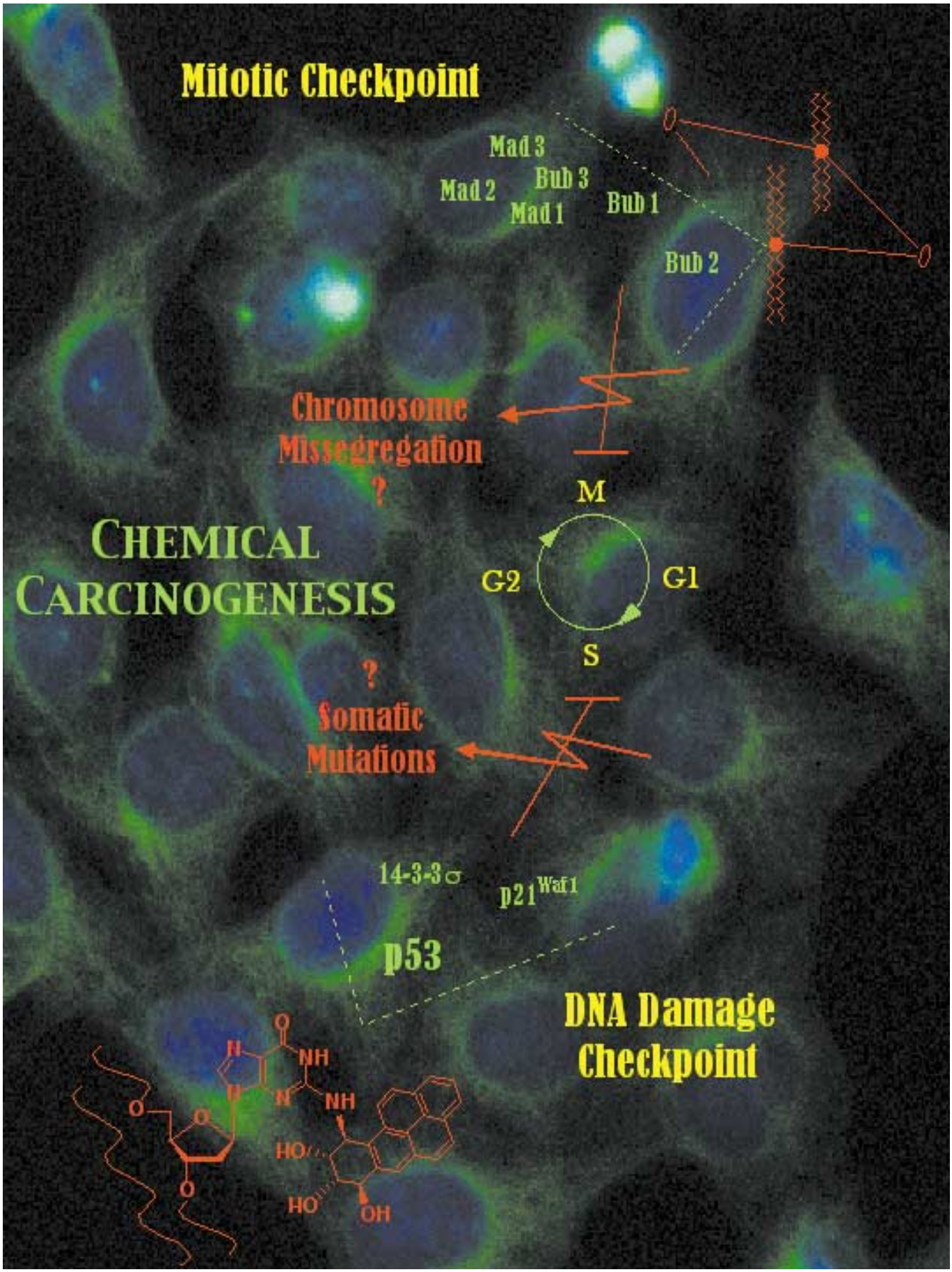

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Cell Cycle Control and Cell Division: Implications for Chemically Induced Carcinogenesis**

Andreas Luch*[a]

Eukaryotic cells proceed through an ordered series of events constituting the cell cycle, during which their chromosomes are duplicated and one copy of each daughter chromosome segregates to each daughter cell (mitosis). A precise and stringent regulation of this cell cycle is absolutely necessary for normal development of multicellular organisms; loss of cell cycle control, however, may ultimately lead to the generation of tumors. The present article provides an overview on the molecular mechanisms constituting the two most important checkpoints within the cell cycle of eukaryotic cells, that is, the spindle/mitotic checkpoint and the DNA damage checkpoint. It will be discussed how these checkpoints may be impaired by chemical carcinogens and how these interactions may contribute to the generation of aneuploidy and accumulation of somatic mutations, two major characteristics of human tumor cells.

KEYWORDS:

carcinogenesis · cell cycle control · DNA damage · mitosis · signal transduction

1. Introduction

Cell cycle checkpoints regulate progression between distinct steps within the cell cycle (G1 →S →G2 →M; Figure 1) to guarantee high fidelity during protein-catalyzed passage of "critical events" like segregation of chromosomes (the spindle/mitotic checkpoint), DNA replication (the DNA replication checkpoint), and DNA damage-induced cell cycle arrest and apoptosis (the DNA damage checkpoint).[1] Disturbance of these cellular checkpoints, for example, by chemically induced mutations or chemical inhibition of contributing protein factors, may therefore lead to chromosome missegregation (aneuploidy), accumulation of DNA mutations, and subsequently to "genomic instability" and generation of tumors.[2]

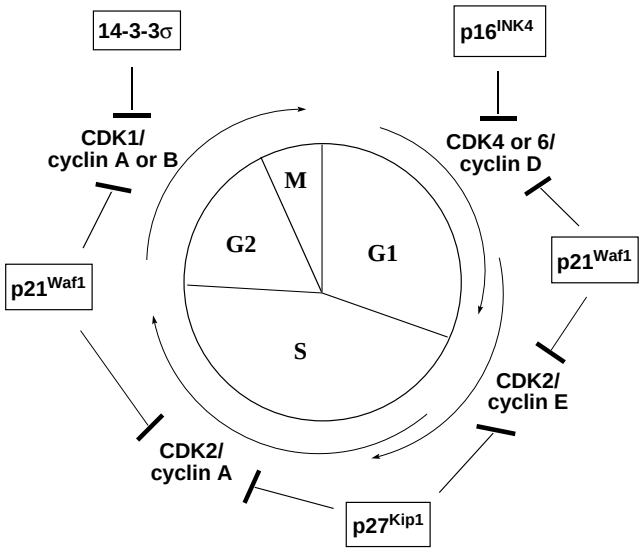

Figure 1. *Schematic representation of the cell cycle of somatic cells from higher vertebrates and humans. Fast-replicating cells from higher vertebrates proceed through an entire cell cycle within 24 hours: M phase (mitosis) ≈ 30 min, G1 phase (first "gap" phase) ≈ 9 h, S phase (synthesis phase = DNA replication) ≈ 10 h, G2 phase (second "gap" phase) ≈ 4.5 h. Immediately after mitosis, initiation of cell division (cytokinesis) occurs. Mitosis can be subdivided into prophase, metaphase, anaphase, and telophase (see Figure 2). The period in between two M phases is called interphase (= G1 + S + G2). The figure also displays the most important cell cycle phase-dependent CDK/cyclin complexes and their most relevant cellular inhibitors (see the text for explanations).*

