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## Cellular models for fundamental and applied biomedical research

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# Chapter 1

General introduction and outline of thesis

## Background

Cell culture models play an important role in biomedical research and will continue to do so given the growing opposition to vivisection and the limited predictive value of animal models for human disease. Moreover, cell culture models can be easily established to mimic physiological or pathological processes, which is difficult to accomplish using *in silico* models. While non-cellular *in vitro* models are highly suitable for studying simple biochemical processes, cell culture models recapitulate many of the complex regulatory circuits governing protein activity *in vivo* and hence allow investigation of diverse physiological processes. Also, cell culture models offer the possibility to address fundamental research questions in a much more simplified, specific and controllable manner than can be achieved using *in vivo* models.

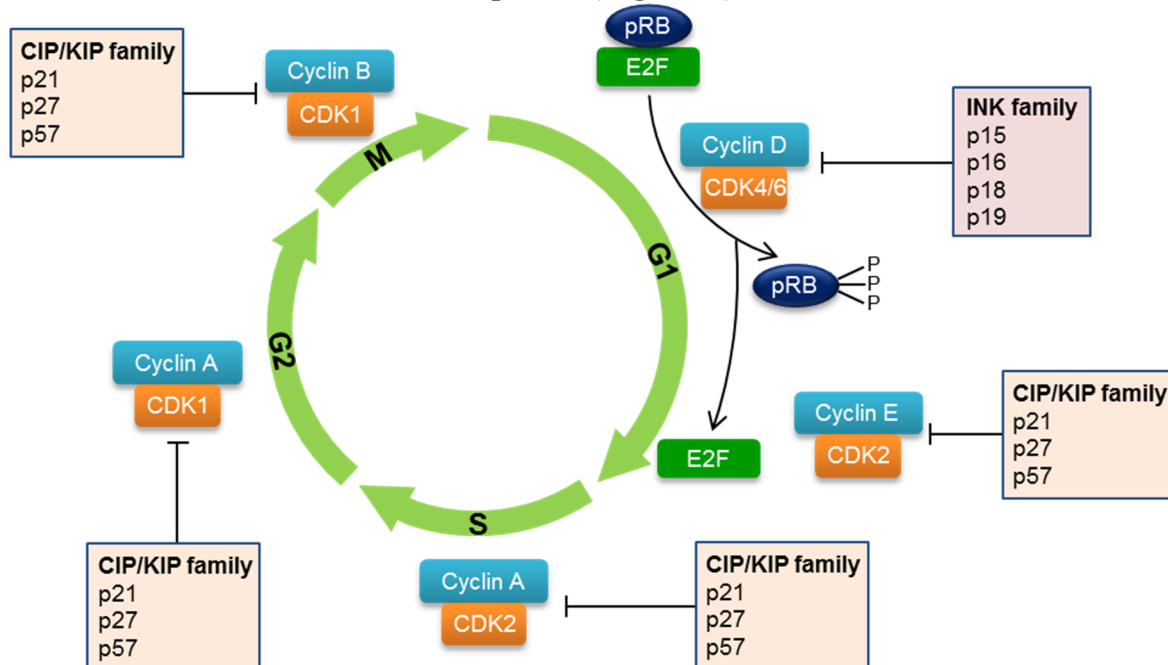
Aging is a complex and multifactorial process driven by genetic and environmental factors and govern by various interacting molecular pathways that lead to the physiological decline of biological systems. A variety of experimentally tractable cellular models were employed in recent years to study the basic mechanisms of aging, knowledge of which is essential to the development of effective therapeutic interventions against age-related diseases<sup>1</sup>. Due to the complexity of striated muscle structure and function, standardized cell culture models are a real asset for studying skeletal and cardiac muscle biology and disease. Cultures of skeletal myoblasts or cardiomyocytes (CMCs) can mimic physiological or pathological conditions, making them well-suited for proof-of-concept studies and for developing novel therapeutic interventions for specific diseases<sup>2,3</sup>. Especially, cultured cardiac cell monolayers have become a popular model system for electrophysiological studies<sup>4</sup> into the mechanisms of cardiac arrhythmias<sup>5-7</sup> as well as drug-induced ventricular pro-arrhythmic effects<sup>8</sup>. Brown adipose tissue (BAT) represents a potential therapeutic target to treat obesity and associated metabolic disorders because of its capacity to ingest and combust glucose and fatty acids for thermoregulation<sup>9</sup>. The properties of BAT has been investigated using cultures of primary brown preadipocytes (BPAs). Although these studies have yielded new insights into adipocyte biology<sup>10-12</sup>, the full potential of BAT culture models has not been utilized due to the scarcity of starting material and the limited proliferation capability of primary BPAs. A potential solution to this problem would be to establish lines of BPAs by cellular immortalization. Although cell immortalization offers the possibility to produce virtually unlimited cell sources by inducing cell cycle reentry and by bypassing cell senescence, generation of robust cell lines that can recapitulate (most of) the properties of the primary cells from which they were derived requires a thorough understanding of the mechanisms of cell quiescence, proliferation, differentiation, senescence and apoptosis.

## Cell cycle

To divide, a cell must grow, copy its genetic material (DNA), and physically split into two daughter cells. Cells perform these tasks in an orderly and tightly regulated series of events known as the cell cycle, which is divided into two major phases, the interphase and the mitotic phase. During the interphase, the cell grows and duplicates its nuclear DNA. The interphase is divided in three subphases. During gap phase 1 (G1 phase), the cell grows physically larger, copies organelles except for the nucleus

and synthesizes the molecular building blocks needed for the next subphase. In the synthesis (S) phase, the cell copies its chromosomal DNA and duplicates a microtubule-organizing structure called the centrosome, which helps to separate the DNA during the mitotic phase. During gap phase 2 (G2 phase), the cell grows more, makes additional macromolecules and organelles, and begins to reorganize its contents in preparation for mitosis. In the mitotic phase, the cell's chromosomal DNA is equally separated into two new daughter nuclei and the cell's cytoplasm and organelles are split after the formation of a cleavage furrow producing two new daughter cells. The segregation of duplicated chromosomes into daughter nuclei is called karyokinesis; the separation of cytoplasm and organelles is called cytokinesis.

The cell cycle is controlled by the activity of cyclin-CDK (cyclin-dependent kinase) complexes. CDKs are always present but need to be activated by phase-specific cyclins. Mitogens induce the synthesis of cyclin D early in the G1 phase. Cyclin D associates with CDK4 and CDK6 resulting in the hyperphosphorylation of the retinoblastoma protein pRB and the disruption of complexes between this pocket protein and several members of the E2F family of transcription factors. This allows these E2F family members to form a complex with the transcription factor DP (dimerization partner) and to induce the expression of various genes involved in cell cycle progression including the gene encoding cyclin E which is necessary for G1/S transition. CDKs are further activated during cell cycle by cyclin A and B to drive transition from S phase to G2 phase and from G2 phase to M phase, respectively. The activities of cyclin-CDK complexes are regulated by CKD inhibitors including the INK4 family (p15, p16, p18 and p19) and CIP/KIP family (p21, p27 and p57) which bind to CDKs and inactivate the complexes (**Figure 1**).

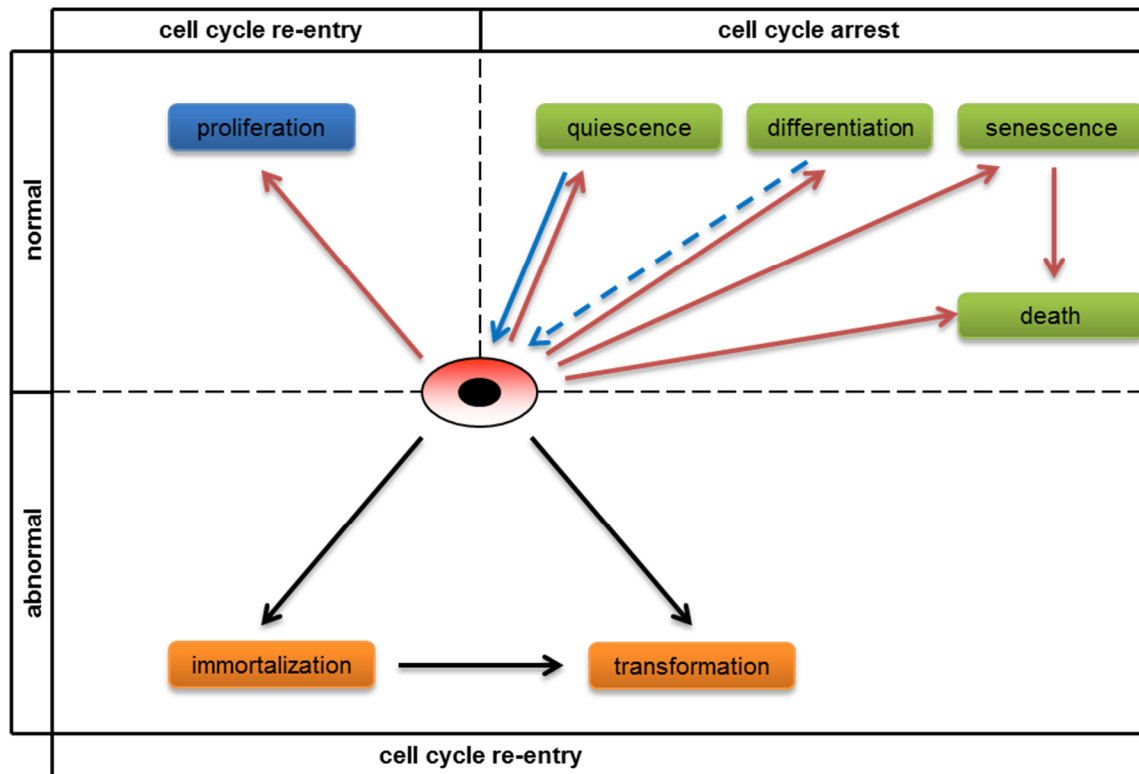


**Figure 1. Cell cycle regulation.**

The CDK-cyclin complexes promote cell cycle progression, while the CDK inhibitors stop it. CDK4/6-cyclin D is activated in phase G1, CDK2-cyclin E in phase G1/S, CDK2/1-cyclin A in phase S/G2 and CDK1-cyclin B in phase M. The CIP/KIP family (p21, p27 and p57) inhibit a wide range of cyclin-CDK complexes, yet the INK4 family (p15, p16, p18 and p19) specifically diminish CDK4/6 activity.

