

# Cell cycle control and cancer

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**Cancer is a multifaceted disease, but a common feature of most tumours is that they harbour one or more genetic mutations that allow them to proliferate outside their normal growth restraints. Proliferation is normally restrained through control of the cell division cycle, which in turn, is regulated by the Cdk family of serine/threonine kinases and their regulatory partners the cyclins. Here, the roles of the Cdk/cyclin complexes in cell cycle control are described followed by a review of the genetic lesions in these and associated proteins which may contribute to tumour progression.**

ONE of the most common questions asked about cancer must be, what exactly is it? At the most basic level, it is a disease where cellular proliferation is no longer under normal growth control. Eventually, this unrestrained growth and division of the cancer cells interferes with the normal functioning of the body, either at the site of origin or through spreading to another location, eventually resulting in the death of the sufferer. There are of course other characteristics that cancer cells may possess, such as the ability to induce vascularization of the tumour in order to receive oxygen and nutrients (angiogenesis) or to disperse from the site of origin and travel to a distant part of the body (metastasis), and also to suppress programmed cell death (apoptosis). But at the end of the day it is the unrestrained proliferation of these cells, which is at the heart of the disease. And so to understand cancer we need to understand what is cell proliferation and how is it controlled?

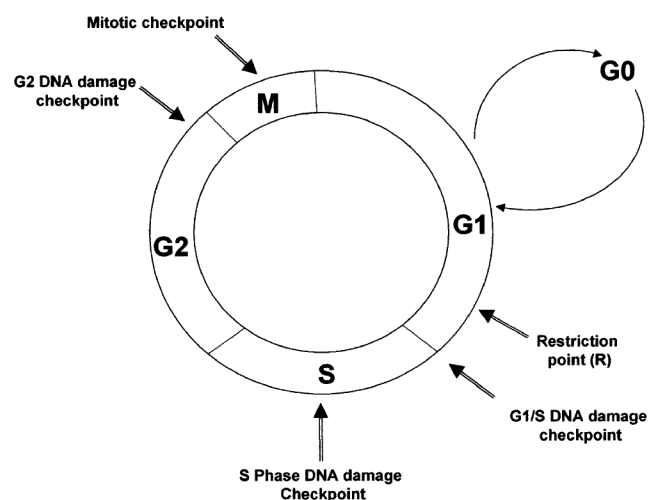
## Cell division cycle

At the centre of cellular proliferation is the cell division cycle, the process by which a cell grows, replicates its DNA and then divides to give two daughter cells. This process is divided into four sequential phases (Figure 1). It is often considered that the two most important of these are S phase, when DNA replication occurs and mitosis (also known as M phase), when the cell undergoes division to give two daughter cells. In fact a key concept of the cell cycle is that S phase must always follow M phase and that M phase must not start until S phase has been

completed. In other words, DNA replication must not commence until mitosis is complete and mitosis must not begin until the previous round of DNA replication has ended, thus, the integrity of the genome is maintained. In-between S and M phase are two gaps G1 and G2. G1 follows on from mitosis and is a time during the cell cycle when the cell is responsive to both positive and negative growth signals. G2 is the gap after S phase, when the cell prepares for entry into mitosis. Finally, there is a fifth state, G0 (also known as quiescence) into which the cell may reversibly exit from G1, if it is deprived of the appropriate growth-promoting signals.

## Cell cycle checkpoints

Movement through each phase of the cell cycle and transition from one phase to the next is regulated at a number of positions within the cell cycle known as checkpoints. Hartwell and Weinert first defined the term cell cycle checkpoint as a mechanism that maintains the observed order of events of each cell cycle<sup>1</sup>. Or, put in another way, checkpoints are sensor mechanisms within the cell which monitor the cellular environment and determine whether appropriate conditions have been fulfilled before it may progress further through a cell division cycle. Consequently a major function of these checkpoints is to see that the integrity of the genome remains intact throughout the cell cycle. Each checkpoint is made up of three components. The first is a sensor mechanism that detects



**Figure 1.** Checkpoints and the cell cycle.

aberrant or incomplete cell cycle events such as DNA damage. This is followed by a signal transduction pathway, which carries the signal from the sensor to the third component, the effector that can invoke a cell cycle arrest until the problem has been resolved. The major cell cycle checkpoints are depicted in Figure 1. The first of these occurs at the G1/S phase transition and is a major sensor of DNA damage. The cell may also arrest later in S phase due to incomplete DNA replication or again, damage to the DNA. Next comes the G2/M checkpoint, which monitors the fidelity of DNA replication and like the G1/S checkpoint is an important sensor of DNA damage. This is followed by the spindle checkpoint, which is invoked during mitosis if a functional mitotic spindle has not been formed correctly.

