Summary

Organisms on earth develop in the presence of gravity. A good opportunity to study the effects of gravity on organisms is to expose organisms or cells to conditions of altered gravity, such as microgravity in space. Microgravity has been described to affect numerous processes that take place in organisms. Effects of exposure to microgravity conditions were also described on a cellular level, such as changes in signal transduction cascades, modification of gene expression, and changes in cell morphology. On the basis of results from previous research, it has been suggested that many of these changes observed in cells result from a changing actin cytoskeleton. This thesis further explores the effects on the actin cytoskeleton in cells that are exposed to microgravity conditions.

In cells, a dynamic network of filaments is formed by several types of proteins. This results in the formation of a kind of skeleton that is referred to as the cytoskeleton of cells. Actin is a major component of this cytoskeleton, in addition to tubulin and intermediate filaments. Actin is present in cells as polymerized actin (F-actin) and non-polymerized actin (G-actin). Actin filaments are continuously formed and degraded, a process that is regulated by actin-binding proteins and specific signal transduction routes. This results in the local formation of several subcellular actin structures that play specific roles in cells. Actin has various functions, such as determining the shape of cells, facilitating cellular motility, supporting the compartmentalization of cellular processes, as well as playing a role in communication and transport in cells and conducting several other functions (Chapter 2).

This thesis describes experiments that exposed cells to microgravity conditions for both short and long duration. Microgravity was achieved during rocket missions in space and was simulated on-ground. Initially, the aim of these experiments was to study the behavior of actin in cells upon exposure to conditions of microgravity. To this end, several experimental models were selected in which actin is very dynamic, i.e. cell cycle progression, membrane ruffling, and cellular movement. The presence of a dynamic actin cytoskeleton implies that regulating signal transduction cascades are activated. This provides an opportunity to study the behavior of actin as well as the regulating signal transduction routes. To be able to compare results obtained in microgravity with the situation at 1g, these models were first extensively studied at 1g on-ground. In the first part of this thesis, the models are described (Chapters 3, 4, and 5). Subsequently, experiments are described that were conducted both in simulated microgravity and in conditions of real microgravity (Chapter 6).

Microgravity has been described to affect actin dynamics as well as cellular proliferation. Actin was suggested to play a wide variety of roles during cellular proliferation, such as involvement in signal transduction and transcription and as a structural protein, for example during cytokinesis and during the rounding and flattening of cells. The regulation of cellular proliferation is dependent on signal transduction routes that are activated by external signal molecules, such as growth factors and extracellular matrix components. Depending on the activation of these signal transduction networks, cells continue proliferation or, alternatively, stop cell-cycle progression and undergo differentiation, apoptosis, or quiescence. This decision is taken during the G1 phase of the cell cycle. To study the relation between the behavior of actin and the proliferation of cells, cells were synchronized in mitosis and subsequently studied in the early G1 phase of the cell cycle. Subsequently, the behavior of actin in relation to the activation of signal transduction cascades at the early G1 phase of the cell cycle was studied (Chapter 3). Results indicate that actin and activated signal transduction are intimately linked to each other at the early G1 phase. Actin is involved in the formation of blebs at the cell membrane that are temporally present directly after completion of mitosis in the early G1 phase. These blebs contain several active key signal transduction proteins, such as phosphorylated FAK and phosphorylated MAP kinase, which are known to regulate progression through the cell cycle. The formation of membrane blebs filled with active signal transduction proteins is not specific to rounded, mitotic-like cells before cell spreading at the early G1 phase of the cell cycle. Similar features were observed in rounded trypsinized cells, just before renewed cell spreading. Inhibition of signal transduction did not affect membrane bleb formation, suggesting that the membrane blebs were formed independent of signal transduction.

The interaction between growth factor-induced signaling and actin was further studied in serum-starved mouse fibroblasts (Chapters 4 and 5). These cells induce spectacular actin structures upon stimulation with the growth factor PDGF (platelet-derived growth factor). In these structures, polymerized actin is pushing the cell membrane outwards locally, thereby forming dorsal circular ruffles. This process is regulated by specific signal transduction routes that start at the activated PDGF receptor. Though the subcellular localization of PDGF β-receptors in serum-starved cells is non-homogenous, the observed pattern of receptors does not explain the local formation of actin structures. That is why the initiation of local rearrangements of the actin cytoskeleton cannot be explained by the subcellular distribution of PDGF β-receptors.