## Cell fates

The fate of a cell is determined by the changes in its gene expression pattern and associated activities, which will lead to cell proliferation, quiescence, differentiation, senescence or death (**Figure 2**). Mitotic cells such as cells in the skin and gut repetitively progress through phases G1, S, G2 and M of the cell cycle. Mitotic cells can also enter the G0 phase of the cell cycle. This state of cell cycle arrest is observed in quiescent, (terminally) differentiated and senescent cells. Quiescent cells such as adult stem cells, fibroblasts and lymphocytes are temporarily arrested in G0 phase and readily reenter the cell cycle when exposed to mitogens. Dormant cells are quiescent cells showing very low metabolic activity, which is frequently interpreted as being in a deeply quiescent state. Cells like adipocytes, cardiomyocytes and neurons are believed to lose the capacity to enter the cell cycle and to permanently reside in the G0 phase of the cell cycle once they are fully differentiated. However, recent studies have shown that both self-renewal of cardiac tissue and regeneration upon damage mainly results from the cell cycle reentry and proliferation of existing cardiomyocytes<sup>13-18</sup>. In general mitotic cells will stop dividing and become senescent after a limited number of divisions. Senescent cells cannot replicate anymore because of telomeres shorting, irreparable DNA damage, nutritional deficiency, harmful chemicals, oxidative stress or physiologic stress. Except for the stem, progenitor and precursor cells that escape from death by being converted in more differentiated cell types, all other cells finally die because of natural turnover or due to injury, including the death of the organism. Cell deaths can occur by various different mechanisms. Two of the major types of cell death are apoptosis and necrosis<sup>19</sup>. Apoptosis, also known as programmed cell death, is a normal physiological, non-inflammatory process to remove unwanted cells. In this process, apoptotic bodies are formed to confine cell organelles and other parts, which will be phagocytosed by macrophages. Necrosis, also known as unprogrammed cell death, is a less orderly process than apoptosis, in which cells and organelles swells resulting in membrane rupture. As a consequence, the cellular content is released in the extracellular space triggering an inflammatory response and possible causing further damage to adjacent cells<sup>20</sup>. A major difference between normal somatic cells and transformed cells is their proliferation potential. Normal somatic cells in culture have a limited ability to multiply themselves known as the Hayflick limit (see below), while transformed cells have evaded normal cellular senescence due to mutations resulting in acquisition of unlimited proliferation ability, evasion of apoptosis, growth factor independency, non-responsiveness to growth inhibitors, promotion of angiogenesis and invasive capacity. Immortalized cells have not necessarily undergone neoplastic transformation, but have accumulated sufficient mutations to proliferate unlimitedly. Upon transplantation immunocompromised mice, transformed cells will form tumors in contrast to immortalized cells. More details about cellular senescence, quiescence and immortalization are described below.



**Figure 2. Possible cell fates.**

Under normal conditions, cells adopt one of five distinct cell fates: they can remain in the cell cycle and continue to proliferate or leave the cell cycle and become quiescent, differentiated or senescent or undergo apoptotic or necrotic cell death. Under pathological conditions, cells can escape from senescence or apoptosis and become immortalized or transformed following the activation of endogenous oncogenes and/or the inactivation of endogenous tumor suppressor genes. Cell immortalization can also be brought about by oncogenic viruses or via genetic engineering.

## Cellular senescence

### Senescence as a common stress response

Normal mammalian somatic cells such as fibroblasts, myoblasts and preadipocytes, grown in culture fail to proliferate after a finite number of divisions and ultimately encounter senescence. This phenomenon was first found in human fibroblast cultures by Hayflick<sup>21</sup> and later referred to as replicative senescence<sup>22-24</sup>. Replicative senescence is thought to be the result of progressive telomere shortening as a cell-intrinsic mechanism<sup>25,26</sup>. Cellular senescence can also be stimulated by various types of cell stress like lack of nutrients, harmful chemicals (e.g. cytostatic drugs), oxidative stress or physiologic stress (e.g. caused by aberrant expression of tumor suppressor or oncogenes and excessive growth factor stimulation), which block the proliferation of cells<sup>27-29</sup>. This type of senescence is referred to as premature or induced senescence<sup>30</sup>. These findings have led to a distinction between “replicative senescence” which is induced by a cell-intrinsic mechanism, and “stress-induced premature senescence” which is triggered by extrinsic factors<sup>31</sup>. In many cases, replicative and premature senescence are interconnected. Telomere shortening is, for instance, accelerated by oxidative stress. Actually, cells may suffer cumulative damage from multiple stresses during culture. Senescence will be triggered when cellular damage has reached a

certain threshold, whatever has caused the damage. Senescent cells undergo dramatic changes in morphology and function. They generally become enlarged and flattened, have an increased lysosomal content, suffer from mitochondrial dysfunction and secrete increased amounts of inflammatory, growth-promoting, and remodeling factors. They may also form senescence-associated heterochromatin foci, often display a chronic DNA damage response, are incapable of synthesizing DNA for mitosis, show high resistance to apoptosis and are unresponsive to mitogenic stimuli. However, senescent cells are still metabolically active for months or even years<sup>27,32-35</sup>.

