

Adhesion and Cytokinesis in Mammalian Cell Division

Background

On entry into mitosis a cell rounds up and loses adhesion to the extracellular matrix and contact with adjacent cells. This process is reversed after cytokinesis in the process of cell spreading and cell adhesion is re-established via formation of focal adhesion complexes through integrin signaling. If cell adhesion or integrin function is perturbed during mitosis then cells fail to undergo correct cytokinesis. It has been found by Dr Lindon's lab that the mitotic ubiquitin ligase APC/C-Cdh1 plays a role in reorganizing the actin cytoskeleton in G1 phase.

Aurora B is a mitotic kinase that is involved in chromosome condensation, the spindle checkpoint and is essential for cytokinesis. This explains its localization to microtubules near kinetochores then to the spindle midzone of mitotic cells. Aurora B is known to be degraded by the APC/C (controlled by Cdh1) after cytokinesis but recently it has been discovered, in Dr Lindon's lab, that some is seen to relocalise to the cell cortex at the points of cell spreading.

Alpha-Actinin is an actin binding protein that is cytoplasmic early in mitosis, relocalising to the cleavage furrow at cytokinesis, then to focal adhesions on their formation and along stress fibers, indicating an involvement in re-establishing cell adhesion after mitosis and hence its localization can be used to time the formation of cell adhesions after mitosis.

Anillin is a contractile ring protein that localizes to the cleavage furrow and is required for maintaining the localization of Rho A and active myosin to the cleavage furrow required for cytokinesis. Anillin expression can be used to time the formation of the cleavage furrow and the completion of cytokinesis.

Aims

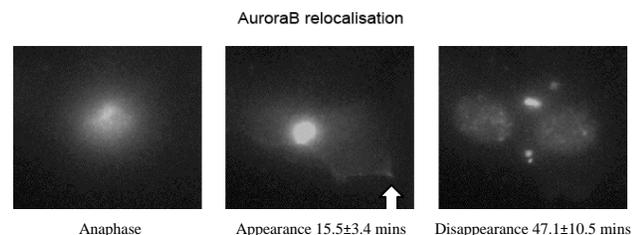
1. Use live cell imaging to investigate the link between cytokinesis and adhesion in mammalian cells by altering conditions to favor or inhibit cell adhesion.
2. Test the hypothesis that APC/C-mediated proteolysis regulates focal adhesion formation after mitosis and is involved in assisting cytokinesis.

Methods

1. Cell lines, already established, expressing stable Aurora B-venus (UTOS cells), anillin-venus and alpha-Actinin-venus (RPE cells) constructs will be used to time cytokinesis and adhesion formation in living cells exiting mitosis.
2. The effect of plating cells arrested in mitosis onto dishes coated with either fibronectin (favors cells adhesion) or polyHEMA (inhibits adhesion) on these timings will be measured.
3. The effect of inhibitors of Aurora B (ZM447439), ROCK (Rho kinase, a second known cleavage furrow kinase), polymerization of microtubules (nocodazole) and the actin cytoskeleton (cytochalasin D) on cytokinesis and adhesion will be assessed.
4. Addition of proteasome inhibitors (MG132) and Cdh1 siRNA treatment of cells under different adhesion conditions mentioned above will be used to test if ubiquitin-mediated proteolysis regulates focal adhesion complex formation on mitotic exit.

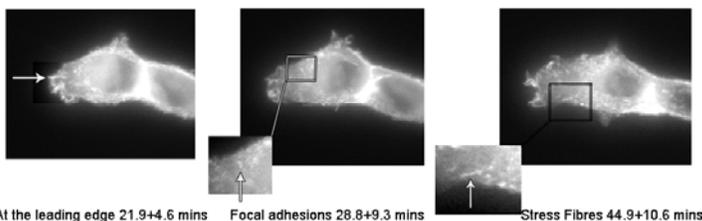
Results

The timing of Aurora B relocalisation to the cortex and its subsequent disappearance were established as shown in the picture adjacent and likewise for the localization of alpha-actinin at focal adhesions and on stress fibers in the picture below (all timings are minutes after anaphase). *These timings show that Aurora B relocalisation is within a very specific window of time.* An effect of cell density on Aurora B



relocalisation was observed and further experiments, involving diluting Aurora B-venus expressing cells in different amounts of wild type U2OS cells, revealed that *when cells are at a higher density appearance at the cell cortex is consistently later and for a shorter period of time.* A density effect was not observed for alpha-actinin localization in a similar experiment.

alpha-actinin stress fibre formation

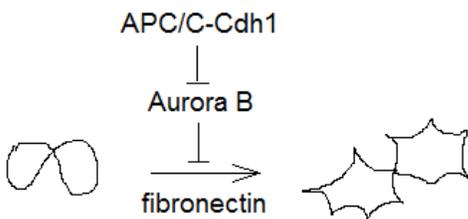


effect on Aurora B relocalisation as an increase in cell density with *appearance at the cortex later and for a shorter time.* By analyzing the timings of alpha-actinin cells it was found that on fibronectin *focal adhesions and stress fibers form earlier*, with stress fibers forming around 20 minutes earlier than in cells not on fibronectin. When cell adhesion was inhibited by seeding cell onto polyHEMA (and poly-lysine to inhibit cell movement) a lot of Aurora B was seen to relocalise to the cortex and it remained here as cells could not establish adhesion to the extracellular matrix showing the *ability of cells to adhere is important for the degradation of this cortical Aurora B and suggests this degradation is important for formation of stress fibers.*

