Genome partitioning

Excerpted and adapted from the Ph.D thesis entitled:

Human metastatic melanoma in vitro

in which it appeared as Appendix J.

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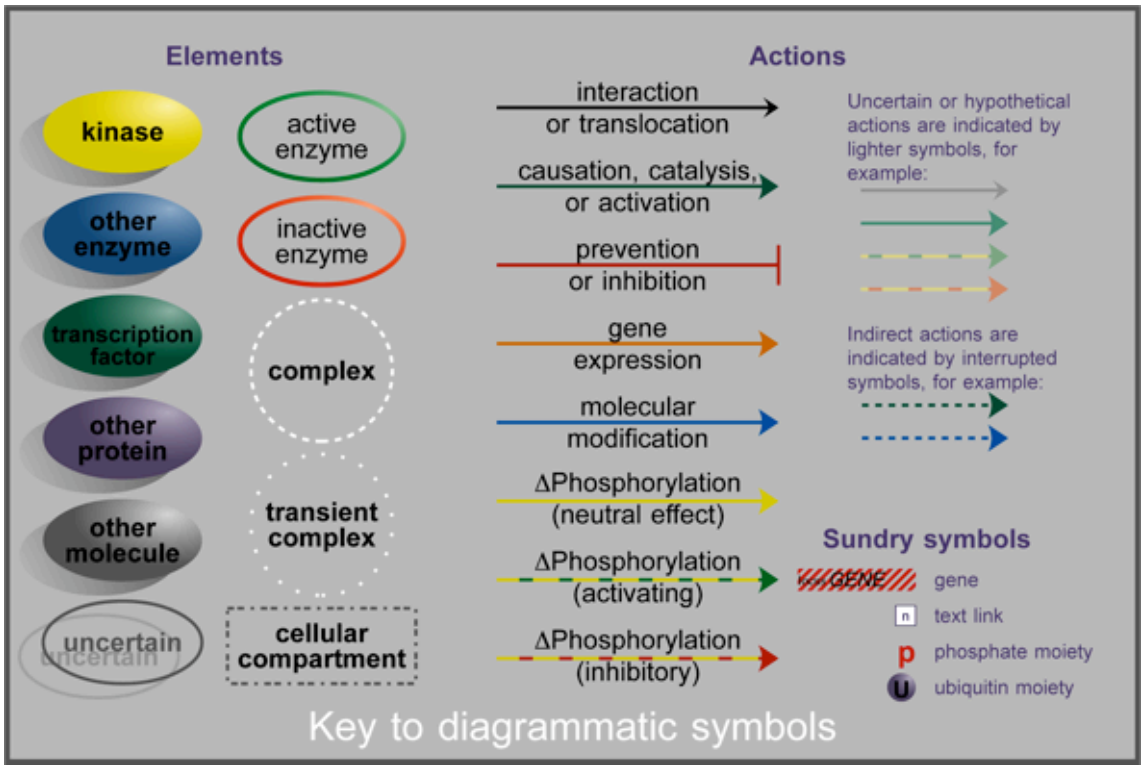
Symbols used:

§	Data from a non-human model, generality uncertain
®	Reference thus tagged is a review article
[*number*]	Reference to the correspondingly labelled part of the table or figure last cited in the text


Elements
kinase
other enzyme
transcription factor
other protein
other molecule
uncertain
active enzyme
inactive enzyme
complex
transient complex
cellular compartment
Actions
interaction or translocation
causation, catalysis, or activation
prevention or inhibition
gene expression
molecular modification
ΔPhosphorylation (neutral effect)
ΔPhosphorylation (activating)
ΔPhosphorylation (inhibitory)
Uncertain or hypothetical actions are indicated by lighter symbols, for example:
Indirect actions are indicated by interrupted symbols, for example:
Sundry symbols
GENE
gene
n
text link
P
phosphate moiety
U
ubiquitin moiety
Key to diagrammatic symbols

Genome partitioning

The critical process of spatially aligning replicated genomes during cell division is the province of the centrosome. Where this fails, the maintenance of stable ploidy is compromised, often with adverse consequences for the newly divided cells. Where they are viable, their genetic complement may be imbalanced and in consequence, their inherent activities and their sensitivity and responsiveness to external influences may be aberrant. If this leads to a dysregulation of proliferation, there can be dire consequences for the organism as a whole. The very frequent observation of centrosomal anomalies and ploidy changes in cancer attests to this.

1 Introduction

The maintenance of cellular viability and of species identity in diploid organisms depends on the reliable partitioning of the replicated genome between the two cells that result from cellular division. Without this, tissue differentiation and function could not be maintained, nor would the reliable hereditary transmission of beneficial genetic changes be possible. Failure of the first would make survival of a multi-cellular diploid organism impossible, and failure of the second would remove a critical component of the evolutionary process. Without evolution, there would be no basis for the generation of distinct species. Clearly, much hinges on the fidelity of this partitioning.

For any process involving the study or control of motion, whether of stars or chromosomes, a frame of reference is essential. The establishment of the mitotic spindle provides this within the dividing the cell, laying down the spatial context of the coming events. It defines the axis of chromosomal motion during anaphase and the location of the division during cytokinesis. In multicellular organisms, where the fidelity of genome partitioning is vital, a supervisory subsystem is present that orchestrates this: the centrosome[®44].

2 Centrosome structure

Morphology

The centrosome is a cytoplasmic structure comprising two centrioles, interconnecting fibres, and associated amorphous pericentriolar material {Figure 1}. Each centriole, measuring ~200 nm by ~500 nm, is composed of nine triplets of parallel co-planar microtubules arranged parallel to a common axis. One end of the centriole appears from electron-microscopic studies to be closed, and one to be open. There is evidence of a central structure aligned with the axis and connected to the middle microtubule of each triplet, and adjacent triplets are also connected. When viewed from the open end, each triplet is oriented at a rotation of ~30° clockwise to the tangential.

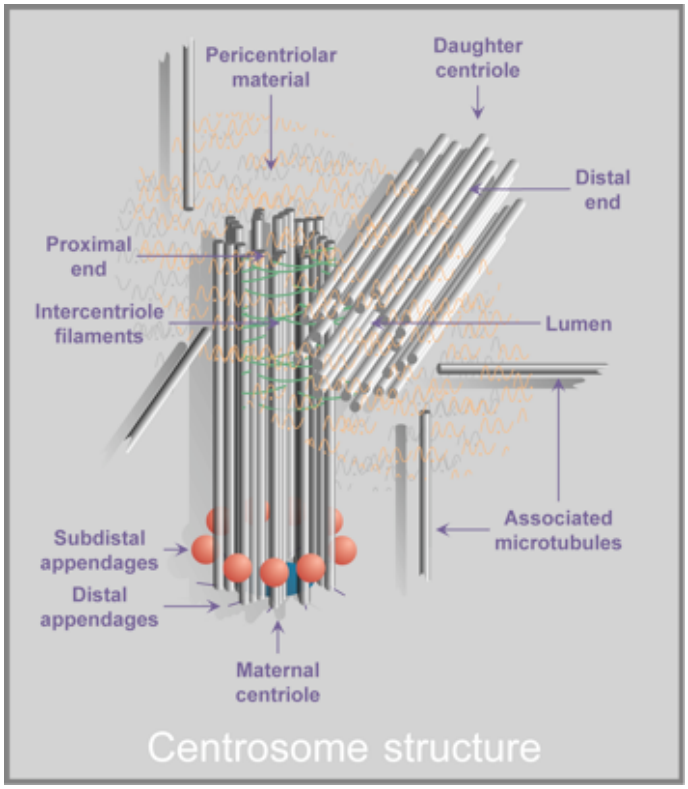


Figure 1: Centrosome structure

The centrioles generally lie perpendicular to one another, with the open ends in proximity, hence their designation as proximal, and that of the other ends as distal. The two centrioles are distinguishable in that one, referred to as maternal, has both distal and subdistal appendages, lacking in the daughter centriole. The entire structure is associated with the slower-growing, minus-ends of cytoplasmic microtubules, connected chiefly via the pericentriolar material.

Composition

Investigations in yeast, *Drosophila*, *Xenopus*, mouse, and human cells have brought to light a number of probable molecular components of the centrosome and its regulators, many listed in Table 1. The investigation of the functions and interactions of these proteins is at present developing rapidly and many have been implicated in specific steps of the centrosome cycle {*See 'The centrosome cycle', below*}. However, given the inchoate state of our knowledge, any mechanistic analysis requires a degree of speculation to compensate for an economy of data.

3 Centrosome function and dysfunction

Despite a century of investigation, the precise role of the centrosome is yet to be determined. Our understanding is based mainly on inferences drawn from coincidences of position and timing with visible cellular events. The association of centrosomes with the foci of the spindle microtubules at the cell poles during mitosis is strong circumstantial evidence for involvement in anaphase. The nature of this involvement has been difficult to investigate as micromanipulative removal of centrosomes was possible only during interphase, and cells so treated did not enter mitosis, in itself an interesting observation. Alternatives, such as antibody injection, could not be guaranteed to obliterate all function.

This changed with the work of Khodjakov et al.[81 §103 §104], who, by incorporating green fluorescent protein into centrosomes, were able to ablate one or both with laser microsurgery at various points in the cell-cycle and observe the consequences[*]. Their innovative approach led to results that have laid the cornerstone for our current understanding of centrosome function. Firstly, they found that destruction of one or both centrosomes in prophase did not interfere with the assembly of the mitotic spindle or, directly, with the process of anaphase. Where one centrosome was left intact, cytokinesis was essentially normal, but where both were ablated, 30% – 50% of cytokineses failed. The proximal cause of this was the failure of the mitotic spindle to maintain its orientation perpendicular to the cellular equator. In consequence, the segregation of chromatids was at times constrained by a reduced cellular diameter; misalignment caused incorrect chromosomal partitioning, even to the extent of generating one binuclear and one anuclear daughter cell; and obstruction of cleavage furrow propagation sometimes caused total failure of cytokinesis, also resulting in polyploidy. They went on to follow the fate of the acentrosomal daughter cell that resulted from the division of a cell in which one centrosome had been destroyed. Quite unexpectedly, they discovered that such cells never again commenced the synthesis of DNA, being trapped forever in a pseudo-G_1 state. Khodjakov et al. have therefore defined a two-fold function for the centrosome: to guide the process of anaphase, and to endow the daughter cell with proliferative potential. This is an extremely elegant method for ensuring that cells which would otherwise suffer a failure of cytokinesis, never get the opportunity to do so. There is also some poetry in the way this recapitulates the contribution by the sperm to the ovum of a functional centriole[176].

