Adhesion-dependent mechanisms regulating mitosis

DEEPESH KUMAR GUPTA
Abstract

Integrin-mediated cell adhesion is required for normal cell cycle progression during G1-S transition and for the completion of cytokinesis. Cancer cells have ability to grow anchorage-independently, but the underlying mechanisms and the functional significance for cancer development are unclear. The current thesis describes new data on the adhesion-linked molecular mechanisms regulating cytokinesis and centrosomes.

Non-adherent fibroblast failed in the last step of the cytokinesis process, the abscission. This was due to lack of CEP55-binding of ESCRT-III and its associated proteins to the midbody (MB), which in turn correlated with too early disappearance of PLK1 and the consequent premature CEP55 accumulation. Integrin-induced FAK activity was found to be an important upstream step in the regulation of PLK1 and cytokinetic abscission. Under prolonged suspension culture, the MB disappeared but septin filaments kept the ICB in the ingressed state. Upon re-plating on fibronectin, such cells were found to divide through traction-based abscission. Non-adherent cytokinetic cells maintained septin filaments around the ICB for >24 hours, but septin was gradually depolymerized later on and furrow-regressed binucleated cells were generated (<15%). Binucleated non-transformed cells were halted in G1 and became senescent, possibly via PIDDosome formation by two centrosomes merging. In contrast to normal fibroblasts, ras-transformed fibroblasts were able to recruit the ESCRT-III-associated protein ALIX to MB under non-adherent condition. Live-cell imaging and septin-7 immuno-staining showed that cytokinetic abscission occurred in non-adherent ras-transformed fibroblast.

Non-adherent fibroblasts, as well as adherent cells lacking FAK expression, were also delayed in early stages of mitosis progression and exhibited defect centrosome separation and abnormal spindle formation. Our data show that integrin-dependent FAK activity promotes centrosome separation via a FAK-PLK1-Eg5 route during early mitosis.

Keywords: Cytokinesis, Centrosome, Septin, Abscission, Mitosis, Integrin, Adhesion, Binucleation

Deepesh Kumar Gupta, Department of Medical Biochemistry and Microbiology, Box 582, Uppsala University, SE-75123 Uppsala, Sweden.

© Deepesh Kumar Gupta 2019

ISSN 1651-6206
ISBN 978-91-513-0531-8
urn:nbn:se:uu:diva-368422 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-368422)
Dedicated to my parents
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


Contents

Introduction ................................................................................................... 11

1. Integrin ...................................................................................................... 12
   1.1 Integrin role in cell cycle................................................................. 12
   1.2 Integrin signaling ............................................................................. 12
      1.2.1 FAK-Src signaling ................................................................. 13
      1.2.2 ERK signaling ........................................................................ 13

2. Cytoskeleton ............................................................................................. 14
   2.1 Actin ................................................................................................... 15
      2.1.1 Actin polymerization ............................................................... 15
      2.1.2 Actin functions ...................................................................... 16
   2.2 Microtubules ....................................................................................... 16
      2.2.1 Microtubule structure .............................................................. 16
      2.2.2 Microtubule dynamics ............................................................. 17
      2.2.3 Microtubule nucleation ........................................................... 18
      2.2.4 Microtubule functions ............................................................. 19
   2.3 Septins ................................................................................................ 19
      2.3.1 Septin structure ...................................................................... 20
      2.3.2 Septin protomer arrangement .................................................. 20
      2.3.3 Septin mammalian functions ................................................... 21
      2.3.4 Septin synthesis and degradation .......................................... 22

3. Cell cycle ................................................................................................... 22
   3.1 Cell cycle regulation ......................................................................... 22
      3.1.1 Interphase ................................................................................ 23
      3.1.2 Mitosis phase ......................................................................... 23
      3.1.3 Cytokinesis ............................................................................ 25
         3.1.3.1 Cleavage furrow formation and ingression .................... 25
         3.1.3.2 Midbody formation ......................................................... 27
         3.1.3.3 Abscission .................................................................... 28
         3.1.3.4 Anillin and septin in cytokinesis .................................. 30
         3.1.3.5 Abscission checkpoint .................................................. 30

4. Centrosome ................................................................................................ 31
   4.1 Centrosome duplication cycle .......................................................... 31
   4.2 Centrosome separation ...................................................................... 32
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Cause of aneuploidy</td>
<td>32</td>
</tr>
<tr>
<td>5.1 Defects at centrosome level</td>
<td>32</td>
</tr>
<tr>
<td>5.2 Defects at cytokinesis level</td>
<td>33</td>
</tr>
<tr>
<td>6. Present investigations</td>
<td>34</td>
</tr>
<tr>
<td>6.1 Paper I</td>
<td>34</td>
</tr>
<tr>
<td>6.2 Paper II</td>
<td>35</td>
</tr>
<tr>
<td>6.3 Paper III</td>
<td>36</td>
</tr>
<tr>
<td>6.4 Paper IV</td>
<td>37</td>
</tr>
<tr>
<td>7. Future perspectives</td>
<td>39</td>
</tr>
<tr>
<td>8. Acknowledgement</td>
<td>41</td>
</tr>
<tr>
<td>9. References</td>
<td>44</td>
</tr>
</tbody>
</table>
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>FAK</td>
<td>Focal adhesion kinase</td>
</tr>
<tr>
<td>CDK</td>
<td>Cyclin-dependent kinase</td>
</tr>
<tr>
<td>PCM</td>
<td>Pericentriolar material</td>
</tr>
<tr>
<td>PLK</td>
<td>Polo-like kinase</td>
</tr>
<tr>
<td>ALIX</td>
<td>ALG-2-interacting protein X</td>
</tr>
<tr>
<td>ESCRT</td>
<td>Endosomal sorting complexes required for transport</td>
</tr>
<tr>
<td>TSG101</td>
<td>Tumor susceptibility gene 101</td>
</tr>
<tr>
<td>CHMP4B</td>
<td>Charged multivesicular body protein 4B</td>
</tr>
<tr>
<td>NEK</td>
<td>NIMA-related protein kinase</td>
</tr>
<tr>
<td>ICB</td>
<td>Intercellular bridge</td>
</tr>
<tr>
<td>MB</td>
<td>Midbody</td>
</tr>
</tbody>
</table>
Introduction

Cancer represents the second most lethal group of diseases worldwide. It is characterized by uncontrolled proliferation and spreading of cells that originated from one single cell. Such changes developed by damage on the genome by external factors, inherited mutations, or stochastic errors during the replication. According to WHO data, 3.7 million new cancer cases are observed in Europe each year and among that 1.9 million fail to survive. A long-standing effort is to detect the diseases at early stages of its progression. Disturbance either in oncogenes or tumor-suppressor genes can cause tumor initiation. The changes in such genes that contribute to carcinogenesis are called “cancer driver” mutations. In addition to gene mutations, most tumor samples have cells with chromosomal abnormality features such as altered copy numbers, breaks, or translocations. Aneuploidy usually arises after a previous tetraploidization event and by chromosomal instability (CIN). Since aneuploidy and CIN are believed to contribute to progression of tumors into more malignant stages, and in some cases to tumor initiation, it is important to examine the cause of such phenotypes.

