"Does the Cdc25A C-terminal tail selectively regulate the activity of Cdk/cyclin complexes?"

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Aims

1) To determine if a C-terminal fragment of Cdc25A is sufficient to mediate Cdc25A binding to cyclin A
2) To confirm whether mutation of residues within the Cdc25A tail blocks the formation of a complex between Cdc25A and cyclin A
3) To test whether Cdc25A contains a cryptic recruitment site binding motif to bind to cyclin A

Introduction

- The cell cycle is the sequence of events which a cell undergoes to allow growth and replication
- This series of events is controlled by the sequential activation of members of the CDK family
- CDKs are regulated in part by the Cdc25 proteins which modulate their activity by inhibitory phosphorylation
- In cancer cells the cell cycle can become unregulated, leading to inappropriate cell growth
- The current model suggests that Cdc25A can bind to cyclins A and E using an RXL recruitment motif in the N-terminus of Cdc25A (Saha et al, 1997)
- An alternative model would suggest that cyclins B, A and E can also bind to a recruitment motif thought to be located on the C-terminus of Cdc25A (Chen et al, 2003)
- Previous studies have implicated two groups of residues C terminal and N terminal to T507 in the Cdc25A C terminal tail which when mutated caused a reduction in binding of Cdc25A to cyclins (Chen et al 2003, Uto et al 2004)

Methods

- Site-directed mutagenesis to create the Cdc25A mutants
- Expression of Cdc25A constructs and cyclin A WT and W217K (to inactivate the recruitment site) in *E.coli* cells:

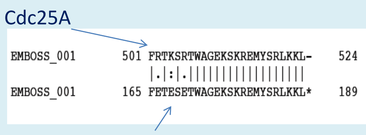
Protein	Residue range	MW (Da)
Cdc25A	336-523	22417.9
Cdc25A N term to E	336-523	22417.9
Cdc25A C term to E	336-523	22417.9
Cdc25A C term to A	336-523	22417.9
Bovine cyclin A	131-426	29798.4
W217K bovine cyclin A	131-426	29798.4

Site-directed mutagenesis:

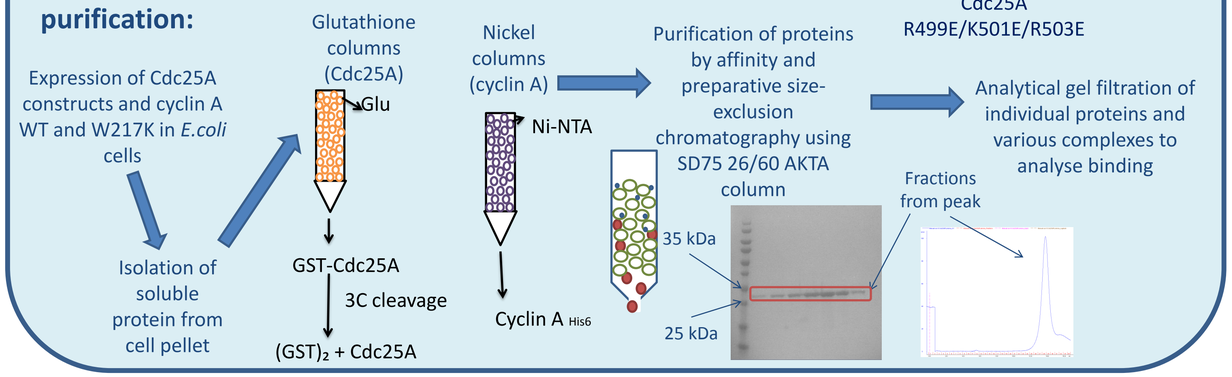
Mutations introduced

Construct	Mutations introduced
Cdc25A N term to E	R499E/K501E/R503E
Cdc25A C term to E	K514E/R520E
Cdc25A C term to A	K514A/R520A

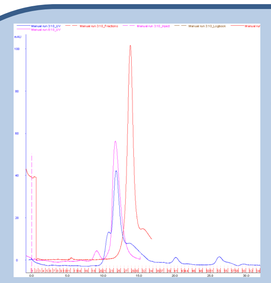
Sequence verification:
Comparison of WT and mutant Cdc25A sequences



Expression and purification:



Results



The peak fractions were run on an agarose gel to confirm they were from Cdc25A and cyclin A

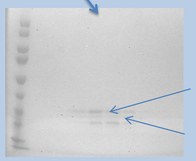


Figure 1- Cdc25A (red line), WT bovine cyclin A (pink line) and a stable Cdc25A/cyclin A complex (blue line).

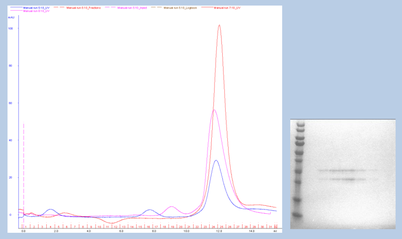


Figure 2

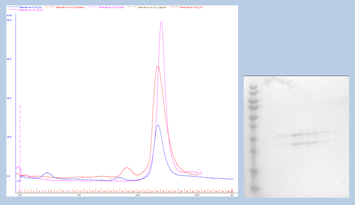


Figure 3

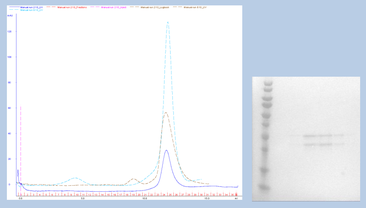


Figure 4

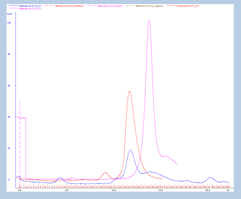


Figure 5

Figure	Cdc25A construct	Cyclin A construct	Result	Conclusion
1	WT	WT	Shift in peak	Stable complex
2	K514A/R520A	WT	No shift	No interaction
3	K514E/R520E	WT	No shift	No interaction
4	R499E/K501E/R503E	WT	No shift	No interaction
5	WT	W217K	Peak shift	Cdc25A does not engage cyclin recruitment site

Discussion/future work

- Cdc25A run in a complex with cyclin A caused the peak of the chromatogram to shift to the left, whilst Cdc25A mutants run with cyclin A did not cause a shift. This suggests that introducing mutations in the Cdc25A C terminal tail affects binding of Cdc25A and cyclin A.
- Mutant W217K cyclin A run with FL Cdc25A did cause a shift in the peak, behaving as wild type cyclin A. This suggests that the sequence is not using the recruitment site, however this needs to be confirmed.

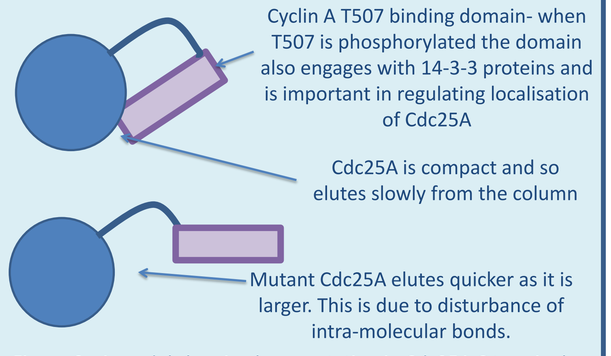


Figure 6- A model showing how mutation in Cdc25A C terminal residues may disrupt binding

Future work:

- Verify these results using another technique
- Verify the Cdc25A model
- Crystallisation to confirm the structure of Cdc25A
- Surface Plasmon Resonance (titrate in the different Cdc25A constructs and see which are most effective at displacing the peptide bound to cyclin A)
- Check to see if purified proteins bind through the cyclin A RXL recruitment site or a novel site

Conclusions

- Mutations in the Cdc25A C terminal tail result in a change in the shape of Cdc25A as determined by SEC suggesting disruption of intra-molecular bonds.
- The mutants support the model that the Cdc25A C-terminal tail binds to cyclin A
- Cdc25A can still bind cyclin A when the cyclin recruitment site is inactivated, suggesting that Cdc25A binds to cyclin A at a novel site.

References:

1. Saha P, Eichbaum Q, Silberman ED, Mayer BJ, Dutta A, 1997. p21CIP1 and Cdc25A: competition between an inhibitor and an activator of cyclin-dependent kinases. Molecular and Cellular Biology, 17(8):4338-4345-
2. Chen M, Ryan CE and Piwnica-Worms H, 2003. Chk1 Kinase Negatively Regulates Mitotic Function of Cdc25A Phosphatase through 14-3-3 Binding. Molecular and Cellular Biology, 23(21):7488-7497
3. Uto K, Inoue D, Shimuta K, Nakajo N, Sagata N, 2004. Chk1, but not Chk2, inhibits Cdc25 phosphatases by a novel common mechanism. The EMBO Journal, 23(16):3386-96

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