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Cancer and the role of cell cycle checkpoints

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There exists an intricate network of proteins that continuously monitor each phase of the cell cycle to ensure proper replication. This network of proteins, termed checkpoints, first detects cellular abnormalities, and then coordinates their repair before the cell divides. The malfunction of these checkpoints often results in the proliferation of potentially damaged cells, and thus a tremendous susceptibility to cancer. This review will focus on the mechanisms by which checkpoints prevent the proliferation of damaged cells through each phase of the cell cycle, and how this understanding can provide novel targets for anticancer therapy.

The classic definition of cancer is "uncontrolled cell division." In a large, multi-cellular organism, uncontrolled cell division will soon result in large masses of rapidly growing cells (tumors), which cause significant damage to surrounding tissues. When tumors spread, they can damage vital organs and eventually cause death. In fact, cancer is currently the second leading cause of death in the United States, and thus cures for it would be of incalculable value. Current treatments of cancer involve exposing the patient to relatively nonspecific toxins, chemotherapy, in the hope that it will kill more cancer cells than normal cells. This type of medicine is a modern equivalent of 18th century bleeding treatments for bacterial infections. However, if clear biochemical differences between cancer cells and normal cell are discovered, chemotherapy could be improved considerably. Much as antibiotics only harm bacteria, novel anticancer drugs that only harm cancer cells can be developed through research. Since cancer is essentially the loss of cell division control, it seems prudent to search in these regulatory mechanisms for distinguishing characteristics of cancer cells. This review will present the general mechanisms which drive the cell cycle and what is currently known about the regulatory pathways that control it. It will then discuss how current anticancer therapies are taking advantage of cell cycle research.

The Cell Cycle

The Nobel Prize in Medicine or Physiology was recently awarded to three men, Leland Hartwell, Tim Hunt, and Paul Nurse, "for their discoveries of key regulators of the cell cycle" (www.nobel.se). Essentially every topic discussed in this review was in some way pioneered by these three men. The details they helped uncover may seem at first glance rather cumbersome, but it is these

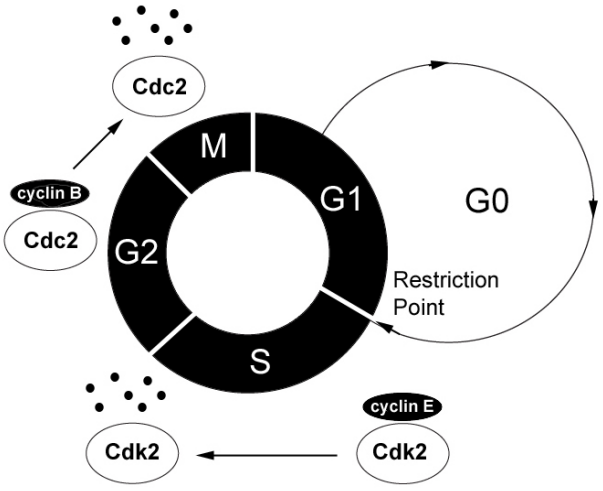

Figure 1. The Cell Cycle

The cell progresses through its division cycle, G1>S>G2>M, in the presence of growth signals and active CDK/cyclin complexes specific for each cell cycle stage. G0, a non-dividing but functional phase, occurs in the absence of growth signals. Finally, cyclin degradation is illustrated by the S-phase cyclin/Cdk complex (cyclin E/Cdk2) and the G2/M cyclin/Cdk complex (cyclin B/Cdc2), where each must be synthesized and degraded systematically for proper cell cycle progression into the next phase.

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very details that will eventually enable the development of targeted anticancer therapies.

Many of our cells can be triggered to divide upon the proper mitogenic stimulus (Cross and Dexter, 1991). A growth factor for example, can trigger a highly regulated unidirectional program that, when run successfully, results in the proper replication and division of the cell. This program, the cell cycle, must copy the parent cell's chromosomes and seal them in a safe daughter cell with all of the essential components needed for the daughter cell to function on its own. The human cell cycle accomplishes this in approximately 24 hours through four major phases: G1 (gap 1), S (DNA Synthesis), G2 (gap 2), and M (mitosis) (Lodish et al., 2000). Each phase serves a specific function to ensure proper cell division.

