Chapter 1

General introduction

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During the last decades a wide variety of space flight experiments have indicated that gravity has significant effects on whole organisms, organs and tissues, resulting for example in bone and muscle mass reduction, the occurrence of cardiovascular malfunctioning, as well as many other processes (Carmeliet et al., 2001; Fitts et al., 2001; Fritsch-Yelle et al., 1996). In addition, the virtual absence of gravity was demonstrated to have profound effects on the cellular and molecular level, including changes in cell morphology (Rijken et al., 1991; Hughes-Fulford et al., 2003), modification of gene expression (de Groot et al., 1991a; Hammond et al., 1999; Liu and Wang, 2008), changes in signal transduction cascades (de Groot et al., 1991b; Ullrich et al., 2008) and even changes in the self-organization of the cytoskeletal protein tubulin (Papaseit et al., 2000; Glade et al., 2006; Tabony et al., 2007). That is why the underlying mechanism for the observed physiological responses in microgravity might be on the molecular level within cells.

Previous studies of adherent A431 epithelial cells in microgravity using sounding rockets revealed that growth factor-induced signal transduction is sensitive to microgravity. EGF-induced (Epidermal Growth Factor) early gene expression of c-fos and c-jun was demonstrated to decrease under microgravity conditions (de Groot et al., 1990; de Groot et al., 1991a). Subsequently, EGF signaling was investigated upstream of this early gene expression by studying receptor clustering, ligand binding and by studying EGF-induced intracellular signaling cascades. Receptor clustering and ligand binding were demonstrated not to alter under microgravity conditions. However, selective transduction pathways were identified to be susceptible to changes in gravity conditions whereas other pathways originating from the EGF receptor were not (Rijken et al., 1992).

Furthermore, it was demonstrated during sounding rocket flights that microgravity exposure of A431 cells resulted in increased actin polymerization and cell rounding (Rijken et al., 1991). Cell rounding is dependent on actin. Interestingly, the signaling cascade that was susceptible to changes in gravity – PKC-mediated signal transduction – was also demonstrated to depend on actin. That is why the actin cytoskeleton may represent a gravity-sensitive component in cells (Boonstra, 1999).

Actin is a main component of the cytoskeleton in eukaryotic cells that provides structure and determines the shape of cells. The cytoskeleton consists of proteins that dynamically interact with one other. Next to actin, the cytoskeleton consists of tubulin and intermediate filaments. Actin is present in the form of individual actin proteins known as

globular actin (G-actin) as well as filamentous polymers (F-actin). F-actin filaments consist of multiple G-actin subunits that interact with one another and are continuously assembled and disassembled. Actin microfilaments organize themselves in dynamic structural components in cells that, for instance, determine the shape of cells, provide the infrastructure for intracellular transport and facilitate processes such as cell motility and cell cycle progression (for a review, see Boonstra and Moes, 2005).

As indicated above, it was hypothesized that actin may represent a microgravity-sensitive component in mammalian cells. Microgravity might affect the behavior of actin directly or indirectly via actin-binding proteins. This thesis describes experiments that aim to identify the role of actin in sensing microgravity.

It was reported that cell proliferation and consequently cell cycling is affected by microgravity conditions (Vassy et al., 2003). Progression through the cell cycle is dependent on the activation of signal transduction cascades that originate from growth factors as well as from the attachment of cells (Hulleman et al, 1999a, b). Both cell attachment and signal transduction were demonstrated to alter under conditions of microgravity and are known to depend on actin. That is why the actin dynamics during the attachment of cells at the early G1 phase of the cell cycle represent an interesting model for studying actin functionality under different gravity conditions. By doing so, microgravity-induced changes in actin behavior that induce modifications in cell proliferation might be identified.