Also shown in the first Figure is the restriction point (R), which occurs between mid and late G1. This is the point at which the cell ascertains whether it has received the necessary growth signals (largely extracellular in origin) so that it can pass out of G1 into S phase, replicate its DNA and complete one round of cell division<sup>2</sup>. If they are sufficient, the cell will pass the R point and for the remainder of that cell cycle will not require these signals. If however, the cell has not received the appropriate cues, it will not pass the restriction point, and will instead enter G0. The restriction point therefore differs from the other checkpoints in that it does not specifically determine whether the genome is intact. However, it is an essential control point in that it restrains cell proliferation if the necessary growth signals have not been received.

If these cell cycle checkpoints are not in place then inappropriate proliferation can occur – the hallmark of cancer. We also now know that probably all human tumours harbour genetic alterations in the genes that control cell cycle progression and checkpoint function. Therefore to understand the links between cell cycle checkpoints and cancer, we must first understand the molecular machinery which drives cell cycle progression.

## Control of the cell division cycle

At the core of the mammalian cell division cycle is the cyclin dependent kinase (Cdk) family of serine/threonine kinases<sup>3</sup>. The name Cdk describes the fact that the full activity of each of these kinases is dependent on its association with a regulatory subunit known as a cyclin. In mammalian cells, different Cdks are active and required at different phases of the cell cycle. And, whilst the expression of the Cdk subunit is generally constant throughout the cell cycle, the expression of each cyclin (of which there is a whole family) tends to be cell cycle dependent so that a specific Cdk will have full activity when its cyclin partner is expressed (Figure 2).

The role of the Cdks is to control cell cycle progression through phosphorylation of proteins that function at spe-

cific cell cycle stages (Figure 2). For example, the product of the retinoblastoma tumour suppressor gene, pRb is a key regulator of G1 progression and possesses 16 potential sites of Cdk phosphorylation<sup>4</sup>. In early G1, pRb is found in a low (hypo) phosphorylated state and tightly binds and represses the activity of the E2F family of transcription factors which are functionally required for the expression of genes necessary for S phase<sup>5</sup>. During G1, pRb becomes phosphorylated at the Cdk consensus sites, disrupting its interaction with the E2F proteins allowing E2F dependent transcription to occur. This is required in order for the cell to pass through the restriction point late in G1. The phosphorylation of pRb at the Cdk consensus sites appears to be a sequential process, initiated by Cdk4 and Cdk6 each acting in association with one of three closely related cyclin subunits, D1, D2 and D3. This allows expression of cyclin E by disrupting the interaction of pRb with proteins known as histone deacetylases (HDACs), which are involved in chromatin remodelling<sup>6</sup>. Expression of cyclin E allows the formation of active Cdk2/cyclin E complexes that then continue the phosphorylation of pRb. This leads to disruption of the pRb–E2F interaction such that E2F is transcriptionally active, a requirement for the cell to progress from G1 into S phase<sup>6</sup>.

Whilst pRb appears to be the primary substrate for the cyclin D-dependent kinases Cdk4 and Cdk6, Cdk2/cyclin E is known to phosphorylate several distinct types of proteins, at least *in vitro*<sup>7,8</sup>. As cells progress into S phase, cyclin A is expressed and becomes the primary cyclin associated with Cdk2. This switching of cyclin partner allows Cdk2 to also switch substrate specificity so that it can now target a new set of proteins during S phase. These include Cdc6, a protein required for initiation of replication which when phosphorylated by Cdk2/cyclin A, relocalizes from the nucleus to the cytoplasm and the transcription factor E2F (refs 9, 10). Progression from G2 into mitosis requires the activity of the Cdk, Cdc2 (also known as Cdk1) complexed with cyclin B, which has

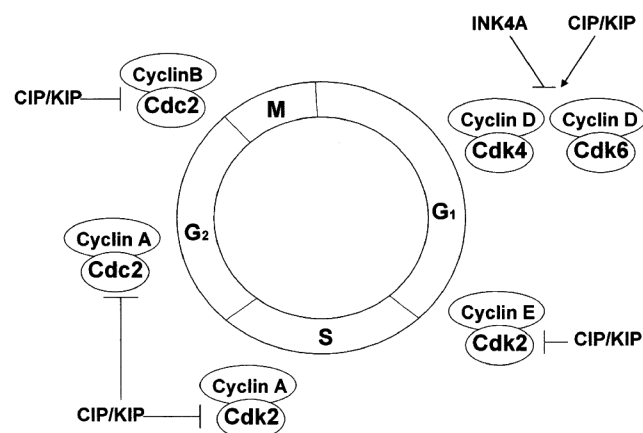


Figure 2. Cyclins, Cdks and the cell cycle.

been shown to phosphorylate proteins regulated during mitosis<sup>11–13</sup>.

