The cell cycle overturned

Mechanisms by which viruses interfere with key components of the cell cycle progression

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Abstract

The cell cycle is regulated by a complex network of signaling pathways and molecular machineries aimed at the faithful division of the complete genome into two identical daughter cells. Errors in this regulation can lead to aberrant chromosome segregation and often cause a form of chromosomal instability (CIN). This cellular phenotype is frequently found in cancer and other malignancies and can arise through mutations in important regulatory tumor suppressor genes or the overactivation of oncogenes. Besides cellular oncogenes, numerous *viral oncogenes* have been described over the last decades. The targets of these viral proteins are often important regulators of mitosis. This viral influence on mitotic regulation can interfere with normal chromosome segregation, increasing the risk of CIN. This review discusses how viral proteins interfere with the cellular machinery that ensures faithful chromosome segregation, using a selection of examples. Viruses can deregulate cell cycle progression by interacting with proteins involved in cell cycle checkpoints or DNA repair, activating or inhibiting the anaphase promoting complex (APC/C), causing centrosome overduplication, (de)stabilizing microtubules, or disturbing cytokinesis. Examples are provided for each of these interactions, intermittently focusing on retroviruses and especially the Human Immunodeficiency Virus 1 (HIV-1). Implications are drawn for cancer research and virology, and a possibility for the use of CIN-inducing viral proteins as a mechanism to enhance oncolytic viral therapy is considered. The interplay between viruses and the cell cycle is an important field that requires more interdisciplinary efforts in research and can contribute greatly to our knowledge of the origins of cancer and possible treatments.

Stopping the cycle: using viruses to treat cancer

Writing assignment Infection & Immunity, plain language summary by Meira Zubčević

Viruses are extremely small microorganisms that can infect all forms of life. They cannot reproduce on their own but must hijack the machinery of living cells to multiply themselves. When a virus infects a cell, it often disrupts the cell's normal controls over its use of nutrients, the production of new substances, and more. In all these influences, the virus manipulates the cell to prioritize the production of more viruses over any cellular needs. This review focuses on a particular form of viral control over the host cell's growth and division, known as the cell cycle.

Some viruses can do this so effectively that the cell completely loses control over its growth. Incidentally, this is a key step in the development of cancer. Indeed, many viruses are known to be *oncogenic*, meaning that they can lead to cancer formation. One of the characteristics of cancer is *chromosomal instability*, which describes how the cell's chromosomes - the form that the cell's DNA has during cell division - are unequally divided after cell division. This has severe consequences for the cell's normal functioning, because it can lead to a loss or gain of gene products, creating more waste and possibly leading to uncontrolled cell division. Thus, chromosomal instability can be both a consequence and a cause of abnormal cell division and cancer.

In this review, I describe the different mechanisms by which viruses can disrupt the cell cycle and its regulation. For example, some viruses can directly damage the DNA and override the *checkpoints* that the cell has in place to halt the cell cycle until the damage is repaired. Other viruses interfere directly with the cell's machinery responsible for the equal division of all chromosomes. The link between viral infection, cell cycle disruption, and cancer is remarkable but also highly complex. It is important to develop a deeper understanding of these mechanisms, because they are at the basis of many different diseases, including cancer. Interestingly, it may be possible to use viral *oncogenic* mechanisms as a tool against cancer. Indeed, the use of (modified) viruses to specifically target and destroy cancer cells has already been investigated before. It led to the discovery of *oncolytic* viruses, which reproduce better in fast-dividing cells. The aim of *oncolytic virus therapy* is to kill the cancer cells by infection. However, complementing this with viral proteins that induce chromosomal instability may greatly improve this type of therapy. This would work as follows: although cancer cells have chromosomal instability, the level of this instability appears to be low enough for the cells to survive. Yet, when introducing a viral *oncoprotein* (that disrupts the cell cycle) to such cells, the level of chromosomal instability may become too high for the cells to survive. In this way, the viral protein destines the cancer cell to die through extreme chromosomal instability. It is therefore important to find more of these viral proteins and to test how they can be used most safely. Together with other anticancer therapies, such as immunotherapy, this novel virus-based intervention may successfully and selectively treat various kinds of cancer.

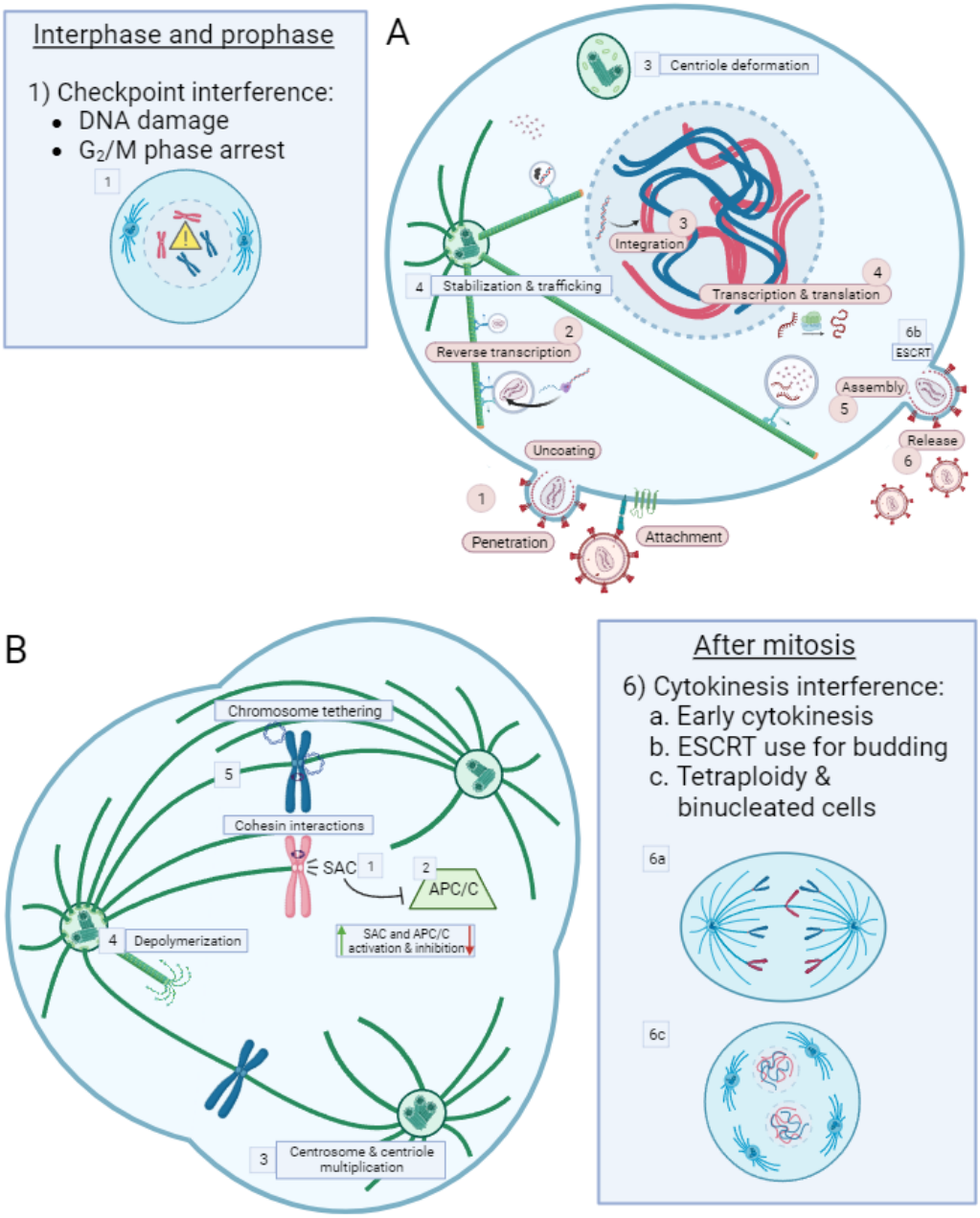

Figure 1: The influences of viruses on mitosis, an overview of the whole review.
The stages of viral infection are depicted in early prophase, but viral infection can also occur during interphase and mitosis. ***A)*** *The HIV-1 viral life cycle: (1) Viral entry, (2) Reverse transcription and trafficking to the nucleus, (3) Viral genome integration, (4) Transcription and translation of viral genes, (5) Assembly of the virion, (6) Viral exit/release by budding. The influences here include [1] checkpoint interference leading to G_2/M-phase arrest, [3] Centrosome deformation (e.g., centriole elongation), [4] Rewiring of the microtubule network, and [6b] the use of the cytokinesis mechanism ESCRT for viral budding.* ***B)*** *A cell under viral influence that undergoes multipolar mitosis due to centrosome overduplication [3]. The other influences that are shown: [1] checkpoint interference of the Spindle Assembly Checkpoint (SAC) in particular, [2] APC/C activation or inhibition (may be via the SAC), [3] Centriole multiplication, [4] Depolymerization of microtubules that constitute the mitotic spindle, [5] Chromosome tethering and cohesin interactions, [6a] premature anaphase & cytokinesis, [6c] Tetraploidy & binucleated cells.*

Introduction

Viruses are intracellular parasites that need resources of a host cell for their continued existence. When successfully establishing infection, viruses repurpose cellular machineries for their own use [1]. In doing so, they can alter numerous processes, including transcription, translation, and the cell cycle. It is important to study the mechanisms that viruses use for this, as it may reveal vulnerable points of normal cell cycle control and help in understanding how the cell cycle can become subverted in (viral) disease. The most serious impact of cell cycle deregulation is the aberrant segregation of chromosomes, which leads to abnormalities in chromosome structure and number. Chromosomal instability (CIN) describes a cellular phenotype in which the genomic material is unequally distributed over two daughter cells after cell division [2]. This can give rise to genomic instability and cells with an imbalanced number of chromosomes (aneuploidy). CIN and aneuploidy are typically associated with cancer. Interestingly, viruses can actively induce CIN by targeting components of the cell cycle. Indeed, it is estimated that 15% of all human cancers worldwide are virus-related [3]. Therefore, it is important to understand the mechanisms by which viruses can disrupt mitotic fidelity.

