nature cell biology

Perspective

https://doi.org/10.1038/s41556-025-01784-w

Biological clocks keep a watch on mitosis

Received: 16 February 2025

Accepted: 3 September 2025

Published online: 06 January 2026

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Accurate chromosome segregation is vital for organismal development and homeostasis, with errors in this process strongly associated with tumourigenesis. A network of safeguard clocks preserves mitotic fidelity by detecting and eliminating cells dividing outside the stereotyped duration of successful mitosis. This Perspective examines recent advances in our understanding of mitotic timing mechanisms, presents emerging evidence for novel mitotic clocks and proposes a conceptual framework for how cells integrate temporal cues to preserve genomic integrity.

Human development requires tens of trillions of cell divisions[1,2], with an additional 300 billion divisions daily to replace damaged or dying cells[3]. This extraordinary scale of proliferation underscores the immense biological challenge of maintaining genomic stability throughout an organism's lifespan. Despite the precision of the mitotic apparatus, errors are inevitable, and are linked to developmental defects, ageing and cancer[4,5]. To mitigate these risks, cells deploy numerous mitotic safeguards to detect and eliminate the progeny of aberrant divisions.

Accurate chromosome segregation is a complex bio-mechanical process, requiring cells to faithfully distribute a complete set of chromosomes into 2 daughter cells within an approximately 30-minute mitotic period. Although modest defects in cell division can have substantial consequences, direct chromosome counting presents a notable biological challenge: mis-segregation of just one or a few chromosomes results in only subtle changes in total DNA content that is difficult to sense directly. Consequently, cells largely rely on indirect indicators to detect and respond to cell division errors (Fig. 1).

Large-scale errors such as multipolar divisions often lead to the loss of essential genes, triggering apoptosis. Mechanical distortions in the nuclear envelope driven by mis-segregation errors activate the tumour suppressor p53[6], while substantial chromosomal imbalances induce protein misfolding and proteotoxic stress, prompting a robust cellular response[7]. The DNA-damage response (DDR) also identifies mitotic errors through various mechanisms. Lagging chromosomes during mitosis may be physically severed by the cytokinesis machinery[8] and often become encapsulated in micronuclei, which are prone to DNA damage[9,10]. Aneuploid cells frequently accumulate DNA damage due to replication stress caused by imbalanced or insufficient replication machinery[11]. Finally, immune responses are activated in reaction to cytosolic DNA[12] or persistent endoplasmic reticulum stress[13]. Together, these pathways mitigate the adverse consequences of the most severe mitotic errors.

Whole-genome doubling (WGD), resulting from mitotic slippage or cytokinesis failure, produces daughter cells containing twice the normal genetic content. Although genome-doubled cells maintain balanced gene-expression patterns, they are frequently associated with the early stages of cancer development[14,15], underscoring the necessity of detection systems for this specific defect. In addition to doubling genetic content, WGD cells also double the number of centrioles, a microtubule-based organelle that organizes the interphase microtubule network and anchors the poles of the mitotic spindle during cell division. A fail-safe pathway known as the PIDDosome is triggered by the clustering of supernumerary centrioles in the G1 cell-cycle phase and halts the proliferation of WGD cells[16–18]. Nonetheless, despite multiple fail-safe systems, chromosome mis-segregations may still evade detection[19,20], allowing unchecked cellular proliferation and increasing the risk of malignant transformation.

Temporal cues are broadly integrated into developmental, homeostatic and safeguard pathways and, in the past decade, mitotic duration has emerged as a critical proxy for identifying and responding to mitotic errors[21]. Mitotic clocks offer distinct advantages over other safeguard mechanisms. First, healthy cells have a stereotypical mitotic duration due to the spindle assembly checkpoint (SAC), which delays anaphase until chromosomes are correctly attached to the mitotic spindle[22,23]. As such, SAC-induced mitotic delays effectively report on compromised mitotic machinery as a risk factor for chromosome segregation errors[24]. Second, cellular homeostasis is dramatically altered during mitosis, including attenuated transcription[25], reduced translation[26], hyper-phosphorylation[27], the use of alternative translational isoforms[28] and the regulated degradation of specific proteins[29–31]. These alterations form reliable 'START' and 'STOP' signals for mitotic timekeeping mechanisms. Lastly, mitotic clocks can respond directly to compromised mitotic machinery, making them uniquely positioned to triage problematic cells before the loss of genome integrity[24].

