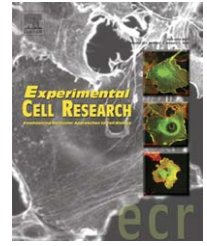


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Review Article

Structure meets function—Centrosomes, genome maintenance and the DNA damage response

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ABSTRACT

Centrosomes are cytoplasmic organelles playing a fundamental role in organizing both the interphase cytoskeleton and the bipolar mitotic spindle. In addition, the centrosome has recently come into focus as part of the network that integrates cell cycle arrest and repair signals in response to genotoxic stress—the DNA damage response. One important mediator of this response, the checkpoint kinase Chk1, has been shown to negatively regulate the G₂/M transition via its centrosomal localization. Moreover, there is growing evidence that a centrosome inactivation checkpoint exists, which utilizes DNA damage-induced centrosome fragmentation or amplification to provoke a “mitotic catastrophe” and eliminate damaged cells. Candidate regulators of this centrosomal checkpoint include the checkpoint kinase Chk2 and its upstream regulators ATM and ATR. In addition, a growing number of other proteins have been implicated in centrosomal regulation of the DNA damage response, e.g. the tumor suppressor p53, the breast cancer susceptibility gene product BRCA1 and mitotic regulators such as Aurora A, Nek2 and the Polo-like kinases Plk1 and Plk3. However, many missing links and discrepancies between different model systems remain.

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Introduction

One of the most common properties of cancer is genomic instability enabling cancer cells to overcome selection barriers [1]. Genomic instability can be classified into microsatellite instability (MIN) and chromosomal instability (CIN) leading to aneuploidy [2]. As early as one century ago, Theodor Boveri proposed that cancer commonly arises through aneuploidization linked to abnormal mitoses, and postulated the underlying cause of this phenotype to be aberrant centrosomes [3]. Nowadays, it is well established that both numerical and structural centrosome aberrations are common in many types of human malignancy and are indeed associated with aneuploidy [4,5]. Moreover, centrosome aberrations were shown to be correlated to clinical and prognostic parameters [4,5].

Centrosomes are tiny ($1\text{--}2\ \mu\text{m}^3$) cytoplasmic organelles consisting of two centrioles which are surrounded by pericentriolar material [6]. In animal cells, the centrosome serves as the microtubule-organizing center (MTOC) and plays a fundamental role in organizing both the interphase cytoskeleton and the bipolar mitotic spindle, ensuring correct segregation of chromosomes to prevent aneuploidy [5,6]. For this purpose, the single centrosome needs to be duplicated exactly once per cell cycle. This highly ordered and tightly regulated process takes place during S phase, concurrent with DNA replication, in a semiconservative fashion, with one daughter centriole arising from each of the two mother centrioles. This generates two pairs of centrioles, which separate at the onset of mitosis to form two distinct centrosomes that build up a bipolar mitotic spindle. In the following G_1 phase, each cell contains one centrosome, the mother and daughter centriole of which split to become two mature mother centrioles for the next duplication cycle. While the normal centrosome cycle and its regulation have been reviewed elsewhere [4–6], this review will focus on the emerging role of centrosomes in the DNA damage response.

Spatial organization of the DNA damage response network

The integrity of the genome can be altered by many ways, e.g. DNA double-strand breaks (DSBs) due to ionizing radiation, DNA cross-links due to ultraviolet radiation or mitomycin C, or stalled replication forks due to hydroxyurea treatment [7–9]. Cells are able to arrest the cell cycle under such conditions, which has been termed the DNA damage checkpoint(s) [7–9]. This provides time for repair of damaged DNA and, optionally, can lead to senescence or apoptosis of cells with irreversibly damaged genomes [7–9]. The total of reactions to genotoxic stress has been named the DNA damage response [7–9]. In addition, cell cycle checkpoints can be triggered not only by external damage, but rather by any threat to orderly cell cycle progression, e.g. depletion of deoxyribonucleotide pools [10]. After all, in accordance with its protective function, there is recent evidence that the DNA damage response may serve as an anti-cancer barrier in early human tumorigenesis, as in clinical specimens, early premalignant lesions commonly ex-

press markers of an activated DNA damage response – likely due to oncogene-induced DNA-damage – which are at least partially lost in advanced cancer [11,12].

Early approaches to the DNA damage response led to definition of simple, linear pathways, but more and more evidence points to a complex DNA damage response network built upon temporal and spatial restrictions that are essential for adequate, orderly reactions to any genotoxic stress [13]. For example, one possible spatial restriction is cytoplasmic sequestration of nuclear regulators [14–17]. Another principle of spatial organization is the interplay between locally restricted reaction centers and mobile messengers—e.g. the DNA DSB as reaction center and Chk2 as mobile messenger in ATM-dependent checkpoint signaling [18]. Increasing evidence suggests that the centrosome might be another such reaction center—the “command center for cellular control” as recently postulated [19–21]. Two fundamental roles of the centrosome in the DNA damage response can be envisioned (Fig. 1): it could firstly serve as spatiotemporal organizer where checkpoint components come into proximity to each other in a defined manner or are, on the other hand, sequestered from undesirable reaction partners. This role could be independent of the “classical” function of the centrosome as MTOC ensuring correct chromosome segregation in mitosis. Secondly, the centrosome cycle might be subjected to regulation by DNA damage, so that the centrosome might serve as an effector of the DNA damage response. This would link the “classical” centrosomal functions to the DNA damage response network, and would be independent of other checkpoint effectors like the replication and transcription machinery.

