

Chapter 2

Cell Cycle: The Life Cycle of a Cell

Abstract “Where a cell arises, there must be a previous cell”. This early statement of Rudolf Virchow already points to the process that is called cell cycle. It describes a series of events leading to cell division and duplication and can be sectioned into phases that are controlled by a collection of proteins interacting with each other, the cyclins and the cycline-dependent kinases. It is mandatory that DNA replication is conservative meaning that its structure and sequence remain unaltered while the DNA is duplicated before the cell actually divides. Checkpoints are responsible for the supervision, proteins such as p53 and RB being the key protagonists in cell cycle control. Upon DNA damage recognized repair programs are activated or if repair fails the cell is driven into a programmed cell death to remove the damaged cell. The transfer of DNA mistakes from the mother cell to the daughter cells can lead to tumor formation. So p53 and RB are key tumor suppressor proteins.

Keywords Cell cycle · Cell cycle control · Cell cycle checkpoints · Cyclins · Cyclin-dependent kinases · Tumor suppressor proteins · Apoptosis · Cellular senescence

In 1858 Rudolf Virchow stated his famous cell doctrine “Where a cell arises, there must be a previous cell, just as animals can only arise from animals and plants from plants”, meaning that cells are generated from cells, and only when the existing cells divide, more cells are arising (Virchow 1858). The cell cycle, also called cell-division cycle, describes a series of events that occur in a cell leading to its division and duplication and is, therefore, an identical replication. Eukaryotic cells, such as cells from mammalian organisms, do have a special organization structure, where the nucleus containing the genetic information (genome) can be distinguished from the surrounding cytoplasm containing different organelles and a huge assembly of proteins, the executors of the functions of the cell. During cell division an original cell (“mother cell”) divides into two new cells (“daughter cells”) in a highly controlled and organized manner (Fig. 2.1).

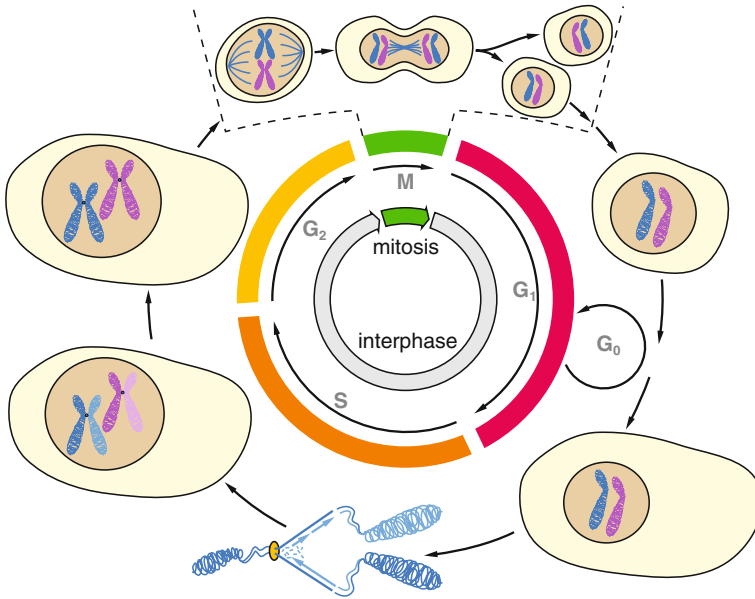


Fig. 2.1 Eukaryotic cell cycle. The division cycle of a cell can be sectioned into different pre-(S, G₂) and postmitotic (G₁, G₀) interphases. Chromosome doubling and synthesis of other cell components are necessary before mitosis where the actual separation of a mother cell into two identical daughter cells occurs (M-phase). Cells that temporarily or reversibly escape the division cycle are in G₀ phase (e.g. neurons; modified after Müller-Esterl 2011)