2. Cell Division: The Spindle or Mitotic Checkpoint

Many tumor cells are aneuploid.[3] Studies of colorectal cancer cell lines indicate that aneuploidy can result from an error-prone distribution of chromosomes during mitosis.[4] Mutations within genes encoding the proteins that constitute the spindle or mitotic checkpoint (Figure 2) may often—but not exclusively—be etiologically related to this process.[5] The resulting "chromosomal instability" may facilitate the additional loss of tumor-suppressor genes and subsequently may lead to formation and selection ("evolution") of malignant cells.[6]

Microtubule inhibitors like taxol, colchicine, nocodazole, or vinca alkaloids (Scheme 1) interfere with the assembly and

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[**] The frontispiece background image of fluorescent asynchronous human cervical carcinoma HeLa S3 cells was kindly provided by Jon Hoyt and Randall W. King, Harvard Medical School, Boston. A glossary can be found at the end of the text.

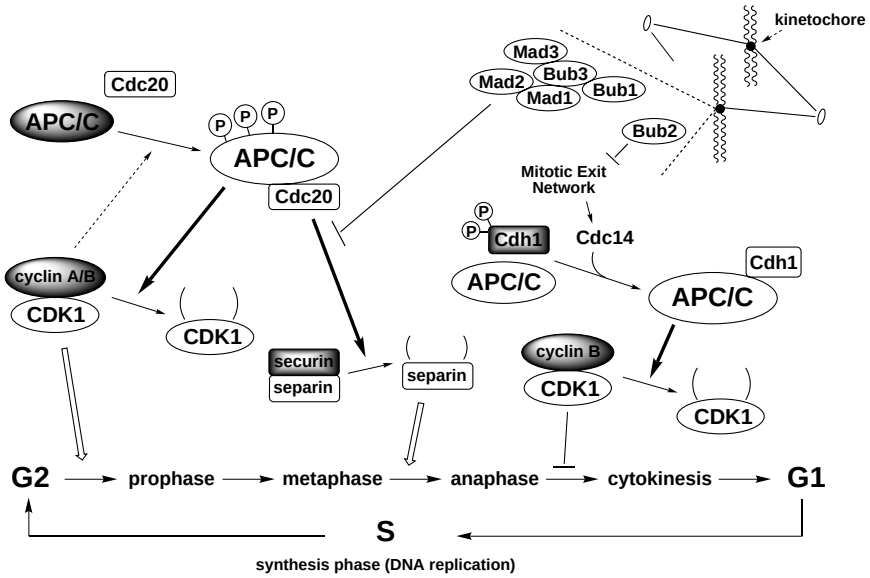
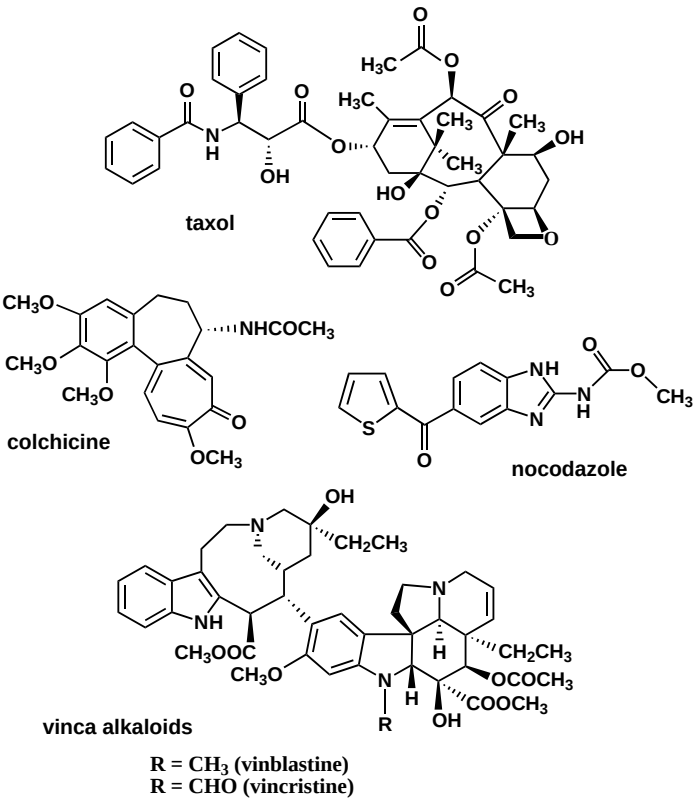


Figure 2. *The spindle checkpoint. APC/C-dependent regulation of cell cycle progression and function of the spindle checkpoint in animal cells (see the text for explanations). For clearness reasons, the final stage in mitosis, traditionally termed as 'telophase', is not depicted in this figure (see Figure 1).*

degradation of the mitotic spindle apparatus and therefore affect mitotic segregation of chromosomes at the transition from metaphase to anaphase. Exposure to these compounds results in a cellular delay/inhibition of the onset of anaphase (by activation of the spindle checkpoint) and/or in induction of apoptosis.[7] The spindle checkpoint is constituted by a signal transduction cascade that inhibits the onset of anaphase until all spindle microtubules are correctly attached to the kinetochores of each individual chromosome. Progression in mitosis remains blocked even in the case where just a single chromosomal connection is missing.[8] The mitotic arrest caused by unattached chromosomes can be overcome by laser ablation of the kinetochore.[9] Thus, the signal for checkpoint-dependent arrest arises from the kinetochores.

