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REVIEW

CDC6: from DNA replication to cell cycle checkpoints and oncogenesis

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Cell division cycle 6 (*CDC6*) is an essential regulator of DNA replication in eukaryotic cells. Its best-characterized function is the assembly of prereplicative complexes at origins of replication during the G_1 phase of the cell division cycle. However, *CDC6* also plays important roles in the activation and maintenance of the checkpoint mechanisms that coordinate S phase and mitosis, and recent studies have unveiled its proto-oncogenic activity. *CDC6* overexpression interferes with the expression of INK4/ARF tumor suppressor genes through a mechanism involving the epigenetic modification of chromatin at the INK4/ARF locus. In addition, *CDC6* overexpression in primary cells may promote DNA hyperreplication and induce a senescence response similar to that caused by oncogene activation. These findings indicate that deregulation of *CDC6* expression in human cells poses a serious risk of carcinogenesis.

Introduction

The process of genome replication that takes place in every mitotic cell cycle has always been considered a topic of 'basic research'. However, defects in DNA replication can be linked to >40 human diseases, including many types of cancer and probably underlie the process of ageing (1). DNA replication proteins serve as diagnosis markers of several neoplastic conditions and are the target of widely used antiviral and anticancer drugs. It is time that the connection between DNA replication and human disease is emphasized and its translational aspects explored in more detail.

The main concept behind the control of DNA replication is the classic 'replicon hypothesis': specific DNA sequences serve as 'origins of replication' and are activated by soluble factors called 'initiators'. A 'replicon' is the stretch of DNA duplicated from a single origin. In this manner, the replication process can be regulated by the frequency of initiation events (2). The genomes of many viruses, bacteria and Archaea are duplicated in a single replicon, whereas eukaryotic cells contain multiple origins of replication that, saving rare exceptions, are not defined by specific DNA sequences but rather by structural chromatin contexts that remain poorly understood. The overall speed of genome duplication is regulated at different developmental stages by the abundance of initiator proteins and the number of active origins. In addition, DNA replication is carefully coordinated with cell division in order to maintain genomic integrity. Under challenging circumstances, cells use 'checkpoint' mechanisms to slow down or arrest DNA replication and also to prevent mitosis when their DNA has been underreplicated, overreplicated or is damaged beyond possible repair. The regulation of eukaryotic DNA replication and its connection to the cellular checkpoints have been extensively reviewed in recent years (3–8).

In this review, we concentrate on the cellular functions and oncogenic properties of initiator protein cell division cycle 6 (*CDC6*). First, we discuss its biochemical roles in the activation of replication origins and the coordination of S phase and mitosis. Then, we address the effects of *CDC6* overexpression in mammalian cells, recapitulate the known examples of *CDC6* deregulation in human cancer and consider different mechanisms that could explain its oncogenicity.

Abbreviations: CDC6, cell division cycle 6; CDK, cyclin-dependent kinase; DDR, DNA damage response; MCM, minichromosome maintenance; ORC, origin recognition complex; pre-RC, pre-replication complex.

Lessons from the model systems: CDC6 is an essential DNA replication factor

CDC6 was originally identified in a genetic screen aimed at finding mutations that arrested the budding yeast cell cycle (9). In the pre-genomics era, it took a long time to clone the corresponding gene (10,11) and to identify its homologs in fission yeast (12) and human cells (13). Actually, *CDC6* is conserved in every eukaryotic organism, and *CDC6*-related genes are found in Archaea (14). The human *CDC6* gene is located at chromosome 17q21.3 and its expression is controlled by the E2F/retinoblastoma transcription factors that regulate S-phase-promoting genes (15–17).

The first evidence for the participation of *CDC6* in DNA replication stems from the analysis of *Cdc6-1*, a temperature-sensitive yeast *CDC6* mutant that arrested cells at the G_1–S transition (18). *CDC6* mutants displayed defects in plasmid maintenance that could be overcome by the addition of extra origins of replication (19). The expression of *CDC6* at the end of mitosis suggested a role of the protein during G_1 (20,21), which was confirmed using a 'conditional knockout (KO)' yeast strain. Without *CDC6*, cells rapidly accumulated with a 1C DNA content and could not initiate DNA replication. Remarkably, *CDC6*-null cells still proceeded to mitosis, indicating a failure in the checkpoint mechanism that prevents cell division in the absence of replication [(20); discussed below].

In the early 1990s, a yeast origin of replication was used as a bait to isolate the six-subunit 'origin recognition complex' [ORC; (22)]. It was quickly noted that ORC associated with other proteins in larger structures called pre-replication complexes (pre-RCs), which could be visualized by genomic footprinting at origins of replication during G_1 (23). The main biochemical achievement during pre-RC formation (also referred to as 'origin licensing') is the assembly of the helicase machinery required to separate the two DNA strands during S phase. In eukaryotic cells, this function resides in a family of proteins called minichromosome maintenance (MCM), six of that are essential for both initiation and elongation of DNA replication (24).

CDC6 is a key factor during origin licensing, because pre-RCs could not be detected in the conditional KO strain without *CDC6* (25). Indeed, three convergent studies established that *CDC6* was responsible for the loading of MCM proteins onto origins of replication. One of them used a biochemical fractionation protocol to prove that MCM proteins could not associate with the chromatin in the absence of *CDC6* (26). The second one analyzed the binding of MCM proteins specifically at origins of replication, confirming the need for *CDC6* and also showing that cyclin-dependent kinase (CDK) activity could inhibit MCM loading (27). The third study added a genetic twist to the plot, identifying 'gain-of-function' *CDC6* mutations that increased the loading of MCMs and caused persistent initiation of DNA replication (28).

Shortly after its identification, it was confirmed that human CDC6 was also essential for initiation of DNA replication. When hCDC6 levels were downregulated in G_1 cells by antibody microinjection, cells could not progress into S phase (15,17). Later on, it was found that CDC6 downregulation by RNA interference (RNAi) prevented cell proliferation and promoted apoptosis (29,30).

