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REVIEW ARTICLE

Virus manipulation of cell cycle

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Abstract Viruses depend on host cell resources for replication and access to those resources may be limited to a particular phase of the cell cycle. Thus manipulation of cell cycle is a commonly employed strategy of viruses for achieving a favorable cellular environment. For example, viruses capable of infecting nondividing cells induce S phase in order to activate the host DNA replication machinery and provide the nucleotide triphosphates necessary for viral DNA replication (Flemington in J Virol 75:4475–4481, 2001; Sullivan and Pipas in Microbiol Mol Biol Rev 66:179–202, 2002). Viruses have developed several strategies to subvert the cell cycle by association with cyclin and cyclin-dependent kinase complexes and molecules that regulate their activity. Viruses tend to act on cellular proteins involved in a network of interactions in a way that minimal protein–protein interactions lead to a major effect. The complex and interactive nature of intracellular signaling pathways controlling cell division affords many opportunities for virus manipulation strategies. Taking the maxim "Set a thief to catch a thief" as a counter strategy, however, provides us with the very same virus evasion strategies as "ready-made tools" for the development of novel antivirus therapeutics. The most obvious are attenuated virus vaccines with critical evasion genes deleted. Similarly, vaccines against viruses causing cancer are now being successfully developed. Finally, as viruses have been playing chess with our cell biology and immune responses for millions of years, the study of their evasion strategies will also undoubtedly reveal new control mechanisms and their corresponding cellular intracellular signaling pathways.

Keywords Viruses · Cell cycle · Cyclins · DNA damage · Nucleolin

Introduction

Viruses are intracellular parasites which have evolved multiple mechanisms to manipulate host cell biology and immune defense responses. They use the host cell machinery and metabolism for their establishment and propagation.

For a virus to survive, it must continuously infect susceptible hosts. Once established in the host, a virus can either cause an acute infection or pass from acute to persistent with periodic reactivation. If, during an acute infection, the virus is cleared by the host immune response, then it must have the capacity to rapidly infect a new host or to survive in the environment. Viruses producing only acute infections can survive in nature by constant infection of the same host, for example mumps, or by infecting more than one host species, like *rabies*. Some acute viruses, such as poxviruses, have the capacity to survive in the environment outside an organism until they contact a susceptible host.

A wide variety of viruses, both RNA or DNA type, can establish long-term persistent infections, typically starting as acute infections which then progress to latent or persistent forms with periodic reactivation and transmission to new hosts. Persistent viruses are a major cause of disease in man; for example the human immunodeficiency virus (HIV) responsible for the acquired immune deficiency syndrome or the wide variety of human herpesviruses

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responsible for infectious mononucleosis, shingles, buccal and genital sores, and Kaposi's cell sarcoma. These are only two of the many examples of diseases caused by virus persistence, the study of which is a priority area in virology.

For a virus to persist in the host, it must be able to maintain its viral genome in the host without creating a cytopathic effect and without being detected by the many mechanisms of the host immune system, yet, at the same time, maintaining a strategy for transmission to another host. Virus persistence in a noninfectious form between episodes of viral reactivation and shedding of infectious virus is named latency, with herpesviruses providing typical examples. In contrast to latency, there other forms of persistence where there is a continuous cycle of productive infection and reinfection, with alternating cycles of viral persistence and production of infectious virus (Redpath et al. 2001; Sullivan and Pipas 2002).

Whatever strategy a virus employs for its propagation, all viruses have evolved appropriate and complementary mechanisms for the manipulation and subversion of the biology of the infected cell and the many defensive mechanisms of the host innate and acquired immune system. Indeed, the coevolution of virus and other pathogens and their hosts has shaped the immune system. This in turn has provided the selection pressure for the development of more pathogen strategies for evading host defenses (Vossen et al. 2002). Thus the complexity of our immune system reflects its coevolution with evolving pathogen host evasion strategies. Basically, therefore, the study of these strategies not only provides novel approaches for the control of viruses and other pathogens but can also lead to discoveries in our cell biology and immune system. For example, normal cells possess elaborate pathways that receive and process growth-stimulatory or growth-inhibitory signals transmitted by other cells in the tissues or organism. Much of what we know about these pathways comes from the study of the cellular genes activated or transduced by viruses.

Manipulation of the host cell cycle is a frequent virus strategy for host evasion, presumably in order to achieve a cellular environment favorable for their replication. For example, small DNA viruses capable of infecting nondividing cells induce S phase in order to activate and utilize the host DNA replication machinery. In contrast, herpesviruses encode their own DNA polymerase and accessory factors and do not require the environment of an S phase for viral replication (Flemington 2001; Lu and Shenk 1999; Song et al. 2000; Sullivan and Pipas 2002; Sunil-Chandra et al. 1992). An important aspect of the effects of viruses on cell cycle dynamics are the consequences for neoplastic transformation. This has been a major area of research, as it offers a rational approach to the control of virus-associated cancers.