### **p53 and pRB as key regulators of senescence**

Molecular pathways of senescence are complex involving multiple layers of regulation with cooperative changes. The p53 tumour suppressor protein and the pocket proteins (*i.e.* pRB, p107 and p130) have been shown to play critical roles in the induction of senescence. The p53 protein is an important cell cycle regulator of both the G1/S and G2/M checkpoints by activating CDKN1A transcription. CDKN1A codes for p21 protein, which is a member of the CIP/KIP family of tumor suppressor proteins. These proteins inhibit various cyclin-CDK complexes required for promotion of the cell cycle (**Figure 3**). Hypophosphorylated pRB controls the G1/S checkpoint by directly binding to and inhibiting various E2F family members thereby repress gene transcription required for transition from the G1 to S phase of the cell cycle. In actively dividing cells, the suppressive effect of pRB on E2Fs is lost due to hyperphosphorylation of pRB by cyclin D-CDK4 and cyclin D-CDK6 complexes. In senescent cells the activity of these complexes is inhibited by members of the INK4 family of tumor suppressor proteins<sup>36</sup>. (**Figure 3**).

Numerous studies have explored the roles of p53 and pocket proteins in the induction and maintenance of senescence in mouse embryo fibroblasts (MEFs). Suppression of p53 expression allows cells to escape from senescence and leads to their indefinite expansion<sup>37</sup>. Inactivation of pRB alone is not enough to ease senescence of MEFs, while accompanying ablation of other pocket proteins including p107 and p130 strongly increased their proliferation capacity<sup>38,39</sup>. This demonstrates that the inactivation of either p53 or all three pocket proteins is sufficient to block the initiation of senescence. Suppression of either p53 or pRB in senescent MEFs is not enough to maintain senescence but leads to reactivation of the cell cycle<sup>37,40</sup>. Collectively, these findings suggest that p53 and the pocket proteins control cellular senescence in a hierarchical manner, in which the activation of pocket proteins is downstream of that of p53. Indeed, as mentioned before, p53 activates p21, which in turn inhibit cyclin-CDK complexes involved in the phosphorylation and inactivation of the pocket proteins. However, MEFs lacking p21 still undergo senescence and have a lifespan similar to wild-type cells<sup>41</sup>, which shows that p21 is not the only link between p53 and pocket protein activity in the regulation of senescence (**Figure 3**).

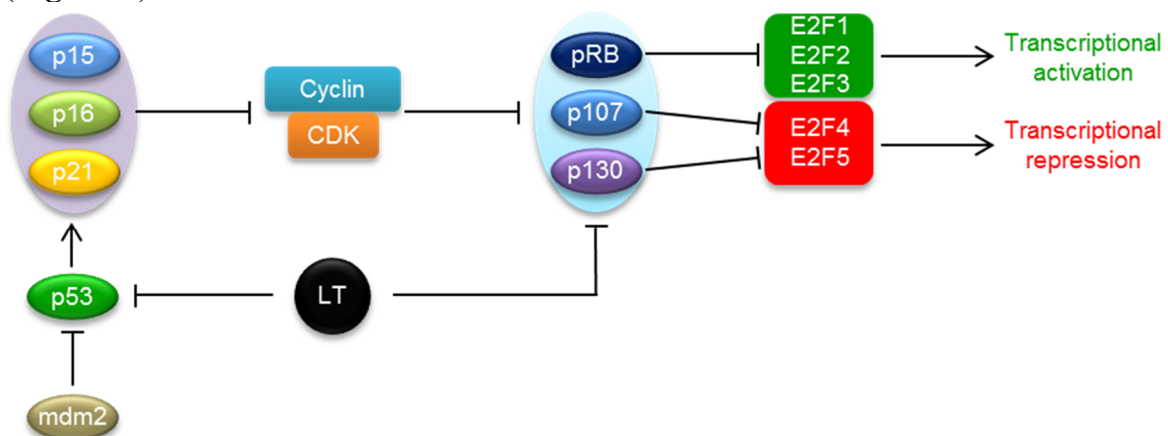
## **Cellular quiescence**

As indicated above, cells can enter into either of three different G0 states. While senescent and (terminally) differentiated cells have permanently exited the cell cycle (irreversible G0 arrest), quiescent cells only temporarily reside in the G0 phase



(reversible G0 arrest) and reenter the cell cycle in response to normal physiological stimuli<sup>42</sup>. The notion that terminally differentiated cells are irreversibly arrested in G0 has been challenged by the finding that mature hepatocytes dedifferentiate and reenter the cell cycle after injury of the liver and contribute to its regeneration<sup>43-46</sup>. Similar observations have recently been made for terminally differentiated cardiomyocytes<sup>13-18</sup>. Hence, not only quiescent cells but also terminally differentiated cells can exit the G0 phase and resume cell division.

