Chromosome segregation: A jigsaw puzzle of protein interactions

A study of the interactions between proteins involved in chromosome segregation in *Bacillus subtilis* using the yeast-two-hybrid system

Laura Walker*, Clare Willis, Ling Juan Wu, Jeff Errington

L.walker7@ncl.ac.uk, Biochemistry (Integrated Master's) MSci, 150212940

Aims and introduction

Project aim: To characterise protein-protein interactions between proteins ComN, DivIVA, MinD, MinJ, RacA, Soj and Spo0J from bacterium *Bacillus subtilis* using a yeast-two-hybrid technique.
Global aim: To understand chromosome segregation during sporulation

Introduction:
Chromosome segregation is essential for all forms of life.

Bacteria form spores in starvation conditions to aid survival[1]. *Bacillus subtilis* sporulation is used as a model for chromosome segregation because bacteria are quick to grow and this soil-dwelling bacterium is non-harmful!

In sporulation, one copy of DNA is moved into the prespore at one end of the cell, while the other copy remains in the mother cell.

Various *Bacillus subtilis* proteins act on DNA at the origin (the start point of DNA replication) to ensure DNA is transferred to the prespore (figure 1).

But how do these proteins interact with one another?

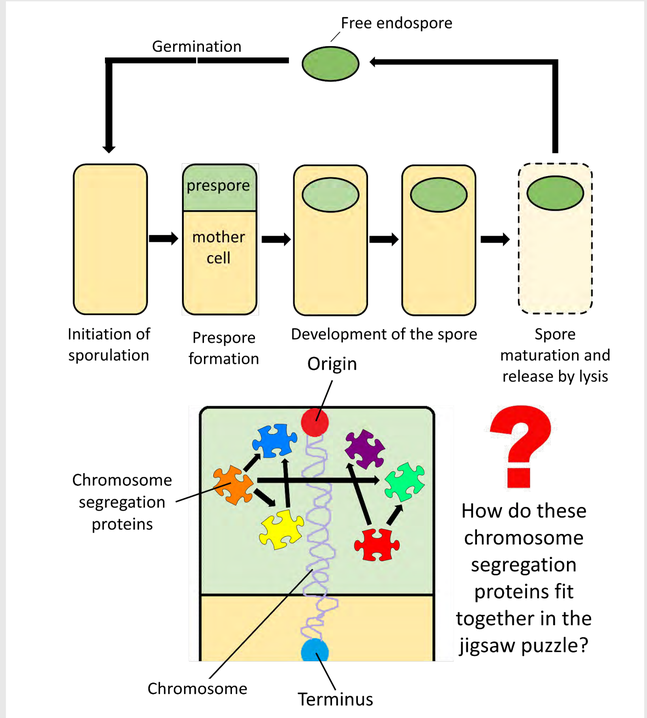


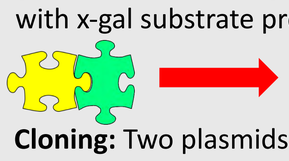
Figure 1: Chromosome segregation during sporulation in *Bacillus subtilis*.

Methods

Yeast-two-hybrid: tests the interaction between two proteins.

Each plasmid has a protein fused to a genetic element. When these bind to the corresponding genetic element on DNA it brings two proteins close together. If these proteins interact it allows the *lacZ* gene to be produced.

The gene *lacZ* encodes for the protein β-galactosidase which gives the colour blue with x-gal substrate present (figure 2/3).



Cloning: Two plasmids (small circular DNA molecules) were created for each test protein by inserting the gene for each protein into each plasmid (figure 4). A total of 20 plasmids were created in this project.

Two plasmids were inserted into yeast cells. Yeast which successfully took up the plasmid were selected for on media lacking amino acids leucine and tryptophan (figure 5).

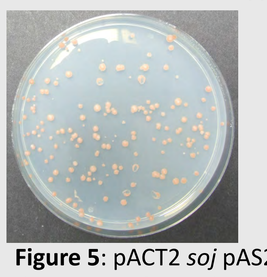


Figure 5: pACT2 *soj* pAS2-1 divIVA transformed yeast.

Yeast successfully transformed with both plasmids on SD/-leu-trp media

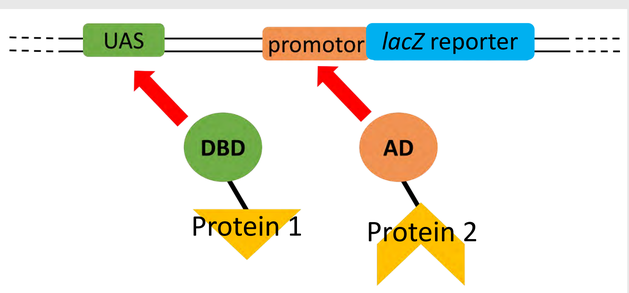


Figure 2: Where each plasmid fused to protein binds.