The complex and interactive nature of intracellular signaling pathways controlling cell division affords many opportunities for virus manipulation strategies. Taking the maxim “Set a thief to catch a thief” as a counter strategy, however, provides us with the very same virus evasion strategies as “ready-made tools” for the development of novel antivirus therapeutics. The most obvious are attenuated virus vaccines with critical evasion genes deleted. Similarly, vaccines against viruses causing cancer are now being successfully developed. Finally, as viruses have been playing chess with our cell biology and immune responses for millions of years, the study of their evasion strategies will also undoubtedly reveal new control mechanisms and their corresponding cellular intracellular signaling pathways. This chapter will review the essentials of the cell cycle and focus on host evasion mechanisms for cell cycle manipulation evolved by viruses, in particular herpesviruses.

The cell cycle

The eukaryotic cell cycle is operationally divided into four phases: G1, S, G2, and M. The G1 phase is the first *gap* during which cells organize themselves prior to DNA replication. Decisive events during G1 phase determine whether the cell proceeds to division, pauses, or exits the cell cycle and enter the programmed cell death pathway. The S phase is the stage at which DNA synthesis, and hence duplication of the genome, occurs. During the second *gap*, or G2, the cell prepares for the process of mitosis, and the associated cell division, when the replicated chromosomes are segregated into separated nuclei and cytokinesis, occurs to form two daughter cells. Once again, this second gap phase provides an opportunity for vigilance, such as recognition and repair of damaged DNA. Thus progression to DNA replication and mitosis is signaled by the intracellular checkpoints at the G1 and G2, respectively.

Regulation of cell division is critical for the normal development and maintenance of multicellular organisms. Loss of control of cell division ultimately leads to cancer, killing many people every day. Although the basic elements controlling the eukaryotic cell cycle were first described for yeast in the late 1980s, it became clear that the essential molecular processes are similar in all eukaryotic cells, from yeast to mammals. The understanding of this basic mechanism, coupled with research with diverse organisms, each with its own particular experimental advantage, has led to a better understanding of how the molecular events required for cell division are controlled and coordinated.

The key element in the regulation of the eukaryotic cell cycle is the periodic synthesis and destruction of cyclins, proteins that associate with and activate cyclin-dependent kinases (Cdks). The related sequential activation and inactivation of cyclin-dependent kinases provide the basis

for cell cycle regulation. At least 16 cyclins and nine Cdks have been identified in mammalian cells (Johnson and Walker 1999). All cyclins contain a homology domain known as the "cyclin box," which functions to bind and activate Cdks. However, not all cyclins and Cdks necessarily regulate the cell cycle. Other functions, such as regulation of transcription, DNA repair, and apoptosis, have been attributed to them (Johnson and Walker 1999).

In addition to their interaction with cyclin, other levels of regulation also exist for controlling the activity of Cdks during the cell cycle. For example, the cyclin-dependent kinase inhibitors (CdkIs) coordinate internal and external signals, thereby impeding proliferation at several key points, leading to both positive and negative regulation of kinase activity. In addition, it is now clear that ubiquitin-mediated proteolysis plays a crucial role in cell cycle control by targeting cyclins and other regulators for destruction at critical time points during the cell cycle. The irreversibility of proteolysis provides a strong directionality to the cell cycle forcing it to go forward at several critical points (Kastan and Bartek 2004).

Progression through the cell cycle

The levels of D cyclins, which associate with and activate Cdk4 and Cdk6, are regulated by growth factors such as epidermal growth factor or insulin-like growth factor I. The sequential activation of the two kinase complexes, Cdk4/6–cyclin D and Cdk2–cyclin E, is the key event that leads to cell cycle progression (Fig. 1). These activated complexes phosphorylate the retinoblastoma protein (pRB), first by the Cdk4/6–cyclin D complex and subsequently by the Cdk2–cyclin E complex. Phosphorylation of the Rb protein causes its dissociation from E2F, allowing the activation of proteins leading to progression into the S phase. This Cdk2–cyclin E complex participates in the maintenance of pRB in the hyperphosphorylated state and is involved in a positive feedback loop for the accumulation of active E2F. In addition, the Cdk2–cyclin E complex also phosphorylates other substrates since cells deficient in Cdk2–cyclin E still require an active cyclin complex (Lukas et al. 2004). Like other Cdk–cyclin complexes, Cdk2–cyclin E phosphorylates histone H1, which is important for the chromatin rearrangement required during replication of the genome. Another cyclin, cyclin A, is in part regulated by E2F, accumulating at the G1/S transition and persisting through S phase (Fig. 2). Cyclin A initially associates with Cdk2 and then, in late S phase, associates with Cdk1 (cdc2). The kinase activity of the cyclin A complexes is needed for entry into S phase, completion of S phase, and entry into M phase. The cyclin A-associated kinases phosphorylate the E2F heterodimerization partner DP1, resulting in an inhibition of E2F DNA binding activity. Thus, whereas cyclin E positively regulates E2F activity, cyclin A participates in a negative feedback loop for E2F regulation (Fig. 1).