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https://doi.org/10.1038/s41556-025-01784-w

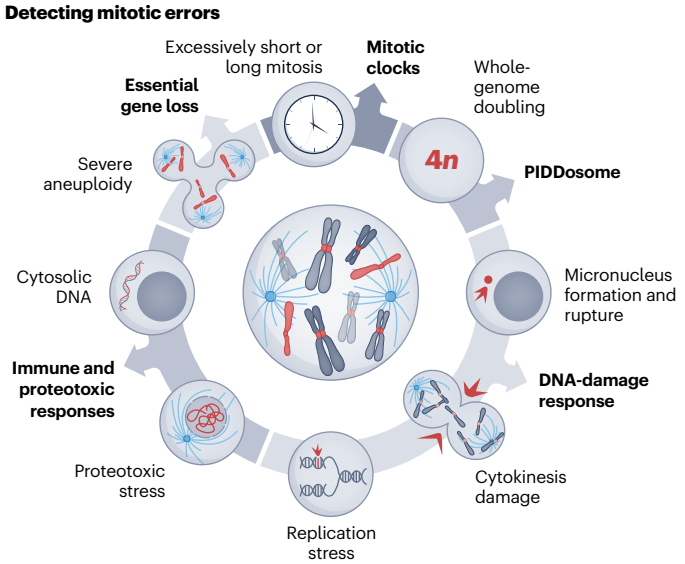

Fig. 1 | Cellular responses to mitotic errors. Mitotic error response pathways. Mitotic clocks (top) respond to abnormally short or long mitotic durations. The PIDDosome (top right) identifies whole-genome doubling by detecting supernumerary centrioles. The DNA-damage response (bottom right) recognizes DNA insults caused by both mechanical stress (that is, cytokinesis damage) or replication stress induced by protein-dosage imbalances. Multiple innate immunity and proteotoxic pathways (bottom left) respond to both protein misfolding and cytosolic DNA. Cell death is driven by loss of essential genes (top left) resulting from severe aneuploidy.

This Perspective examines three specific mitotic timing mechanisms: the 'apoptotic timer', the 'minimum duration of mitosis' clock and the 'mitotic-stopwatch pathway'. We discuss the current mechanistic understanding of these pathways, highlighting how biology incorporates time sensing into existing cell-death and cell-cycle checkpoint pathways. Finally, we explore emerging evidence linking these timing mechanisms to developmental disorders and cancer.

The apoptotic timer

The apoptotic timer is a surveillance mechanism that activates the intrinsic (mitochondrial) apoptotic pathway in response to prolonged mitotic arrest, typically following disruption of normal spindle dynamics. The threshold for timer activation varies between individual cells, with apoptosis onset observed between 10 hours and 30 hours of sustained mitotic arrest[32,33]. Intrinsic apoptosis is a form of programmed cell death most associated with environmental stressors, such as toxins, poor nutrient availability and ultraviolet irradiation[34]. In this pathway, BH3-only proteins (BIM, BID, NOXA, PUMA and so on) sense cellular stress and inhibit pro-survival BCL-2 family members, including MCL-1[35]. This in turn releases pore-forming proteins BAX and BAK, which drive mitochondrial outer membrane permeabilization, initiating caspase activation and apoptotic cell death[36].

MCL-1 is both the molecular timekeeper and effector protein of the apoptotic timer. In this pathway, MCL-1 is gradually degraded over several hours, initiating apoptosis once its levels drop below the threshold necessary to restrain the pro-apoptotic proteins BAX and BAK[37,38] (Fig. 2, right). Despite its critical role, the precise mechanism governing MCL-1 degradation during mitosis remains contentious. A leading model implicates the anaphase-promoting complex/cyclosome (APC/C), bound to its co-activator CDC20, as a primary regulator of MCL-1 turnover[33,37]. This E3 ubiquitin ligase complex orchestrates mitotic progression by targeting its key substrates cyclin B1 and securin for degradation. Degradation of cyclin B1 deactivates the master mitotic kinase CDK1, while securin loss activates the anaphase-promoting activity of the protease separase[39]. The activity of APC/C–CDC20 towards these substrates is tightly regulated by the SAC, which suppresses APC/C–CDC20 function until all chromosomes achieve proper kinetochore–microtubule attachment[22,23]. Interestingly, MCL-1 degradation is initiated at mitotic entry and proceeds independently of SAC signalling. This process is mediated by an atypical degron motif that renders MCL-1 insensitive to SAC-mediated inhibition[32,33], akin to early mitotic APC/C–CDC20 substrates, such as cyclin A and Nek2A, which are also degraded despite SAC signalling[40–42].