Cyclin subunit association is not the only form of regulation imposed on the Cdks. There is also timed proteolytic degradation of the cyclins, phosphorylation on both the Cdk and cyclin subunits, and interaction with other regulators. Proteolytic degradation of the cyclins occurs through ubiquitin-mediated proteolysis, a process whereby each protein is targeted to the 26S proteasome for degradation by the attachment of multiple ubiquitin molecules at one or more lysine residues<sup>14</sup>. This process is known as polyubiquitination and involves a cascade of three enzymes, E1, E2 and E3 (ref. 15). Ubiquitin initially becomes attached to the E1 enzyme via an ATP-dependent reaction. It is then transferred to one of 12 or so E2 enzymes and is finally added to one or more lysine residues on the target protein via the E3 ligase enzyme. There are two known types of E3 ligase, the SCF complex and the anaphase promoting complex (APC). For the G1 cyclins D1 and cyclin E, polyubiquitination is carried out through the SCF ubiquitin ligase system and is dependent on phosphorylation of a specific threonine residue on each protein<sup>14</sup>. In contrast, cyclins A and B are polyubiquitinated by the APC<sup>14</sup>. This is mediated by a sequence motif present at the N-terminus of these and other cyclins known as the 'destruction box', which acts as a signal for the timed degradation of these proteins<sup>14</sup>.

Phosphorylation of the Cdk subunit can have both positive and negative effects on its activity. Phosphorylation at a specific threonine residue towards the centre of the protein (T161 in Cdc2) is required in order for the Cdk/cyclin complex to have full activity<sup>16</sup>. However, phosphorylation of a pair of adjacent threonine and tyrosine residues at the N-terminus of the Cdk exemplified by threonine 14 (T14) and tyrosine 15 (Y15) of Cdc2, inhibits Cdk activity even when it is phosphorylated at T161 (ref. 17). Phosphorylation on T14 of Cdc2 is performed by the Myt1 kinase, whilst Y15 is predominantly phosphorylated by the Wee1 kinase<sup>18,19</sup>. This allows the complex to be present, but inactive during G2 until entry into mitosis is required whereupon the dual specificity phosphatase Cdc25C can dephosphorylate both residues<sup>20</sup>. Interestingly, whilst Cdk2 has both the threonine and a tyrosine equivalent to T14 and Y15 of Cdk2, the cyclin D-dependent kinases Cdk4 and Cdk6 only possess the tyrosine residue.

Two protein families have been identified that can bind to and inhibit the Cdks. The first identified was the INK4 (Inhibitors of Cdk4) family, through the cloning of the first member p16<sup>INK4A</sup> and its identification as a Cdk4 inhibitor 21 (Figure 2). Three other family members have subsequently been identified p15<sup>INK4B</sup>, p18<sup>INK4C</sup> and p19<sup>INK4D</sup> all of which specifically bind to and inhibit the cyclin D-dependent kinases, Cdk4 and Cdk6 (ref. 22). In contrast, the CIP/KIP (Cdk Interacting Protein/Kinase Inhibitory Protein) family of proteins p21<sup>CIP1</sup>, p27<sup>KIP1</sup> and

p57<sup>KIP2</sup> can bind to a much broader range of Cdks that includes Cdk4, Cdk6, Cdk2 and Cdc2 (ref. 22, Figure 2). Now, although the CIP/KIP family were originally identified as Cdk inhibitors, it has recently come to light that at least in the case of the cyclin D-dependent kinases they may actually promote the activity of these Cdks by stabilizing the Cdk–cyclin subunit interaction<sup>23,24</sup>. However, they still strongly inhibit Cdk2 activity. The 'sequestration model' of G1 Cdk/cyclin activation provides one possible explanation for their contrasting behaviour towards the cyclin D-dependent kinases versus Cdk2 (ref. 25). In this scenario, as the D-type cyclins are expressed they bind to Cdk4 and Cdk6. This assembly is promoted through stoichiometric association with CIP/KIP proteins, such that these complexes are still active and can initiate phosphorylation of pRb. A second function of the cyclin D-dependent kinases is that they consequently sequester the CIP/KIP proteins away from Cdk2/cyclin E, thus promoting Cdk2 kinase activity, which can then continue phosphorylation of pRb leading to E2F-dependent transcription. In conclusion, these multiple forms of regulation are indicative of the fact that Cdk activity is a critical regulator of cell cycle progression and so must itself be under tight control.