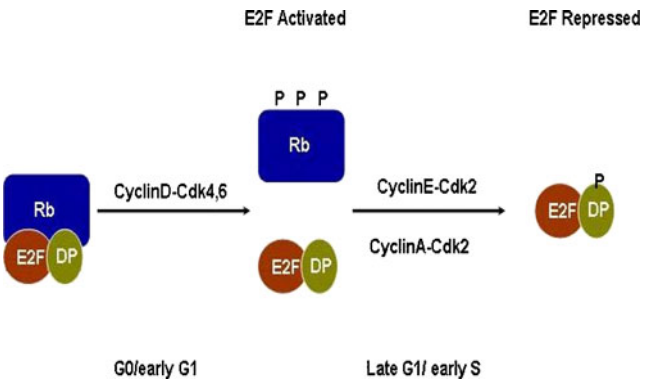

Fig. 1 Progression through the cell cycle. Viruses examples that modulate this pathway included in the text are HTLV-I, HPVs, KSHV, EBV, and HCV

Virus modulation of the cell cycle

Viruses have evolved mechanisms for the manipulation of the host cell cycle in order to benefit their replication; for example, the synthesis of proteins that associate with cyclin and cyclin-dependent kinase complexes (cyclin–Cdk), and/or molecules that regulate their activity. Typically, viruses have evolved proteins with the emphasis or "economy" so that minimal protein–protein interactions lead to a major effect on intracellular signaling networks.

The impact of viruses on cell cycle dynamics and their consequences for neoplastic transformation has been a

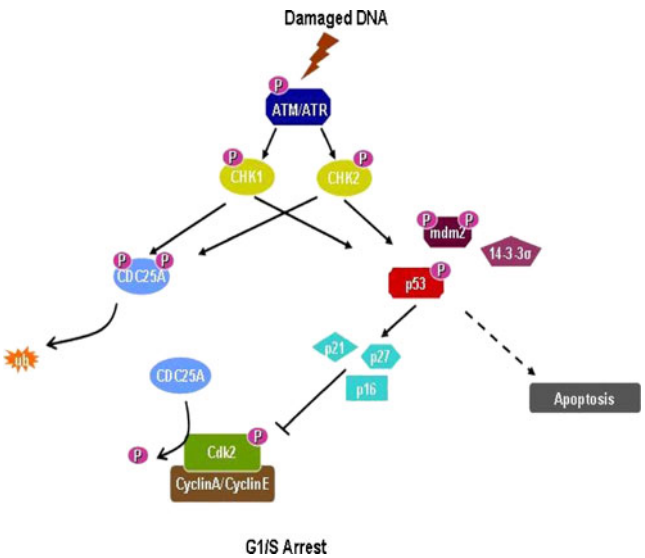

Fig. 2 A simplified scheme of cell cycle G1/S checkpoint induced in response to DNA damage. Viruses that modulate this pathway include the HPVs, HSV-1, and HCMV

major area of research. Indeed, development of anticancer vaccines directed at viruses associated with tumorigenesis, such as papilloma (Knox and Shannon 1988), is a very positive area of research.

Human T-cell leukemia virus type I (HTLV-I) is associated with malignancies characterized by an excessive proliferation of T cells. The transcription transactivator factor Tax, of the HTLV-1, agent of adult T-cell leukemia, interacts with Cdk4 and Cdk6 enhancing binding to cyclin D2. This binding leads to an accumulation of phosphorylated pRB accelerating S phase entry (Iwanaga et al. 2008). However, recent studies suggest that Tax expression is not sufficient for a sustained and active proliferation in the context of HTLV-I infection. In fact, HTLV-I p30 interacts with cyclin E, reducing its ability to form the functional Cdk2–cyclin E complex and so delays the cell cycle before entry into the S phase (Baydoun et al. 2010). This dual mechanism to ensure that S phase arrest is typical of many virus evasion strategies is in fact a "belt and braces" approach. Another strategy has been evolved by human papilloma virus (HPV), responsible for one of the most common sexually transmitted diseases, and uterine cervical cancer. Certain strains of HPV associated with a higher risk of cervical cancer encode several proteins that promote cell transformation, one being the E7 protein. The interaction of E7 with pRB, being analogous to Cdk-mediated phosphorylation, results in release of active E2F and stimulation of S phase entry (Gatza et al. 2005). While viruses capable of infecting nondividing cells induce S phase in order to activate the host DNA replication machinery and thus provide the nucleotide triphosphates necessary for viral DNA replication, many large DNA virus, such as herpesviruses, code for their own viral DNA polymerase and do not require S phase environment to support viral replication (Flemington 2001; Sullivan and Pipas 2002).