Coordinated ATP hydrolysis by CDC6 and ORC promotes the loading of the MCM helicase onto the DNA

CDC6 belongs to the AAA^+ family of ATPases with chaperone-like activities related to the assembly, activity and disassembly of protein

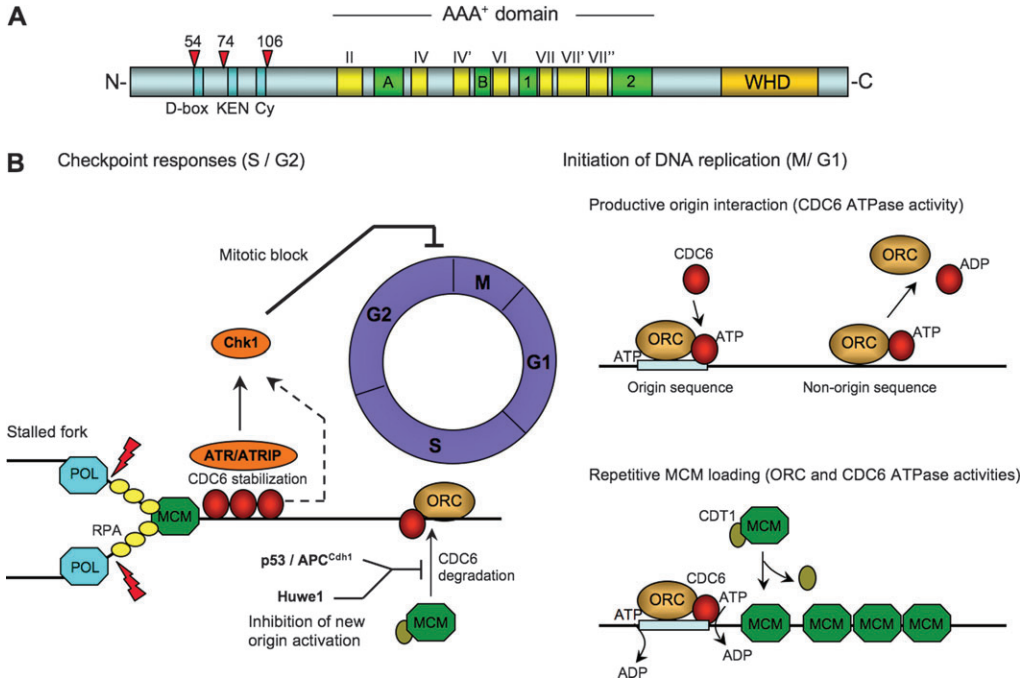

Fig. 1. Functions of CDC6 in DNA replication and checkpoint activation. (**A**) Main conserved motifs in human CDC6 protein. The positions of the three serine residues phosphorylated by CDKs are indicated with red arrows. D-box and KEN are 'protein degradation' motifs. Cy, cyclin-binding box. The different conserved AAA$^+$ boxes are indicated in yellow, except Walker A, Walker B, Sensor 1 and Sensor 2 that are indicated in green. WHD, winged-helix fold domain. (**B**) Schematic of the potential functions of CDC6 at different stages of the cell division cycle. This cartoon includes CDC6 functions derived from different experimental systems. Not all of them may apply to human CDC6. See text for details.

complexes [see Figure 1A; (31)]. In yeast, mutations that impair the ATPase activity of CDC6 are hypomorphic, lethal and even dominant negative (32–35). At least two functions of CDC6 rely on its ATPase activity (Figure 1B). The first one, which so far has only been demonstrated in yeast, relates to the ability of CDC6 to influence the specificity with which ORC associates with the DNA (36). In the presence of ORC and non-origin DNA, CDC6 rapidly hydrolyses ATP and promotes the dissociation of both proteins from the DNA. In contrast, the interaction with specific origin sequences delays ATP hydrolysis, stabilizing the interaction with the DNA until MCM proteins are loaded (37). Human origins of replication are more complex than their yeast counterparts and it remains to be tested whether hCDC6 influences the choice of hORC-binding sites. Interestingly, the targeting of hCDC6 to a plasmid is sufficient to create an 'artificial' origin of replication, and this capacity depends on an intact ATP-binding site (38).

The second function requiring CDC6 ATPase is the actual loading of MCM proteins. In the current model of pre-RC formation (Figure 1B), the hexameric ring formed by MCM proteins, along with their associated factor CDT1, is attracted to the origin by ORC and CDC6, without becoming immediately engaged with the DNA. ATP hydrolysis by CDC6, possibly induced by the interaction with MCM–CDT1, is coupled to the topological association of the MCM ring with the DNA, while CDT1 is released. ORC is also an ATPase whose activity is not required for the loading of the first MCM complex but becomes essential for the repeated loading of additional MCMs. This suggests that CDC6-mediated ATP hydrolysis regulates the effective engagement of MCM with the DNA, whereas ORC-mediated ATP hydrolysis is coupled to the release of each loaded MCM unit from the ORC–CDC6 'loading machine' (39,40). The loading of several MCMs at a single origin is not surprising, as they are found on the chromatin in large excess relative to other initiator proteins (41). The excess MCM complexes are believed to activate 'dormant' origins of replication under conditions of replicative stress (42).

Human CDC6 protein is also capable of ATP binding and hydrolysis, and both can be abolished by mutations in the Walker A/B motifs (43). CDC6 protein carrying a Walker A mutation that impairs ATP binding prevented S phase when it was microinjected in G_1 cells, whereas a Walker B mutation that allows ATP binding but impairs hydrolysis allowed the G_1–S transition but impaired S-phase progression (43). An independent study revealed that a mutation in the ATP-binding site reduced the ability of CDC6 to cooperate with Cyclin E in the induction of DNA replication in quiescent, serum-starved cells. This effect was linked to a defective loading of MCM proteins onto the chromatin (44). Therefore, human CDC6, as its yeast counterpart, functions as an ATP-dependent DNA helicase loader prior to DNA replication.

An interesting structural parallelism has been drawn between CDC6 and 'clamp loaders' such as replication factor C or the γ-complex of *Escherichia coli* DNA polymerase III (32), multisubunit complexes that use the energy derived from ATP hydrolysis to engage ring-shaped molecules such as proliferating cell nuclear antigen (PCNA) or the *E.coli* β-dimer with the DNA (45,46). Similarly, the loading of MCM proteins by CDC6 and ORC could be achieved by a series of conformational changes, coupled to ATP binding and hydrolysis, which open and close the MCM ring around the DNA. Indirect support for this hypothesis comes from the three-dimensional map of yeast ORC in complex with CDC6, generated from electron microscopy images (47). The overall shape and dimensions of the ORC–CDC6 complex match the structure of a ring-shaped archaeal MCM complex (48). Additional structures of ORC, CDC6 and MCM, ideally in complex with each other, will be required to construct a structural model for the DNA helicase loading reaction.

The only CDC6 high-resolution structure available so far is that of an archaeal homolog (49). This 2 Å crystallographic structure has revealed at atomic detail the characteristics of the ATP-binding domain and has unraveled another interesting structural motif, the winged-helix domain that is present in several DNA-binding proteins, including ORC1, the largest subunit of ORC. Mutations in the winged-helix domain of fission yeast CDC18 (the CDC6 homolog) impaired the function of the protein *in vivo* (49).

