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## Deregulation of Chromosome Segregation and Cancer

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**Keywords**

spindle assembly checkpoint, kinetochore-microtubule network, oncoviruses, chromosome segregation defects, genome instability, protein complexes

**Abstract**

The mitotic spindle assembly checkpoint (SAC) is an intricate cell signaling system that ensures the high fidelity and timely segregation of chromosomes during cell division. Mistakes in this process can lead to the loss, gain, or rearrangement of the genetic material. Gross chromosomal aberrations are usually lethal but can cause birth and development defects as well as cancer. Despite advances in the identification of SAC protein components, important details of the interactions underpinning chromosome segregation regulation remain to be established. This review discusses the current understanding of the function, structure, mode of regulation, and dynamics of the assembly and disassembly of SAC subcomplexes, which ultimately safeguard the accurate transmission of a stable genome to descendants. We also discuss how diverse oncoviruses take control of human cell division by exploiting the SAC and the potential of this signaling circuitry as a pool of drug targets to develop effective cancer therapies.

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**Spindle assembly checkpoint (SAC):**

an evolutionarily conserved regulatory mechanism of higher organisms that monitors and corrects chromosome segregation defects upon cell division

**Chromosomal passenger complex (CPC):**

a complex constituted by aurora B kinase, borealin, the inner centromere protein (INCENP), and survivin that acts as a master regulator of mitosis

**Mitotic checkpoint complex (MCC):**

the assembly constituted by BUBR1, BUB3, CDC20, and MAD2 that acts as an inhibitor of the APC/C

**Anaphase-promoting complex/cyclosome (APC/C):**

a multiprotein E3 ubiquitin ligase for which substrate selection is driven through binding of one of two protein cofactors (CDH1 and CDC20) in a cell cycle-dependent manner

**Pseudokinase:**

a protein that adopts the canonical kinase fold but lacks one or more of the amino acids required to phosphorylate protein substrates

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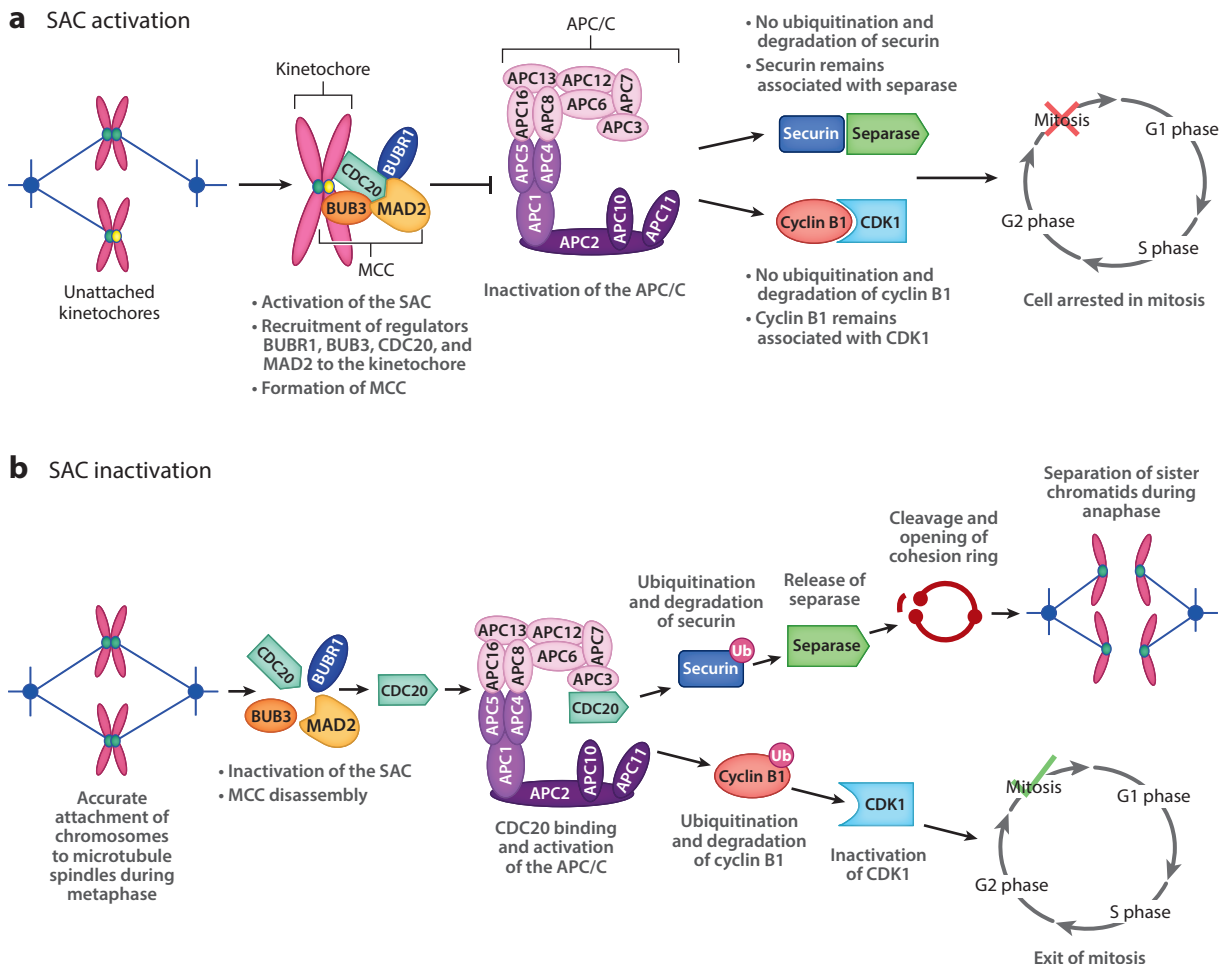
## 1. INTRODUCTION

### 1.1. A Brief History of the Mitotic SAC

The exquisite regulation of the mitotic spindle assembly checkpoint (SAC) is a fine example of how the remodeling of protein complexes in time and space has evolved as an effective strategy to increase selectivity of signals in the cell with a minimal margin for error. Mistakes in the process of cell division can lead to the rearrangement, loss, or gain of chromosomes (aneuploidy); genome instability; and cancer. SAC signaling requires communication with the kinetochore, a large macromolecular assembly that acts as the site for attachment of chromosomes to microtubule polymers that pull sister chromatids apart during cell division (**Figure 1**). A critical aspect of SAC signaling is that connection of the centromere to microtubules of the mitotic spindle by the kinetochore must be strong enough to sustain the pulling forces during anaphase, whereas it must also be sufficiently dynamic to ensure proper chromosome alignments at the metaphase plate. This is coordinated through a sophisticated network of protein-DNA and protein-protein interactions. Different biochemical and mechanical models of the formation of SAC protein assemblies in response to spindle attachment defects have been proposed. On the one hand, biochemical-oriented models postulate that sensing and signal initiation relies on binding competition between SAC and spindle proteins for microtubule binding sites at the kinetochore (Hiruma et al. 2015, Ji et al. 2015). On the other hand, mechanical models postulate that proteins in close proximity to each other in unattached kinetochores define a mechanical switch for chromosome-microtubule attachment sensing and signal initiation, where protein separation after attachment silences the signal (Aravamudhan et al. 2015). More recently, a new model of SAC signaling initiation that integrates biochemical and mechanical principles has been proposed (Joglekar & Aravamudhan 2016). Considerable progress has been made in understanding the composition of the SAC and the recruitment hierarchy of its components [recent excellent revisions of this fascinating topic have been reported (Dou et al. 2019, Gelens et al. 2018, Pesenti et al. 2016, Saurin 2018)]. However, important aspects of SAC initiation and its regulation have proved elusive even though they are clearly indispensable for a deeper understanding of the control of chromosome segregation and a prerequisite to envisage new ways to manipulate SAC signaling for the treatment of cancer.