[*] Khodjakov et al. provided an excellent video supplement to their seminal paper that demonstrates graphically the consequences of centrosome dysfunction. It is available at: *http://www.jcb.org/cgi/content/full/153/1/237/DC1*

Protein	Observations
14-3-3	Stratifin and 14-3-3γ are centrosomal. They are lost from the centrosome upon serum starvation[§154]
AKAP9	Associates with centrosomes and the cleavage furrow[177]
ATR	*ATR* duplication is associated with centrosome amplification and aneuploidy[189]
BRCA1	Mutation is associated with excess centrosomes, unequal chromosome segregation, and aneuploidy[40]
BRCA2	Mutation is associated with excess centrosomes and micronucleation[202]
CDC16	Centrosomal throughout the cell-cycle[201]
CDC2	Centrosomal throughout the cell-cycle. Present within the pericentriolar material and on centrioles themselves[156]
CDC20	Required for centriole splitting[§204]
CDC25	Required for daughter centriole assembly[§204]
CDC27	Centrosomal throughout the cell-cycle[201]
CDK2	Function essential for centrosome duplication[§134 138] Critical centrosomal regulator[®214]
CEP2	Target of NEK2; important in centriole cohesion[57]
CUL1	SCFC component associated with the centrosome; essential for centriole separation[§56]
Cyclin-A	Centrosomal from preprophase to metaphase[9] Function is essential for centrosome duplication[§138] Necessary for microtubule nucleation[§22]
Cyclin-E	Over-expression is associated with chromosomal instability[191] Over-expression cooperates synergistically with *TP53* deletion[143]
Dynein proteins	Interaction with dynactin is necessary for centrosome duplication and separation[§126] Dominant negative dynein allows spontaneous centrosome assembly, decoupling nuclear and centrosomal cell-cycles[§13] With dynactin, involved in delivery of γ-tubulin and pericentrin for microtubule nucleation[§219]
E2F2, E2F3	Function is essential for centrosome duplication[§138]
GADD45	Deletion is associated with aneuploidy, chromosome aberrations, gene amplification, and excess centrosomes[§84]
HRAS	Ectopic expression results in excess centrosomes, chromosome misalignment, and micronucleation[175]
HSP90	Core centrosomal protein[§112]
MDM2	Over-expression is associated with excess centrosomes and chromosomal instability[27]
MEK1	Ectopic expression results in excess centrosomes, chromosome misalignment, and micronucleation[175]
MRE11A	Non-expression results in excess centrosomes[§218]
NEDD8	Modifier of centrosomal SKP1[§56]
NEK2	Centrosomal throughout the cell-cycle; over-expression causes centrosome splitting and dispersal[58]. Binds and inhibits PP1[75]. Probably anchors CEP2 to centrosome during interphase[57]
NM23	Centrosomal disposition[§172]
NPM1	Associates with unduplicated centrosome; target of CDK2 causing loss of association; detachment is required for centrosome duplication [149 200]
NUMA1	Associates with separating centrosomes in early mitosis[§221]
p21	Reduction is associated with excess centrosomes and polyploidy[130 197]
p27	Injection of p27 inhibits centrosome duplication[§110]
p53	Deletion is associated with excess centrosomes, aneuploidy, gene amplification, and apoptosis[60] Cooperates synergistically with cyclin-E over-expression[143]
PARP	Centrosomal disposition[91]
PLK	Required for centrosome maturation[145]
PP1	PP1α is a target of CDK2[121], and PP1γ is a target of NEK2[75]. PP1α[139] and PP1γ[75] are centrosomal
SKP1	Centrosomal throughout the cell-cycle[69] SCFC component associated with the centrosome; essential for centriole separation[§56]
SKP2	Targeted disruption results in excess centrosomes, polyploidy, enlarged nuclei, and apoptosis [§144]
STK15	Gene amplification is associated with excess chromosomes and aneuploidy[223]
STX8	Associates with centrosomes and mitotic spindle. Binds cyclin-B1 and p21[137]
TTK	Mouse homologue Mps1p is required for centrosome duplication; target of CDK2; associates with centrosomes beginning in S-phase; over-expression is associated with excess centrosomes [§54]. Human protein is not implicated[194]
XRCC2	Deletion is associated with centrosome fragmentation and chromosome missegregation[68]
XRCC3	Deletion is associated with centrosome fragmentation and chromosome missegregation[68]
zyg-1	Required for daughter centriole formation[§147]

Table 1: Proteins implicated in centrosomal regulation

The centrosome may yet prove to have a further indispensable cellular function. As a cell divides, the last physical link between nascent daughter cells is an intercellular bridge that derives from the spindle midbody. In what appears to be a final, critical step in their separation[153], this bridge is visited by a maternal centriole [127], very likely implementing the last checkpoint on cell division. Its arrival signals that it has been released from its duty in anchoring the mitotic spindle by the breakdown of the latter in telophase, and that no impediment remains to the culmination of cytokinesis.

While loss of centrosomal function has dire consequences for cellular propagation, excessive functionality, in the form of supernumerary centrosomes, is no less deleterious. This is principally because despite their being unnecessary for spindle formation, they are not without influence on its structure. When, for whatever reason, excess centrosomes are present, the centrosome's microtubule organising capacity overrides the default bipolar spindle geometry rather than reinforcing it. In consequence, multipolar spindles can form, and at anaphase, two sets of chromosomes will attempt to segregate in three or more directions with resultant chaos. With the number of pronuclei at odds with the normal two-fold symmetry of cleavage furrow propagation, cytokinesis is also chaotic. With three centrosomes, the cell may well divide into three, and such behaviour has been observed in CHO cells[§100], with the production of cells of unequal size accompanied by micronucleation[179]. Thus, the failure of centrosome numerical control may well lead to the generation of cells likely to contain one or two thirds of the normal chromosome complement. Coupled with the possibility of aborted cytokinesis, centrosome functional failure can readily account for triploidy and derivatives thereof, as reported here, and previously by others in human melanoma tumours[12 141 150] and cell-lines[37 106 118].

4 The centrosome cycle

Overview

The centrosome and the nucleus share the distinction of being under numerical control during cell division. Each is duplicated exactly once every cell-cycle[188], and each daughter cell receives exactly one of each. In either case, were this not a fundamental requirement for the survival of the cell or its descendants, it is unlikely that this degree of control would have come into existence, or if it did, have endured. Why this is so for the nucleus is well established, but the critical role of the centrosome remains enigmatic.

The centrosome derives its name from its predominantly perinuclear location, but as implied above, this alters in synchrony with the cell-cycle. With the commencement of S-phase, centrosome duplication begins, and is essentially complete by late G_2. Immediately prior to the onset of mitosis, the now duplicated centrosomes separate and migrate to opposite poles of the cell associating closely with the forming mitotic spindle. Each remains at this location until late in telophase, when, with the disassembly of the mitotic spindle, a single centriole moves to the midbody that connects the two incipient daughter cells. When cytokinesis is complete, the centriole returns to a perinuclear location.

Molecular biology

Interphase

During interphase, the centrosome is to be found in its perinuclear location {Figure 2}. The centriole pair is tethered closely by NPM1 [1], a ribonuclease[149] better known as a nucleolar ribosome assembly factor[82]. The pair is more loosely attached via the NEK2 kinase and the CEP2 protein [2]. Of these, only NEK2 remains centrosomal throughout the cell-cycle[58]. They are closely associated with a catalytic subunit of the PP1 protein phosphatase[75]. Both the alpha[139] and gamma[75] isoforms have been reported to be centrosomal, but nothing appears to be known of which regulatory subunits may be involved. At this

stage, PP1 is unphosphorylated and therefore active [3], inhibiting the aurora-family kinase, STK15. NEK2 may also be a PP1 substrate, but whether or not this is the case, it is inactive for want of phosphorylation.

Initiation of centriole replication

The activation of CDK2 {Figure 3} [1] at the G_1–S-phase transition appears to be the critical event triggering the onset of centrosome duplication[134], and provides synchronisation between the nuclear and centrosomal cell-cycles[214]. Whether the activating partner for CDK2 is cyclin-E, cyclin-A, or either, is not clear. There is strong evidence from *Xenopus* that cyclin-E is critical[§80], and this is supported by a role for p27 in regulating duplication, and the association seen between cyclin-E over-expression and genomic instability[191]. However, in mammalian cells, cyclin-A has been strongly implicated[§138].

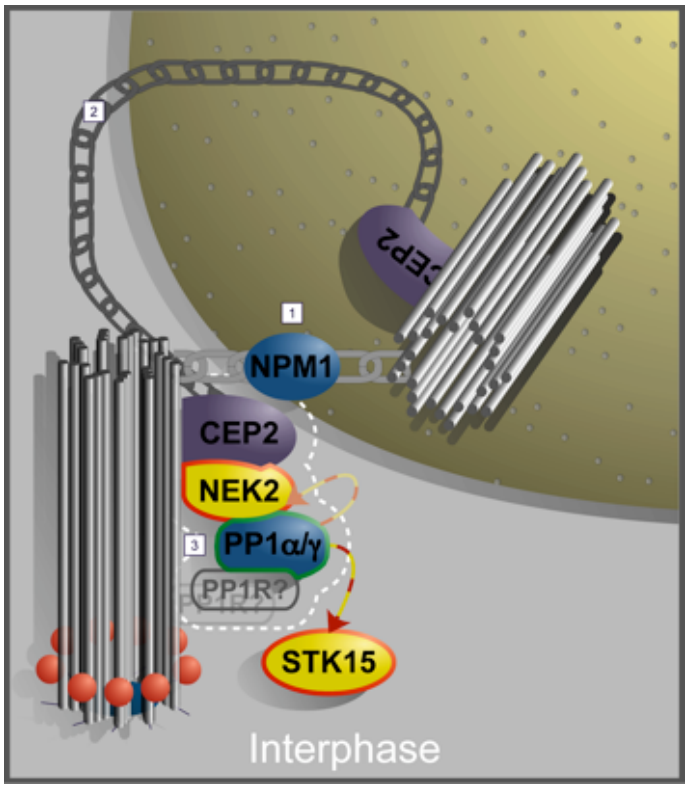


Figure 2: The centrosome in interphase

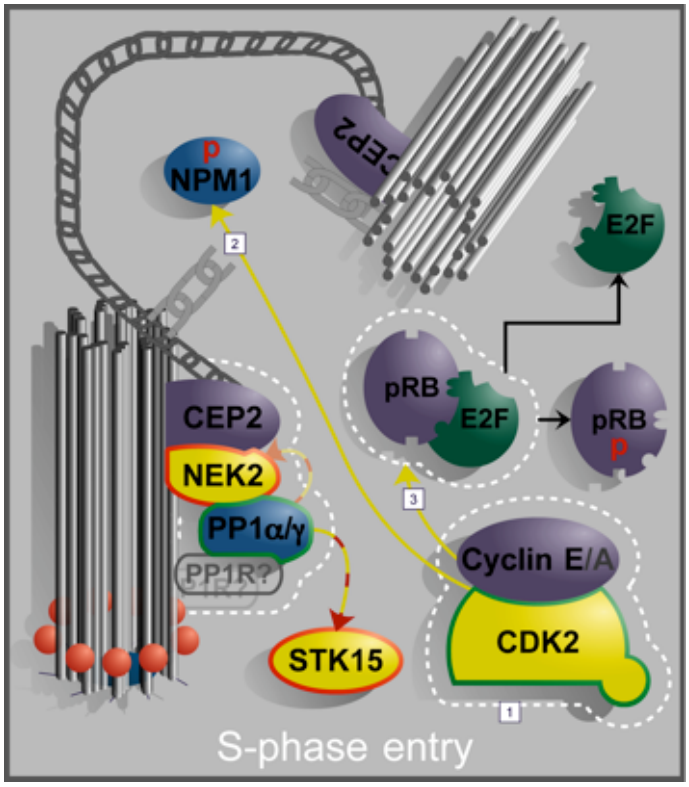


Figure 3: The centrosome at S-phase entry

Activated CDK2 phosphorylates NPM1, dislodging it from the centrosome and breaking the close association between centrioles [2] in a step critical for the progression of centrosome replication[200]. The NEK2–CEP2 linkage remains intact, however, keeping the separated centrioles in proximity. A second CDK2 substrate, at least in the mouse[§54], is the Mps1p kinase. While phosphorylation increases protein stability and allows Mps1p to associate with the centrosome, the consequences for Mps1p enzyme function, and what its substrates may be are yet to be determined. Recent work has suggested that its homologue in humans, TTK, while being necessary for the spindle assembly checkpoint, is dispensable for centrosome duplication[194]. Finally, the apparent requirement for E2F-dependent transcription to support centrosome duplication[138] implies that the well-characterised role of CDK2 phosphorylation of pRB [3] may have consequences beyond fostering S-phase entry.

At this point, centriole replication can commence. Whether the disruption of the strict orthogonal geometry of the centriole pair attendant upon the departure of NPM1 represents the limiting factor in this process is unclear, as are the details of procentriole establishment and growth. Given that centrioles can assemble *de novo* in cells where no maternal centriole is present, albeit in *Chlamydomonas*[§131], an attractive hypothesis is that the component molecules are able to self-assemble, ultimately achieving the lowest energy state with the effective co-crystallisation of a new centriole. If so, where a maternal centriole was present, it may act as a centre of nucleation, accelerating the process and dictating the place at which it occurs.