2.1 Phases of the Cell Cycle

Only cells with a nucleus undergo cycling and basically this eukaryotic cell cycle can be divided in two major phases, interphase and mitosis. While the interphase describes the time when the cell accumulates material and nutrients and subsequently doubles its genome, the mitophase summarizes the processes during which the cell actually splits into two distinct but identical daughter cells (identical DNA material, identical genome). The mitophase ends with the cytokinesis when the original (mother) cell is completely divided and the two daughter cells are on their own. Cycling of cells is a vital process for the whole body and the power of this event is best exemplified when looking at the first hours after a single-celled fertilized egg starts to develop, ultimately, into differentiated tissue and step by step into a full organism. But also many tissues in the adult body constantly go through cell cycling, a process that can be observed, for instance, when thinking of the renewal of hair, skin, blood cells and tissue of internal organs (e.g. hepatocytes in the liver). Cycling of the cell and ongoing or halted cell division is strongly related to cell and organism aging. It should be mentioned here that in studies on the life span of different species, the maximum life span of different animal models is put into relation with, for instance, body size, metabolisms, movement, and type of reproduction (sexual vs.

asexual). The hydra is a small freshwater polyp related to jellyfish that can achieve an indefinite life span if it reproduces asexually. Similar to yeast, this is possible via the budding off of daughter cells from the mothers body wall. So in these animals there is an unlimited high proliferative activity and cell cycling constantly going on (Deweerd [2012](#)).

Coming back to the biochemical process of the cell cycle. The major goal of this series of events is the doubling of the cell, for instance, for the replacement of damaged cells. Here, a major requirement is that the daughter cells are intact which relates mostly to the genomic material, the DNA. Since the mandatory process of DNA doubling (DNA replication) is a molecular process that underlies faults and errors as every biological process does, and because biomolecules are highly susceptible to chemical changes as, for instance, induced by UV light or radioactive radiation, DNA itself can be damaged during the process of cell cycling or errors occur during DNA replication. To maintain the correct function of the individual cell (e.g. skin cell, melanocyte) the DNA as the basis for all proteins must be maintained in its integrity, stability and function. This goal is reached via the execution of a tight control represented by DNA-repair enzymes etc., which are integral parts of the cell cycle. A look at the exact sequel of the steps of cell cycling will demonstrate the complexity of this process and will allow us to identify control mechanisms and checkpoints that may decide to either let the cell complete the cycle or let it run into death.

Many cells in our organism are permanently in cycle. Again, the division itself describes the process when one “mother cell” splits off into two genetically identical “daughter cells”. Before this physical division, the so called phase of mitosis, the whole cell material needs to be duplicated. While the amount of protein material is increased by enhanced protein synthesis rates, the doubling of the cellular genome via DNA replication is complex and highly regulated. Roughly, there are two general major sequences in the cell cycle, the phases before and the phases after mitosis (M), called G-phases, where G stands for gap (Fig. [2.1](#)). The gap phases are used to monitor the intracellular but also the extracellular conditions to make sure that everything is in order before actually proceeding to the next cycle phase. The G1-phase directly follows cell division and is frequently also called post-mitotic pre-synthesis phase. The cell starts to grow, the content of the cell (cytoplasm) with the functional machineries (organelles) is formed. In addition, the synthesis of mRNA takes place, histone proteins and the enzymes of the DNA replication machinery necessary for the next phase are generated. In a constantly dividing cell the G1-phase regularly takes approximately 3 h depending on the particular cell type. The S- or synthesis phase is characterized by the process of DNA replication (doubling of the cellular genome) and a major effort to produce histone proteins that are finally needed for the packaging of the genomic DNA. In average the S-phase takes about 7 h. The G2-, premitotic or post-synthetic phase is the time when the cell prepares to split off in two cells, the actual division. As part of a certain tissue cells loosen up the direct contact to neighboring cells, they usually round up and increase in general size. The synthesis of RNA and proteins concerns the main players needed for the mitosis and may take up to 4 h. Finally, in the M- or mitosis-phase division occurs, the doubled DNA organized in

