Spo0J, the ParB spreading protein in chromosome partitioning system

Bo-Wei Chen, Ming-Shing Lin, Chen-Hsi Chu, Chia-En Hsu and Yuh-Ju Sun*

Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu, Taiwan

Spo0J (stage 0 sporulation protein J, a member of the ParB superfamily) is an essential component of the ParABS related bacterial chromosome segregation system. ParB and its regulatory protein, ParA, act cooperatively through *parS* DNA to facilitate chromosome segregation. ParB binds to chromosomal DNA at specific *parS* site as well the neighboring non-specific DNA sites. Various ParB molecules can associate together and spread along the chromosomal DNA. ParB oligomer and *parS* DNA interact together to form a high order nucleoprotein that is required for the loading of the SMC (structural maintenance of chromosomes) proteins onto the chromosome for chromosomal DNA condensation. In this report, we characterized the binding of *parS* and Spo0J from *Helicobacter pylori* (*Hp*Spo0J) and solved the crystal structure of the Ct-*Hp*Spo0J-*parS* complex. Ct-*Hp*Spo0J folds into an elongated structure that includes a flexible N-terminal domain for protein-protein interaction and a conserved DNA-binding domain for *parS* binding. Two Ct-*Hp*Spo0J molecules bind with one *parS*. Ct-*Hp*Spo0J interacts vertically and horizontally with its neighbors through the N-terminal domain to form an oligomer. These adjacent and transverse interactions are accomplished via a highly conserved arginine patch, "RRLR". These interactions might be needed for molecular assembly of a high order nucleoprotein complex and for ParB spreading. We also solve the molecular envelope of full-length Spo0J by SAXS, The model were combined with *Hp*Spo0J and *Ep*P1ParB C-terminal domain. A structural model for ParB spreading and chromosomal DNA condensation that lead to chromosome segregation is proposed. The study of full length *Hp*Spo0J and *parS* interaction in the chromosome partitioning system is undergoing.

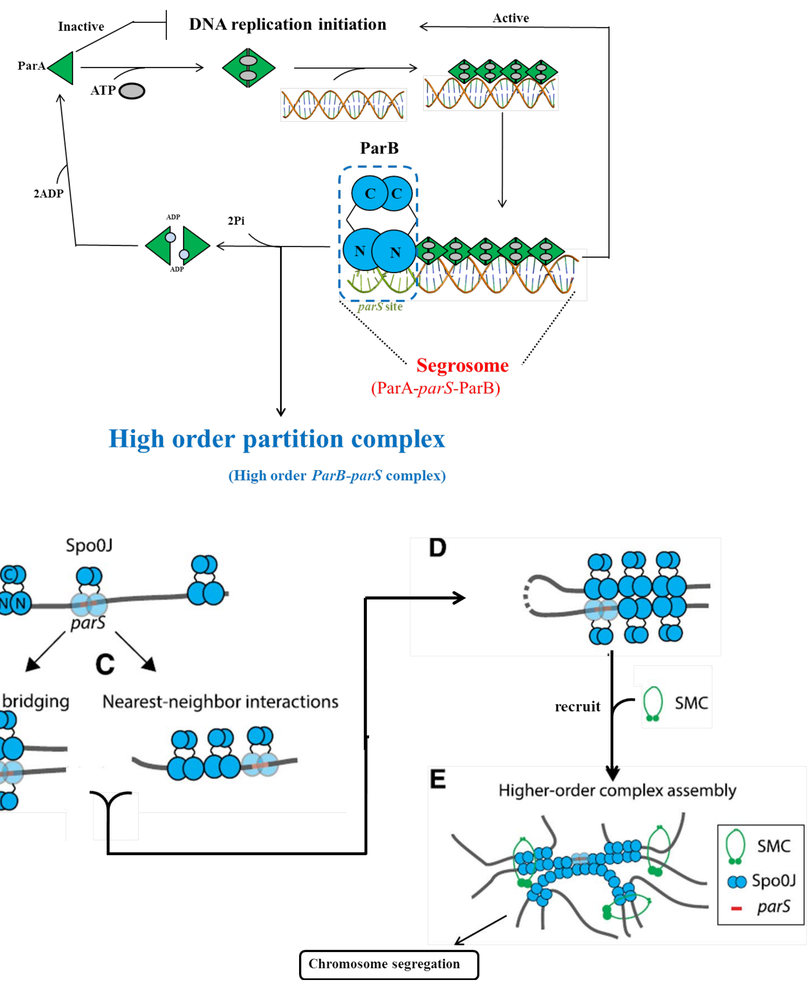

Fig1: The Role of the Soj and Spo0J in the chromosome replication and segregation (Top) The pathway to describe Soj ATP binding, dimerization, cooperative Spo0J-DNA binding. (Bottom) ParB spreads along chromosome DNA by binding at specific *parS* and non-specific DNA sites to form a high order partition complex. This partition complex is required for the loading of SMC onto the chromosome DNA. **(2014 Genes Development 28(11):1140-1142.)**