In quiescent cells, p53 has been considered as main molecular regulator of cell cycle arrest<sup>47</sup>. Similar to senescence, the mechanism by which p53 mediates quiescence is dependent on the activation of p21, a CDK inhibitor and critical component of the pRB-E2F pathway<sup>48</sup>. In addition to p53, quiescent cells typically feature lower levels of pRB-E2F pathway activators, such as cyclin D<sup>49-51</sup> and high levels of pathway repressors, such as p21<sup>48</sup>. The disruption of all three pocket proteins and the acute loss of pocket protein function in quiescent cells lead to cell proliferation<sup>38-40,52</sup>. pRB deficiency in quiescent skeletal stem cells leads to an increase of proliferation and a loss of terminal differentiation capacity<sup>38,53</sup>. All of the above suggest that the RB-E2F pathway plays a pivotal role in quiescence. Among the E2F family of transcription factors, E2F1, -2 and -3 are considered “E2F transcriptional activators” and E2F4 and -5 are deemed “E2F transcriptional repressors”<sup>54</sup>. In cell cycle-arrested cells, hypophosphorylated pRB prevents cell cycle reentry by binding and repressing E2F1, -2 and -3, which activate genes required for the transition from G1 into S phase. In addition, hypophosphorylated p130 and p107 form complexes with E2F4 and -5. The complexes bind to the promoters of E2F target genes and recruit chromatin remodeling factors that block transcription<sup>55,56</sup>. In quiescent cells that are stimulated with mitogens, cyclin-CDK complexes phosphorylate pRB thereby releasing E2F1, -2 and -3. Similarly, phosphorylation of p130 and p107 abolishes their interaction with E2F4 and -5 repressors<sup>57,58</sup>. Especially E2F4-p130 complexes are very prominent in resting (*i.e.* G0-arrested) cells suggesting that they play a pivotal role in the maintenance of cell quiescence<sup>59-61</sup> (**Figure 3**).



**Figure 3. Participation of cell cycle regulators in cell cycle arrest.**

p53 activates CDK inhibitors (e.g. p15, p16 and p21), which in turn disrupts cyclin-CKD complexes involved in the inactivation of the pocket proteins (pRB, p107 and p130). This allows the pocket proteins to arrest cell in a non-proliferative state by blocking the transcription of E2F target genes. LT, large tumor antigen. mdm2, mouse double minute 2.

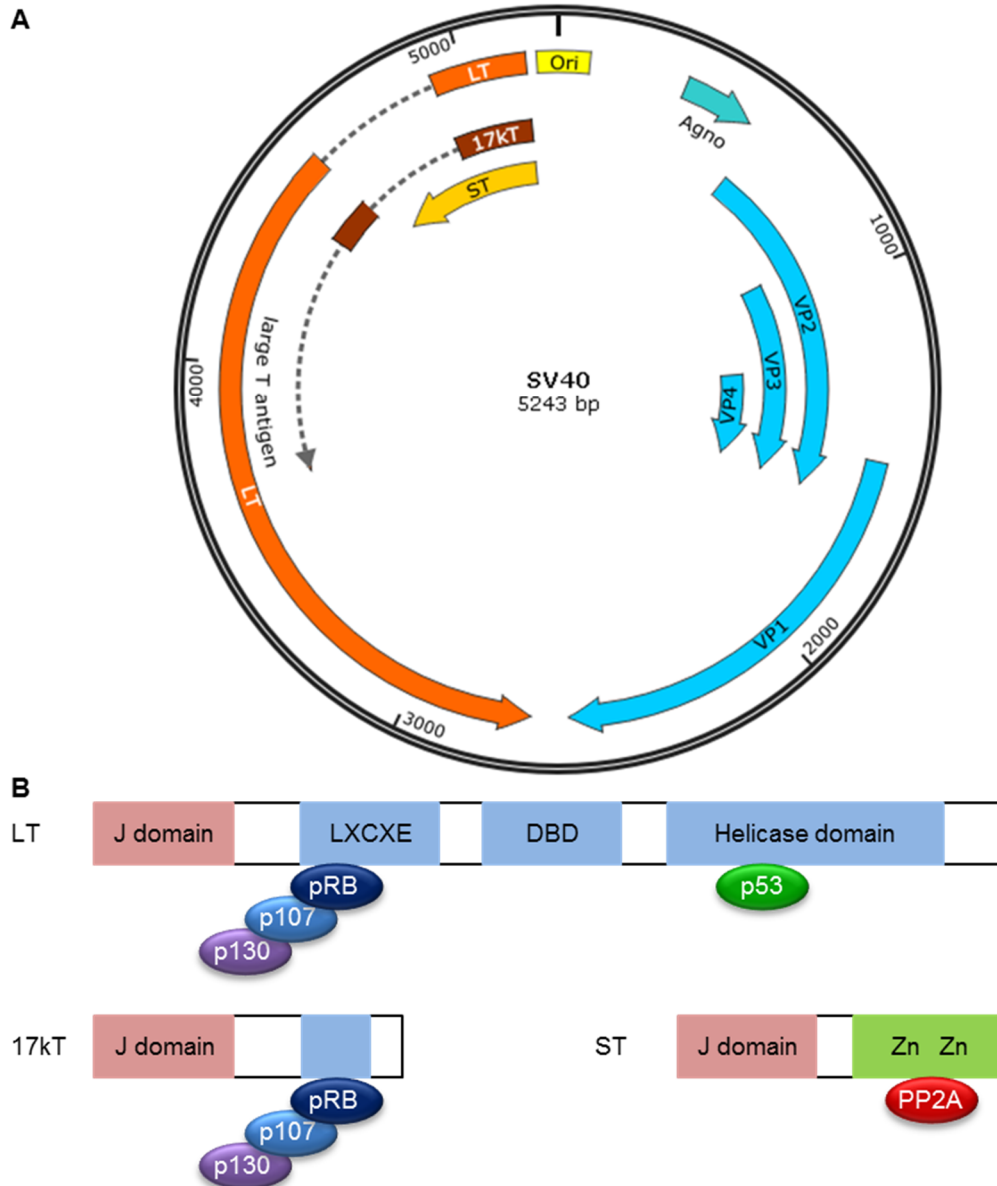
## Simian virus 40 LT antigens & cell immortalization