Regulation of CDC6 protein in the cell cycle

In the budding and fission yeasts, the 'execution point' of CDC6 goes from late mitosis until late G_1, before the activation of the S-phase-promoting CDKs. In both cases, CDKs phosphorylate CDC6/CDC18, targeting it for ubiquitination and degradation by the proteasome

shortly after the formation of pre-RCs (50–54). This regulation may be important to avoid origin reactivation within the same cell cycle, and at least in *Schizosaccharomyces pombe* the ectopic expression of CDC18 causes DNA rereplication and hyperploidy (55). In contrast, CDC6 deregulation does not lead to origin reactivation in *Saccharomyces cerevisiae*, due to overlapping mechanisms that involve the inactivation of additional initiator proteins (56).

In rapidly proliferating cells, human CDC6 is also destabilized in G_1 by the action of the ubiquitin ligase APC^{Cdh1} and the proteasome (57,58), but its steady-state levels increase again before S phase and remain constant until mitosis (13). Several studies that made use of epitope-tagged versions of CDC6 converged on the idea that hCDC6 is translocated from the nucleus to the cytosol at the G_1-S transition, thereby reducing the risk of origin reactivation (59–61). A dissenting note came from other reports that indicated that a fraction of endogenous CDC6 was nuclear and remained associated with the chromatin at all stages of the cell cycle (57,62,63). In a more recent attempt to clarify this issue, it was found that endogenous CDC6 is indeed nuclear throughout the cycle, whereas overexpressed CDC6 may suffer the nuclear–cytosolic translocation (64). This interesting observation cleared the way to understand the participation of CDC6 in additional cellular functions, including checkpoint mechanisms and mitotic entry (see below).

Although mammalian cells do not eliminate CDC6 in S phase and G_2, it is worth noting that alternative mechanisms have evolved to prevent the untimely formation of pre-RCs. One such mechanism involves ORC1, the largest subunit of the ORC complex, which is inactivated after the G_1–S transition either by polyubiquitination by SCF^{Skp2} and destruction by the proteasome (65) or by monoubiquitination and dissociation from the chromatin (66). The 'switched regulation' between ORC1 and CDC6 in yeast and mammalian cells may seem surprising, but both proteins are highly related and probably share a common ancestor. Indeed, some Archaeal organisms have a unique *ORC1/CDC6* gene that plays the functions of both (14). Cells also prevent DNA rereplication by exerting a tight control over CDT1, which is essential for MCM loading. CDT1 levels are controlled by proteolytic regulation mediated by SCF^{Skp2} or the $Cul4\text{-}DDB1^{Cdt2}$ ubiquitin ligases. In addition, CDT1 activity is controlled by an inhibitor protein called geminin (67).

Coupling DNA replication and mitosis: CDC6 and the S-M checkpoint

Checkpoints are signaling pathways that ensure the fidelity of cell division and prevent cell-cycle progression when DNA integrity is compromised. The S–M checkpoint is triggered by the accumulation of stalled replication forks caused by replicative stress, e.g. under conditions of low deoxynucleotide concentration or in the presence of an inhibitor of DNA polymerases. It involves a multipurpose response that stabilizes stalled forks, slows down the progression of ongoing forks, prevents the firing of late origins and inhibits mitotic division. These responses are elicited through the orchestrated action of multiple sensors, transducers or effectors. For instance, the mitotic block is achieved by the activation of Ataxia-Telangiectasia-mutated (ATM)/Ataxia-Telangiectasia-mutated-related (ATR) kinases, which phosphorylate and activate Chk2/Chk1 kinases and these in turn inactivate CDC25 phosphatase, an essential activator of mitotic kinase CDK1.

Strikingly, both *S.cerevisiae* and *S.pombe CDC6*-null cells undergo mitosis after having failed to replicate their DNA (12,20). The nature of the signal sent by CDC6 to prevent premature entry into mitosis is independent from the origin-activating role, as a CDC6 mutant that did not support DNA replication still blocked mitosis (34). Mitotic inhibition by this mutant did not rely on the checkpoint kinase Rad53 (the Chk2 homolog), but it rather involved the inhibition of mitotic CDK through a direct interaction with CDC6 N-terminal domain (68). Therefore, the functions of CDC6 during G_1 include an inhibitory action over CDK activity that may facilitate pre-RC formation and prevent mitosis at the same time.

The human cell division cycle is more complex but the potential of human CDC6 to restrain mitosis seems conserved, at least when the protein is overexpressed in G_2 cells (69). In this case the mechanism involves the activation of checkpoint kinase Chk1, because the mitotic block could be overridden by UCN-01 (a chemical inhibitor of Chk1) or by overexpression of the CDC25 phosphatase. Intriguingly, the mitotic block was maintained in the presence of caffeine, an ATM/ATR inhibitor, suggesting a direct activation of Chk1 by CDC6 (69). In another study, RNAi-mediated downregulation of CDC6 in cells synchronized in S phase resulted in inefficient DNA replication and prevented firing of new origins. Despite this fact, CDC6-depleted cells did not activate the ATR–Chk1 checkpoint and progressed into mitosis, causing aberrant chromosomal segregation and increasing the frequency of apoptosis (30). This result strongly argues that CDC6 plays a role in the S–M checkpoint in human cells.

Recent studies have provided insights about the mechanism of checkpoint activation by CDC6. During chromosomal replication in *Xenopus* cell-free extracts, the affinity of CDC6 for the chromatin initially drops after the loading of MCM proteins but it increases again when replication forks are established. The pool of chromatin-associated CDC6 is required to activate Chk1 in the presence of stalled replication forks. In this case, ATM–ATR checkpoint kinases are also involved (70). Another study done in *S.pombe* brings us one step closer to an actual mechanism. It was known that CDC18/CDC6 was required to activate the checkpoint kinase Cds1 (Chk2/Rad53) and maintain the block over mitosis in S-phase-arrested cells (71). Now it has been found that during an S-phase arrest, CDC18/CDC6 is stabilized on the chromatin and serves as a receptor for the Rad3–Rad26 complex, the homologs of mammalian ATR and ATR-interacting protein (ATRIP) [Figure 1B; (72)]. Rad3 and Rad26 are essential to maintain the block on mitosis for the duration of the arrest. Whether this ATR–interacting protein (ATRIP) 'anchoring' function is conserved in human CDC6 remains to be tested, but it is interesting to note that ATRIP interacts with MCM7 (73). It could be speculated that chromatin-bound CDC6 attracts MCM7, which in turn recruits ATR–ATRIP to initiate the checkpoint signaling in S-phase-arrested human cells.