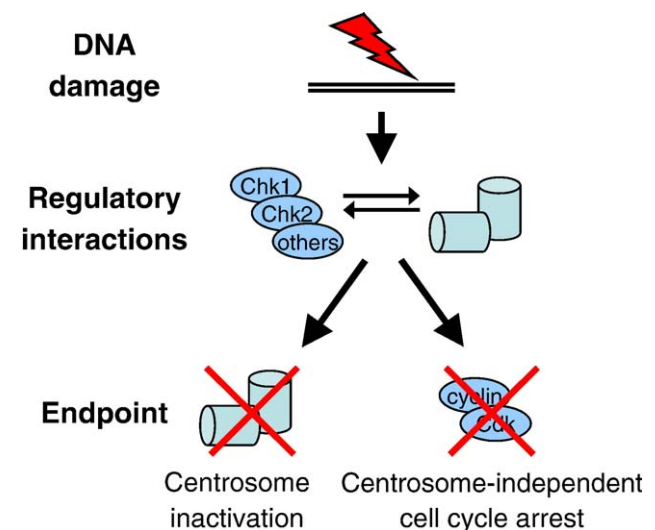


Fig. 1 – Two possible endpoints of centrosomal checkpoint signaling in response to DNA damage: DNA damage induces (in addition to centrosome-independent checkpoint signaling) interactions between checkpoint regulators and centrosomal structures. These presumably regulatory interactions may either target the centrosome itself as an effector of the DNA damage response – e.g. centrosome inactivation – or lead to centrosome-independent regulatory effects like cell cycle arrest via inhibition of cyclin/Cdk complexes.

Centrosomal regulation of the G₂/M transition

To understand possible roles of centrosomes in DNA damage response, it should be noted that the strict discrimination between unperturbed cell cycle and checkpoint regulation has come into question in several ways. For example, the checkpoint kinase Chk1, once seen as a prototypic checkpoint mediator, seems to have a basal activity during unperturbed cell cycles, which is upregulated in reaction to DNA-damaging insults [22]. This basal activity maintains a physiological level of the positive cell cycle regulator Cdc25A and protects cells against aberrantly increased initiation of DNA replication and DNA breakage, thereby acting as an “integrated cooling system” against an overheated cell cycle.

During unperturbed cell cycles, mitotic entry requires active cyclin B/Cdk1 [23,24]. The initial activation of cyclin B/Cdk1 in prophase, including Cdk1 dephosphorylation at Tyr15 and cyclin B phosphorylation at Ser126 and Ser133, has been shown to occur at the centrosome [25]. Thereby, Cdc25B seems to be an activator of cyclin B/Cdk1, as it is capable of activating centrosomal cyclin B/Cdk1 in vitro [26] and temporarily localizes to the centrosome during mitosis after being phosphorylated at Ser353 by Aurora-A [27], which correlates in vivo with cyclin B/Cdk1 activation and nuclear translocation [28]. Recently, this centrosomal regulator club was joined by Chk1, which was shown to localize to the centrosome during interphase, from where it dissociated at the onset of mitosis [29]. Furthermore, chemical Chk1 inhibition led to loss of centrosome-associated inhibitory Tyr15 phosphorylation of Cdk1, premature centrosome separation and accelerated entry into mitosis, while centrosome-targeted Chk1 expression – by fusion to the centrosomal targeting domain of AKAP450 – prevented centrosome separation and induced polyploidization by skipping of mitosis [29]. As expected, centrosome-tagged kinase-dead Chk1 led to premature Cdk1 activation. In addition, centrosome-tagged Chk1 was shown to target endogenous Cdc25B to the centrosome [29].

In summary, a model was proposed in which Cdc25B is positively regulated by Aurora-A but negatively regulated by Chk1, which, by means of its centrosomal localization, might prevent access of Cdc25B to centrosomal Cdk1 before mitotic entry [19]. Accordingly, this function of Chk1 as regulator of mitotic entry seems to be directly linked to its centrosomal localization. How the centrosomal localization of Chk1 itself is regulated remains an open question. It is tempting to speculate that the amount or activity of centrosomal Chk1 could be regulated by checkpoint pathways: this might be another component of the G₂/M checkpoint—and another example of a basal function of Chk1 during unperturbed cell cycles which can be upregulated ‘on demand’. Indeed, according to preliminary findings in human fibroblasts, Chk1 accumulates at centrosomes in response to DNA damage induced by ultraviolet radiation or hydroxyurea treatment (H. Löffler and A. Krämer, unpublished).

Another recent report showed that both centrosomal Aurora-A activity and Ser353 phosphorylation of centrosomal Cdc25B are inhibited by the G₂/M checkpoint, which was induced by etoposide treatment [28]. Treatment with the Chk1 inhibitor UCN-01 abolished this checkpoint response, hinting

at Chk1 being upstream of both Aurora-A and Cdc25B. However, a direct demonstration that centrosomal Chk1 is involved in this G₂/M checkpoint response is still missing, and further research on this issue is needed.

A completely different aspect was revealed by a study on mammalian embryonic stem cells [30]. Compared with fibroblasts, stem cells seem to lack a G₁ checkpoint, apparently due to the absence of Chk2-dependent degradation of Cdc25A after ionizing radiation. This was associated with sequestration of Chk2 to centrosomes. In addition, ectopic expression of Chk2 rescued the G₁ checkpoint [30]. Therefore, the centrosome seems to be not only a site where checkpoint reactions take place, but also a site where checkpoint components are sequestered to prevent reactions.