Many new and detailed insights on the control of the transition between metaphase and anaphase have been achieved during recent years by investigation of yeast cells (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*).[10] However, the mechanistic relationship between activation of the spindle checkpoint and induction of apoptotic cell death still remains unclear. Apoptosis may be a direct result of the activation of proteins involved in the checkpoint; alternatively, apoptosis may also result from an arrest in metaphase or may be induced by proapoptotic structures accumulating in cells that undergo aberrant mitosis (exit from mitosis in the absence of anaphase or cytokinesis).[7, 11]



Scheme 1. *Important inhibitors of microtubules and mitosis: taxol (from* Taxus brevifolia*), colchicine (from* Colchicum autumnale*), nocodazole (methyl-[5-(2-thienylcarbonyl)-1H-benzimidazol-2-yl]-carbamate), and vinblastine or vincristine (from* Catharanthus roseus*).*

Andreas Luch studied biology, chemistry, and medicine in Mainz and Munich. After receiving his PhD in toxicology at the University of Mainz in 1995, he moved to the Technical University of Munich, where he continued his studies on chemically induced DNA damage and mutagenicity at the Institute of Toxicology and Environmental Hygiene (Director: Prof. Dr. H. Greim). He was appointed as an Expert Toxicologist of the German Society of Pharmacology and Toxicology (DGPT) in 1996 and as a Eurotox Registered Toxicologist in 1998. After completing his Habilitation on the molecular mechanisms of initiation of chemical carcinogenesis in 1999, he became a lecturer in "pharmacology and toxicology" and finished with his medical studies by receiving an MD degree in 2001. In January 2002, he moved to the Department of Cell Biology at Harvard Medical School, where he joined the laboratory of Prof. Dr. R. W. King. As a research fellow of the Deutsche Forschungsgemeinschaft, he now continues to focus on the interactions between chemical carcinogens and the regulatory pathways involved in cell cycle control and cell division in human cells.

2.1. Cyclin-dependent progression through mitosis

Progression through the eukaryotic cell cycle is controlled by heterodimeric protein kinases consisting of a regulatory subunit (cyclin) and a catalytic subunit (cyclin-dependent kinase, CDK; in yeast, Cdc stands for cell division cycle; Figure 2). Mitosis is initiated by a maturation- or mitosis-promoting factor (MPF) consisting of cyclin B and Cdc2 (in fission yeast), Cdc28 (in budding yeast), or CDK1 (in mammals).[12] Phosphorylation reactions catalyzed by mitotic CDK complexes that accumulate during interphase are responsible for condensation of chromosomes (condensin), nuclear-envelope breakdown (nuclear lamins), and assembly of the mitotic spindle apparatus (microtubule-associated proteins). Inactivation of mitotic CDK complexes is induced by the polyubiquitination-triggered degradation of their cyclin subunits; this starts in late anaphase and is catalyzed by the anaphase-promoting complex/cyclosome (APC/C).[13] APC/C is a complex ubiquitin ligase (12 subunits) that targets substrate proteins for rapid degradation by the proteasome.

2.2. The role of the anaphase-promoting complex

The APC/C-catalyzed degradation of mitotic CDK complexes in late anaphase is required for cellular progression into G1 phase. Complex cellular events like the breakdown of the spindle apparatus, decondensation of chromosomes, cytokinesis (cell division), reassembly of the nuclear envelope, and formation of prereplication complexes occur during the transition between mitosis and interphase. Segregation of chromosomes (metaphase →anaphase transition), however, is induced by the APC/C-catalyzed degradation of securins (inhibitors of chromosome segregation during anaphase; Figure 2). The presence of securins (for example, Pds1) leads to sequestration of initiators of anaphase, the separins (such as Esp1). During metaphase, sister chromatids are connected to each other by a multiprotein complex named "cohesin". Esp1-dependent proteolysis of Scc1, an important component among proteins composing the cohesin complex, directly facilitates segregation of sister chromatids.[10, 13] Initiation of chromosomal segregation is therefore realized by a multistep protein cascade activated by APC/C-catalyzed degradation of securins. Consistently, mutations within subunits of APC/C lead to metaphase arrest in the cell cycle.[14]

Accessory factors regulate the oscillating activity of APC/C.[10, 13, 15] Biochemical studies with oocytes from the frog *Xenopus laevis* have shown that transition to anaphase is initiated in early M phase by degradation of securins only after CDK-catalyzed phosphorylation of APC/C subunits and subsequent binding of Cdc20 (APC/C^{Cdc20}) (Figure 2). Binding of Cdh1, another activator of APC/C in yeast and somatic cells, however, occurs during late M/early G1 phase and constitutes the complex APC/C^{Cdh1} that leads to polyubiquitination (and finally degradation) of cyclin B (telophase →G1 transition through cytokinesis; Figure 2).

2.3. Protein components constituting the spindle checkpoint

Conversely, the activity of APC/C is controlled by components of the spindle checkpoint.[1, 10] Genetic screening of *S. cerevisiae* led to the identification of a number of contributing genes named *mad1 – 3* (stands for mitotic-arrest defective) or *bub1 – 3* (stands for budding uninhibited by benzimidazole).[10] Cloning of homologous genes from animals or humans and analysis of the gene products revealed that protein kinase Bub1 is localized at the kinetochore and activated by the spindle checkpoint. Together with another kinase (Mps1), Bub1 leads to phosphorylation of Mad1 in a Bub3- and Mad2-dependent manner (Figure 2), subsequently resulting in activation of Mad2 and inhibition of APC/C^{Cdc20} through formation of a ternary Mad2/APC/C^{Cdc20} complex (the so-called Bub/Mad pathway). This cascade leads to an arrest of the cell in metaphase through securin-dependent sequestration of the separin Esp1.[10]

On the other hand, Bub2 is probably activated by as yet unknown mechanisms that are initiated through microtubule depolymerization rather than by signals originating from unattached kinetochores. Results from studies with yeast cells demonstrated that Bub2 is localized at the spindle poles (centrosomes). As soon as chromosomes arrive at the spindle poles in late anaphase, cytokinesis is initiated. It is likely that this process is initiated by association of Bub2 with Byr4 because the resulting GTPase-activating protein complex (Bub2/Byr4) inhibits the multistep protein cascade called the "mitotic exit network" (MEN). Since MEN normally activates the function of APC/C^{Cdh1} through Cdc14-catalyzed dephosphorylation of Cdh1, Bub2-dependent inhibition of MEN finally results in a blockage of the transition into G1 (the Bub2/Byr4 pathway, Figure 2).[10]

2.4. Mitotic cyclins in mammalian cells

In somatic cells from higher vertebrates two different major mitotic cyclins, cyclins A and B, were identified (Figures 1 and 2). Cyclin A is required for both S phase progression and the early steps in mitosis, whereas cyclin B is required for entry into mitosis. Cyclin A is degraded by APC/C^{Cdc20} at the time of nuclear envelope breakdown and not stabilized by activation of the spindle checkpoint (Figure 2).[16] In contrast to the situation in yeast cells or oocytes from *Xenopus laevis* (see Section 2.2.), cyclin B degradation in somatic cells is started by APC/C^{Cdc20} during metaphase and is finally finished by APC/C^{Cdh1} after anaphase (Figure 2).[17] Although somatic cells express both APC/C activators known to date (Cdc20 and Cdh1) and also components of the MEN,[18] the physiological role of the APC/C activators and MEN remains to be exactly characterized.