Viruses are broadly classified by their path towards the +-stranded mRNA that is used for translation [4]. This classification distinguishes RNA viruses, DNA viruses, and retroviruses (+RNA or dsDNA). The viral genome can be single- or double-stranded (ss/ds). RNA viruses mostly replicate in the cytoplasm, because they do not require DNA-to-RNA transcription. In contrast, DNA viruses replicate inside the nucleus, since they mostly do not bring their own RNA polymerases. Retroviruses establish intranuclear infections, because they integrate their genome into that of the host cell. DNA- and retroviruses have many mechanisms to interfere with their host's cell cycle and will get special attention in this review. Although the life cycles of all viruses vary, some steps are always required. In **Figure 1A**, the viral life cycle of the human immunodeficiency virus 1 (HIV-1) is depicted as an example. Viral entry (1) consists of three steps: attachment, penetration, and uncaging. The next steps would be replication, transcription, and translation (4), although HIV-1 first needs to perform reverse transcription (2), and genome transportation through the nuclear membrane followed by integration (3). The last steps of the viral life cycle are assembly of new viral particles (5) and release from the host cell (6).

The cell cycle consists of a long interphase (G_1, S, and G_2), followed by the short but crucial phase of mitosis (M-phase, **Figure 1B**). The progression towards mitosis is tightly regulated by the G_2/M-checkpoint that checks for DNA damage and correct genome replication. If the conditions are met, specific cell cycle kinases initiate mitotic entry. Crucial steps of mitosis are the formation of the mitotic spindle, consisting of two centrosomes and microtubules, the condensation of the chromosomes and their alignment in the middle of the cell, correct chromosome segregation, and separation of the two daughter cells. In the middle of mitosis, between metaphase and anaphase, the spindle assembly checkpoint (SAC) monitors whether all chromosomes are correctly positioned and attached to microtubules. When the SAC is turned off, the anaphase-promoting complex or cyclosome (APC/C) is activated. The APC/C then drives the separation of the sister chromatids, which is crucial for a faithful division of all genomic material. This review highlights mechanisms used by different viruses to compromise many of the components involved in mitosis (**Table 1**). Furthermore, the value of studying these interactions will be discussed in the context of cancer, with a special focus on the potential to harness the CIN-inducing capacity of these viral proteins in new cancer treatments.

Table 1: All viruses discussed in this article, arranged in alphabetical order.
Full names of the viruses: *AAV: Adeno-Associated Virus, CAV: Chicken Anemia Virus, HBV/HCV: Hepatitis B/C Virus, HIV-1: Human Immunodeficiency Virus-1, HPV: Human PapillomaVirus, HSV-1: Herpes Simplex Virus-1, HTLV-1: Human T-LymphoTropic virus 1, MCV: Merkel Cell Virus, ODV: Occlusion Derived Virus, Rous SV: Rous Sarcoma Virus, SV40: Simian Virus 40, ZIKV: Zika virus.*
Full names of diseases: *AIDS: Acquired ImmunoDeficiency Syndrome, ATL: Adult T-cell Leukemia, HCC: HepatoCellular Carcinoma, MCC: Merkel Cell Carcinoma.*
Influences on mitosis: *AC: APC/C, CH: Chromatin or cohesin, CP: Checkpoints, CS: Centrosomes, CK: Cytokinesis, MT: Microtubules*
**This list is not exhaustive and only serves as a representative overview.*
***ODV is one form of baculovirus AcMNPV (*Autographa californica *nucleopolyhedrovirus), optimal for primary infection. The other form is the Budded Virus.*

Virus	Genome	Viral proteins and their influence on mitosis*	Cancer-associated?
AAV	- ssDNA - 4.7 kb	E4orf4 (AC)	No
CAV	- Circular ssDNA - 2.3 kb	apoptin (AC)	No
HBV/HCV	- Partially circular dsDNA - ~3.2 kb	HBx, LHBs (CS)	Yes (HCC)
HIV-1	- Linear ssRNA(+) - 9.75 kb)	Vpr (CP, AC, CS, CH) Tat (MT) Gag (MT)	Yes, indirectly (AIDS-associated cancer)
(high risk) HPV	- dsDNA - 8 kb	E4 (CP) E6 (CS) E7 (CS)	Yes (cervical cancer, anogenital cancer)
HSV-1	- Linear dsDNA - 152 kb	ICP0 (MT)	Yes
HTLV-1	- Linear ssRNA(+) - 8.5 kb	Tax (CP, AC, CS)	Yes (ATL and more)
MCV	- Circular dsDNA - 5.4 kb	small t-antigen (CP)	Yes (MCC)
ODV**	- Circular dsDNA - 80-180 kb	EC27 (CP)	No
Rous SV	- ssRNA(+) - ~7.2 kb	v-Src (CK)	Yes (sarcoma)
SV40	- Circular dsDNA - 5 kb	Large T-antigen (CP, MT)	Not confirmed
ZIKV	- ssRNA(+) - 10.8 kb	NS2B-NS3 (CK)	No

1. Cell cycle checkpoints alterations: G2/M transition and SAC

Cell cycle checkpoints are networks of interacting proteins that regulate the 'decision' of the cell to move into the next cycle phase. The most important checkpoints for mitosis are the G2/M-checkpoint and the spindle assembly checkpoint (SAC) at the metaphase-anaphase transition. Two important classes of cellular proteins that mediate cell cycle progression are cyclin proteins and cyclin-dependent kinases (CDKs). Different CDK-cyclin complexes phosphorylate specific sets of substrates, including transcription factors to change gene expression patterns. This creates a unique environment depending on the type of cyclin that is available in different phases of the cell cycle. Many viral proteins can disrupt cell cycle regulation at this level. Constitutive activation or premature silencing of a cell cycle checkpoint has far-reaching consequences.

G_2/M-phase transition

The main function of the G_2/M-checkpoint is to prevent mitotic entry in the presence of DNA damage, or when the genome is not yet (correctly) replicated. Excessive DNA damage can result in a G_2/M-phase arrest, termination of mitosis altogether, or even (p53-independent) apoptosis. Unrepaired DNA can lead to inter-chromatid fusions around the site of any (virally induced) dsDNA breaks. These result in 'anaphase bridges', which can also form around the telomeres, persist throughout mitosis, and may even continue to connect the two daughter cells after cytokinesis [5].

Transition into M-phase involves the activation of CDK1 through its association with cyclin B, the 'mitosis-cyclin' (**Figure 2**). The levels of cyclin B rise throughout the G_2-phase. Once the CDK1-cyclin B complex enters the nucleus, it is inhibited by WEE1 kinase. Dephosphorylation occurs later in the cytoplasm by Cell Division Control 25 (CDC25), which only works when it is itself released from the inhibitory 14-3-3 protein. Once activated, CDK1-cyclin B complexes accumulate in the nucleus to establish mitotic entry by initiating chromosome condensation and breakdown of the nuclear envelope. Viruses can interact with all these proteins in different ways [6]. Here, only a few exemplary interactions will be covered: viral mechanisms that affect CDK1 regulation, and those that disrupt the DNA damage response (DDR).

Viral influence of Cdk1 activation

The baculovirus Occlusion-Derived Virus (ODV) envelope protein EC27 is a functional homologue of cyclin B and –D [7]. The CDK1-EC27 complexes are constitutively active, as EC27 cannot be degraded (**Figure 2**). Although the exact mechanisms are yet unknown, it causes a G_2/M-arrest in which the nuclear envelope remains partially intact, yet much more fluid than in a normal interphase [7]. This has been speculated to contribute to formation of the ODV envelope through the overabundance of nuclear envelope material. The nuclear entry and accumulation of active Cdk1-cyclin B complexes can also be inhibited. For example, Human PapillomaVirus 16 (HPV-16) E4 protein can sequester the dephosphorylated Cdk1-cyclin B complexes to keratin networks [8], [9].