The evidence outlined above indicates that CDC6 stabilization mediates the activation of the checkpoint response that prevents mitosis before DNA replication is complete. The fact that the CDKs that drive the main cell-cycle transitions are inactivated by the same checkpoint creates a potentially dangerous situation because an excess of CDC6 in the absence of CDK activity could result in origin hyperactivation and DNA overreplication. Actually, in some instances CDC6 protein is downregulated by the cellular responses to DNA damage, probably to prevent further activation of replication origins. CDC6 stability relies on the phosphorylation of three N-terminal serine residues by CDKs, which protects it from ubiquitination and degradation (74,75). Therefore, checkpoint responses that inhibit CDK activity are probably to destabilize CDC6. Indeed, CDC6 levels are reduced after ionizing radiation by a cellular response that involves p53 (74). Actually, the increase in the number of replicating cells observed after p53 knockdown is reversed by the simultaneous downregulation of CDC6 (74). Whether a non-degradable form of CDC6 would be sufficient to override the G_1–S arrest induced by p53 after DNA damage remains to be tested. p53-independent targeted destruction of CDC6 has also been found after treatment of the cells with adozelesin (76), methyl methane sulfonate or UV irradiation (77). Degradation of CDC6 by these alternate pathways involves CDC6 ubiquitination by Huwe1, which adds another layer of control on CDC6 protein levels when APC^{Cdh1} is not active (77).

Therefore, in a situation of cellular stress, CDC6 is at the crossroads of two seemingly opposing pathways, one that stabilizes it in order to activate the S-M checkpoint signaling and another one that destabilizes it in order to avoid further initiation events (Figure 1B). Several possible solutions to this intriguing paradox can be considered. First, CDC6 may be regulated differently depending on the extent of DNA damage. In the context of stalled replication forks caused by inhibitors of DNA replication, cells could stabilize CDC6 to induce the mitotic block, whereas in the context of extensive DNA damage cells would opt to degrade it to prevent origin activation, while relying on alternative pathways (e.g. ATM–Chk2) to inhibit mitosis. A second

possibility is that both responses follow a temporal program: upon an S-phase arrest, CDC6 could be stabilized for a short period of time, sufficient to activate the S-M checkpoint, and be targeted for degradation immediately afterward. A third model is that different cellular fractions of CDC6 are regulated separately. The fraction of CDC6 located at origins of replication, possibly labeled by specific post-translational modifications, would be targeted for degradation, whereas the rest of CDC6 would remain tightly associated with the chromatin to facilitate the S-M checkpoint response. A comprehensive analysis of the posttranslational modifications of CDC6 and its stability in different cellular contexts may be needed to clarify this issue.

CDC6 in human cancer

Considering the functions of *CDC6* in DNA replication and S-M coordination, its deregulation is expected to have a negative impact in genomic integrity. Actually, certain 'oncogenic features' can be inferred from experiments carried out in tissue culture cells, i.e. the accelerated G_1–S transition observed in G_1 nuclei incubated with S-phase extracts supplemented with CDC6 (78), the cooperation of CDC6 with Cyclin E to induce DNA replication in quiescent cells (44) or the DNA overreplication observed in tumor cells upon ectopic expression of CDC6 and CDT1 (79). Only in special cases, CDC6 overexpression and stabilization may serve a physiological function, such as the polyploidization of megakaryoblastic cells (80).

Because the retinoblastoma–E2F transcriptional pathway is frequently deregulated during cell transformation, genes like *CDC6* and *MCM2-7* are prone to be overexpressed in cancer cells. The levels of the corresponding proteins may be kept close to physiological levels by the action of their normal regulatory mechanisms, but in same cases they reach abnormally high concentrations. Hence, high levels of CDC6 protein have been reported in 55% of brain tumors in a study that included tumors of neuroepithelial tissue, vestibular schwannomas, meningiomas and pituitary adenomas (81). CDC6 is also overexpressed in ∼50% of non-small cell lung carcinomas, the most common lung malignancy (82), and in a subset of mantle cell lymphomas (83). Interestingly, high levels of CDC6 do not necessarily correlate with increased proliferation within a tumor sample. In the case of non-small cell lung carcinomas, no direct correlation was observed between the levels of CDC6 and proliferation marker Ki67 (82). Another intriguing observation is that CDC6 is downregulated in aggressive prostate cancer (84). This could be an *in vivo* example of a CDC6 loss-of-function situation leading to aberrant cell proliferation.

Because CDC6 and MCM proteins are normally absent in quiescent and differentiated cells, their immunohistochemical detection can be used for the early detection of malignancies. The crisp nuclear signals detected with CDC6 or MCM antibodies in premalignant lesions of the cervical squamous epithelium have opened the way for an efficient 'immunoenhanced' Pap smear test (85). Additional diagnostic applications based on CDC6 and MCM immunodetection are being developed, including population screenings of bladder, colorectal, anal, lung and oral cancers (86). In many cases, higher level of replication proteins correlates with poor prognosis (87).

Oncogenic activity of CDC6: the INK4/ARF link

The 50 kb INK4/ARF locus encodes three important tumor suppressor genes: $p16^{INK4a}$ and $p15^{INK4b}$, both activators of the retinoblastoma pathway, and ARF, an activator of p53. Inactivation of this locus is one of the most frequent events in human cancer (88). A recent study has revealed that CDC6 deregulation may cause the inactivation of the INK4/ARF locus (89). A short, conserved genomic element located upstream of the INK4b gene contains an active origin of replication, as confirmed by nascent strand polymerase chain reaction analysis and chromatin immunoprecipitation experiments with pre-RC components. Surprisingly, CDC6 overexpression in tissue culture cells blocked the expression of the INK4/ARF genes, as measured by reverse transcription–polymerase chain reaction, and led to a significant reduction in the levels of the corresponding proteins. Furthermore, CDC6 overexpression increased colony formation and cooperated with oncogenic Ras to transform mouse embryo fibroblasts. INK4/ARF repression was mediated by the recruitment of histone deacetylases HDAC1 and HDAC2 that modified the epigenetic signature of the locus, leading to its eventual 'heterochromatinization' (89). This mode of gene repression associated to a neighboring replication origin had an interesting precedent in the control of the yeast mating type locus, which involves the ORC-mediated recruitment of histone deacetylase silent mating type information regulation (SIR) (90,91).

Is the oncogenic potential of CDC6 exclusively mediated by its effect on the INK4/ARF locus? We believe that this is unlikely because CDC6 deregulation dramatically affects the cell cycle of model organisms in which these tumor suppressor genes are not conserved. Other mechanisms may apply, starting with the basic functions of CDC6 in DNA replication and S-phase/mitosis coordination.

Alternative mechanisms of CDC6-driven oncogenesis

The notion that aberrant DNA replication is a driving force of genomic instability is supported by several studies showing, both in yeast and mammalian cells, that the interference with pre-RC formation leads to inefficient S phase and increases aneuploidy and the frequency of gross chromosomal rearrangements (92–95). Therefore, the oncogenic properties of *CDC6* could also arise from the genomic instability associated to imperfect DNA replication.