Several gammaherpesviruses code for a viral cyclin (v-cyclin) with homology to the cellular D-type cyclin (Upton et al. 2005; van Dyk et al. 1999; Verschuren et al. 2004). Reminiscent of cellular cyclins, v-cyclin interacts with, and thus activates, the Cdk4 and Cdk6 kinases. Although the v-cyclin is functionally similar to cellular cyclin, it is resistant to inhibition by p21 and p16 and is not regulated by the cyclin-inhibitor kinase (CdkIs) as are the cellular Cdks (Direkze and Laman 2004). Herpesviruses have evolved highly sophisticated interactions with host cell cycle machinery in a way to support efficient viral replication. They not only modulate the cell cycle at a precise point in the cycle that favors viral replication but also modulate the levels of Cdk–cyclin function to specific levels to support efficient viral DNA replication (Flemington 2001).

Human oncogenic herpesviruses such as Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are closely linked to a variety of malignancies including nonkeratinizing nasopharyngeal carcinoma, gastric adenocarcinoma, Burkitt's lymphoma, Kaposi's sarcoma, primary effusion lymphoma, multicentric Castleman's disease, and various forms of lymphoproliferative disorders. Both EBV and KSHV are latent residents in B lymphocytes and show sporadic reactivation in lymphoepithelial tissues. EBNA3C, one of the EBV-encoded latent antigens, is essential for primary B-cell transformation. Previously, EBNA3C was shown to bind to cyclin D1 in vitro along with cyclin A and cyclin E (Knight and Robertson 2004). Recently it was demonstrated that EBNA3C forms a complex with cyclin D1 in human cells stabilizing cyclin D1 through inhibition of its polyubiquitination (Saha et al. 2011). The EBNA3C (Planelles et al. 1996) together with Cdk6–cyclin D1 complex also efficiently nullifies the inhibitory effect of pRB on cell growth. Thus EBNA3C can stabilize as well as enhance the functional activity of cyclin D, thereby facilitating the G1–S transition in EBV-transformed lymphoblastoid cell lines.

There are several examples of genes expressed during latency by gammaherpesviruses which have a strong cell cycle promoting activity and thus potential role in the induction of the neoplastic Kaposi's tumor. One candidate for KSHV tumorigenesis is the viral latency-associated nuclear antigen (LANA-1). This viral antigen is responsible for ensuring equal segregation of host chromosomes and viral episome into daughter cells, but also interacts with pRB leading to an activation of E2F and progression of cell cycle (Moore and Chang 1998).

While some viral factors activate proteins that normally promote cell cycle progression, others elicit cell cycle arrest. For example, the hepatitis C virus (HCV) as an RNA virus also modulates the host cell cycle progression. Recently, the HCV RNA-dependent RNA polymerase, NS5B, was demonstrated to induce cell cycle delay in the S phase through interaction with a new host protein, the cyclin-dependent kinase 2-interacting protein (Wang et al. 2011). This is an interesting example of how the study of cell cycle manipulation by viral genes can also contribute to our basic understanding of cell biology and illustrates how the identification of novel virus strategies affecting different cell cycle phases and checkpoints may lead to identification of key alternative cellular regulatory factors.

Viral modulation of cell cycle checkpoints

Cells can temporarily arrest at cell cycle checkpoints to allow for the repair of cellular damage, the dissipation of an exogenous cellular stress signal or the absence/availability of essential growth factors, hormones, or nutrients. Checkpoint arrest may also result in activation of pathways leading to programmed cell death if cellular damage cannot be precisely repaired. There are two checkpoints in the cell

cycle, G1/S and G2/M. The mechanisms for halting cell cycle progression at the G1/S and G2/M checkpoints are generally conserved and unique, although many phosphatases and kinases are shared between the two checkpoints.

Damage to DNA is a common occurrence through exposure to a variety of environmental stresses, such as exposure to abnormally low levels of oxygen or nutrients, and constant attacks to DNA from external and internal agents that can directly damage DNA. Cells can respond directly to DNA damage by repairing DNA breaks or alternately by halting cell cycle progression and/or by undergoing programmed cell death (apoptosis). Extensive damage, as it generally leads to apoptosis, is less of a problem than limited damage which can have potentially disastrous consequences, for example cancer, and so eukaryotic organisms have evolved mechanisms to sense and respond to infidelity in DNA replication. Although cells use different signaling pathways to deal with different microenvironment stresses, there are common elements, such as the "sensor" molecules, stimulated in response to DNA damage (Lukas et al. 2004).