The present doctorate thesis focuses on cell adhesion-dependent mechanisms that control the mitosis (-M) phase of the cell cycle and how defects in this regulation may generate aneuploidy. It also aims to clarify the mechanisms underlying anchorage in-dependent cancer cell growth.
1. Integrin

Integrins were discovered as cell – ECM adhesion receptors in early 1980s and are found in all metazoans. The integrin receptor family consists in vertebrates of 24 heterodimers of α and β subunits [1]. While most integrins bind to adhesion proteins in the extracellular matrix (ECM), some integrins mediate cell-cell adhesions. In addition to their anchoring function they also transduce signals from the environment to the cell [2-4].

1.1 Integrin role in cell cycle

Cell proliferation occurs through the amazingly complex cell cycle. The cell cycle is an ordered and strictly regulated chain of events where specific cyclin-dependent protein kinases (CDKs) are required in order to progress into different cell cycle phases. Cell adhesion has so far been found to be involved in the regulation of the cell cycle at two stages. The G1-S phase checkpoint is regulated synergistically by signals from integrins and growth factor receptors [5, 6]. Integrins contribute to the transition of this checkpoint via cyclin D1-dependent CDK4/6 and cyclin E-dependent CDK2 kinase to trigger the initiation of DNA synthesis [7]. Additionally, the last step of the mitosis phase, cytokinesis, is controlled via integrin-based signaling [8, 9].

1.2 Integrin signaling

Integrins got their name because they connect the ECM with intracellular filament systems. However, in order to bind extracellular ligands integrins first have to undergo a large conformational change that is induced by binding of talin and kindlin to the cytoplasmic domain. Binding of ECM ligands, e.g. fibronectin and collagens, to such activated integrins induce further conformational changes in the extracellular domain of integrins [10, 11] and clustering of the receptors. The integrin clusters recruit several cytoplasmic enzymes and adaptor proteins and thereby form so called focal complexes. The focal complexes have the dual function as signaling centers and as attachment sites for actin filaments. Talin is a main actin-binding protein in focal complexes and is thus a direct link between integrin and actin cytoskeleton [12, 13]. This arrangement also allows talin to function as a sensor of mechanical stimuli on cells by exposing cryptic binding sites after stretch-induced unfolding.
1.2.1 FAK-Src signaling

FAK is a protein tyrosine kinase that is composed of a N-terminal FERM domain, a central kinase domain and a C-terminal focal adhesion-targeting (FAT) domain [14]. FAK and its associated proteins mediate diverse signaling pathways downstream of integrins, and it is involved in the control of many cellular processes including migration, proliferation and survival. Upon ligand binding, clustered integrins initially promote recruitment of FAK to adhesion site via interaction with integrin-associated proteins such as talin and paxillin [15]. Ligand-induced integrin clustering thus brings FAK molecules together and allows FAK trans-autophosphorylation at Tyr397 [14], which becomes a high affinity binding site for the SH2 domain of Src tyrosine kinase. The now activated Src can in turn phosphorylate FAK at the kinase domain (tyrosines 576 and 577) and C-terminal domain (tyrosines 861 and 925). By these phosphorylations FAK become both an active kinase and an adaptor protein for recruitment of additional proteins.

The activated FAK-Src activates downstream several proteins, one example is p130CAS which after phosphorylation binds to Crk, leading to Rac-1 activation and lamellipodia formation. An important FAK ligand is PI3 kinase, which like Src can bind to phosphorylated Tyr397 in FAK and thereby generates long-term anti-apoptotic signals required for anchorage-dependent cell survival (“anoikis”).

1.2.2 ERK signaling

The ERK pathway can be activated independently by growth factors and integrin-based cell adhesion, but a strongly enhanced ERK activation is obtained when both types of stimuli act in synergy. Cell adhesion contribute to ERK activation at several levels of this MAPK pathway. FAK-Src in adhesion complexes induces PAK1 activation via GTP-loading and translocation of Rac to the membrane, and PAK1 then phosphorylates MEK at Ser 298 which contributes to the activation of MEK [16]. At another level, Raf-1 activity, downstream of GTP-Ras, is stimulated by PAK1 via phosphorylation at Ser338. In addition, Src makes an essential phosphorylation of Raf-1 at Tyr34 [17, 18]. Integrins and growth factors are known to cooperate in the regulation of many cellular processes, and ERK activation is a relatively well understood example of that [19].
Figure 1: Integrin signaling
2. Cytoskeleton

The cytoskeleton consists of the four filament systems microtubules (MT), actin, intermediate filaments and septin. It is a main structural component of cells and performs diverse cellular functions. It supports the architecture and most mechanical processes of an animal cell and it also serves as a platform for binding and assembly of signaling protein complexes. The mitosis (-M) phase of the cell cycle requires extensive cytoskeletal transformation. During interphase-mitosis transition cells change their architecture from flattened to round, depolymerize the intermediate filaments, and re-organize actin, MT and septin for distinct functions throughout mitosis. The current thesis background focuses mainly on the septin filament system and briefly describes the actin and microtubules.

![Figure 2: The four cytoskeletal filament systems](image)

2.1 Actin

The actin, one of the major cytoskeletal components, is highly conserved among all eukaryotes. It is present in cells as globular, monomeric actin (G-actin) and filamentous actin (F-actin) [20]. Actin has been shown to participate in several physiological functions in a cell i.e. cell contacts, cell migration, proliferation.

2.1.1 Actin polymerization

Similar to MT, actin polymerization involves two steps, nucleation and elongation. Nucleation is the rate-limiting step for actin filament formation
and is regulated via different nucleation proteins, including the Arp2/3-complex and formin proteins. The Arp2/3-complex mimics the structure of a polymerizing filament end and induces a branch from existing actin filaments, while formins are located at the plasma membrane and generates linear filaments. Elongation of filaments is promoted by the ENA/VASP protein family at the plasma membrane, as well as by formins. The filaments have a polarity based on the monomer structure and they are further asymmetric based on the distribution of bound ATP/ADP. ATP-bound actin monomers (G-actin) is added on to the fast-polymerizing end (barbed end) while the other end contains ADP-actin (pointed end). The filament dynamics are regulated by cycle of ATP hydrolysis, depolymerization at the pointed end, ADP exchange for ATP in the released G-actin [21], a process described as treadmilling. Filament formation and stability is regulated by many actin-binding proteins that affect the rate of polymerization, cross-linking and severing the actin filament [22].

Figure 3: Schematic representation of actin turnover.

2.1.2 Actin functions

The actin filament network system performs diverse key functions in the cell. During interphase when the cells are flat and spread onto ECM, actin filament are anchored to focal adhesion sites, signaling interfaces between ECM, integrins and actin [23, 24]. When cells reach to mitosis, actin is reorganized into the contractile ring required for cleavage furrow formation and ingression during cytokinesis [25]. Some actin filaments remain associated with the adhesion sites, which keep the cells attached during mitosis although they have rounded up.
The actin filament system works in close collaboration with motor proteins, i.e. myosins, and with the other filament systems. GEFs and their downstream signaling GTPases, such as RhoA, Rac, cdc42, play important roles in the regulation of actin functions, including cell migration and cytokinesis [26].

2.2 Microtubules

Microtubules (MT) constitute highly dynamic cytoskeletal filament system in all eukaryotic cells. It has several cellular functions, e.g. intracellular transport and chromosome segregation etc. [27]. These regulations of MTs are specialized and affected by cell-extracellular matrix (ECM).