G1 – Gap 1

G1 takes about 9 hours to prepare the cell for DNA synthesis (S-phase) (Lodish et al., 2000). Though preparing the cell for S-phase may seem relatively uneventful, G1 is actually the most important regulatory phase in the cell cycle. It is in this phase that the cell decides whether to irreversibly continue the cell cycle through mitosis, or to enter G0, a quiescent phase during which the cell can function but not divide (Figure 1). This decision, called the restriction point in mammalian cells, is made in late G1 based predominantly on external growth signals (Lodish et al., 2000). Another key feature of late G1, which contributes to the unidirectionality of the cell cycle progression, is the priming of replication origins with MCM (minichromosome maintenance) proteins. The binding of these proteins to origins of replication is required for the initiation of DNA synthesis, but they can only bind to DNA in late G1 (Young and Tye, 1997). Thus, DNA synthesis is only initiated once – right at the G1/S transition.

S – DNA Synthesis

Once the cell has passed the restriction point, proteins synthesized in late G1 initiate the DNA replication machinery of S-phase. This delicate process of copying the parent cell's genome takes approximately 10 hours, and yields a single cell with two sets of each chromosome (sister chromatids) (Lodish et al., 2000).

G2 – Gap 2

G2 lasts about 4.5 hours and serves as a buffer to ensure the completion of DNA synthesis before the cell physically divides in mitosis (Lodish et al., 2000). Now with two sets of each chromosome, cell growth continues in order to double its size such that upon division two fully functional cells will result. A large amount of information is known about the G2/M transition, and it is discussed in detail below. This phase serves to synthesize proteins required for nuclear envelope breakdown, chromosome condensation, spindle formation, and other processes required for entry into mitosis (Maller et al., 1989). Many of these mitosis-promoting functions cannot be initiated until DNA synthesis is completed, thus serving as a buffer phase to prevent premature cell division.

M - Mitosis

Mitosis (nuclear division) is comprised of four substages during which specific events occur to separate daughter chromosomes from the parent's and enclose them in a new cell. Mitosis typically takes about 30 minutes in human cells, which is rather fast considering the complexity of this phase (Lodish et al., 2000). The first substage to occur is Prophase, in which the proteins synthesized in G2 break down the nuclear envelope of the parent cell, condense its chromosomes, and initiate spindle formation. Prometaphase follows as a transition period during which the sister chromatids shuffle until they align in the middle of the cell, which is then termed Metaphase. The sister chromatids then separate to opposite poles of the cell in Anaphase, which is followed its physical division in Telophase.

Cyclins and Cyclin-Dependent Kinases

The proper transition from each cell cycle phase to the next is dependent on two classes of proteins called cyclins and cyclin-dependent kinases. As the name suggests, cyclins are a class of proteins which are periodically synthesized and degraded, and the coordination of proper cyclin levels at the right time is essential for successful cell cycle progression. Cyclin-dependent kinases (Cdk) are a class of kinases whose catalytic activity is dependent on complexing with an appropriate cyclin. Once this complex is formed, the Cdk kinase activity is activated which results in the phosphorylation of many downstream effectors (reviewed in Udvardy, 1996). This phosphorylation serves to regulate the activity of the downstream effector, typically by activating or inhibiting it. One of the best studied cyclin/Cdk complexes involves cyclin B and Cdc2, also called MPF (Maturation Promoting Factor), and was first discovered by Yoshio Masui and Clement Market (Masui and Markert, 1971). It has been shown to serve many crucial roles in cell cycle progression such as nuclear envelope degradation and sister chromatid condensation in early mitosis (Maller et al., 1989) (Figure 1). These MPF-dependent processes are essential for the cell to efficiently divide the genetic information into daughter cells, and thus the activity of MPF is tightly regulated. If the cell allowed MPF to remain active through the latter stages of mitosis when the nuclear

envelopes are reforming, cell division would be prevented altogether. The way the cell deals with this problem is by ubiquitin-mediated proteolysis of cyclin B in late mitosis (Murray et al., 1989). Thus, the actively regulated levels of cyclin B mediate mitotic entry and exit. Without the synthesis of cyclin B prior to the G2/M transition, the kinase activity of Cdc2 remains inactive, and the cell can not enter mitosis. Without the subsequent degradation of cyclin B, the kinase activity of Cdc2 remains active and prevents the exit from mitosis.