The G1/S checkpoint

Entry of cells with damaged DNA into S phase is prevented by activation of the two transducing kinases, ATM/ATR and Chk1/Chk2, which then target the CDC25A phosphatase and the p53 transducing molecule, thus activating two distinct branches of the G1 checkpoint, respectively. Despite the fact that activation of both branches occurs simultaneously, the CDC25A pathway has a faster inhibitory impact on the cell cycle machinery, probably because the CDC25A cascade is dependent on phosphorylation and desphosphorylation events and does not require transcription or accumulation of newly synthesized proteins (Lukas et al. 2004). The kinases Chk1 and Chk2 phosphorylate multiple serine residues of the phosphatase CDC25A prior to its enhanced ubiquitination and consequent proteosome-mediated degradation. As CDC25A dephosphorylates and activates Cdk2, the catalytic subunit of the Cdk2–cyclin E and Cdk2–cyclin A kinases, in the absence of these kinase complexes, there is no loading of CDC45 onto chromatin and thus no initiation of DNA synthesis.

Activation of the Chk1/Chk2–CDC25A pathway delays G1/S transition for only a few hours, whereas a p53-dependent mechanism can prolong G1 arrest further. The important multifunctional regulator of cell division, p53, is not only phosphorylated, by Chk1/Chk2, but also by ATM/ATR kinases, particularly on serine 15. The ubiquitin ligase MDM2, which binds and ensures p53 turnover, is also inactivated by ATM/ATR after DNA damage. This and the ATM/ATR-mediated phosphorylation of p53 lead to an accumulation of p53. Association of p53 with p300 results in acetylation and increased transcription factor activity of p300. These multiple modifications of p53 are important in controlling the transcriptional activation program of genes involved in cell cycle arrest and/or apoptosis. The resulting decision to enter cell division or apoptosis is dependent on both qualitative and quantitative evaluation of the extent of DNA damage in the cell.

The most studied genes downstream of p53 induction are p21 for cell cycle arrest and Bax for apoptosis. However, many others, such as 14-3-3 sigma protein, GADD45, and FAS, are also involved in the p53-mediated induction of a variety of cellular responses. These, in turn, are controlled by specific signaling responsive elements in the DNA regulatory sequences of either p21, Bax, or other genes (Castedo and Kroemer 2002; Castedo et al. 2002; Yu and Zhang 2005). The direction of p53 signaling between the possible outcomes is determined by its availability and the affinity of its downstream interactions. Accumulation of p21 silences the G1/S progression, promoted by Cdk2–cyclinE kinase expression. This causes G1 arrest through failure to start DNA synthesis and through the preservation of the Rb/E2F pathway, in the active growth-suppressing mode. This mechanism complements and eventually replaces the transient inhibition of Cdk2 through the CDC25A degradation pathway, leading to a sustained cell cycle arrest in G1.

The small DNA human papillomaviruses are the causative agents of cervical cancer (Moody and Laimins 2010). They encode oncoproteins E5, E6, and E7, and as previously mentioned, E7 binds Rb leading to an inhibition of cell cycle progression and apoptosis through a p53-dependent pathway. In contrast, the E6 protein recruits the cellular E3 ubiquitin ligase E6-associated protein and p53 to a trimeric complex, leading to the ubiquitylation and proteasomal degradation of p53 (Camus et al. 2007). Any remaining undegraded p53 could, however, still be activated in response to cellular stresses. As a strategy to inhibit this, the viral E6 protein is also able to bind directly to p53 and block its transcription by interfering with its binding activity (Fu et al. 2010). This is one of many examples of multitasking viral genes impacting at multiple levels in a way to control one specific pathway of the host cell machinery.

Herpes simplex virus 1 (HSV-1), an alphaherpesvirus, codes for the immediate early transcription factor ICP0 that promotes cell cycle arrest by inducing the tumor suppressor p53 and its downstream target proteins (p53, p21, GADD45, and MDM2), even in the absence of p53; once again, an example of a multitasking virus host evasion mechanism activating more than one pathway to lead to cell cycle arrest (Hobbs and DeLuca 1999; Kawaguchi et al. 1997). The human cytomegalovirus (HCMV), a betaherpesvirus, similarly blocks the G1/S cell cycle transition, employing a mechanism involving at least two genes, the

tegument protein UL69 (Lu and Shenk 1999) and the immediate early IE2 protein (Wiebusch and Hagemeier 1999). In addition to promoting cell cycle arrest, HCMV also activates several factors that normally would induce cell cycle progression. Finally, it is important to stress that the interaction between herpesviruses and cell cycle regulatory mechanisms is complex, with some viral factors eliciting cell cycle arrest and others promoting cell cycle progression. Thus, herpesviruses induce cell cycle arrest in the precise cell cycle phase which most favors their replication, preventing competition with cellular DNA replication (Flemington 2001).