The centrosome inactivation checkpoint

Recently, it has been reported that exposure to DNA damage during mitosis results in centrosome inactivation or fragmentation in both *Drosophila* embryos and somatic mammalian cells [31,32]. In syncytial *Drosophila* embryos, centrosome disruption in mitosis is triggered by damaged or incompletely replicated DNA—this effect can be experimentally induced by a variety of different insults including UV light, X-rays, bleomycin, camptothecin, etoposide, VM-26, aphidicolin, and hydroxyurea [31]. Centrosome fragmentation generates defects in spindle assembly and anaphase chromosome segregation. The damaged nuclei drop from the cortex into the interior yolk and are thereby eliminated from the embryo [31]. Noteworthy, a null mutation in the *Drosophila* checkpoint kinase 2 homolog (DmChk2) blocks this mitotic response to DNA lesions and prevents loss of defective nuclei from the cortex, while DNA damage leads to increased loading of Chk2 to centrosomes, arguing for a direct involvement of Chk2 in centrosome fragmentation in *Drosophila* [33]. These results led to the definition of a centrosome inactivation checkpoint where induction of “mitotic catastrophe” is utilized for eliminating cells with damaged DNA [31,33]. Consecutively, a similar phenotype of DNA damage-induced centrosome fragmentation leading to multipolar spindles and severe division errors was described in Chinese hamster ovary (CHO) cells [32]. New data supporting a role for the mammalian checkpoint kinase Chk2 in this phenotype have been reported recently: in human HCT116 cancer cells, DNA damage-dependent mitotic catastrophe occurred in Chk2-expressing but not in Chk2^{-/-} cells (W. Theurkauf, oral presentation at EMBL, Heidelberg, Germany, September 2005).

In agreement with this finding, Chk2 was reported to localize to centrosomes also in somatic human cells [34]. Interestingly, even in the absence of DNA damage, Chk2 was present at centrosomes in its presumably activated form phosphorylated at Thr68, Thr26, and Ser28. This phosphorylation might be caused by the Polo-like kinase 1 (Plk1), which colocalized with Chk2 at centrosomes in early mitosis [34]. In contrast, another study argues for centrosomal recruitment of activated Chk2 phosphorylated at its autophosphorylation site Thr387 only after ionizing radiation but not in undamaged cells [29]. This would favor a model in which, similarly to the interplay between nuclear Chk1 and Chk2 [35], centrosomal

Chk1 and Chk2 cooperate as checkpoint mediators – with Chk1 being the “workhorse” that is active even during unperturbed cell cycles and Chk2 being the “amplifier” that is switched on after DNA damage – but this is hypothetical as yet.

More data on mammalian centrosomal Chk2 came from experiments using fusions between nonsynchronized human cells to explore mechanisms of cell death [36,37]. In this model, cell fusion led to the formation of heterokarya which transiently activate cyclin B/Cdk1 and enter prophase, then downregulate cyclin B, stop cycling, activate p53, disorganize centrosomes and undergo apoptosis [37]. Downregulation of cyclin B under these conditions was preceded by Chk2 activation [36]. Moreover, chemical inhibition of Chk2 or expression of dominant-negative Chk2 maintained both high cyclin B levels and high Cdk1 activity, which led to stabilization of centrosomes, mitotic progression beyond prophase and cell death during metaphase [36]. The authors concluded that Chk2 is a negative regulator of mitotic catastrophe by preventing mitotic entry via G₂/M arrest [36]. Hence, in contrast to embryonic *Drosophila* cells where DmChk2-dependent checkpoint activation induces a “mitotic catastrophe” phenotype [33], somatic mammalian cells might possess an additional, Chk2-dependent checkpoint acting at an earlier point of the cell cycle to prevent mitotic catastrophe, which might reflect species-specific or cell type-specific differences.

New surprising aspects arose from experiments using chicken DT40 cells conditionally lacking Rad51 recombinase activity, which leads to high levels of spontaneous DNA damage [38]. This induced a prolonged G₂ phase arrest, consecutive centrosome amplification and cell death due to aberrant mitosis. The authors proposed that DNA damage-induced centrosome amplification might ensure the deletion of cells with damaged DNA that escaped from G₂ arrest by preventing productive mitosis and the production of viable progeny [38]. Interestingly, G₂/M checkpoint abrogation by caffeine or wortmannin suppressed centrosome amplification, hinting at ATM and/or ATR being necessary for this phenotype [38]. ATM and ATR, two out of five mammalian members of a conserved protein family related to phosphatidylinositol 3-kinase, are the apical kinases upstream of Chk1 and Chk2 in the DNA damage response [39]. Involvement of ATM in centrosome amplification was further supported by the demonstration that ATM disruption by gene targeting suppressed this phenotype [38]. In an independent approach, ATR gene duplication, which was induced by transferring isochromosome 3q into myoblasts, was shown to result in centrosome amplification as well [40]. Finally, centrosome amplification in response to ionizing radiation was reported to be abolished by UCN-01 treatment of human cells and absent in Chk1^{-/-}DT40 chicken cells, and this could be restored by Chk1 expression, hinting at Chk1 being the mediator of ATM/ATR-dependent centrosome inactivation (E. Bourke and C. Morrison, poster presentation at EMBL, Heidelberg, Germany, September 2005).

Another question raised by these data was whether or not this centrosome amplification phenotype is different from centrosome fragmentation described by others [31–33]—the argument for centrosome amplification rather than fragmentation was the detection of multiple centrioles using both anti-centrin immunofluorescence and electron microscopy [38].

However, current evidence concerning the ultrastructural details of centrosome amplification versus fragmentation might not yet be sufficient to truly discriminate between these two phenotypes.

Finally, it should be noted that centrosome amplification has been described under a variety of conditions, e.g. in p53-deficient mouse fibroblasts [41] and as response to ionizing radiation in human cancer cells [42,43]. Mostly, centrosome amplification has been interpreted not as regulatory event, but rather as a result of a cellular defect and associated with genetic instability and malignant transformation [4,5,44]. As one major route to centrosome amplification in this context, tetraploidy checkpoint failure leading to polyploidization of both DNA and centrosomes has been identified [44–46], which seems to be distinct from the mechanisms described for the centrosome inactivation checkpoint. Alternatively, activation of the DNA damage response at an early stage during cancer evolution [11] might as well trigger centrosome amplification.