chromosomes is separated, the cellular nucleus divides (= karyokinesis) as does the rest of the cell (cytokinesis). The M-phase itself may take approximately 30–60 min and can itself be divided into five phases (pro-, prometa-, meta-, ana-, telophase; for details see Alberts et al. 2007). In proliferating tissue with cells undergoing constant divisions after mitosis then the next G1-phase occurs. Cells that are fully differentiated having special functions and roles in the tissue permanently remain in the G1-phase which is then called G0- or quiescence-phase. G0 represents a specialized resting and quiescent state of the cell. Nerve cells, muscle cells and red blood cells (erythrocytes) are the most prominent examples of cells in G0-phase. G0 is not only the state for differentiated cells so that they can fulfill their tasks in the tissue. Cells also enter G0 when the extracellular microenvironment is not in favor for further cycling, for instance when growth factors or nutrients are lacking that are necessary for the S-phase. In the context of this book it should be noted that a permanent arrest of cells in G0 is not identical with the senescence or the physiological aging of a cell and not all cells in G0 are on the road to death. Upon appropriate stimulation some cell types may re-enter the cell cycle. Damage to the cellular DNA and other significant changes provoke the quiescent state of the cell. Therefore, it is important to note that the status of senescence and of quiescence are different things, since once a cell is entering the senescence process this is a point of no return, ultimately leading to controlled cell death (apoptosis). Quiescence on the other hand is reversible. The state of a shorter or longer lasting quiescence is not only observed at the cellular level but also in whole organisms. One key model organism of aging research, the nematode worm *Ceanorhabditis elegans* (*C.elegans*) is developing in four larval phases. When the environmental conditions (e.g. low nutrition supply, too many further larvae) are not in favor of further proceeding in the development, the second larval state is changing into a permanent one, called *dauer*-state (dauer larvae) which can last up to 3 months. Interestingly, this quiescent state in *C.elegans* is induced by a specialized steroidal hormone (the so-called dauer-pheromone). The dauer-quiescence is actually a survival strategy of the worm development to overcome special or unfavourable conditions. While most nerve cells permanently remain in the G0-phase some cell types are in G0 for weeks and months and eventually reenter the cell cycle, including liver cells (hepatocytes) and lymphocytes. To do so cells need to be stimulated by special conditions and external growth factors. The knowledge about such external stimulatory signals that bring cells back into cycling or keep them permanently in the cell cycle is of pivotal importance for the understanding of various human disorders. Constant cycling triggered by uncontrolled growth input is a hallmark of cancer cells. As the understanding of any medical problem starts with the understanding of the basic molecular mechanisms and key control switches of pathological processes, here, the main executors of cell cycling should be shortly summarized.

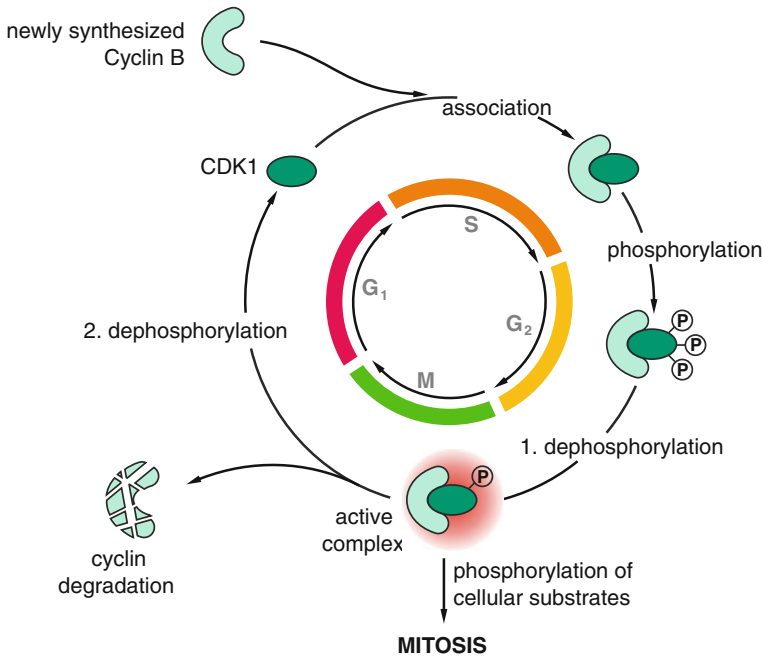


Fig. 2.2 Regulation of the cell cycle by cyclins/CDKs Cyclins and cyclin-dependent kinases (CDKs) interact physically. A sequence of phosphorylation and dephosphorylation represents respective cell cycle phases. While after second dephosphorylation the CDKs enter a new round of cell cycling, cyclins become degraded and newly synthesized when needed (by the example of CyclinB/CDK1; modified after Müller-Esterl 2011)

2.2 About Cyclins and Cyclin-Dependent Kinases: Proteins that Trigger Cell Cycle Phases

The process of the cell cycle, the exact order of the phases as well as the metabolic and synthetic effort in the respective single phases needs to be strictly controlled. And cellular functions as well as their control and supervision are executed by specialized proteins. Two groups of proteins are essential for this cell cycle control, so-called cyclins and cycline-dependent kinases, CDKs (Fig. 2.2).