The G2/M checkpoint

Before cells enter mitosis, the G2 phase provides a delay in cell cycle progression to detect DNA damage and, if necessary, to instigate DNA repair. The G2/M checkpoint responds to DNA damage occurring during G2, or due to a failure to repair DNA in the previous G1/S phase, by inducing G2 arrest prior to entry into mitosis. Mechanistically similar to the G1 checkpoint, the G2 arrest is the result of a combination of rapid response mechanisms operating via posttranslational modifications of diverse proteins, and a more delayed and sustained mechanism that involves transcription.

The key downstream target of G2 arrest is the mitosis promoting kinase complex Cdk1–cyclin B. Depending on the type of DNA damage, the ATM-Chk2 signal transducing pathway and/or the ATR/Chk1 pathway is activated to arrest the cell cycle in G2. Activation of Cdk1–cyclin B is prevented by ATM/ATR and Chk1/Chk2-mediated sequestration and/or inhibition of CDC25C phosphatase, an enzyme that normally activates Cdk1 at G2/M transition. The inhibition of Cdk1 activity is achievable through phosphorylation of the inhibitory sites on tyrosine 15 and threonine 14 by the kinases Wee1/Myt1, by inhibiting specific phosphatases (CDC25C) that remove the phosphorylated residues, or by sequestering the Cdk1–cyclin B complex in the cytoplasm.

The CDC25C phosphatase is normally localized in the cytoplasm and translocates to the nucleus just before mitosis. When it is bound to 14-3-3 proteins, however, it remains cytoplasmic and is unable to translocate to the nucleus (Smits and Medema 2001). The region of CDC25C interacting with 14-3-3 proteins contains a phosphorylation site, serine 216, that is not phosphorylated during mitosis. The increased phosphorylation of CDC25 observed during G2 arrest prevents its translocation to the nucleus and thus the subsequent activation of Cdk1–cyclin B (Fig. 3). Both Chk1 and Chk2 kinases are able to phosphorylate CDC25C on serine 216 and, although structurally unrelated, are functionally overlapping serine/threonine kinases that are

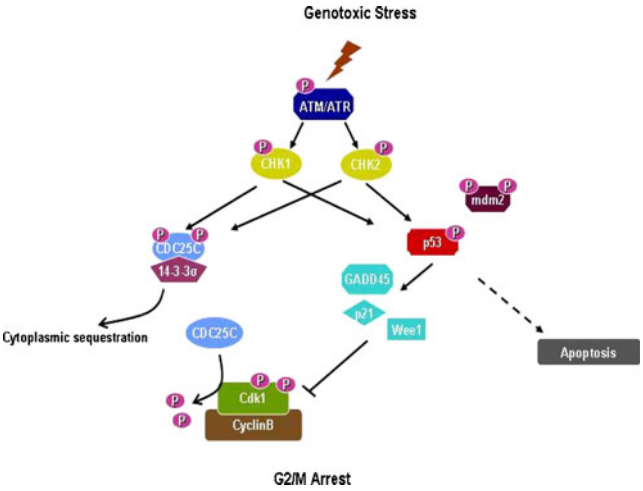

Fig. 3 A simplified scheme of cell cycle G2/M checkpoint induced in response to genotoxic stress (example: UV or IR). Viruses that modulate this pathway are HPVs, HIV-1, HHV-6A, HHV-6B, HCMV, and B19V

activated after DNA damage and transduce signals received from the ATM/ATR kinases to the cell cycle machinery. The ATM/ATR kinases not only phosphorylate Chk1 and Chk2 but also p53 on its serine 15. Phosphorylation of p53 following DNA damage prevents interaction with MDM2, thereby stabilizing p53 by preventing its ubiquitin-mediated degradation (Lukas et al. 2004; Pietenpol and Stewart 2002).

One mechanism that contributes to long-term silencing of the Cdk1–cyclin B complex is through the p53 pathway. This protein not only upregulates the Cdk inhibitor p21 at the G1 checkpoint but also GADD45 (growth arrest and DNA damage-inducible 45) and 14-3-3 sigma proteins. In addition, other upstream regulators of CDC25C and Cdk1–cyclin B, such as polo-like kinases and BRCA1, seem to be targeted to the G2 checkpoint by DNA damage. The fact that cells lacking p53 still accumulate in G2 after DNA damage indicates that additional pathways of induction of p21 and GADD45 may be mediated by other molecules, like BRCA-1.