Linking centrosome amplification to a DNA damage-dependent checkpoint response would be a new interpretation of an old phenomenon—which does not necessarily contradict the older interpretation: centrosome amplification may normally work as a backup checkpoint mechanism, but may also lead to chromosomal instability and malignant transformation under certain circumstances. One factor facilitating cancer evolution in the context of centrosomal aberrations may be the recently described phenotype of centrosomal clustering, which prevents formation of multipolar mitotic spindles by functionally silencing extra centrosomes [47,48]. Cancer cells exhibiting centrosomal clustering are thus able to undergo bipolar mitosis at a high frequency, but the remaining chromosomal instability may be just enough to boost cancer evolution. Thus, one might speculate that molecular mechanisms involved in centrosomal clustering may also take part in centrosomal checkpoints.

Candidate regulators of a centrosomal checkpoint

Given the accumulating evidence that a centrosomal checkpoint exists, the question about regulators participating in this cascade is arising. The above cited principle works regarding centrosomal checkpoint mechanisms have identified Chk1 and/or Chk2 as likely checkpoint mediators, and there is at least some evidence that ATM and/or ATR might lie upstream of them. Still, a wide range of other checkpoint regulators working at the centrosome have come into view as candidate regulators of a centrosomal checkpoint.

Two out of three members of the Plk family, not only Plk1, which might lie upstream of centrosomal Chk2 (see above), but also Plk3, have been identified as Chk2 interactors [34,49,50]. Plks are centrosomal kinases that form a part of the regulatory circuit controlling entry into mitosis [51]. *Xenopus laevis* Plx1 was originally isolated from egg extracts in the search for activators of the Cdc25 phosphatase [52], and recent data indicate that both Plk1 and Plk3 bind, phosphorylate, and thereby regulate Cdc25 [52–55] and Myt1 [56,57]. Plk1 also phosphorylates cyclin B1 at Ser133 and possibly Ser147, but the consequences of this phosphorylation are not yet known [25,58]. Intriguingly, both Plk1 and Plk3 have recently

been implicated as targets of the DNA damage checkpoint pathway. DNA damage during G₂ phase and mitosis seems to inhibit Plk1 activity in an ATM-dependent manner, leading to a block in mitotic exit [59,60]. Plk3, on the other hand, seems to become activated in response to DNA damage-dependent phosphorylation mediated by ATM [49,50]. Surprisingly, Plk3 was found to interact with both Chk2 and p53 and to phosphorylate Ser20 of p53 in response to DNA damage in an ATM-dependent manner [49,50]. Whether Chk2 serves as an upstream regulator or a downstream target of Plk3 following DNA damage, remains to be established. Taken together, these data strengthen the hypothesis that centrosomes might not only act as regulation centers for entry into mitosis, but that this function might be also under DNA damage-responsive checkpoint control.

Another component of the DNA damage response network, the breast cancer susceptibility gene product BRCA1, has been demonstrated to localize to centrosomes during mitosis [61]. After DNA damage, this centrosomal BRCA1 is phosphorylated at Ser988, a Chk2 phosphorylation site, the consequences of which are unknown [62]. Moreover, Aurora-A, an established centrosomal regulator of the G₂/M transition and the proposed counter-actor of centrosomal Chk1 in Cdc25B regulation (see above), was shown to phosphorylate BRCA1 at Ser308 *in vitro* and *in vivo* [63]. This Ser308 phosphorylation increased in early M phase and could be abolished by ionizing radiation. Furthermore, expression of S308N mutant BRCA1 in BRCA1-deficient mouse fibroblasts led to a decrease in mitotic cell number as compared to wild-type BRCA1, resembling the effect of ionizing radiation in the wild-type controls [63]. Thus, BRCA1 phosphorylation by Aurora-A seems to play a role in G₂/M transition, and this seems to be under DNA damage checkpoint control. On the other hand, it has been reported that BRCA1 localizes to centrosomes throughout the cell cycle and functions to inhibit formation of supernumerary centrosomes (J. Parvin, oral presentation at EMBL, Heidelberg, Germany, September 2005). This might depend on the ubiquitin ligase activity of BRCA1 in conjunction with its partner BARD1, and a critical step for suppression of centrosome amplification might be monoubiquitination of γ -tubulin [64]. In contrast, another study using EGFP-BRCA1 fusion proteins led to the conclusion that BRCA1 is not a centrosomal protein, and the observation of centrosomal BRCA1 might rest upon fixation artifacts [65]. The authors proposed that, in the case of dysfunctional BRCA1, centrosome amplification might be a nonspecific effect of G₂/M checkpoint failure [65]. Against this notion, new arguments came from a study demonstrating by cell synchronization that BRCA1 inhibition causes centrosome amplification between late S and G₂/M phase before cell division [66]. In summary, the issue of centrosomal BRCA1 appears still controversial.

Above all, the tumor suppressor and checkpoint mediator p53 has been shown to associate with centrosomes in unperturbed mitotic cells [67]. Transient inhibition of mitotic spindle assembly by nocodazole triggers activation of p53 leading to a p53-dependent G₁ arrest, and during this nocodazole treatment, the centrosomal localization of p53 is abolished [67]. Interestingly, AT cells, which lack functional ATM, an upstream activator of p53, are deficient not only for this nocodazole-induced G₁ arrest, but also for centrosomal

localization of p53 during mitosis, which can be restored by ATM expression [68]. In addition, centrosomal association seems to be dependent on phosphorylation at the ATM-dependent phosphorylation site Ser15 of p53, as p53-S15A does not localize to centrosomes [68]. Most recently, also activated ATM phosphorylated at Ser1981 was reported to localize to centrosomes during unperturbed mitosis but not during nocodazole treatment [69]. Taken together, these correlations suggest that activation of p53 by ATM in response to spindle damage, leading to G₁ arrest, might somehow be regulated by the centrosomal localization of p53 and ATM. However, a clear mechanistic proof for this hypothesis is missing, so it still seems obscure how the centrosome might be involved in p53-mediated checkpoint regulation.

