosphorylation, blocking the binding of adaptors to the APC/C, forming an inactive complex with the APC/C, and degradation of proteins essential for the APC/C activity. Another means of inhibiting the APC/C is exemplified by Emi2, which associates with the APC/C and blocks transfer of ubiquitin from ubiquitin-charged E2 [26].

Viruses target the APC/C

As described above, the timely activation and inactivation of the APC/C occurs through complex and intricate controls. Deregulation of these events frequently leads to a defective G2 DNA damage checkpoint, loss of control of genome replication, aberrant M phase exit or premature G1/S phase transition, and can result in genomic instability [27]. The APC/C has also been shown to associate with transcription regulatory machineries such as pRB and CBP/p300 [28,29]. Therefore, manipulation of the APC/C has the potential to profoundly effect cell cycle regulation and other key aspects of cell physiology. These properties mark the APC/C as an attractive target for viruses in their battle to create an intracellular environment supportive of viral replication.

Human cytomegalovirus (HCMV)

HCMV is a large double-strand DNA virus capable of infecting terminally differentiated cells [30]. HCMV induces a cellular environment favorable for viral replication, in part by expression of immediate early (IE) proteins, such as IE72 and IE86 that disable pRB-family proteins with the consequent expression of E2F-responsive S-phase genes [30]. HCMV also expresses factors that interrupt the APC/C function and even disassemble the complex [31,32]. The inhibition appears to be partly due to the activity of a viral kinase, UL97, that phosphorylates Cdh1, a modification which in uninfected cells is known to inhibit Cdh1 binding to the APC/C [31,33]. In addition, the APC3 subunit was found to redistribute to the cytoplasm of infected cells whereas APC1 was retained within the nucleus and APC4 and APC5 underwent proteasome-mediated degradation (Figure 4) [32]. The viral factors responsible for this disassembly and partial degradation remain unknown. The retention of APC1 in the nucleus might indicate the need for this subunit of the APC/C in viral replication. Indeed, different localization patterns of the APC/C subunits have been observed during mitosis, suggesting different subunits of the APC/C might function independent of the APC/C [34].

HCMV does not express TK or TMPK, both of which are substrates of the APC/C, and are required for DNA metabolism [31]. Therefore, by disrupting the APC/C, HCMV may increase the availability of these cellular enzymes and obtain crucial dNTPs for its own DNA synthesis. Furthermore, at least in part by targeting the APC/C, HCMV manipulates quiescent cells into a pseudo-S phase supportive of viral replication while at the same time blocking cellular DNA synthesis through the expression of other viral factors [35].

Human papillomavirus (HPV) E2

HPVs are small DNA tumor viruses that exclusively infect epithelial cells of the skin or mucosa, inducing hyperproliferative lesions. They are classified into high risk types that are associated with cervical cancer and low risk types that form benign growths. Productive replication with high-level amplification of the viral genome occurs in differentiated, non-dividing cells and depends almost entirely on host factors, requiring the virus to manipulate the host cell into providing an environment suitable for DNA replication. The HPV E2 protein has well established roles as a viral transcription and replication factor. Bellanger and colleagues showed that high risk HPV E2 also disrupts the APC/C function by interacting with Cdh1 and Cdc20, inducing G2/M arrest and eventually metaphase-specific apoptosis [36]. Genome instability was