## Cdks, cell cycle checkpoints and cancer

Having described the molecular machinery which drives cell cycle progression, and in particular the Cdks, it is now possible to outline how cell cycle checkpoints operate and their relationship with cancer. As we do this, it will become clear that there are a number of key genes that participate in multiple cell cycle checkpoints, which are also frequent targets of genetic alteration in cancer. It should also be pointed out that much of the work identifying the key players in these checkpoints has been carried out in fission and budding yeast (*S. pombe* and *S. cerevisiae* respectively) and also in the *Xenopus laevis* biochemical system. Consequently a number of the genes described here are homologues of those first identified and characterized using these model systems. Due to space limitations it is not possible to go into this but many excellent reviews can be found in the literature.

## G1

The G1 phase of the cell cycle is a critical time where extracellular signals both positive and negative are integrated into regulation of cell cycle progression. This occurs until the restriction point at which time the cell becomes committed to one round of cell division. If the cell does not receive the correct cues during G1, it cannot pass the restriction point and will instead enter the quiescent state, G0. At the molecular level, it is the cyclin D-dependent kinases that act as integrators of these

extracellular signals. An example of this is that cyclin D1 expression can be induced by both the Ras and PI3 kinase signalling pathways, thus promoting G1 progression<sup>26,27</sup>. Therefore, the cyclin D-dependent kinases, their regulators and pRb are a focal point of control for G1 progression and so not surprisingly, their links with cancer are very strong. This link actually starts with the signalling pathways which regulate cyclin D-dependent kinase activity. For instance, the *Ras* genes themselves, the *PIK3CA* gene encoding the p110 $\alpha$  subunit of PI3 kinase and the tumour suppressor gene *PTEN* which acts as a lipid phosphatase and reverses the PI3 kinase reaction, have all been shown to be mutated in cancer<sup>28,29</sup>. All these genetic alterations have the ability to cause activation of the cyclin D-dependent kinases leading to inappropriate phosphorylation of pRb and misregulation of the restriction point. Downstream the cyclin D-dependent kinases, their regulators, as well as the gene encoding pRb itself (*RB*) are all cancer targets. Indeed, most tumours contain a genetic alteration in one of these genes. Cyclin D1 was first identified as the *BCL1* gene, found at the t(11; 14) translocation in mantle cell lymphoma and also as *PRAD1/CCND1* the gene at the inversion of part of chromosome 11, inv(11)(p15; q13) found in parathyroid adenoma<sup>30</sup>. Amplification of the cyclin D1 locus 11q13, has also been identified in a number of cancer types including breast, lung and glioma. The tumour suppressor gene *CDKN2* encoding the p16<sup>INK4A</sup> protein is also found deleted, mutated or its expression silenced in multiple cancers<sup>30</sup>. Amplification of *CDK4* has also been reported, whilst low levels of the CKI p27<sup>KIP1</sup> protein have been shown to indicate poor prognosis in both colon and breast cancer<sup>30,31</sup>. Looking at all these genetic alterations it is clear to see that the one common denominator is that they all have the ability to promote inappropriate phosphorylation and inactivation of pRb. As for the *RB* gene itself, mutation or deletion is a common occurrence in cancer, thus directly abrogating the requirement for cyclin D-dependent kinase activity during G1 (ref. 30). One final thing to note is that in some tumour types there appears to be a mutually exclusive behaviour in the genetic alterations on the p16<sup>INK4A</sup>/cyclin D/pRb pathway<sup>30</sup>. An example of this is in lung cancer where tumours tend to harbour either deletions or mutations in *RB* or *CDKN2* encoding p16<sup>INK4A</sup>, but not in both<sup>30</sup>. This suggests that in certain circumstances, genetic alteration in one member of this pathway is a sufficient contribution to tumour progression.