2.5. Inhibition of proteins of the spindle checkpoint

Selective inhibition of Bub1, Bub3, or Mad2 in mice demonstrated that these checkpoint proteins are also important for mitotic chromosome segregation in mammalian cells.[19] In addition to increased frequencies of chromosomal missegration (aneuploidies) and apoptotic cells, elevated levels of lung tumors were observed in mice that had been knocked-out for only one

mad2 allele. Analogous or similar effects were also seen after mutational alteration of other proteins associated with the kinetochore, for example, the tumor-suppressor gene responsible for a hereditary precancerous stage of colon cancer called *Adenomatosis Polyposis Coli*.[20] The tumor-suppressor protein, which was discovered by investigating the molecular pathology of this disease, accumulates at the chromosomal kinetochores during mitosis by forming complexes with Bub1 and Bub3. In summary, these observations together with the detection of mutations found in the spindle checkpoint components of tumor cells[5] clearly underscore the importance of chromosomal missegregation and formation of aneuploid daughter cells for the generation of cancer.

3. Cell Cycle Progression in Interphase

3.1. Control of DNA replication

Cell cycle progression from G1 to S phase was investigated and characterized in yeast before it became clear that proteins with similar structural and functional properties are also expressed in cells from vertebrates or mammals.[21, 22] The transition between G1 and S phase is realized through the synthesis of CDKs and cyclins specific to late G1 or S phase (CDK2 and cyclins E and A in animals) and subsequent activation of the complexes formed (Figure 1). Prior to activation of these complexes, CDK inhibitors like Sic1 (budding yeast) or $p27^{Kip1}$ (mammals) must be destroyed at the proteasome. This process is cooperatively catalyzed by the ubiquitin-conjugating enzyme Cdc34 and the Cdc53-dependent ubiquitin-ligase SCF (the Cdc34-dependent ubiquitination pathway). After degradation of the inhibitors, S phase CDKs become catalytically active and contribute to activation of prereplication complexes by phosphorylation reactions. Prereplication complexes are already formed during G1 phase at origins of replication within DNA and consist of a number of proteins (Cdc6, Cdc45, and MCMs) associated with a multisubunit origin recognition complex.[23] After replication, the same CDKs prevent re-formation of prereplication complexes until mitosis has occurred and specific G1 phase conditions have been restored.

In mammalian cells, synthesis of CDK2/cyclin E or A complexes and other important proteins (for example, dihydrofolate reductase, thymidine kinase, DNA polymerase α) during G1 phase is induced by members of the transcription factor family E2F (such as E2F-1). Through phosphorylation of the retinoblastoma tumor-suppressor protein (the pRB pathway), the activity of the E2F proteins is indirectly controlled by G1-specific and mitogen-induced CDK/cyclin complexes formed from CDK4 or 6 and cyclins of the D family (D1 – D3; Figures 1 and 3).[22, 24] Cyclin D dependent activity of CDK4 and 6 is blocked by specific inhibitors (the INK4 proteins p15, p16, p18, and p19). Overexpression of these inhibitors leads to an arrest of the cell cycle in G1 phase. On the other hand, overexpression of cyclin D1 or loss of function of $p16^{INK4}$ not only leads to an elevated rate of cell proliferation but may also contribute to the generation of tumors in experimental set-ups or in humans.[22, 25]

3.2. The p53-dependent cell cycle progression

Cell cycle progression from G1 to S phase is further controlled by an additional group of CDK inhibitors, the Cdk inhibitory proteins (Cip) $p21^{Waf1}$, $p27^{Kip1}$ (see Section 3.1.), and $p57^{Kip2}$ (Figure 1).[1, 22] The CDK inhibitor $p21^{Waf1}$ is of particular interest because of its p53-dependent regulation (Figure 3). The p53 protein acts as a tumor-suppressing transcription factor that is frequently mutated in human tumors.[26] In contrast to the CDK-inhibitor $p16^{INK4}$, $p21^{Waf1}$ inhibits various cyclin-dependent kinases and initiates a cell cycle arrest at the G1 to S phase transition as well as between G2 phase and mitosis. Direct inhibition of transcription factors of the E2F family by $p21^{Waf1}$ may be another mechanism involved in this regard (Figure 3).[27, 28] In epithelial cells there also exists a p53-dependent expression of the 14-3-3σ protein that is capable of inducing a cell cycle arrest at the transition between G2 phase and mitosis (Figure 1).[27, 29] 14-3-3σ-

