Chapter 7

Summarizing discussion

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A number of studies have indicated that gravity affects mammalian cell growth and cell differentiation. Altered gravity values were described to result in alterations in signaling cascades, ranging from the activation of cells – such as human lymphocytes – to the expression of genes. Furthermore, gravity was demonstrated to affect both cellular morphology and the morphology of the actin cytoskeleton. For example, it was demonstrated that the amount of F-actin increased under microgravity conditions. This indicates that either the polymerization of actin is increased or the depolymerization of actin is inhibited under microgravity conditions. The mechanisms that induce these effects in microgravity in cells are unknown. However, based on several observations made in (simulated) microgravity, it was hypothesized that actin might be the microgravity-sensitive component in mammalian cells (Boonstra, 1999).

Interestingly, it was demonstrated that not all intracellular signal transduction cascades are sensitive to conditions of microgravity. The activity of specific signaling routes – such as protein kinase C signal transduction – was demonstrated to be affected by microgravity (De Groot et al., 1991). Other signaling molecules were demonstrated to behave not significantly different under microgravity conditions compared to the situation at 1g. This rules out the idea that all molecular interactions in cells are affected in microgravity. Thus, the polymerization dynamics of actin might represent a specific cellular response to microgravity conditions. Interestingly, the self-organization of microtubules was demonstrated to depend on gravity (Papaseit et al., 2000; Glade et al., 2006; Tabony et al., 2007). The self-organization of actin might be sensitive to altered gravity conditions accordingly. In addition, the mechanism that underlies the sensitivity of actin polymerization in cells to microgravity conditions might result from an altered interaction with actin-binding proteins that regulate the dynamics of actin in cells. In this thesis, experiments were described that were aimed at studying the behavior of actin in microgravity.

The overview of the actin metabolism in mammalian cells that was listed in Chapter 2 illustrated the versatile and complex role of actin in cells. Actin is localized throughout the whole cell and is present in various conformations that interact with numerous actin-binding proteins. The local and dynamic interplay with actin-binding proteins enables actin to fulfill functions in cells that vary locally and over time. The metabolism of actin is tightly regulated to control this dynamic and versatile functionality of actin. Thus, the network comprising actin metabolism is linked to a complex network of signal

transduction cascades that regulate numerous cellular functions. In fact, many of the observed changes in cellular behavior in microgravity can, in theory, be related to an altered behavior of actin in cells. For example, the observed alterations in gene expression, signal transduction, attachment, motility and cell cycle progression could all result from alterations in actin dynamics in cells exposed to altered gravity conditions.

The versatile and complicated interactions of actin with other molecules in cells, and consequently the versatile functioning of actin in cells, makes it difficult to interpret any observed changes in actin behavior. That is why experimental models were studied extensively at 1g before exposing cells to altered gravity conditions. In Chapter 3-5 of this thesis, three cellular processes were characterized that display a specific role of actin in cells. The appearance of actin was studied in cell cycle progression, membrane ruffling and cellular movement.

Actin appears intimately linked to cell-cycle progression (see Chapter 2). In Chapter 3 we focused on the appearance of actin after completion of mitosis during the early G1 phase of the cell cycle. This phase of the cell cycle is of particular interest since during the G1 phase, cells are susceptible to external signals that regulate cell proliferation, such as external matrix components and growth factors. Actin acts as a structural protein in facilitating the spreading of post-mitotic rounded cells. In addition to determining alterations in cellular morphology, actin is involved in signal transduction and attachment (see Chapter 2 and 3). Furthermore, actin appears involved in the formation of membrane blebs that are temporarily present at the cell surface during the spreading of rounded post-mitotic cells. These blebs are shielded with F-actin and contain activated $cPLA_2\alpha$. Therefore it is tempting to suggest that actin interacts with $cPLA_2\alpha$ to form these membrane blebs, similar to the interaction that was demonstrated for other local actin and cell membrane dynamics (see Chapter 5). The blebs may act as temporal storage for membrane that is required during the growth of cells during the G1 phase. In addition, the blebs contain tyrosine-phosphorylated proteins, such as phosphorylated FAK and phosphorylated MAPK. This is indicative of the activity of integrin-induced as well as growth factor-induced signaling in these blebs. Newly-formed focal adhesions appear directly after the disappearance of the membrane blebs. Therefore, these blebs may act as temporal local signaling centers that facilitate the formation of new focal adhesions.