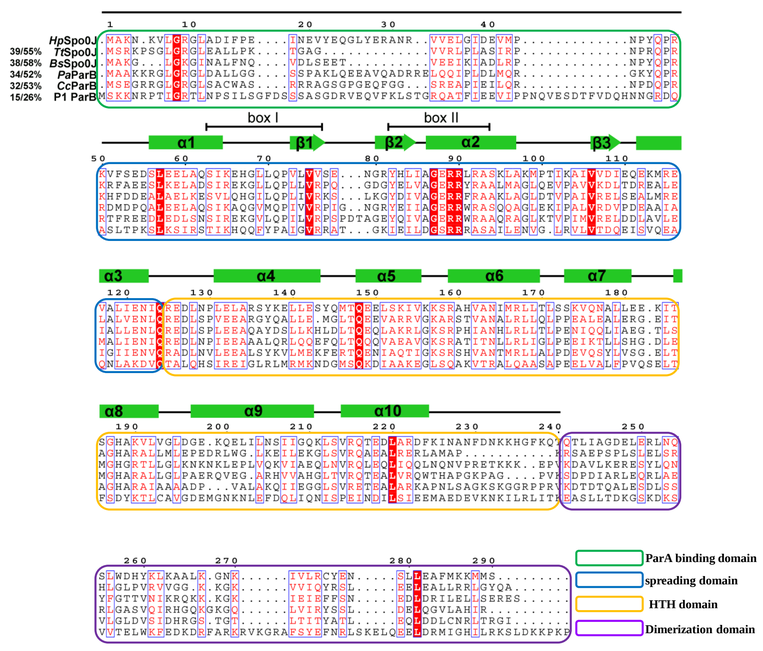

Fig2: Multiple sequence alignments of the ParB superfamily
Multiple sequence alignments of the ParB superfamily members from *Helicobacter pylori* (*Hp*Spo0J), *Thermus thermophilus* (*Tt*Spo0J), *Bacillus subtilis* (*Bs*Spo0J), *Pseudomonas aeruginosa* (*Pa*ParB), *Caulobacter crescentus* (*Cc*P1ParB) and *Enterobacteria phage* P1 (P1 ParB). Completely conserved residues are boxed in red, and conservatively exchanged residues are shown in red text. The residue numbering uses that of *Hp*Spo0J. Secondary-structure elements of *Hp*Spo0J are as cylinders and arrows for its α-helices (α1-10) and β-strands (β1-3), respectively. The box I (S63-V76) and box II (Y82-A93) are indicated.

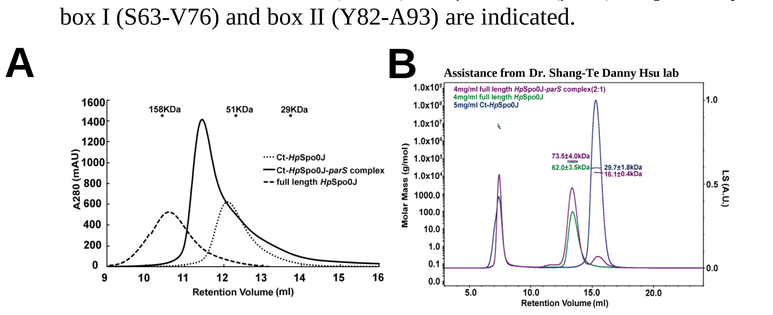

Fig3: Full length *Hp*Spo0J, *Hp*Spo0J, *Hp*Spo0J-*parS* complex (**A**) Size exclusion chromatography of full length *Hp*Spo0J (dash line), *Hp*Spo0J (dot line) and *Hp*Spo0J-*parS* complex (solid line). (**B**) MALS of full length *Hp*Spo0J (green) and the *Hp*Spo0J-*parS* complex (purple) at concentrations of 4 mg/ml. MALS of *Hp*Spo0J (blue) at concentration of 5 mg/ml. (**C**) Fluorescence anisotropy of *Hp*Spo0J and DNA binding. (**D**) Electrophoretic mobility shift assays of full length *Hp*Spo0J (lane 2-4) and *Hp*Spo0J (lane 6-8) were incubated with *parS*.