### 1.2. The Central SAC Protein Components

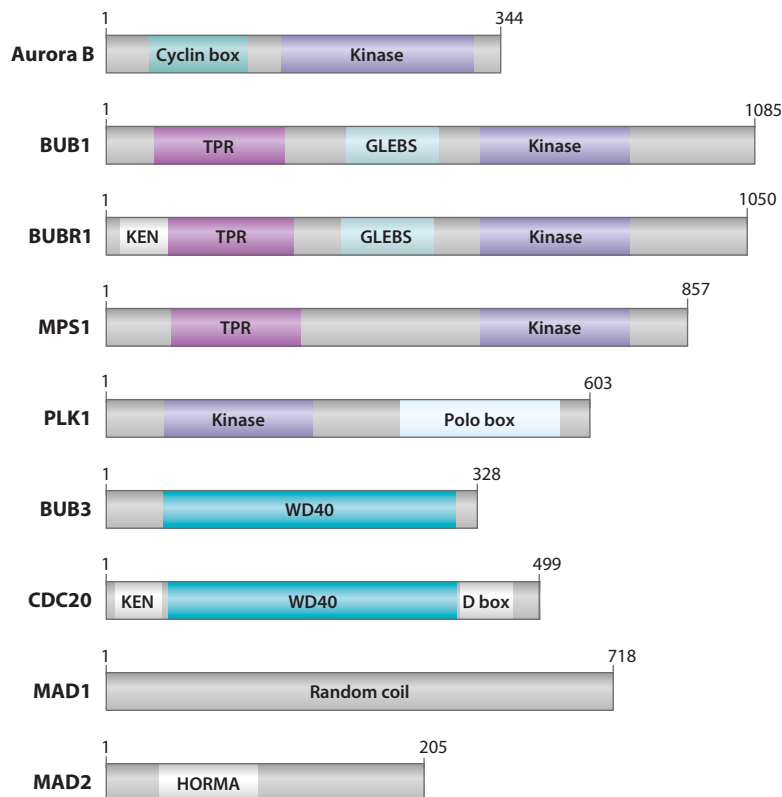
Central protein components of the SAC harbor specific protein motifs and domains that dictate protein functionality and stability (**Figure 2**). For instance, BUB1 (budding uninhibited by benzimidazoles 1), BUBR1 (BUB-related 1), MPS1 (monopolar spindle 1), PLK1 (polo kinase 1), and aurora B are all multidomain serine/threonine protein kinases with essential roles in the SAC. BUB1 is required for the proper assembly of the inner centromere (Boyarchuk et al. 2007) and recruitment of the chromosomal passenger complex (CPC) and SGO1 (shugoshin 1) to the centromere. Moreover, BUB1 kinase activity is important for the establishment and maintenance of productive attachment to spindle microtubules (Elowe 2011). BUBR1, together with BUB3 (budding uninhibited by benzimidazoles 3), MAD2 (mitotic arrest deficient 2), and CDC20 (cell division cycle 20), forms part of the mitotic checkpoint complex (MCC), which is assembled in response to improper chromosome attachment to the mitotic spindle to inhibit the anaphase-promoting complex/cyclosome (APC/C) (Barford 2011, Foe & Toczyski 2011, Herzog et al. 2009, Yamano 2019, G. Zhang et al. 2016). At least in mitosis, BUBR1 functions as a pseudokinase that plays important roles in chromosome segregation, DNA repair, ciliogenesis, and neuron differentiation (Bolanos-Garcia & Blundell 2011). PLK1 is a mitotic protein kinase that contributes to mitotic entry, bipolar spindle formation, and the establishment of stable kinetochore-microtubule



**Figure 1**

Basis of SAC activation and inactivation during mitosis. (a) Unattached kinetochores catalyze recruitment of SAC proteins. MAD2 binds BUBR1, BUB3, and CDC20, forming the MCC, which subsequently inhibits the APC/C. Cells are arrested in mitosis as a result of APC/C inhibition until all chromosomes are accurately attached to microtubule spindles. (b) When this ensues, the MCC disassembles, allowing CDC20 to bind and activate the APC/C, resulting in the ubiquitination and subsequent degradation of the APC/C targets securin and cyclin B1. Degradation of securin releases the protease separase, which cleaves its specific substrate cohesin, enabling sister chromatids to separate, while the timely degradation of cyclin B1 by the proteasome inactivates CDK1, driving mitotic exit. Abbreviations: APC/C, anaphase-promoting complex/cyclosome; MCC, mitotic checkpoint complex; SAC, spindle assembly checkpoint.

attachments (Lenart et al. 2007, Liu et al. 2012, Steegmaier et al. 2007, von Schubert et al. 2015). PLK1 localizes to kinetochores by interacting with several kinetochore proteins (Amin et al. 2014, Qi et al. 2006) and is also involved in the maintenance of SAC activity through its physical interaction with BUB1, which supports BUB1 localization to the kinetochore and the phosphorylation of MPS1 and KNL1 (kinetochore scaffold 1) (Ikeda & Tanaka 2017). When PLK1 is depleted or inhibited, cells arrest in mitosis due to SAC activation by defective kinetochore-microtubule attachments (Lenart et al. 2007, Steegmaier et al. 2007). MPS1 (also known as TTK) is the most upstream regulator of the SAC signaling pathway (Pachis & Kops 2018, Saurin 2018). The



**Figure 2**

A simplified view of the functional domains and motifs of the central SAC protein components. The highlighted functional modules mediate the various catalytic (kinase activity) or specific protein recognition functions of the SAC proteins, whereas degrons such as the KEN box and D box motifs are responsible for their timely degradation throughout mitosis. For simplicity, other functional motifs such as the ABBA motif and KI motif of BUB1 and BUBR1 are not shown. Abbreviations: D, destruction; KEN, lysine-glutamate-asparagine; KI, lysine-isoleucine; SAC, spindle assembly checkpoint.

phosphorylation consensus sequence of MPS1 seems to be similar to that of PLK1 (Dou et al. 2011): MPS1 phosphorylates the kinetochore organizer protein KNL1, a posttranslational modification that promotes the recruitment of the SAC proteins BUB1, BUBR1, and BUB3 to the kinetochore (Ji et al. 2017, Primorac et al. 2013, Vleugel et al. 2015, Zhang et al. 2014). Aurora B phosphorylates substrates at the kinetochore to inhibit microtubule attachment, including NDC80, which is the principal microtubule attachment complex at kinetochores (Cheeseman et al. 2006, Ciferri et al. 2008, Cordeiro et al. 2018, Wei et al. 2007).