An alternative model proposes that SCF–FBXW7, a Skp–cullin–F-box (SCF) E3 ubiquitin ligase complex, targets phosphorylated MCL-1 for degradation[32,43]. In this model, the mitosis-restricted kinase complex CDK1–cyclin B1 phosphorylates MCL-1 to activate a phospho-degron that is recognized by SCF–FBXW7. However, evidence for the involvement of APC–CDC20 and SCF–FBXW7 in MCL-1 degradation is inconsistent across different experimental systems, and studies have supported the involvement of one, both or neither of these ligases in mitotic MCL-1 degradation[32,33,38,43,44]. Despite these mechanistic uncertainties, the gradual loss of MCL-1 is widely recognized as a temporal checkpoint that ensures that cells unable to resolve mitotic errors are eliminated, thereby safeguarding genomic integrity.

The minimum duration of mitosis clock

Surprisingly, MCL-1 has also been implicated in a minimum duration of mitosis timer that safeguards against premature mitotic exit before 15–20 minutes[45] (Fig. 2, left). Early in mitosis, the mitotic kinase Nek2A phosphorylates MCL-1, sensitizing it to cleavage by the protease separase, which is active only at mitotic exit[45]. Typically, Nek2A is rapidly degraded within the first 30–40 minutes of mitosis, independently of SAC signalling, creating a restricted window during which phosphorylated MCL-1 is present[40,45,46]. If separase is activated by premature mitotic exit during this time, MCL-1 is cleaved into pro-apoptotic fragments that initiate apoptosis via mitochondrial outer membrane permeabilization. Under normal mitotic conditions, this apoptotic pathway is not triggered, as minimal phosphorylated MCL-1 remains by the time mitotic exit occurs. A parallel mechanism has been observed for the related anti-apoptotic protein BCL-XL, which also undergoes Nek2A-dependent phosphorylation and subsequent separase-mediated cleavage. However, the cleavage products of BCL-XL appear less effective at inducing apoptosis during mitosis.

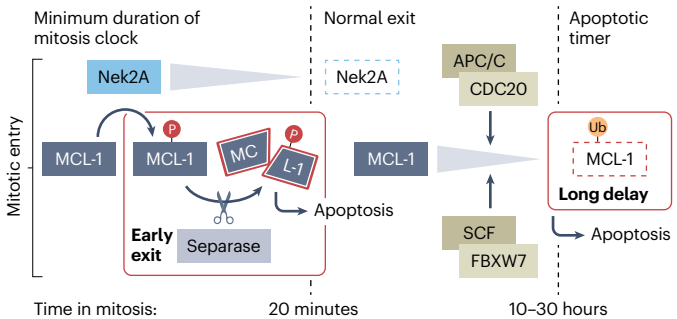

Fig. 2 | Apoptotic mitotic clocks. Left: minimum duration of mitosis clock (<20 minutes in mitosis). Early in mitosis, the pro-survival BCL-2 family member MCL-1 is phosphorylated by high levels of Nek2A. If mitotic exit occurs while phosphorylated MCL-1 is still present, separase activity cleaves MCL-1 into pro-apoptotic fragments, leading to cell death. Rapid degradation of Nek2A constrains phospho-MCL-1 to the first 20 minutes of mitosis, thereby creating a restricted window for activation of the minimal duration of the mitosis timer. Right: apoptotic timer (>10 hours in mitosis). During prolonged mitosis, MCL-1 is progressively targeted for degradation by the APC/C–CDC20 and/or SCF–FBXW7 ubiquitin ligase complexes. The slow depletion of MCL-1 diminishes the anti-apoptotic buffering capacity, shifting the balance towards cell death. Once MCL-1 levels fall below a critical threshold, typically between 10 hours and 30 hours in mitosis, the apoptotic timer is activated, ensuring elimination of cells that fail to complete division in a timely manner.