In more molecular detail: kinases are specialized enzymes that transfer phosphate groups mostly from ATP (adenosine triphosphate, generally speaking, the energy currency in cells) to specific substrates and are, therefore, phosphotransferases. While kinase is the name of the enzyme, the process itself is called phosphorylation and is one of the most frequent occurring post-translational modifications of proteins and essential for the regulation of the function of many proteins. More than 500 kinases are known in mammalian cells being key intracellular signal transmitters. In general, the residues of three amino acids in protein sequences are phosphorylated in most cases. Based on their biochemical structure these amino acids are threonine,

serine and tyrosine. Kinase-driven phosphorylation can occur on multiple sites in proteins. And exactly this is happening in cell cycle control since a timely controlled transfer of phosphate groups and their removal which is biochemically performed by enzymes called phosphatases, actually controls the activity state of the cell cycle-associated proteins, the cyclins. Cyclins and CDKs are closely associated in the cell and the actual phosphorylation and dephosphorylation state of the cyclin is the central regulation signal for the individual cell cycle phase. Today, at least eight types of cyclins (cyclin A-H) and nine different CDK variants (CDK1-9) are described. Based on a huge amount of data, it is known that mainly cyclin A-E and CDK1, -2, -4, -6 are directly affecting the cell cycle.

The cyclins can be divided into general classes that are each defined by the stage of the cell cycle in which they bind CDKs. The three classes that are essential in eukaryotic cells are: (1) G1/S-phase-cyclins that bind CDKs at the end of G1 and lead the cell to the replication of its DNA, (2) S-phase-cyclins binding CDKs in the S-phase and being essential for turning on the DNA replication, and (3) mitose-cyclins that promote and drive the process of mitosis. Since cell cycle control is excellently presented and discussed in major text books of molecular biology and only the basics can be addressed here, a short summary can be as follows: The actual cell cycle phase is determined by three major parameters, (1) the exact stoichiometric ratio of cyclins to CDKs, (2) the biochemical activity of these proteins determined by the phosphorylation state, and (3) the actual direct molecular (physical) interaction of these proteins (shown for cyclins in Fig. 2.2). The activity of the CDKs can be switched off by phosphorylations with inhibitory effects as well as by proteins, so called CDK inhibitor proteins (CKI, eg. p21 is a potent cyclin-dependent kinase inhibitor) that resemble the “controllers of the controllers” (for details, see Alberts et al. 2007). The control and monitoring systems of the cell cycle has been studied in molecular detail in yeast and the results are transferable to human cells. The cell cycle control proteins are highly conserved during evolution, so proteins of lower organisms and cells (e.g. yeast) can perform their function in mammalian cells and vice versa. So, we now know that the intimate cooperation combined with reversible phosphorylation of cyclins mediates and determines the actual cell cycle phase and, consequently, an upstream regulatory mechanism is necessary to control these cell cycle executors. Intracellular proteolysis is such a regulatory mechanism that controls the presence of proteins in cells. Most importantly, cyclin-CDK complexes are inactivated by regulated proteolysis that occurs via the ubiquitin-proteasome system, one of the two key protein degradation pathways in cells, which will be discussed also later in the context of their role for cell function and aging. The cell cycle-associated proteins are undergoing cyclical proteolysis to control their actual intracellular levels.