Another link between the DNA damage response and the centrosome seems to involve the serine/threonine kinase Nek2, the mammalian homologue of Never in Mitosis A (NIMA), an *Aspergillus* protein required for mitosis [70]. Nek2 is an established regulator of centrosome separation [70]. During DNA damage-induced G₂ arrest, centrosome separation is inhibited [71], which could be secondary to cell cycle arrest. However, premature centrosome splitting induced by Nek2 overexpression is inhibited by ionizing radiation, arguing for Nek2 as a direct effector targeting the centrosome in the G₂/M checkpoint [71].

Conclusions and open questions

There is accumulating evidence to speculate that the centrosome might be part of the DNA damage response network. However, current knowledge about the details is still fragmentary, and many points are controversial. It would well fit into the established functions of the checkpoint kinases that both Chk1 and Chk2 cooperate in a centrosomal checkpoint. However, a role of centrosomal Chk1 has to date only been established during unperturbed cell cycles—up to now, data on its checkpoint reactivity are rather weak. For Chk2, involvement in a centrosomal checkpoint has been established in *Drosophila*, but data on its centrosomal activation state in mammalian cells are somewhat conflicting, and there is still no mechanistic link between mammalian Chk2 and a centrosomal checkpoint. Are ATR and/or ATM upstream of Chk1/Chk2 in the centrosomal checkpoint? There is at least some evidence that ATM plays a role in centrosome amplification during G₂ arrest, but whether this centrosome amplification is simply a reaction to the prolonged duration of G₂ phase due to an ATM-dependent checkpoint, or ATM itself stimulates centrosome amplification in a linear pathway, remains to be seen. For ATR, on the other hand, a similar role in centrosome amplification seems possible, but the data supporting this are merely based upon isochromosome 3q transfer. Finally, one should keep in mind that the described phenotypes of centrosome fragmentation and centrosome amplification are ill defined as yet.

As the list of components of the DNA damage response that associate with centrosomes is growing, and more and more established centrosomal regulators are turning out to be DNA damage-responsive, the question remains whether and how all these regulators, or many of them, collaborate in a single

pathway, or in several different pathways, and whether these regulators target the centrosome as checkpoint effector, or simply temporarily associate with the centrosome as “meeting point”. After all, the list of missing links seems to be growing faster than the list of established interactions, so the future challenge should be to work on mechanistic models unifying some of the present fragmentary data.

Current data on centrosomes as part of the DNA damage response network focus on the regulation of mitosis and mitotic entry. What about the other cell cycle phases? We know that the regulators of the centrosome cycle are, at least partly, identical with the “classical” cell cycle regulators—namely Cdk2 in conjunction with cyclin A or E for the initiation of DNA as well as centrosome replication [4,6]. Accordingly, the centrosome cycle should be equally affected by most cell cycle checkpoints, which should lead not only to general cell cycle, but also to centrosome cycle arrest. On the other hand, it is feasible to experimentally separate the centrosome cycle from the rest of the cell cycle: prolonged treatment of CHO cells with aphidicolin or hydroxyurea, leading to an S phase arrest, allows for multiple centrosome duplication rounds in the absence of DNA replication or cytokinesis [72,73]. This means that under certain circumstances, centrosome amplification might occur in cell cycle phases other than G₂, but the outcome should be the same: damaged cells cannot survive mitosis.

Coming back to the above stated alternatives: is the centrosome a “meeting point” for checkpoint regulators that do not affect the centrosome itself, or is the centrosome and its cycle a checkpoint effector? The latter seems clear, as centrosomal fragmentation or amplification seems to be an endpoint of a checkpoint cascade in many settings, leading to “mitotic catastrophe” in order to eliminate damaged cells. The former possibility is supported by the finding that Chk1 shields Cdk1 from being activated at the centrosome, which makes Cdk1 another putative effector of a centrosomal checkpoint—this does not necessarily affect the centrosome itself and its function as organizer of the mitotic spindle. As yet, there is no clear experimental evidence for a link between the Chk1/(Cdc25B)/Cdk1 cascade regulating mitotic entry and the centrosome inactivation checkpoint, but this might well change in the future—a common centrosomal checkpoint might have both Cdk1 inhibition and centrosomal inactivation as effective endpoints. One might speculate that Cdk1 inhibition might be a transient way to provide time for repair, while centrosome inactivation might work as a backup mechanism if Cdk1 inhibition fails or damage is too extensive. Surely, the next few years will bring exciting new data that help us understand the complex interconnection between the functional network of the DNA damage response and the dynamic structural organization of eukaryotic cells through centrosomes.

