Investigating the regulation of Cdk1-Cyclin B1 complex at kinetochores during mitosis

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Aim

- To investigate Cdk1-Cyclin B1 complex regulation at the kinetochores during prometaphase of the cell cycle using a rapamycin-inducible system to tether Cyclin B1 to kinetochores.

Background

Mitotic cell division is vital for organisms to grow or for the maintenance of healthy tissues. Defects in cell division underlie developmental problems, infertility, ageing, cancer, and other diseases. Therefore, proper control of cell division is vital for health. The components of cells known as “Cyclin-dependent kinases” are master coordinators of the processes that ensure accurate cell division. In this project, we aim to find out how Cdk1-Cyclin B1, which is a vital regulator of mitosis, is itself regulated at kinetochores.

In order to study the regulation of Cdk1-Cyclin B1 complex at kinetochores, a system is required in which Cyclin B1 can be chemically tethered to the kinetochores , as depicted in diagram below. Cyclin B1 is fused to FRBP and the kinetochore protein is fused to FRB. Upon addition of rapamycin, FRBP and FRB dimerise, forcing Cyclin B1 to localise at kinetochores. In this experiment, we investigate the efficiency of our system in localising Cyclin B1 to kinetochores in the absence and presence of rapamycin.

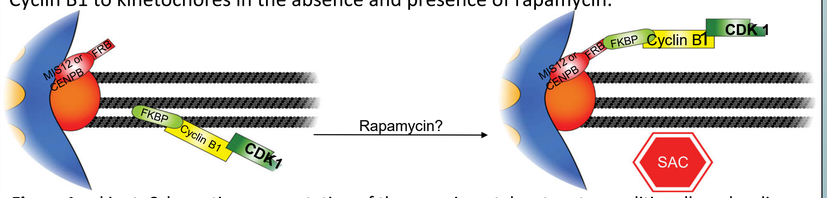
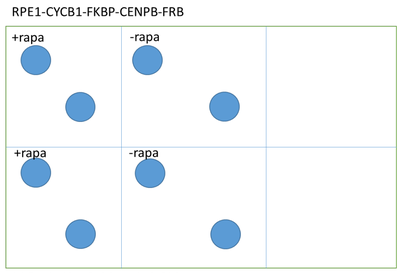


Figure 1: a kinetoSchematic representation of the experimental system to conditionally re-localise CDK1/Cyclin B1 to chores [Moderated graph from DOI 10.1007/s00412-014-0458-9]

Method

Figure 2: Plate set-up



Seeding
-In a six-well plate, both RPE1-CYCB1-FKBP-CENPB-FRB and RPE1-CYCB1-FKBP-MIS12-FRB were seeded at 100,000 cells/well.
-Cells were incubated overnight at 37°C

Treatment with Doxycycline
-Doxycline was added at 1μg/ml to all the wells
-cells were Incubated overnight at 37°C

Treatment with Rapamycin
-Nocodazole was added at 2μM for 3hrs, incubated at 37°C
- Rapamycin was added at 0.5μM for 1hr, incubated at 37°C
-Cells were Pre-extracted with X1 PHEM 0.5% Triton for 5min at 37°C
-Cells were Fixed with 4%PFA for 10mins at room temperature
-Wells were washed with PBS, and left overnight in the fridge at 4°C

Antibody Staining
-PBS was taken out from wells, 10% BSA 2ml/well was put and shaken for 1hr
-Coverslips were stained with CYCB1 ab 1:500 and CENP-C 1:1000 for 3 hrs at 37°C
-The coverslips then washed with PBS, two times rinse with PBS 5mins each on shaker
-Coverslips were further stained with 488 anti-rabbit ab -1:1000 and 647 anti-gp ab 1:1000 for 1hr at 37°C
-Coverslips then washed with PBS, three times rinse with PBS 5mins each on shaker, with foil covered on top
-Coverslips set on slides, ready for microscopy.

Results

Figure 3.1: RPE1-CYCB1-FKBP-MIS12-FRB -rapa
Low level of Cyclin B1 observed, suggesting that low level of CDK1/Cyclin B1 complexes are recruited to the kinetochores

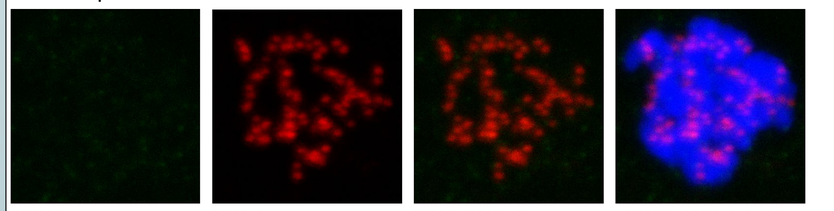


Figure 3.2: RPE1-CYCB1-FKBP-MIS12-FRB +rapa
Cyclin B1 and CENP-C co-localises, suggesting that CDK1/Cyclin B1 complexes are recruited to the kinetochores