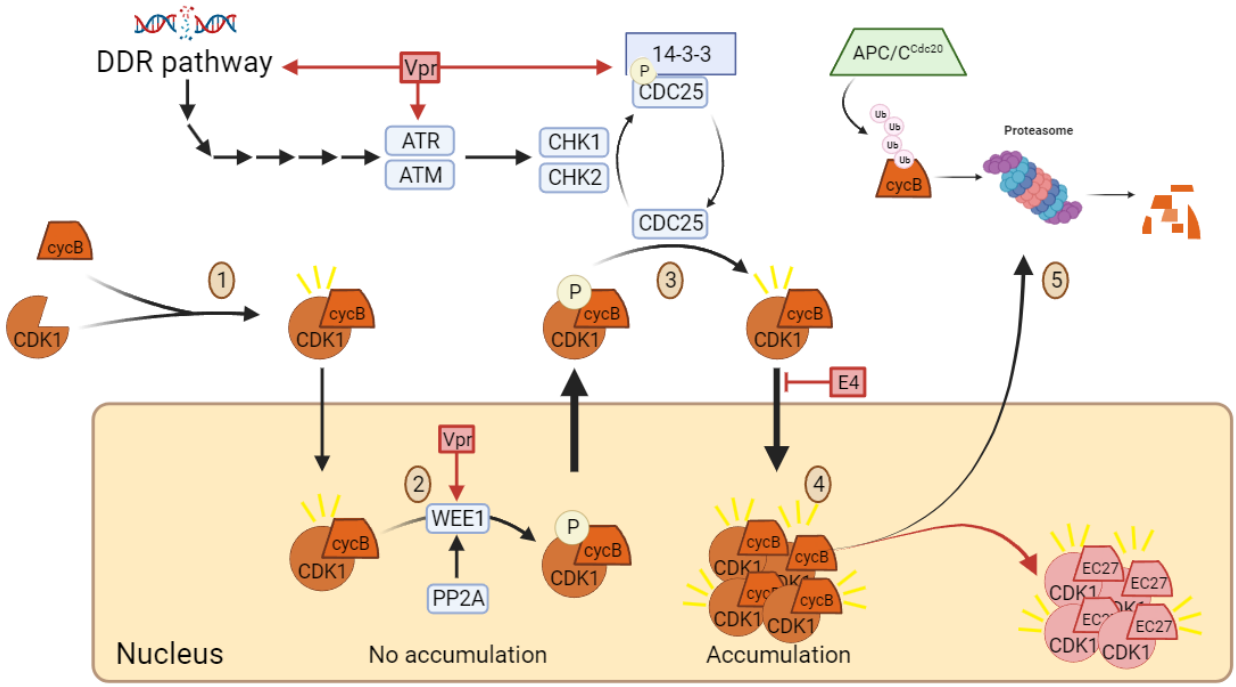

Figure 2: The G_2/M-phase transition is regulated by CDK1-cyclin B

(1) CDK1-cyclin B complexes form throughout the G_2-phase. (2) In the nucleus, they are inactivated by WEE1 kinase (which can be targeted by HIV-1 Vpr), and (3) in the cytoplasm they can be reactivated by CDC25 phosphatase. (4) When active CDK1-cyclin B complexes accumulate in the nucleus, they initiate mitotic entry. The simplified depiction of the G_2/M-checkpoint in this figure shows how the DNA damage response (DDR) pathway leads to activation of kinases that inactivate CDC25, which is sequestered by 14-3-3 to prevent activation of CDK1-cyclin B (and mitotic entry) when there is unresolved DNA damage. Viral genomes and -proteins such as Vpr can prolong this arrest. The export of inactive CDK1-cyclin B at first exceeds the import of active CDK1-cyclin B to prevent nuclear accumulation of these complexes until the G_2/M-checkpoint is relieved. At that moment, nuclear import of CDK1-cyclin B is increased, and the accumulation starts. HPV-16 E4 protein inhibits this by sequestering active CDK-cyclin B to keratin networks in the cytosol. (5) Over time, the accumulation declines when cyclin B is degraded under the influence of APC/C^{Cdc20}. ODV EC27 protein prevents this by remaining associated with CDK1 as a cyclin B analogue. APC/C: Anaphase-Promoting Complex/cyclosome, ATM: Ataxia-Telangiectasia Mutated kinase, ATR: Ataxia-Telangiectasia and Rad3-related kinase, CDC: Cell Division Control protein, CDK: Cyclin-Dependent Kinase, CHK1/2: cell cycle checkpoint kinases 1/2, cycB: cyclin B, HPV: Human PapillomaVirus, PP2A: Protein Phosphatase 2A

Human Immunodeficiency Virus (HIV-1) Viral protein r (Vpr) is an important viral accessory protein involved in many stages of the viral life cycle (**Box 2**). For an extensive review covering the structure and interactions of various HIV-associated proteins and their implications, the reader is referred to the following book: [10]. Vpr binds to several targets involved in cell cycle progression, including 14-3-3, CDC25, WEE1, and Protein Phosphatase 2A (PP2A, chapter 2) [10]. In addition, Vpr alters the gene expression of genes in the MAPK pathway, which has also

been implicated in mitotic entry regulation, although the exact mechanism is not clear yet [6]. Also, Vpr influences the transcription of p21, a CDK1/cyclin B inhibitor normally regulated by transcription factor and well-known tumor suppressor protein p53.

DNA damage response/repair (DDR) pathway

The DDR pathway is employed when there is DNA damage that requires more time for repair. It consists of a network of DNA damage sensing proteins interacting with downstream effectors. The DDR is also involved in cell cycle regulation throughout the cell cycle. Recently, it was found that HIV-1 Vpr can activate the DDR pathway by directly inducing DNA damage [11]. Furthermore, Vpr binding to chromatin and/or splicing factors leads to activation of ATR, which phosphorylate CDC25-inactivating kinases CHK1 and CHK2 (**Figure 2**) [6], [12]. When CDC25 is inactive, the CDK1-cyclin B complexes remain in the cytoplasm in a phosphorylated, inactive state.

The main mechanism by which Vpr interferes with the DDR is via the DDB1-CUL4A-DCAF1 ($CUL4A^{DCAF1}$), an E3 ubiquitin ligase complex that is also involved in DNA repair [12]. $CUL4A^{DCAF1}$ and another DCAF1-complex will be discussed in the next chapters. Ubiquitin ligases destine their target substrates for proteasomal degradation through polyubiquitination. When a ubiquitin ligase is Vpr-bound, it has more possible targets and can degrade its natural substrates prematurely. In this case, Vpr uses $CUL4A^{DCAF1}$ to degrade at least three key DNA repair proteins in distinct pathways: a DNA repair helicase (Helicase-Like Transcription Factor, HLTF), a base excision repair enzyme (Uracil N-Glycosylase 2, UNG2), and a crossover junction endonuclease (MUS81) [13]. Thus, Vpr not only induces DNA damage, but also hinders host cell mediated repair mechanisms. This is part of a defense of the virus against the intracellular innate immune response against viral DNA [13].

Box 1: The versatility of viral proteins - Vpr

Many of the viral proteins discussed in this review (e.g., large T antigen, Tax oncoprotein) exhibit remarkable versatility in their functions, often serving multiple roles in the viral life cycle and interacting with various host cell components. HIV-1 Vpr is a prime example of this. The normal function of this 11.3 kDa protein is the nuclear import of the viral pre-integration complex (PIC) that contains the viral RNA genome [54]. Additionally, besides its dysregulation of the cell cycle, its influence extends towards (among others) apoptosis induction, and immune evasion [10]. Vpr and other versatile viral proteins provide viruses with a significant selective advantage due to their adaptability and multifunctionality. Principally, encoding a single protein with multiple functions allows for an efficient use of the virus's restricted genome coding capacity [22]. Additionally, versatile proteins can be very adaptable, which provides several advantages. First, inbuilt redundancy ensures that when the host cell or some therapeutic intervention blocks one function, another function of the versatile viral protein can take over and have the same downstream effect. Second, the flexibility of these proteins may allow viral survival/replication in multiple different host species. Versatile viral proteins can thus contribute to the long-term persistence of the virus in the population by adapting quickly to changing host environments and evolving to overcome new challenges posed by host defenses.

Spindle assembly checkpoint (SAC)

For accurate chromosome segregation, all chromosomes establish proper connections with microtubules via their kinetochores during mitosis. The process of this attachment is tightly monitored by the spindle assembly checkpoint (SAC), a signaling cascade that is locally activated by free kinetochores (**Figure 1B**). Ultimately, the SAC functions to inhibit the anaphase-promoting complex (APC/C, discussed in the next chapter). By inhibiting the APC/C, the SAC prevents the degradation of key mitotic regulators, including cyclin B (**Figure 2**) and securin, which is an inhibitory subunit that prevents enzymatic removal of the cohesin complex that holds sister chromatids together (chapter 4). The SAC is very sensitive, as even one unattached kinetochore is enough to halt the cell cycle [14]. The checkpoint is activated by Monopolar Spindle 1 (MPS1) kinase at unattached kinetochores. This activation leads to the recruitment of several factors, most notably Mitotic Arrest Deficiency 1 (MAD1) and Budding Uninhibited by Benzimidazole 1 (BUB1), which contribute to the formation of the Mitotic Checkpoint Complex (MCC), which diffuses into the cytoplasm and acts as the final APC/C inhibitor. The MCC consists of the proteins BUB3, MAD2, BUB-related 1 (BUBR1), and CDC20 [15].

The Simian Virus 40 (SV40) large T antigen (LT) interacts with BUB1 [16]. Interestingly, this interaction does not necessarily result in disruption of the SAC, but in stimulation of the DDR, leading to the activation of CHK1, CHK2, and p53. This demonstrates the involvement of BUB1 in both pathways [17]. It also illustrates that cellular networks rarely function independently. Another protein that has been reported to interfere with SAC-associated proteins is human T lymphotropic virus type 1 (HTLV-1) transactivator protein Tax. Tax functionally inhibits MAD1 by preventing its homodimerization, which is required for the MAD1-MAD2 interaction [15]. This leads to a cellular phenotype with multiple nuclei along with micronuclei, in which kinetochores could be found. Besides this, Tax inhibits the DDR-proteins that LT activates (p53, CHK1, and CHK2) [5]. Both LT and Tax are examples of versatile viral proteins (**Box 1**).

Viral interference with the cell cycle checkpoints greatly impacts the infected cell. Cell cycle arrest after S-phase, when the DNA is replicated, will result in (multinucleated) cells with a >2n genome [18]. This can lead to genotoxic stress and ultimately cell death [19]. For viruses, cell cycle arrest is initially beneficial, because it stalls the cell in a stage in which more resources are available. For retroviruses, the arrest may be even more important for stable genome integration in the early stages of their life cycle. However, the virus must also be able to release enough viral particles before the cell eventually undergoes apoptosis. Interestingly, G_2/M-arrest could assist in viral budding and/or spreading to other tissues, because the adherence of cells is lower when their cell cycle is arrested [20]. Contrary to arresting the cell cycle, viruses can also override the SAC, leading to premature anaphase and chromosomal instability in the daughter cells. Since some level of chromosomal instability is permissible and even enhances cell survival (e.g., cancer), this type of cell cycle interference is not lethal and allows for viral persistence in these cells over longer periods of time. For both strategies there are more than enough examples. All mechanisms discussed in this chapter and throughout this review are but a small selection to underscore the diverse ways in which viruses can manipulate the host cell cycle.

2. Interference with the APC/C: activation & inhibition

The Anaphase Promoting Complex or 'cyclosome' (APC/C) plays an important role throughout the cell cycle. It is a 1.5 MDa complex E3 ubiquitin ligase that can coordinate the transition from G_1- to S-phase, from metaphase to anaphase in mitosis (SAC), and mitotic exit (return to G_1). The APC/C specifically targets over 30 protein substrates for polyubiquitination and proteasomal degradation (**Figure 2**). It consists of three subcomplexes: the enzymatic unit (APC2/-10/-11) and the specificity arm (APC3/-6/-7/-8/-12/-13/-16), connected with the bridge (APC1/-4/-5) [21]. The function of the APC/C is dependent on the type of coactivator protein that it is associated with: CDC20 in M-phase and CDC20-homologue 1 (CDH1) in G_1-phase. For example, APC/C^{CDH1} targets cyclin A, and, later, APC^{CDC20} targets cyclin B for degradation (**Figure 2**). In this way, the APC/C switches between different types of CDK activity, modulating cell cycle progression. The most important function of the APC/C is the initiation of chromosome segregation [22].

Viral inhibition of the APC/C

There are several examples of viruses that directly target APC/C. Most of these function as inhibitors and lead to G2/M arrest and ultimately a p53-independent form of apoptosis. Viral APC/C inhibitors target different parts of the complex and attain the effect in different ways [22]. For example, the Chicken Anemia Virus (CAV) protein apoptin inhibits APC/C through association with the bridge subunit APC1. In this way it causes dissociation of APC/C itself and stabilization of its substrates. Secondly, apoptin causes the formation of promyelocytic leukemia nuclear bodies (PML) and relocates the APC/C to them [20].

HIV-1 Vpr also interacts with APC1, however, its inhibition occurs in a different fashion. As mentioned earlier, Vpr can recruit a ubiquitin ligase complex, DDB1/CUL4A-associated factor 1 complex (DCAF1com), and use it to mediate the destruction of many cellular proteins, including DNA repair proteins. It has been shown that the Vpr-DCAF1com complex also targets the APC1 bridge subunit of APC/C for proteasomal degradation [12]. Other interaction partners of HIV-1 on APC/C include the two coactivators: mostly CDH1, but also CDC20. So far, only the destruction of APC1 (not of the coactivators) is confirmed. Interestingly, whereas the other interactions of Vpr-DCAF1com clearly lead to G_2/M cell cycle arrest, its effect on APC/C remains without a clearly defined function for HIV-1 itself. Vpr variants that can bind to DCAF1com without causing cell cycle arrest were still able to induce an efficient APC1 depletion [12]. Additionally, Vpr variants that cannot degrade APC1 anymore had no significant impact on HIV-1 replication in primary CD4+ T cells or macrophages [12]. One theory for the function of Vpr-mediated APC/C inhibition, is that it might increase the levels of APC/C-targets thymidine kinase (TK1) and thymidylate kinase (TMPK). These kinases yield higher amounts of dTTP relative to UTP, which is useful for HIV-1, because its reverse transcriptase (RT) is unable to distinguish between dTTP and UTP. Thus, by increasing the chance to incorporate a thymine instead of a uracil, the virus avoids uracil-associated mutations or detection of its genome by the host as 'non-self' [12]. Other viral accessory proteins may also play a role, e.g., Viral infectivity factor (Vif) has been reported to mediate the degradation of PP2A phospho-regulators [23]. PP2A is known to play a role in the regulation of mitotic events and will be discussed more in the next section.

Viral activation of the APC/C

Viral interaction with the APC/C is not always inhibitory but can also be activating (**Figure 1B**). For example, HTLV-1 oncoprotein Tax prematurely activates APC/C^{CDC20} in the S/G_2-phase through direct binding, although it is still unclear to which APC/C domain Tax binds [24]. In this way, Tax mediates the destruction of cyclin B, cyclin A, and securin. The pre-mitotic reduction of cyclin B levels inhibits the accumulation of cyclin B throughout G_2-phase, which slows the progression towards mitosis. Additionally, securin should be present before the DNA is condensed into chromosomes, to prevent immediate separation. Therefore, in the presence of Tax, the separation of chromosomes may happen too early through cohesin-removal in the absence of securin. It is also probable that this action of Tax is one of the reasons behind the chromosomal aberrations observed in HTLV-1-infected cells.

The adenovirus protein Early region 4 open reading frame 4 (E4orf4) targets PP2A, a major Ser/Thr cellular phosphatase holoenzyme, and recruits it onto the APC/C, followed by the inactivation of the whole complex. E4orf4 is another versatile viral protein with many different effects on host and viral gene expression and protein phosphorylation [25]. Interaction of E4orf4 with PP2A occurs mainly via the Bα/B55/Cdc55 subunit, and interaction with the APC/C is via subunit APC-6 (CDC16). An early study demonstrated that this led to inhibition of APC/C^{Cdh1} as well as Cdc28/Cdk1 complex activation which would partly counteract the inhibition. The conflicting signals would cause cell cycle arrest and ultimately apoptosis [25]. A second study found that E4orf4 inhibits APC/C^{Cdh1}, but also prematurely activates APC^{Cdc20}, leading to early anaphase entry and aberrant chromosome segregation [26]. The difference between these studies may in part be explained by the fact that they have been performed in different phases of the cell cycle, while E4orf4's function may vary over time.

3. Centrosome amplification: direct & indirect effects

The centrosome is the microtubule-organizing center in mitosis, playing an important role in the formation and organization of the mitotic spindle. The centrosome is an organized matrix of proteins (the pericentriolar material, PCM) surrounding two orthogonally positioned centrioles. The PCM contains gamma-tubulin ring structures, from which microtubules grow (nucleate). The centrioles are bundles of nine microtubules connected with a linker. One of the centrioles (the 'mother centriole') has a large protein complex at its proximal end (within the PCM): the torus. In early G_1-phase, the cell has only one centrosome, but during mitosis two centrosomes on opposite sides of the cell organize the mitotic spindle. The centrosome needs to be duplicated before mitosis (**Figure 3**). Already in the G_1-phase, the centrioles disengage from each other and drift apart (centriole disengagement). They both become new mother centrioles when, in S-phase, the new daughter centrioles start to nucleate. Elongation of these 'procentrioles' and maturation of the new centrosomes occurs in the G_2-phase. Just before mitosis, the connecting linker is removed (centrosome disjunction) and, in prophase, the centrosomes travel to opposite sides of the cell to form the bipolar mitotic spindle [26]. For accurate cell division, it is essential that centrosome duplication occurs exactly once, and at the right time (in G_1/S-phase). The cell employs numerous levels of regulation for this.