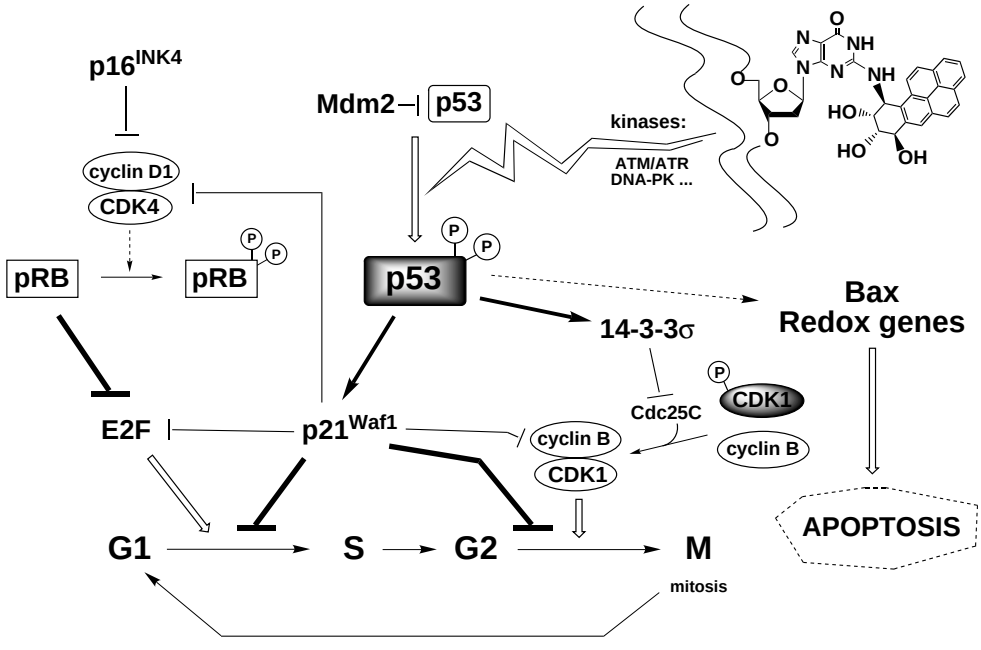


Figure 3. *The DNA damage checkpoint. Regulation of cell cycle progression at the transitions between G1 → S and G2 → mitosis in the M phase (see the text for explanations). The figure summarizes the DNA damage checkpoint signaling in response to the formation of a bulky DNA adduct lesion. The DNA adduct shown is formed through covalent interaction between guanosine and the "ultimately" carcinogenic metabolite of the polycyclic aromatic hydrocarbon benzo[a]pyrene ((+)-anti-BPDE; see Scheme 3). Cellular kinases inducible by DNA damage (see Figure 4): ATM = mutated in the disease* Ataxia Teleangiectasia, *ATR =* Ataxia Teleangiectasia*-related kinase, and DNA-PK = DNA-dependent protein kinase.*

dependent sequestration of the Cdc25C phosphatase prevents dephosphorylation of CDK1 and thus inhibits the formation of active CDK1/cyclin B complexes that would be required for entry into mitosis (see Section 2.4.; Figure 3).

3.3. The p53-dependent apoptosis

In addition to controlling the transitions between different cell cycle stages, the p53 protein may also intitate the induction of apoptotic cell death under certain circumstances (Figure 3).[1, 30] Programmed cell death is induced by transactivation of various proapoptotic proteins, for example, the Bax protein, a member of the Bcl-2 protein family that is localized within mitochondria. Bax promotes the release of mitochondrial cytochrome *c* into the cytosol. Subsequently, the elevated level of cytosolic cytochrome *c* triggers an activation cascade of proteolytic caspases (cysteine-dependent aspartases) that catalytically promote the degradation of cellular constituents and hence result in the demise of a cell. Other p53-dependent death-promoting proteins, for example, enzymes that contribute to the generation of reactive oxygen species (ROS, such as $HO^{\bullet}$, $O_2^{-\bullet}$) or "death-signal" receptor cascades were also identified (Figure 3).[30]

3.4. The DNA damage checkpoint

Since the tumor-suppressor protein p53 is probably the most important cellular key protein that integrates signaling pathways involved in cellular growth, division, and death, its steady-state level (half-life time of $\approx$20 min) is tightly and precisely controlled. The Mdm2 protein is a key mediator of p53 protein stability. Mdm2 functions as an ubiquitin ligase that targets p53 for polyubiquitination-mediated proteolysis once it has been bound to the amino-terminal transactivation domain of the tumor suppressor (Figures 3 and 4).[27] On the other hand, the cellular level of the p53 protein increases in response to cellular stress signals, such as hypoxia, genotoxic compounds, or spindle damage, or as a result of overexpression of various oncogenes (such as *ras* or *myc*).[27, 31]

Studying the reaction of cells to DNA damage induced by UV radiation or chemically modifying (alkylating) carcinogens (DNA damage checkpoint; Figure 3) revealed that cellular accumulation of p53 results from inhibition of its Mdm2-mediated proteolysis. Immediately after DNA damage occurs, various kinases (for example, ATM, ATR, DNA-PK, and Chk 1) that catalyze multiple phosphorylation reactions at the Mdm2-binding amino terminus of the p53 protein become activated (Figure 4).[27] Eight phosphorylation sites at the amino terminus have been identified that are enzymatically modified upon induction of various types of cellular DNA damage. Among those, phosphorylation of Ser 15 has been found to occur in murine Swiss 3T3 cells after exposure to benzo[*a*]pyrene and subsequent induc-

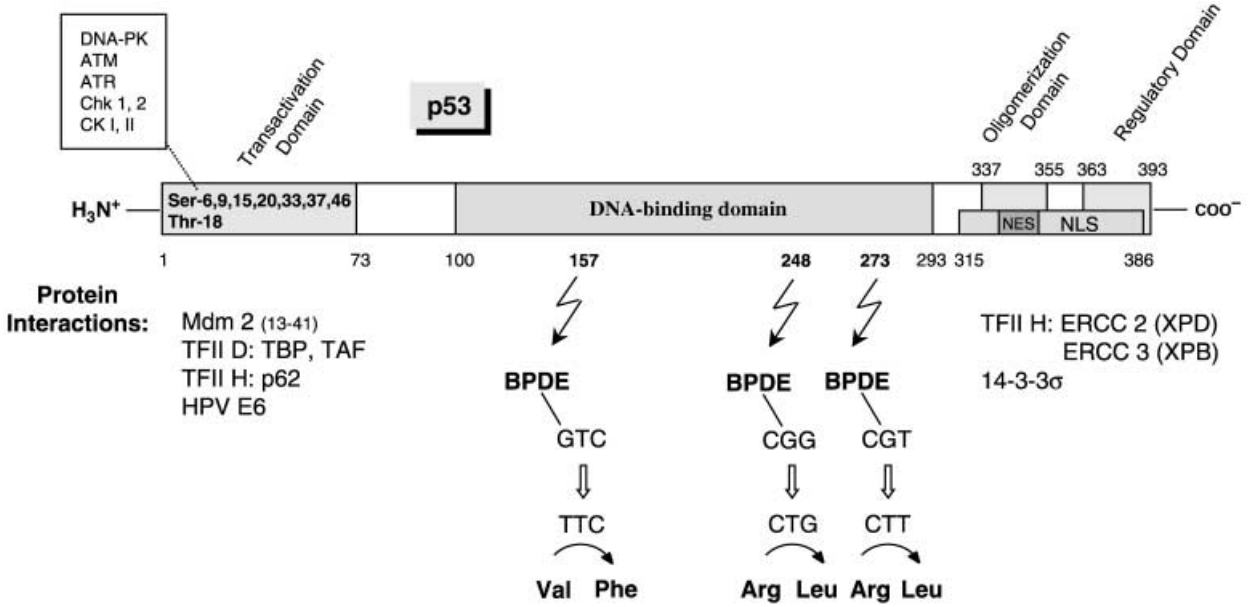
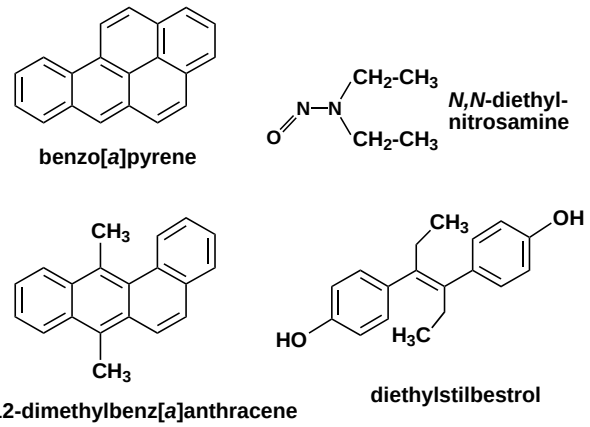