In order to study the interaction between growth factor-induced signal transduction and actin, the formation of membrane ruffles was investigated in Chapters 4 and 5. In mouse fibroblasts, stimulation with the growth factor PDGF induces spectacular

rearrangements of actin within minutes. Newly-formed actin structures locally push the cell membrane outwards, resulting in the formation of ruffles such as circular dorsal ruffles. In Chapter 4, the initiation of this response was investigated by studying the localization of the PDGF β-receptor. A mutual interaction between the PDGF β-receptor and actin was found that might explain how cells become less sensitive to PDGF during stimulation. Activated PDGF β-receptors were demonstrated to co-localize with newly-formed actin structures in dorsal circular ruffles that were induced upon stimulation with PDGF. These circular ruffles contract and move across the cellular surface. The activated PDGF β-receptors follow the movement of the circular ruffles and stay connected to the induced actin and membrane dynamics. The concentration of activated PDGF β-receptors in circular ruffles may facilitate efficient internalization of receptors.

The formation of membrane ruffles was further studied in Chapter 5. It was demonstrated that $cPLA_2\alpha$ plays an important role in facilitating actin-directed membrane ruffling. Active $cPLA_2\alpha$ is recruited at circular ruffles, and the exposure to $cPLA_2\alpha$ inhibitors blocked the formation of ruffles. The interplay between actin and $cPLA_2\alpha$ may have a broader significance, as both $cPLA_2\alpha$ and phosphorylated $cPLA_2\alpha$ were localized at several other protrusions of the cell membrane that exhibit active actin and membrane dynamics, such as lammelipodia, linear ruffles and leading edges. The leading edge represents a region of intense actin and membrane dynamics that promotes motility in cells (Ridley, 2011). Thus, $cPLA_2\alpha$ appears involved in facilitating actin and membrane dynamics in cells, such as membrane ruffling and cell migration.

The suitability of the experimental models for studying actin in microgravity depends on several factors. In all experimental models, i.e. at early G1, after stimulation with PDGF and during cell migration, the actin cytoskeleton is highly dynamic. The presence of a highly dynamic actin cytoskeleton ensures that regulators of the actin metabolism are locally activated. Therefore, these models are suited to study actin dynamics including the activity of regulators of the actin metabolism in cells. But the suitability of the model is also determined by the duration of experiments in microgravity. For short-duration experiments, such as conducted on a sounding rocket, the induction of circular ruffles upon the addition of the growth factor PDGF form a good model. To study the effects of actin in cell cycle progression and cellular movement, longer-duration missions are required.

In addition to experiments in real microgravity, experiments were conducted in simulated microgravity (Chapter 6.1). Simulation of microgravity conditions is a much-

needed addition to research in real microgravity due to the limited access to space flights. Two methods that had been described to simulate weightlessness were studied, i.e. random positioning and magnetic levitation. The use of both methods was demonstrated to result in effects in cells that have previously been described in real microgravity (Chapter 6.1). However, further analysis of both random positioning and magnetic levitation is required, as side effects were observed that complicate relating the cellular responses observed to simulation of weightlessness. Simulation experiments revealed a transient response in cells. Cells initially responded by cell rounding that was followed by renewed spreading and attachment. This transient response is facilitated by a dynamic actin cytoskeleton in combination with the disappearance and reappearance of focal adhesions. Because cells exhibit a transient response, one must be rather careful in comparing the results of experiments that expose cells to microgravity conditions for different durations.

For a first series of experiments in real microgravity, during the Dutch Soyuz Mission, the formation of ruffles induced by actin upon stimulation with the growth factor PDGF was investigated (Chapter 6.2). Mouse fibroblasts were exposed to conditions of microgravity for several days and subsequently stimulated with PDGF. Based on the results of the simulation experiments (Chapter 6.1), the duration of exposure to microgravity conditions was sufficient for the adaptation of cells. The results of the ground control experiments demonstrated that the experimental set-up worked properly. Unfortunately, there were no results in microgravity due to a combination of malfunctioning hardware and temperature requirements that were not met. In a subsequent series of experiments in real microgravity during the MASER-10 mission, cells were stimulated with PDGF directly upon exposure to microgravity, meaning cells that had not yet adapted to microgravity conditions were stimulated with PDGF to induce a response of actin in microgravity. Subsequently cells were chemically fixed after six minutes. Analysis of recorded critical parameters indicated that the experiment was successful and that the developed hardware functioned properly. Unfortunately, there were no results in microgravity due to an uncontrolled landing of the rocket.

Since experiments in space are both scarce and not without risks, it is essential to further develop methods that allow the routine performance of experiments in simulated microgravity. Combined with new experiments in real microgravity, this would certainly clarify the mechanisms that induce effects in cells that are exposed to conditions of altered gravity.

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