Several viral inducers of G2/M cell cycle arrest have been described. Examples of such proteins include the E2 from human papilloma virus (Goodwin et al. 1998), adenovirus E4orf4 (Kornitzer et al. 2001), and the human immunodeficiency virus type I (HIV-1) vpr, which is conserved among the primate immunodeficiency viruses (Fukumori et al. 2000). In fact, cells infected with HIV-1 do not proliferate and their arrest by vpr in G2 phase is associated with an increase in virus production (Fukumori et al. 2000). The vpr protein acts as a bridging factor between the 14-3-3 and CDC25C, irrespective of the phosphorylation state of CDC25C (Kino et al. 2005).

Recent studies with HHV-6A, one of the less studied herpesvirus, demonstrated that T cells infected with HHV-

6A arrest at the G2/M phase due to the inactivation of the Cdk1–cyclin B1 complex and an increased expression of the p21 protein in a p53-dependent manner. The same report demonstrated an increased phosphorylation of the checkpoint kinases Chk1 and Chk2, which suggested that HHV-6A infection might activate the DNA damage response. As this activation was only observed at later time points (72 h postinfection), however, it is likely that the G2/M arrest is not a direct result of the activation of DNA damage-associated protein checkpoint kinases, but possibly due to a later event of virus infection, one that maintains the inactivation of the Cdk1–cyclin B1 complex and thus the subsequent cell cycle arrest (Li et al. 2011). Manipulation of the cell cycle was also described for HHV-6B (Øster et al. 2005), and for HHV-6A infection in cord blood mononuclear cells (De Bolle et al. 2004), but with different results, suggesting that the regulatory pathways and mechanisms induced by HHV-6 infection might be different according to the type of infected cells. For the HHV-6A virus, it has been suggested that G2 arrest serves to block the clonal expansion and proliferation of the anti-HHV-6 virus specific T cells (Li et al. 2011). It has also been reported that noncycling cells are relatively refractory to killing by cytolytic T cells (Nishioka and Welsh 1994) and so HHV-6 may employ a double strategy to avoid being killed by cytolytic T cells.

The discovery of viral proteins inducing G2/M arrest raises the pertinent question of how does this arrest facilitate viral replication. One plausible hypothesis is that viruses require an intact intracellular organization for their assembly and egress, and this is lost during mitosis.

DNA damage and viral replication

The checkpoint signaling cascade does not only lead to arrest of the cell cycle. It can also lead to activation of the pathway leading to programmed cell death. When the DNA damage can no longer be repaired, the response may vary between organisms. Unicellular organisms resume the cell cycle despite DNA damage. However in multicellular organisms, the health of the organism takes priority over an individual cell. The manipulation of the cell cycle by viruses is closely related to activation of the DNA damage response, including double-strand break repair pathways. Viral infection confronts the host cell with large amounts of exogenous genetic material that might be recognized as abnormal and damaged DNA and so may precipitate the premature apoptosis of the virus-infected cells (Weitzman et al. 2004). Thus, in order to establish a productive infection, it is essential that viruses defend themselves from the host cell DNA damage response machinery. Paradoxically, recent reports indicate that the DNA damage response may have a beneficial role in viral replication.

Simian virus 40 replication is dependent on ATM-mediated phosphorylation of large tumor antigen, an essential viral protein involved in viral replication (Shi et al. 2005). HPV infection also induces an ATM response in both undifferentiated and differentiated cells. Importantly, ATM kinase activity is required for viral genome amplification in differentiating cells but not for episome maintenance in undifferentiated cells. This suggests that activation of the DNA damage signaling response by HPV is tailored to different requirements, depending on the differentiation stage of the host cell (Moody and Laimins 2009). Infection with human parvovirus B19 (B19V), on the other hand, induces a broad range of DNA damage responses by phosphorylation of the three upstream kinases: ATM, ATR, and DNA-PKcs. Disruption of either the ATR or DNA-PKcs, but not ATM, signaling pathway significantly reduced the efficiency of B19V replication without affecting the cell cycle arrest characteristic of B19V infection, indicating that a DDR-independent checkpoint is responsible for the arrest of B19V-infected cells at the G2/M transition of the cell cycle (Luo et al. 2011). Adenovirus, however, has evolved mechanisms to inhibit DNA repair during infection, by degradation and mislocalization of the Mre11–Rad50–NBS1 complex, thus preventing activation of DNA damage checkpoints and viral DNA concatemerization. The model proposed is that the DNA damage response results in the masking of the origins of adenovirus DNA replication such that viral replication proteins are unable to gain access (Stracker et al. 2002).