Figure 4. *Schematic representation of the p53 protein (adapted from the papers of May and May[46], Stewart and Pietenpol,[27e] and Denissenko et al.[36b]). Wild-type p53 regulates the transcription of gene products involved in growth arrest, DNA repair, and apoptosis. (The human genome is estimated to contain approximately 200 – 300 p53-binding consensus sites.) P53-dependent transactivation is realized by its central DNA binding domain in conjunction with its amino-terminal transactivation domain. Most of the mutations found in the p53 gene of tumor cells are located in the central DNA binding domain. Denissenko et al.[36b] have shown that exposure of human lung epithelial cells to benzo[a]pyrene results in the formation of (+)-*anti*-BPDE – DNA adducts (see Figure 3 and Scheme 3), predominantly at codons 157, 248, and 273. The same positions were characterized as mutational hotspots in human lung cancer cells. Stabilization of wild-type p53 in response to DNA damage (the DNA damage checkpoint) occurs through multiple phosphorylation reactions catalyzed by checkpoint kinases (for example, DNA-PK, ATM, ATR, or Chk1) at the amino terminus of the protein (see the text). The figure also depicts some examples of important interactions between the N or C terminus of the p53 protein and transcription factors (TFII, ERCC), the E6 antigen of human papilloma viruses (HPV E6), and the checkpoint proteins Mdm2 and 14-3-3σ (see Figure 3). P53 physically interacts with Mdm2 at its sequence between residues 13 and 41.*

tion of stable DNA adducts (see Figure 3). Phosphorylation of Ser15, Thr18, or Ser20 induced by DNA damage leads to dissociation of the p53/Mdm2 interaction due to charge – charge repulsion forces. As a result, the p53 protein becomes stabilized and its cellular steady-state level increases. Thus, the DNA lesion triggers induction of a kinase-dependent inhibition of the interaction between Mdm2 and p53 that finally results in an elevated p53 protein level of the particular cell.

Loss of wild-type p53 function through mutation or chemical alteration impairs the DNA damage checkpoint of cells that are further unable to sufficiently respond on DNA damage with induction of an arrest in cell cycle progression. Since the cell cycle arrest enables cellular DNA repair systems (for example, nucleotide or base excision repair) to remove particular DNA lesions or alternatively may induce signaling cascades responsible for apoptotic cell death, both different cellular defense mechanisms would finally fail to respond on DNA damage in an adequate manner. Consequently, the cells are prone to accumulate DNA mutations that may contribute to genomic instability and ultimately to the generation of tumors.[26] Paradoxically, in the case of chemically induced carcinogenesis it is obvious that escaping these well-regulated and complex p53-triggered defense mechanisms is initially required for cellular acquisition of increasing chemical susceptibility through mutations within the corresponding gene or within one of the elements involved in the corresponding signal cascade.



Scheme 2. *Some examples of carcinogenic chemicals.*

4. The Role of Cell Cycle Checkpoints in Chemical Carcinogenesis

4.1. Mutations within genes of cellular checkpoints

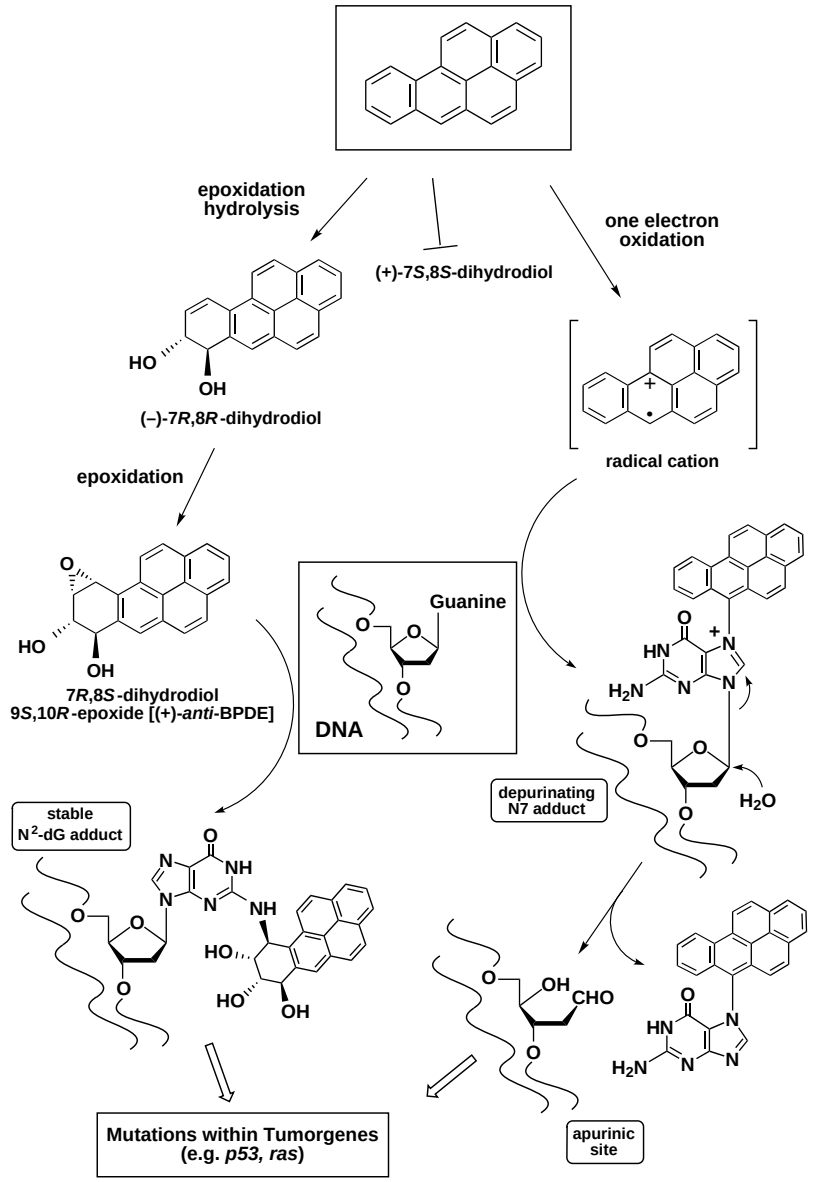
Cancer researchers presently favor the dogma that accumulation of mutations in various tumor-relevant genes is prerequisite in the formation and progression of human tumor cells.[32] This view is now further supported by a recent study that reports the generation of human cancer cells in an experimental set-up by blocking two different cellular checkpoints (the pRB pathway and p53-dependent DNA damage checkpoint; see Figure 3) together with simultaneous overexpression of growth-promoting genes (oncogene *ras* and telomerase).[33]