https://doi.org/10.1038/s41556-025-01784-w

While this alternative MCL-1-based timer provides a compelling model for protecting against premature mitotic exit, its physiological significance remains unresolved. Activation of this pathway is contingent on the premature activation of separase, an event most likely triggered by acute and penetrant failure of the SAC[45]. Under such conditions, cells are at risk of WGD, a state that typically triggers p53 activation through the PIDDosome pathway[16–18]. Partial SAC defects, which more commonly lead to chromosome mis-segregation rather than catastrophic mitotic exit, are unlikely to shorten mitosis sufficiently to trigger this apoptotic mechanism[47]. Thus, although separase-mediated cleavage of MCL-1 has been observed in a range of human and non-mammalian vertebrate cell lines, further investigation is needed to determine whether this pathway functions as an intrinsic mechanism of mitotic quality control or represents a context-dependent, stress-activated apoptotic response.

The mitotic-stopwatch pathway

The apoptotic timer and minimum duration of mitosis clock are robust, fail-safe mechanisms that protect cells from severe mitotic errors. These pathways are activated under only extreme conditions, such as SAC failure or premature sister chromatid disjunction, resulting in persistent SAC signalling. Consistently, the effects of these pathways are sensed and executed acutely and show limited sensitivity to subtle or chronic mitotic disturbances. By contrast, the mitotic-stopwatch pathway (MSP), also known as the mitotic surveillance pathway or mitotic timer, functions as a highly sensitive monitor of mitotic duration[48,49]. Uniquely, it responds not only to moderately extended mitoses (typically 90 minutes to 2 hours) but also to chronic, subthreshold delays across successive generations[50–53] (Fig. 3a,b). Upon activation, the MSP halts the cell cycle of daughter cells in the subsequent interphase, distinguishing it from the immediate mitotic death triggered by MCL-1-based mitotic clocks.

Discovery and mechanistic insights into the MSP

Since its discovery in 2010, the MSP has emerged as a critical mitotic clock that safeguards cells by activating p53 in response to moderate mitotic delays[50]. The MSP operates independently of the underlying cause of the mitotic delay, and responds to a wide range of perturbations, including small-molecule disruption of the mitotic spindle, centriole loss and proteasome inhibition[48,50,52,54]. Although the precise timing mechanism of the MSP remains unresolved, early genome-wide CRISPR–Cas9 knockout screens have identified key components of this pathway, including the DDR scaffolding protein 53BP1, the deubiquitylating enzyme USP28, p53 and its transcriptional target p21[51–53].

The MSP is one of many pathways that engage the linchpin tumour suppressor protein p53[50,54]. Under unstressed conditions, p53 levels are maintained at low levels through continuous degradation by the E3 ubiquitin ligase MDM2[55]. In response to cellular insults, such as DNA damage, oncogene activation or metabolic stress, p53 is stabilized either through MDM2 inhibition or post-translational modifications that render p53 resistant to MDM2 degradation[56]. Stabilized p53 selectively induces the transcription of a wide set of effector genes depending on specific post-translational modifications, which can either stimulate cell-repair pathways, initiate negative feedback regulators, drive cellular senescence or execute cell death[57,58]. Despite sharing several components with the DDR pathway, the MSP is a distinct signalling axis. This has been demonstrated using separation-of-function mutations[53] and the insensitivity of the MSP to canonical DDR inhibitors[51]. Moreover, genetic ablation of DDR-specific proteins fails to rescue cells from MSP-mediated growth arrest after mitotic delay[51–53].

Central to MSP function is the temporally regulated formation of the 53BP1–USP28–p53 trimeric stopwatch complex[49,59]. The formation of this complex depends on the tandem BRCT domains of 53BP1, which simultaneously interact with USP28 and p53[60–62]. Crucially, MSP activation relies on the catalytic activity of USP28[53], suggesting that USP28