Among many others, regulator proteins PLK4 and SAS-6, both part of the torus, are involved in the nucleation of the procentrioles. The presence of these proteins is under the control of several SCFs, which are cell-cycle-dependent E3 ubiquitin ligases. For example, SAS-6 is targeted by SCF^{FBXW5}, which is inhibited by PLK4, while PLK4 itself can be ubiquitinated by $SCF^{\beta\text{-}TrCP}$, thereby keeping centriole duplication in check (**Figure 3**). Indeed, PLK4 overexpression has been confirmed by many studies to induce centrosome overduplication [26]. If centrosomes are overduplicated, multipolar mitosis can be avoided by centrosome clustering. In this case, a 'pseudobipolar' spindle is formed, in which one (or both) spindle pole(s) consists of more than one centrosome [26]. Although this reduces the risk of multipolar mitosis, merotelic kinetochore attachment could still occur. This means that a kinetochore is connected to microtubules from more than one spindle pole. The effect of this is the 'lagging' of chromosomes in metaphase, which can lead to chromosome segregation errors (chapter 1).

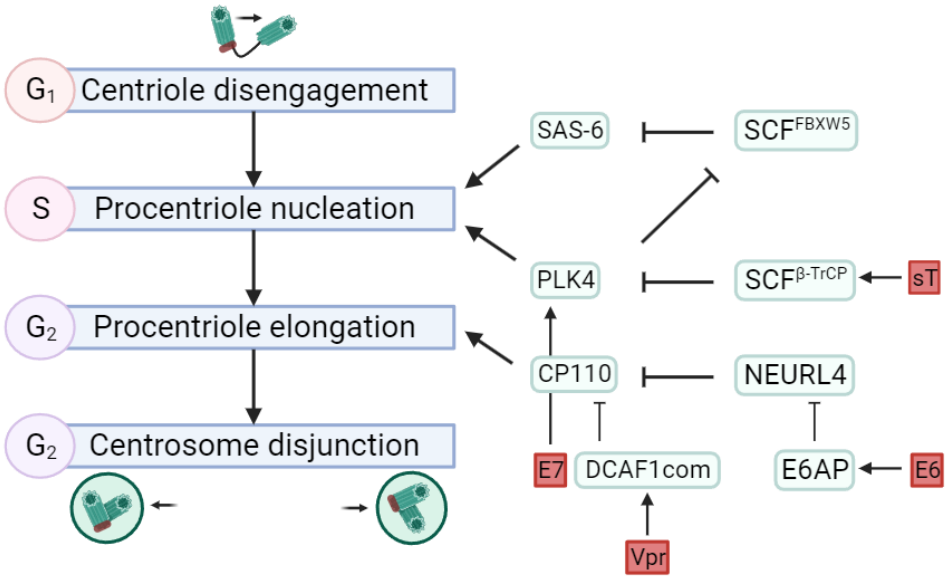

Figure 3: The process of centrosome duplication.
This scheme shows the interactions of viral proteins especially with the proteins that influence procentriole nucleation and procentriole elongation. Abbreviations: CP110: Centriolar coiled-coil protein 110, DCAF1com: DDB1-CUL4A-associated factor complex, E6AP: E6-associated protein, NEURL4: Neuralized E3 Ubiquitin pRotein Ligase 4, PLK4: Polo-Like Kinase 4, SCF: Skp, Cullin, F-box containing complex, SAS-6: Spindle ASsembly abnormal protein 6 homologue, sT: small T antigen (from Merkel Cell Virus, MCV)

Viral influence on centrosome number: direct & indirect

There are various mechanisms by which viruses can cause centrosome amplification. It can be by directly interfering with proteins involved in centrosome duplication (overduplication), or as a byproduct of another process (**Figure 1B**). Interference with other parts of the cell cycle can result in cells with supernumerary centrosomes. For example, in G_2/M-arrested cells that already had two centrosomes, premature centriole disengagement of both centrosomes was observed, resulting in four centrosomes in one cell [26]. Aberrant cytokinesis (chapter 6) also leads to centrosome amplification. In addition, many proteins involved in cell cycle control, such as p53, CDK1, cyclin B, CHK1/2, among others, also play a role in centrosome duplication [5]. Therefore, viruses that interfere with these proteins not only influence the cell cycle, but also centrosome duplication.

The Hepatitis B virus (HBV) encodes two viral oncoproteins that both are known to *indirectly* influence centrosome duplication. HBV viral protein X (HBx) uses the Ras-MEK-ERK pathway, which has been implicated in different human cancers by promoting deregulated cancer progression. Large surface protein (LHBs) was suggested to cause ER stress, leading to truncation of cyclin A, which could then constitutively interact with CDK2 and be involved in centrosome overduplication [27], [28]. HBx also directly interacts with HBx-interacting protein (HBXIP), a cellular protein required for centrosome disjunction and possibly migration [29]. (Induced) HBXIP overexpression leads to multipolar spindle formation. High-risk HPV E6 interacts with E6-associated protein (E6AP), a ubiquitin ligase that interacts with several centriole-associated proteins, some of which are ubiquitin ligases themselves (e.g., NEURL4) (**Figure 3**). The effect of this is that some proteins will get degraded, while others consequently are not degraded anymore and can stabilize and help the formation of new centrosomes [26].

Centriole multiplication

Besides duplication of entire centrosomes, viruses can also deform existing centrosomes in other ways. For example, some viruses can change centrosome morphology by recruiting PCM (HPV-16 E7) or fragmentation (HTLV-1 Tax) [30], [31]. Another example is 'centriole multiplication', done by high-risk HPV E7 and Merkel Cell polyomaVirus (MCV) small T antigen (sT) [32]. This is a rapid form of centriole duplication that yields aberrant centrosomes with one mother and two or more daughter centrioles (**Figure 1B**). HPV E7 causes excessive activation of PLK4 by the CDK2/cyclin E complex and the transcriptional activation of cyclin A and PLK4. PLK4 moves close to mother centrioles to start procentriole nucleation. MCV sT has the same effect as E7, but this protein inhibits key SCF E3 ubiquitin ligases that normally ensure destruction of cyclin E and PLK4. Thus, both HPV and MCV induce higher levels of PLK4, which leads to centriole multiplication but not centrosome duplication.

HIV-1 Vpr has been known to cause both centriole elongation and centrosome duplication, but it does so through different mechanisms. Centriole elongation is achieved by interacting with a DCAF1-associated multi-subunit ubiquitin ligase: EDD-DYRK2-DDB1^{DCAF1} (DCAF1com) [33]. Viral association with another DCAF1-associated ubiquitin ligase has been discussed in the previous chapters. In this case, the target is centriole-associated protein CP110 and the effect of this degradation is the overelongation of centrioles (**Figure 1A**). Functionally, longer centrioles affect the overall shape of the centrosome, which leads to increased nucleation of microtubules from more gamma-tubulin rings. Vpr's effect on CP110 was independent of G_2/M arrest, as was verified experimentally with a DCAF1com-binding Vpr variant that does not induce G_2/M arrest (see chapter 2) and supported by the observation that the complex is located around the centrosome throughout the cell cycle. The other effect of Vpr is cell cycle dependent: centrosome amplification. This does not happen via CP110 depletion, because centrosome amplification correlates with higher levels of CP110. Although the exact mechanism is still unknown, it might involve other targets of DCAF1com. Interestingly, it has been found that proteins involved in the DDR pathway (including ATR, ATM, and CHK1) also cluster around the centrosome and that DNA damage alone is sufficient to induce centrosome amplification [34]. Since Vpr also directly targets ATR and ATM (chapter 1), this protein may induce centrosome amplification via multiple pathways independently.

4. Rewiring of the microtubule network: stabilization & destabilization

The mitotic spindle consists of microtubules, dynamic structures consisting of long polymers of alpha- and beta tubulin. Microtubules are indispensable for the intracellular transport within the cell, and during mitosis they constitute the framework of the mitotic spindle. They are dynamically instable structures, meaning that they continuously form and reform. However, some microtubules may be more stabilized than others, e.g., often used routes for endosomal trafficking between different organelles. Different proteins play a role in microtubule stabilization. The end-binding protein (EB1) recruits other proteins that stabilize the microtubules, which is done by, for example, detyrosination or acetylation of tubulin [35].

Viral influence on microtubules

For viruses, the ability to control microtubule dynamics offers clear opportunities. After uncoating, viruses need to orchestrate the formation of new copies of themselves, using their own genome, self-proteins, and host-borrowed proteins and -complexes for replication, transcription, and/or translation. They can achieve this by interfering with the host cell microtubule network. For instance, the creation of new stabilized microtubules towards the plasma membrane may facilitate microtubule-mediated transport of viral proteins there. For this type of rewiring, other stable microtubules need to be broken down. Viral influence on microtubules can thus be stabilizing or destabilizing.

