ESRF	**Experiment title:** Bacteriophage phi29 connector Project carried out : Plasmid R388 Helicase TrwC	**Experiment number:** LS-1065
Beamline: ID02B	**Date of experiment:** from: 13.11.1998 to: 15.11.1998	**Date of report:** 31.8.1999
Shifts: 6	**Local contact(s):** Bjarne Rasmussen	*Received at ESRF:* 1 SEP. 1999

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Report:

Bacterial conjugation and rolling-circle replication are two processes of bacterial DNA metabolism that share the characteristic of generating a ssDNA molecule. In both processes, a key enzyme plays the dual role of initiator and terminator by introducing a site-specific nick at the origin of transfer/replication in the strand to be displaced and resealing the nick after a round of transfer/replication. In conjugation as well as in rolling-circle replication there is a need for a DNA helicase activity to displace the nicked strand after the initial nicking event. In the case of phage or plasmid replication this is assumed to be a function supplied by the host, since no specific DNA helicases are encoded by the plasmids of phages. In conjugation, however, two conjugative plasmids, F and R388, belonging to distant incompatibility groups have been shown to code for an oriT-specific relaxase that in addition has DNA helicase activity. These proteins are TraI for F and TrwC for R388. Both proteins show conservation of the DNA relaxase motifs in their N-terminal 200 amino acid residues, and a set of motifs shared by a family of DNA helicases in their C-terminal halves. Both proteins further display another particularity: they are able to nick oriT-containing supercoiled plasmids at the nick site *in vitro* in the absence of any other protein. TrwC is therefore a bifunctional enzyme that displays two biochemical activities essential for plasmid R388-conjugation: oriT-specific DNA strand-transferase and DNA helicase activities. Different regions of the protein TrwC were overproduced and purified, showing that the nicking-closing and DNA helicase activities could be isolated and lay in separate regions of the protein. Moreover, it was found that the fragment

of TrwC required to cut and reseal oligonucleotides encompassing the *nic* site was smaller than the fragment required to produce a nick in a supercoiled dsDNA substrate.

The sequence of TrwC and the predicted secondary structure don´t match any previously solved protein structure, and we expect to observe a new three-dimensional protein fold. A protein fragment comprising the 275 N-terminal residues (for which ability to catalyze DNA cleavage and strand-transfer reactions has been shown) has been crystallized. Crystals are thin plates and required ESRF ID02B synchrotron radiation to render a 2.9 Å native dataset. These data have been processed and belong to space group $P2_1$ with cell constants a= 51.9 Å, b= 64.5 Å, c= 78.6 Å, β= 97.9 °. A second dataset obtained by soaking native crystals in a mercury-containing solution could be also measured at ID02B and processed. This crystal is isomorphous to the native one with cell constants a= 52.0 Å, b= 65.1 Å, c= 78.8 Å, β= 97.8 °. A difference Patterson analysis revealed the position of one heavy-ion site.

In parallel, SeMet-containing TrwC (N275) could be produced and SeMet-derivative crystals have been obtained from conditions analogous to those that rendered native crystals. Therefore, MAD beamtime at BM30 is requested in order to solve the structure of this interesting target.