As mentioned above, signal transduction that is induced by growth factors has been demonstrated to alter under conditions of microgravity. In order to focus on the relation between growth factor-induced signal transduction and the behavior of actin, the dynamics of actin were studied in mouse fibroblasts that were stimulated with PDGF (Platelet-Derived Growth Factor). PDGF induces spectacular rearrangements of actin in mouse fibroblasts within minutes. This implies that regulators of the actin metabolism become activated. Therefore, this model is suited to study actin dynamics, including the activity of regulators of the actin metabolism, in cells that are exposed to microgravity conditions for a matter of minutes. This model of PDGF-induced actin dynamics in mouse fibroblasts was not only selected for the spectacular response of the actin cytoskeleton, but also because of the ability of these cells to survive the harsh conditions that are unavoidable when doing experiments in real microgravity.

In addition to ruffle formation, actin is well-known for its role in facilitating motility responses in cells upon stimulation by growth factors. Interestingly, cell migration was

reported to be affected by changing microgravity conditions (Meloni et al., 2011). Hence, the relation between PDGF-induced motility responses and actin dynamics also represents an interesting model for studying actin functionality in microgravity.

Thus, the first part of this thesis describes these three cellular processes that display a specific role of actin in cells. The appearance of actin was studied in cell cycle progression, cellular movement and membrane dynamics and in relation with growth factor-induced signal transduction. In the future, these models may be used to investigate the influence of microgravity on specific functions of actin. The second part of this thesis describes experiments that were conducted in both real and simulated microgravity.

This thesis has the following outline:

Chapter 2 provides an overview of the actin metabolism in mammalian cells. The various roles of actin in cells are described and the key players involved in the actin metabolism in mammalian cells are listed. The focus of this review is on the role of actin in the regulation of G1-phase progression.

Chapter 3 describes the appearance of actin during cell cycle progression, in particular during the early G1 phase of the cell cycle. Though it is clear that actin plays important roles in the regulation of cell cycle progression, the underlying molecular mechanisms remain to be elucidated. The experiments in Chapter 3 focused on the local activation of signal transduction – as directed by cellular attachment and growth factors – and actin during the early G1 phase of the cell cycle. Interestingly, various key signal transduction proteins were found in blebs at the cell membrane within 30 minutes after mitosis. These membrane blebs appeared in round, mitotic-like cells and disappeared rapidly during spreading of the cells in the G1 phase.

The relation between growth factor-induced signal transduction and the behavior of actin is studied in **Chapter 4**. The growth factor PDGF induces spectacular rearrangements of actin in mouse fibroblasts within minutes. This results for instance in the formation of dorsal circular ruffles. The formation of these dorsal circular ruffles was investigated in detail. Furthermore, a mutual interaction between growth factor signaling and actin is described that might explain how cells become less sensitive towards PDGF stimulation during the stimulation itself.

In **Chapter 5**, the role of actin in membrane dynamics such as growth factor-induced membrane ruffling and motility is further investigated. A new relation between $cPLA_2\alpha$ and actin-directed cell migration is described that may eventually be studied in

(simulated) microgravity. Upon stimulation of ruffling and cell migration by growth factors, endogenous cPLA$_2$α and its active phosphorylated form relocate at protrusions of the cell membrane involved in actin and membrane dynamics. Inhibition of cPLA$_2$α activity with specific inhibitors blocked growth factor-induced membrane and actin dynamics, suggesting an important role for cPLA$_2$α in these processes.

In **Chapter 6** the models described above are studied in microgravity. First, an overview is provided of research that was conducted in the past in (simulated) microgravity in relation to the actin cytoskeleton. Based on previous microgravity experiments and current knowledge of actin behavior at 1g, it was hypothesized that the actin microfilament system is the microgravity-sensitive component in mammalian cells (Boonstra, 1999). Next, Chapter 6 describes experiments that were performed in both simulated and real microgravity. In **6.1** two different ways to simulate microgravity conditions on earth are explored: random positioning and magnetic levitation. In **6.2a** experiments that were performed in real microgravity during the Dutch Soyuz Mission are described. In **6.2b** experiments that were conducted in real microgravity during the MASER-10 mission are described.

Chapter 7 discusses the results in relation to previous research performed in (simulated) microgravity and in relation to current knowledge of the behavior of actin at 1g on earth.

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