REFERENCES

- [1] D.P. Cahill, K.W. Kinzler, B. Vogelstein, C. Lengauer, Genetic instability and Darwinian selection in tumours, *Trends Cell Biol.* 9 (1999) M57–M60.
- [2] H. Rajagopalan, C. Lengauer, Aneuploidy and cancer, *Nature* 432 (2004) 338–341.
- [3] T. Boveri, *Zur Frage der Entstehung maligner Tumoren*, Gustav Fischer Verlag, Jena, 1914.
- [4] A. Krämer, K. Neben, A.D. Ho, Centrosome replication, genomic instability and cancer, *Leukemia* 16 (2002) 767–775.
- [5] E.A. Nigg, Centrosome aberrations: cause or consequence of cancer progression? *Nat. Rev., Cancer* 2 (2002) 815–825.
- [6] S. Doxsey, Re-evaluating centrosome function, *Nat. Rev., Mol. Cell Biol.* 2 (2001) 688–698.
- [7] B.B. Zhou, S.J. Elledge, The DNA damage response: putting checkpoints in perspective, *Nature* 408 (2000) 433–439.
- [8] J.H. Hoeijmakers, Genome maintenance mechanisms for preventing cancer, *Nature* 411 (2001) 366–374.
- [9] M.B. Kastan, J. Bartek, Cell-cycle checkpoints and cancer, *Nature* 432 (2004) 316–323.
- [10] J. Bartek, C. Lukas, J. Lukas, Checking on DNA damage in S phase, *Nat. Rev., Mol. Cell Biol.* 5 (2004) 792–804.
- [11] J. Bartkova, Z. Hořejší, K. Koed, A. Krämer, F. Tort, K. Zieger, P. Guldberg, M. Sehested, J.M. Nesland, C. Lukas, T. Ørntoft, J. Lukas, J. Bartek, DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis, *Nature* 434 (2005) 864–870.
- [12] V.G. Gorgoulis, L.V. Vassiliou, P. Karakaidos, P. Zacharatos, A. Kotsinas, T. Liloglou, M. Venere, R.A. Dittullo Jr., N.G. Kastrinakis, B. Levy, D. Kletsas, A. Yoneta, M. Herlyn, C. Kittas, T.D. Halazonetis, Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions, *Nature* 434 (2005) 907–913.
- [13] J. Lukas, C. Lukas, J. Bartek, Mammalian cell cycle checkpoints: signalling pathways and their organization in space and time, *DNA Rep.* 3 (2004) 997–1007.
- [14] J. Liang, J. Zubovitz, T. Petrocelli, R. Kotchetkov, M.K. Connor, K. Han, J.H. Lee, S. Ciarallo, C. Catzavelos, R. Beniston, E. Franssen, J.M. Slingerland, PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest, *Nat. Med.* 8 (2002) 1153–1160.
- [15] B.O. Petersen, J. Lukas, C.S. Sørensen, J. Bartek, K. Helin, Phosphorylation of mammalian CDC6 by cyclin A/CDK2 regulates its subcellular localization, *EMBO J.* 18 (1999) 396–410.
- [16] I. Shin, F.M. Yakes, F. Rojo, N.Y. Shin, A.V. Bakin, J. Baselga, C.L. Arteaga, PKB/Akt mediates cell-cycle progression by phosphorylation of p27(Kip1) at threonine 157 and modulation of its cellular localization, *Nat. Med.* 8 (2002) 1145–1152.
- [17] G. Viglietto, M.L. Motti, P. Bruni, R.M. Melillo, A. D’Alessio, D. Califano, F. Vinci, G. Chiappetta, P. Tschlis, A. Bellacosa, A. Fusco, M. Santoro, Cytoplasmic relocalization and inhibition of the cyclin-dependent kinase inhibitor p27(Kip1) by PKB/Akt-mediated phosphorylation in breast cancer, *Nat. Med.* 8 (2002) 1136–1144.
- [18] C. Lukas, J. Falck, J. Bartkova, J. Bartek, J. Lukas, Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage, *Nat. Cell Biol.* 5 (2003) 255–260.
- [19] S. Doxsey, W. Zimmerman, K. Mikule, Centrosome control of the cell cycle, *Trends Cell Biol.* 15 (2005) 303–311.
- [20] S.J. Doxsey, Centrosomes as command centres for cellular control, *Nat. Cell Biol.* 3 (2001) E105–E108.
- [21] A. Krämer, J. Lukas, J. Bartek, Checking out the centrosome, *Cell Cycle* 3 (2004) 1390–1393.
- [22] C.S. Sørensen, R.G. Syljuåsen, J. Falck, T. Schroeder, L. Rönnstrand, K.K. Khanna, B.B. Zhou, J. Bartek, J. Lukas, Chk1 regulates the S phase checkpoint by coupling the physiological turnover and ionizing radiation-induced accelerated proteolysis of Cdc25A, *Cancer Cell* 3 (2003) 247–258.
- [23] E.A. Nigg, Mitotic kinases as regulators of cell division and its checkpoints, *Nat. Rev., Mol. Cell Biol.* 2 (2001) 21–32.