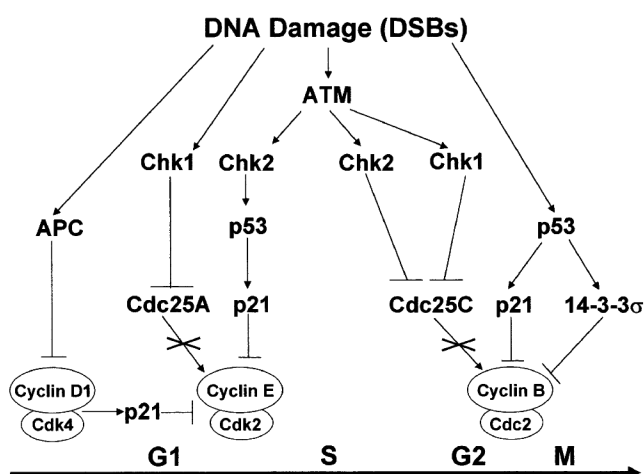
Although many cellular stresses can invoke cell cycle checkpoints (hypoxia, nucleotide deprivation and DNA damage, to name but a few), the checkpoint pathways described next for G1/S, S, and the G2/M transition are those which are invoked in response to DNA damage caused by double strand breaks in the DNA (DSBs). This type of DNA damage can be brought about by a number of agents of which ionizing radiation, genotoxic chemicals

and reactive oxygen species are three major culprits. The cell cycle checkpoints are described in the context of these forms of insults, as DSBs affect the integrity of the genome, which if not maintained correctly can lead to cancer.

## G1/S

A primary response of the normal cell to DSBs is activation of cellular pathways which induce cell cycle arrest at the G1/S transition. This is so that cells which are in G1 and have suffered DNA damage do not enter S phase. Induction of this arrest appears to be a two-step process, a rapid initiation of the arrest followed by a slower maintenance (Figure 3). To date, two cellular events have been identified which can participate in initiating the G1/S checkpoint. The most recently described is activation of cyclin D1 degradation<sup>32</sup>. This leads to a release of p21<sup>CIP1</sup> from Cdk4 to inhibit Cdk2. The second is an increase in the inhibitory phosphorylation of Cdk2 at the site equivalent to tyrosine 15 of Cdc2 (ref. 33). This is due to degradation of the Cdc25A phosphatase, which dephosphorylates this site. Degradation of Cdc25A is induced by activation of a serine/threonine checkpoint kinase known as Chk1 (ref. 33).

Maintenance of the G1/S cell cycle checkpoint is dependent on the product of the tumour suppressor gene *TP53*. This gene is mutated or deleted in over half of all sporadic cancers making genetic changes in *TP53* the most common defect in human cancer<sup>34</sup>. One explanation of this is the role that the product of the *TP53* gene, p53, plays as 'Guardian of the Genome'<sup>34</sup>. The p53 protein performs this function by acting as a receiver of stress signals (including DNA damage) that cause activation and accumulation of p53 protein in the cell. This then transcriptionally induces the expression of genes that can invoke cell cycle arrest and apoptosis. One of these is the CIP/KIP family member, p21<sup>CIP1</sup>, which on induction by



**Figure 3.** The G1/S and G2/M DNA damage checkpoints.

p53, binds to Cdk2/cyclin E causing cell cycle arrest at the G1/S transition<sup>35</sup> (Figure 3). One of the ways in which upregulation of p53 expression is induced is by blocking its degradation. Like the cyclins, p53 is degraded via ubiquitin-mediated proteolysis, in this case instigated by the E3 ligase Mdm2 (ref. 36). One route by which degradation can be abrogated leading to upregulation of p53 levels and induction of p21<sup>CIP1</sup>, is via activation of the cell cycle checkpoint serine/threonine kinase Chk2 (also known as hCds1). Chk2 is activated in response to DNA damage and phosphorylates serine 20 of p53 (ref. 37). This disrupts the interaction of p53 with Mdm2, p53 degradation is blocked and consequently the protein is upregulated, leading to expression of p21<sup>CIP1</sup> (ref. 36). Chk2 is activated through phosphorylation of threonine 68 by the ATM protein kinase, a key player in activation of cell cycle checkpoints<sup>38</sup>. The *ATM* gene is responsible for the autosomal recessive disorder Ataxia Telangiectasia (AT), characterized by cerebellar ataxia, oculocutaneous telangiectasia and most interestingly, extreme sensitivity to ionizing radiation which causes DSBs and a predisposition to certain forms of cancer. Also, ATM-deficient cells show reduced responses to genotoxic agents that cause DSBs, highlighting the importance of defective cell cycle checkpoints in cancer<sup>39</sup>. Mutations in Chk2 have also been found in a subset of patients with Li-Fraumeni syndrome a familial cancer phenotype usually associated with mutation of the *TP53* gene, providing genetic evidence that p53 and Chk2 lay on the same pathway<sup>40</sup>.