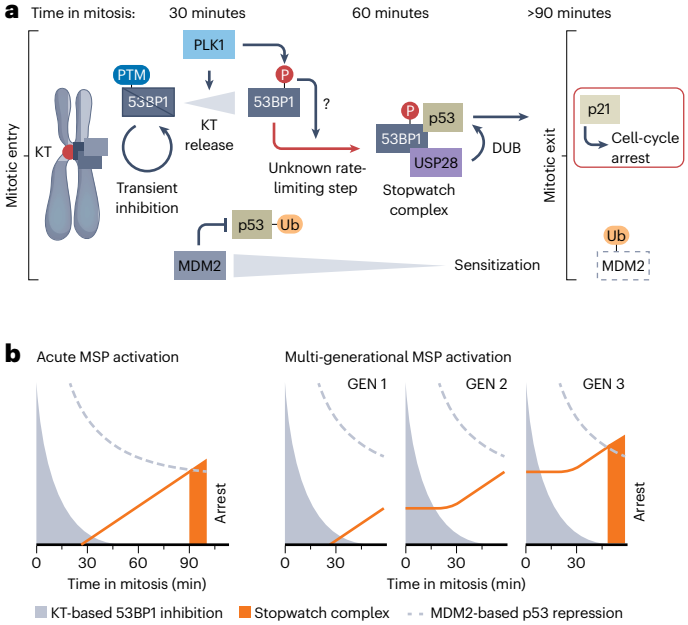

Fig. 3 | The mitotic-stopwatch pathway. a, During early mitosis, 53BP1 localizes to the kinetochore (KT), where it is functionally inhibited, probably through post-translational modifications (PTM). As mitosis progresses, PLK1-mediated phosphorylation of an unknown substrate displaces 53BP1 from the KT, which in turn licenses the assembly of USP28–53BP1–p53 trimeric stopwatch complexes. Within these complexes, USP28 deubiquitylates and stabilizes p53, leading to the transcriptional activation of p21 and the induction of cell-cycle arrest in the subsequent interphase. Approximately 90 minutes into mitosis, the MSP is activated due to the critical accumulation of stabilized p53. Concomitantly, the gradual degradation of MDM2, a negative regulator of p53 stability, cooperates with the MSP to trigger p53 activation. DUB, deubiquitinase. **b**, Time-course of KT-based 53BP1 inhibition (grey region), stopwatch complex formation (orange line) and MDM2-based p53 repression (dotted line) during MSP activation. Threshold levels of stopwatch complex formation can occur in response to a single prolonged mitosis (left) or after multiple cell cycles experiencing moderate mitotic delays (right). GEN, generation.

may use the stopwatch complex to directly stabilize p53 through deubiquitination, paralleling mechanisms that are induced pharmacologically by stabilizing p53 with the MDM2 inhibitor nutlin-3[63]. Stopwatch complexes accumulate progressively during prolonged mitosis and remain stable throughout the subsequent interphase, allowing for persistent signalling[49]. This prolonged stability is particularly important for MSP activation in response to centriole loss, where cells experience repeated, subthreshold mitotic delays over successive divisions, rather than a single, overtly prolonged mitosis[51–53]. However, robust detection of stopwatch complexes remains an ongoing experimental challenge, with negligible complex formation being observed before 4 hours in mitosis—well beyond the approximately 90-minute threshold for MSP activation[49,53,59]. Therefore, clarifying the temporal dynamics and regulatory mechanisms that govern the formation and persistence of this complex remains a crucial area of investigation.

Post-translational regulation and the role of PLK1

Biological clocks often function through changes in abundance or post-translational modification state of timekeeper molecules[21]. In the context of the MSP, current evidence suggests that protein abundance is not the primary determinant of mitotic timing, as the abundance of the stopwatch complex components remains relatively stable throughout mitosis[49], and the MSP has been demonstrated to be insensitive to proteasome inhibition[50]. Given these findings, post-translational modification has been proposed as the primary mechanism driving the gradual licensing of stopwatch complex formation and MSP activation.

Supporting this model, 53BP1 is extensively post-translationally modified during mitosis, and undergoes a striking shift in the subcellular localization over the course of mitotic progression. Immediately following mitotic entry, 53BP1 is strongly enriched at kinetochores, the protein complexes responsible for tethering chromosomes to the mitotic spindle microtubules[64]. As mitosis proceeds, 53BP1 gradually dissociates from kinetochores into the cytoplasm, possibly due to a shift in its post-translational state[51,53,64]. This spatiotemporal change led to speculation that the release of 53BP1 from kinetochores may license the formation of the stopwatch complex and trigger MSP activation[48].

Recent studies have identified the kinase PLK1 as a critical regulator of 53BP1-kinetochore release and MSP function. Notably, inhibition of PLK1 activity results in sustained kinetochore association of 53BP1, prevents stopwatch complex formation and abrogates cell-cycle arrest following prolonged mitosis[49,59]. However, kinetochore release of 53BP1, while necessary, is not sufficient for MSP activation. Cells with a mutation in the kinetochore receptor CENP-F, which prevents 53BP1-kinetochore localization altogether, exhibit normal MSP function, suggesting that release is required but not rate limiting[59].