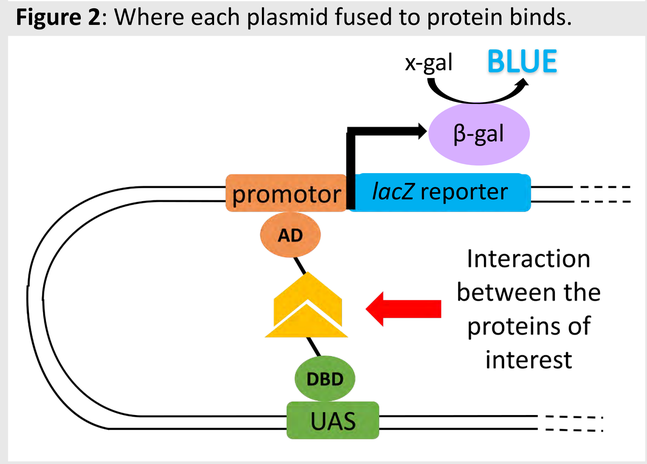


Figure 3: Interaction of two proteins and the expression of *lacZ* to produce β-gal resulting in formation of blue.

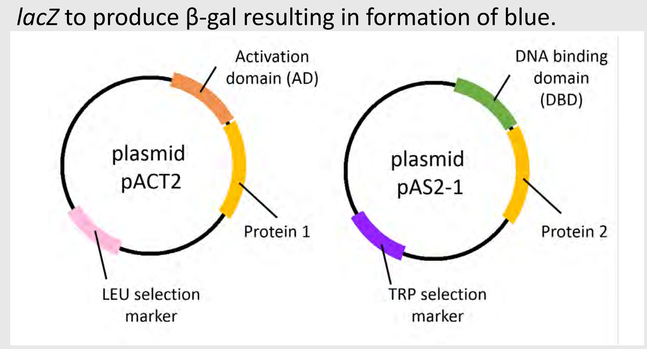


Figure 4: Cloned plasmids pACT2 and pAS2-1.

Conclusions and Future Work

Conclusions:
Previously published interactions between DivIVA-DivIVA and ComN-DivIVA have been replicated[2]

Further evidence to the idea that that ComN and MinD interact, as previously published and shown using bacterial-two-hybrid[3]

Provided two interesting interactions for further investigation: Spo0J-DivIVA (not thought to interact in *B.subtilis*) and Soj-SojG12V (SojG12V is a mutant trapped as an empty monomer without ATP and so this interaction may imply Soj can dimerise without needing ATP[4])

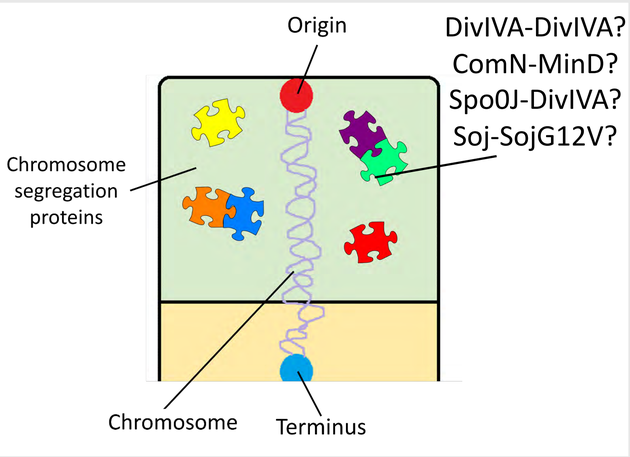


Figure 9: Key preliminary protein-protein interactions determined.

Future work
Confirm interactions by repeats and addressing the problems with the pACT2-Soj construct by varying the length of the linker.

Results: Colony filter lift assay

Production of the *lacZ* gene encodes the protein β-galactosidase. This breaks down the sugar galactose in X-gal to give the colour blue in a colony filter lift assay.

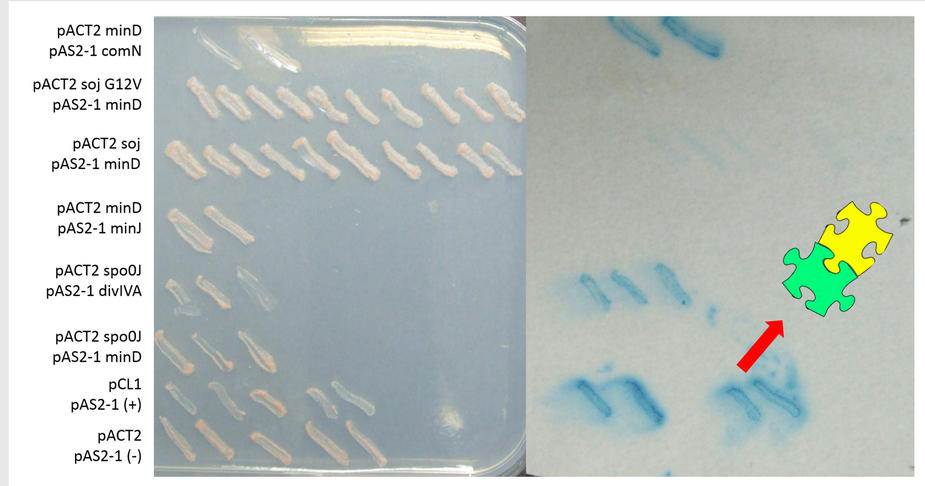


Figure 5: Yeast transformation streaks against their colony filter lifts.