2.2.1 Microtubule structure

The basic unit of MT filament, called protofilament, is the arrangement of the $\alpha$ and $\beta$ heterodimers. Such linear protofilaments are associated laterally and build 25 nm hollow cylindrical structures called microtubules [27]. Both subunits of the heterodimers contain GTP, however only $\beta$-tubulin-bound GTP is hydrolyzable to GDP upon polymerization and is exchangeable to GTP after depolymerization [28]. Such GTP-bound heterodimers are then ready to be incorporated into MT in the next round of polymerization. The hydrolysis of GTP is involved in MT dynamics regulation by affecting the structure of the tubulin dimer. Recent observations show that GTP-bound dimers are straight while GDP-bound dimers are under a mechanical strain that lead to a curved appearance in the whole protofilaments [29]. These MT arrangements generate structural asymmetry where one end exposes an alpha subunit while other end has a beta subunit.

2.2.2 Microtubule dynamics

The structural asymmetry of MTs creates different polymerization and depolymerization rates at both ends, a property shared with actin filaments. Net polymerization of free tubulin dimers occurs at the so called (+) end, while the (-) end has net depolymerization [27]. MT length fluctuations between growth and shrinkage in a stochastic manner generate dynamic instability [27]. Such property allows cells to rearrange their MT meshwork. This regulation is controlled via microtubule-associated proteins (MAPs) that are classified into four groups: MT nucleation promoters, disassembly promoters, tubule modifying enzymes and cross-linkers.

MT’s dynamic instability is characterized by four parameters: MT growth and shrinkage speed, rescue frequency and catastrophe frequency [30]. MAPs regulate MT dynamics by altering one or several of the above mentioned parameters. The kinetics are also regulated by a so called GTP cap at the MT tip. According to the GTP cap model, the growing MT is loaded with a protein complex containing GTP-$\beta$-tubulin, which stabilizes the MT.
plus end and keep it in growing phase. As the GTP cap is lost, a GDP-bound β-tubulin is exposed which induces depolymerization of the MT. However, the mechanism of dynamic instability is still not well understood [31].

Figure 4: Schematic representation of microtubule dynamics

2.2.3 Microtubule nucleation

In vitro, MT assembly is a slow process and progresses only above a critical concentration of oligomers (i.e. protofilament) [32]. In cells, multiprotein complexes containing γ-tubulin act as a template for MT assembly, thus fasten the nucleation process [33]. Such de novo synthesis of MTs is located to microtubule organizing centers (MTOCs) close to the nucleus, i.e. centrosomes and basal body [34]. γ-tubulin was first identified in the fungus Aspergillus nidulans and is highly evolutionary conserved among a variety of organisms. Thus, it is considered as a universally required component for the nucleation of MT.

γ-tubulin is present in two forms in cells that differ in their size and compositions: γ-tubulin small complexes (γ-TuSC, 300 kDa) and ring complexes (γ-TuRC, 2 MDa). Each γ-TuSC is a hetero-tetramer consisting of GCP2, GCP3, and two γ-tubulin. Each γ-TuRC consists of seven γ-TuSCs arranged in a helical manner and resembles the template for MT nucleation. γ-TuRC and its associated proteins are spatio-temporally regulated at MTOC during the cell cycle and transformed into a structural template for MT nucleation. MTOC dynamics varies between the cell types and organisms, and the regulation of their composition and signaling within the cell are still under debate.

In many eukaryotes, it has also been found that the nucleation of MTs can occur at other sites than MTOC [35]. This is guided by γ-TuRC attached laterally along the surface of existing MTs. Attachment of γ-TuRC to the MT surface is mediated by augmin complexes and further involves the recruitment factor NEDD1 and the assembly factor TPX2 [36, 37].
Figure 5: Microtubule nucleation and organization at centrosome

2.2.4 Microtubule functions
The MT filament system (MFS) is very dynamic and thus has ability to reorganize in the cells to perform diverse functions and to serve the distinct needs in interphase and mitosis of the cell cycle. During interphase, MFS contributes to directional intracellular cargo transport and directional cell migration events [38]. During mitosis it is required for faithful chromosome segregation and cytokinesis [39, 40].

During mitosis, the MT spindle is formed from the two centrosomes and the MTs will bind to the floating chromosomes by a search-and-capture mechanism [41]. When all chromosomes are captured they are aligned to the central plate in a process called congression. The dragging to the center is mainly performed by motor proteins associated with the chromosome arms. Dynemin moves toward the spindle pole (MT minus end) while centromere protein E (CENP-E) and polar ejection force (PEF) moves away from the spindle pole.

MFS is also important for the cytokinesis process by specifying the division plane and by bringing MT-associated proteins to the emerging midbody. The initial phase of chromosome segregation is followed by elongation of antiparallel polar MT and formation of the central spindle MT. These structures induce further pole-pole separation by pushing force from motor proteins. These events partially overlap with the early cytokinesis process, including division plane specification by astral MT. Later, polar microtubules are re-organized and participate in the cytokinesis process (explained later in detail).
2.3 Septins

The septins were initially identified during screening for temperature sensitive budding yeast mutants that were defect in cell cycle progression; they are called cell division cycle (cdc) mutants. Certain of the yeast mutants found (cdc3, cdc10, cdc11 and cdc12) were described to have a long neck-like filamentous structure between mother cell and bud, and to show cytokinesis failure [42, 43]. By electron microscopy 10 nm thick filaments were observed in the neck region. Later, fluorescence study confirmed the localization of gene products cdc3, cdc10, cdc11 and cdc12 along the bud neck-like septum, and thus they were named as “septins” by John Pringle.

Since the identification of septins in yeast, homologous protein sequences were found in all animals, including humans. In Drosophila, septin-like protein (Pnut) was found to be involved in eye development [44] and its mutant form caused defects in cytokinesis. Since then septins have received high attention for its role in different cellular processes, including cytokinesis [45, 46].

2.3.1 Septin structure

Septins are highly conserved among eukaryotes except plants [47, 48]. They are GTP-binding proteins and belong to the phosphate-binding loop (P-loop) superfamily, similar to RAS-like GTP-binding proteins. Humans have 13 septin genes, SEPT1 to SEPT12 and SEPT14, and further protein variability is generated by alternative splicing [49]. Based on the sequence similarities, septins are categorized into four groups [50]: SEPT2, SEPT6, SEPT7 and SEPT8. Septin filaments consist of components from either 3 or 4 groups, which form hetero-polymer structures. The “Kinoshita rule” explains how different septins can be combined during filament formation. Recent information suggests the possible existence of 60 different combinations of septins from the four septin groups.

2.3.2 Septin protomer arrangement

In human, septins assemble into oligomer complexes of hexamers or octamers in palindromic order as shown in the figure 6B. Septins consist of three distinct regions: N-terminal extension (NTE), central core, C-terminal extension (CTE), and they use two different interfaces to interact with their neighbours and form non-polar filaments. These interfaces are between two GTP-binding domains (G-G surface) and between one NTE and one CTE (NC-surface). High order complexes are arranged with alternative G-G and NC-surfaces which give rise to different structures such as filament bundles, rings, and gauzes [51, 52]. Among all septin combinations, septin-7 is the only member, which is present in all septin filaments and plays an essential role in stabilizing the other core subunit.
2.3.3 Septin mammalian functions

The septin research area is quite new and many questions remain to be clarified. Due to the diverse subunit arrangement and combinations, septin filaments are associated with several functions e.g. as scaffold and as diffusion barrier [45, 53].