DNA replication stress may be critical at the early stages of tumorigenesis (see Figure 2). It has been proposed that precancerous cells undergo a transient burst of proliferation in which they lose control over DNA replication, resulting in multiple firing of the same origins and inefficient fork progression (96). The high frequency of stalled or collapsed replication forks could activate the DNA damage response (DDR) and drive the cells into a senescence state (96,97). This cellular response, similar to the premature senescence caused by activation of oncogenes like H-RasV12 (98), could be a first defense mechanism to avoid proliferation and transformation. In fact, the active forms of ATM and Chk2 have been detected in a variety of human precancerous lesions (99,100). To explain how origin refiring could lead to activation of the DDR, it should be noted that DNA rereplication in *Xenopus* extracts results in the accumulation of multiple 'nascent DNA' fragments, probably caused by head-to-tail collision of forks operating on the same DNA template (101). If this phenomenon also occurred in somatic cells, it could be sufficient to activate the DDR.

Oncogene expression in tissue culture cells seems to activate *CDC6*, which could be involved in the abnormal DNA replication phase that ensues (96). At least in one study, CDC6 overexpression in human primary fibroblasts triggered the DDR and drove the cells into senescence (97). This observation is at odds with the report that CDC6 overexpression represses the INK4/ARF locus and promotes proliferation (89). The reason for these contrasting results is unclear, but an interesting possibility is that CDC6 overexpression induces a rapid repression of the INK4/ARF locus (89), facilitating a burst of cell proliferation that could eventually lead to the accumulation of DNA damage and entry into senescence (97). In this regard, it is worth noting that the repression of the INK4/ARF locus was observed as early as 72 h after CDC6 overexpression, whereas the senescence-associated β-GAL assays were performed after a 3-week selection for CDC6-expressing cells. A more trivial possibility is that different levels of CDC6 overexpression could tip the balance toward one outcome or the other.

The deregulation of other DNA replication proteins has been linked to cancer in animal models. Overexpression of MCM7 in the basal layer of the epidermis increased the incidence and prevalence of chemically induced papillomas and led to a higher frequency of malignant tumor conversion (102). In addition, a genetic screening designed to identify mouse genes implicated in chromosomal instability found a recessive mutation, Chaos3, which turned out to be a viable but hypomorphic mutant of MCM4. $Mcm4^{Chaos3/Chaos3}$ embryonic fibroblasts were highly susceptible to chromosome breaks at common fragile sites during replication stress induced by aphidicolin. This

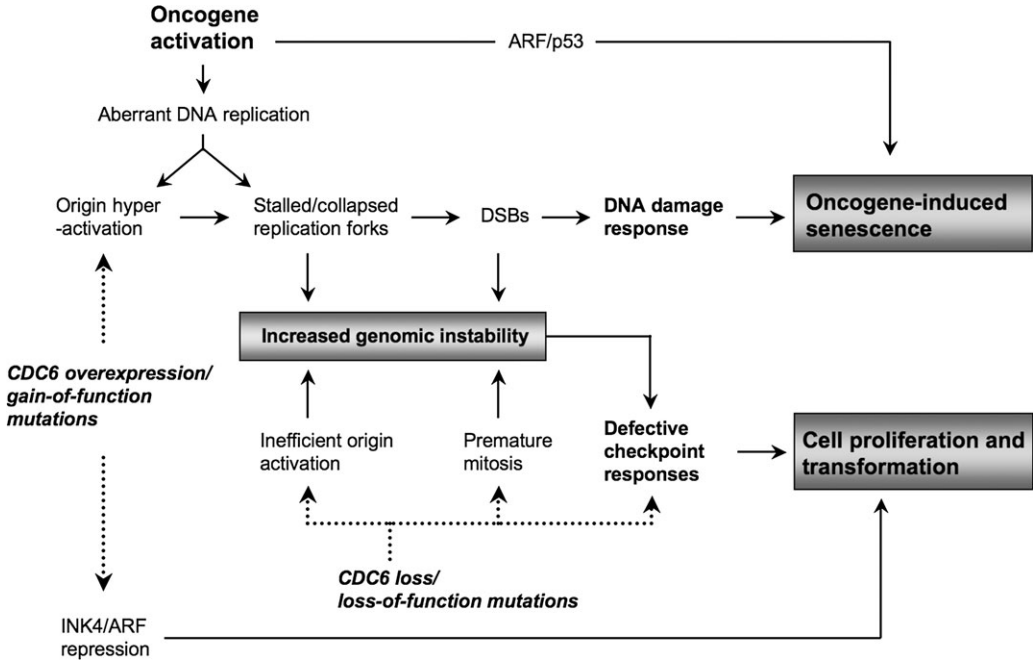

Fig. 2. Early cellular response to oncogene activation. The early cellular response consists in hyperproliferative burst characterized by aberrant DNA replication (96). The accumulation of DNA double strand breaks (DSBs) triggers the DDR but also increases genomic instability. Inactivation of the DDR upon a sustained oncogenic stimulus leads to cell transformation and proliferation. CDC6 deregulation has the potential to interfere at different levels in these pathways, as indicated with dashed arrows.

effect was accompanied by a very high incidence of spontaneous mammary adenocarcinomas in $Mcm4^{Chaos3/Chaos3}$ females (103). With these precedents, it will be very interesting to evaluate the effects of CDC6 overexpression *in vivo* (see 'Questions for the future' below).

The possible pathways of CDC6-mediated oncogenesis described above do not need to be exclusive. In CDC6-overexpressing non-small cell lung carcinomas, aberrant DNA replication is likely to occur because the MCM-associated factor CDT1 also appears overexpressed in the same set of tumors (82). On the other hand, a statistically significant inverse correlation between high levels of CDC6 and low levels of $p16^{INK4a}$ in these tumors suggests that the mechanism of INK4a/ARF repression by CDC6 is also in place (89).

Questions for the future

The recent discovery of *CDC6* oncogenic properties should prompt a systematic search for genetic alterations in *CDC6* (gene amplification, loss or mutation) in a wide range of tumor samples. It will be interesting to learn whether *CDC6* mutations that affect one specific protein function, such as origin activation, the ability to block mitosis or to repress the INK4/ARF locus are detected in human tumors. On the other hand, the different functions played by CDC6 during the cell division cycle suggest a very fine regulation that is probably achieved by multiple posttranslational modifications. The study of these modifications, and the proteins responsible for them, will be decisive for our complete understanding of CDC6 functions.

CDC6 oncogenic potential, clearly shown in tissue culture cells, ought to be confirmed in animal models. For this purpose, we have recently generated a mouse strain that allows for the inducible overexpression of CDC6 in the skin epithelia. Our preliminary results suggest that overexpression of CDC6 over a period of several weeks is sufficient to induce tissue hyperplasia (C.L.Sgarlata, L.R.Borlado and J.Méndez, unpublished results). The detailed characterization of this mouse model will certainly contribute to understand the molecular mechanisms underlying CDC6-mediated cell transformation.