It was already mentioned that to maintain genome stability the DNA of the cycling and dividing cell needs to be transmitted without any changes or damage. Therefore, as soon as the cycle is started mistakes absolutely have to be avoided because otherwise changes in the genome would be transferred directly to the daughter cells. Consequently, all steps and phases of the cell cycle are closely monitored and several control and restriction points exist mediated by different classes of proteins. The main task of this control mechanisms is the prevention of the propagation of DNA

misinformation that can lead to uncontrolled cell proliferation, ultimately causing tumor formation. To underline the key importance of cell cycle control mechanisms and its enduring significance for the understanding of pivotal processes in modern medicine and the development of disease the Nobel Prize in Physiology or Medicine has been given to Leland H. Hartwell, Tim Hunt and Paul M. Nurse in 2001 who all contributed to a better understanding of this fundamental process.

2.3 Better Save than Sorry: The Complex Control of the Cell Cycle

The control of cell cycling is a hot topic of molecular research although many details are already known. Here, the two main control proteins will be introduced, the retinoblastoma protein (Rb) and the protein 53 (p53). A lot of insight into cell cycle control has been gained by cancer research since, obviously, cells with unlimited proliferation as seen in cancer are the result of escaping from this control and any type of restrictions. Looking closer into cancer cells and their cell cycle it is found that frequently the control of G1 progression and S-phase initiation is disrupted, ultimately leading to an open and unrestrained entry into the cell cycle and proliferation. One important player in this context is a gene regulatory protein called E2F (a transcription factor) that binds to specific DNA sequences in promoters of genes that encode proteins necessary for the cell's entry into S-phase, including G1/S-cyclins and S-cyclins. Upstream its transcriptional activity the protein E2F itself is controlled by a direct interaction with a protein called Rb. Rb stands for retinoblastoma which are tumors of the human retina originally detected in an inherited form of eye cancer in children. Mutations in Rb lead to tumor formation, non mutated, i.e. wildtype Rb is suppressing tumor formation by interfering indirectly with the cell cycle (via E2F). Therefore, functionally intact Rb is a classical inhibitor of cell cycle progression. As shown in Fig. 2.3, during the G1-phase non-phosphorylated (active) Rb protein is associated with E2F. The Rb-E2F binding at the DNA inhibits the transcription of genes of the S-phase. In cells that are stimulated to proliferate, e.g. by extracellular growth factors, cyclin-CDK complexes accumulate leading to the phosphorylation and inactivation of Rb. The phosphorylation of Rb then causes a decrease in its affinity to E2F and finally the complete dissociation. E2F protein liberated from Rb leads to the activation of the transcription of S-phase genes. Taken together, by directly interacting with E2F and modulating its function, Rb is an inhibitor of cell-cycle progression preventing uncontrolled proliferation. Due to this prominent activity Rb is also called a tumor suppressor protein (Lombard et al. 2005). Considering that a single mutation in the gene coding for Rb leads to cancer development as seen in retinoblastoma underlines the key role for Rb as tumor suppressor. Rb does not stand for a single protein but rather for a family of proteins. But recently, the view of Rb as well known tumor suppressor controlling cell cycle progression and cell proliferation was extended. Interestingly, it has been found that Rb plays also a role

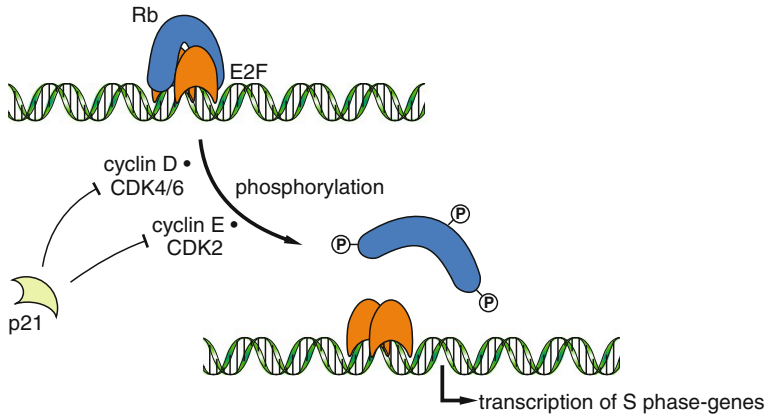


Fig. 2.3 The tumor suppressor Rb: Mode of action. The active retinoblastoma protein (Rb) binds to the transcription factor E2F. This Rb-E2F protein complex at the DNA blocks transcription of S-phase genes. Following stimulation Rb becomes phosphorylated (by the Cyclin D-CDK4/6 complex or CyclinE-CDK2 complex) leading to its displacement from E2F and the DNA. This dissociation allows transcription of S-phase genes (modified after Müller-Esterl 2011)

in the maintenance of genomic stability. Dysfunctional Rb protein drive the instability of chromosomes and aneuploidy, meaning an abnormal number of chromosomes (Manning and Dyson 2012).