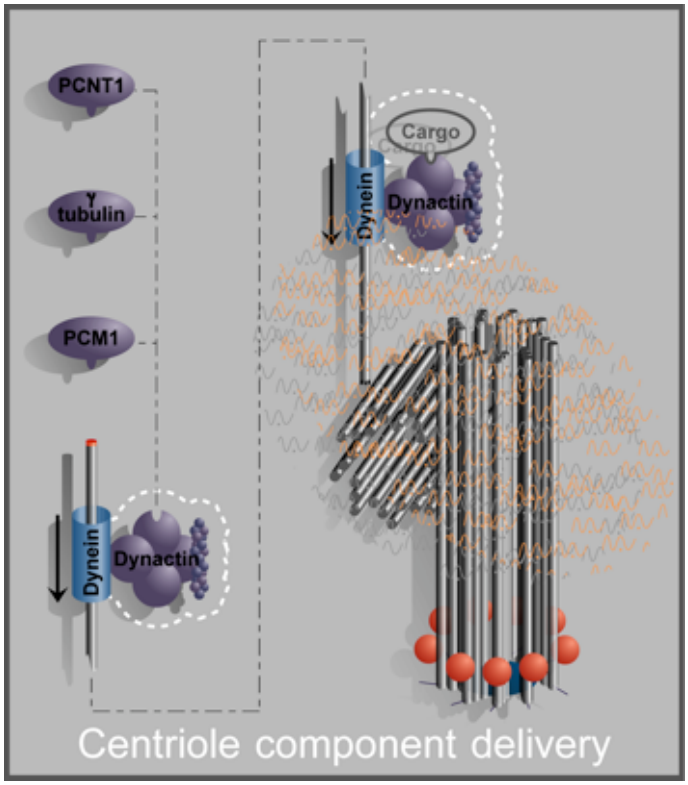


Figure 4: Centriole component delivery

A more active role for a pre-existing centriole relates to supply logistics. Functional and immunocytochemical studies have established that the minus-end directed cytoplasmic dynein/dynactin microtubule motor is required for centriole assembly[§126]. Its role appears to be as a transport system for delivery of centriole components including PCM1[§10], pericentrin[219], γ-tubulin[219], and dynactin itself {Figure 4}. By increasing the local concentration of these by virtue of being at the hub of a microtubule network, the maternal centriole would greatly enhance the rate of daughter centriole assembly. The other, and possibly preferred theory, is that the maternal centriole acts as a template, but nothing has been established concerning how this may occur.

Once started, centriole assembly continues until halted by the onset of mitosis. There does not appear to be any inherent mechanism arresting assembly after one round of duplication. One consequence of this is that where S-phase is extended, centrosome amplification can occur[§10]. This is normally prevented by a mechanism involving p53 and BRCA1 {*See 'p53: Guardian of the centrosome?', below*}, but where this is defective, or not triggered by the particular event, a failure of numerical control can occur. The mechanism is not fail-safe. The recent implication of the *Caenorhabditis elegans zyg-1* gene[147] in this numerical control may lead to a greater understanding of this, as it encodes a kinase that appears to inhibit procentriole establishment until after centriole separation.

Centrosome severance

Late in G_2, the centrosomes separate and migrate to the cell poles to form the prophase asters. This is one of the points in the molecular regulation of centrosome replication where only fragmentary information is available and inference and speculation must serve instead. Centrosome severance appears to be linked to the commitment to enter mitosis since the study cited above involving an extended S-phase found that under these circumstances the centrosomes remained linked.

What is clear is that at or about this time, PP1 is phosphorylated and deactivated. Two kinases are known to be able to perform this: STK15[97] and NEK2[75]. An intriguing relationship therefore exists

between PP1 and STK15 in that each is able to inactivate the other[97] {Figure 5} [1]. The consequence of this functional antagonism is that at any time, one of the pair will be dominant, suppressing the function of the other, and this state will endure in the absence of any external perturbation. To borrow a term from digital electronics, this could be said to form a bi-stable kinase-phosphatase oscillator. This fosters the suggestion that PP1 and NEK2 may form another such bi-stable element [2], particularly in light of their direct physical association and the ability of NEK2 homodimers to effect reciprocal trans-phosphorylation[75], thereby maintaining dominance. This would be a logical inference from a mechanistic viewpoint, but its proof must await the demonstration of an inactivating dephosphorylation of NEK2 by PP1.

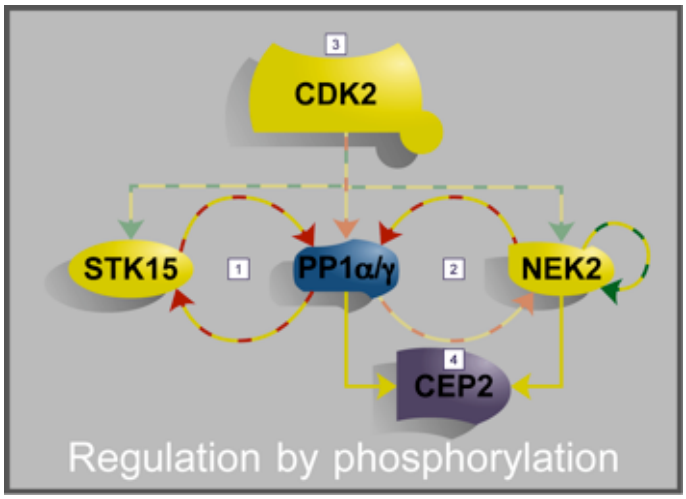


Figure 5: Centrosomal regulatory phosphorylations

Also to be determined is the nature of the external perturbation that triggers the state change. This may take the form of a kinase targeting NEK2 or STK15 and thereby opposing their deactivation by PP1. Conceivably, PP1 may itself be the kinase target if the inherent autophosphorylation capacity of NEK2 were sufficiently strong. An obvious candidate kinase is activated CDK2 [3]. As centrosome separation usually occurs late in G_2, cyclin-A presents a more attractive choice of activating partner for CDK2 that does cyclin-E. Such a change may hold significance for substrate preference, allowing events to be initiated in their proper sequence. This model is lent some credence by the reported ability of CDK2 to phosphorylate and inhibit the PP1 alpha[121] catalytic subunit, although this has not been demonstrated in a centrosomal context. CDK2 could therefore serve to prime the state transition, being the external perturbation necessary to upset the status quo, and STK15 and NEK2 maintain this state beyond the inactivation of CDK2 upon the loss of its cyclin partner during mitosis. It seems likely that PP1 is targeted by multiple kinases, ensuring that it remains inhibited until the last of them becomes inactive. Ultimately, a prime target of this control mechanism is CEP2, as it is a substrate of both NEK2 and PP1[75] [4]. The significance of this becomes clear when it is recalled that CEP2 is a critical component in the linkage between duplicating centrosomes[136].

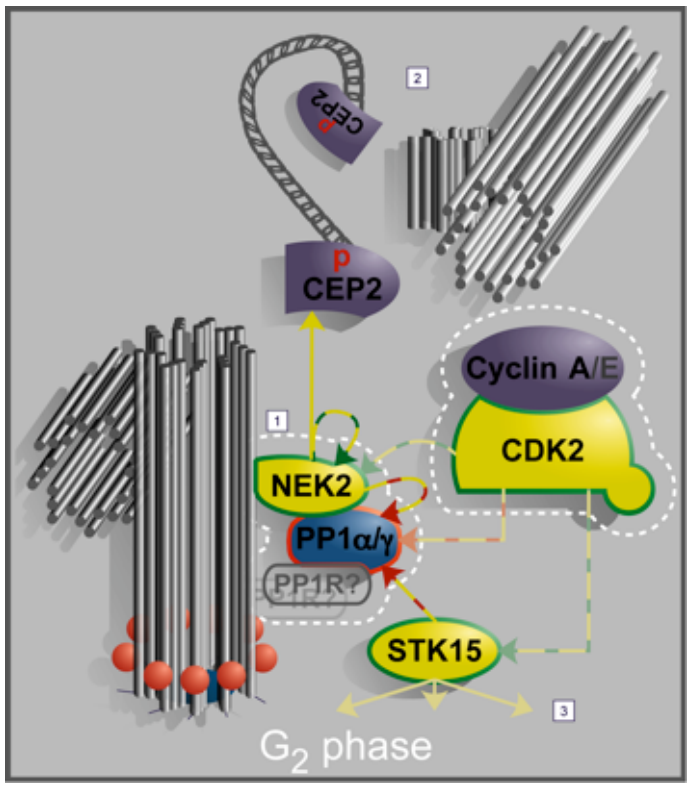


Figure 6: Centrosomes in G_2

Upon activation of NEK2 {Figure 6} [1], CEP2 is phosphorylated causing it to dissociate from NEK2, thereby severing its

centrosomal link and triggering centrosome separation [2]. There are probably additional STK15 substrates yet to be identified, providing scope for further consequences of its activation [3].

Centrosome separation

Once the centrosomes are fully detached, they are free to relocate to the cell poles. This is an active process that is dependent on cytoplasmic microtubules and motor proteins, but there is as yet no broad agreement on how these elements contribute to the process. Consideration of the known activities and spatio-temporal associations of these components suggests a number of possible mechanisms to generate the required separative force {Figure 7}. Cytoplasmic dynein, perhaps the most common minus-end directed microtubule motor protein[®5], has been shown to be essential for centrosome migration in *Drosophila melanogaster*[169] and *Caenorhabditis elegans*[66]. In both cases, the observation was that centrosomes failed to become diametrically opposed at the nuclear surface prior to nuclear envelope breakdown (NEB), and that this asymmetry resulted in poor spindle alignment. However, the centrosomes did separate substantially after NEB, even if the geometry was imperfect. Therefore, while dynein may be important in centrosomal positioning prior to separation, it does not appear to provide the major separative force. As it is a minus-end directed motor, and microtubules are arranged radially around centrosomes with the minus-ends in the pericentrosomal material, the only direction that dynein could travel with respect to a centrosome would be towards it. For this to result in separation of centrosomes implies that it must be anchored at the cellular cortex, and act to pull the centrosomes outward [1]. In support of this model, 'astral-pulling' has been reported[7 211], there is evidence that dynein participates in cortical microtubule anchoring[24], and disassembly of microtubules, particularly at their plus end, is well established. The NUMA1 protein[221] could play a role here as it associates with both microtubule minus-ends and the dynein minus-end directed motor protein. In so doing it can organise randomly oriented microtubule into asters with minus foci, and concentrate dynein at their centres, precisely what is seen at the spindle pole. As it stands, this model cannot account for specific bipolarity or separation of centrosomes beginning prior to the extension of microtubules to the cortex. It is in the resolution of the first that centrosomes come into their own as microtubule organising centres.