The extent of the role of PLK1 in the MSP beyond facilitating the kinetochore release of 53BP1 remains unclear. Mutating three PLK1-mediated phosphorylation sites on 53BP1 to alanine has been shown to impair the binding of p53 to 53BP1[49]. However, the physiological significance of these phosphorylation sites remains unresolved due to a lack of data regarding the nuanced timing of these phosphorylation events and the absence of functional experiments with phosphomimetic mutants. By contrast, a separate study found that disruption of MSP function by PLK1 inhibition was limited to cells in which 53BP1 could be recruited to kinetochores. Consequently, cells harbouring CENP-F mutations that prevent 53BP1-kinetochore localization were able to activate the MSP regardless of whether PLK1 was inhibited[59]. Even though the clonogenic assays used in these latter experiments lacked sensitivity for detecting subtle changes in the MSP timer threshold, these findings support a model in which PLK1 primarily influences timer function by releasing 53BP1 from kinetochores.

How kinetochores prevent 53BP1 integration into the stopwatch complexes during early mitosis remains unclear. Bulk sequestration of 53BP1 has been proposed as one possible mode of action[59], although the comparatively large amount of cytosolic 53BP1 relative to the small total surface area of kinetochores as a binding site makes this unlikely. Alternatively, kinetochore-mediated phosphorylation may transiently inhibit 53BP1, with subsequent cytosolic dephosphorylation allowing complex formation, although compelling evidence supporting either of these models is still lacking.

A major unresolved issue is whether stopwatch complex formation is itself the timer. The low abundance of complex detected within the first 2 hours of mitosis[49,59] indicates that either the MSP is highly sensitive to minimal amounts of stopwatch complex assembly, or a self-sustaining mechanism exists whereby initial complex formation triggers further assembly that persists through mitotic exit and into the subsequent interphase. Addressing these questions will require advanced, highly sensitive assays to resolve subtle temporal variations in complex assembly and signalling dynamics.

Mechanisms of crosstalk

A recent study evaluating the consequences of MDM2 degradation in mitosis highlights another important consideration for MSP function[65]. In this work, the authors observed that reduced MDM2 synthesis during mitosis, due to both transcriptional repression[25] and targeted translational inhibition[66], leads to decreased levels of this short-lived protein[65]. In cells undergoing prolonged mitosis, MDM2 levels can fall below a critical threshold, triggering p53 activation[65,66]. Indeed, this mechanism was initially proposed as a potential MSP timing mechanism before the discovery of stopwatch complex components[67]. However, this hypothesis was largely disfavoured because of several key findings.

BOX 1

Model cell lines, biological variability and physiological relevance

Despite extensive research in cell lines, few observations have been made regarding the physiological activation of mitotic timing mechanisms at the organismal level. This limitation has largely confined our understanding of these pathways to their synthetic activation in model systems. For example, investigations into the minimum duration of mitosis timer have been primarily restricted to HeLa and HEK cells, without direct evidence of in vivo activation[45]. Similarly, while cell death mediated by the apoptotic timer has been observed in various cell lines and is linked to the response to anti-mitotic cancer therapies[44], both the threshold for activation and therapeutic efficacy vary across and within cell lines[33,99,100]. The majority of the foundational MSP work was conducted in RPE1 cells[51–53]. However, MSP function across other cell lines has proved inconsistent, even among those expressing all known MSP components[49]. Moreover, studies in ex vivo neurons revealed substantial variability in the MSP activation threshold compared with measurements from RPE1 cells in vitro[68]. These findings suggest that mitotic timing pathways may exhibit cell-type-specific fidelity and activation thresholds, which are not adequately captured by the current literature.

First, proteasome inhibition was shown to neither alter the timing threshold of the mitotic timer nor prevent MSP activation[50,53]. Second, deletion of core MSP components, such as USP28 and 53BP1, was sufficient to rescue cell proliferation following mitotic delays[51–53]. Finally, the gradual degradation model of MDM2 fails to account for the 'memory' aspect of the MSP; specifically, the ability to trigger cell-cycle arrest after repeated, subthreshold mitotic stress events across multiple generations. Nonetheless, given that p53 is a component of the stopwatch complex, reduced MDM2 levels will inevitably bias the system towards p53 stabilization, effectively lowering the activation threshold of the MSP. This suggests a functional crosstalk between MDM2 degradation and stopwatch complex assembly, with both processes potentially acting in concert to enforce the strict activation threshold of the MSP.

