Introduction:

- In order to grow and divide, a cell goes through one round of the cell cycle and divides to generate two daughter cells.
- To control this process, a family of proteins known as cyclin-dependent kinases (CDKs) are required.
- CDK2/cyclin E phosphorylates a number of substrates during the cell cycle that are required to ensure DNA replication is initiated properly.
- CINP (CDK2 interacting protein) binds to CDK2/cyclin E (CDK2E) at DNA replication origins to provide a functional and structural link between CDK2 and Cdc7 to ensure appropriate origin firing during DNA replication.

Hypothesis:

The hypothesis to be investigated is that CINP interacts with CDK2/cyclin E through a novel interacting region to control the interaction between CDK2 and Cdc7 that is essential for appropriate initiation of DNA replication.

Aims:
- To identify the best conditions to support optimal soluble expression of the GST and MBP-tagged CINP protein variants shown in table:

<table>
<thead>
<tr>
<th>Insert</th>
<th>Residue range</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>CINP1</td>
<td>1-212</td>
<td>pGEX6P-1</td>
</tr>
<tr>
<td>CINP2</td>
<td>K12-212</td>
<td>pGEX6P-1</td>
</tr>
<tr>
<td>CINP3</td>
<td>1-E206</td>
<td>pGEX6P-1</td>
</tr>
<tr>
<td>CINP4</td>
<td>K13-E206</td>
<td>pGEX6P-1</td>
</tr>
<tr>
<td>CINP5</td>
<td>1-212</td>
<td>pET21dMBP3C</td>
</tr>
<tr>
<td>CINP6</td>
<td>R12-212</td>
<td>pET21dMBP3C</td>
</tr>
</tbody>
</table>

- To express and purify cyclin A, cyclin E and CDK2 protein
- To assess the interaction between CINP and CDK2/cyclin E
- SDS-PAGE shows more soluble protein in pull downs from Rosetta cells grown in TB

Methods:

- CINP DNA was cloned into the vectors using infusion, then transformed into competent DH5α E.coli cells and grown up overnight on LB + Amp agar plates.
- CINP was expressed and purified on a large scale, using Rosetta cells grown in TB. Then CDK2, cyclin A and cyclin E were expressed and purified on a large scale.
- CDK2A and CDK2E complexes were made and the interactions between CINP2 and CINP3 were tested with both complexes using spin columns.
- From the plates starter cultures of LB were prepared overnight so CINP DNA could be extracted using spin columns and sent for sequencing.
- CINP DNA was inserted into expression strains of E.coli: BL21, Rosetta, Artic and Tuner and a multiple test expression was carried out in 24 well-plates.
- Finally the GST-tag was removed from CINP1, CINP4 and CDK2E. An analytical gel filtration was run to test the interaction between CINP1 and CDK2E. Then trays were set up for crystallisation of CINP4.

Results:

Multiple Parallel Expression of CINP in E.coli:

Key:
- R – Rosetta cells grown in TB
- A – Artic cells grown in LB
- T – Tuner cells grown in LB
- BA – BL21 cells grown in LB
- BL2 – BL21 cells grown in AIM
- PL – Protein ladder
- PL T3 T4 T5 T6 BL2 BA2 BL3 BA3

- SDS-PAGE shows more soluble protein in pull downs from Rosetta cells grown in TB
- SDS page shows successful expression and purification of CDK2, Cyclin A and Cyclin E and that complexes of CDK2/cyclin E and CDK2/cyclin A have been made

Conclusion:

- The most effective form of heterologous expression of the 6 CINP constructs was achieved by transforming into Rosetta E.coli cells and growing in TB.
- Full length CINP doesn’t bind to CDK2/Cyclin E (Cyclin E residues 96-378) however this lack of interaction could be due to the truncated version of Cyclin E expressed.

Future Research:

- As it has been shown that there is no interaction between CDK2/cyclin E and CINP1 alone this suggests the residues 1-96 of the cyclin E, or Cdc7 could be required for the interaction between the two.

Analytical Gel Filtration of CINP1 and CDK2E:

- The chromatogram on the left shows the GST tag being removed from CDK2E and the graph on the right shows the GST tag being removed from CINP4
- The first peak on each graph shows the protein in the sample and the second peak shows the GST

Clean-up of GST-tagged CDK2E and CINP4:

- The analytical gel filtration of GST-cleaved CINP1 and CDK2E using Superdex 200 column
- The analytical gel filtration of GST-cleaved CDK2E using Superdex 200 column
- The analytical gel filtration of CINP1 and CDK2E using Superdex 200 column