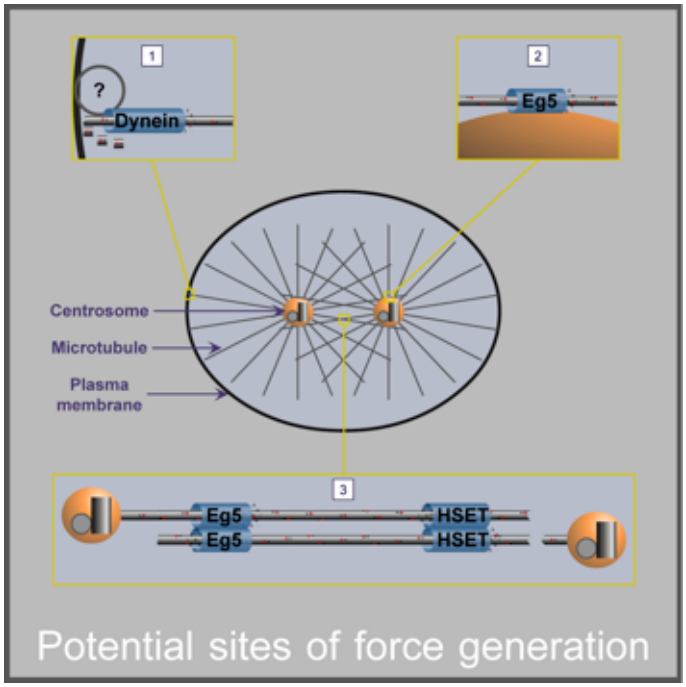


Figure 7: Generation of inter-centrosome force

A more likely candidate to provide motive force is the plus-end directed kinesin-like protein, Eg5. By its nature, it distances itself from the centrosome anchoring the microtubule to which it is attached, and it is known to be required for centrosome separation[®145]. To harness the force generated by Eg5 to promote separation requires only that it be physically coupled to the centrosome that is not anchoring the microtubule on which it travels. An obvious mechanism for this is the direct attachment of Eg5 to one centrosome where it engages a microtubule radiating from the other centrosome [2]. Studies of Eg5 location during mitosis show, however, that it does not remain centrosomal, but rather associates with

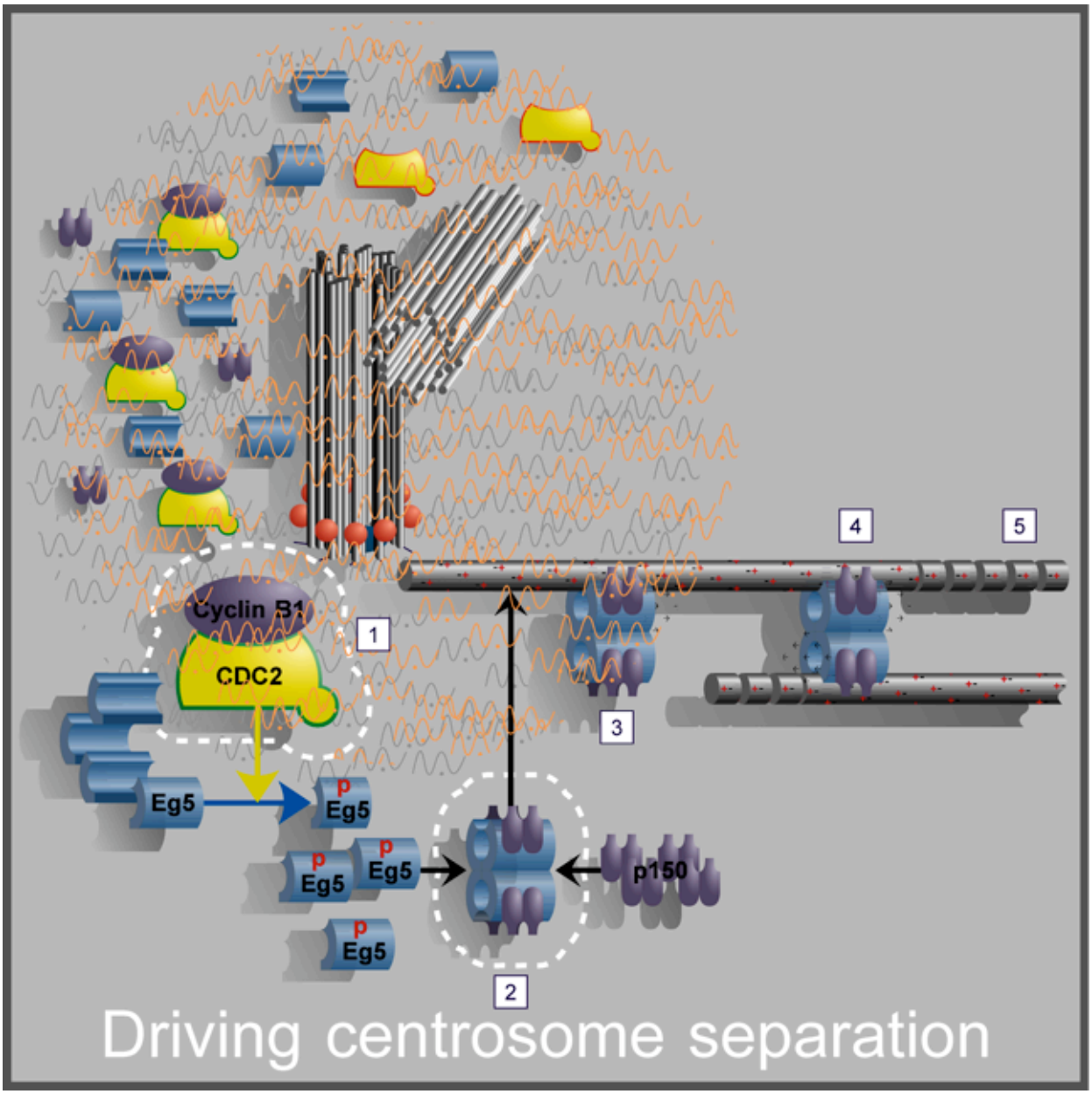


Figure 8: Centrosome separation

the full length of the microtubules of the forming mitotic spindle[203], and furthermore, moves upon them[213]. This leads to the third, and most favoured model of centrosomal force generation, wherein Eg5 promotes the relative motion of antiparallel microtubules, with each being translated in the minus direction [3]. However, not all workers find this to be consistent with experimental observations[211]. A particularly attractive aspect of this model is that it spontaneously gives rise to bipolar symmetry since the net force generated will be directed along a line linking the centrosomes. The situation is complicated by the existence of a related kinesin-like motor protein, HSET, which has been demonstrated to cross-link microtubules directly, but is minus-end directed[142], and therefore works in opposition to Eg5. The net effect is therefore likely to depend on the relative activities of the various elements, and this balance is likely to be under an active control that is still to be characterised.

The synchronisation of the commencement of centrosome separation with the start of mitosis parallels the mechanism that synchronises centrosome replication with S-phase: the activation of a CDK. In this case {Figure 8}, it is CDC2, most probably in conjunction with cyclin-B1 [1]. CDC2 is a constitutive part of the centrosome, being distributed throughout the pericentriolar material and present at the surface of

centrioles[156]. Phosphorylation of Eg5 by activated CDC2 dramatically affects its cellular disposition and binding properties causing it to accumulate in prophase at the centrosomes from a state of cytoplasmic dispersal[212]. This is probably due to an increased affinity for the p150 subunit of dynactin[15 16] [2], already there as a result of dynein mediated component delivery. In conjunction with dynein, dynactin is thought to act as an adaptor, linking the dynein motor to its cargo.

In addition to domains mediating interactions with dynein and cargo, each component of the usual p150 dimer contains one that binds microtubules. These domains are thought to augment the affinity of the attached motor unit, be it dynein or Eg5, for microtubules and possibly maintain contact during any temporary detachment of the motor during procession. Eg5 most probably adopts a conformation similar to its *Drosophila melanogaster* homologue Krp130, that of a bipolar homotetramer[8 94], ideally suited for the interlinking of antiparallel microtubules. Whether as dimers or tetramers, the assembled Eg5 complex, with its associated p150, is then able to form a stable association with the centrosomally anchored microtubule, and it begins its motion toward the plus-end [3]. During its progression, it may encounter a microtubule of opposite polarity to which the available Eg5/p150 site can bind. More symmetrically, dimeric unipolar Eg5 motors may form at each centrosome, and upon encountering one another, engage to bring about the same structure. Once the cross-link is in place, and assuming that the microtubules are rigid and non-compressible, a force tending to separate the centrosomes will be developed [4]. During this period, microtubule growth at the plus-end is also favoured [5], providing increasingly long connecting rods that the Eg5 complex can use to displace the attached centrosomes.

Ultimately, separation must be constrained by the physical size, flexibility, and strength of the plasma membrane in order to prevent its rupture, but the manner in which this is regulated is unknown. Ideally, once the maximal tolerable extension has been reached, further extension should be suppressed, but this should not be at the expense of the stability of the assembled mitotic spindle. Two simple mechanisms for achieving this goal would be the modulation of Eg5 activity or of plus-end microtubule extension. Both may occur via active mechanisms, and physical contact with the forming metaphase plate would constitute a suitable synchronising trigger. Alternatively, the Eg5 motor may simply stall when the translational force it is able to exert on a microtubule is counter-balanced by the compressive force ultimately generated by plasma membrane containment, and defined by its elasticity and cohesiveness.

Post-mitotic relocation

As telophase is completing, the advancing cleavage furrow constricts the equator of the dividing cell resulting in the formation of a bridge interconnecting the incipient daughter cells. The mitotic spindle and polar microtubules have been disassembled, and the centrosome is no longer required at the cell pole. The fate of the centrosome at this stage has best been described by Piel et al.[153], who followed events with fluorescently tagged centrosomes and time-lapse video phase-contrast microscopy. They found that one, and occasionally both centrosomes split into their separate centrioles once again, that the maternal centriole moved rapidly across the cell to the intercellular bridge, and upon its arrival, a narrowing of the bridge was observed. They demonstrated that the arrival of the maternal centriole at the bridge, and its subsequent departure, were both necessary precursors to cellular abscission, and, by synchronised nocodazole addition, that movement in both directions was microtubule dependent. By serial-section electron microscopy, they determined that it was the subdistal appendages of the centriole that were implicated in the bridge interaction. The motive force behind this relocation is unknown, but the presence of dynein and dynactin at the cleavage furrow and midbody[93] may go some way toward an explanation. In 70% of cells observed, only one centrosome split, and only one maternal centriole visited

the bridge. This raises the question of the basis for this asymmetry, an aspect not addressed in their paper.

It seems unlikely that there could be any communication between centrosomes located at opposite sides of nearly completely separated cells, so the distinction in abscission mediating function must be inherent within each, and guaranteed to exist in only one. A plausible model to explain this can be developed from the hypothesis that centrioles progress through three stages of functional maturity. The first is the partially or newly formed nascent centriole, incapable of either fostering further centriole assembly or of sponsoring cellular abscission. In the second stage, the centriole achieves a fully active status, being able to perform both functions. Finally, the centriole becomes cytokinetically passive, being able to promote centriole assembly, but not mediate abscission. The first corresponds to the current definition of a daughter centriole, and the last two to subdivisions of maternal status. The established involvement of the maternal subdistal appendages with cellular abscission suggests that this may be the site where the distinction between active and passive states is made. If the appendages possessed a one-time abscission mediating function, the transition from nascent to active could correspond to their synthesis, and from active to passive, to their use and disablement. The transitions between these stages and their associations with cellular events are depicted in Figure 9. Early in G_1 [1], the cell has a single centrosome consisting of one nascent centriole, and one which for the moment is assumed to be passive, having been the agency behind the recent abscission. During G_1 [2], the nascent centriole achieves active status, and in S-phase, the centrosome splits, and new nascent centrioles are formed [3]. During mitosis [4], the centrosomes separate and move to the cell poles where they reside until the completion of telophase. At this time, the single active centriole separates from its partner and moves to the inter-cellular bridge [5] where it stimulates abscission, and in so doing, loses its active status and becomes passive. Cytokinesis completes [6] with two daughter cells each containing centrosomes that are again in their initial state, ready for the next cycle.