Cell Cycle Control: Checkpoints

A cell cycle checkpoint is a general term used to describe a cellular process that stops or slows the cell cycle in conditions unfavorable for cell division (Hartwell and Weinert, 1989). This review will focus on the DNA damage cell cycle checkpoint. Since our cells undergo continuous bombardment by DNA damaging agents such as UV light and by-products of cellular metabolism, there exists an elaborate and evolutionarily conserved cellular DNA damage response that coordinates cell cycle progression with the repair of potentially mutagenic DNA damage (reviewed in Zhou and Elledge, 2000). Many of the proteins involved in the mammalian DNA damage response act as tumor suppressors and suggest that there are newly evolved repair and/or checkpoint genes critical in the maintenance of genome integrity, which highlights an additional importance of checkpoint control in mammals. The DNA damage response, like most cellular signaling pathways, involves first sensing a signal and then transducing it to downstream effectors that elicit the appropriate response. This review will present the recent studies, including some previously unreviewed, which have provided tremendous insight into many key components of this signal transduction pathway (outlined in Table 1).

Sensors

It is still unclear precisely how the cell senses damaged DNA, but a group of four fission yeast checkpoint proteins, Rad1, Rad9, Hus1, and Rad17, have been implicated along with their respective homologues in other organisms (reviewed in Lowndes and Murguia, 2000). It has been proposed that Rad1, Rad9, and Hus1 form a trimeric checkpoint-sliding clamp (CSC) similar in structure to the DNA polymerase clamp PCNA (Proliferating Cell Nuclear Antigen) (Venclovas and Thelen , 2000). By analogy to the loading of PCNA onto DNA by the RFC_{1-5} pentamer, the CSC is loaded onto DNA by $Rad17/RFC_{2-5}$, where Rad17 is a checkpoint protein that replaces RFC_1 in

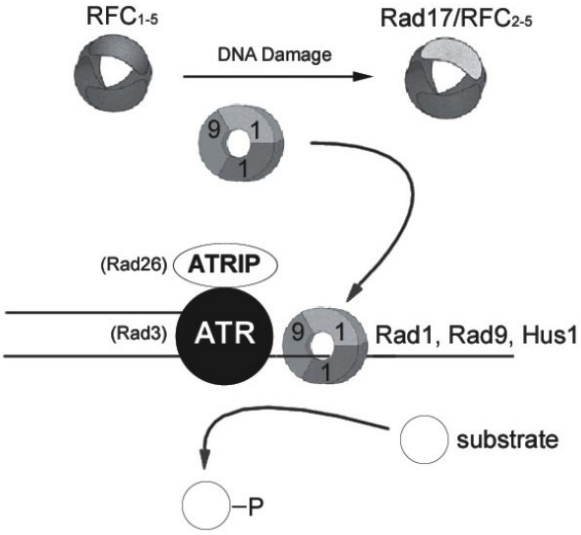

Figure 2. Substrate recruitment model for sensing DNA damage

Both ATRIP (Rad26) and the checkpoint sliding complex (CSC) Rad1, Rad9, Hus1, bind to the same sites of DNA damage. The CSC is loaded onto chromatin after DNA damage in a $Rad17/RFC_{2-5}$ catalyzed reaction. Once chromatin bound, the CSC recruits substrates of the ATR (Rad3) signal transducer kinase to propagate the DNA damage response.

the pentamer (Venclovas and Thelen , 2000). Once the CSC is loaded onto DNA, it could serve not only to recruit DNA polymerase but also to signal the activation of downstream DNA damage checkpoint proteins. In addition to the CSC and Rad17, the checkpoint protein Rad26 has also been implicated as a DNA damage sensor because it binds to and is phosphorylated by the Rad3 kinase independently of all other checkpoint proteins (Edwards et al., 1999). Thus, Rad26 has been proposed to be a sensor or at least far upstream in the DNA damage response. A recent model, the substrate recruitment model (Melo et al., 2001), provides some insight into the behavior of the putative DNA damage sensors Rad26/Rad3 and CSC/Rad17 in budding yeast (Figure 2). The model proposes that each complex is independently recruited to the same sites of DNA damage, and work in tandem for proper DNA damage checkpoint activation (Kondo et al., 2001; Melo et al., 2001). After the budding yeast CSC homologue is loaded onto DNA near sites of damage, it recruits various substrates of its Rad3 homologue to drive the signal transduction pathway of the DNA damage response. Though this model seems quite convincing in budding yeast, little is known about DNA damage sensing in mammalian cells (Melo et al., 2001). Recently, it was shown that the activation of both G1/S and G2 DNA damage checkpoints requires the phosphorylation of hRad17 by ATR (a Rad3 related kinase in mammals) and possibly ATM as well (Bao et al., 2001;

Post et al., 2001). However, unphosphorylated hRad17 can still load hRad9 of the CSC onto chromatin (Zou et al., 2002), so the significance of this phosphorylation remains unclear. Also, a recently cloned human protein, ATRIP, has some homology to the putative DNA damage sensor Rad26 (Cortez et al., 2001). ATRIP also seems to have many functional similarities to Rad26 including its tight association with and its phosphorylation by ATR (Rad3 related kinase), and a role in the G2/M checkpoint. Thus, human homologues of both of the putative DNA damage sensors in yeast seem to play similar roles in the human DNA damage response.