SV40 LT does not associate with the microtubules themselves, but it binds to Transforming Acid Coiled-Coil protein 2 (TACC2), a centrosomal protein involved in microtubule stabilization [36]. TACC2 belongs to a family of three proteins (TACC1-3) and is thought to play a role in stable microtubule nucleation at the centrosomes, probably together with other, yet undefined, regulatory proteins. LT may *destabilize* microtubules by interfering with this cooperation. Again, considering the interactions of SV40 LT with key mitotic checkpoint proteins (chapter 1), it is possible that TACC2-inhibition works synergistically with these interactions to induce mitotic defects. Interestingly, towards the end of the viral life cycle, the SV40 major capsid viral protein 1 (VP1) fulfills an antagonizing role by *stabilizing* microtubules [37]. This results in a G_2/M cell cycle arrest and preservation of intracellular trafficking and general cell morphology.

Herpes Simplex Virus 1 (HSV-1) Infected Cell Protein 0 (ICP0) is a ubiquitin ligase that functions in two different ways as viral infection progresses. In the immediate early phase of the infectious cycle, ICP0 stimulates the expression of many viral genes. However, later in the infectious cycle, it can dismantle the host cell's microtubule network [38] (**Figure 1B**). Once ICP0 leaves the nucleus and enters the cytoplasm, microtubules are bundled and dispersed into small α-tubulin globular bodies. ICP0 colocalizes with α-tubulin, but it is unclear whether they interact directly. It seems not to function as a typical ubiquitin ligase here, since the colocalization endures far longer than the other interactions that it undergoes [38]. The goal of this action is to clear the way for HSV-1 to be synthesized and/or to egress.

Retroviral influence on microtubules

For retroviruses, the genomic viral RNA (gRNA) is transported out of the cell nucleus and into the cytoplasm for translation. Many retroviruses do this by interacting with components of the cell's mRNA nuclear export machinery, which occurs in a microtubule-dependent manner [39]. Retrovirus HIV-1 takes advantage of microtubules in a different way. The HIV-1 matrix protein

(MA) can direct microtubule stabilization by associating with the EB1-binding motor protein Kinesin family 4 (Kif4) (**Figure 1A**) [35]. Additionally, HIV-1 uncoating also depends on the action of motor proteins, along with intact, stable microtubules [40]. The motor proteins appear to pull the viral capsid in opposite directions, resulting in mechanical disassembly along with a net minus-end-directed transport towards the centrosomes (i.e., closer to the nucleus to prepare for nuclear entry) (**Figure 1A**).

HIV-1 Trans-Activator of Transcription (Tat) has a variety of effects on microtubules, both direct and indirect. Its mechanism of action may depend on the phase in the viral life cycle or the host cell cycle. Tat associates directly with tubulin and can enhance microtubule polymerization, but also shortening [34]. Furthermore, like HSV-1 ICP0 discussed above, it can bundle microtubules [41]. In neurons, Tat recruits the proteasome to microtubules to degrade both the microtubules themselves, as well as the microtubule stabilizing protein Microtubule-Associated Protein 2 (MAP2) [42]. Besides direct microtubule interaction, Tat also binds to cellular Ribosomal Protein S3 (RPS3) that in turn interacts with α-tubulin and causes microtubule disassembly. Tat-binding to RPS3 causes its aberrant localization throughout the cell, leading to longer and denser α-tubulin filaments in metaphase. This ultimately affects mitotic spindle organization, chromosome segregation, and abscission (chapter 6). Studies concerning this accessory protein could perhaps give more insight into the origin of the high levels of apoptosis in HIV-1 infected individuals, especially in non-infected cells [39], [41]. Indeed, Tat is secreted by the infected T cells, after which it can be taken up by neighboring healthy T cells and affect their microtubule network, leading to apoptosis. In this way, Tat plays a clear role in HIV-1 immune evasion.

In summary, HIV-1 and other viruses can use their (in)direct influence on microtubule stability to control intracellular trafficking of their own components (stabilization), but also to induce aberrant mitotic spindle formation (destabilization). It remains to be elucidated how different viruses coordinate both stabilization and destabilization of the host cell microtubules to assure the most optimal environment for viral replication without detection. Microtubule network rewiring could be detrimental for the cell, for it affects not only the mitotic spindle, but also the motor protein-mediated trafficking of numerous cellular components.

5. Host chromatin-interactions: tethering & altered chromatin organization

During mitosis, the cell's chromatin is aligned in the middle of the mitotic spindle as pairs of identical ultra-condensed sister chromatids that are held together by the cohesin complex until all kinetochore-microtubule attachments have been made. As discussed in chapter 1, viruses can interact with the host cell DNA, e.g., by introducing DNA damage to induce cell cycle arrest via the DDR. This chapter focuses on another aspect of viral interactions with the host DNA: physical tethering to chromatin during mitosis (**Figure 1B**). Viruses can have many reasons for positioning themselves in or around the host chromatin. For example, association of viral genomes to both sides of a chromatid-pair allows the viral genome to be "inherited" by both daughter cells after cell division. Additionally, for some DNA viruses and retroviruses, chromosome tethering during mitosis enables them to later end up in the new nuclei of the daughter cells. In this way, they bypass the need to cross the nuclear envelope [43]. Lastly,

retroviruses may use tethering for integration. Since only DNA viruses and retroviruses perform replication and transcription near host cell DNA, chromosome tethering is predominantly found in those viruses, although there are exceptions for some intranuclear RNA viruses (e.g. orthobornaviruses [44]).

Viral persistence (episome maintenance)

The viral genome of (latent) viruses can manifest itself as episomes: circular plasmids existing outside of the host cell DNA. The general mechanism of chromosome tethering is the attachment of episomes to the host chromatin via a viral episome maintenance protein (EMP). These proteins are extremely versatile (**Box 1**). They can have a direct DNA-binding portion, or they can bind to various DNA-associated host proteins, like kinetochores, histones, histone-associated or other chromosomal binding proteins, and cohesin (below) [45]. Different EMPs assist in each stage, e.g., docking, attachment and stabilization. In some cases, the mode of interaction can change over time depending on the needs of the virus. For example, in mitosis a firmer association would be required to prevent dissociation due to the physical forces pulling on the chromosomes. In contrast, genome tethering during interphase can be looser to allow for viral transcription and/or replication. Regulated association and release are thus critical to maintain viral infectivity [1]. Chromosome tethering is not merely beneficial for the virus, but can also impact the cell, e.g., when it occurs via an important cell cycle regulating protein. For example, an EMP of high-risk HPV (E2), binds to the APC/C coactivators CDH1 and CDC20 [46].

Cohesin interactions

Cohesin is a complex that holds sister chromatids together during mitosis. The cohesin complex consists of three core subunits: Structural Maintenance of Chromosomes proteins (SMC1 and -3) and RAD21. Within cohesin, RAD21 interacts with another complex: Stromal Antigen 2 (SA), SCC3, Wings APart-Like (WAPL), and PDS5 (SA/SCC3-WAPL-PDS5), in which WAPL is a cohesin releasing factor [47]. The cohesin from the chromosome arms is removed by WAPL during mitosis, but the centromeric cohesin is protected by Shugosin protein (SGO1). This results in selective cohesin accumulation around the centrosomes, which is crucial to keep the two sister chromatids together during chromosome alignment.

Until the onset of anaphase, centromeric cohesin remains protected by Shugosin (SGO1). Many viruses can disrupt the cohesin complex (**Figure 1B**). HIV-1 Vpr interacts with the Histone Acetyl Transferase (HAT) p300, which leads to the selective acetylation of lysine 9 in histone H3 (H3K9Ac). This is also where heterochromatin protein 1 (HP1) binds to the chromosomes to regulate centromeric cohesin. Ultimately, the effect of (Vpr-induced) p300/HAT-mediated acetylation is the displacement of RAD21 and SGO1 and premature sister chromatid separation [48]. Intriguingly, the Hepatitis C virus (HCV) also influences cohesin via p300/HAT, but in this case the H3K9-acetylation occurs around the promoter of the *RAD21* gene, leading to RAD21 overexpression. At the same time, HCV induces the downregulation of releasing factor WAPL. These actions result in more cohesin around the chromosomes, leading to chromosome hypercondensation. In conclusion, viral interactions with cohesin can cause aberrant chromosome segregation and chromosomal instability.

