

## **Using co-localization to measure the kinetics of cell division complex assembly in *Bacillus subtilis***

Meira, G.L.S., and Gueiros-Filho F.J.

Departamento de Bioquímica, IQ, USP.

Septum formation in bacteria is carried out by a large protein complex known as the divisome. The protein responsible for the initiation of septum construction is FtsZ, the bacterial tubulin. This protein self-assembles into a structure known as the Z-ring that triggers the association of other division proteins into the divisome. There are a dozen proteins that participate in divisome formation, but how this complex assembles is still unclear. In Sbbq 2008 we showed that we can study the temporal sequence of assembly of different division proteins into the *Bacillus subtilis* divisome by dual color fluorescence microscopy. For that, we fused FtsZ to mCherry (red reporter) and other division proteins fused to GFP (green reporter). By measuring the frequency of co-localization between the Z ring and other division proteins we can establish the sequence of events during divisome assembly. The higher the co-localization the faster this protein arrives at the divisome. Here, we have expanded on previous results and found that ZapA, EzrA, YpsB, DivIVA and MinC co-localized with the Z-ring in 97%, 98%, 50%, 42%, 32% of the cells, respectively. ZapA and EzrA like to assemble into the divisome immediately after Z ring formation. On the other hand, YpsB, DivIVA and MinC, seem to exhibit a delay in their association. The different co-localization frequencies among “late proteins” suggest that these do not assemble at the same time. Thus, the complex does not seem to be divided simply between “early proteins” and “late proteins”. Instead, the divisome seems to mature in a more gradual way, with each protein arriving at the complex with a different kinetics.

Financial Support: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP)