The Interaction Between CDK2/Cyclin E and CINP

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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.
- Reference:
  Grishina, I. and Lattes, B. (2005) A novel Cdk2 interactor is phosphorylated by Cdc7 and associates with components of the replication complexes. Cell Cycle 4,1120-6.

Hypothesis:

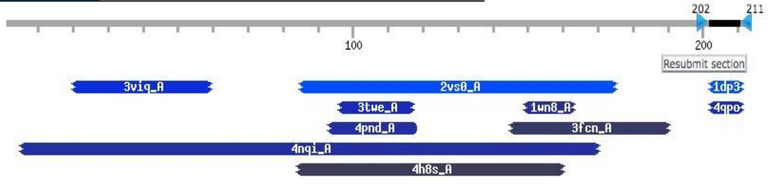
The hypothesis to be investigated is that CINP interacts with CDK2/cyclin E through a novel interacting region to control the interaction between CDK2 and Cdc7 that is essential for appropriate initiation of DNA replication

Aims:

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

Insert	Residue range	Vector
CINP1	1-212	pGEX6P-1
CINP2	K12-212	pGEX6P-1
CINP3	1-E206	pGEX6P-1
CINP4	K13-E206	pGEX6P-1
CINP5	1-212	pET21dMBP3C
CINP6	R12-212	pET21dMBP3C

- To express and purify cyclin A, cyclin E and CDK2 protein
- To assess the interaction between CINP and CDK2/cyclin E



- Figure 1 shows the CINP sequence (grey line) run through the HHpred structure prediction algorithm (http://toolkit.tuebingen.mpg.de/hhpred). High scoring structures all share an α-helical content and a number have coiled-coil structures.

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.

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.

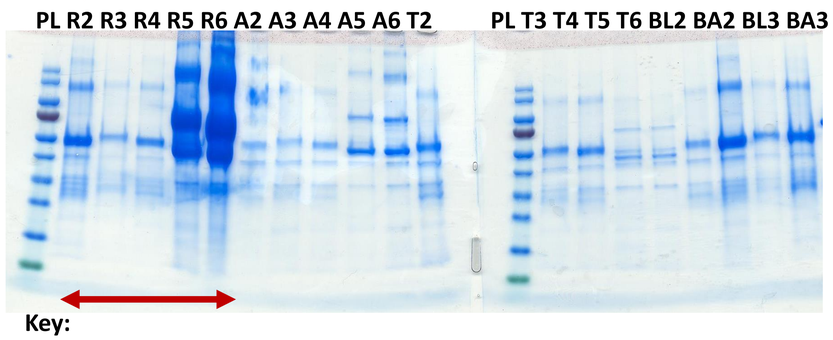
CINP was expressed and purified on a large scale, using Rosetta cells grown in TB. Then CDK2, cyclin A and cyclin E was expressed and purified on a large scale.

CDK2A and CDK2E complexes were made and the interactions between CINP2 and CINP3 were tested with both complexes using spin columns.

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:

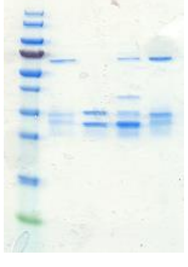
Multiple Parallel Expression of CINP in E.coli:



Key:
R – Rosetta cells grown in TB **A** – Artic cells grown in LB **T** – Tuner cells grown in LB **BL** – BL21 cells grown in LB **BA** – BL21 cells grown in AIM **2-5** – CINP constructs 2-5 **PL** – protein ladder

- SDS-PAGE shows more soluble protein in pull downs from Rosetta cells grown in TB

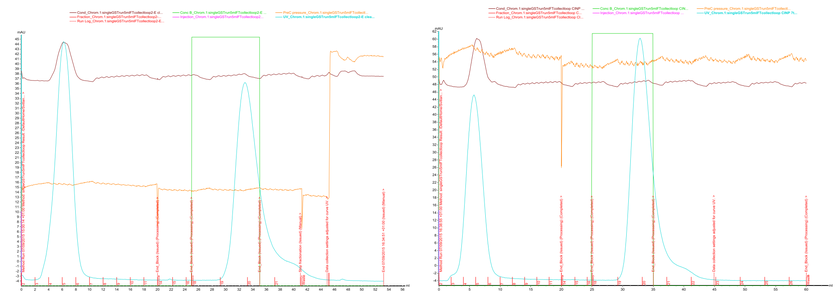
Expression and Purification of Cyclin A, Cyclin E and CDK2:



Key:
PL – Protein ladder
2 – CDK2
E – Cyclin E
2E – CDK2/Cyclin E
2A – CDK2/Cyclin A

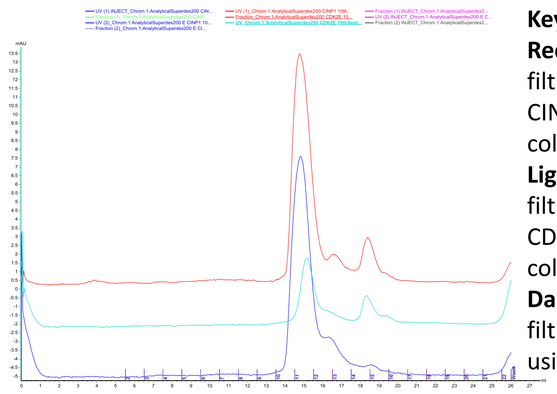
- SDS page shows successful expression and purification of CDK2, Cyclin A and Cyclin E and that complexes of CDK2/cyclin E and CDK2/cyclin A have been made

Clean-up of GST-tagged CDK2E and CINP4:



- 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

Analytical Gel Filtration of CINP1 and CDK2E:



Key:
Red line – Analytical gel filtration of GST-cleaved CINP1 using Superdex 200 column
Light blue line – Analytical gel filtration of GST-cleaved CDK2E using Superdex 200 column
Dark blue line – Analytical gel filtration of CINP1 and CDK2E using Superdex 200 column

- The analytical gel filtration of GST-cleaved CINP1 and CDK2E shows that there is no interaction between CINP1 and CDK2E, as there is no shift in the curve to left on the chromatogram

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.