In conclusion, current evidence supports an inhibitory role of kinetochores in stopwatch complex formation, with PLK1-dependent release of 53BP1 from kinetochores serving as a prerequisite for MSP activation. While direct phosphorylation of 53BP1 by PLK1 may further enhance its association with p53, such effects appear relatively modest. Importantly, cells incapable of localizing 53BP1 to kinetochores exhibit apparently normal MSP function[59], suggesting that the kinetochore release of 53BP1 is not rate limiting. This finding implies the presence of a yet-unidentified cytosolic mechanism that serves as the primary MSP timekeeper.

Challenges and considerations when studying mitotic clocks and cell death

Cell-cycle decisions following stress are influenced by numerous factors, with the p53 pathway representing a particularly complex and highly interconnected network whose responses vary by cell type, tissue and cellular stress[58]. Crucially, crosstalk between the p53 pathway and apoptotic signalling pathways can lead to either senescence or apoptosis in response to activation of the MSP[51–53,68] (Box 1). Although the main players in the MSP have been identified, it is becoming increasingly evident that the MSP integrates multiple signalling

events that result from a broad shift in both the mitotic proteome[69] and the phospho-proteome[27,70]. For example, a recent study uncovered a non-canonical role for the double-stranded DNA sensor cyclic GMP–AMP synthase (cGAS) in promoting cell death during prolonged mitosis[71]. Although chromatin binds cGAS with high affinity, it also largely suppresses its catalytic activity, rendering chromosomes refractory to innate immune detection during normal mitosis. However, when cells are delayed in mitosis, incomplete cGAS inhibition leads to the accumulation of pro-apoptotic signalling. This mechanism probably acts in concert with the degradation of mitotic MCL-1 and MDM2, shifting the balance towards cell-cycle arrest.

The intertwined regulation of mitotic arrest and apoptosis presents notable challenges in interpreting experimental models of prolonged mitosis. Perturbations that deplete critical pro-proliferative proteins, such as MCL-1 or MDM2, inherently push cells towards death. However, such experiments might not inform on whether these proteins are the primary mediators of death following delayed mitosis. Conversely, synthetically stabilizing or overexpressing these proteins can create artefacts, whereby pathways such as the MSP fail to activate due to exaggerated pro-survival signalling (for example, excessive MDM2-mediated p53 degradation). Therefore, deriving unambiguous conclusions from perturbation experiments depends on carefully modulating protein expression and activity within physiologically relevant ranges. This can be achieved through precise genetic mutations, selective chemical inhibitors or degraders that enable the acute loss of target proteins.

Manipulating core mitotic regulators that also serve as components of mitotic clocks can inadvertently disrupt mitotic entry, progression or exit, thereby confounding interpretations of mechanistic experiments. Indeed, several proteins involved in mitotic clocks, including APC/C–CDC20, SCF–FBXW7 and PLK1, have critical roles in the precise execution of mitosis[23,72,73]. Similarly, genome-wide CRISPR–Cas9 knockout screens, although powerful, are limited in their ability to assess essential genes, which will exclude many core mitotic regulators from analysis. As a result, the identification of mitotic clock components through experimental perturbation remains challenging, and results must be interpreted with caution, considering the pleiotropic effects and technical limitations inherent with each experimental approach.

Broader impacts of mitotic clocks

Mitotic clocks are critical guardians of cell fate, ensuring accurate chromosome segregation and preventing aberrant cell proliferation. Alongside other mitotic fail-safe pathways, they play a key role in maintaining genomic stability. However, the timing and extent of their activity during development remain incompletely understood. Errors in early embryonic cell divisions can result in mosaic genetic disorders, such as mosaic trisomy 21, mosaic Klinefelter syndrome and mosaic Turner syndrome[74]. Furthermore, mutations in some SAC genes can cause mosaic variegated aneuploidy, which is associated with a variety of developmental defects and an increased risk of cancer[75]. While early mitotic errors clearly cause these disorders, no direct association with mitotic clock dysfunction has been established.