Another key protein that prevents the uncontrolled proliferation of cells and, therefore, also acts as tumor suppressor, is the protein p53 (53 meaning its molecular weight of 53 kilodalton). Acting in a completely different way than Rb, p53 represents a checkpoint protein halting the cell cycle upon DNA damage. Cells are permanently confronted with a variety of external challenges. Of special interest is radiation, in particular, the ongoing UV and ionizing radiation caused by the atmosphere (“Höhenstrahlung”) that both can cause direct damage to the DNA. Moreover, chemicals and toxins such as compounds that may intercalate into the double helix structure of the DNA may cause DNA damage, subsequently disturbing processes linked to the genomic DNA (e.g. transcription, replication). The most prominent and evident lesions at the DNA are crosslinks of the DNA helix double strand and other structural damage such as breaks of the DNA strand (discussed also later in the book). Beyond the disturbance of replication and transcription DNA damage can lead to changes in the DNA sequence, i.e. mutations that are if not removed conservatively inherited from the mother cell to the daughter cells which may again directly cause cellular deterioration, dysfunction and tumor formation. Since such DNA damaging events are rather frequent, cells do have intrinsic DNA repair mechanisms that can reverse damage and maintain the correct DNA structure and sequence. To allow this machinery which will be introduced later in some more detail the time for repair, upon DNA damage, cells in cycle are halted at the p53 checkpoint in the late G1-phase. p53 is a cellular key player that upon genotoxic and other stresses is activated by the upregulation of its protein level as well as by regulatory modulation (e.g. phospho-

rylation). p53 is involved in several pivotal signaling pathways, its specificity being governed by the interaction with other cellular proteins. Most important, p53 acts as a transcription factor inducing the expression of genes mediating growth arrest, DNA repair and apoptosis (see below). The G1 checkpoint is accomplished by p53 transactivating the CKI (cyclin-dependent kinase inhibitor) protein p21 that blocks G1/S-CDK complexes (Vousden and Lu 2002; Vogelstein et al. 2000; Tokino and Nakamura 2000). Moreover, it was shown that p53 takes direct part in the repair of double strand breaks by controlling the fidelity of recombination processes and thus exhibits functions counteracting carcinogenesis beyond cell cycle checkpoint control (Bertrand et al. 2004; Gatz and Wiesmüller 2006).

2.4 Last Exit Apoptosis

Mutations in the p53 gene leading to a dysfunctional gene product are very frequent in human tumors and observed in approximately half of all cancer pathologies. In case that the repair mechanisms are not sufficiently working or the DNA damage is just too severe, the intrinsic p53 control mechanism develops another function. Since during evolution the concept was successful that the health of the whole organism is more important than the survival of an individual cell which accumulates DNA damage and, subsequently, inherits mutated tumorigenic DNA putting the organism in danger, p53 activity can push such a cell into controlled cell death called apoptosis (Fig. 2.4). In fact, this particular function of p53 is a key for the prevention of cancer formation. It also demonstrates why in so many cancer types mutated and mal- or dysfunctional p53 is observed. Taken together, the correct function of p53 and Rb blocks uncontrolled cell division and actively prevents tumor formation. At the molecular level p53 and Rb are linked via the protein p21 (Figs. 2.3 and 2.4). The p53-mediated process to drive a damaged cell into the controlled suicide (apoptosis) can be seen as *exit strategy* to get rid of potential tumor precursor cells with accumulated DNA damage and to rescue the rest of the organism. In more recent literature it is also emphasized that “It is being recognized as a critical feature of mammalian cells to suppress tumorigenesis, acting alongside cell death programs” (Kuilman et al. 2010).