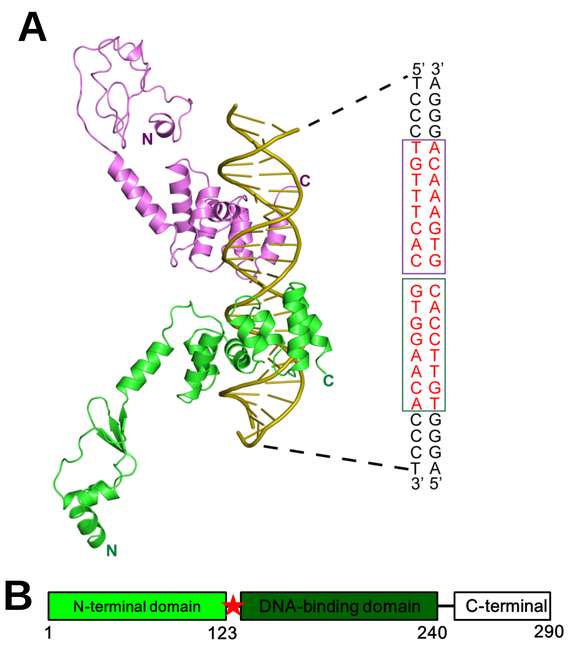

Fig4: The protein-DNA interaction of *Hp*Spo0J-*parS* complex and Ct-*Hp*Spo0J monomer.
(A) Two Ct-*Hp*Spo0J molecules and one 24-bp *parS* DNA are shown. The two binding sites on a *parS* (16-bp, red), one each for the *Hp*Spo0J A (green) and B (magenta) molecules, are boxed. **(B)** A ribbon diagram of Ct-*Hp*Spo0J is shown. The molecule contains 10 α helices (α1-10) and three β strands (β1-3). The N-terminal domain (green) and the DNA-binding domain (darker green) are shown.

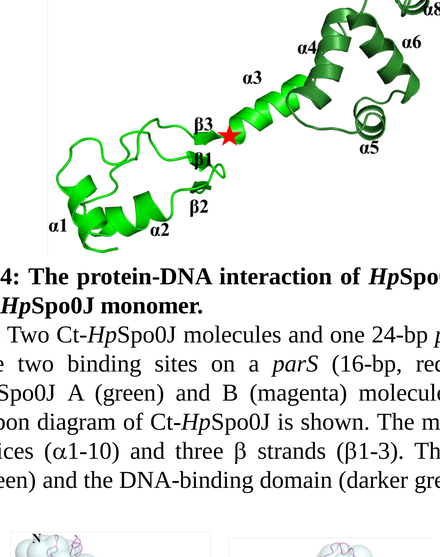

Fig7: A structural model of the full-length *Hp*Spo0J. The superposition of the Y shaped SAXS envelop and the structural model of the full-length *Hp*Spo0J are shown. *Hp*Spo0J is shown as a dimer. The N-terminal and DNA-binding domain of the two *Hp*Spo0J molecules are colored separately in green and magenta. Their C-terminal domains are shown as a dimer and colored in grey.