Simian virus 40 (SV40) is a small double-stranded non-enveloped DNA virus with a circular genome and an icosahedral capsid in the family of Polyomaviridae. The SV40 genome is composed of three main regions: (i) an early region encoding large tumor antigen (LT), 17kT and small tumor antigen (ST), (ii) a late region encoding the viral coat proteins (VP1, VP2, VP3 and VP4) and the agnoprotein (Agno) and (iii) the regulatory region containing the early and late promoter and the origin (Ori) of replication (**Figure 4A**). LT, 17kT and ST are produced from alternative splice products of the early viral pre-mRNA and are co-expressed in infected cells. The amino-terminus of LT contains a J domain which contributes to viral DNA replication<sup>62</sup>. Downstream of the the J domain, there is a LXCXE motif that can directly bind to the pocket proteins (*i.e.* pRB, p107 and p130)<sup>63,64</sup>. The J domain cooperates with the LXCXE motif to disrupt the interaction between E2F family members and the pocket proteins<sup>63</sup>. The DNA-binding domain (DBD) of LT recognizes Ori sequences and this interaction is essential for the initiation of viral DNA replication. The helicase domain works as DNA helicase to unwind the dsDNA template for viral replication and it also interacts with p53<sup>65</sup>. ST contains a J domain followed by a cellular protein phosphatase 2A (PP2A) binding region which contains two zinc fingers. ST binds the A and C subunits of PP2A and displaces the B subunit, thereby inhibiting the pro-apoptotic activity of PP2A<sup>66,67</sup>. 17kT shares several regulatory domains with LT that mediate some of its most important functions including stimulation of cell proliferation by binding to pocket proteins<sup>68</sup> (**Figure 4B**).

LT plays an essential role in multiple steps of the viral life cycle. After infection, LT induces host cells to enter the S phase thereby creating optimal conditions for the replication of viral DNA. Meanwhile, LT works as the DNA helicase and then utilizes the host's replication machinery to replicate the viral DNA. Besides, LT is also involved in transcription and virion assembly. Given the capability of LT to incite cell proliferation, LT has been successfully used to immortalize multiple primary cell types with no/limited proliferative capacity *in vitro*<sup>69-71</sup>. Our current understanding indicates LT's ability to drive cell proliferation is mainly due to its interaction with the pocket proteins and p53<sup>72,73</sup>. Quiescent cells including differentiated cells are trapped in G0 by the pocket proteins<sup>42</sup>. Hypophosphorylated pocket proteins bind to E2Fs and thereby block gene expression required for nucleotide synthesis, DNA replication, cell cycle progression and apoptosis. When cells are mitogenically stimulated, pocket proteins become phosphorylated allowing E2F-mediated S phase gene transcription. Instead of disrupting E2F-pocket protein complexes by pocket protein hyperphosphorylation, LT binds to hypophosphorylated pocket proteins setting free the pocket protein-bound E2Fs resulting in E2F-dependent gene expression and cell cycle reentry<sup>74</sup>. The p53 level in normal cells remains very low because it binds to Mdm2 which induces ubiquitination and subsequent degradation of p53<sup>72,75</sup>. Besides Mdm2, many other proteins affect the biological activity and stability of p53 through various types of post-translational modifications. Different cellular stress conditions lead to stabilization of p53 allowing it to act as a transcriptional activator of genes mediating apoptosis or cell cycle arrest<sup>76,77</sup>. LT inactivates p53 by covering its DNA-binding domain and thus block the expression of

p53-dependent genes, which leads to cell cycle reentry and avoids cell cycle arrest as well as apoptosis<sup>78</sup> (**Figure 3**).

Exposure of SV40 particles to chemical mutagens has resulted in the recovery of several temperature-sensitive LT mutants (ts-LTs) that can cause cell immortalization/transformation at permissive temperatures ( $\leq 34^{\circ}\text{C}$ ) but fail to do so at nonpermissive temperatures ( $\geq 37^{\circ}\text{C}$ )<sup>79,80</sup>. These ts-LTs have been used to control the activity of LT allowing conditional immortalization of primary mammalian cells.



**Figure 4. Structure of SV40**

(A) Genomic structure of SV40. The circular double-stranded DNA genome contains three main regions: the regulatory region, an early region encoding LT, 17kT and ST, and a late region encoding the viral coat proteins VP1, VP2, VP3 and VP4 as well as the agnoprotein. (B) The functional domains of SV40 T antigens. The J domain is shared by all three antigens. LT also contains a pocket protein-binding LXCXE motif, a DNA-binding domain (DBD), a p53-binding helicase domain. 17kT shares several regulatory domains with LT including a pocket protein binding domain. ST contains a J domain followed by a PP2A-binding region with two zinc-fingers.