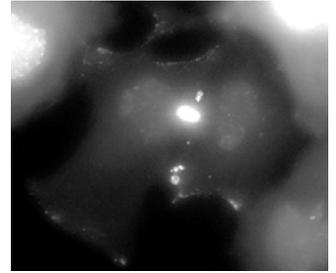
Addition of nocodazole to Aurora B cells appeared to inhibit any relocalisation to the cell cortex and caused loss of any that may have already moved there before addition of the drug showing *microtubules are required for the initial relocalisation and sustaining this localization of Aurora B.* Addition of Cytochalasin D caused sustained localization of Aurora B at the

cortex in post mitotic cells and also some recruitment there in interphase cells. This may be due to stabilization of microtubules in response to destabilization of actin filaments. Addition of the Aurora B inhibitor often caused cells to fail cytokinesis, mostly no relocalisation of Aurora B to the cell cortex is seen and as with nocodazole any seen at the cortex is quickly lost. In cells in which relocalisation to the cortex does occur it is not sustained for very long. Alpha-actinin expression shows that formation of focal adhesions and stress fibers occurs earlier as on fibronectin in cells where Aurora B is inhibited. The Rho kinase inhibitor seemed to have no effect of Aurora B relocalisation suggesting it, and Rho A, act downstream of Aurora B or in another pathway.

Addition of MG132 (proteasome inhibitor) to Aurora B cells caused a larger amount of Aurora B to arrive at the cell cortex (see figure to the right) and it is sustained here for up to two hours. When MG132 was added to alpha-actinin expressing cells focal adhesion complexes



appeared to form at the same time as in uninhibited cells but stress fibers took a lot longer to form (normally over 90 minutes after anaphase), this result was also observed in cells transfected with Cdh1 siRNA but could be rescued by addition of nocodazole or partially by inhibition of Aurora kinase activity. Addition of MG132 had no additive effect. *This shows that stress fiber formation is mediated by the ubiquitin mediated proteolysis of Aurora B as inhibiting degradation inhibits stress fiber formation.*



Finally I had a go at fixed cell Immunostaining on Aurora B-venus expressing cells for actin, microtubules and poly-ubiquitin to see if there was co-localization of Aurora B with the cytoskeleton at the cortex. Unfortunately the staining didn't work perfectly and it was hard to see if there was any co-localization or not.

Future Directions

1. If I had a little more time in the lab I would have investigated Cdh1 siRNA in Aurora B expressing cells, hopefully this would have yielded the same result as addition of MG132, the proteasome inhibitor. Also I would have liked to look at the difference between Cdh1 knock-down cells on and not on Fibronectin, expecting to see prolonged localization of Aurora B to the cell cortex and later formation of stress fibers even in cells on Fibronectin, confirming stabilization of Aurora B at the cortex inhibits stress fiber formation.
2. To look at the exact role of Aurora B at the cell cortex immunostaining of fixed cells for known targets of Aurora B could be carried out to see if Aurora B co-localizes with any of these targets at the cell cortex. The function of any targets found to interact with it here could give an idea of its role which looks likely to be in helping/inhibiting re-establish cell adhesion after cytokinesis. This could also help in understanding if the effects of Aurora B on the actin cytoskeleton are controlled via microtubule stability.
3. To look at an exact link between the timing of Aurora B disappearance from the cortex and appearance of stress fibers it would be good to express the alpha-actinin-venus construct in U2OS cells and establish timings here to see if the two events coincide as this cannot be concluded when comparing two different cell lines.

Departures from Original Proposal

- The Aurora B expressing cell line established in Dr Lindon's lab was recently found to relocalise to the cell cortex after cytokinesis at the points of cell spreading and hence I decided to use it as one of my markers due to its interesting localization in dividing cells.
- The plasmid expressing the anillin-venus construct was found to be toxic to cells and hence fluorescent cells were not frequent enough to gain enough results. Anillin expression was not therefore used as one of the timing markers.
- In initial experiments to find out the timings of Aurora B relocalisation an effect of cell density was noticed so further experiments were carried out to investigate this effect using U2OS cells not expressing the Aurora B-venus construct to create a higher density of cells whilst still being able to distinguish the cortex of individual cells.
- On trying to film cells plated onto polyHEMA (inhibits cell adhesion) it was found that they moved out of view due to lack of adhesion to the extracellular matrix, poly-lysine (introduces an electrostatic interaction) was therefore added to the plate as well to minimize cell movement.

Value of the Studentship

To the student: This studentship has been invaluable to me for both my final year at university and my future career plans. I now feel far more comfortable in a laboratory environment and have learnt various tissue culture, microscopy, data handling and presentation skills which I will be able to use in my research project in my final year and any further study in this area. I have learnt also that a lot of research is about patience and accepting that things will not always go to plan, but it is very rewarding when they finally do. This studentship has also encouraged me to apply to study for a PhD after I have finished my undergraduate degree, something I was not sure whether or not to do before this summer.

To the lab: *This studentship has supported a series of experiments that form a key addition to recent work from the Lindon lab. Data from these experiments link different strands of research in the lab by showing that the role of cell adhesion in promoting cell spreading involves both proteolysis and Aurora B activity. A publication is in preparation, and Nicola will be a co-author on the paper.*

Nicola's studentship will also be of lasting value to the lab in having introduced some technological advances: Nicola pioneered the use of 8-well slides for live cell work, increasing the number of conditions that can be tested in a single experiment, as well as the reproducibility of data. She also developed a number of new protocols (such as co-coating dishes with polyHEMA and poly-L-lysine) that will be useful in the future.