BUB1 and BUBR1 share a common architecture: a conserved N-terminal domain, a middle nonconserved domain that contains the binding region for the mitotic checkpoint protein BUB3, and a C-terminal serine/threonine kinase domain. The N-terminal regions of BUB1 and BUBR1 (and also MPS1) contain a divergent arrangement of the TPR (tetratricopeptide repeat) motif (Bolanos-Garcia et al. 2009, D’Arcy et al. 2010, Lee et al. 2012) that mediates recruitment of the proteins to the kinetochore (Bolanos-Garcia et al. 2011; Krenn et al. 2012, 2014; Marquardt et al. 2016; Nijenhuis et al. 2013; Thebault et al. 2012). The middle domain of BUB1 and BUBR1 contains the GLE2p-binding sequence (GLEBS)-like motif, which is located within the region

implicated in BUB3 binding. In BUB1 and MPS1, the C-terminal domain contains a catalytically active kinase domain, whereas in BUBR1, the equivalent region harbors a domain that, at least in the SAC, acts as a pseudokinase (Suijkerbuijk et al. 2012a, Tomoni et al. 2019).

In addition to its role as an MCC component, BUB3 binds the GLEBS motif of BUB1 and BUBR1 (**Figure 2**), resulting in at least two tightly bound, constitutive protein subcomplexes throughout the cell cycle: BUB3-BUB1 and BUB3-BUBR1. It seems that the GLEBS-mediated interaction with BUB1 and BUBR1 is crucial for the recruitment of the later protein kinases to the kinetochore in a process that involves the recognition of KNL1 MELT-phosphorylated motifs by MPS1 (Faesen & Musacchio 2015; Ghongane et al. 2014; Overlack et al. 2015; Primorac et al. 2013; Vleugel et al. 2013, 2015). However, other KNL1 residues that define the lysine-isoleucine motifs also contribute to BUB1 and BUBR1 recruitment to the kinetochore (Bolanos-Garcia et al. 2011, Krenn et al. 2012, S. Zhang et al. 2016).

MAD1 (mitotic arrest deficient 1) is a 718-residue coiled-coil protein (**Figure 2**), the depletion of which severely affects proper SAC signaling in mammalian cells (Luo et al. 2018). MAD1 forms a stable complex with MAD2 in vitro (De Antoni et al. 2005, Fava et al. 2011, Ji et al. 2018, Yu 2006). Disruption of one *MAD2* gene allele causes enhanced rates of chromosome loss and impaired checkpoint function and tumorigenesis (Michel et al. 2001). Mad2 overexpression results in an overactive mitotic checkpoint where cells show a much more aggressive and wider range of tumors compared to mice with low Mad2 levels. MAD2 exhibits the distinctive HORMA (HOP1, REV7, and MAD2) domain (**Figure 2**).

CDC20 constitutes the target of the branch of the spindle checkpoint that monitors kinetochore behavior via its direct interaction with BUBR1 (Diaz-Martinez et al. 2015, Han et al. 2013, Lischetti et al. 2014). CDC20 is largely organized in a N-terminal region of low structural complexity and a single domain that defines the WD40 fold (Larsen et al. 2007). The N-terminal disordered region contains C box, KEN box, and CRY box motifs, whereas the C-terminal tail proceeding the WD40 domain contains an isoleucine-arginine (IR) motif (**Figure 2**). Both the C box and the IR motif mediate CDC20 binding to the APC/C complex (S. Zhang et al. 2016), the ultimate effector of cell cycle progression from metaphase to anaphase (Alfieri et al. 2017, Watson et al. 2019). APC/C is an E3 ubiquitin ligase with its activity tightly regulated by multiple mechanisms including acetylation, phosphorylation, the binding of inhibitors, subcellular localization, and destabilization of its activators or acetylation of its component subunits (Craney et al. 2016, Hein et al. 2017, Höckner et al. 2016, Touati et al. 2018). Tight regulation of cell cycle progression by the APC/C requires its physical and sequential interaction with its two coactivators, CDH1 (CDC20 homolog 1) and CDC20, which form the APC/C-CDH1 and APC/C-CDC20 complexes, respectively. Essentially, the APC/C-CDH1 complex is primarily active during the end of mitotic exit and early G1 phase of the cell cycle, whereas the APC/C-CDC20 complex regulates the transition from metaphase to anaphase and mitotic exit.

## 2. SAC SIGNALING RELIES ON TRANSIENT COMPLEXES

Earlier molecular details of the dynamic nature of the network of interactions underpinning SAC signaling came from the crystal and nuclear magnetic resonance structures of members of the MAD protein family. For instance, the structure of the MAD1-MAD2 complex revealed a so-called safety belt mechanism underlying the regulation of the interactions between MAD2-MAD1 and MAD2-CDC20 (Luo et al. 2002, Sironi et al. 2002, Skinner et al. 2008, Zhang & Nilsson 2018), where a large conformational rearrangement of MAD2 from an open to a closed conformation acts as the rate-limiting step in cells mounting an SAC response (De Antoni et al. 2005).

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### E3 ubiquitin ligase:

a class of enzymes that catalyze the final step of ubiquitination, namely, the transfer of ubiquitin from an E2 ubiquitin-conjugating enzyme to a protein substrate

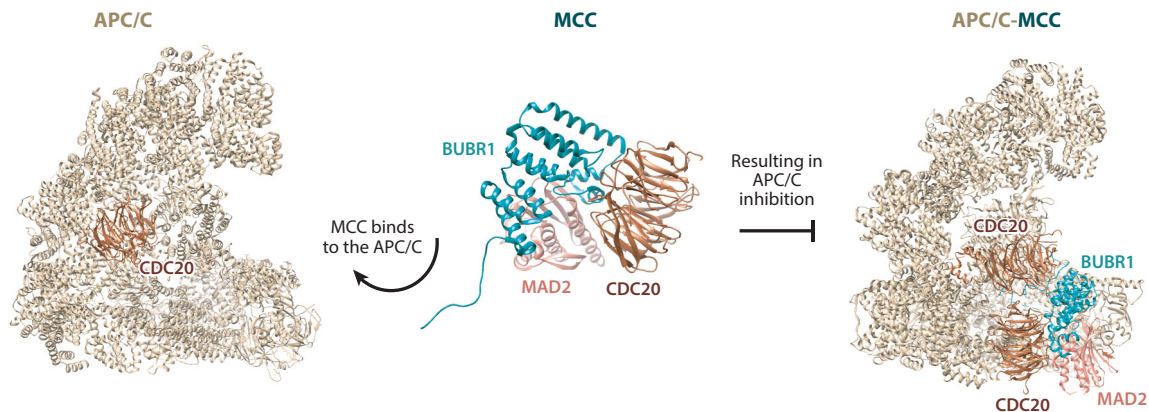
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MPS1 activity is highly dynamic on kinetochores and its activation involves autophosphorylation (Kang et al. 2007, Mattison et al. 2007). Activated MPS1 promotes the association of SAC protein components on unattached kinetochores by phosphorylating the kinetochore protein KNL1 at the MELT repeats, a posttranslational modification that is required for BUB1-BUB3 recruitment to the kinetochore. This is followed by recruitment of other SAC components such as BUBR1, MAD1, and MAD2. SAC signaling is antagonized by the protein p31<sup>comet</sup> (Yang et al. 2007), which binds to the same MAD2 and BUBR1 interface, implying that there is competition between p31<sup>comet</sup> and BUBR1 to bind MAD2 (Fava et al. 2011, Mapelli et al. 2006, Yang et al. 2007). An additional layer of regulation of the p31<sup>comet</sup>-MAD2 interaction is conferred by p31<sup>comet</sup> phosphorylation (Date et al. 2014).