During HCMV infection, the localization of various checkpoint proteins normally organized near the site of damage is altered, inhibiting their normal function. Thus, although HCMV infection results in phosphorylation of ATM and H2A.X and the downstream proteins Chk2 and p53, the DNA damage signaling pathway is disrupted due to “mislocalization” of checkpoint proteins (Gaspar and Shenk 2006). Although previously it was concluded that ATM is not relevant for HCMV replication (Luo et al. 2007), recent results indicate that the DNA damage response mediated by E2F1 transcription factor contributes to replication of HCMV (E et al. 2011).

It is still not clear if virus-induced DNA damage involves the recognition of existing double-strand breaks. The replication of viral DNA genomes, such as herpesviruses, is synthesized in a rolling circle manner to produce head-to-tail concatemers that are subsequently cleaved into unit-length genomes that may be recognized as double-strand breaks and trigger a DNA damage response (McVoy and Adler 1994). In the case of HCMV infection, although the mechanism of E2F1-induced DNA damage response is still unknown, the inactivation of Rb and subsequent deregulation of E2F1 results in double-strand breaks in human fibroblasts (Pickering and Kowalik 2006). Interest-

ingly, prolonged binding of DNA repair factors to chromatin can elicit DNA damage response in an ATM- and DNA-PK-dependent manner in the absence of DNA lesions (Soutoglou and Misteli 2008). Thus, it is possible that the trigger of virus-induced DNA damage response is not the recognition of viral DNA as double-strand breaks or actual damage to DNA, but it is the recruitment of DNA damage repair factors observed during viral infection.

Virus and nuclear structures affecting cell cycle

Viruses also target nuclear bodies inducing modifications in these nuclear substructures, including promyelocytic leukemia bodies, Cajal bodies, and nucleoli. The nucleolus is the best studied nuclear body to be described as a target of viral subversion of host cell cycle regulation. This nuclear body is formed around rRNA genes and has as its primary function ribosome biogenesis (Boisvert et al. 2007). The most abundant nucleolar proteins are nucleolin and B23, also called nucleophosmin, and these are involved in many functions. Recent studies reveal that nucleoli contain multifunctional proteins that play many roles in different cellular pathways (Andersen et al. 2005). There is a concurrent growth in the knowledge of nucleolus protein function and its relevance in viral infection. A recent publication has shown that nucleolin is required for efficient nuclear egress of HSV-1 nucleocapsids, although the mechanism by which nucleolin acts remain to be determined (Sagou et al. 2010).

Infection with HSV-1 results in dramatic alterations to nuclear structure and organization, including changes in the morphology of nucleoli (Besse and Puvion-Dutilleul 1996). In cells infected with HSV-1, staining for nucleolin reveals a diffuse distribution throughout the nucleus, in contrast to the large, prominent spots of nucleolin observed within the nucleus of mock-infected cells. During infection with HSV-1, UL24 family gene homologues are detected predominantly in the nucleus and transiently localize in the nucleoli (Hong-Yan et al. 2001; Nascimento and Parkhouse 2007; Pearson and Coen 2002; Wang et al. 2000, 2004). Expression of the N-terminal domain of UL24 is sufficient to induce the redistribution of nucleolin in the nucleus (Bertrand et al. 2010; Bertrand and Pearson 2008). However, cells infected with two independent UL24-deficient viruses, UL24XB and UL24XG, retain the prominent foci of nucleolin staining, although the pattern was not identical to the uninfected cells. This result indicates that this alteration of the nucleoli in HSV-1-infected cells is partially dependent on UL24 (Lymberopoulos et al. 2011). The fact that deletion of UL24 conserved homology domains, including the putative endonuclease motifs, resulted in loss of nucleolin and B23 dispersal activity suggests that this function may be shared among all herpesviruses and must be relevant for the viral life cycle (Bertrand et al. 2010; Bertrand and Pearson 2008; Lymberopoulos et al. 2011). Indeed, a study with UL24 homologues from human herpesviruses representative of each subfamily (HSV-1 UL24, HCMV UL76, and KSHV ORF20) showed that all these genes are able to induce cell cycle arrest followed by apoptosis. Expression of UL24 homologues results in an increase in Cdc2 phosphorylation increase at the Tyr-15 inactivation site and consequent inhibition of the mitotic complex cdc2–cyclin B (Nascimento et al. 2009). The mechanism by which this cell cycle arrest is induced or if the nucleolin is involved in this phenotype is not described. Extensive reviews on viral proteins impacting on nucleolus have been published. In conclusion, the multifunctionality of virus host evasion genes may provide important tools to unravel the complex interactions underlying the regulation of intracellular signaling.

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