Structural basis of plasmid addiction

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Plasmid addiction systems ensure the stable maintenance of low-copy number plasmids in bacterial populations by killing plasmid-free segregants. They consist of a stable toxin and an unstable antidote. The cells are protected from the toxin by the antidote, with which the toxin forms a non-covalent complex. If the plasmid is lost, no antidote can be produced, and after degradation of the remaining antidote molecules, the toxin can act on its target. The complex between toxin and antidote not only stabilizes the antidote, but also binds to it promotor DNA, regulating expression.

The ccd system on plasmid F is the best characterized (Dao-Thi et al. 2000 and references therein). Recently, we determined the structure of the toxin (CcdB) of this system (Loris et al., 1999). This was the first crystal structure of a plasmid addiction protein. In the past year, we measured data on the complex between CcdB and a 59kD fragment of the A subunit of gyrase (GyrA59). For several crystals diffraction was observed to about 4 Å on beamline ID14-2 (compare with 8 Å obtained at other synchrotron sources), and data was collected. Unfortunately, all crystals tested turned out to be perfect merohedral twins (twinning fraction of 0.5). Therefore, the structure cannot be determined from the current data.

Crystals of an antibody against the antidote Maze of the MazEF addiction system were also tested. These crystals diffracted to 2.75 Å on beamline ID14-2 but had a very high mosaicity, which was reduced somewhat by annealing the crystals. The resulting mosaicity remained, however, very high (about 2.5°). Data were nevertheless collected. The crystals belong to space group P1 (unit cell a= 41.1Å, b= 57.6Å, c=61.3Å, alpha= 97.9°, beta=105.1°, gamma = 110.8°) and contain three molecules in their asymmetric unit. The data are of acceptable quality (R_{merge} 15.1%, <I/Sig(I)> 8.1). We are currently attempting to solve this structure by molecular replacement using different camel VHH antibody fragments as search models.

References

Dao-Thi M.-H., Messens, J., Wyns, L., Backmann, J. (2000) The thermodynamic stability of the proteins of the ccd plasmid addiction system. *J. Mol. Biol.* **299**, 1373-1386.

Loris R., Dao-Thi M.-H., Bahassi E.M., Van Melderen L., Poortmans F., Liddington R., Couturier M., Wyns L. (1999) Crystal Structure of CcdB, a topoisomerase poison from *E. coli*. *J. Mol. Biol.* **285**, 167-1676.