In addition to KNL1, the MIS12 and NDC80 complexes define the KMN (KNL1/MIS12/NDC80) network, a multiprotein macromolecular assembly that is essential to organize correct kinetochore-microtubule attachments (Aravamudhan et al. 2015). Although the assembly of the kinetochore is a crucial event in cell division, the precise sequence of events underlying the process remains obscure. KNL1 integrates SAC kinase and phosphatase activities and contributes to the formation of kinetochore-microtubule attachments (Przewlaka & Glover 2009). In the human, the MIS12 complex (also known as the MIND complex) consists of the proteins MIS12, DSN1, NNF1, and NSL1, the last of which links together the MIS12 and the NDC80 complexes (Petrovic et al. 2010). This interaction enhances the binding of the NDC80 complex to microtubules (Kudalkar et al. 2015). The NDC80 subcomplex is composed of four subunits: NDC80 (the subunit that gives its name to the entire subcomplex), NUF2, SPC24, and SPC25 (Ciferri et al. 2008, Wan et al. 2009, Wei et al. 2007). The association of the SPC24-SPC25 heterodimer is required for NDC80 binding to both the KNL1 and the MIS12 complex, while the formation of the NUF2-NDC80 assembly is required for NDC80 complex binding to microtubules (Cheeseman et al. 2006, Ciferri et al. 2008, Joglekar & DeLuca 2009, Kiyomitsu et al. 2007, Wan et al. 2009, Wei et al. 2007). We have argued that multiprotein complexes that assemble cooperatively are less likely to be formed fortuitously (Bolanos-Garcia et al. 2012). The cooperative association of SAC signaling complexes resulting from binary interactions supports this notion, as the interactions have proved to be specific and of low affinity, enabling the amplification of specific signals to mount an effective SAC response.

### 3. POSTTRANSLATIONAL MODIFICATIONS FINE-TUNE THE SAC

In addition to the specific functions of SAC protein components conferred by the motifs and domains depicted in **Figure 2**, additional layers of SAC regulation rely on diverse posttranslational modifications. The concerted phosphorylation, acetylation, and ubiquitination of specific SAC protein components greatly affects their stability, and their coordinated assembly/disassembly into protein subcomplexes ultimately ensures the proper segregation of the chromosome upon cell division. For instance, in prometaphase BUBR1 is acetylated by the histone acetyltransferase PCAF (P300/CBP-associated factor) at the BUBR1 residue K250, a posttranslational modification that is important to protect BUBR1 degradation by APC/C-CDC20 (Choi et al. 2009). Once the checkpoint is satisfied by the proper attachment of microtubules to the kinetochores, BUBR1 is deacetylated and becomes a substrate of APC/C-CDC20-dependent proteolysis. Thus, coordinated BUBR1 acetylation/deacetylation operates as a molecular switch that regulates the conversion of BUBR1 from an inhibitor of the APC/C complex to its substrate. Furthermore, BUBR1 amino acid residue K668 seems to be acetylated by the acetyltransferase CBP and deacetylated by SIRT2 (North et al. 2014). Unlike the acetylation of BUBR1 K250, acetylation at K668 in



**Figure 3**

Structural details of the MCC-APC/C interaction. The MCC exists in a free state and in complex with the APC/C. The MCC binds and inhibits CDC20-dependent activation of the APC/C, forming a larger assembly that contains two copies of CDC20. The loading of CDC20 onto APC/C is regulated through APC/C phosphorylation by CDK1. Fine-tuned cycles of controlled CDC20 synthesis and degradation confer further regulation to APC/C activity and, therefore, to mitosis progression, in response to the status of the mitotic spindle. Abbreviations: APC/C, anaphase-promoting complex/cyclosome; MCC, mitotic checkpoint complex.

BUBR1 promotes the ubiquitination and degradation of BUBR1, providing further evidence of the exquisite regulation of the SAC.

Tight regulation of SAC components also involves complex phosphorylation and dephosphorylation cascades (Bajaj et al. 2018, Faesen et al. 2017, Foss et al. 2016, Gelens et al. 2018, Ikeda & Tanaka 2017, Jia et al. 2016, Manic et al. 2017, Moura et al. 2017). BUBR1 undergoes auto-phosphorylation when the SAC is unsatisfied and is the substrate of PLK1 and CDK1 (cyclin-dependent kinase 1) (Combes et al. 2017, Elowe et al. 2007, Suijkerbuijk et al. 2012b, Wong & Fang 2007). In prophase, phosphorylation of APC/C by CDK1 kinase primes this ubiquitin E3 ligase to preferentially bind the coactivator CDC20 to form an APC/C-CDC20 complex (**Figure 3**). Six CDC20 residues are phosphorylated *in vitro* by BUB1, but not by BUBR1, MAPK, or several other kinases (Ge et al. 2009, Tang et al. 2001, G. Zhang et al. 2016). The mutation of such CDC20 phosphorylation sites to alanine results in reduced checkpoint arrest in mitosis. Phosphorylation of CDC20 by PLK1 is important for the ubiquitination and destruction of CDC20 and impairs the CDC20-dependent activation of UBE2S by APC/C. The inhibitory phosphorylation on CDC20 is released by kinetochore-bound PP2AB56 phosphatase. PP2AB56 is recruited to the kinetochore by BUBR1, and a single mutation in CDC20 (D464R) disrupts binding to BUBR1, thus impairing the dephosphorylation of CDC20 by PP2AB56. Further control of CDC20 functions are provided by the KEN and the CRY boxes, which act as two independent degrons. APC/C ubiquitin ligase activity is modulated through conformational changes that disrupt the architecture of the substrate-binding site (Herzog et al. 2009), a mode of regulation that closely resembles the regulation of protein kinases. For instance, recruitment of the MCC to APC/C-CDC20 blocks access to the APC/C substrate binding site, thus acting as an allosteric regulator of this ubiquitin E3 ligase (**Figure 3**). Concerted conformational changes of the CDC20-MCC complex and the APC/C regulate the UBE2C-dependent ubiquitination of CDC20 bound to the MCC, enabling an exquisite control of the process that monitors and corrects chromosome segregation errors upon cell division (Fujimitsu et al. 2016, G. Zhang et al. 2016). In summary, control of APC/C-CDC20 activity in response to the status of the mitotic spindle involves tuneable cycles

**Degrans:** specific amino acid sequences or structural motifs that regulate protein degradation rates; some SAC proteins contain multiple degrons

of CDC20 synthesis and degradation, MCC assembly and disassembly, and the coordinated association/dissociation of the MCC with the APC/C-CDC20 complex.