*Septin role in cytokinesis*

Septin was studied initially for its role in cytokinesis [45]. During furrow ingression, septin acts as bridge between the contractile ring and the plasma membrane. Recently, it was also shown to be involved in intercellular bridge (ICB) maturation, which generates a platform for the final cytokinesis step, abscission [54]. In mammalian systems, depletion of SEPT2, SEPT11 or SEPT7 causes cleavage furrow defect in HeLa cells, while SEPT9 depletion causes abscission defect [46]. Similarly, mouse fibroblasts lacking septin-7...
maintain the ICB structure, indicative of abscission failure [55]. In conclusion, septins are an essential back-bone for the cytokinesis process.

Figure 7: Septin filament acts as a bridge between cell membrane and contractile ring formed during early cytokinesis.

Other septin functions
In addition to its characteristic localization during the mitosis phase, septin exhibits various interphasic distribution patterns both at cortical and cytosolic locations. Septin filaments sometimes show co-distribution with actin fibers to which it can be cross-linked via myosin-II. These cytoskeletal filament systems also share similarities in their RhoA-dependent regulation, further suggesting interdependent functions in vivo [56, 57].

Septins can also associate with the microtubule machinery [58-60]. Further, phospholipid interactions with septins promote septin filament assembly. It has also been suggested that membrane phospholipids accelerate the septin higher-order assembly formation. We still need to understand much more about the formation of mammalian septin assemblies and how it is temporally regulated during a cell cycle.

2.3.4 Septin synthesis and degradation
Compared to other cytoskeletal filaments, septin filament turnover is poorly understood. The non-polar arrangement of septin monomers may explain its stability since there is no unstable (-) end. Septin turnover is believed to be regulated by post-translational modifications such as phosphorylation, sumoylation, or ubiquitylation. The yeast model has been widely used to study such questions and has identified the involvement of protein kinases in septin assembly, while sumoylation causes septin disassembly at the bud neck. A few interesting articles have reported that E2 and E3 SUMO enzymes are involved as septin binding partners in mammals/human. Recently, it was found that sumoylation on specific septins (i.e. SEP6, SEPT7 and SEPT11) are involved in septin filament assembly and required for cell division [61].
3. The cell cycle

The cell cycle is commonly separated into two main phases, i.e. interphase and mitosis (M phase) where cells grow and DNA duplicates and then segregates into two cells with identical genomes, respectively. Both interphase and M phase are further divided into sub-phases.

3.1 Cell cycle regulation

In order to progress into different phases of the cycle, cells require a commitment signal to cross into the following phases via checkpoint regulation. The cell cycle progression is driven mainly by several cyclins and cyclin-dependent protein kinases (CDKs). Cyclins are regulatory subunits that undergo synthesis and degradation in each cell cycle phase while CDKs are catalytic subunits having protein kinase activity specifically at serine and threonine residues. Checkpoint regulations are executed via cyclin-binding to CDK and the subsequent phosphorylation of the target proteins.

Cyclin levels are mainly controlled by ubiquitin-dependent proteolysis. The ubiquitin ligases Skp1-Cullin-F-boc (SCF) and anaphase promoting complex (APC) control cyclin level in G1-S phase and M phase, respectively. In addition to cyclin synthesis and degradation, CDK activity is regulated by phosphorylation/dephosphorylation of CDK and by binding of CDK inhibitor proteins (CKIs) [62].

3.1.1 Interphase

Most proliferating cells spend around 95% of their time in interphase, which is used for cell growth. It is further sub-divided into three phases, i.e. G1, S, and G2 phases based on its regulation by checkpoints. During G1, which is the longest phase of the cell cycle, the amount of cytoplasmic constituents increases. S phase is the period when DNA synthesis occurs; it is the second longest phase. G2 phase is the period following S phase. The G1 and G2 phases are collectively called gap phases, and represent periods where the cell monitor itself before it commits to move into S phase and mitosis, respectively. Most cells in our body are in the G0 state, which means that they are withdrawn from cell cycle and have reached into a differentiated state.
3.1.2 Mitosis phase

M phase is the period for the actual cell division, which consists of two major processes: karyokinesis and cytokinesis. Karyokinesis is the process where duplicated chromatids are segregated to their respective pole powered by MT-associated motor proteins in the mitotic spindle. Cytokinesis splits the emerging daughter cells apart. The M phase is a continuous process but commonly described as four stages: prophase, metaphase, anaphase, and telophase. Here cytokinesis is described as a separate phase.

Prophase

At very early stage of mitosis the condensation of chromatin occurs to form chromosomes. The chromosomes consist of two sister chromatids, which meet at a constricted region, the centromere. These sister chromatids are glued together throughout their length via multiprotein complexes called cohesin. At the end of prophase, the nuclear envelope disorganizes and the nucleus content merges with the cytoplasm. Moreover, cytoplasmic MTs depolymerize and the mitotic spindle is formed from the centrosomes.

Figure 8: Prophase; nuclear envelope breakdown and centrosome separation

Metaphase

Metaphase is a highly dynamic stage of mitosis where mitotic spindle MTs find a correct attachment site at chromosomes. Each chromatid has one MT binding site, the kinetochore, to which spindle MT can attach by a mechanism called “trial and error”. The sensing of correct attachment is based on tension from the spindle attached to two sister chromatids.

Figure 9: Metaphase; spindle assembly and chromosome equatorial alignment
Anaphase
Metaphase to anaphase transition is tightly regulated at the so called spindle checkpoint by the anaphase-promoting complex (APC). When all chromatids are connected to microtubules, APC is activated and induces the cleavage of cohesion between sister chromatids by the cysteine protease separase. Then each chromatid is pulled towards opposite poles with the same speed, 1 \( \mu m \) per minute, but during two independent and overlapping sub-phases i.e. anaphase A and anaphase B.

In anaphase A kinetochore microtubules are shortened, and associated chromatids progress to the spindle pole region. The movement is further driven by dynein at kinetochores which cause poleward movement of chromatids as it is a MT minus end-directed motor protein. Anaphase B depends on motor proteins associated with the pole and astral microtubules, which push the poles apart. The MT plus end-directed kinesin proteins cross-link between overlapping antiparallel astral spindle MTs and thereby push the spindle poles.

Figure 10: Anaphase; chromosome segregation and cytokinesis specification

Telophase
At this stage, the segregated chromosomes reach to opposite poles and kinetochore MTs are disassembled. Chromatin starts to de-condense and the nuclear envelope starts to reform. Astral and central MTs are still in elongated form and will later contribute to the formation of the midbody (MB).

Figure 11: Telophase; nuclear envelope reformation and cytokinesis cleavage furrow
3.1.3 Cytokinesis
Cytokinesis is the process where two physically connected nascent daughter cells split apart. The following sections describe the cytokinesis process in detail, and are divided into three steps:

3.1.3.1 Cleavage furrow formation and ingression
3.1.3.2 Midbody formation
3.1.3.3 Cytokinetic abscission

3.1.3.1 Cleavage furrow formation and ingression
The cytokinesis process is initiated shortly after anaphase onset in animal cells and is tightly regulated in spatiotemporal manner. It is inaugurated by inactivation of CDK1 through proteolytic degradation of mitotic cyclin B [63, 64]. Such termination of CDK1 control drives cleavage furrow formation and ingression, which involves the following steps:

*Central spindle assembly*

*Division plan specification*

*Contraction of actomyosin ring*

In early cytokinesis, de novo formation of MTs from existing MT is supported by the augmin complex. This generates antiparallel MTs with overlapping (+) ends in the central part of mitotic cells [65] which is called central spindle/spindle midzone. The organization of the spindle midzone is dependent on MT-associated motor proteins, which are regulated by mitotic kinases [66-68]. The spindle midzone specifies the division plan axis, and later it will provide a platform for the assembly of the midbody (MB). The spindle midzone MTs are remodeled by MT bundling factors, among which PRC1 and centralspindlin are the best studied. PRC1, a non-motor MAP, is kept inactive until early anaphase via CDK1 and PLK1-mediated phosphorylation and prevents dimerization. After CDK1 decline, activated PRC1 cross-links anti-parallel MTs and recruits the motor protein KIF4 to maintain the MTs’ overlapping region [69, 70]. In addition to PRC1, a key structural and signaling scaffold of the central spindle is centralspindlin [71]. It is a heterotetramer consisting of a pair of kinesin MKLP1 and a pair of the Rho family GTPase-activating protein (GAP) CYK-4 (also known as MgcRacGAP). Centralspindlin form larger clusters which migrate along the MTs towards the (+) end [72, 73] and promote MTs bundling[68, 74]. Centralspindlin cluster formation is initially prevented by binding of protein 14-3-3 to MKLP1 but this protein is later released due to Aurora B activity dur-
ing cytokinesis\cite{75}. Additionally, CDK1 phosphorylates MKLP1 and thereby reduces its affinity for MTs\cite{65}.

\textit{Division plan specification}

The first step towards cleavage furrow ingression is cross-talk between astral MTs and the cellular cortex in a process called division plan specification. The astral MTs mediate the transport of the RhoGEF ECT2 via centralspin-dlin to the cortex, and thereby determine the site where active RhoA will accumulate\cite{76}.

\textit{Contraction of actomyosin ring}

After the establishment of cleavage furrow formation site, RhoA and its downstream signaling maintain initial furrow ingression. RhoA is an enzyme that requires interaction with GAP to stimulate its GTPase activity and GEF to load it with GTP. GTP-loaded RhoA has a conformation that can act on several effector targets, including formins, ROCK, citron kinase and anillin. RhoA binding to the amino end of the formin mDia induces polymerization of actin bundles \cite{77, 78}. RhoA also controls the myosin-II activity via the serine-threonine kinase ROCK that phosphorylates myosin light chain (MLC) and MLC phosphatase (among other substrates). The pulling on actin by myosin drives the cleavage furrow ingression \cite{79-81} and anillin and its associated proteins further support the process, which bridge between the plasma membrane and the contractile ring. Still, actomyosin-based furrow ingression is not well understood in term of its regulation at the processing site.

\textbf{Figure 12: Early cytokinesis; Rho A equatorial accumulation}
3.1.3.2 Midbody formation

The MB starts to form when the contraction of the cleavage furrow has reached a diameter of 1-2 μm. It is a protein complex that serves as a platform for the ordered recruitment of proteins required for the final cell division. The MB formation process can be separated into the following parts:

- **Reorganization of MB components**
- **Stabilization of ingressed cleavage furrow**
- **Maturation of midbody**

**Reorganization of midbody components**

Several actomyosin ring components, including anillin, septin, and RhoA are reorganized to the center of the narrow intercellular bridge formed by the ingestion [82, 83]. The central spindle-associated proteins PRC1 and KIF4 keep their localization at the area of overlapping central microtubules whereas the previous centromere-binding partners PLK1, CENP-E and Aurora B now localize at the microtubules flanking the forming midbody [84, 85].

**Stabilization of ingressed cleavage furrow**

Upon complete ingestion of cleavage furrow, the narrow intercellular bridge is stabilized by the linking of the plasma membrane to the forming MB via several proteins, including anillin, septin, MKLP1 and CYK-4 [86]. Anillin, septin and CYK-4 have a membrane binding domain at their C-termini while MKLP1 associates with the membrane through the small GTPase Arf6. The plasma membrane enclosing the midbody is enriched with PtdIns(4,5)P2 which directs the localization of several of these proteins [87].

**Maturation of midbody**

When centralspindlin (MKLP1/CYK-4) has become enriched at the (+) ends of overlapping anti-parallel MTs of the central spindle it can recruit centrosomal protein 55 (CEP55) through a binding to MKLP1. Polo-like kinase 1
(PLK1) exerts a tight temporal regulation at this stage by phosphorylating CEP55 at Ser436 and thereby preventing its premature localization at the MB until late telophase [88]. CEP55 is a key adaptor protein, which make the MB a platform where the abscission machinery can act and finish the process of cytokinesis.

PLK1 controls several events in the cell cycle progression from G2-M phase transition to cytokinesis [89]. In the early phase of mitosis, it is localized on centrosomes and kinetochores where it helps in the establishment of a mitotic spindle [90, 91]. Later in anaphase it relocates to the central spindle and initiates cleavage furrow ingression [92-94]. PLK1 is gradually degraded as cells progress towards mitotic exit and is absent at the time of late cytokinesis [95].

![Midbody formation; recruitment of scaffold protein CEP55](image)

**Figure 14:** Midbody formation; recruitment of scaffold protein CEP55

### 3.1.3.3 Cytokinetic abscission

Abscission is achieved by first clearing of central spindle MTs from the ICB, followed by fusion of the two closely apposed ingressed membranes. At the end of the abscission process, the ICB is constricted further and associated with 17 nm diameter helical filaments composed of ESCRT-III subunits [96, 97]. ESCRT-III is a protein complex evolutionary conserved from archaea to human, which drives various fundamental cellular processes i.e. virus budding, multivesicular body biogenesis and cellular abscission[98]. Abscission is regulated by a series of ESCRT complexes and involves the following steps:

*Early steps in cytokinetic abscission*

*Membrane deformation*

*Final Cut*

*Early steps in cytokinetic abscission*

CEP55 recruits ESCRTs and their binding proteins in a sequential manner to the MB region. ESCRT-I binds to CEP55 via the subunit TSG101. CEP55
also binds ALIX, and both ESCRT-I and ALIX can independently recruit the ESCRT-III complex to complete cytokinesis process [99, 100].

**Membrane deformation**

The ESCRT-III complex consists of 12 subunits known as CHMPs. It has the ability to deform the membrane via polymerization of multiple ESCRT-III subunits. Initially, CHMP4B is recruited and oligomerized into a ring at the MB, and later it is converted into coiled helical spiral polymers of decreasing diameter upon binding of additional membrane-deforming factors, i.e. CHMP2 and CHMP3[101].

**Final cut**

The CHMP4B spirals promote further narrowing of the intercellular bridge and bring the two membranes close for fusion[102]. In addition, MTs are disassembled by the MT-severing AAA-ATPase spastin. Spastin is targeted to the constriction region through binding to the ESCRT-III subunit CHMP1B [103, 104], which allows the abscission to be done. The actual membrane fusion mechanism is not yet understood.

3.1.3.4 **Anillin and septin in cytokinesis**

During the cytokinesis process it is crucially important that the contractile ring properly connects to the plasma membrane and the central spindle. Anillin is one of the linker proteins that perform such function [87]. Anillin was initially isolated together with F-actin from Drosophila and was thus considered as an actin-binding protein. Later, it was observed to interact with multiple partners, including septin in human. During interphase, anillin is packed
into the nucleus and it is released to the cytoplasm after nuclear envelope breakdown during mitosis. Prior to karyokinesis, anillin is distributed all along the cell membrane and later it becomes concentrated to the cleavage furrow ingression site. Further, it has been shown that anillin binds and recruits septin to the furrow site [87].