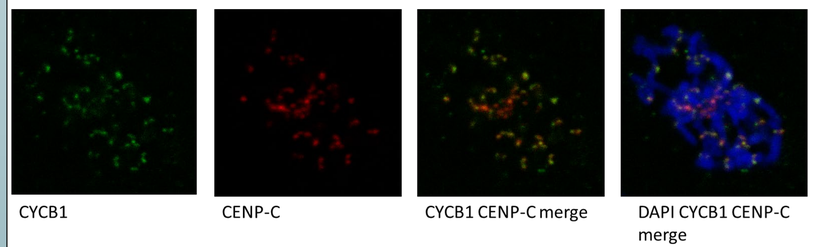


Figure 3.3: RPE1-CYCB1-FKBP-CENPB-FRB -rapa
Low level of Cyclin B1 observed, suggesting that low level of CDK1/Cyclin B1 complexes are recruited to the kinetochores

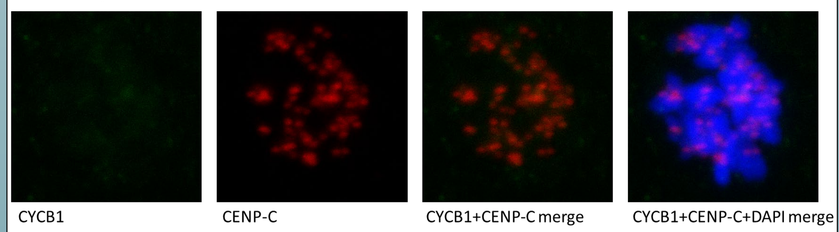
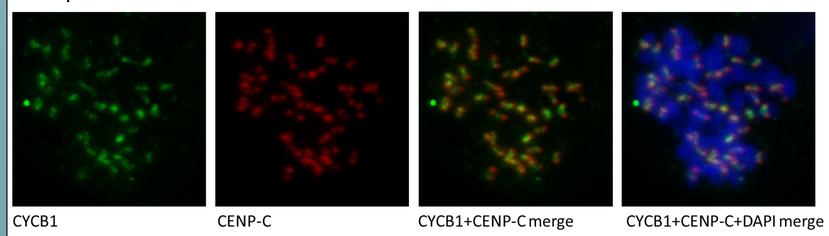


Figure 3.4: RPE1-CYCB1-FKBP-CENPB-FRB +rapa
Cyclin B1 and CENP-C co-localises, suggesting that CDK1/Cyclin B1 complexes are recruited to the kinetochores



[Antibody staining of CDK1/Cyclin B1 complex, CENP-C and DNA. Processed images from Fiji Image j]

Processed Results and Discussion

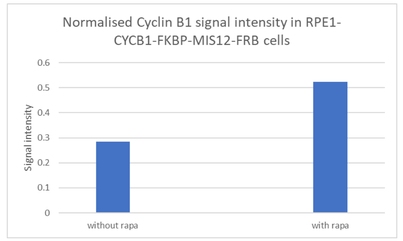


Figure 4

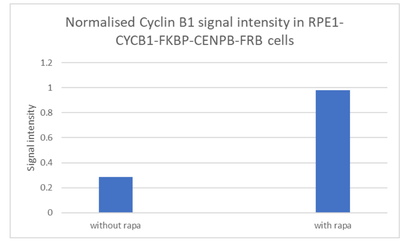


Figure 5

[Excel Graphs of converted experimental data]

Figure 4 shows the normalised Cyclin B1 fluorescent signal in the absence or presence of rapamycin in RPE1-CYCB1-FKBP-MIS12-FRB cells . In the absence of rapamycin, there is some level of Cyclin B1 recruitment at kinetochores. This is due to the normal localisation of Cyclin B1 during prometaphase. Moreover, the signal intensity for Cyclin B1 is increased higher by 1.8 fold when rapamycin is present, as expected.

Calculation for Fig. 4
Average signal intensity of Cyclin B1 with rapa / Average signal intensity of CENP-C with rapa
=22.31468 / 43.57329 = 0.524147417 = 0.524 to 3 s.f.
Average signal intensity of Cyclin B1 without rapa / Average signal intensity of CENP-C without rapa
=26.39311 / 92.62806 = 0.284936444 = 0.285 to 3 s.f.

Figure 5 shows the normalised Cyclin B1 fluorescent signal in the absence or presence of rapamycin in RPE1-CYCB1-FKBP-CENPB-FRB cells . The level of Cyclin B1 recruitment to kintechores is increased by 3.5 fold in the presence of rapamycin, as compared to no rapamycin. This indicates that this system is more efficient in localising Cyclin B1 to the kinetochores.

Calculation for Fig.5
Average signal intensity of Cyclin B1 with rapa / Average signal intensity of CENP-C with rapa
=65.91963 / 67.29716 = 0.979530637 = 0.980 to 3 s.f.
Average signal intensity of Cyclin B1 without rapa / Average signal intensity of CENP-C without rapa
=25.43641 / 89.36477 = 0.284635769 = 0.285 to 3 s.f.

Further Experiments

In the future, we could investigate the substrates of Cdk1-Cyclin B1 complex at kinetochores and their role in modulating mitotic checkpoint. Since deregulation of mitotic checkpoints lead to mitotic diseases, it is essential to determine the mechanisms that regulate these checkpoints.

Conclusions

- Rapamycin is enhances the localisation of CDK1/Cyclin B1 complex to the kinetochores during prometaphase.
- RPE1-CYCB1-FKBP-CENPB-FRB cell line is more efficient in targeting Cyclin B1 to the kinetochores.