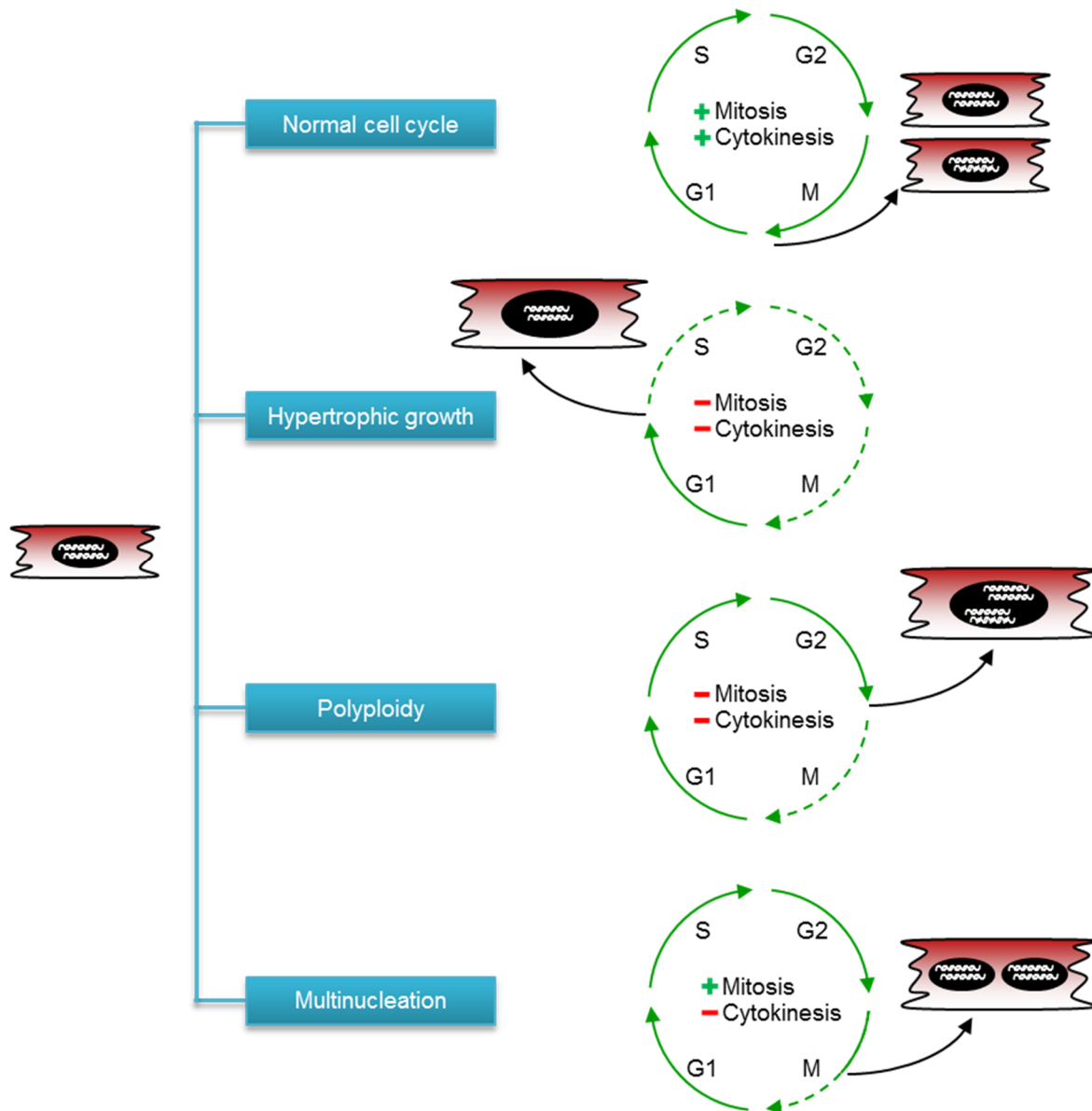
For instance, ts-LT tsA58, which carries an alanine to valine substitution at amino acid position 438 that abolishes its binding to and inhibition of p53 at nonpermissive temperatures<sup>81</sup> was expressed in transgenic mice to generate conditional immortalized cell lines from different tissues<sup>82,83</sup>.

## Cell cycle regulation in cardiomyocytes

In the embryonic and fetal hearts of vertebrates, including mammals, cardiomyocytes have high cell cycle activity and are capable of DNA synthesis, karyokinesis and cytokinesis resulting in cardiomyocyte division and an increase of cardiomyocyte number. However, the proliferative capacity of postnatal hearts differs from various species. Zebrafish have cardiomyocyte proliferative capacity throughout life. A complete regeneration of heart tissue lost from the resection of the ventricular apex can be managed in adult zebrafish through the proliferation of pre-existing cardiomyocytes<sup>15,84</sup>. In contrast, mammalian cardiomyocytes lose their proliferative capacity shortly after birth and the expansion of postnatal mammal heart driven by non-mitotic growth produces multinucleated or polyploid cells and hypertrophic cells<sup>85-88</sup>. In mice, during the first week of postnatal period, the majority of cardiomyocytes undergo karyokinesis in the absence of cytokinesis, which generates a population of binucleated cardiomyocytes<sup>85</sup>. In this event, cardiomyocytes enter the M phase of the cell cycle and perform nuclear division, but they do not undergo cytokinesis<sup>86,88</sup>. In contrast, the timeline of human cardiomyocyte proliferative capacity is not clear, however, more than half of all adult human cardiomyocytes are polyploid as a result of DNA duplication without nuclear division<sup>87</sup>. The polyploidization results from shuttling between G1, S and G2 phase without entering into the M phase. Although damage-induced cardiomyocyte proliferation in adult mammals has been observed<sup>13,89,90</sup>, the growth of stressed and injured mammalian hearts is largely caused by hypertrophy. Under physiological and pathological hypertrophy-stimulating conditions, cardiomyocytes enter the G1 phase of cell cycle where they grow in size by synthesis of extra RNAs and proteins, but they fail to undergo S phase<sup>91-93</sup> (**Figure 5**).