Another relevant question stems from the ability of CDC6 to modulate the expression of genes located in the vicinity of origins of replication. If CDC6 overexpression is capable of attracting HDAC activities and modulate chromatin structure, why should the transcriptional effect be restricted to the INK4/ARF locus? This point has been partially addressed by examining the transcriptional effects on four other genes located close to origins of replication, and the preliminary conclusion is that the repressor effect is not universal (89). A more straightforward approach to this issue will require the analysis of genome-wide expression changes after CDC6 overexpression.

In summary, CDC6 is an essential DNA replication protein that also participates in the activation and maintenance of checkpoints and in some cases affects gene expression. After the remarkable progress of the last 20 years, a new student entering the field may wonder whether there are any mysteries left in CDC6. The answer can be learnt from the inspiring biography of Francis Crick (104), who 'as a small boy was haunted by a fear that by the time he grew up everything would have been discovered'. Luckily, his mother came up with a hopeful answer: 'Don't worry, ducky. There will be plenty left for you to find out'.

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References

1. DePamphilis,M.L. (2006) *DNA Replication and Human Disease*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

2. Jacob,F. *et al*. (1963) On the regulation of DNA synthesis in bacteria: the hypothesis of the replicon. *C. R. Hebd. Seances Acad. Sci.*, **256**, 298–300.
3. Branzei,D. *et al*. (2005) The DNA damage response during DNA replication. *Curr. Opin. Cell Biol.*, **17**, 568–575.
4. DePamphilis,M.L. *et al*. (2006) Regulating the licensing of DNA replication origins in metazoa. *Curr. Opin. Cell Biol.*, **18**, 231–239.
5. Mendez,J. *et al*. (2003) Perpetuating the double helix: molecular machines at eukaryotic DNA replication origins. *Bioessays*, **25**, 1158–1167.
6. Schwob,E. (2004) Flexibility and governance in eukaryotic DNA replication. *Curr. Opin. Microbiol.*, **7**, 680–690.
7. Sclafani,R.A. *et al*. (2007) Cell cycle regulation of DNA replication. *Annu. Rev. Genet*, **41**, 237–280.
8. Stillman,B. (2005) Origin recognition and the chromosome cycle. *FEBS Lett.*, **579**, 877–884.
9. Hartwell,L.H. (1976) Sequential function of gene products relative to DNA synthesis in the yeast cell cycle. *J. Mol. Biol.*, **104**, 803–817.
10. Lisziewicz,J. *et al*. (1988) Cloning and characterization of the *Saccharomyces cerevisiae* CDC6 gene. *Nucleic Acids Res.*, **16**, 11507–11520.
11. Zhou,C. *et al*. (1989) Molecular cloning of *Saccharomyces cerevisiae* CDC6 gene. Isolation, identification, and sequence analysis. *J. Biol. Chem.*, **264**, 9022–9029.
12. Kelly,T.J. *et al*. (1993) The fission yeast cdc18+ gene product couples S phase to START and mitosis. *Cell*, **74**, 371–382.
13. Williams,R.S. *et al*. (1997) A human protein related to yeast Cdc6p. *Proc. Natl Acad. Sci. USA*, **94**, 142–147.
14. Barry,E.R. *et al*. (2006) DNA replication in the archaea. *Microbiol. Mol. Biol. Rev.*, **70**, 876–887.
15. Hateboer,G. *et al*. (1998) Cell cycle-regulated expression of mammalian CDC6 is dependent on E2F. *Mol. Cell. Biol.*, **18**, 6679–6697.
16. Ohtani,K. *et al*. (1998) Regulation of cell growth-dependent expression of mammalian CDC6 gene by the cell cycle transcription factor E2F. *Oncogene*, **17**, 1777–1785.
17. Yan,Z. *et al*. (1998) Cdc6 is regulated by E2F and is essential for DNA replication in mammalian cells. *Proc. Natl Acad. Sci. USA*, **95**, 3603–3608.
18. Bueno,A. *et al*. (1992) Dual functions of CDC6: a yeast protein required for DNA replication also inhibits nuclear division. *EMBO J.*, **11**, 2167–2176.
19. Hogan,E. *et al*. (1992) Addition of extra origins of replication to a minichromosome suppresses its mitotic loss in cdc6 and cdc14 mutants of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **89**, 3098–3102.
20. Piatti,S. *et al*. (1995) Cdc6 is an unstable protein whose *de novo* synthesis in G1 is important for the onset of S phase and for preventing a 'reductional' anaphase in the budding yeast *Saccharomyces cerevisiae*. *EMBO J.*, **14**, 3788–3799.
21. Zwerschke,W. *et al*. (1994) The *Saccharomyces cerevisiae* CDC6 gene is transcribed at late mitosis and encodes a ATP/GTPase controlling S phase initiation. *J. Biol. Chem.*, **269**, 23351–23356.
22. Bell,S.P. *et al*. (1992) ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. *Nature*, **357**, 128–134.
23. Diffley,J.F. *et al*. (1994) Two steps in the assembly of complexes at yeast replication origins *in vivo*. *Cell*, **78**, 303–316.
24. Labib,K. *et al*. (2000) Uninterrupted MCM2-7 function required for DNA replication fork progression. *Science*, **288**, 1643–1647.
25. Cocker,J.H. *et al*. (1996) An essential role for the Cdc6 protein in forming the pre-replicative complexes of budding yeast. *Nature*, **379**, 180–182.
26. Donovan,S. *et al*. (1997) Cdc6p-dependent loading of Mcm proteins onto pre-replicative chromatin in budding yeast. *Proc. Natl Acad. Sci. USA*, **94**, 5611–5616.
27. Tanaka,T. *et al*. (1997) Loading of an Mcm protein onto DNA replication origins is regulated by Cdc6p and CDKs. *Cell*, **90**, 649–660.
28. Liang,C. *et al*. (1997) Persistent initiation of DNA replication and chromatin-bound MCM proteins during the cell cycle in cdc6 mutants. *Genes Dev.*, **11**, 3375–3386.