Recent work has provided evidence of mitotic clock function in a physiological context. In a murine model of primary microcephaly, reduced brain size was traced to chronic embryonic activation of the MSP triggered by centrosome loss[68]. Conditional knockout of the stopwatch complex components *USP28*, *53BP1* and *TP53* in neural cells restored normal brain size and architecture. These findings suggest that aberrant MSP activation promotes microcephaly pathogenesis, indicating that mitotic clocks are active during early neural development.

Beyond development, the MSP has been proposed to primarily serve a tumour-suppressive function. Aneuploidies resulting from mitotic errors are an oncogenic driver[76,77], and the frequent inactivation of the MSP has been reported in cancer cell lines[49]. In a panel of p53-proficient cancer cell lines, two-thirds exhibited partial or complete loss of MSP activity, often associated with mutations in *USP28*, *53BP1* or disruptions in p53 signalling. These results suggest that MSP impairment may facilitate tumour progression. Supporting this model, large-scale bioinformatics studies have highlighted the tumour-suppressive influence of *USP28* and *53BP1*, although not explicitly linked to mitotic clock activity[78–81].

Deciphering the specific contribution of mitotic clock activity to tumour-suppressor pathways is non-trivial, as all known clock components are either essential genes, and/or involved in multiple effector pathways. Overexpression of the oncogene *MCL-1* is common in cancer and associated with poor prognosis[82,83], as this gene is broadly involved in suppressing the apoptotic response beyond its role in the apoptotic timer[84,85]. Similarly, although MSP dysfunction is often observed in cultured cancer cells[49], MSP proteins also participate in several other tumour-suppressive processes, which makes their MSP-specific contribution to tumour suppression difficult to evaluate. The multifunctionality of key components, such as p53, which integrates signals from DNA damage, oncogene activation, the PIDDosome and the MSP[86], complicates attribution of tumour-suppressive effects to mitotic clock activity alone. While 53BP1 is known for its role in the DDR[63,81] and USP28 stabilizes numerous tumour-suppressor and oncogenic proteins[87,88], only 53BP1 loss has been linked to increased aneuploid tumour formation in mice[89,90]. USP28 loss, by contrast, has no known association with spontaneous tumourigenesis in murine models[91,92]. These findings underscore that MSP disruption alone is insufficient to initiate cancer, and that sensitized models will be necessary to clarify the MSP's protective role against malignant transformation.

Whether deeper mechanistic insights into mitotic clocks will yield viable therapeutic targets for cancer remains an open question. Despite the early success of therapies based on vinca alkaloids and taxanes, which disrupt the microtubule-based mitotic spindle, the next-generation of anti-mitotic compounds stalled in the clinic due to their indiscriminate killing of healthy proliferative tissues[93]. Moreover, it is now understood that therapeutic taxane doses induce only low-level mitotic errors rather than full arrest[94]. Given that aneuploidy, a frequent hallmark of cancer, is associated with prolonged mitosis, strategies to activate or restore mitotic clock function may provide therapeutic benefit. This approach may be especially promising in combination with treatments that exploit mitotic vulnerabilities in genomically unstable tumours[95–98].

Concluding remarks

Mitotic clocks serve as critical surveillance mechanisms that detect and respond to problematic cell divisions. These safeguards are sensitive to a wide range of mitotic mistiming and trigger corrective responses to preserve genome integrity. The apoptotic timer represents a simple clock architecture, utilizing MCL-1 to both sense time and regulate cell death, likely contributing to the tumour-suppressive role of this protein. By contrast, the MSP trades simplicity for sensitivity, as it can respond to both prolonged and repeated moderate mitotic delays. Recent studies underscore both physiological and pathological roles for the MSP, particularly in development and tumour suppression. However, the precise molecular timing mechanisms that govern mitotic clock activity remain incompletely understood and are the focus of ongoing investigation. A deeper understanding of mitotic clock biology may ultimately unlock new strategies for targeting cancer and other diseases rooted in chromosomal instability.

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Acknowledgements

This work was supported by National Institutes of Health grant 2T32HL007534-41 to C.R.G.

Author contributions

A.J.H. and C.R.G. conceived of and wrote this paper.

Competing interests

The authors declare no competing interests.

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Peer review information *Nature Cell Biology* thanks Jonathon Pines and Adrian Saurin for their contribution to the peer review of this work.