This different behavior of antenatal and postnatal cardiomyocytes is due to the developmental changes in cell cycle activity<sup>86,94</sup>. The cell cycle of cardiomyocyte is tightly controlled by transcription factors and regulators thereof like the pocket proteins, which play an essential role in cell cycle exit and differentiation of cardiomyocyte<sup>95</sup>. For example, p107 and p130 interact with E2F family members to inhibit DNA synthesis in the fetal and neonatal cardiomyocytes, respectively<sup>96</sup>. Moreover, pRB silences multiple proliferation-promoting genes in adult cardiomyocyte to maintain their post-mitotic state by interaction with heterochromatin protein 1 $\gamma$ <sup>97,98</sup>. Apart from the pocket proteins, transcription factors such as Meis1, MEF2D, FoxO1, and FoxO3, inhibit cell cycle progression by increasing the expression of p21, p27, p15, and p16<sup>99-102</sup>. In contrast, transcription factors, such as E2F1, E2F2, E2F4, CASZ1, GATA4, Tbx20, and FoxM1, stimulate cell cycling by increasing the expression of cyclins<sup>99,101,103-106</sup>. Besides, PI3K-AKT, Wnt/ $\beta$ -catenin and YAP signaling pathways play important roles in cardiomyocyte proliferation<sup>107-111</sup>. Manipulation of the cell cycle in post-mitotic cardiomyocytes may be an attractive strategy to obtain large numbers of cardiomyocytes for *in vitro* studies, cardiac cell

therapy and the regeneration of injured hearts from inside by re-awaking cell cycling in cardiomyocytes surrounding the site(s) of myocardial damage.



**Figure 5. Cell cycle activities in cardiomyocytes.**

In a typical cell cycle, cell successively enters the G1 phase (for synthesis of RNAs and proteins), S phase (for DNA synthesis), G2 phase (for additional growth) and M phase (for karyokinesis and cytokinesis) resulting in two individual cells. In the presence of hypertrophic stimuli, cardiomyocytes enter the G1 phase and grow in size by synthesis of RNAs and proteins, but they fail to enter the S phase. Polyploidization results from shuttling between G1, S and G2 phase without entering into the M phase. Multinucleation occurs when cardiomyocytes enter the M phase and perform karyokinesis, but are withdrawn from the cell cycle before cytokinesis.

## Aim and outline of thesis

The aim of this thesis is to develop and use cell culture models for fundamental and applied biomedical research. Cell immortalization is a promising approach to produce large number of cells from same amounts/scarce primary starting material by inducing cell cycle reentry or bypassing cell senescence. Accordingly, a major part of this thesis was dedicated to the generation of robust cellular model systems by cell immortalization for addressing fundamental and applied research questions in a reproducible, flexible and standardized way.

Bypassing cellular senescence is a prerequisite for cell immortalization. It is therefore important to have a better understanding of the mechanism of senescence before immortalizing cells. A variety of proteins and pathways were found to be involved in senescence. However, the role of heterochromatin proteins and their contribution to abnormal chromatin organization in cell senescence is largely unknown. **Chapter II** of this thesis uses premature MEFs as a senescence model system which is derived from progeria disorder mice and studies the role of heterochromatin proteins and the associated chromatin organization in DNA damage response (DDR)-induced senescence.

In order to get familiar with the properties of immortalized cells including their culture conditions, proliferation and differentiation, an existing line of immortalized human myoblast is cultured and its differentiation is investigated in **Chapter III**. With the aid of this cell line, a novel non-destructive quantitative assay to monitor cell fusion is developed.

Cardiac cell therapy, tissue engineering and *in vitro* studies into cardiac arrhythmias or for testing the potential cardiotoxicity of drugs require large numbers of functional and homogeneous cardiomyocytes. Progress in these areas of research is impeded by the limited availability of heart tissue and the very low mitotic activity of cardiomyocytes highlight the urge to develop new/rich source of cardiomyocytes for both basic and translational research. **Chapter IV** describes the generation and characterization of a immortalized atrial myocyte cell line with preserved cardiomyogenic differentiation capacity. Through controlled expansion and differentiation of this cell line, large numbers of functional cardiomyocytes are generated, hence providing an attractive cell model for cardiac research.

BAT is regarded as a potential target to treat obesity and associated metabolic disorders because of its capacity to take up and combust glucose and fatty acids for thermoregulation. However, its cellular and molecular investigation has been hampered due to high cellular heterogeneity and a limited availability of cell material. In **Chapter V**, monoclonal lines of immortalized BPAs are generated by using mouse BAT as starting material. The long-term proliferation and high adipogenic capacity of the cell lines provide an attractive model system for fundamental and applied research into BAT.

Besides immortalized cell lines, primary cardiomyocytes were also used to solve research questions. In **Chapter VI**, neonatal rat ventricular myocyte cultures are established as an *in vitro* model for investigating the action potential-prolonging and associated proarrhythmic effects of Kv11.1 blockers. In this study, a newly designed

and synthesized compound LUF7244 is shown to prevent drug-induced proarrhythmic effects as by an allosteric mechanism.

Finally, **Chapter VII** provides the summary and conclusions drawn from each study of this thesis, as well as future perspectives related to the application of immortalized cells.

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