Site-directed mutagenesis of cell division proteins for contraction generation in vitro

Don Praveen Amarasinghe¹, Claire Dow¹, David Roper², Alison Rodger¹

¹MOAC Doctoral Training Centre & ²School of Life Sciences, The University of Warwick, Coventry, CV4 7AL, U.K.

Method

Figure 3 (right) outlines the mutagenesis protocol.

- Four residues in each protein were chosen to be mutated into cysteines.
- The QuikChange online primer calculator was used to build the DNA primers required to instigate the mutation.
- DpnI was used to remove the original methylated DNA from plasmid mixtures after PCR.
- BL21(DE3) and C43(DE3) cells were used to over-express mutants for protein structure-function studies.
- IPTG was used to induce over-expression via the Lac operon.

Results

Three mutations were successful according to sequencing results:

- FtsZ residue 21 – Glycine to Cysteine.
- ZipA residue 328 – Alanine to Cysteine.
- A residue coded in the plasmid upstream of ZipA – Phenylalanine to Cysteine.

However, although a wide range of DNA polymerases (PfuUltra™, Phusion™ and Q5™), annealing temperatures (50 to 72°C) and annealing times (30 to 90 seconds per kb), a very small number of mutagenesis experiments yielded the required mutation.

Next steps

- Carry out the mutagenesis with the SPRINP method (Edelheit et al. (2009)), to improve mutagenesis yield.
- Protein verification.
  - MALDI to check the amino acid sequence is the one desired.
  - Circular dichroism to check that the secondary structure is still the same as in the wild-type.
- Linear dichroism to ascertain if the mutated FtsZ will still polymerise.
- Attach fluorescent tags to the cysteine residues generated in mutated FtsZ and ZipA and to a cysteine residue already present in another Z-ring protein, ZapA.
- Construct “artificial cells” using the SMALP method outlined in Knowles et al. (2009) containing tagged FtsZ and ZapA in the cytosol and tagged ZipA in the membrane.
- Observe the binding of FtsZ polymers to the membrane-bound ZipA (with the aid of ZapA) using confocal microscopy.

Acknowledgements

The authors would like to thank EPSRC and BBSRC for their continued support and funding.

Special thanks go to fellow staff and students at MOAC and the structural biology laboratory in the School of Life Sciences.

References