		pAS2-1						
		soj	spo0J	minD	racA	divIVA	comN	soj G12V
pACT2	soj	5/5 +	0/5 -	6/10 +	5/5 +	5/5 ++	5/5 +	1/1 +++
	spo0J	0/1 -	0/5 -	0/3 -		3/3 +++		
	minD	0/5 -	0/3 -	0/5 -	0/10 -		2/2 +++	0/1 -
	racA	0/1 -		0/9 -		1/1 ++	1/1 ++	
	divIVA	0/3 -		0/5 -	0/4 -	5/5 +++	1/1 ++	
	comN	0/10 -	0/10 -	0/5 -	0/5 -	2/5 +++		0/10 -
	soj G12V	0/5 -		0/10 -			0/5 -	0/10 -

No interaction
Weak interaction
Moderate Interaction
Strong Interaction

Figure 6: Colony filter lift results with the degree of positive interaction shown using a blue colour gradient and negative interactions shown in yellow. The number of transformanys showing interaction are indicated as a fraction.

Figure 5 shows some examples of successful yeast transformations streaked onto agar plates with corresponding colony filter lifts on the right.

The presence of blue on the colony filter lift indicates that the proteins transformed into the yeast are interacting, such as minD with comN. Figure 6 shows all tested protein-protein interactions. Preliminary positive protein-protein interactions are shown in blue, with degree of interaction shown using an increasing colour gradient.

Interesting protein-protein interactions are as follows:

- DivIVA-DivIVA
- ComN-DivIVA
- Soj-SojG12V
- Spo0J-DivIVA

Results: α-galactosidase assay

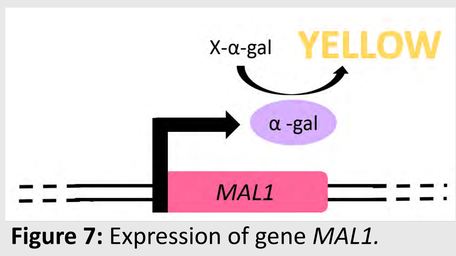


Figure 7: Expression of gene *MAL1.*

Yeast also produces the gene *MAL1* when proteins interact. *MAL1* encodes the protein α-galactosidase which gives the colour yellow with x-α-Gal substrate present (figure 7). This can be used to quantify the level of interaction between proteins.

Three positive protein-protein interactions determined from the colony filter lift assay were tested by this method to determine the level of protein-protein interaction compared to a positive and negative control. A yeast transformed with just the pACT2-soj construct was also tested as previous results suggested the construct had a basal level of gene activation.

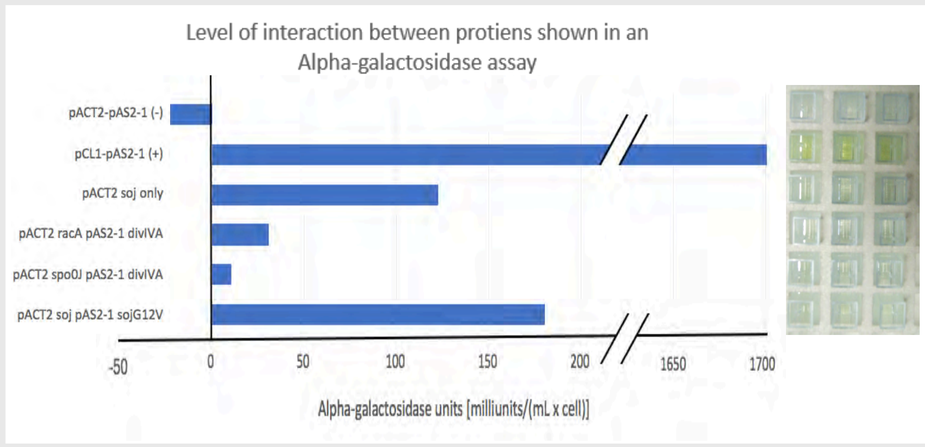


Figure 8: Results of the Alpha-galactosidase assay.

Soj-SojG12V showed weak protein interaction even with the 'leaky' pACT2-Soj construct.

RacA-DivIVA and Spo0J-DivIVA both showed weak protein-protein interaction (figure 8).

These results support results obtained from the colony filter lift assay.

References and Acknowledgements

References:
1. Errington J. *Microbiology.* 2010;156(1):1-13
2. Santos VTD *et al. Journal of Bacteriology*. 2012;194(14):3661-3669
3. Errington J, Wu, LJ *et al*. *Molecular Microbiology*. 2016;101(2):333-350
4. Errington J *et al*. *EMBO Journal*. 2012;31(6);1542-1555

Acknowledgements:
I would like to express my gratitude to my project supervisor Clare Willis for all her help and guidance throughout the project. Thanks are also due to Ling Wu Juan, David Roberts, Jeff Errington and everyone at CBCB for providing myself with the resources to undertake the project. Lastly I would like to thank Wellcome Trust for providing funding to make my project possible.