## S Phase

DNA damage during S phase does invoke a cell cycle checkpoint, although our understanding of how it functions is limited. After exposure to DNA damaging agents, the rate of DNA synthesis is slowed, but in contrast to the G1/S checkpoint described earlier there is not a complete arrest. Instead it appears that there is a slow down of replication and S phase is lengthened<sup>41</sup>. This has led to the suggestion that the S phase DNA damage checkpoint does not actually stop replication in order to complete DNA repair, but instead slows replication if damage has occurred<sup>42</sup>. In mammalian cells four proteins have been shown to be involved in this checkpoint. These are, the protein kinase ATM, Nibrin (also known as NBS1), a novel DNA double strand break repair protein which is mutated in Nijmegen breakage syndrome (NBS), Mre11 which is mutated in an AT-like disorder and Rad50<sup>43</sup>. In the cell, NBS1, Mre11 and Rad50 are found in a complex together that can carry out ATP dependent DNA unwinding and hairpin cleavage, a process required for DNA repair<sup>44</sup>. A link between the checkpoint protein ATM and DNA repair is provided by Nibrin, which can be phosphorylated by ATM<sup>45</sup>.

Since exposure to ionizing radiation causes an increase in the levels of the p21<sup>CIP1</sup> and Cdk inhibition, it has been suggested that p21<sup>CIP1</sup> may also play a role in the S phase checkpoint. In support of this proposal, it has been shown that p21<sup>CIP1</sup> can slow down DNA replication by inhibiting Cdk activity<sup>46</sup>. On the other hand, in a cell line in which the p21<sup>CIP1</sup> gene, *CDKN1A* has been knocked out, the S phase DNA damage checkpoint is intact suggesting that p21<sup>CIP1</sup> is not essential for this function<sup>47</sup>. Finally, whether p21<sup>CIP1</sup> is required or not, in mouse cells it appears that the S phase DNA damage checkpoint is dependent on dephosphorylation of pRb to block S phase progression<sup>48</sup>.

## G2/M

The G2/M DNA damage checkpoint functions late in G2 and involves many of the players that participate in the G1/S checkpoint (Figure 3). However, their target this time is not Cdk2/cyclin E, but the Cdc2/cyclin B complex, which is required for progression from G2 into mitosis. Consequently, the main function of the checkpoint is to maintain Cdc2/cyclin B1 in an inactive state. A major route by which this is accomplished is by upholding inhibitory phosphorylation on the T14 and Y15 residues of Cdc2. This is achieved by blocking the function of the Cdc25C phosphatase, which dephosphorylates these sites on Cdc2. Again ATM plays a role, by mediating phosphorylation and activation of the Chk1 and Chk2 checkpoint kinases<sup>39,49</sup>. Both these kinases can phosphorylate Cdc25C at serine 216, which promotes its association with 14-3-3 proteins. This leads to sequestration of Cdc25C in the cytoplasm, where it cannot dephosphorylate the nuclear localised Cdc2/cyclin B. There is also evidence that p53 may play a role at this checkpoint to sustain the G2 arrest (Figure 3). Expression of p53 is upregulated at the G2 checkpoint leading to induction of p21<sup>CIP1</sup>, which can bind to and inhibit the activity of Cdc2/cyclin B in the nucleus<sup>50</sup>. Upregulation of p53 also induces expression of the 14-3-3σ protein<sup>51</sup>. This does not bind to Cdc25C, but instead sequesters CDC2/cyclin B in the cytoplasm away from its nuclear targets. Thus p53 provides a double insurance policy that Cdc2/cyclin B activity is strongly inhibited.

In terms of cancer, the relationship between many of these proteins and tumourigenesis has already been outlined. However, there are a few exceptions. Although a connection has been made between Chk2 and cancer<sup>40</sup>, the same is not true for the functionally related Chk1 kinase. At this stage though, it is difficult to determine whether this is because Chk1 mutations do not occur in tumours or whether they have yet to be discovered. In the same way, inactivating mutations of p21<sup>CIP1</sup> have not been identified in tumours. However, perhaps this is redundant, since its chief regulator, p53, is mutated in 50% of human tumours<sup>34</sup>. Interestingly, it has recently been shown that in

breast cancers the function of p21<sup>CIP1</sup> can be disrupted via phosphorylation that causes relocalization of p21<sup>CIP1</sup> from the nucleus to the cytoplasm<sup>52</sup>. The phosphorylation is carried out by the proto-oncogene Akt, found overexpressed in Her2/neu overexpressing breast cancers and provides a novel mechanism by which the function of p21 in a tumour can be removed without gene mutation.