In the case of many chemical carcinogens (Scheme 2) and their metabolites, extraordinarily strong genotoxic and mutagenic potencies have been observed in various experimental test systems.[34] Among the class of polycyclic aromatic hydrocarbons some carcinogenic and environmentally relevant member compounds (for example, benzo[*a*]pyrene) were intensively investigated and it was found that—to some extent—their carcinogenic potency correlates well with the mutagenic activity of their genotoxic and DNA-binding metabolites (Scheme 3).[35] Detection of mutations within tumor-relevant genes like *p53* (Figure 4) or *ras* after exposure to these compounds also supports the importance of the corresponding proteins in chemically induced tumors.[36] More recent results demonstrated that carcinogenic polycyclic aromatic hydrocarbons may escape a p53-triggered cell cycle G1 phase arrest (see Figure 3). Exposure of cells to low but still DNA damaging and probably more environmentally relevant doses of these compounds resulted in an accumulation of wild-type p53 that correlated well to the exposure dose and to the level of DNA damage that was induced.[37] However, subsequent to a weak $p21^{Waf1}$ induction, cells proceeded into S phase[38] or were found to arrest in G1 phase only after treatment with higher doses of the compound.[39] Consequently, genomic mutations may be formed within the following replication period (S phase) through mispairing of chemically modified nucleobases (DNA adducts) or due to misincorporation of nucleotides at apurinic sites (see Scheme 3). Based on these observations "stealth effects" have been attributed to carcinogenic polycyclic aromatic hydrocarbons.[40]

4.2. Aneugenic properties of carcinogenic chemicals

Together with these stealth effects and their role in escaping a G1 phase arrest after DNA damage has occurred, aneugenic (aneuploidy-inducing) properties of carcinogenic polycyclic aromatic hydrocarbons may also contribute to nongenotoxic (epigenetic) activities of these compounds. Induction of chromosomal aberrations and aneuploidy has been described after treatment of cells or animals with polycyclic aromatic hydrocarbons and other chemical carcinogens, including known human carcinogens such as asbestos, cadmium, and diethylstilboestrol (Scheme 2).[41] For instance, aneuploidy occurs in cells in culture and in early stages during skin, muscle, and liver carcinogenesis after exposure of rodents and hamsters to 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene, or to *N*,*N*-diethylnitrosamine (Scheme 2).[42] Induction of aneuploidy in cell lines that lack sufficient enzymatic activity for activation of parental polycyclic aromatic hydrocarbons to genotoxic (DNA-reactive) intermediates (for example, dihydrodiol epoxides; Scheme 3)[41, 43] provides evidence for the contribution of epigenetic mechanisms during induction of chromosomal mis-segregation in these systems.

In addition to karyotyping of cells, "genetic toxicology" researchers routinely use the well-established micronuclei assay along with fluorescence in situ hybridization (FISH) or antibody-based techniques for kinetochore-specific staining to test whether putative carcinogens also display aneugenic activi-



Scheme 3. *Metabolic activation and DNA binding of the carcinogen benzo[a]pyrene. Benzo[a]pyrene is an environmental pollutant that may be enzymatically converted in vivo to yield DNA-reactive dihydrodiol epoxides or radical cations. Stereoselective generation of the 7*R*,8S-dihydrodiol 9S,10*R*-epoxide ((+)-*anti*-BPDE) is catalyzed by cytochrome-P450-dependent monooxygenases (P450) in conjunction with epoxide hydrolases. Alternatively, aromatic radical cations are formed through P450- or peroxidase-dependent one electron oxidation reactions. Subsequent reaction of these electrophilic intermediates with genomic DNA may produce stable adducts between dihydrodiol epoxides and the exocyclic amino group of guanosine. On the other hand, apurinic sites may emerge within the phosphodiester backbone of genomic DNA as a result of depurination of instable adducts that are formed from radical cations and the N7 position in purine bases. Both kinds of DNA lesions may be converted into mutations within the following replication cycle unless repair of damaged DNA restores the integrity of the genome. Lack of sufficient DNA repair, however, may lead to mutations within tumor-suppressor genes (for example,* p53*) and oncogenes (such as* ras*). This has been widely demonstrated by application of various experimental in vitro and in vivo approaches where living cells have been exposed to benzo[*a*]pyrene (see Figure 4 and the text).*

ties.[41, 44] Since the process of chromosomal segregation depends on many different subcellular components and is controlled by multiple signaling pathways (see Figure 2), epigenetically acting aneugenic carcinogens may be capable of producing disturbances to chromosome segregation by interfering with a variety of cellular targets (for example, microtubules, microtubule-associated proteins, kinetochore proteins, or centrosomes). Despite of, or even because of, the variety of potential targets, there is presently only a poor understanding of the molecular mechanisms involved in particular and compound-specific interactions and processes.[41, 44] On the other hand, the potency of genotoxic carcinogens or metabolites in inducing mutations within tumor-relevant genes like *p53* (Figure 4), *ras*, or others indicates that similar effects on important components of the cellular spindle checkpoint may be also expected—although experimental verification is still missing. Particularly in the case of nongenotoxic and aneugenic carcinogens that may be activated toward DNA-reactive intermediates through cellular enzyme systems (for example, benzo[*a*]pyrene →benzo[*a*]pyrene 7,8-dihydrodiol 9,10-epoxide; Scheme 3), there is some evidence for a synergistic cooperation between epigenetic mechanisms and somatic mutations during the process of induction of chromosomal missegregation.

Despite these restrictions in a mechanistic understanding, a variety of genotoxic and nongenotoxic carcinogens have been identified as being capable of inducing aneuploidy in experimental systems both in vitro and in vivo. Since chemically induced aneuploidy resembles the highly heterogenous karyotypes found in virtually all cancers at early stages, direct or indirect (through gene mutations) generation of aneuploid daughter cells may contribute to "genomic instability" and to the generation of tumors, as it is assumed from somatic mutations within checkpoint-relevant tumor-suppressor and oncogenes. Therefore, an irreconcilable collision of both theories on cancer induction seems not to be justified.[45]

5. Summary and Prospects

Various cell cycle checkpoints in animal or human cells are able to sense and to respond to disturbing signals that may originate from endogenous or exogenous sources. In general, the checkpoint-dependent biochemical response enables cells to proceed through phases of growth and division in a well-regulated manner, and thus the underlying mechanisms contribute to an error-free reduplication of individual cells. During chemically induced generation of tumors, however, single somatic cells gradually acquire a malignant phenotype by accumulation of somatic mutations within tumor-suppressor and oncogenes ("genetic instability"). In addition, the

numbers and structures of individual chromosomes may become altered, thus leading to heterogenous karyotypes of the particular cells ("chromosomal instability").