Box 2: HIV-1 as a model virus

Human Immunodeficiency Virus 1 (HIV-1) is a lentivirus and part of the family of retroviruses. It is the causative agent of Acquired ImmunoDeficiency Syndrome (AIDS), currently affecting > 30 million people worldwide, with already > 40 million casualties since the beginning of the epidemic in 1981 (data: WHO). HIV-1 primarily infects CD4+ T cells and macrophages. It has a linear ssRNA genome of 9.75 kb and encodes 15 mature (functional) proteins: 3 structural (poly)proteins, 2 regulatory proteins, 4 accessory proteins, and 6 proteins processed from some of the polyproteins (structural components and enzymes) [54]. In this review, Vpr is discussed most (**Box 1**), but other HIV-1 proteins are also involved in deregulation of the cell cycle. This makes HIV-1 a perfect illustration of the many possibilities of viral interference with the cell cycle. Vpr interferes with cell cycle regulation through its interaction with proteins and pathways involved in key checkpoints, along with inducing irreparable DNA damage, which can lead to G2/M cell cycle arrest and apoptosis. Moreover, Vpr inhibits the Anaphase-Promoting Complex/Cyclosome (APC/C) by recruiting the ubiquitin ligase complex DCAF1com to degrade an essential structural APC-component. At the same time, accessory protein Tat extends and shortens, and mostly destabilizes microtubules, while the structural matrix protein MA restabilizes them. In this way, HIV-1 orchestrates the most optimal environment for itself. Because of the severity of AIDS and the discovery that some cancers are related to HIV-1 infection [52], a great effort has been put into studying the mechanisms of HIV-1 proteins. The abundance of HIV-1 cell cycle deregulatory mechanisms seems to suggest that this virus is a master-deregulator of the cell cycle. However, this mountain of knowledge may also be due to the intensity with which this virus is investigated [6]. It would therefore be good to direct more research efforts towards the other viruses mentioned in this review. For example, since 2005 it has still not been elucidated to which APC/C domain the HTLV-1 Tax oncoprotein binds [24]. Expanding our knowledge in this area will be of great benefit for the development of treatments for both cancer and infectious diseases.

6. Cytokinesis, viral budding, and septins

Cytokinesis is the process of daughter cell separation after chromosome segregation. It begins with invagination of the membrane and formation of a contractile ring in the middle of the cell that starts to close in and separate the new nuclei into two daughter cells [49]. While they move further apart, the midbody, a membranous bridge-structure, connects the two cells. The last step of cytokinesis is abscission [19]. Cytokinesis is regulated in multiple layers [49]. The formation of the contractile ring is regulated by two major master regulatory complexes: the centralspindlin complex, and the chromosomal passenger complex (CPC). The cytoskeletal components actin (for the contractile ring), and microtubules (for the midbody) are needed, along with motor proteins that position the master regulatory complexes. Septins are GTP-binding proteins that are also important for the organization of the contractile ring [19]. For the lasts steps, a key set of complexes is the Endosomal Sorting Complexes Required for Transport (ESCRT). This cellular machinery consists of four multiprotein complexes [50]. The centralspindlin complex eventually leads to the recruitment of ESCRT-III, which is involved in the last step of midbody scission.

Cytokinesis failure typically results in tetraploid cells with supernumerary centrosomes (chapter 2). Before discussing how viruses impact cytokinesis, it is interesting to note that certain viruses utilize components of the cytokinetic machinery for their own viral release (**Figure 1A**). Indeed, 'budding', the final step of the viral life cycle, requires the same mechanisms and proteins involved in abscission. The process of cytokinesis appears to be less targeted than the other processes discussed in this review. This could be because it is difficult to prove whether a defect in cytokinesis is caused by direct interference with this step or simply followed from a cell cycle disruption in an earlier phase. It may also be that there are no clear influences on the mechanisms of cytokinesis itself, but that cytokinesis is impaired by the viruses that hijack the ESCRT mechanism when infecting mitotic cells, although this remains to be explored.

Viral influence on cytokinesis

The Zika virus (ZIKV), an RNA virus that infects neural progenitor cells in the brain, can cause microcephaly characterized by aneuploidy. This occurs through a direct influence on cytokinesis. One of ZIKV's protease complexes, NS2B-NS3, cleaves host cell septin 2 [19]. The NS2B-NS3-mediated cleavage of septin 2 led to disruption of the septin complex around the midbody that also contained septin 7. Consequences include delayed cell division, increased cellular apoptosis, and the formation of multipolar spindles [19].

Another example is the alpharetrovirus Rous sarcoma virus v-Src, which can cause cytokinesis failure through delocalization of mitotic regulators, notably Aurora B kinase and one of its substrates in the centralspindlin complex, Mitotic Kinase-Like Protein 1 (MKLP1) [51]. v-Src is the viral counterpart of cellular Substrate of RhoA-binding kinase C-terminal (c-SRC), which has been associated with oncogenic signaling and stimulation of the PI3K and ERK pathways (chapter 1). c-SRC is normally tightly regulated and only activated in response to extracellular signals, but v-Src is constitutively activated and can cause cytokinesis failure, resulting in the familiar phenotype of 4n binucleated cells with multiple centrosomes (**Figure 1B**).

Discussion

There is a variety of viruses that interact with different cellular components during mitosis and thereby influence cell cycle progression. The most prominent effect of aberrant mitosis is the incorrect segregation of sister chromatids, which leads to chromosomal instability (CIN) followed by aneuploidy in the two daughter cells. Intriguingly, CIN has also been linked to (aneuploid) cancers [2], [26]. Most of the viruses discussed in this review are associated with neoplasm formation (**Table 1**). Even the model cell cycle deregulating virus, HIV-1 (**Box 2**), has been linked to the development of 'AIDS-associated cancers' [52]. Furthermore, even when a virus does not cause cancer, it can still cause other malignancies that are characterized by CIN, e.g., ZIKV-related microcephaly [19].

Although there are ample exceptions, the typical profile of a virus that interferes with faithful chromosome segregation is either a DNA oncovirus (e.g., HPV, HBV, and MCV) or an oncoretrovirus (e.g., HIV-1, HTLV-1). The most important reason for this is their prominent involvement in what happens in and around the host cell DNA, since they require nuclear entry for their replication. Indeed, some viruses selectively infect mitotic cells to enter the nucleus before the nuclear envelope is restored after mitosis [43]. The most targeted components are proteins/complexes that operate at the intersection of multiple regulatory pathways, for example the APC/C. This 'intersection complex' is regulated by the SAC *and* by microtubule dynamics. Aberrant APC/C-activation can lead to CIN by inducing anaphase *and* by causing centrosome overduplication [21], [22], [26]. This interconnectedness can also be dangerous for the virus, because some coincidental effects may not be beneficial, or even threatening, to its survival. For example, direct or indirect viral influence on centrosome overduplication may lead to multipolar mitosis, which produces levels of CIN that are too serious to overcome. When those daughter cells undergo apoptosis before the viral life cycle is complete, viral propagation is stopped as well. Therefore, viruses need to find the right balance in cell cycle dysregulation while avoiding cell death. In the case of centrosome duplication, the cell can prevent multipolar mitosis by centrosome clustering [26]. Indeed, it has been speculated that viruses can aid in centrosome clustering, although this has not been verified yet [26].

There are logical reasons for viruses to induce a cancer-like state in the cells that they infect. Since cancer cells are often metabolically highly active, there are enough resources for the virus to exploit for replication and virion-reassembly. The same effect is obtained when viruses cause a G_2/M cell cycle arrest, which leads to a more S-phase like environment. Moreover, accelerating cell division can help viruses by generating more host cells to reside in [6]. Considering this last reason, arresting the cell cycle seems counterintuitive. However, for viruses like HIV-1 and HTLV-1, that infect immune cells, expansion of the host cell population may work against the virus [6], especially when the virus is not passed down to both daughter cells.

Antiviral therapy – a broader perspective

For many of the viruses discussed here, antiviral drugs have been developed [53]. Current anti-HIV-1 antiviral therapy is dominated by inhibitors of the first two steps of the viral life cycle: attachment (entry), polyprotein proteolysis and reverse transcription [54]. However, these steps represent only a fraction of HIV-1's life cycle, with a $t_{1/2}$ of 0.62 and 5.5 hours, respectively. In contrast, the average time that the virus would spend in the nucleus (for integration and

transcription) is ~35 hours [55]. For some antivirals, however, treatments that were initially successful were later met by drug-resistant viral strains. Resistance can arise through beneficial mutations that either block the function of the drug or provide a way for the virus to propagate despite the drug's effect. Interestingly, another mechanism of "resistance" development is t*olerance by synchronization*: viral populations can reset their life cycles to complete the steps targeted by the antiviral in the timeframe in which the drug concentrations are at a minimum [53]. These findings underscore the importance of multi-drug combination therapies such as Highly Active AntiRetroviral Therapy (HAART) [54], [55]. Although for most viruses the influence on mitosis is not essential for the completion of their life cycle, it does provide a survival advantage and could be considered for targeting, especially since there would be a broader timeframe of the viral life cycle in which such drugs would be effective. Moreover, if this type of influence really is the main mechanism that drives the development of infection-associated cancer, this type of inhibition may be especially helpful in preventing tumorigenesis. For example, HPV proteins E2 and HBx are thought to be important drug targets to prevent cancer development upon infection [21]. The most interesting targets may be the proteins that some DNA-viruses use for association to host chromatin, because that may inhibit their entry into the nucleus and disturb their entire life cycle.