**Biallelic:** pertaining to both alleles (both alternative forms of a gene) of a single gene (paternal and maternal)

## 4. SAC AND CANCER

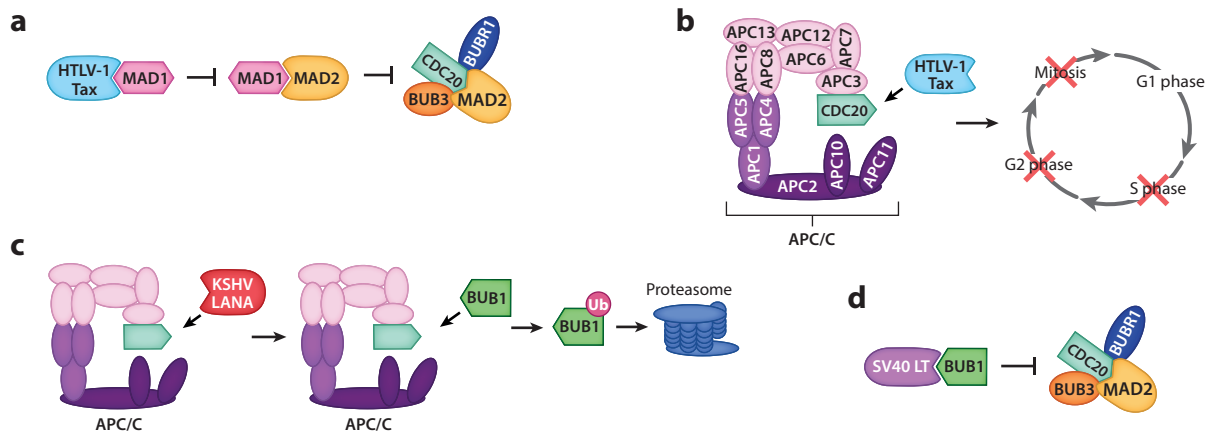
### 4.1. SAC Misregulation

Aneuploidy is a common characteristic among cancer cells. Deletions, insertions, and point and silent mutations that are associated with aneuploidy, chromosome instability (CIN), and cancer occur throughout the *BUB1* and *BUBR1* gene sequences. A role for BUB1 in oncogenesis is suggested by the occurrence of *BUB1* mutations, differential *BUB1* gene expression, and BUB1 protein levels in cancer tissues and cell lines (Piao et al. 2019, Pinto et al. 2008, Ricke et al. 2011, Wada et al. 2008), as well as the formation of spontaneous cancers in transgenic mice that express a dominant interfering fragment of BUB1 (Cowley et al. 2005). An oncogenic role for BUBR1 abnormal expression levels, which often results in aneuploidy and gastric cancer progression, is also well documented (Bolanos-Garcia & Blundell 2011, Chen et al. 2015, Kawakubo et al. 2018, Lira et al. 2010). Furthermore, *BUBR1* truncating and missense mutations have been identified in families with mosaic-variegated aneuploidy (MVA), a syndrome characterized by growth and mental retardation and microcephaly (Hanks et al. 2004, Ochiai et al. 2014, Simmons et al. 2019). The MVA biallelic mutations have provided important insights into cancer development, as these mutations were the first to relate germline mutations in a spindle checkpoint gene with a human disorder (Hanks et al. 2004). Nearly 50% of the BUB1 amino acid substitutions associated with cancer can be mapped onto regions predicted to be mostly disordered, suggesting that substitution of these residues impairs specific protein-protein interactions that are critical for SAC signaling. Compared to the *BUB1* gene, a larger number of *BUBR1* mutations associated with cancer have been reported to date. Like with BUB1, the majority of BUBR1 amino acid residue substitutions that are associated with different classes of cancer can be mapped onto regions of predicted low structural complexity (Bolanos-Garcia & Blundell 2011). The mutations that are mapped onto the kinase domain are the second most frequent followed by those located in the kinetochore-binding region. Although studies on BUB1 and BUBR1 mutants suggest that cancer formation is linked to a weakened SAC, the precise roles of these mutants in tumor formation remain unclear.

MPS1 has been identified in the signature of the top 25 genes overexpressed in CIN and aneuploid tumors (Carter et al. 2006, Kops et al. 2005) and is upregulated in several tumors of different origins including bladder, anaplastic thyroid, breast, lung, esophagus, and prostate. MPS1 protein levels and kinase activity peak upon spindle checkpoint activation. However, abnormally high and low MPS1 levels cause aneuploidy and SAC signaling defects. Similar to BUB1, BUBR1, and MPS1, abnormal levels of BUB3, MAD1, MAD2, and CDC20 are significantly elevated in diverse cancer tissues (details of this are presented in **Supplemental Tables 1–7**). The relative level of CDC20 overexpression is associated with an aggressive course of breast cancer and is correlated with extremely poor outcomes (Karra et al. 2014). CDC20 is also highly expressed in certain lung cancers and associated with pleural invasion (Kato et al. 2012), whereas suppression of its expression not only in such tumors but also in hepatocellular carcinoma cells inhibited cell growth, induced G2/M cell cycle arrest, and retarded colony formation (Li et al. 2014). CDC20 overexpression has also been reported in gastric and pancreatic cancer and positively associated with tumor size, histological grade, and lymph node metastasis (Chang et al. 2012, Taniguchi et al. 2008). Because of its association with clinical stage, CDC20 has been proposed as a prognostic biomarker of human non-small-cell lung and colorectal cancer (Kato et al. 2012).

**Supplemental Material** >



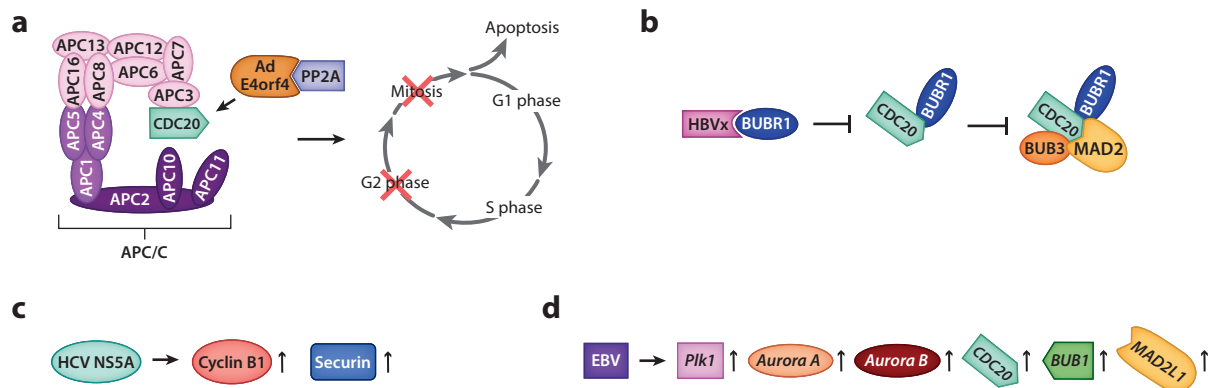


**Figure 4**

Manipulation of the SAC by oncoviruses. (a) HTLV-1 Tax binds MAD1, causing loss of the MCC, and (b) interacts with and prematurely activates the APC/C, subsequently inducing aneuploidy. (c) KSHV LANA associates with the APC/C for subsequent ubiquitination and degradation, which impedes SAC activity. (d) SV40 LT binds BUB1, disrupting the SAC. Abbreviations: APC/C, anaphase-promoting complex/cyclosome; HTLV-1, human T cell lymphotropic virus type 1; KSHV, Kaposi's sarcoma–associated herpesvirus; LANA, latency-associated nuclear antigen; LT, large tumor antigen; MCC, mitotic checkpoint complex; SAC, spindle assembly checkpoint; SV40, simian virus 40.