Upon PDGF exposure, cells exhibit a changing subcellular distribution of PDGF β-receptors. PDGF β-receptors stay connected to the induced actin structures and accumulate in the newly formed dorsal circular ruffles as a result. The presence of both macropinosomes and clathrin in the induced circular ruffles suggests that the accumulation of PDGF β-receptors in circular ruffles results in the efficient internalization of PDGF β-receptors.

In addition to the translocation of PDGF β-receptors, stimulation with PDGF induces a relocation of $cPLA_2\alpha$ (Chapter 5). $cPLA_2\alpha$ is a member of a family of enzymes that catalyzes the cleavage of fatty acids from the sn-2 position of membrane phospholipids that results, amongst other things, in the release of arachidonic acid. Arachidonic acid is involved in several important physiological processes; for instance, it is a precursor of molecules that are involved in inflammatory responses. In mouse fibroblasts that are stimulated with PDGF, $cPLA_2\alpha$ translocates from the cytoplasm to protrusions of the cell membrane involved in actin and membrane dynamics, such as ruffles and lamellae. In migrating mouse fibroblasts as well as migrating human umbilical vein endothelial cells (HUVEC), $cPLA_2\alpha$ was also localized at the leading edge, a zone of intense actin and membrane dynamics that facilitates cellular motility. In addition, the active phosphorylated form of $cPLA_2\alpha$ was localized in ruffles, lamellae, and leading edges. Inhibition of $cPLA_2\alpha$ activity with specific inhibitors blocked growth factor-induced membrane and actin dynamics, such as the formation of ruffles. This suggests an important role for $cPLA_2\alpha$ in these processes. Since $cPLA_2\alpha$ was also localized at the membrane blebs of rounded cells that were in the process of spreading (Chapter 3), $cPLA_2\alpha$ seems to play an important role in various forms of local actin and membrane dynamics.

Simulation of microgravity conditions is a much-needed addition to research in real microgravity due to the limited access to space flights. Previously, it had been described that in real microgravity conditions, the human epidermoid cell line A431 exhibits specific changes in the actin cytoskeleton, ultimately resulting in the rounding of cells. This rounding of A431 cells was studied in detail during exposure to two methods that have been described to simulate microgravity, i.e. Random Positioning Machine (RPM) rotation and magnetic levitation (Chapter 6.1). In the RPM, samples are mounted on a platform that randomly changes position in three dimensions by driving two independent frames that rotate independently in random directions and at random speed. The random rotation in all directions results in a net force of zero. So the RPM is based on the principle

that the direction of gravity is randomized. In case of magnetic levitation, magnetic forces are exerted on cells by positioning them in a strong gradient magnetic field. Since diamagnetic objects, such as cells, are repelled by magnetic fields, this results in a magnetic force that can be used to counterbalance the gravitational force, resulting in stable levitation and the stimulation of microgravity. The use of both methods was demonstrated to result in effects on the actin morphology that had previously been described in real microgravity. Upon exposure to conditions of simulated microgravity, a transient process of cell rounding and renewed spreading was observed over time. The rounding and flattening of cells was illustrated by a changing actin cytoskeleton and variations in the presence of focal adhesions. Actin and focal adhesions are key players in facilitating the attachment of cells. Despite the fact that the results in simulated microgravity matched the effects that were observed in real microgravity, further characterization of both methods is required. Side effects of both methods can easily lead to false linking of cellular responses to simulated microgravity.

The reorganization of the actin cytoskeleton that takes place upon stimulation with the growth factor PDGF was studied during an initial series of experiments in real microgravity (Chapter 6.2). During the DELTA Soyuz mission of 2004, cells were exposed to microgravity for several days, in contrast to the MASER-10 mission of 2005 that subjected cells to microgravity conditions for just six minutes. Both experiments aimed at studying the behavior of actin upon stimulation with PDGF. However, the simulation experiments indicated that cells transiently respond to microgravity conditions. Short-duration experiments, such as the MASER missions, allow the study of cells that are adapting to microgravity conditions, whereas long-duration experiments allow the study of cells that might have adapted to the changed environment. Neither experiment in real microgravity provided any results. In case of the MASER-10 mission, the experiment was successful but the results were lost as a result of the uncontrolled landing of the rocket. In case of the DELTA mission, there were no results in microgravity due to a combination of malfunctioning hardware and temperature requirements that were not met.

Microgravity based research is hampered by a very limited access to space flights making routine laboratory research impossible. In addition, it can be concluded that the opportunities are not without risks. That is why it is essential to further develop methods that allow the routine performance of experiments in simulated microgravity. In combination with new experiments in real microgravity in space – for example with the

models described in this thesis – this would certainly shed light on the mechanisms that induce effects in cells exposed to microgravity conditions.