MS07-P13 | Enzyme Activation by a Flavoprotein Redox Network

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Ribonucleotide reductases (RNRs) reduce ribonucleotides to deoxyribonucleotides by employing radical chemistry. In class Ib RNRs, reduced NrdI, a flavodoxin-like protein, is essential for the activation of a dimanganese center in the radical generating RNR β subunit. It has been proposed that NrdI is recycled *in vivo* by an NrdI reductase, but no such reductase had been identified. Ferredoxin/flavodoxin-$NADP^+$oxidoreductases (FNRs) are probable reductants of NrdI. We identified three FNRs in the genome of *Bacillus cereus*and carried out structural and functional studies in order to characterize their ability to reduce NrdI. By comparing reduction kinetics, binding affinities, redox potentials and 3D structure,we have shown that one FNR reduces NrdI at a much higher rate than the two remaining FNRs. Using this FNR as an NrdI reductase, we were also able to activate the RNR β subunit under aerobic conditions, mimicking cellular conditions. Altogether, our observations suggest that this FNR might be the superior NrdI reductase *in vivo*, and hence, an essential activator of the class Ib RNR system.

In addition to NrdI, *B. cereus* encodes two flavodoxins (Flds). We have performed biochemical and structural investigations of the full FNR-Fld/NrdI redox network, and identified that one FNR-Fld pair is more efficient than the others. By studying the interactions between proteins in flavoprotein networks, which are poorly characterised in bacteria, we aim to map defining features that govern recognition and selectivity for electron transfer between flavoproteins.