It is a key feature of tumor cells that they are running out of control and keep on passing through the cell cycle and mitosis. But in most (non transformed) mammalian cells the potential to divide and, therefore, to run through cell cycles is limited indicating that there is a potential physiological endpoint of cellular life, the cellular senescence. This limited replicative potential of cells has been shown in primary cells put out of an organism into the culture dish and is called the “Hayflick limit” of mammalian cells (Hayflick and Moorhead 1961). In 1965 Leonard Hayflick further hypothesized that “the finite lifetime of diploid cell strains in vitro may be an expression of aging or senescence at the cellular level”. Considering this view cellular senescence means a stable and long-term loss of proliferative capacity, despite continued cellular viability and metabolic activity (Hayflick 1965; for review: Kuilman

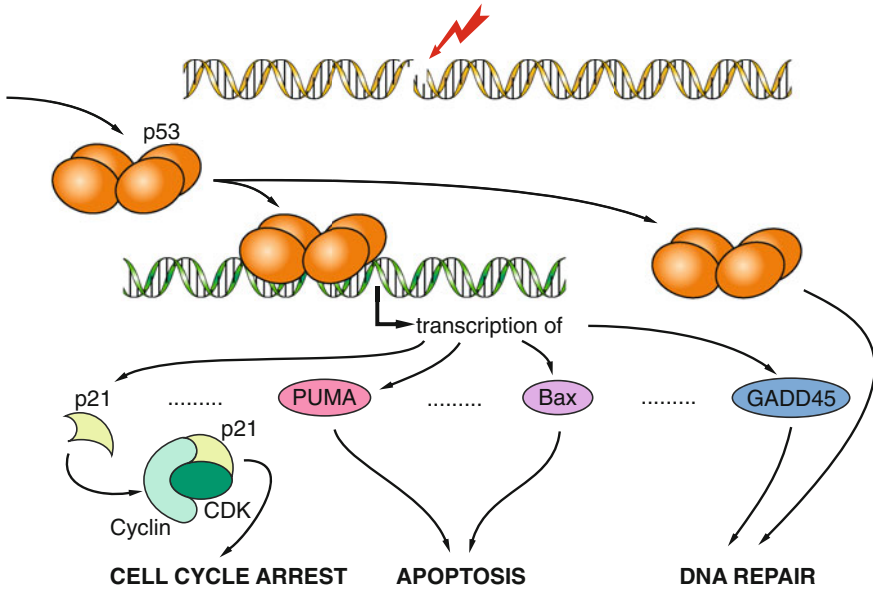


Fig. 2.4 The tumor suppressor p53: simplified mode of action. Upon DNA damage (e.g. strand breaks) as caused, for instance, by UV or ionizing radiation p53 is activated and induces the transcription of genes controlling cell cycle arrest (e.g. p21), apoptosis (e.g. Bax, PUMA) and DNA repair (e.g. GADD45). In addition p53 can also directly affect DNA repair

et al. 2010). Senescent cells can be identified also in tissues *in vivo*, where the actual cause why these cells become senescent is not exactly known. It is speculated that frequently senescence may be the result of the activities of activated oncogenes. The fate of senescent cells *in vivo* was unknown for long time, but recent new evidence suggests that these cells are cleared and removed by the innate immune system. Therefore, senescence and apoptosis can be also addressed as pathways occurring in parallel. Via both processes significantly damaged cells are eliminated from the body. Nevertheless, it is reported that some senescent cells persist in tissues and the number is increasing with age. It is currently hypothesized “that these persistent senescent cells have adverse effects on tissue function. If so, senescence may be an example of antagonistic pleiotropy, providing an anticancer mechanism in early life but having adverse effects on tissue function in late life” (Hornsby 2010).

In trying to explain the molecular basis of the clockwork of replicative senescence as consequence of the Hayflick limit one realizes that the limitation of the number of cell divisions may lie in the power and effectiveness of the enzymatic machineries of DNA replication and the particular physical structure of the chromosomes, namely their end structures, the telomeres. And this, actually is also the rational and basis of an important theory of aging, the *telomere theory of aging* that can be lined up as one key theory of aging which will be introduced in the next chapter.

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