## Mitosis

Mitosis is the time in a cell when newly replicated DNA (as condensed sister chromatids) is segregated so that the subsequent two daughter cells will have identical genomes. This is achieved through a highly organized series of events starting with chromosome condensation and culminating in cell division. A critical point occurs when the condensed sister chromatids become attached to the bipolar spindle emanating from the two centrosomes at opposite sides of the cell (metaphase). This attachment is via protein complexes on the chromatids called kinetochores. The two sets of identical sister chromatids are then pulled to opposite poles (anaphase), a nuclear envelope reform around each set of chromatids (telophase) ready for cell division (cytokinesis). If mis-segregation of the sister chromatids occurs, then the resulting daughter cells will have the incorrect number of chromosomes (aneuploidy), a phenotype commonly observed in cancer and believed to contribute to the malignant phenotype of the tumour<sup>53</sup>. Thus mitosis would be hypothesized to be an important target of genetic mutation in cancer and recent discoveries bear this out.

Phenotypic defects in the centrosomes, the organizing centres of the bipolar spindle, have been reported in many forms of cancer. One possible cause of this is the Aurora2/STK15/BTAK kinase, which is centrosome associated during interphase and both centrosome and spindle associated during mitosis<sup>54</sup>. The gene is overexpressed in colorectal carcinomas and maps to a region on chromosome 20 found frequently amplified in a number of tumour types. *Aurora2* can also act as an oncogene, transforming both Rat-1 and NIH3T3 fibroblasts *in vitro*. Its function remains unclear although antisense experiments have shown that depletion of *Aurora2* causes a mitotic arrest with an intact bipolar spindle but a defect in chromatid attachment<sup>54</sup>.

Chromatid attachment to the bipolar spindle is essential for correct mitosis and if defective, invokes the mitotic spindle checkpoint at the metaphase–anaphase transition. During normal mitosis, as the last chromatid becomes attached to the bipolar spindle, the APC E3 ligase becomes active<sup>55</sup>. This requires association with the Cdc20 regulatory subunit and phosphorylation by CDC2/cyclin B (ref. 56). Once active, APC/Cdc20 initiates degradation of securin, a protein associated with the mitotic protease, Separin. After degradation of Securin,

Separin is released and cleaves protein(s) involved in sister chromatid cohesion, thus allowing their separation<sup>55</sup>. Human Securin is identical to the product of the pituitary tumour-transforming gene (PTTG), which is overexpressed in some tumours and causes cellular transformation when overexpressed in NIH3T3 cells<sup>57</sup>. No direct link between separin and cancer has yet been identified.

If the chromatids do not become correctly attached to the bipolar spindle (due to DNA damage or a mitotic spindle inhibitor), the mitotic checkpoint will be invoked. Several homologues of proteins participating in the yeast mitotic spindle checkpoint have been identified in mammalian cells<sup>55</sup>. The first of these Mad1 and Mad2, bind to phosphorylated kinetochores on unattached chromatids. Mad2 then inhibits the APC/Cdc20 complex via association with Cdc20, thus blocking degradation of securin and subsequent sister chromatid separation<sup>55</sup>. Normally as the last kinetochore becomes attached, the recruitment of Mad2 to APC/Cdc20 ends and chromatid separation then occurs as the Cdc20/Mad2 interaction dwindles<sup>58</sup>. Three other players in the mammalian mitotic checkpoint are the two related protein kinases hBub1 and hBub1R, and a third protein Bub3. All three are attached to kinetochores at mitosis and probably act upstream of Mad1 and Mad2 in the mammalian mitotic checkpoint<sup>55</sup>. The importance of this checkpoint in maintaining genome integrity is emphasized by the fact that many of these proteins have links with cancer. The human T cell leukemia virus type 1 abrogates the mitotic spindle checkpoint by targeting Mad1 with the Tax oncoprotein<sup>59</sup>. Deletion of one allele of the *MAD2* gene in either human tumour cells or murine primary embryonic fibroblasts results in a defective mitotic checkpoint. Additionally, Mad2<sup>+/−</sup> mice develop lung tumours at high rates after long latencies, connecting defects in the mitotic checkpoint to tumorigenesis<sup>60</sup>. Two inactivating mutations of Bub1 have been found in colon cancer and shown to cause an abnormal mitotic checkpoint<sup>61</sup>. Mutations of the related kinase BubR1 have also been found in colorectal cancer although the functional consequences of these mutations are yet to be determined<sup>61</sup>.