During furrow ingression, septin-anillin is arranged along the ICB as a collar around the MT bundle. At the onset of abscission, anillin and septin re-organize into three rings: a central ring around the midbody and two flanking rings. It appears as the flanking rings mark the sites to which the narrowing CHMP4B spirals become directed and thereby form the constriction sites for abscission. This model illustrates the indirect, but very central, role of anillin-septin in the abscission step of cytokinesis [54].

### 3.1.3.5 Abscission checkpoint

In case there are defects disturbing the cytokinetic process, i.e. the presence of chromatin material in the ICB or nucleopore complex defects, a so-called abscission checkpoint will be activated which triggers abscission delay[105] and sometimes lead to bi-nucleation. Abscission arrest is mainly regulated via the mitotic protein kinase Aurora B and CHMP4C. CHMP4C phosphorylated by Aurora B binds to ALIX and thereby prevents the polymerization of ESCRT-III subunits at the MB.
4. Centrosome

The centrosome is a complex structure and consists of paired barrel-shaped centrioles surrounded by dense material called pericentriolar matrix (PCM). Centrioles are known to perform unique functions in microtubule organizing centres (MTOCs), i.e. centrosomes and basal bodies[106]. Centrosomes have several cellular functions, such as in cell polarity organization, cell migration and cell division. Centrosome structure and function fluctuate during the cell cycle [107]. In this thesis, the role of centrosomes during mitosis will be discussed. Accurate chromosome segregation is achieved through proper centrosome duplication, centrosome separation, spindle formation, and cytokinesis [108]. Aberrant function at centrosome or centriole level correlates with several diseases, including cancer [109].

4.1 Centrosome duplication cycle

During the G1 phase of cell cycle, cells have one centrosome with two centrioles: a “mother” and a “daughter” centriole. These centrioles are disengaged but still connected via a flexible linker formed by C-Nap1 and rootletin [110]. At this stage, centrioles are licensed to duplicate their structure in parallel with DNA replication in S phase. A new daughter centriole is formed perpendicular to each one of the existing centrioles at their proximal end. During G2, the daughter centrioles get elongated and the new young mother centriole (previous daughter centriole) develops appendages, a sign of maturity. Upon G2-M transition, the flexible linker between the two mother centrioles are dissolved and centrosomes separate to form the MT spindle[111].
Figure 16: The centrosome duplication cycle. Centrioles are disengaged and licensed for duplication upon mitotic exit. During G1-S transition, the daughter centriole transforms into mother centriole with the loss of daughter centriole proteins. The two mother centrioles then start to assemble a daughter centriole each. In the G2 phase, daughter centrioles get elongated equal to the length of the mother centrioles and further mature by accumulation of pericentriolar matrix (PCM). Furthermore, appendages are added to the newly developed mother centriole.

4.2 Centrosome separation

Centrosome separation occurs in distinct steps: centrosome maturation, centrosome disengagement, and actual separation. This process has been extensively studied and involves various mitotic regulatory proteins, including kinases and motor proteins. Centrosome maturation involves recruitment of PCM and appendages formation on the new mother centriole. The mitotic kinases PLK1 and Aurora A localise to centrosomes during G2-M and recruit essential proteins in a sequential manner that further contributes to the development of interphasic centrosomes to mitotic centrosome. An important such event in G2 is the translocation of γ-TuRCs from the cytoplasm to centrosomes and the anchoring to the centriole [112-114]. The duplicated centrosomes then disengage by linker disruption in a process induced by Nek2 kinase activity. Subsequently, the actual separation of centrosomes starts late in G2 and finishes before metaphase. Centrosome separation is guided by MT and is driven by MT-associated motor proteins. Eg5, a MT (+) end motor protein which has an important role, is activated by Nek kinases downstream of PLK1 and CDK1. Eg5, together with some other motors move along MTs and generate pushing and pulling force to bring centrosomes apart, a prerequisite for spindle formation [115, 116].
5. Causes of Aneuploidy

Cell growth and division are tightly regulated and impairment can cause genomic instability. A defect where the chromosome number deviates from the normal is called aneuploidy [117]. In all organisms, aneuploidy is frequently linked with disease, and aneuploidy cells are found in 90% of solid tumors. In spite of efficient control mechanisms, there are many chances for mistakes to happen during each cell division. Here, two possible causes of aneuploidy in human cells are described: defect at centrosome and cytokinesis levels, respectively.

5.1 Defect at centrosome level

Accurate segregation of chromosomes is a mechanical event, which requires a bi-polar MT spindle. Duplicated centrosomes in S phase undergo separation in early mitosis at prophase and pro-metaphase and then form the spindle. This centrosome migration is regulated by kinase-dependent motor proteins (Eg5 and dynein). Defect centrosome separation, centrosome over-replication, or centrosome fragmentation cause malformation of the spindle and defect chromosome segregation. It has been suggested that most centrosome defects promote merotelic MT attachment to chromosomes (a kinetochore binds to MTs derived from both centrosomes) resulting in lagging chromosome at the ICB region [118].

5.2 Defect at cytokinesis level

As described above cytokinesis can be divided into three stages on the basis of their regulation: cleavage furrow ingression, MB formation and abscission. Defects in either of these steps could cause cytokinesis failure. Defects at early stages of cytokinesis have been discussed in several articles and shown to lead to furrow regression[119]. Furrow regression results in cells containing both duplicated genome and duplicated centrosomes. This situation can give rise to aneuploidy in the following cell cycles by two mechanisms: 1) formation of multipolar spindle due to too many centrosomes. 2) loss of chromosomes because tetraploidy is an unstable condition.
6. Present investigations

Paper I

Aim: Anchorage-independent proliferation is a characteristic feature of cancer cells. Adhesion-dependent cell cycle regulation is governed at two stages in non-transformed cells: at the G1-S transition and at cytokinesis. Previous studies from our group show that transiently detached cells progress through S phase to M phase and segregation of chromosome, but they are not able to complete cytokinesis in the absence of integrin-mediated cell adhesion to extracellular matrix. This observation raised two main questions: 1) How far do suspended mitotic cells progress into the cytokinesis process, i.e. what is the failing step? 2) How are integrin signals linked to cytokinesis?

Results

Observations of non-transformed human fibroblasts (BJhTERT) by live-cell imaging confirmed that these cells were unable to complete cytokinesis under non-adherent conditions while the adherent cells completed the process in 2-3 hours. Immunofluorescence analyses revealed that the cytokinesis process progressed in the non-adherent cells until the recruitment of CEP55 to the MB, but the late MB components ALIX, TSG101 and CHAMP4B did not appear at this site. Further studies indicated that the failed cytokinesis may result from aberrant temporal regulation of PLK1 and CEP55; a too early degradation of PLK1 was accompanied with a premature accumulation of CEP55 at the MB as expected, a condition which is known to interfere with cytokinetic abscission. To identify signaling events upstream of PLK1 which could be regulated by integrins we used inhibitors of known major integrin signaling pathways, i.e. FAK-Src, ROCK, MEK etc. We found that PLK1-CEP55 were spatiotemporally regulated via FAK-Src signaling, and inhibitors of these kinases blocked cytokinetic abscission of adherent cells.
**Paper II**

**Aim:** Our previous studies showed that cytokinetic abscission is blocked in non-transformed fibroblast cells under non-adherent culture conditions. Cytokinetic abscission failure results in tetraploid cells. As it is known that tetraploidy can be an early step for the initiation of cancer and promotes tumor progression, we investigated the fate of bi-nucleated cells generated after a transient detachment period.