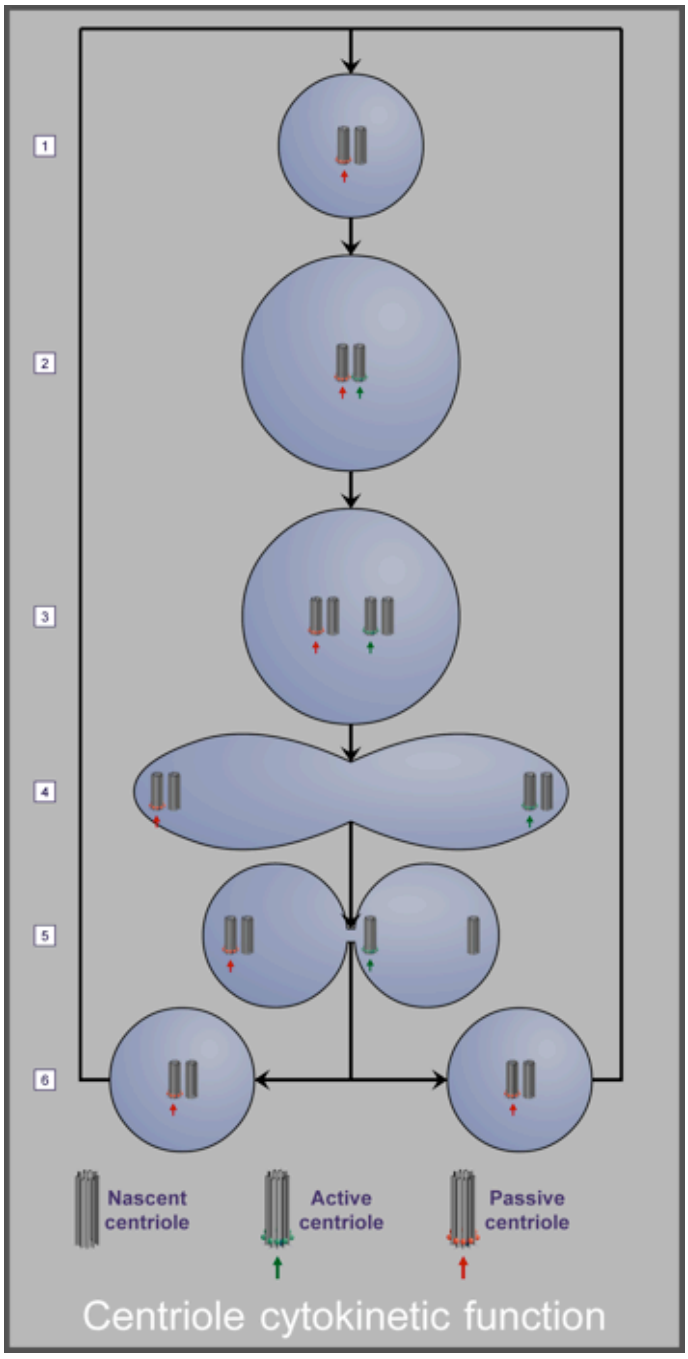


Figure 9: Centriole peregrination

This model neatly accounts for the activation of a single centriole during each cytokinesis. How then are the 30% of cases where two centrioles are activated to be accounted for? One possibility rests with the experimental system in which the key observations were made: the HeLa human cervical adenocarcinoma cell-line. This line is aneuploid[2], contains HPV18 DNA sequences[67], and possibly as a result, only weakly expresses p53. This suggests that centrosomal regulation may be abnormal in this cell-line, and the 30% incidence of multiple maternal centriole activation may simply be a consequence of this {*See 'p53: Guardian of the centrosome?', below*}. Examination of this scenario within the context of the model just described brings to light a further aspect worthy of consideration. If each centrosome contains an active centriole at the completion of telophase, then both will detach and migrate to the intercellular bridge, and both will then become inactive. Irrespective of whether one or two centrioles were active, after cytokinesis the disposition of the centrioles in the daughter cells is identical. The system spontaneously reverts to generating exactly one active centriole per cytokinesis.

Upstream regulation – the cyclin-dependent kinases CDK2 and CDC2

A tacit assumption in the preceding discussion was that the regulation of the synchronising kinases CDK2 and CDC2 was being performed correctly. However, given their crucial role, this must be expanded upon, as flaws in this process can and do influence centrosome regulation and may therefore impact on the maintenance of euploidy. Three major modes of regulating CDK kinase activity are known[®152].

Regulation by cyclin association

The first mode of CDK regulation provided the basis for the name of the class to which they belong: cyclin-dependency. Only with the cooperation of an activating partner can any CDK function as a kinase. In the case of CDK2, activation has been reported in conjunction with cyclins A[47], B1[41], D2[195], and E[107]. Interestingly, while it binds to cyclin-D1, it is inhibited, rather than activated by it[59 79], and opinion is divided over the effect of cyclin-D3 binding[32 49]. The most important physiological CDK2 cyclin partners appear to be cyclin-A and cyclin-E. A non-cyclin activating partner, RINGO, has recently been identified in *Xenopus laevis*, and CDK2 so activated is less susceptible to the other regulatory modes[§92]. In the case of CDC2, the activating cyclin must be either a cyclin-A or cyclin-B isoform.

One central theme of this dependency is that it lays down the broad sequence of CDK activation during the cell-cycle. With the disinhibition of E2F1 late in G_1, synthesis of cyclin-E commences and the activation of CDK2 becomes possible. Later, in a poorly understood process involving E2F and pRB-related pocket proteins, cyclin-A expression increases. The availability of a second activating cyclin for CDK2 may have implications for kinase substrate specificity[®140]. When levels of cyclin-A grow beyond that of its preferred partner CDK2, the excess may commence the activation of CDC2 late in S-phase or in G_2. This is soon overtaken by the increasing availability of cyclin-B1, which in conjunction with CDC2 mediates the majority of M-phase activities.

Regulation by alteration of phosphorylation status

The second mode of CDK regulation involves alterations to the phosphorylation status of three residues conserved both evolutionarily and among the CDKs. Representative proteins with close homology to CDK2 or CDC2 are shown in Table 2.

In general, the effect of phosphorylation of <T14> or <Y15> inhibits kinase function[®14], whereas phosphorylation of <T160> is mandatory for activity[70]. The kinases and phosphatases responsible for regulation of these sites in vivo have not been identified unequivocally, but in some cases, very good

Species	Protein	<T14>	<Y15>	<T160>
Saccharomyces cerevisiae	Cdc28	T18	Y19	T169
Schizosaccharomyces pombe	CDC2	T14	Y15	T167
Dictyostelium discoideum	crp	T14	Y15	S159
Arabidopsis thaliana	p34(cdc2)	T14	Y15	T161
Caenorhabditis elegans	p34cdc2	T32	Y33	T179
Drosophila melanogaster	cdc2c (cdk2)	T18	Y19	T162
	cdc2	T14	Y15	T161
Xenopus laevis	CDK2 (Eg1)	T14	Y15	T160
	CDC2	T14	Y15	T161
Mus musculus	Cdk2	T14	Y15	T160
	Cdc2A	T14	Y15	T161
Homo sapiens	CDK2*	T14	Y15	T160
	CDC2	T14	Y15	T161

* Multiple splice variants exist, including an N-terminal extension with T17/Y18 (XP_049150).

Table 2: Conservation of CDK regulatory phosphorylation sites

candidates have been suggested. For the most part, these too share a high degree of homology among species.

From structural studies, it is known that the <T14> or <Y15> residues are positioned within the catalytic cleft of the kinase domain, and inhibition is probably through exclusion of ATP by the resident phosphate groups {*See 'Regulation by inhibition', below*}. The kinase responsible for <Y15> phosphorylation may be <WEE1> [215], but that for <T14> has not been established with any certainty and may be *PKMYT1*[18]. In both cases however, the associated phosphatase appears to be CDC25. In vertebrates, where multiple CDC25s and CDKs exist, CDC25A[19 180 ®146] appears to participate predominantly in the regulation of CDK2, and CDC25C, that of CDC2.

Despite the similarities among CDKs, differences in regulation by phosphorylation are known[18 159], and generalisations must be viewed with caution. Indeed, studies in *Drosophila melanogaster* have suggested that the phosphorylation state of T14 and Y15 of cdc2c is functionally irrelevant[§111], and a paradoxical Cdk2 Y15 phosphorylation in conjunction with stimulus to proliferate has been reported in mouse cells expressing human CDC25A[§181].

The critical T-loop T160 phosphorylation significantly alters CDK2 conformation and thereby facilitates substrate binding[21 85 174], and a similar situation almost certainly prevails in the case of CDC2[161]. In vivo, the CAK complex, or a related kinase, performs the activating phosphorylation of <T160>[89], but there is evidence that significant differences exist in this function between yeast and vertebrates[88], with the possibility in the latter of an influence by p53[178]. The identity of the antagonistic phosphatase is unresolved, with PP2[31] and KAP[160] being implicated.

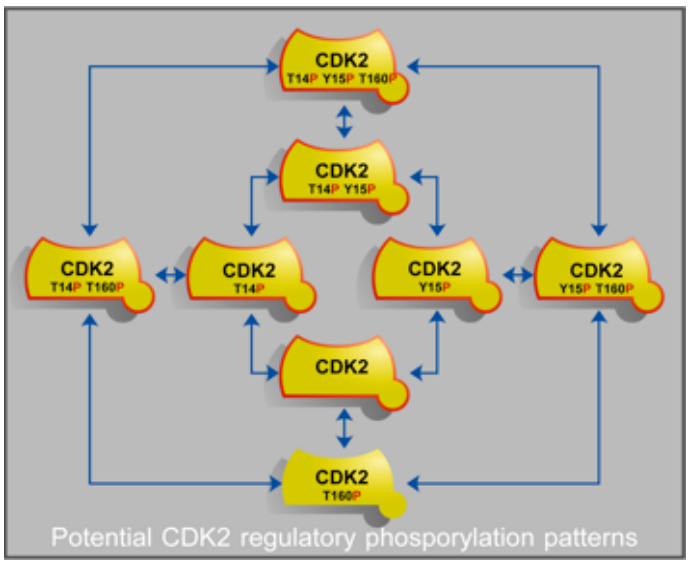


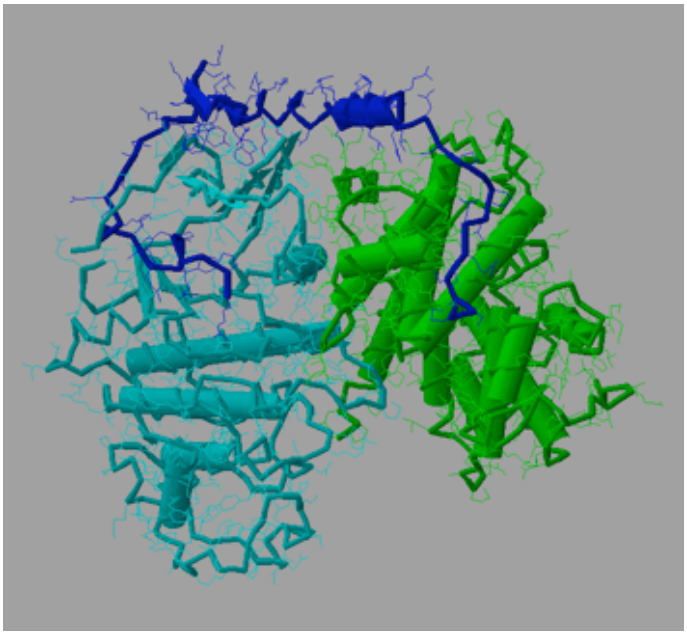
Figure 10: CDK2 phosphorylation states

The presence of multiple phosphorylation sites, potentially independently regulated, implies numerous unique combinations and transmutations {Figure 10}. Some patterns and transitions have been detected

experimentally and some can be inferred to exist. A thorough analysis of possible interactions among these states is yet to be reported. What does seem to be clear is that only that molecular species phosphorylated on <T160> alone has the potential to become active.

Regulation by inhibition

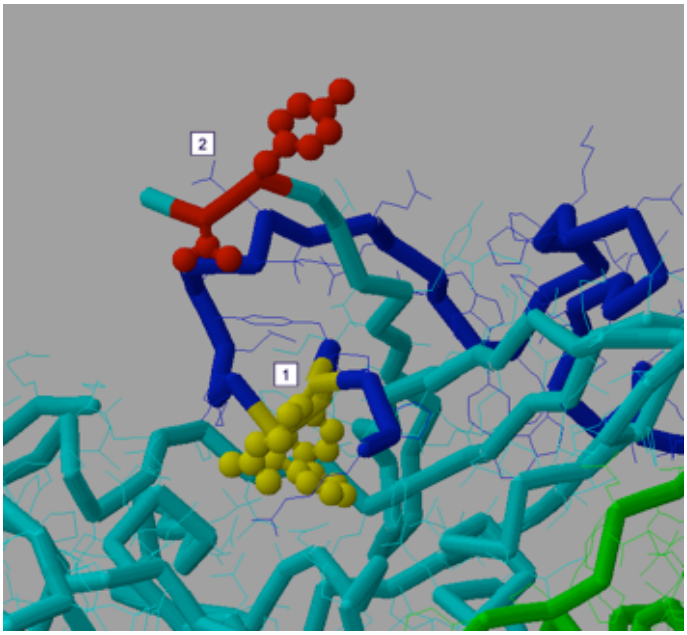
The third mode of CDK regulation is via the actions of inhibitory proteins. Members of one class, the p16-related family, are specific inhibitors of CDK4/6, and so have no direct role in the regulation of CDK2 or CDC2, or consequently, centrosome regulation. In contrast, members of a second class, characterised by homology to the p21$^{CDKN1A®20}$ protein, are of direct relevance, particularly p27^{CDKN1B}.