Transducers

Once DNA damage is sensed, the cell must transduce this signal down to its appropriate effector. In human cells, the activation of two kinases is essential for the proper transduction of DNA damage: ATM (Ataxia Telangiectasia Mutated) and ATR (ATM and Rad3 Related) (reviewed in Elledge, 1996; Zhou and Elledge, 2000). Little is known about precisely how ATM and ATR are activated, but a great deal has been uncovered about their respective roles in coordinating the DNA damage response. ATM was identified from a rare mutation found in the disease ataxia telangiectasia and leads to chromosomal instability and a high susceptibility to cancer (Savitsky et al., 1995). There are no known pathologies with ATR mutations, and ATR knockout mice die in early embryogenesis (Brown and Baltimore, 2000; de Klein et al., 2001). Upon activation, these similar kinases phosphorylate a number of target proteins, which transmit the DNA damage signal downstream, eventually arresting the cell cycle, initiating DNA repair, or, if necessary, causing cell death (apoptosis) (reviewed in Zhou and Elledge SJ, 2000) (Figure 3).

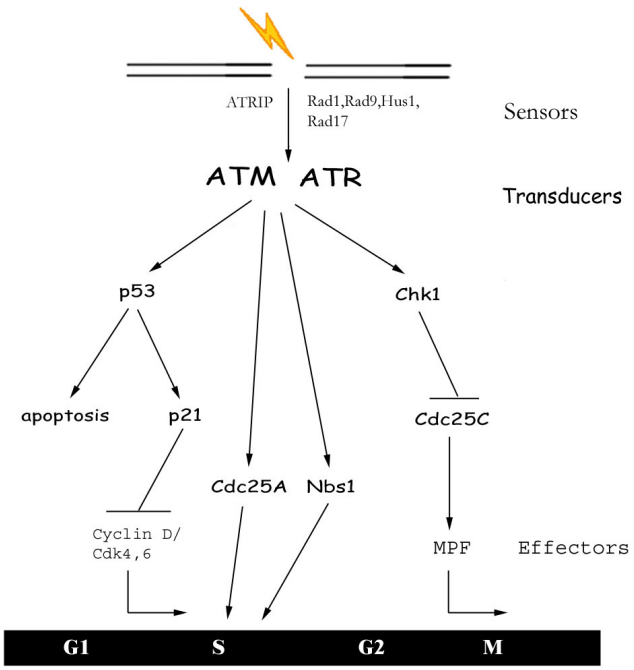

Figure 3. The DNA Damage Response

DNA damage is first detected by sensor proteins which in turn activate transducers in the signal cascade. These transducers then mediate the activation or inhibition of downstream effectors which can arrest the cell cycle or cause apoptosis.

Depending on when the DNA damage is sensed, ATM/ATR will activate a different axis of proteins. For G1 damage, ATM/ATR will phosphorylate p53, which then acts as a transcription factor for the synthesis of p21 (Cdk inhibitor) (Canman, et al., 1998; Banin et al., 1998; Tibbetts et al., 1999). Upon p21 expression, the G1/S transition is inhibited, preventing the synthesis of damaged DNA (Li et al., 1994). If damaged DNA is sensed during S-phase, the cell needs to slow DNA synthesis to provide time for repair. An ATM dependent pathway exists for an intra S-phase checkpoint in which ATM activation leads to the degradation Cdc25A (Falck et al., 2001). Since Cdc25A drives the initiation of DNA synthesis (Vigo et al., 1999), its degradation would allow synthesis to slow during S-phase and provide the necessary time for repair. This intra-S phase checkpoint is also controlled by the ATM phosphorylation of Nbs1 (Lim et al., 2000; Zhao et al., 2000; Wu et al., 2000), but the precise molecular mechanism remains elusive. Interestingly, Nbs1 is a protein involved in DNA repair as well, so this functional link between ATM and Nbs1 provides evidence of a high level of coordination between cell cycle progression and DNA repair. DNA damage incurred after S-phase results in the activation of the G2/M checkpoint to prevent entry into mitosis with damaged chromosomes. ATM/ATR also mediate this pathway by phosphorylating Chk1 in response to DNA damage (Chen et al., 1999; Zhao et al., 2001). This phosphorylation of Chk1 appears to enhance its kinase activity, which in turn phosphorylates Cdc25C (Sanchez et al., 1997). Cdc25C is inactivated by this phosphorylation and can no longer mediate entry into mitosis.