**Results**

We subjected the cells to prolonged suspension treatment for different durations (up to 3 hours) to chase MB proteins and found that all analyzed mid-body proteins i.e. Aurora B, MKLP1, CEP55 disappeared with time from the intercellular bridge (without cytokinetic abscission as expected). When such bi-nucleated cells were re-plated on fibronectin-coated surfaces we found that most of them were able to complete cytokinesis within 9 hours (observed by live-cell imaging) although none of the MB proteins appeared in the intercellular bridge during this period. Also microtubules, the central MB assembly platform, remained absent from the intercellular bridge. Time-lapse movies suggested that the abscission was achieved by mechanical tension when the cells moved apart. To verify this interpretation, we re-plated the cells on soft (0.5 kPa) and stiff matrix (64 kPa) after the 3-hour suspension period; the MB-independent abscission was strongly promoted by the stiff matrix. Notably, cells exposed to cytochalasin D to inhibit furrow ingression did not undergo the tension-induced abscission, and instead became permanently tetraploid. Exposure of cells to a brief cytochalasin D treatment at different times during the cytokinesis process indicated that the furrow can regress until the maturation of the intercellular bridge has reach a late stage. Furrow regression generated tetraploid cells while cells with narrow intercellular bridge underwent tension-dependent abscission. In order to investigate how the latter cells maintained a narrow intercellular bridge without involvement of linker proteins between MB and plasma membrane, such as centralspindlin, we analysed the localization of septin-7, a protein known to be involved in the formation and stabilization if the intracellular bridge. Septin-7 was found to be associated with the intercellular bridge also when the MB was absent after incubation of the cells in suspension for 3 hours. However, it was not present in the midzone when furrow ingestion was prevented by cytochalasin D.

Together these results show that adhesion-mediated tension along septin-stabilized intercellular bridge is sufficient to complete cytokinetic abscission without the assembly of a MB protein complex.
Paper III

Aim: Paper II described that septin filaments maintain the ingressed intercellular bridge (ICB) in detached cells although the midbody disassemble/disappear within 3 hours. In this study we investigated the kinetics of ICB regression and the long-term fate of bi-nucleated cells in non-transformed (BJhTERT) and transformed (BJ-LT and BJ-LT-Ras) fibroblasts.

Results

Septin was shown to persistently stabilize the ICB for long time (>24 hours) under non-adherent culture condition and did not allow furrow regression. BJhTERT fibroblasts maintained a bi-lobular/bi-nucleated structure while BJ-LT fibroblast passed the G1-S checkpoint and became tetra-lobular/tetra-nuclear as determined by immunofluorescence and live-cell imaging. For BJ-LT-Ras cells initial data showed that the ESCRT-III-associated protein ALIX was present at the MB region (marked by Aurora B) during the expected abscission time period when the cell were kept in suspension. Furthermore, live-cell imaging and septin-7 immuno-staining supported the possibility that abscission was completed in the Ras-transformed cell under non-adherent condition, but uncertainty remained since the two daughter cells adhered to each other in suspension. Absence of septin-7 distribution along ICB could be due to either abscission or furrow regression in the cells. To clarify whether the cells divided in suspension or became tetraploid, they were re-plated on fibronectin. We found that the bi-lobular BJhTERT and BJ-LT fibroblasts migrated apart and divided by the traction-based abscission mechanism after 5-6 hours as described in paper II, while BJ-LT-Ras cell migrated apart within 40 min, confirming that they had undergone abscission already during the suspension period.

After prolonged culture of BJhTERT cells in suspension the septin-staining at the ICB gradually decreased, which correlated with an increasing number of bi-nucleated cells after re-plating on fibronectin (approximately 10% after 42 hours in suspension). As bi-nucleation potentially can induce tumor initiation, we chased the fate of such cells. Our data showed that bi-nucleation generated in non-transformed cells causes G1-S arrest, possibly due to centrosome fusion-dependent p53 activation, and these cells became senescent. In contrast, cells with suppressed G1 checkpoint, such as BJ-LT, progress into cell cycle and divide as tetraploid cells with high risk of becoming aneuploid.

The results of this study show that normal cells have several mechanisms, which prevent them from uncontrolled proliferation during/after transient detachment. However, detachment of cells after certain virus infections may generate chromosomal instability.
Paper IV

Aim: Our published data showed that cell-adhesion to ECM is required for cytokinetic abscission. Moreover, our initial observations showed that there was also a delay in early mitosis progression in detached cells, which affected spindle formation and chromosome segregation. We asked then three basic questions: 1) Is the delayed mitosis progression linked to centrosomes? 2) How does cell adhesion affect centrosome functions during mitosis? 3) How are integrin signals linked to centrosome regulation?

Results

We first synchronized exponentially growing BJhTERT cells with nocodazole to collect cells in early mitosis by tapping off the rounded cells. The cells were then cultured either under adherent or non-adherent conditions for different time periods (from zero-180 minutes) and mitosis progression was monitored from prophase to cytokinesis by staining for tubulin, DNA, and pericentrin (centrosome marker). We found that most of the mitotic adherent cells reached to cytokinesis stage in 90 minutes while non-adherent cells were scattered among different mitotic stages with 30% in prophase. In addition to delayed mitotic progression, we found centrosomal abnormalities: 1) defects in centrosomal separation and spindle formation 2) fragmented centrosomes. To investigate how integrin signalling was involved in mitotic progression and the centrosome abnormalities, we looked at signalling mediators downstream of integrins, first focusing on focal adhesion kinase (FAK). After collecting mitotic cells upon nocodazole treatment, we immediately added FAK inhibitor (PF-562271) and then re-plated the cells on fibronectin-coated surface while control cells were cultured in the presence of DMSO. Our data showed that the FAK inhibitor strongly induced similar features as under non-adherent condition, i.e. delayed mitosis, spindle assembly defects, poor separation of the centrosomes, and centrosome fragmentation. Further, it has been reported that PLK1 and CDK1 are required for centrosome separation and spindle formation upstream of Eg5, a mitotic motor protein. To study whether PLK1 or/and CDK1 activity was linked to cell adhesion-dependent centrosome regulations, the effect of the FAK inhibitor on the level of PLK1pThr210 and pEg5 (pSer1033 and pThr296) was analysed. The FAK inhibitor-treated cells had reduced activity of PLK1 as monitored by low PLK1pThr210 and Eg5pSer1033 levels while Eg5 phosphorylation at Thr296 by CDK1 was not affected. To exclude side effects of the FAK inhibitor, we performed similar experiments with Tet-FAK MEF cells in which the FAK expression can be turned off by tetracycline. Under FAK ON condition, cells were progressing in mitosis normally whereas FAK OFF cells showed a similar phenotype as BJhTERT cells treated with FAK inhibitor. These results suggest that integrin signals are required for proper centrosome separation to form a correct mitotic spindle, and that the regulation is maintained through a FAK-PLK1-Eg5 axis.
7. Future perspectives

The research work presented in the current thesis has generated novel findings and raises several unsolved questions at the same time. Some major questions are listed below:

- In paper I, we showed that the scaffold protein CEP55 remained “immature” in non-adherent cells and could not recruit the abscission proteins ALIX and ESCRT III to the midbody. Integrin-linked FAK signaling was required to drive the maturation and the subsequent membrane fusion event, abscission. Further investigations are required in order to understand
  
  - What protein modifications or additional proteins are required for CEPP55 to function as scaffold protein for the abscission machinery?
  
  - the connecting link between integrin-FAK activation and PLK1-CEP55 regulation.