Key: CDK2 = light blue; cyclin-A = green; p27 (N-terminal 69 amino acids) = dark blue. Data from Russo et al.[173] Rendered by Cn3D.

Figure 11: Complex of cyclin-A, CDK2, and p27

p27 has been implicated in cell-cycle arrest in response to the presence of inhibitory cytokines (IL4[122 208], TGFβ[166], IL1-α[220]), the absence of stimulatory cytokines (PDGF[182], FGF2[182], IL2[208], IL3[163], IL10[208]), hypoxia[63], and in anchorage dependency[74 109 187], contact inhibition[42 77 86 116 158 192], and myeloid cell differentiation[36]. It can bind CDK2 and cyclin-A or cyclin-E either individually, or in a ternary structure {Figure 11} through multiple protein interaction domains. Its major inhibitory function {Figure 12} is mediated by the insertion into the kinase catalytic site of three amino acids, F87, Y88 and R90 [1], which mimic the interactions of ATP. This model is supported by studies of the related p57 protein[73]. The immediate adjacency of the CDK2 T14 and Y15 regulatory sites [2], displaced by the presence of p27, suggests that the same underlying inhibitory mechanism is employed by both: the occupation of the ATP binding site.



Key: as for Figure 11. p27 amino acids shown in yellow mimic ATP binding. CDK2 amino acids shown in red are those subject to regulatory phosphorylation.

Figure 12: Mechanism of CDK2 inhibition by p27

The level and functionality of p27 are under post-transcriptional control via at least three degradative mechanisms, operative in different cell-cycle phases and physiological conditions[128 186]. The first, which dominates in G_1, involves the ATP-dependent proteolytic cleavage of the N-terminal cyclin-binding domain, resulting in a reduced affinity of p27 for cyclin–CDK

complexes[186]. The second, operative in S and G_2, hinges on T187 phosphorylation by CDK2 and ubiquitin-directed proteolysis[205].

This presents an apparent conundrum in that p27 is a substrate of the very enzyme it inhibits. One mechanism that could account for this would require that p27 be phosphorylated by a CDK2 other than that to which it is bound, and therefore inhibiting. One implication of this would be that a possibly large fraction of CDK2 would be bound and inhibited by unphosphorylated p27, there being an increasing scarceness of active kinase. This does not accord well with the efficient degradation of p27 at the appropriate time. The likely resolution of this paradox is both simpler and more elegant {Figure 13}. The key lies in the physical and temporal separation of the binding event and the inhibition event. Avid binding of p27 depends on its interaction with both the cyclin and the CDK in a complex [1]. It does not, however, appear to depend on any interaction between its inhibitory domain and the ATP-binding site of the CDK. Furthermore, there is no evidence, nor does it appear likely, that p27 could displace a resident ATP. Particularly in light of the extended, flexible structure of p27, it is reasonable to infer that p27 binds an active cyclin–CDK2 complex, already charged with ATP, and merely awaiting the docking of a substrate [2]. The C-terminal region of p27 provides an immediate target [3]. Thus, with the execution of its function, CDK2 discharges the resultant ADP molecule freeing the docking site. This vacancy is immediately filled by the p27 inhibitory domain that is immediately available [4], completing the process.

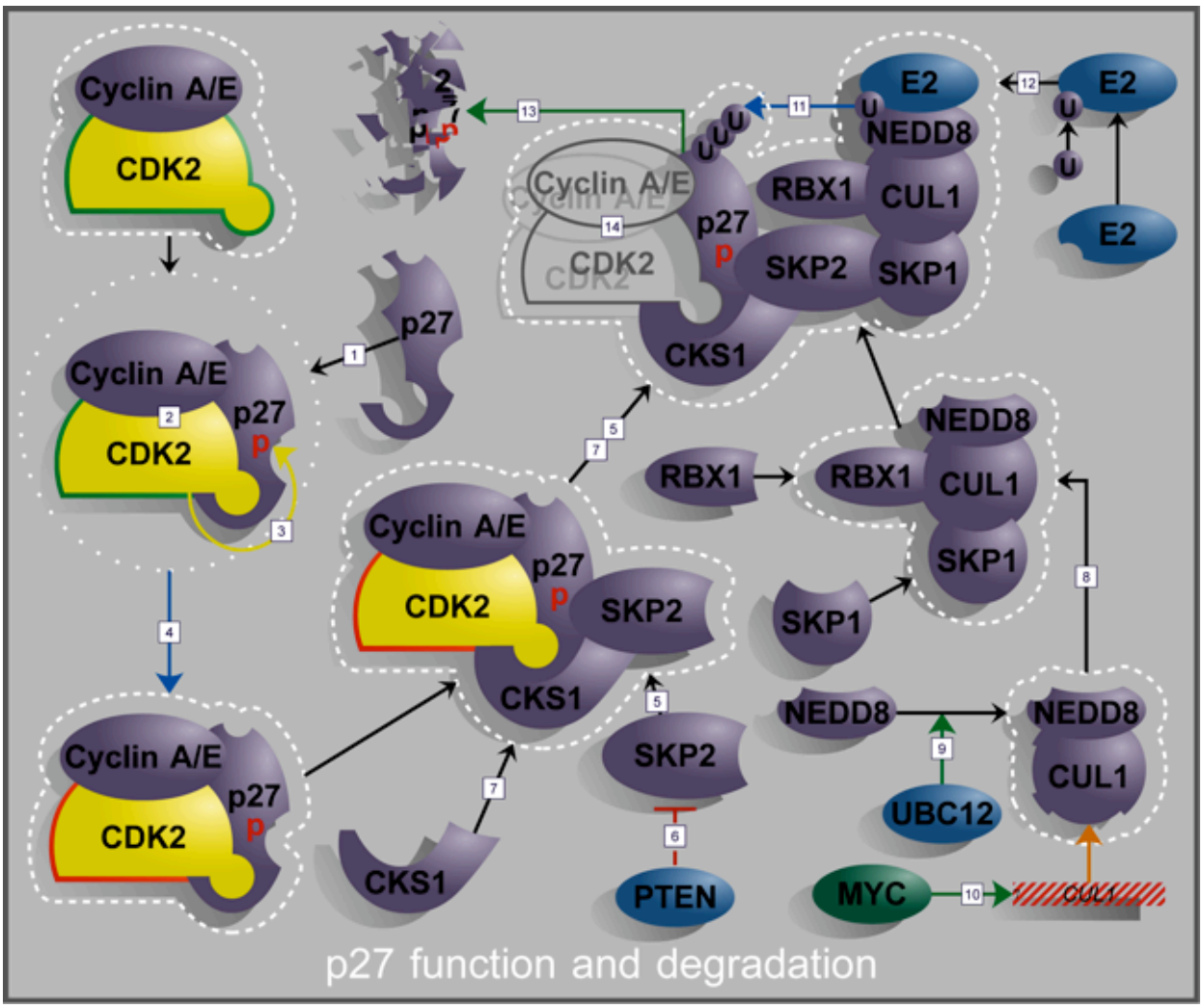


Figure 13: p27 function and degradations

The subtleties of this proposed mechanism extend further. For the phosphorylation of p27 to occur, the kinase must be active, implying that p27 is joining a cyclin-CDK2 complex, rather than either element alone, and that the T14/Y15/T160 phosphorylation state necessary for kinase activity exists. If the first condition is not met, the joining of the remaining partner is unlikely to result in p27 phosphorylation. If the second is not met, then the subsequent modification of CDK2 phosphorylation status will not be sufficient to activate it, indeed in the presence of p27 the activating kinase, CAK, is thought to be denied access to the T160 site[164]. Overall, the implication is that the inhibition of an active CDK2 is easier to reverse by ubiquitin-directed proteolysis than is the inhibition of an inactive CDK2. While it may be a pedantic distinction, it would be more accurate and potentially less misleading, to refer to p27 and its kin not as inhibitors, but rather as activational repressors.

The third mechanism for post-translational modification of p27 function involves the caspase-dependent cleavage of the C-terminal region of p27, which includes both the nuclear-localisation signal (NLS) and the T187 residue whose phosphorylation triggers ubiquitin-directed degradation[125]. The combined consequences of this are unclear. Loss of T187 should render p27 immune to ubiquitin-directed degradation, making it a more effective repressor of CDK2, and potentially other CDKs. However, the loss of the NLS may constrain it to the cytoplasm. CDKs may therefore be differentially repressed depending upon their cellular location. This is particularly noteworthy considering the role played by CDK2 in centrosome regulation. The caspase-dependency also suggests a role in apoptosis, but this too is unclear as p27 is considered to have anti-apoptotic properties, even after cleavage[51].

Consignment of p27 for degradation by the proteasome is achieved by the ubiquitin ligase action of the SKP1–Cullin–F-box complex (SCFC) {Figure 13}. The best characterised mechanism for delivery of p27 to the SCFC for ubiquitinylation is mediation by the F-box protein SKP2, although a SKP2-independent mechanism is known[71]. SKP2 is able to bind both T187-phosphorylated p27 and SKP1 simultaneously [5], and, notably, SKP2 levels are modulated via the PTEN/PI3K signal transduction channel[129] [6], often perturbed in cancer[®26]. The affinity of SKP2 for p27 is significantly enhanced by the accessory protein CKS1[62] [7], better known for its CDK-binding ability[®206]. Efficient recruitment of CUL1 to SCFC [8], and therefore enhanced p27 degradation, depends upon its conjugation to the NEDD8 ubiquitin-like protein, a process possibly catalysed by UBC12[157] [9]. *CUL1*, the gene for the third core component of the SCFC is itself a transcriptional target of MYC[148] [10], linking oncogenic transformation to the activation of the SCFC. SCFC acts as an E3 ubiquitin ligase, assisting the transfer of activated ubiquitin from an E2 ubiquitin-conjugating enzyme [11] to the target protein [12]. The identity of the E2 enzyme has not been established unequivocally, with one report showing that either UBC2 or CDC34 could perform this function in vitro, while UBC4 is inactive[151], and a second making a strong case for UBC4, particularly in conjunction with NEDD8[98]. By whichever mechanism it is achieved, once p27 has been ubiquitinylated, it becomes eligible for proteasomal degradation [13]. In light of the context of this discussion, it is noteworthy that the SCFC complex is centrosomal[56 69], associates directly with the 26S proteasome, also possibly via NEDD8[90], and most conclusively, that centrosomes associate with functional 20S and 26S proteasomes[52]. It appears that monomeric p27 is not a subject of this process, and that it is the trimeric complex that is the target[217]. Whether this is a substrate specificity, or simply due to phosphorylated p27 only existing in these complexes is unclear. Little is known of the fate of the complex. It may be degraded *in toto*, or a de-repressed cyclin–CDK complex may survive [14].

The similarity between p21 and p27 is strongest in the N-terminal regions, implicated, as discussed, in cyclin and CDK interaction. Like p27, p21 also prevents access to the critical T160 residue by CAK[164],

but whether p21 also directly interferes with ATP binding is not known. Of the three residues implicated in ATP mimicry in p27, only that corresponding to Y88 is conserved in p21, so until the analogous crystal structure for p21 is reported, the question remains open. The C-terminal regions of the two proteins are quite dissimilar. In p21, there is a domain that binds and inhibits PCNA, and a further cyclin-binding domain homologous to that near the N-terminus, neither present in p27. The critical p27 T187 phosphorylation site governing SKP2 binding and thence degradation has no analogue in p21. Notwithstanding this, p21 is phosphorylated on T145, with consequences for its PCNA inhibitory function[170], however, it seems unlikely that CDK2 is the responsible kinase. Interestingly, while p21 is labile in vivo, and is both ubiquitinylated and degraded via the proteasome, its degradation is independent of its ubiquitinylation status[183]. The manner and biological significance of this ubiquitinylation are yet to be elucidated.