- [24] P. Nurse, Universal control mechanism regulating onset of M-phase, *Nature* 344 (1990) 503–508.
- [25] M. Jackman, C. Lindon, E.A. Nigg, J. Pines, Active cyclin B1-Cdk1 first appears on centrosomes in prophase, *Nat. Cell Biol.* 5 (2003) 143–148.
- [26] C.P. De Souza, K.A. Ellem, B.G. Gabrielli, Centrosomal and cytoplasmic Cdc2/cyclin B1 activation precedes nuclear mitotic events, *Exp. Cell Res.* 257 (2000) 11–21.
- [27] S. Dutertre, M. Cazales, M. Quaranta, C. Froment, V. Trabut, C. Dozier, G. Mirey, J.P. Bouche, N. Theis-Febvre, E. Schmitt, B. Monsarrat, C. Prigent, B. Ducommun, Phosphorylation of CDC25B by Aurora-A at the centrosome contributes to the G2-M transition, *J. Cell Sci.* 117 (2004) 2523–2531.
- [28] M. Cazales, E. Schmitt, E. Montembault, C. Dozier, C. Prigent, B. Ducommun, CDC25B phosphorylation by Aurora-A occurs at the G(2)/M transition and is inhibited by DNA damage, *Cell Cycle* 4 (2005) 1233–1238.
- [29] A. Krämer, N. Mailand, C. Lukas, R.G. Syljuåsen, C.J. Wilkinson, E.A. Nigg, J. Bartek, J. Lukas, Centrosome-associated Chk1 prevents premature activation of cyclin-B-Cdk1 kinase, *Nat. Cell Biol.* 6 (2004) 884–891.
- [30] Y. Hong, P.J. Stambrook, Restoration of an absent G1 arrest and protection from apoptosis in embryonic stem cells after ionizing radiation, *Proc. Natl. Acad. Sci. U. S. A.* 101 (2004) 14443–14448.
- [31] O.C. Sibon, A. Kelkar, W. Lemstra, W.E. Theurkauf, DNA-replication/DNA-damage-dependent centrosome inactivation in *Drosophila* embryos, *Nat. Cell Biol.* 2 (2000) 90–95.
- [32] H.M. Hut, W. Lemstra, E.H. Blaauw, G.W. Van Cappellen, H.H. Kampinga, O.C. Sibon, Centrosomes split in the presence of impaired DNA integrity during mitosis, *Mol. Biol. Cell* 14 (2003) 1993–2004.
- [33] S. Takada, A. Kelkar, W.E. Theurkauf, *Drosophila* checkpoint kinase 2 couples centrosome function and spindle assembly to genomic integrity, *Cell* 113 (2003) 87–99.
- [34] L. Tsvetkov, X. Xu, J. Li, D.F. Stern, Polo-like kinase 1 and Chk2 interact and co-localize to centrosomes and the midbody, *J. Biol. Chem.* 278 (2003) 8468–8475.
- [35] J. Bartek, J. Lukas, Chk1 and Chk2 kinases in checkpoint control and cancer, *Cancer Cell* 3 (2003) 421–429.
- [36] M. Castedo, J.L. Perfettini, T. Roumier, K. Yakushijin, D. Horne, R. Medema, G. Kroemer, The cell cycle checkpoint kinase Chk2 is a negative regulator of mitotic catastrophe, *Oncogene* 23 (2004) 4353–4361.
- [37] M. Castedo, T. Roumier, J. Blanco, K.F. Ferri, J. Barretina, L.A. Tintignac, K. Andreau, J.L. Perfettini, A. Amendola, R. Nardacci, P. Leduc, D.E. Ingber, S. Druillennec, B. Roques, S.A. Leibovitch, M. Vilella-Bach, J. Chen, J.A. Este, N. Modjtahedi, M. Piacentini, G. Kroemer, Sequential involvement of Cdk1, mTOR and p53 in apoptosis induced by the HIV-1 envelope, *EMBO J.* 21 (2002) 4070–4080.
- [38] H. Dodson, E. Bourke, L.J. Jeffers, P. Vagnarelli, E. Sonoda, S. Takeda, W.C. Earnshaw, A. Merdes, C. Morrison, Centrosome amplification induced by DNA damage occurs during a prolonged G2 phase and involves ATM, *EMBO J.* 23 (2004) 3864–3873.
- [39] Y. Shiloh, ATM and related protein kinases: safeguarding genome integrity, *Nat. Rev., Cancer* 3 (2003) 155–168.
- [40] L. Smith, S.J. Liu, L. Goodrich, D. Jacobson, C. Degnin, N. Bentley, A. Carr, G. Flaggs, K. Keegan, M. Hoekstra, M.J. Thayer, Duplication of ATR inhibits MyoD, induces aneuploidy and eliminates radiation-induced G1 arrest, *Nat. Genet.* 19 (1998) 39–46.
- [41] K. Fukasawa, T. Choi, R. Kuriyama, S. Rulong, G.F. Vande Woude, Abnormal centrosome amplification in the absence of p53, *Science* 271 (1996) 1744–1747.
- [42] H.S. Yoon, A.M. Ghaleb, M.O. Nandan, I.M. Hisamuddin, W.B. Dalton, V.W. Yang, Krüppel-like factor 4 prevents centrosome amplification following gamma-irradiation-induced DNA damage, *Oncogene* 24 (2005) 4017–4025.
- [43] K. Kawamura, K. Fujikawa-Yamamoto, M. Ozaki, K. Iwabuchi, H. Nakashima, C. Domiki, N. Morita, M. Inoue, K. Tokunaga, N. Shiba, R. Ikeda, K. Suzuki, Centrosome hyperamplification and chromosomal damage after exposure to radiation, *Oncology* 67 (2004) 460–470.
- [44] A. Krämer, Centrosome aberrations—Hen or egg in cancer initiation and progression? *Leukemia* 19 (2005) 1142–1144.
- [45] F. Borel, O.D. Lohez, F.B. Lacroix, R.L. Margolis, Multiple centrosomes arise from tetraploidy checkpoint failure and mitotic centrosome clusters in p53 and RB pocket protein-compromised cells, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 9819–9824.
- [46] P. Meraldi, R. Honda, E.A. Nigg, Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53-/- cells, *EMBO J.* 21 (2002) 483–492.
- [47] N.J. Quintyne, J.E. Reing, D.R. Hoffelder, S.M. Gollin, W.S. Saunders, Spindle multipolarity is prevented by centrosomal clustering, *Science* 307 (2005) 127–129.
- [48] B.R. Brinkley, Managing the centrosome numbers game: from chaos to stability in cancer cell division, *Trends Cell Biol.* 11 (2001) 18–21.
- [49] M. Bahassi el, C.W. Conn, D.L. Myer, R.F. Hennigan, C.H. McGowan, Y. Sanchez, P.J. Stambrook, Mammalian Polo-like kinase 3 (Plk3) is a multifunctional protein involved in stress response pathways, *Oncogene* 21 (2002) 6633–6640.
- [50] S. Xie, H. Wu, Q. Wang, J. Kunicki, R.O. Thomas, R.E. Hollingsworth, J. Cogswell, W. Dai, Genotoxic stress-induced activation of Plk3 is partly mediated by Chk2, *Cell Cycle* 1 (2002) 424–429.
- [51] F.A. Barr, H.H. Sillje, E.A. Nigg, Polo-like kinases and the orchestration of cell division, *Nat. Rev., Mol. Cell Biol.* 5 (2004) 429–440.
- [52] A. Kumagai, W.G. Dunphy, Purification and molecular cloning of Plx1, a Cdc25-regulatory kinase from *Xenopus* egg extracts, *Science* 273 (1996) 1377–1380.
- [53] M. Bahassi el, R.F. Hennigan, D.L. Myer, P.J. Stambrook, Cdc25C phosphorylation on serine 191 by Plk3 promotes its nuclear translocation, *Oncogene* 23 (2004) 2658–2663.
- [54] A.E. Elia, L.C. Cantley, M.B. Yaffe, Proteomic screen finds pSer/pThr-binding domain localizing Plk1 to mitotic substrates, *Science* 299 (2003) 1228–1231.
- [55] Y.W. Qian, E. Erikson, C. Li, J.L. Maller, Activated polo-like kinase Plx1 is required at multiple points during mitosis in *Xenopus laevis*, *Mol. Cell Biol.* 18 (1998) 4262–4271.
- [56] H. Nakajima, F. Toyoshima-Morimoto, E. Taniguchi, E. Nishida, Identification of a consensus motif for Plk (Polo-like kinase) phosphorylation reveals Myt1 as a Plk1 substrate, *J. Biol. Chem.* 278 (2003) 25277–25280.
- [57] T. Okano-Uchida, E. Okumura, M. Iwashita, H. Yoshida, K. Tachibana, T. Kishimoto, Distinct regulators for Plk1 activation in starfish meiotic and early embryonic cycles, *EMBO J.* 22 (2003) 5633–5642.
- [58] F. Toyoshima-Morimoto, E. Taniguchi, N. Shinya, A. Iwamatsu, E. Nishida, Polo-like kinase 1 phosphorylates cyclin B1 and targets it to the nucleus during prophase, *Nature* 410 (2001) 215–220.
- [59] M.A. van Vugt, V.A. Smits, R. Klomp maker, R.H. Medema, Inhibition of Polo-like kinase-1 by DNA damage occurs in an ATM- or ATR-dependent fashion, *J. Biol. Chem.* 276 (2001) 41656–41660.
- [60] V.A. Smits, R. Klomp maker, L. Arnaud, G. Rijksen, E.A. Nigg, R.H. Medema, Polo-like kinase-1 is a target of the DNA damage checkpoint, *Nat. Cell Biol.* 2 (2000) 672–676.
- [61] L.C. Hsu, R.L. White, BRCA1 is associated with the centrosome during mitosis, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 12983–12988.
- [62] S. Okada, T. Ouchi, Cell cycle differences in DNA