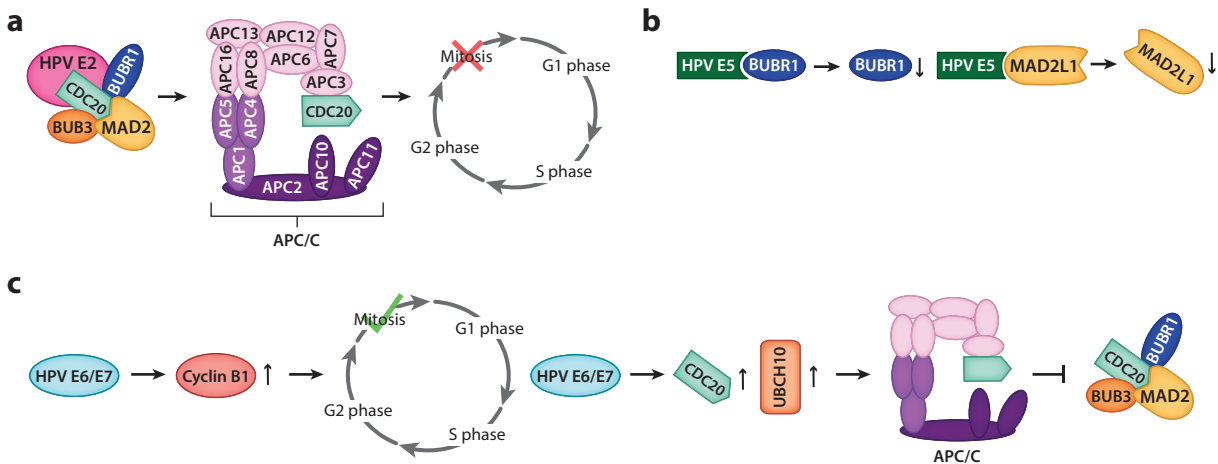
## 4.2. The SAC is a Target for Oncoviruses

Diverse types of oncoviruses manipulate the host cell cycle during infection, making the SAC an attractive target for establishing an intracellular environment favoring viral replication and inducing chromosomal missegregation with subsequent tumorigenesis (Figures 4, 5, and 6). For



**Figure 5**

Manipulation of the SAC by oncoviruses. (a) Ad E4orf4 in complex with PP2A manipulates the APC/C, causing a mitotic block in G2/M phase. (b) HBVx binds BUBR1, impeding the binding of BUBR1 to CDC20 and forming the MCC, causing deregulated APC/C and subsequent aneuploidy. (c) HCV NS5A disrupts mitotic progression with decreased cyclin B1 and securin degradation. (d) SAC genes are upregulated in the presence of EBV, potentially inducing genomic instability. Abbreviations: Ad, adenovirus; APC/C, anaphase-promoting complex/cyclosome; E4orf4, early region 4 open reading frame 4; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; MCC, mitotic checkpoint complex; NS5A, nonstructural protein 5A; PP2A, protein phosphatase 2A; SAC, spindle assembly checkpoint.



**Figure 6**

Manipulation of the SAC by oncoviruses. (a) HPV E2 binds CDC20, BUBR1, and MAD2, potentially forming an E2-MCC complex that prevents SAC inactivation; subsequently APC/C activity is impeded, causing mitotic arrest. (b) HPV E5 interacts with BUBR1 and MAD2, decreasing BUBR1 and MAD2 levels. (c) HPV E6/E7 causes elevated cyclin B1 levels, enabling circumvention of the G2 damage checkpoint and mitosis entry and elevating CDC20 and UBCH10 levels with impulsive APC/C activation and subsequent SAC inactivation and mitotic exit. Abbreviations: APC/C, anaphase-promoting complex/cyclosome; HPV, human papillomavirus; MCC, mitotic checkpoint complex; SAC, spindle assembly checkpoint.

example, retrovirus human T cell lymphotropic virus type 1 (HTLV-1), the etiological agent of aggressive leukemia adult T cell leukemia/lymphoma, targets cell cycle progression control to sustain viral replication and stimulate infected T cell proliferation (Boxus & Willems 2009).

The influence of Tax protein on the SAC was first indicated when MAD1 was identified as a tax interacting partner, which coincided with human MAD1 discovery (Jin et al. 1998). Tax binds MAD1 through the same domain required for heterodimerization with MAD2, causing loss of the mitotic checkpoint (Figure 4a) (Jin et al. 1998). Further studies of Tax-transformed cells showed that MAD1 and MAD2 were mislocalized to the cytoplasm, which correlated with a loss of SAC function and microtubule inhibitor chemoresistance (Kasai et al. 2002). In addition, tax can manipulate APC/C-CDC20, provoking a delay in cell cycle phases S/G2/M by directly interacting and prematurely activating APC/C-CDC20 before the onset of mitosis (Figure 4b).

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV8), expresses latency-associated nuclear antigen (LANA) during latent infection. LANA interacts *in vivo* and *in vitro* with BUB1 (Figure 4c), which is essential for LANA centromeric localization (Xiao et al. 2010). BUB1 KNL and kinase domains are crucial for LANA-BUB1 interaction (Sun et al. 2014, 2015). Moreover, LANA mediates BUB1 degradation by the ubiquitin-mediated proteasome degradation pathway through the association of LANA with the APC/C, which recruits BUB1 to the APC/C for degradation (Sun et al. 2014). It is plausible that this degradation impedes SAC activity with consequential uncontrolled APC/C activity and subsequent CIN.

Simian virus 40 (SV40) large tumor antigen (LT) is a multifunctional phosphoprotein essential for driving viral replication and cell cycle dysregulation that causes efficient immortalization and oncogenic transformation of rodent cells (Cotsiki et al. 2004). LT was shown to induce CIN hallmarks of micronuclei, lagging chromatin and anaphase bridges, in a BUB1-binding-dependent

manner (**Figure 4d**), reflecting SAC disruption (Cotsiki et al. 2004, Hu et al. 2013). Some viruses targeting the SAC are oncolytic rather than oncogenic, preferentially targeting and infecting tumor cells, resulting in apoptotic death. Adenoviruses (Ads) are double-stranded DNA viruses that cause diverse clinical illnesses including upper respiratory infections, conjunctivitis, tonsillitis, and gastroenteritis in mammalian species. Although its oncogenic potential in humans is unknown, human Ad (HAd) can stimulate cell transformation in tissue culture (Fehr & Yu 2013). HAd encodes E4orf4 (early region 4 open reading frame 4), which stimulates a G2/M phase cell cycle block and induces apoptosis of transformed cells (Mui et al. 2010), dependent on E4orf4 binding to PP2A (protein phosphatase 2A). The E4orf4-PP2A complex manipulates APC/C activation (**Figure 5a**), directly interacting with APC/C to alter its activity (Kornitzer et al. 2001, Mui et al. 2010).