With respect to their interaction with CDKs, the salient functional distinctions between p21 and p27 appear to be fourfold. Firstly, p27 can repress <T160>-enabled CDKs through ATP-mimicry, enabling it to modulate CDK activity efficiently even after this phosphorylation. On the other hand, p21 may lack this ability, and would be restricted to the role of inhibition through competitive binding to the cyclin, rendering it less potent at curbing CDKs once activated. Secondly, repression of activated CDK2 by p27 is inherently self-limiting by virtue of T187 phosphorylation and degradation targeting, while p21 is not subject to this. Thirdly, p21 has alternative modes of cyclin binding not available to p27. Binding via the N-terminal domain may result in CDK inhibition, while binding via the C-terminal domain may not, and rather serve to target the CDK kinase function to particular substrates. This 'adaptor' role has been demonstrated with respect to CDK2 and DNA ligase I[108], but the precise mode of p21–CDK2 interaction has not been explored. This model also neatly resolves the continuing controversy in the literature over the stoichiometry of p21 inhibition of CDKs [72 76]. Studies into this aspect have generally involved immunoprecipitations and relative quantitation, and consequently, can provide only a population average of the complexes present. If the p21–CDK interactions were randomly distributed between the two binding modes, only half would result in inhibition, consistent both with the presence of active CDK in immunoprecipitates, extinguishable by the addition of excess p21, observed by some, and the ability of a single p21 to effect inhibition, observed by others. Finally, while the level of p27 appears to be regulated principally by changes in protein stability, that of p21 is under a much greater degree of transcriptional control, and is among the proteins induced by p53[46]. This distinction is of particular interest as it directly links cellular stress responses to centrosome regulation.

Upstream regulation: the response to genomic damage

Overview

In *Schizosaccharomyces pombe*, the need to delay cell-cycle progression in the event of genomic damage is addressed by the regulation of Cdc2 activity[§167]. The presence of DNA damage causes the activation of the Rad3 kinase, which phosphorylates and activates the Cds1 kinase[§196]. This phosphorylates Cdc25 creating a binding site for a 14-3-3 protein, either Rad24 or Rad25[§55], and promoting its exclusion from the nucleus. While this separates Cdc25 from its Cdc2 substrate, the principal means of regulation seems to be direct inhibition[§61 §124]. Cds1 also phosphorylates Wee1, activating its kinase function, at least in vitro[§17]. This achieves a result that complements the deactivation of Cdc25 as they are antagonistic enzymes that both target Cdc2.

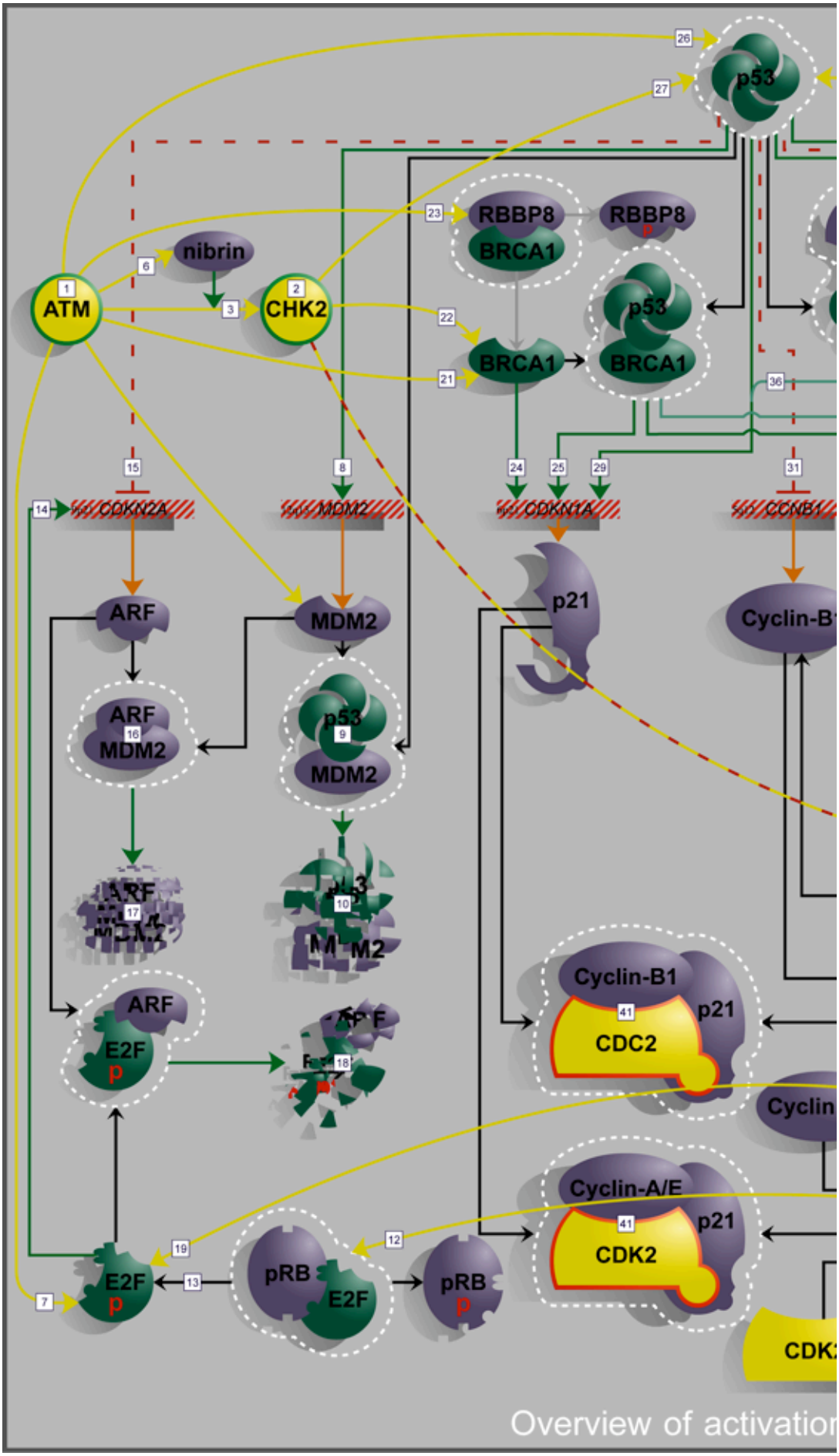


Figure 14: Activation and effect of p53 pertaining to centrosome regulation

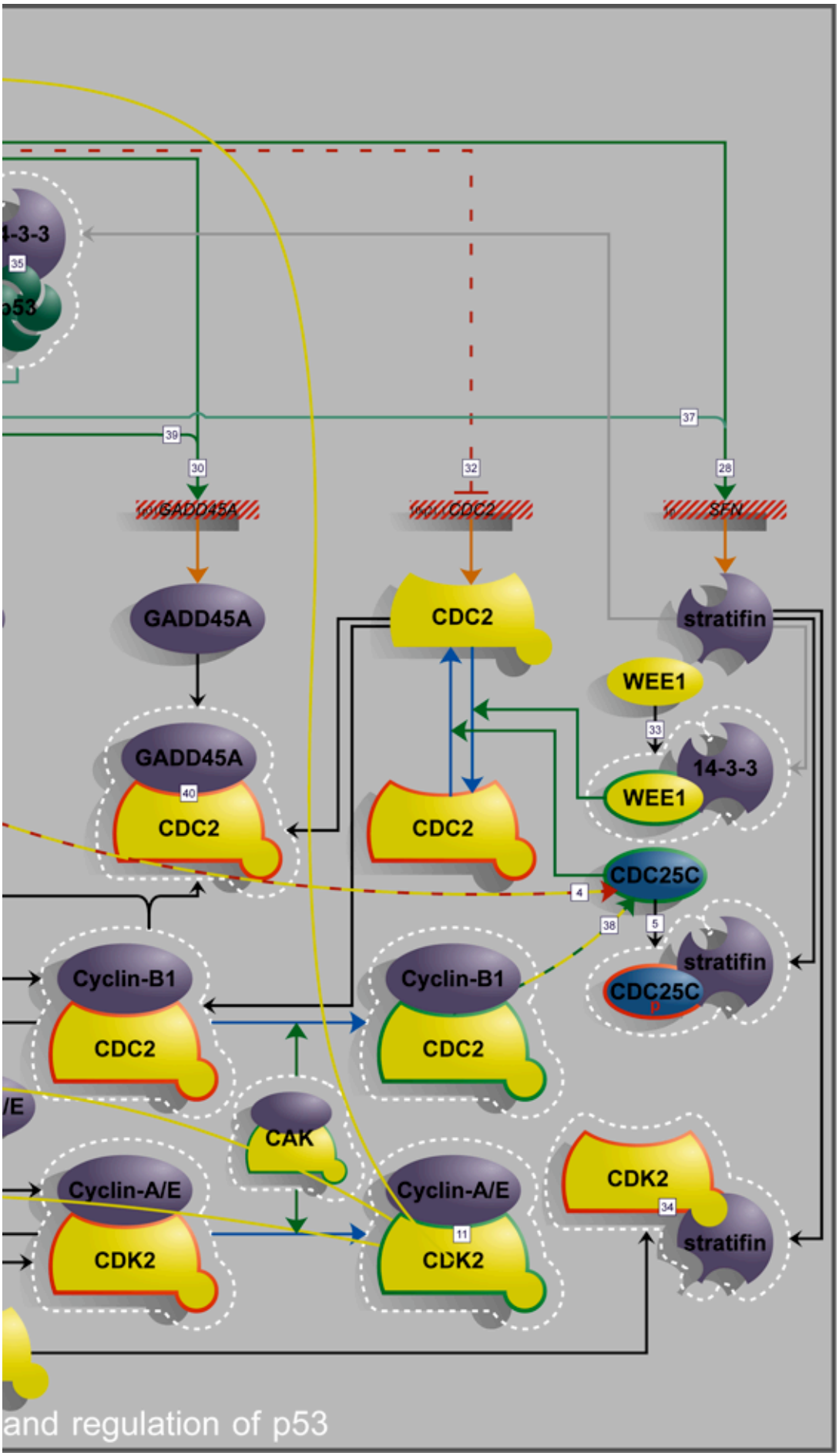


Figure 14 continued

This mechanism is conserved essentially in its entirety in humans {Figure 14}, the homologues of Rad3 and Cds1 being, respectively, ATM [1], the principal kinase of the BRCA1-associated genome surveillance complex (BASC)®95, and CHK2 [2]. The manner of its activation in humans is not fully understood, but by analogy with DNA-dependent protein kinase, is thought to be triggered by the presence of double-strand DNA breaks102. Paralleling the yeast mechanism, ATM phosphorylates T68[1] of CHK2135 [3], activating its kinase function and allowing it to propagate the effects of ATM activity to downstream targets, including both CDC25A53 and CDC25C S16[29][4], with similar consequences: inhibition, association with stratifin, and nuclear exclusion [5]. The phosphorylation of CHK2 occurs only at DNA breaks209 and depends upon the prior phosphorylation by ATM of nibrin23[6], another component of BASC. Direct phosphorylation of WEE1 in humans is yet to be demonstrated for ATM or CHK2, but that by Chk1, a structurally distinct kinase with overlapping function, is suspected in *Xenopus laevis*§114. This phosphorylation is necessary for 14-3-3 association, and this significantly enhances kinase activity171, so the prospect of phosphorylation by ATM or CHK2 seems likely. A further target of activated ATM is the transcription factor E2F1 resulting in its stabilisation and accumulation prior to apoptosis§119 [7].

