

### PROBING INTERACTIONS OF THE *E. COLI* HELICASE AND ITS LOADING PARTNER BY ELECTROSPRAY IONISATION MASS SPECTROMETRY

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The replication of DNA in *E. coli* requires the assembly of many proteins whose non-covalent interactions with each other and cofactors result in the faithful reproduction of the chromosome. In the initial stages of replication, two proteins play an important role in establishing contact with DNA at the origin of replication. These proteins are DnaB and DnaC. DnaB is the principal *E. coli* replicative helicase. It comprises six identical 52 kDa subunits which interact to form a homohexameric enzyme involved in unwinding duplex DNA in a 5' to 3' direction.<sup>1</sup> DnaC is referred to as the DnaB loading partner and interacts in a 1:1 ratio with DnaB monomers, forming a 480 kDa complex. The role of DnaC in replication is thought to be in establishing interactions of DnaB with single-stranded DNA at the origin of replication. This process is driven by the energy released from the hydrolysis of ATP.<sup>2</sup>

Although it is established that these proteins are essential for the cell's replicative machinery, the difficulty in examining large complexes by techniques such as NMR and X-ray crystallography, means that there is little information about the timing of the assembly and disassembly of the proteins, or the contacts between them. In this study we used electrospray ionisation mass spectrometry to examine the behaviour of the DnaB hexamer, DnaC and its interaction with ATP and ADP, and the DnaB-DnaC complex. Using a Waters Q-ToF mass spectrometer, we have carried out experiments aimed at confirming the stoichiometries of the DnaB-DnaC complex.<sup>1,3</sup> Furthermore, we have carried out collision-induced dissociation experiments to probe the mechanism of how these complexes form and dissociate. This information will ultimately be used to understand the role(s) of DnaB and DnaC in replication.

1. Donate, L.E., Llorca, O., Barcena, M., Brown, S.E. Dixon, N.E., Carazo, J.M. (2000) *J. Mol. Biol.* 303, 383-393.
2. Davey, M.J., Fang, L.H., McInerney, P. Georgescu, R.E., O'Donnell, M. (2002) *EMBO J.* 21, 3148-3159.
3. San Martin, C., Radermacher, M., Wolpensinger, B., Engel, A., Miles, C.S., Dixon, N.E., Carazo, J.M., (1998) *Structure* 6, 501-509.