Anticancer therapy – targeting cancer cells with viral proteins

The link between viruses, CIN, and cancer brings several exciting possibilities for therapy. Research can go in two directions: the specific induction of apoptosis in cancer cells, and the over-destabilization of cancer cells towards lethality. Some of the viral proteins discussed in this review have the capacity to induce apoptosis, especially through a cell cycle delay (**Table 2**). Most importantly, this apoptosis-induction occurs in the absence of functional p53. Because this tumor suppressor is mutated in most tumors, it is crucial to identify other pathways that can specifically induce apoptosis in those cells [22]. These viral proteins are all interesting candidates for this type of cancer targeting. Indeed, the APC/C-inhibitor apoptin (CAV) is now under investigation as a selective anti-tumor agent, with other APC/C-regulators likely to follow [21].

Table 2: Viral proteins discussed in this review that can induce (p53-independent) apoptosis
For abbreviations, see the legend of Table 1.

Viral proteins	**Influence leading to apoptosis**
HIV-1 Vpr	activation of the DDR
HIV-1 Tat, HSV-1 ICP0	destabilization of the microtubule network
CAV apoptin, HIV-1 Vpr, AAV E4orf4	inhibition of the APC/C
ZIKV NS2B-NS3	disruption of the midbody complex in cytokinesis

The second possible anticancer therapy using viruses is the aggravation of the already skewed mitosis as found in cancer cells. This is not a novel idea, as other studies to completely turn off mitotic regulation in cancer have been investigated [56]. The rationale is that CIN-displaying cancer cells are just in between a healthy non-CIN cell and a critical threshold beyond which CIN becomes intolerable. In fact, aneuploid cells appear to be more sensitive to inhibition of the SAC, and it is likely that this holds true for other cell cycle deregulating interventions as well [57]. If it would be possible to deliver, for example, the APC/C activating HTLV-1 oncoprotein Tax to cancer cells that already have other deregulated mechanisms (e.g., a deregulated SAC, multiple centrosomes, or dysfunctional cohesin), it may push the level of CIN further towards the lethality threshold (**Figure 4**).

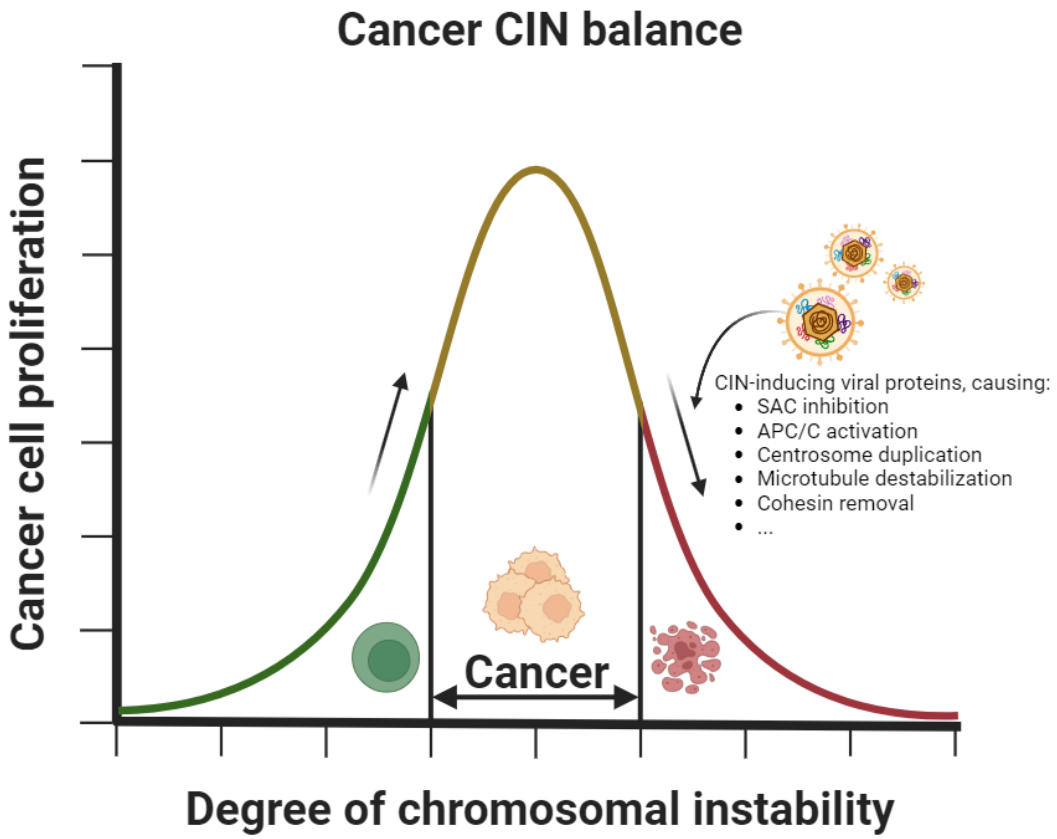

Figure 4: The theoretical distribution of the development of chromosomal instability
It is thought that cancer cells display just enough CIN to allow for rapid uncontrolled cell division, but not so much that they would lose their viability. Theoretically, if a cancer cell were introduced to (viral) proteins that cause CIN, the accumulative effect of the cancer phenotype combined with that of the protein would tip the balance towards a state in which the cancer cells are no longer viable.

Oncolytic virus therapy
The challenge in this type of therapy is to specifically deliver such viral proteins to the cancer cells. One solution could be the use of oncolytic viruses as a vector. Oncolytic viruses are natural or genetically engineered viruses that selectively infect and lyse cancer cells without harming the normal tissues [58]. The first interest in using viruses to combat cancer came from the observation that cancer patients that naturally acquired a viral infection showed an improved

prognosis due to tumor regression. The same reasons for transforming healthy cells into cancer cells, as explained above, might also explain the tendency for some viruses to selectively infect fast proliferating cells. Since the 1960's, different viruses have been repurposed as oncolytic viruses, most notably herpes simplex virus 1 (HSV-1), a variant of which has recently been described to be 'unable to enter into neurons and establish latency', but well capable to function as an oncolytic virus [59]. Another example is the non-tumor associated ZIKV, which also has potential to target specifically difficult-to-treat aggressive central nervous system tumors in infants [60]. It has already been suggested to 'arm' oncolytic viruses with extra transgenes encoding for other helpful antitumor proteins. However, to date, this has only been used for the co-transfection of cancer cells with cytokines, e.g., GM-CSF, IL12, IL18, or B7-1, with the hope to induce a systemic antitumor immunity effect [58]. Considering the potential of CIN-overinduction with viral proteins, engineering these proteins into oncolytic viruses would significantly enhance this therapy. Currently, the biggest concern with oncolytic virus therapy is that the virus may be intercepted by circulating antibodies before reaching the tumor. This is a special risk for those oncolytic viruses that are part of the standard vaccination program.

Challenges with studying viral interference with the cell cycle

For the development of novel anticancer therapies using such CIN-inducing viral proteins, further characterization of the different viral influences on the cell cycle is necessary. However, there are several challenges for this field [6]. First, the regulation of the cell cycle is not a simple, defined, linear pathway, but a complex network of interacting proteins that is under extensive investigation to this day. This makes it difficult to correctly interpret the results of cell cycle experiments, because it cannot be determined with one experiment where in the network the interaction took place. The second problem is the multifunctionality of many viral proteins. This is again a challenge for research, because the traditional knock-out/knock-down experiments will have too many effects to trace back to one aspect of the protein. Protein binding to find the interactions of the viral protein with cellular proteins is also a challenge, since the small size of the viral protein has many overlapping binding sites [1]. Viral protein versatility may also be a problem in therapy-design, for it is difficult to predict all the effects of one protein. This might be overcome by specifically engineering compounds based on known viral mechanisms, e.g., to capture only the premature cohesin removal-capacity of Vpr without Vpr's other functions. Nevertheless, this still requires a thorough understanding of the protein-protein interactions of interest. The third challenge is the choice for a correct model system. Most of the earliest in vitro experiments are performed in transformed cell lines. However, these are often cancer cells, meaning that their cell cycle regulation is already defective. It would be wise to employ a greater number of model systems from different organisms. For example, results in yeast models are not always in line with those in mammalian cells [9].

Future perspectives

Since there is such a clear link between aneuploidy and cancer, it would be expected that viruses that influence chromosome segregation in any way are oncogenic. This hypothesis can fuel research in, for example, virus-related cancers in which the link has so far remained elusive. Additionally, the study of the viral influence on mitosis can teach us more about cell cycle regulation itself. Indeed, some of the first cell cycle regulatory proteins were discovered because they were interaction partners of viral proteins [21]. This research can also be extended beyond viruses and cancer to other CIN-displaying malignancies and other intracellular pathogens. In conclusion, the interactions between viruses and their host in mitotic regulation have far-reaching implications. The resulting aberrant chromosome segregation induces CIN, which can eventually lead to cancer. More research is necessary to unravel the different mechanisms, and especially in developing ways to use those viral proteins to expand our arsenal of weapons in the fight against cancer.

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The WHO data on HIV-1 epidemiology can be accessed via the following web-page: https://www.who.int/data/gho/data/themes/hiv-aids#:~:text=Globally%2C%2039.0%20million%20%5B33.1%E2%80%93,considerably%20between%20countries%20and%20regions

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