Hepatitis B virus (HBV) is a primary etiological factor for hepatocellular carcinoma (HCC) development. A high SAC impairment frequency has been illustrated in HCC cell lines exhibiting CIN, and the aneuploidy observed is likely due to a defective SAC (Saeki et al. 2002). HBV regulatory protein HBx binds and colocalizes with BUBR1 at kinetochores. The HBx-binding domain and the CDC20-binding domain of BUBR1 overlap; thus, HBx-BUBR1 interaction disrupts BUBR1's ability to bind CDC20 and subsequent MCC function by inhibiting APC/C-CDC20 (**Figure 5b**) (Chae et al. 2013, Kim et al. 2008). However, other investigations have not been able to confirm that HBx disrupts the SAC and MCC but instead have shown that HBx binds E3 ubiquitin ligase DDB1 (damaged DNA-binding protein 1), which potentially contributes to CIN (Martin-Lluesma et al. 2008).

Overexpression of hepatitis C virus (HCV) protein NS5A (nonstructural protein 5A) induces mitotic aberrations including unscheduled delay in mitotic exit and multipolar spindles that are associated with CIN and aneuploidy (**Figure 5c**) (Baek et al. 2006). The frequency and extent of CIN was abnormally high in NS5A-expressing cells, even in the absence of the mitotic cell cycle dysregulating stimulus nocodazole, indicating that NS5A directly disrupts the timing of mitotic progression (Baek et al. 2006). NS5A-expressing cells exhibited increased fractions of mitotic cells, reduced G1 cell populations, and decreased cyclin B and securin degradation, indicating that elevated cyclin B and securin levels perturb the normal mitotic cell cycle timing (Baek et al. 2006). Furthermore, HCV infection or expression of the viral core protein alone in various cell types, including hepatocyte culture and transgenic mice, impeded mitotic checkpoint function and induced chromosomal aberrations including polyploidy, indicating that these effects are likely universal (Machida et al. 2009).

PLK1 has been identified as a host factor for HCV replication regulation. PLK1 co-immunoprecipitated and partially colocalized with NS5A and was capable of inducing NS5A basal phosphorylation and hyperphosphorylation in vitro (Chen et al. 2010). PLK1 inhibition with a PLK1 inhibitor or knockdown in vivo caused reduced NS5A hyperphosphorylation and HCV replication, indicating that PLK1 mediates HCV replication regulation through NS5A hyperphosphorylation and its potential as an antiviral target for HCV-associated HCC (Chen et al. 2010).

Epstein-Barr virus (EBV) is etiologically implicated in several cancers including Burkitt's lymphoma, posttransplant lymphoproliferative disease, nasopharyngeal carcinoma, and AIDS-associated lymphomas (Carbone et al. 2008). Expression of a subset of EBV genes in Burkitt's lymphoma-derived cell lines treated with nocodazole and taxol demonstrated SAC disruption and mitotic slippage with subsequent mitotic aberrations, polyploidy, and aneuploidy (Leao et al. 2007). Global gene expression profile analysis revealed that the central SAC-related genes *PLK1*,

*AURORA A*, *AURORA B*, *CDC20*, *BUB1*, and *MAD2* were upregulated in EBV-transformed lymphoblasts (Dai et al. 2012). As EBV-induced aneuploidy has been associated with SAC disruption (**Figure 5d**), upregulation of these SAC genes potentially induces genomic instability, lymphocyte transformation, and aberrant proliferation (Dai et al. 2012).

Human papillomaviruses (HPVs) are small DNA viruses infecting human epithelial skin cells and the cervix, stimulating hyperproliferative lesions resulting in cervical cancer. Different strains are categorized into two types: High-risk strains comprise viruses with malignant properties and are related to cervical cancer, and low-risk strains contain viruses accountable for benign growths. HPV E2 protein as a viral transcription and replication factor has well-ascertained roles. E2 from high-risk HPV strains directly interacts with CDC20, disrupts APC/C function, and stimulates a mitotic block and often subsequent metaphase-specific apoptosis (Bellanger et al. 2005). As HPV E2 is known to stabilize cyclin B, E2 interactions with MCC proteins (**Figure 6a**) potentially prevent SAC inactivation and MCC disassembly at the end of metaphase, resulting in mitotic arrest (Tan et al. 2015).

Immunoprecipitation analysis has demonstrated that BUB1 and MAD2 interact with HPV E5 and may be implicated in decreasing BUB1 and MAD2 expression (**Figure 6b**) (Liao et al. 2012). In E5-transfected cells, BUB1 and MAD2 RNA and protein levels were significantly reduced, with cells exhibiting significantly enhanced proliferation and a greater cell population proportion in S phase (Liao et al. 2012). These findings indicate that HPV E5 crucially contributes to tumorigenesis and could serve as a potential target for cervical cancer therapeutic strategies. HPV E6/E7 gene expression facilitates circumventing the G2 DNA damage checkpoint and the SAC despite the presence of DNA damage. Elevated cyclin B, CDC20, and UBC10 levels were observed in HPV E6/E7-expressing cells (Patel & McCance 2010). It is plausible that elevated cyclin B permits evading the G2 DNA damage checkpoint and enables M phase entry, while high CDC20 and UBC10 levels cause impulsive APC/C activation and ensuing SAC inactivation, with cyclin B degraded, allowing mitotic exit and cell cycle progression (Patel & McCance 2010). Furthermore, HPV E6 expression in noncancerous cells induced lagging chromosomes, multiple chromosomes associated with one or both spindle poles with subsequent aneuploidy, and dramatic mitotic delay (**Figure 6c**) (Shirnekhi et al. 2017).

Despite the fact that viral particles are just beginning to be used as novel tools for studying the SAC, they could prove instrumental in enhancing our understanding of cell division and regulation and exploring the prospects of isolating central SAC regulator functions in MCC assembly, APC/C regulation, and mitotic exit. Future investigations must ascertain the precise mechanisms by which viral proteins manipulate the SAC and identify all central SAC regulators targeted.

### 4.3. SAC and Innovative Cancer Therapies

In the past 40 years, cancer treatment techniques, including chemo- and radiotherapy, have not significantly decreased cancer-related mortality, which is still above 50% of cases. This limited success is due in part to multiple side effects these therapies cause to normal, surrounding cells, including the induction of DNA damage that can promote the development of mutations and neoplastic transformation. This situation has stimulated the search for more specific, efficient, and widely accessible anticancer therapies. One important focus of research is the signaling and metabolic pathways implicated in cancer cell biology and tumor progression. Solid tumors are frequently aneuploid, and many display high rates of chromosome missegregation and CIN. The most common cause of the latter condition is the persistence of aberrant kinetochore-microtubule attachments, as shown by the presence of lagging chromosomes in anaphase. The observations that inhibiting MPS1 with chemical inhibitors kills cultured tumor cells while leaving normal cell