- damage-induced BRCA1 phosphorylation affect its subcellular localization, *J. Biol. Chem.* 278 (2003) 2015–2020.
- [63] M. Ouchi, N. Fujiuchi, K. Sasai, H. Katayama, Y.A. Minamishima, P.P. Ongusaha, C. Deng, S. Sen, S.W. Lee, T. Ouchi, BRCA1 phosphorylation by Aurora-A in the regulation of G2 to M transition, *J. Biol. Chem.* 279 (2004) 19643–19648.
- [64] L.M. Starita, Y. Machida, S. Sankaran, J.E. Elias, K. Griffin, B.P. Schlegel, S.P. Gygi, J.D. Parvin, BRCA1-dependent ubiquitination of gamma-tubulin regulates centrosome number, *Mol. Cell. Biol.* 24 (2004) 8457–8466.
- [65] H.M. Hut, K.P. Rembacz, M.A. van Waarde, W. Lemstra, W.A. van Cappellen, H.H. Kampinga, O.C. Sibon, Dysfunctional BRCA1 is only indirectly linked to multiple centrosomes, *Oncogene* 24 (2005) 7619–7623.
- [66] M.J. Ko, K. Murata, D.S. Hwang, J.D. Parvin, Inhibition of BRCA1 in breast cell lines causes the centrosome duplication cycle to be disconnected from the cell cycle, *Oncogene* 25 (2005) 298–303.
- [67] M. Ciciarello, R. Mangiacasale, M. Casenghi, M. Zaira Limongi, M. D'Angelo, S. Soddu, P. Lavia, E. Cundari, p53 displacement from centrosomes and p53-mediated G1 arrest following transient inhibition of the mitotic spindle, *J. Biol. Chem.* 276 (2001) 19205–19213.
- [68] A. Tritarelli, E. Oricchio, M. Ciciarello, R. Mangiacasale, A. Palena, P. Lavia, S. Soddu, E. Cundari, p53 localization at centrosomes during mitosis and postmitotic checkpoint are ATM-dependent and require serine 15 phosphorylation, *Mol. Biol. Cell* 15 (2004) 3751–3757.
- [69] E. Oricchio, C. Saladino, S. Iacovelli, S. Soddu, E. Cundari, ATM is activated by default in mitosis, localizes at centrosomes and monitors mitotic spindle integrity, *Cell Cycle* 5 (2006).
- [70] A.M. Fry, P. Meraldi, E.A. Nigg, A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators, *EMBO J.* 17 (1998) 470–481.
- [71] L. Fletcher, G.J. Cerniglia, E.A. Nigg, T.J. Yend, R.J. Muschel, Inhibition of centrosome separation after DNA damage: a role for Nek2, *Radiat. Res.* 162 (2004) 128–135.
- [72] R. Balczon, L. Bao, W.E. Zimmer, K. Brown, R.P. Zinkowski, B.R. Brinkley, Dissociation of centrosome replication events from cycles of DNA synthesis and mitotic division in hydroxyurea-arrested Chinese hamster ovary cells, *J. Cell. Biol.* 130 (1995) 105–115.
- [73] P. Meraldi, J. Lukas, A.M. Fry, J. Bartek, E.A. Nigg, Centrosome duplication in mammalian somatic cells requires E2F and Cdk2-cyclin A, *Nat. Cell Biol.* 1 (1999) 88–93.