29. Feng,D. *et al*. (2003) Inhibiting the expression of DNA replication-initiation proteins induces apoptosis in human cancer cells. *Cancer Res.*, **63**, 7356–7364.
30. Lau,E. *et al*. (2006) The functional role of Cdc6 in S-G2/M in mammalian cells. *EMBO Rep.*, **7**, 425–430.
31. Neuwald,A.F. *et al*. (1999) AAA+: a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. *Genome Res.*, **9**, 27–43.
32. Perkins,G. *et al*. (1998) Nucleotide-dependent prereplicative complex assembly by Cdc6p, a homolog of eukaryotic and prokaryotic clamp-loaders. *Mol. Cell*, **2**, 23–32.
33. Wang,B. *et al*. (1999) The essential role of *Saccharomyces cerevisiae* CDC6 nucleotide-binding site in cell growth, DNA synthesis, and Orc1 association. *J. Biol. Chem.*, **274**, 8291–8298.
34. Weinreich,M. *et al*. (1999) The Cdc6p nucleotide-binding motif is required for loading mcm proteins onto chromatin. *Proc. Natl Acad. Sci. USA*, **96**, 441–446.
35. Schepers,A. *et al*. (2001) Mutational analysis of conserved sequence motifs in the budding yeast Cdc6 protein. *J. Mol. Biol.*, **308**, 597–608.
36. Mizushima,T. *et al*. (2000) Cdc6p modulates the structure and DNA binding activity of the origin recognition complex *in vitro*. *Genes Dev.*, **14**, 1631–1641.
37. Speck,C. *et al*. (2007) Cdc6 ATPase activity regulates ORC × Cdc6 stability and the selection of specific DNA sequences as origins of DNA replication. *J. Biol. Chem.*, **282**, 11705–11714.
38. Takeda,D.Y. *et al*. (2005) Recruitment of ORC or CDC6 to DNA is sufficient to create an artificial origin of replication in mammalian cells. *Genes Dev.*, **19**, 2827–2836.
39. Bowers,J.L. *et al*. (2004) ATP hydrolysis by ORC catalyzes reiterative Mcm2-7 assembly at a defined origin of replication. *Mol. Cell*, **16**, 967–978.
40. Randell,J.C. *et al*. (2006) Sequential ATP hydrolysis by Cdc6 and ORC directs loading of the Mcm2-7 helicase. *Mol. Cell*, **21**, 29–39.
41. Hyrien,O. *et al*. (2003) Paradoxes of eukaryotic DNA replication: MCM proteins and the random completion problem. *Bioessays*, **25**, 116–125.
42. Woodward,A.M. *et al*. (2006) Excess Mcm2-7 license dormant origins of replication that can be used under conditions of replicative stress. *J. Cell Biol.*, **173**, 673–683.
43. Herbig,U. *et al*. (1999) The Cdc6 nucleotide-binding site regulates its activity in DNA replication in human cells. *Mol. Biol. Cell*, **10**, 2631–2645.
44. Cook,J.G. *et al*. (2002) Analysis of Cdc6 function in the assembly of mammalian prereplication complexes. *Proc. Natl Acad. Sci. USA*, **99**, 1347–1352.
45. Davey,M.J. *et al*. (2002) Motors and switches: AAA+ machines within the replisome. *Nat. Rev. Mol. Cell Biol.*, **3**, 826–835.
46. Ellison,V. *et al*. (2001) Opening of the clamp: an intimate view of an ATP-driven biological machine. *Cell*, **106**, 655–660.
47. Speck,C. *et al*. (2005) ATPase-dependent cooperative binding of ORC and Cdc6 to origin DNA. *Nat. Struct. Mol. Biol.*, **12**, 965–971.
48. Fletcher,R.J. *et al*. (2003) The structure and function of MCM from archaeal *M. thermoautotrophicum*. *Nat. Struct. Biol.*, **10**, 160–167.
49. Liu,J. *et al*. (2000) Structure and function of Cdc6/Cdc18: implications for origin recognition and checkpoint control. *Mol. Cell*, **6**, 637–648.
50. Drury,L.S. *et al*. (1997) The Cdc4/34/53 pathway targets Cdc6p for proteolysis in budding yeast. *EMBO J.*, **16**, 5966–5976.
51. Drury,L.S. *et al*. (2000) The cyclin-dependent kinase Cdc28p regulates distinct modes of Cdc6p proteolysis during the budding yeast cell cycle. *Curr. Biol.*, **10**, 231–240.
52. Jallepalli,P.V. *et al*. (1997) Regulation of the replication initiator protein p65cdc18 by CDK phosphorylation. *Genes Dev.*, **11**, 2767–2779.
53. Jallepalli,P.V. *et al*. (1998) sud1(+) targets cyclin-dependent kinase-phosphorylated Cdc18 and Rum1 proteins for degradation and stops unwanted diploidization in fission yeast. *Proc. Natl Acad. Sci. USA*, **95**, 8159–8164.
54. Perkins,G. *et al*. (2001) Separate SCF(CDC4) recognition elements target Cdc6 for proteolysis in S phase and mitosis. *EMBO J.*, **20**, 4836–4845.
55. Nishitani,H. *et al*. (1995) p65cdc18 plays a major role controlling the initiation of DNA replication in fission yeast. *Cell*, **83**, 397–405.
56. Nguyen,V.Q. *et al*. (2001) Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. *Nature*, **411**, 1068–1073.
57. Mendez,J. *et al*. (2000) Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. *Mol. Cell. Biol.*, **20**, 8602–8612.
58. Petersen,B.O. *et al*. (2000) Cell cycle- and cell growth-regulated proteolysis of mammalian CDC6 is dependent on APC-CDH1. *Genes Dev.*, **14**, 2330–2343.
59. Jiang,W. *et al*. (1999) Multistep regulation of DNA replication by Cdk phosphorylation of HsCdc6. *Proc. Natl Acad. Sci. USA*, **96**, 6193–6198.
60. Petersen,B.O. *et al*. (1999) Phosphorylation of mammalian CDC6 by cyclin A/CDK2 regulates its subcellular localization. *EMBO J.*, **18**, 396–410.
61. Saha,P. *et al*. (1998) Human CDC6/Cdc18 associates with Orc1 and cyclin-cdk and is selectively eliminated from the nucleus at the onset of S phase. *Mol. Cell. Biol.*, **18**, 2758–2767.
62. Coverley,D. *et al*. (2000) Chromatin-bound Cdc6 persists in S and G2 phases in human cells, while soluble Cdc6 is destroyed in a cyclin A-cdk2 dependent process. *J. Cell Sci.*, **113**, 1929–1938.
63. Fujita,M. *et al*. (1999) Cell cycle regulation of human CDC6 protein. Intracellular localization, interaction with the human mcm complex, and