Late mitosis does not escape attention. The Aurora1 kinase, related to Aurora2, is active late in mitosis during anaphase and possibly telophase<sup>54</sup>. Overexpression of a kinase dead form of Rat Aurora 1 (AIM1) in mink lung epithelial cells creates cells with more than one nuclei caused by a disruption in the cleavage furrow generated at cytokinesis. Human Aurora1 is found highly expressed in many tumour samples and was originally isolated in a PCR screen to identify kinases overexpressed in colon cancer<sup>54</sup>.

To conclude this section a mention should go to the polo-like kinase (Plks) family of conserved serine/threonine kinases<sup>62,63</sup>. This family of kinases function throughout mitosis, starting with activation of Cdc2/cyclin B by phosphorylation and activation of the Cdc25C phos-

phatase. Later they phosphorylate and activate the APC (which has been prephosphorylated by Cdc2/cyclin B), which leads to degradation of cyclin B and mitotic exit. There are also hints that these proteins are involved in cytokinesis<sup>62,63</sup>. In terms of cell cycle checkpoints and cancer, there have been several recent developments. Human Plk1 activity is inhibited in G2 in response to DNA damage<sup>64</sup>. This response requires ATM, and probably functions by inhibiting Plk1 so that it can no longer phosphorylate and activate Cdc25C. DNA damage also causes inhibition of Plk1 in mitosis. This is accompanied by a block in degradation of cyclin B1 and inhibition of mitotic exit, clearly showing that DNA damage does induce a mitotic checkpoint. In addition, antibodies to Plk induce a mitotic abnormality, which contributes to aneuploidy when micro-injected into cells and recently mutations of the *Plk* gene found in human tumour cell lines have been shown to decrease the stability of the protein<sup>65</sup>.

### Genomics, the cell cycle and cancer

It would be inappropriate to complete this review without reference to the recent publications on the first draft of the human genome sequence by the publicly funded international Human Genome Project (HGP) and the privately funded Celera Genomics company<sup>66,67</sup>. One of the most interesting facts to be revealed by both the publications is that the number of genes actually encoded by the human genome is probably a lot less than the one hundred thousand that had been predicted. The Celera project identified 26,500 with around another 12,000 candidates, whilst HGP estimated around 33,000. This of course raises the big question of whether environment therefore plays a bigger part than we anticipated in who we are. On the other hand, our genes are more complex than most eukaryotes, with more splicing variants. However, it is not within the scope of this review to discuss all the consequences of the human genome sequencing project but instead to give a perspective in relation to the cell cycle and cancer.

As mentioned earlier, many of the major discoveries in the cell cycle field have not come from research in mammalian cells, but from both biochemical and genetic studies in other organisms. So now that the human genome sequence is available, what benefits can it have in understanding this process? There are two obvious possibilities. The first is that new members of gene families involved in cell cycle regulation such as Cdks and cyclins, may be identified. The second is that genes found to only play a role in the cell division cycle of other organisms could have homologues in the human genome sequence. An excellent paper by Murray and Marks accompanying the HGP paper in *Nature*, reveals some of these possibilities<sup>68</sup>. Comparison of known cyclin genes with the human genome sequence lead to identification of a human homologue of the chicken cyclin *B3* gene and three novel cyclin

genes, cyclins M, O and P. It also revealed that there appears so far to be no human homologue of the budding yeast Cak1 protein, which phosphorylates the activating threonine on Cdc28, analogous to T161 of mammalian Cdc2. Thus, this initial analysis of the human genome sequence has not provided a leap forward in our understanding of the mammalian cell cycle, and it is evident that understanding the context of how these genes function is essential to continued advancement in this area.

What about cancer? Even from just looking at the role the cell cycle plays in cancer it is clear that in most cases this disease is not one derived from a single genetic mutation, but from alterations in a number of genes arising to give a cancer type. Until now, many of the tumour suppressor genes which play a role in cancer have been identified through the hard slog of mapping a genetic locus to a chromosome and then looking for candidate genes. Will sequencing of the human genome make the job easier? This issue is addressed in a paper by Futreal *et al.* also published along with the HGP paper in *Nature*<sup>69</sup>. Like Murray and Walker, they searched the human genome database, but this time for novel sequences related to known tumour suppressor genes. The idea was that if they identified such a sequence, then it may also be tumour suppressor gene. However none were identified. They also tested whether it was possible to identify gene rearrangements involved in cancer by comparing cancer genome sequences against the human genome sequence. It turned out that they found approximately the same number of such chimaeric sequences in both the normal and cancer sequences, suggesting a high rate of false positives. In conclusion, generation of the initial draft of the human genome sequence is just the first step along the path towards understanding how the whole genome contributes to cancer.

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