growth unaffected (Daniel et al. 2011, Kwiatkowski et al. 2010) and that its partial inhibition creates tumor cells more sensitive to clinical doses of taxol (Janssen et al. 2009) indicate that tumor cell proliferation can be halted through SAC inhibition (Daniel et al. 2011, Kwiatkowski et al. 2010). However, the incomplete inhibition of the SAC can also have the opposite effect of conferring a growth advantage to cancer cells, enabling them to tolerate aneuploidy and to escape from apoptosis (Kawakami et al. 2019, Sinha et al. 2019). Furthermore, the ATP-binding competitor inhibitors of SAC kinases developed to date exhibit relaxed target specificity and cannot truly differentiate between cancer and normal cells. For example, the MPS1 inhibitor reversine is a promiscuous compound that was first recognized as an inhibitor of aurora B, while the MPS1 inhibitors NMS-P153 and SP600125 are known to bind to FAK/PTK2A and Janus kinases, respectively (Schmidt et al. 2005). Therefore, despite sustained efforts by various drug discovery groups in industry and academia, all of the small inhibitors of aurora and PLK1 kinases reported to date have failed to demonstrate good therapeutic activity against solid tumors. An alternative strategy currently being explored is the search of ubiquitin ligase inhibitors that target the E3 ubiquitin ligase activity of the APC/C complex and APC/C regulators (Henriques et al. 2019, Zhou et al. 2016). In addition to upstream SAC kinases and downstream ubiquitin ligases, components of this molecular circuitry with no enzyme activity such as the APC/C activator CDC20 may constitute suitable new drug targets, an idea supported by the reported observations that depletion of CDC20 enhanced pancreatic cancer cytotoxicity upon paclitaxel treatment and increased the effectiveness of gamma irradiation (Taniguchi et al. 2008). Although conventional cytotoxic therapies that cause DNA damage or target cell cycle regulation can induce cell death when checkpoint repair mechanisms fail (Goldstein & Kastan 2015), the acquisition of defects in one or more checkpoint pathways during tumor progression can result in the suboptimal maintenance of genome integrity. This in turn may open up new therapeutic windows for targeted cancer treatments, including approaches that exploit synthetic lethality and oncogene addiction (Amin et al. 2015) to reduce dose-limiting toxicities to normal cells. For example, mutations that result in the activation of oncogenes or the loss of certain tumor suppressors can create new vulnerabilities that are cancer cell specific (Bartkova et al. 2005, Di Micco et al. 2006, Gorgoulis et al. 2005, Halazonetis et al. 2008, Hills & Diffley 2014). Even though the individual inactivation of one out of two compensatory pathways often does not compromise cell viability, the simultaneous disruption of both compensatory pathways can trigger programmed cell death.

In summary, one downside of chemo- and radiotherapy is that cytotoxicity derived from treatment regimens can increase the extent of mutagenesis in subpopulations of cancer cells that have already evaded the mechanism of checkpoint control, resulting in even more genetically unstable, drug-resistant clones. One way to avoid the reemergence of drug-resistant populations is to use combinations of cytotoxic agents that simultaneously attack different DNA damage response pathways. The development of new drugs that can interfere with the tension-sensitive nature of the SAC and its communication with the KMN network in human tumors appears to be an attractive alternative for the selective killing of cells that display CIN. In this regard, one strategy that remains largely unexplored is the targeting of specific protein-protein interactions in regulatory hubs that control chromosome segregation and mitosis progression, which may constitute an important pool of novel cancer drug targets. Similarly, the comprehensive analysis of protein expression profiles in different cancer cell lines will be instrumental to define the contribution of central components of the SAC signaling pathway to chemotherapy drug sensitivity, paving the way for the rational development of more effective anticancer drugs that function on different levels or involve diverse molecular mechanisms of action (see the sidebar titled Artificial Intelligence, Chromosome Segregation, and Cancer).

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**Oncogene addiction:**

the dependency of certain tumor cells on a single activated oncogenic protein or signaling pathway to preserve the malignancy state

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## ARTIFICIAL INTELLIGENCE, CHROMOSOME SEGREGATION, AND CANCER

The enormity of clinical data that oncologists, radiologists, surgeons, and doctors handle proves challenging and complex for analysis (Rabbani et al. 2018). Artificial intelligence (AI), which ultimately aims to computationally model human behavior and thinking processes, is gaining momentum in its application for tumor detection, patient stratification, cancer prognosis, and treatment (Ari & Hanbay 2018, Londhe & Bhasin 2019, Robertson et al. 2018). Recent AI advances and automated capabilities have proved momentous in enhancing clinicians' qualitative expertise in translating intratumoral phenotypic attributes to genotype implications and outcome predictions by allowing researchers to cross-reference individual tumors with databases of potentially limitless comparable cases, collectively leading to earlier interventions and significant diagnosis and clinical care enhancements (Bi et al. 2019). These approaches will soon be implemented in the analysis and treatment of aggressive tumors associated with chromosome segregation defects. AI is currently used in drug discovery programs to aid target identification, drug design, protein engineering, gene expression analysis, and pharmacodynamics modeling for precision medicine development (Chen et al. 2018, Kalinin et al. 2018, Mak & Pichika 2019, Zhang et al. 2017, Zhavoronkov et al. 2019), but has not yet been fully exploited to identify and validate new drugs designed to target the SAC.

### SUMMARY POINTS

1. The spindle assembly checkpoint (SAC) plays a fundamental role in the maintenance of genome stability by ensuring the timely segregation of the genetic material every time a cell divides. Despite recent important advances, crucial details of SAC signaling, including signal initiation and SAC regulation, remain to be fully understood.
2. Diverse types of oncoviruses, ranging from double-stranded DNA parapoxviruses and papilloma viruses to adenoviruses and retroviruses, harness control of chromosome segregation in human cells. The anaphase-promoting complex/cyclosome (APC/C) appears to be a preferred viral target for establishing an intracellular environment favoring viral replication.
3. Defects in the SAC result in cell-specific vulnerabilities that can be exploited for the treatment of aggressive tumors.
4. Several small-size inhibitors of SAC kinases are currently undergoing clinical trials. Further exploration of their use in combination with established cytotoxic agents or radiation biology is needed to develop more effective therapies for the treatment of cancers of poor prognosis.

### FUTURE ISSUES

1. Establishing the underlying SAC mutations in a patient's tumor to identify specific oncogenic drivers remains of paramount importance.
2. Several important mechanistic aspects underlying the control of chromosome segregation remain to be clarified, including the definition of the precise role of BUB3 in the mitotic checkpoint complex (MCC) bound to APC/C-CDC20, and how exactly the various conformational states of APC/C-CDC20 are regulated in the cell.

3. It is equally important to evaluate the global implications that oncoviruses targeting the SAC exert on cellular metabolism in normal cells and tissue-specific tumors.
4. It seems feasible to target central SAC components alone or when engaged in complex formation for the treatment of cancer. Strategies based on fragment-based discovery will be increasingly popular to assess the druggability of the SAC.
5. It remains to be determined whether manipulation of the SAC together with X-ray irradiation/proton beam therapy can sensitize cancer cells to drugs.
6. The utilization of viral particles for cancer therapeutic strategies that target the SAC-kinetochore-microtubule axis is yet to be explored.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Elegantly demonstrates that Mps1 and Plk1 kinases share a similar substrate recognition consensus motif.

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A comprehensive revision of how kinases and phosphatases work together to regulate SAC signaling.

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Discusses the evolution of Bub1 and BubR1 kinases and postulates that BubR1 is a pseudokinase.

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Presents an authoritative revision of APC/C structure, function, and complex mode of regulation.

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Reports Bub1’s role in the long-term persistence of an oncovirus genome in the infected cell.

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## Errata

An online log of corrections to *Annual Review of Cancer Biology* articles may be found at <http://www.annualreviews.org/errata/cancerbio>