CDC2 kinase-mediated hyperphosphorylation. *J. Biol. Chem.*, **274**, 25927–25932.
64. Alexandrow,M.G. *et al*. (2004) Cdc6 chromatin affinity is unaffected by serine-54 phosphorylation, S-phase progression, and overexpression of cyclin A. *Mol. Cell. Biol.*, **24**, 1614–1627.
65. Mendez,J. *et al*. (2002) Human origin recognition complex large subunit is degraded by ubiquitin-mediated proteolysis after initiation of DNA replication. *Mol. Cell*, **9**, 481–491.
66. Li,C.J. *et al*. (2002) Mammalian Orc1 protein is selectively released from chromatin and ubiquitinated during the S-to-M transition in the cell division cycle. *Mol. Cell. Biol.*, **22**, 105–116.
67. Fujita,M. (2006) Cdt1 revisited: complex and tight regulation during the cell cycle and consequences of deregulation in mammalian cells. *Cell Div.*, **1**, 22.
68. Weinreich,M. *et al*. (2001) Binding of cyclin-dependent kinases to ORC and Cdc6p regulates the chromosome replication cycle. *Proc. Natl Acad. Sci. USA*, **98**, 11211–11217.
69. Clay-Farrace,L. *et al*. (2003) Human replication protein Cdc6 prevents mitosis through a checkpoint mechanism that implicates Chk1. *EMBO J.*, **22**, 704–712.
70. Oehlmann,M. *et al*. (2004) The role of Cdc6 in ensuring complete genome licensing and S phase checkpoint activation. *J. Cell Biol.*, **165**, 181–190.
71. Murakami,H. *et al*. (2002) Maintenance of replication forks and the S-phase checkpoint by Cdc18p and Orp1p. *Nat. Cell Biol.*, **4**, 384–388.
72. Hermand,D. *et al*. (2007) Cdc18 enforces long-term maintenance of the S phase checkpoint by anchoring the Rad3-Rad26 complex to chromatin. *Mol. Cell*, **26**, 553–563.
73. Cortez,D. *et al*. (2004) Minichromosome maintenance proteins are direct targets of the ATM and ATR checkpoint kinases. *Proc. Natl Acad. Sci. USA*, **101**, 10078–10083.
74. Duursma,A. *et al*. (2005) p53-Dependent regulation of Cdc6 protein stability controls cellular proliferation. *Mol. Cell. Biol.*, **25**, 6937–6947.
75. Mailand,N. *et al*. (2005) CDKs promote DNA replication origin licensing in human cells by protecting Cdc6 from APC/C-dependent proteolysis. *Cell*, **122**, 915–926.
76. Blanchard,F. *et al*. (2002) Targeted destruction of DNA replication protein Cdc6 by cell death pathways in mammals and yeast. *Mol. Biol. Cell*, **13**, 1536–1549.
77. Hall,J.R. *et al*. (2007) Cdc6 stability is regulated by the Huwe1 ubiquitin ligase after DNA damage. *Mol. Biol. Cell*, **18**, 3340–3350.
78. Stoeber,K. *et al*. (1998) Cdc6 protein causes premature entry into S phase in a mammalian cell-free system. *EMBO J.*, **17**, 7219–7229.
79. Vaziri,C. *et al*. (2003) A p53-dependent checkpoint pathway prevents rereplication. *Mol. Cell*, **11**, 997–1008.
80. Bermejo,R. *et al*. (2002) Regulation of CDC6, geminin, and CDT1 in human cells that undergo polyploidization. *Mol. Biol. Cell*, **13**, 3989–4000.
81. Ohta,S. *et al*. (2001) Cdc6 expression as a marker of proliferative activity in brain tumors. *Oncol. Rep.*, **8**, 1063–1066.
82. Karakaidos,P. *et al*. (2004) Overexpression of the replication licensing regulators hCdt1 and hCdc6 characterizes a subset of non-small-cell lung carcinomas: synergistic effect with mutant p53 on tumor growth and chromosomal instability—evidence of E2F-1 transcriptional control over hCdt1. *Am. J. Pathol.*, **165**, 1351–1365.
83. Pinyol,M. *et al*. (2006) Unbalanced expression of licensing DNA replication factors occurs in a subset of mantle cell lymphomas with genomic instability. *Int. J. Cancer*, **119**, 2768–2774.
84. Robles,L.D. *et al*. (2002) Down-regulation of Cdc6, a cell cycle regulatory gene, in prostate cancer. *J. Biol. Chem.*, **277**, 25431–25438.
85. Williams,G.H. *et al*. (1998) Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. *Proc. Natl Acad. Sci. USA*, **95**, 14932–14937.
86. Coleman,N. *et al*. (2006) *Cancer Diagnosis and DNA Replication. DNA Replication and Human Disease*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
87. Gonzalez,M.A. *et al*. (2005) Control of DNA replication and its potential clinical exploitation. *Nat. Rev. Cancer*, **5**, 135–141.
88. Kim,W.Y. *et al*. (2006) The regulation of INK4/ARF in cancer and aging. *Cell*, **127**, 265–275.
89. Gonzalez,S. *et al*. (2006) Oncogenic activity of Cdc6 through repression of the INK4/ARF locus. *Nature*, **440**, 702–706.
90. Gonzalez,S. *et al*. (2006) A new mechanism of inactivation of the INK4/ARF locus. *Cell Cycle*, **5**, 1382–1384.
91. Triolo,T. *et al*. (1996) Role of interactions between the origin recognition complex and SIR1 in transcriptional silencing. *Nature*, **381**, 251–253.
92. Ekholm-Reed,S. *et al*. (2004) Deregulation of cyclin E in human cells interferes with prereplication complex assembly. *J. Cell Biol.*, **165**, 789–800.
93. Lengronne,A. *et al*. (2002) The yeast CDK inhibitor Sic1 prevents genomic instability by promoting replication origin licensing in late G(1). *Mol. Cell*, **9**, 1067–1078.
94. Sidorova,J.M. *et al*. (2003) Precocious G1/S transitions and genomic instability: the origin connection. *Mutat. Res.*, **532**, 5–19.
95. Tanaka,S. *et al*. (2002) Deregulated G1-cyclin expression induces genomic instability by preventing efficient pre-RC formation. *Genes Dev.*, **16**, 2639–2649.
96. Di Micco,R. *et al*. (2006) Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature*, **444**, 638–642.
97. Bartkova,J. *et al*. (2006) Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature*, **444**, 633–637.
98. Serrano,M. *et al*. (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell*, **88**, 593–602.
99. Bartkova,J. *et al*. (2005) DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature*, **434**, 864–870.
100. Gorgoulis,V.G. *et al*. (2005) Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature*, **434**, 907–913.
101. Davidson,I.F. *et al*. (2006) Deregulated replication licensing causes DNA fragmentation consistent with head-to-tail fork collision. *Mol. Cell*, **24**, 433–443.
102. Honeycutt,K.A. *et al*. (2006) Deregulated minichromosomal maintenance protein MCM7 contributes to oncogene driven tumorigenesis. *Oncogene*, **25**, 4027–4032.
103. Shima,N. *et al*. (2007) A viable allele of Mcm4 causes chromosome instability and mammary adenocarcinomas in mice. *Nat. Genet.*, **39**, 93–98.
104. Ridley,M. (2006) *Francis Crick: Discoverer of the Genetic Code*. Harper-Collins Publishers, New York, NY.

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