These signal transduction pathways (Figure 3) are just a few examples of the complex interacting network of proteins actually involved in processing DNA damage signals. The main idea embedded in these vast yet important details is that proteins (ATM/ATR) are activated upon DNA damage, and then trigger phase-specific cell cycle arrest and DNA repair.

Effectors

Name	Function
PCNA	Trimeric DNA clamp, holds pols on DNA
RFC_{1-5}	Pentameric complex, loads PCNA onto DNA
CSC – Checkpoint Sliding Clamp	DNA damage sensor? Structurally similar to PCNA
(Rad1, Rad9, Hus1)	
Rad17	DNA damage sensor? Complexes with RFC_{2-5} to load CSC onto DNA
Rad26 (fission yeast)	DNA damage sensor?
ATRIP (humans)	
Rad3 (fission yeast)	DNA damage transducer
ATR (humans)	
ATM	DNA damage transducer
p53	Transducer for G1/S checkpoint and apoptosis
p21	Cdk4,6 Inhibitor, involved in G1/S checkpoint
Cdc25A	Initiation of DNA synthesis, S-phase checkpoint
Nbs1	S-phase checkpoint transducer, DNA repair
Chk1	G2/M checkpoint transducer
Cdc25C	G2/M checkpoint transducer
MPF – Mitosis Promoting Factor	Necessary for G2/M transition
(Cyclin B/Cdc2)	DNA damage effector, G2/M checkpoint
Cyclin D/Cdk4,6	Necessary for G1/S transition
	DNA damage effector, G1/S checkpoint

Table 1. Components of the DNA damage response

Cell cycle checkpoint proteins and their respective functions. Putative, but not yet proven functions are followed by question marks.

The halting of the cell cycle is typically elicited by deactivating the Cyclin/Cdk complex involved in a specific phase transition (G1/S or G2/M). For example, p21 which is synthesized in response to DNA damage in G1, directly inhibits Cdk4,6 and thus prevents the transcription of proteins required for DNA synthesis. The final effector in the G2/M checkpoint is the CyclinB/Cdc2 complex (MPF) described earlier as being essential for the transition from G2 into mitosis. Upon DNA damage, the Cdc25C phosphatase can no longer remove inhibitory phosphates from Cdc2, and thus prevents the CyclinB/Cdc2 complex from breaking down the nuclear envelope, condensing chromosomes, and other events that occur in early mitosis.

Cell Cycle Controllers as Anticancer Drug Targets

Thus far this review has focused on the details underlying the control of cell division, but it is important not to lose sight of the exciting applications of this knowledge. For example, about half of all tumors have a damaged copy of the tumor-suppressor protein p53. Now that much of the detailed mechanism by which p53 inhibits tumor growth is understood, drugs can be developed to take advantage of its action. There are several biotechnology companies currently attempting to develop p53 therapies by reconstituting functional p53 back into tumor cells. This would restore the broken signal transduction pathway, and thus prevent tumorigenesis. The DNA damage checkpoint consists of many more pathways than those introduced in this review, and it is difficult to say at this phase which pathways will be of greatest utility in anticancer therapies. Thus, current cancer biology research is directed at better characterizing known pathways and elucidating novel ones involved in the DNA damage response.

About the Author

Will Renthal is currently a third-year Biochemistry Honors student at The University of Texas at Austin. There he conducts research as an Arnold and Mabel Beckman Scholar on the mechanism by which a novel antibiotic kills bacteria and as an NSF Fellow on MAP Kinase signal

transduction. For the previous two summers, he has researched cell cycle checkpoints with Dr. Eva Lee at the University of Texas Health Science Center at San Antonio, Institute of Biotechnology. His research focused specifically on characterizing the functions of a protein which is mutated in Nijmegen Breakage Syndrome (NBS) patients. This is a disease in which patients have an extremely high susceptibility to cancer and chromosomal instability. In his two summers of research, he has helped to clarify some of the subtle points about the NBS gene product, which is involved in both cell cycle checkpoints and DNA repair.

After his undergraduate education he plans to attend either an MD/PHD program or graduate school where he will further study the mechanisms underlying cell growth and development. Following this graduate training and a brief postdoctoral position, he aspires to become a professor at a medical center. There he hopes to conduct high quality basic research with a focus on drug discovery while teaching the next generation of scientists.

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