- In Paper II and Paper III, we described that septin can maintain the ICB even without MB proteins for long time and thereby prevent furrow regression in non-adherent fibroblasts. However, slowly a minor fraction of the cells became bi-nucleated. Bi-nucleated non-transformed cells became senescent and arrested in G1, possibly due to p53 activation after centrosomes moving together. Ras-transformed fibroblasts completed normal abscission (i.e. ESCRT-dependent) in non-adherent culture condition. These findings motivate investigations on
  
  - the septin dynamics along the ICB and the mechanisms regulating septin depolymerization and organization into different structures.
  
  - the mechanism behind centrosome merging in bi-nucleated cells.
• In paper IV, we found that integrin-dependent FAK-PLK1-Eg5 signaling axis is required for centrosome stability, proper spindle assembly and accurate chromosome segregation. These observations on adhesion-dependent early mitosis regulations open up a new research area which includes many key questions, including:

- the cause of centrosome fragmentation in the absence of integrin signals.
- the signaling links between integrin–FAK and pEg5.
Acknowledgement

I would like to thank:

My main supervisor Prof. Staffan Johansson, for recruiting me as a PhD student in the Medical Biochemistry and Microbiology (IMBIM) department. I am very thankful to your fully support during the entire my PhD period. Your kindness and positive nature gave me an excellent opportunity to improve not only my scientific knowledge but knowledge in general. Thanks so much for having patience to listening and appreciating my thought after each experiment. Actually, your passion for doing good science motivates me to be in academia. Honestly, it was right decision to join your research laboratory. Staffan, I will definitely miss my PhD time.

My daily supervisor Dr. Siamak, very thanks for supervising me how to do experiments in the laboratory. Initially, I had an impression that you are really tough teacher since you always said to me that “this is very important experiment”. That is how I improved my practical skills a lot. Thanks for actively involving me in the research projects and give me a nice platform to do the best in research. You are a “precise” scientist and want everything perfect. Sorry for interrupting you all the time with microscopes and research plan. You are actually caring and friendly person as well. Very thanks for PhD thesis cover picture.

Prof. Aris Moustakas and Assoc. Prof. Anna-karin Olsson, very thanks to be my co-supervisor. I am grateful to have such good co-supervisors to support me all the time. Though, it was not much related to scientific collaboration but I learnt a lot of your research lines while attending your research seminars and journal clubs. I wish to have collaboration in near future to share our scientific ideas. Aris, we had good memory hosting a game together last year when we had group activities in Christmas dinner. Anna-Karin, thanks for nice dinners during summer time.

Prof. Carl-henrik Heldin, thanks for discussing my research projects and asking valuable questions in the cell cycle field. I am impressed to see the way you present and think about science.
Dr. Ingvar Ferby, thanks to be part of my committee member during my licentiate examination and sharing knowledge with us.

Prof. Kjellen Lena, very nice to meet you. You have very good communication skill and of course, you are a good singer.

Dr. Mikael Sellin, very thanks for sharing your experiences with septin filament system. Septin is a quite interesting field in the area of cell biology.

Lab colleagues: Thanks Xiofang, it was very nice to meet you. Ying, thanks a lot for helping me with everything in the laboratory. Thanks to Mariela, Chandana, Maria, Oishee and Ali for sharing your knowledge. It was nice experience to work with you. Thanks Alexandra for having nice friendship. Thanks for teaching me some Swedish recipes, cakes and cookies. Thanks Jian for everything. You are really nice and caring person. It was very nice to work with you and thanks for contributing in two projects especially the involvement of RAS in cytokinesis. Thanks Liangwen for your good support and helping us a lot with the projects especially initial parts of binucleation manuscript. You are really smart and clever girl. Thanks Anishia for sharing your knowledge in the laboratory. You are very hardworking and passionate about science. We had very nice discussion on centrosome project during summer time.

I am very thankful to IMBIM department especially to B9:3 and B11:3 corridor friends. Thanks to Swedish “FIKA”. It is really good way to connect with people and at the same time refresh your mind. Very thanks Yanyu for all your support and help during experiments. We always find something interesting to talk and once I missed a time point for my experiment. Happy to share office room with you. Thanks Melanie, Jessica and Anahita for all discussion we had. Thanks to Kavya and Parisa for having you in the office. Kavya, thanks for sharing your career experiences. Hope it will help me a lot in future. Thanks to Ravi for nice friendship. I would like to say thanks all past and present IMBIM members and especially to the new B11:3 corridor. This corridor is really a scientific corridor.

Special thanks to my Indian friend, Priya, Zeeshan and Alisha for having wonderful company in the initial PhD days. Zeeshan and Alisha, you guys are really funny and helpful. We were like a nuclear family in Uppsala. ☺ We had memorable trip to Finland and Russia during PhD first year and excited to see cruise first time.

Very thanks to Narendra, Kalicharan, Sulena, Sujata, Rajesh and Priyanka, I was fortunate to get in touch with you. Again thanks to Narendra, Rajesh and Kalicharan for guiding me about career opportun-
ties. Thanks to Rajesh and Priyanka for nice friendship. Special thanks to Rajesh for helping me with tough experiments especially with suspension live-cell imaging. Furthermore, good discussion afterwards with Staffan. Thanks to Sandeep, Navya, Varun, Pratyusha, Snehangshu, Soumi, Rekha, Tanya aggrawal for all your help and suggestions about life and career. Especial thanks to Ravi shah, Mayank and Tahira for having serious discussion on India, Pakistan, Sweden culture. Thanks Tahira for having lunch together almost everyday since last semester.

Very thanks to my Dear parents, I understand that it was very tough decision to send me abroad. At somepoint I also got scared how will I adapt to the Swedish culture. Now, I realized that it was good decision to come abroad.

Thanks to my brother Prashant and sister-in-law Pallavi for supporting me all the time with everything. Special thanks to Prashant to understand my potential interest for science. Your contribution is very unique in my life.

The special person that I really grateful to thank is my fiance, Shailakshi Gupta. You are charming and happy person. I appreciate your humble and honest behavior. Thanks for being special part of my PhD thesis memories.
References

5. Miyamoto S, Teramoto H, Gutkind JS, Yamada KM. Integrins can collaborate with growth factors for phosphorylation of receptor tyrosine kinases and MAP kinase activation: roles of integrin aggregation and occupancy of receptors. The Journal of cell biology. 1996; 135: 1633-42. doi:
13. Calderwood DA, Zent R, Grant R, Rees DJ, Hynes RO, Ginsberg MH. The Talin head domain binds to integrin beta subunit cytoplasmic tails and regulates integrin activation. The Journal of biological chemistry. 1999; 274: 28071-4. doi:


34. Zheng Y, Jung MK, Oakley BR. Gamma-tubulin is present in Drosophila melanogaster and Homo sapiens and is associated with the centrosome. Cell. 1991; 65: 817-23. doi:


42. Hartwell LH. Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. Experimental cell research. 1971; 69: 265-76. doi:


44. Neufeld TP, Rubin GM. The Drosophila peanut gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. Cell. 1994; 77: 371-9. doi:


64. Echard A, O'Farrell PH. The degradation of two mitotic cyclins contributes to the timing of cytokinesis. Current biology : CB. 2003; 13: 373-83. doi:


88. Bastos RN, Barr FA. Plk1 negatively regulates Cep55 recruitment to the midbody to ensure orderly abscission. Journal of Cell Biology. 2010; 191: 751-60. doi: 10.1083/jcb.201008108.


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)