This article provides an overview of the molecular mechanisms constituting the two most important checkpoints within the cell cycle of eukaryotic cells, that is, the spindle/mitotic checkpoint (Figure 2) and the DNA damage checkpoint (Figure 3). Disturbance or alteration of the protein factors constituting these checkpoints may result in generation of aneuploidy and accumulation of somatic mutations. Therefore, these checkpoints have shifted into the focus of interest of researchers working in the field of molecular carcinogenesis.

A high number of chemical carcinogens are converted into DNA-reactive intermediates through cellular biotransformation reactions (Scheme 3). These intermediates may subsequently covalently modify nucleobases within genomic DNA and may therefore induce somatic mutations through mispairing of nucleotides. A growing list of experimental studies provides evidence for chemically induced mutations of tumor-suppressor genes (for example, *p53*) and oncogenes (such as *ras*). The transcription factor p53 is likely to be the most important cellular node center for regulation of signaling pathways that control cell cycle progression, apoptosis, and DNA repair (Figures 3 and 4). Its essential role within the DNA damage checkpoint may also provide sufficient explanation for the genetic instability found in tumor cells expressing mutated p53 proteins. Moreover, detection of mutations in the *p53* gene of many human tumor cells may support the conclusion that tumor formation in humans relies on similar mechanisms to those observed and characterized in experiments conducted thus far. Due to its function, the p53 protein also ensures the integrity of its own gene. Thus, chemical induction of mutations within the *p53* gene depends on molecular mechanisms that are able to escape the onset of an intact DNA damage checkpoint. Exactly this has recently been described for human cells in culture that are exposed to low concentrations of strongly carcinogenic polycyclic aromatic hydrocarbons.

As again demonstrated by various experimental approaches, many chemical carcinogens also display aneugenic (aneuploidy-inducing) characteristics. However, the underlying mechanisms are only poorly characterized. In addition to many other conceivable explanations, direct inhibition of protein components of the spindle checkpoint as well as induction of mutations within the corresponding genes may contribute to this activity. Both different modes of action may lead to chromosomal missegregation during mitosis. The discovery and molecular characterization of components of the spindle checkpoint (Figure 2) together with recent investigations on its role during tumor formation in animals certainly will provide a better understanding of the mode of action of aneugenic carcinogens in the near future. It is likely that future knowledge may also support the view that both mechanisms together, that is, accumulation of somatic mutations in tumor-suppressor and oncogenes as well as direct or mutation-dependent generation of aneuploidy in daughter cells, essentially contribute to formation of tumors.

Glossary

Aberration: that is, chromosomal aberration; an irregularity in the number or structure of chromosomes that may alter the course of development of the living cell; usually in the form of a gain (duplication), loss (deletion), exchange (translocation), or alteration in sequence (inversion) of genetic material.

Aneugenic: having an aneuploidy-inducing property (chemicals).

Aneuploid: possessing a chromosome number that is not an exact multiple of the haploid number.

Caspase: a cysteine protease that selectively cleaves proteins at sites just to the C-terminal side of aspartate residues.

Centrosome: the cell center; an organelle located near the nucleus of animal cells that is the primary microtubule-organizing center and contains a pair of centrioles; it divides during mitosis to form the spindle poles.

Centriole: a cylindrial organelle containing nine triplets of microtubules arrayed around its edges; centrioles migrate to opposite poles of the cell during cell division and serve to organize the spindles; they are capable of independent replication.

Centromere: the constricted portion of a mitotic chromosome where sister chromatids are attached and from which kinetochore fibers extend toward a spindle pole; it is required for proper chromosomal segregation during mitosis and meiosis; according to its location, a centromere is said to be metacentric (central), submetacentric (off center), or acrocentric (near one end).

Chromatid: one of the paired chromosome strands joined at the centromere, which make up a metaphase chromosome; chromatids result from chromosome reduplication during S phase.

Colorectal: pertaining of or affecting the colon and rectum.

Consensus site: highly conserved DNA sequence located upstream of the start sites of genes whose transcription is controlled by a particular transcription factor.

Karyotyping: determination of the karyotype, the full chromosome set of the nucleus of a cell; by extension, photomicrography of chromosomes arrayed according to a standard classification.

Kinetochore: a multilayer protein structure located at or near the centromere of each mitotic chromosome from which microtubules (kinetochore fibers) extend toward the spindle poles of the cell; it plays an active role in movement of chromosomes toward the spindle poles during anaphase.

MEN: mitotic exit network, proteinaceous network that enables cells to exit mitosis (through initiation of Cdc14-catalyzed dephosphorylation of Cdh1).

Oncogene: a gene capable under certain conditions of causing the initial and continuing conversion of normal cells into cancer cells; the term may be used to denote such a gene occurring in a viral genome (v-*onc*) or a cellular gene derived from (mutational) alterations of a protooncogene (c-*onc*); the corresponding proteins (oncoproteins) interfere with the mechanisms that normally control cell growth, division, and differentiation and thereby contribute to uncontrolled proliferation (cancer; for example, Myc or Ras).

Proteasome: a large multiprotein complex with a hollow cylindrical core that degrades intracellular proteins marked for destruction by attachment of multiple ubiquitin molecules; it is located in the cytosol and catalyzes an ATP-dependent degradation process.

Replication: that is, DNA replication; the production of multiple identical copies of a DNA molecule by unwinding the two strands of the double helix and forming new complementary strands thereto.

Securins: proteins that contribute to inhibition of segregation of chromosomes during anaphase (for example, Pds1).

Separins: proteins that contribute to the onset of anaphase/chromosomal segregation of mitosis (for example, Esp1).

Tumor-suppressor protein: protein that directly or indirectly inhibits progression through the cell cycle and in which a loss-of-function mutation in its corresponding gene may be oncogenic (for example, RB, p53 or APC).

Ubiquitination: covalent attachment of multiple copies of ubiquitin to an eukaryotic intracellular protein; ubiquitin is a 76-residue highly conserved protein that becomes linked to lysine residues in other intracellular proteins; proteins to which a chain of ubiquitin molecules is added are usually degraded in a proteasome.

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