The situation is, however, a great deal more complex in humans than in yeast. In addition to the proteins with close yeast homologues, such as ATM, CHK2, MLH1, MSH2, MHS6, RAD50, and MRE11A, BASC contains, or affects evolutionarily new proteins, including BLM, BRCA1, p53, and nibrin. The existence of an additional control layer is a likely evolutionary concomitant of the transition to multicellular, organ-based animals, with its attendant increased requirement for mitotic fidelity. It seems that the process is not yet complete as the failure of these late additions is often associated with a disease unique to such organisms: cancer. Chief among these evolutionary newcomers is that model tumour-suppressor, p53.

The importance of p53 dysfunction to the process of tumorigenesis may well be the best researched and most widely accepted phenomenon in the field of cancer molecular biology. The regulation of p53 function is therefore of great interest as it may have major and wide-ranging therapeutic implications. This regulation is also among the most complex yet perceived, and while its full elucidation is an enormous challenge, there is potential scope for interventions ranging from the indiscriminate to the extremely subtle. Recent emphasis has been on its roles in facilitating repair of genomic damage and inducing apoptosis. Less well studied is the interaction between p53 activation and centrosome regulation, the aspect of concern here. The brief review that follows bears only on this aspect of p53 function, enabling a causal link to be established. It therefore omits a great deal of p53 molecular biology, but these omissions have been extensively reviewed elsewhere®11 ®34.

Inferred characteristics of p53

The results of *Trp53* knockout studies in the mouse§43 have established that p53 function is dispensable for normal development and survival. However, natural or engineered <*TP53*> defect leads to a disease of general cancer predisposition: in humans, LFS48. The variable onset and spectrum of tumours associated with LFS suggests that p53 defects are not directly causative of cancer, in contrast to the situation with, for example, *RB1*. It seems instead that there is a failure to intervene in the progression toward cancer resulting from arbitrary tumorigenic events. From this can be inferred two characteristics of p53 molecular biology: firstly, that it is continuously active in a monitoring role without adversely affecting cellular physiology; and secondly, that its function is modified in response to a tumorigenic event.

Watchful waiting by p53

In its continuous monitoring role, cellular p53 is maintained at a relatively low level by virtue of having a short half-life[165]. This appears to be mediated principally by the induction of MDM2 by p53 [8] resulting in the formation of p53–MDM2 complexes [9] that are proteolytically degraded [10]. In this way, p53 expression is self-governing, with the actual level being determined by the kinetics of transcription and degradation. It was the failure of this mechanism that caused p53 to be misidentified originally as an oncogene since increased expression was seen to correlate with malignancy. The point of equilibrium of this dynamic balance is sensitive to any external alteration. A relevant example of this occurs with the activation of CDK2 on entry to S-phase [11]. By phosphorylating pRB [12], cyclin-E–CDK2 disrupts its association with E2F1, releasing it from inhibition [13]. In addition to many targets associated with proliferation and apoptosis, E2F1 also induces the beta transcript of *CDKN2A* [14], whose expression is normally held at a low level by p53-dependent repression[168] [15]. The protein product of this expression is ARF, which bears the same relationship to MDM2 as MDM2 does to p53 [16], that is, it hastens its degradation [17]. ARF also binds and inhibits the transactivational capacity of E2F1[50] and may contribute[§132] to its proteasome-dependent degradation once it has been dissociated from pRB[25] [18]. Inversely, MDM2 binds and augments the activity of E2F1[133], perhaps contributing to its own demise by stimulating ARF production. Overall, the entry to S-phase is accompanied by augmented p53 levels, consistent with an increased state of vigilance being appropriate during the critical process of genome replication. The status quo is regained with the deactivation of E2F1 through the elimination of its DNA-binding ability consequent upon phosphorylation by cyclin-A–CDK2[216] [19]. `SER315` of p53 is also a target of CDK2[162] [20], and its phosphorylation results in localisation of p53 to the centrosome[35 198].

The inter-relationships among p53, E2F1, ARF, and MDM2 are complex, and, coupled with the mechanisms for p53 activation, form an extremely dynamic and responsive regulatory network with the potential to support fine nuances of control under a variety of circumstances. The elucidation of these relationships will likely form the core of a new model for cell-cycle regulation.

p53: Guardian of the centrosome?

The activation of p53 from its dormant, surveillance mode to full functionality is mediated in large part by post-translational modification[®3], and can be triggered by diverse environmental stresses[6 120 155], the best-characterised stimulus being the presence of genomic damage. Neatly conforming to the evolutionary progression presented above is the fact that perhaps the two most important 'new' components, BRCA1 and p53, are each targets of both of the most highly conserved 'old' components, ATM and CHK2. This delineates the interface between the old and the new.

Phosphorylation of BRCA1 by ATM after exposure to ionising radiation occurs on `S1387`, `S1423`, and `S1457`[64] [21]; the functional significance of these modifications is unknown. Consistent with the possibility of selective response, different phosphorylation patterns, mediated by the ATM-relative ATR, are observed after UVR[65] exposure. CHK2 and BRCA1 coincide at nuclear foci, but after gamma-irradiation, they separate. This process depends upon `S988` phosphorylation of BRCA1 by CHK2 [22][115]. It will be interesting to learn whether this process is ATM-dependent, and whether it has consequences for transcriptional activation, with or without the involvement of p53. ATM also phosphorylates the BRCA1-binding protein RBBP8 [23], another evolutionary newcomer. The significance of this is currently hotly disputed. On the one hand, Li et al. assert that phosphorylation of RBBP8 `S664` and `S745` by ATM causes dissociation of the BRCA1–RBBP8 complex allowing BRCA1 to participate in transcription[117]. On the other hand, Wu-Baer and Baer found that this complex remained intact after

irradiation[803]. Notwithstanding this controversy, BRCA1 participates in the induction of *CDKN1A*, either independently[190] [24], or in conjunction with p53[28] [25].

In the case of p53, phosphorylation of S15 [26] by ATM[101] augments its transactivational capacity by increasing its affinity for the p300 co-activator[45], while phosphorylation of S20 by CHK2[184] [27] stabilises it by preventing its association with MDM2[30]. Simultaneously, MDM2 is phosphorylated in an ATM-dependent manner, possibly directly[105]. The phosphorylation of p53 directly by ATM, and indirectly via CHK2 would allow the triggering of a subset of subsidiary mechanisms through the activation of CHK2 independently of ATM[®185].

The mainstream of the p53-response is mediated by its influence on gene transcription upon activation. The target genes involved in centrosome regulation are essentially the same as those that bring about cell-cycle arrest since both activities are driven by CDKs. Among these genes are some whose expression is enhanced by virtue of containing specific p53-binding sites[207], such as *SFN* [28], *CDKN1A* [29], and *GADD45A* [30]. Others have their expression reduced, such as *CCNB1* [31] and *CDC2* [32], and this is achieved indirectly, dependent on the prior induction of p21[CDKN1A39]. The favoured explanation, at least in the case of *CDC2*, is that the repression is performed by the binding of p130–E2F4 to the promoter. In the normal course of events, this would be released upon phosphorylation of p130 by a CDK, but this is prevented by the p53-mediated expression of p21[199]. A similar situation may prevail with respect to repression of *CDKN2A*. By whatever mechanism it is achieved, the repression of *CCNB1* and *CDC2* is an important contribution to the reduction of CDC2 kinase activity.

The *SFN* gene encodes the 14-3-3 protein, stratifin, introduced above as part of the 'old' DNA damage response system in which it binds and inhibits CDC25C [5] after phosphorylation of the latter by CHK2. 14-3-3 proteins also participate in the activation of WEE1 [33], and while stratifin has not been specifically identified in this capacity, this is an attractive scenario as p53 could then influence both arms of a major mechanism of CDC2 activation. In tandem with this, stratifin has been implicated in the regulation of CDK2 activity by direct inhibitory binding[113] [34]. Increased expression of 14-3-3 by the 'new' p53 bolsters these useful effects. Furthermore, 14-3-3 proteins are known to associate with p53 [35] and enhance sequence-specific DNA binding[210]. This positively influences transcription of *CDKN1A*[193] [36], and possibly other genes. Assuming stratifin has this capacity, p53 would induce a co-factor that enhances and possibly directs its own function. The 'new' system may modify the 'old' in yet another way as mouse studies suggest that p53-sponsored transcription of *SFN*[78] may also benefit from BRCA1 activity[§4] [37].

While these mechanisms suffice to reduce the level of activation of existing or new CDC2, they do not address the presence of previously activated kinase, and without this, inhibition of CDC2 kinase activity would not be absolute. This is particularly true since CDC25C is itself activated by cyclin-B1–CDC2 phosphorylation[83] [38] forming a self-reinforcing system that facilitates the rapid activation of CDC2 at the entry to M-phase. Even a small residual CDC2 activity could soon be amplified. This possibility is prevented by the induction of GADD45A, or possibly either of its close relatives, by p53[96] [30] assisted by BRCA1[87] [39]. It has the capacity to disrupt cyclin-B1–CDC2 complexes and sequester CDC2 in an inactive state[222] [40]. Other interactions of GADD45 with p21[99] and PCNA[8] are known, but the significance of these is unclear.

Finally, the induction of p21, in addition to mediating the repression of *CDC2* and *CCNB1*, also provides a potent direct activational repressor of CDKs. By binding preformed cyclin-A/E–CDK2 or cyclin-A/B–CDC2 complexes, p21 prevents CDK activation by CAK [41].

Together, these p53-mediated effects expunge all CDC2 and CDK2 activity and prevent its reappearance while p53 remains active. Hence, since these are critical mediators of the centrosome cycle, the case is made that aberrations of p53 regulation may adversely affect centrosome regulation.

Interdependence of nuclear and centrosomal cycle regulation

The need to synchronise commitment to the nuclear and centrosomal cell-cycles is addressed by employing the same key activators: CDK2 and CDC2. Under ideal circumstances that is all that would be required. Unfortunately, there are times when the nuclear cycle either stalls for want of some limiting factor, or must be delayed due to the presence of genomic damage. If this occurs, synchronisation with the centrosome cycle must still be maintained. Due to the commonality of control between the two cycles, no additional provision is required to achieve this. Whatever delays the nuclear cycle by modulating CDK2 and CDC2 activity will perforce delay the centrosome cycle to a corresponding degree.

The converse condition may also prevail, wherein centrosome duplication is stalled, for example, by the presence of a microtubule toxin. Fittingly, a mechanism exists for such an event to trigger an arrest of the nuclear cycle. It has been found that after only a brief treatment with nocodazole, a tubulin depolymerising agent, p53 is released form its centrosomal association and activated[33]. In consequence, the daughter cells arrest in G_1 after cytokinesis with elevated p21 levels. In addition, the activating `Y15` dephosphorylation of CDC2 has been shown to occur first at the centrosome before propagating to the nucleus[38]. If this were the catalyst that commences the self-reinforcing activation of nuclear CDC2, then any delay at the centrosome would delay the onset of mitosis.

Evolution in action

In the present epoch, mechanisms for the organisation of the mitotic spindle appear to be in a state of evolutionary transition. In plants, the odd aberrant mitosis may not be too dramatic. While they engage in fluid transportation, they lack a bona fide circulatory system, and while they have specialised tissues, they have few specialised organs. Their vulnerability to cancer-like disease is limited and a centrosomal system or its equivalent is not required. Yeast, being unicellular, are more vulnerable to failed mitosis in that one fault wipes out an entire lineage. In consequence, they possess a mechanism to improve mitotic fidelity, the spindle pole body. Multi-cellular animals, with complex circulatory and organ systems, whose corporeal life-span far exceeds that of their constituent cells, require still greater control over mitotic fidelity, hence the centrosome. It acts to manage an otherwise error-prone system in order to increase its reliability. For similar reasons, an evolutionary need to enhance the accuracy of genome duplication at the genetic level exists, hence p53.

Each of these systems normally performs well in isolation, but where they interact, or under abnormal circumstances, the few vulnerabilities become manifest. Evolution has brought life to the point where these mechanisms work adequately, but they do not always fail gracefully.

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