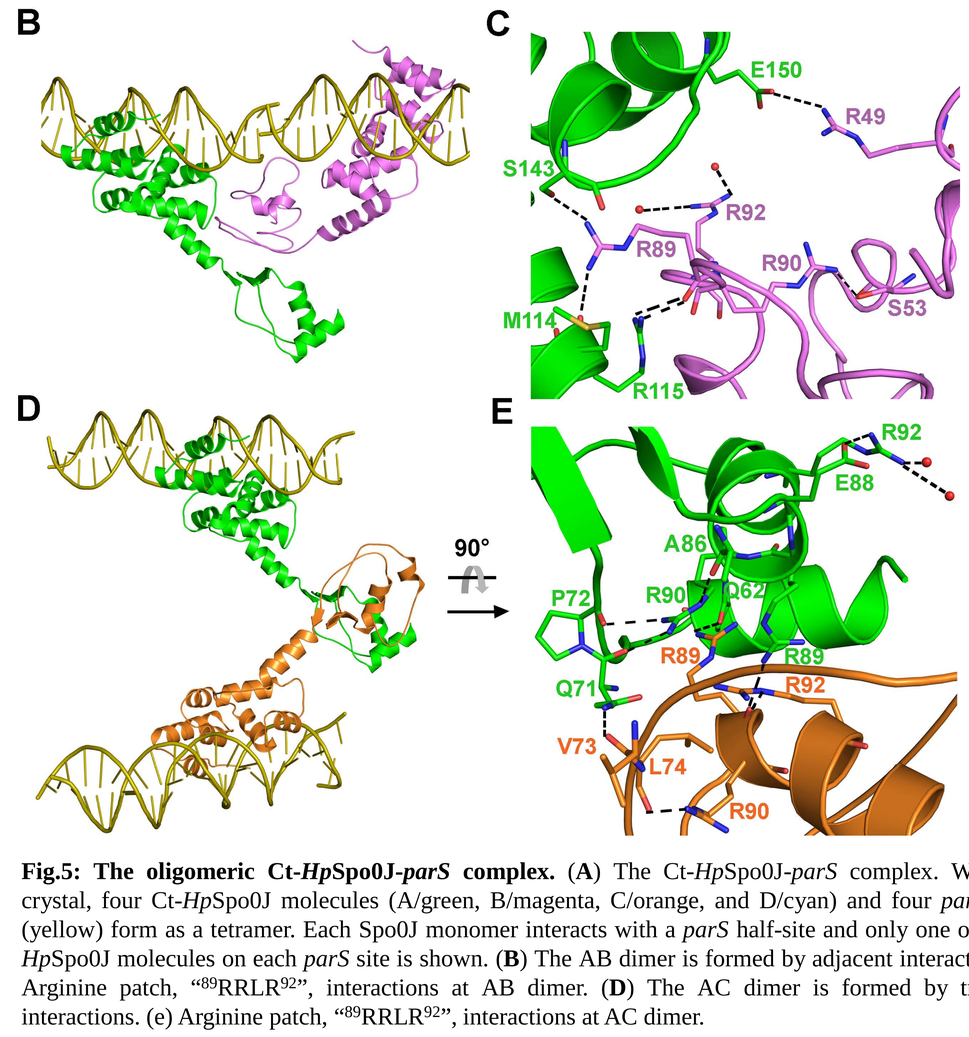

Fig.5: The oligomeric Ct-*Hp*Spo0J-*parS* complex. (**A**) The Ct-*Hp*Spo0J-*parS* complex. Within the crystal, four Ct-*Hp*Spo0J molecules (A/green, B/magenta, C/orange, and D/cyan) and four *parS* DNAs (yellow) form as a tetramer. Each Spo0J monomer interacts with a *parS* half-site and only one of the two *Hp*Spo0J molecules on each *parS* site is shown. (**B**) The AB dimer is formed by adjacent interactions. (**C**) Arginine patch, "^{89}RRLR^{92}", interactions at AB dimer. (**D**) The AC dimer is formed by transverse interactions. (e) Arginine patch, "^{89}RRLR^{92}", interactions at AC dimer.

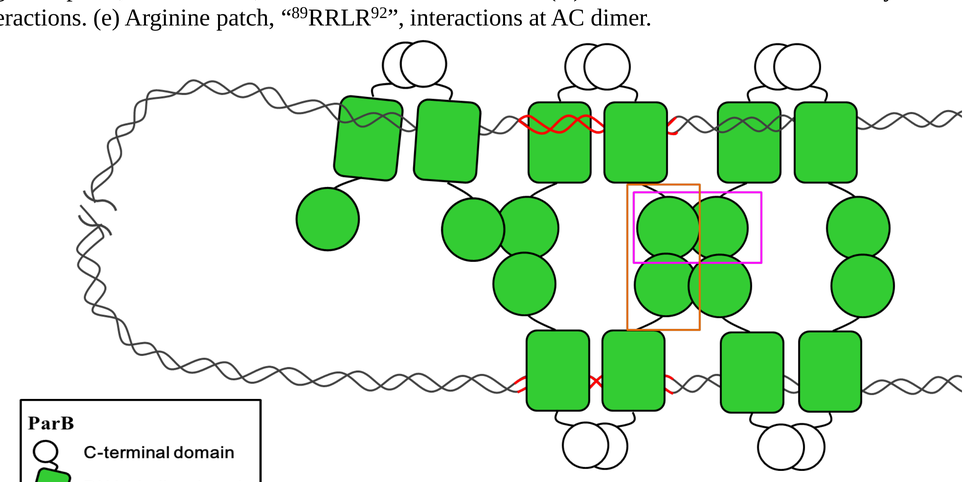

Fig6: ParB spreading model. The C-terminal dimerization (in white sphere), the DNA-binding (in green rectangle) and the flexible N-terminal domains (in green sphere) of ParB are shown. A ParB dimer is formed via the C-terminal dimerization domain. ParB binds chromosomal DNA at specific *parS* site (in red) or non-specific sites (in gray). Multiple ParB molecules spreading along the lengthy and looping chromosomal DNA through the N-terminal domain by adjacent interactions (boxed in magenta) horizontally and transverse interactions (boxed in orange) vertically. Consequently, a high order ParB-DNA complex is formed.