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Comparing system-specific chaperone interactions with their Tat dependent redox enzyme substrates

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ABSTRACT

Redox enzyme substrates of the twin-arginine translocation (Tat) system contain a RR-motif in their leader peptide and require the assistance of chaperones, redox enzyme maturation proteins (REMPs). Here various regions of the RR-containing oxidoreductase subunit (leader peptide, full preprotein with and without a leader cleavage site, mature protein) were assayed for interaction with their REMPs. All REMPs bound their preprotein substrates independent of the cleavage site. Some showed binding to either the leader or mature region, whereas in one case only the preprotein bound its REMP. The absence of Tat also influenced the amount of chaperone–substrate interaction.

Structured summary:
MINT-8047497: *FdhE* (uniprotkb:P13024) and *FdoG* (uniprotkb:P32176) *physically interact* (MI:0915) by *two hybrid* (MI:0018)
MINT-8046441: *HybO* (uniprotkb:P69741) and *HybE* (uniprotkb:P0AAN1) *physically interact* (MI:0915) by *two hybrid* (MI:0018)
MINT-8046375: *DmsA* (uniprotkb:P18775) and *DmsD* (uniprotkb:P69853) *physically interact* (MI:0915) by *two hybrid* (MI:0018)
MINT-8046425: *TorA* (uniprotkb:P33225) and *TorD* (uniprotkb:P36662) *physically interact* (MI:0915) by *two hybrid* (MI:0018)
MINT-8046393: *NarJ* (uniprotkb:P0AF26) and *NarG* (uniprotkb:P09152) *physically interact* (MI:0915) by *two hybrid* (MI:0018)
MINT-8046409: *NapD* (uniprotkb:P0A9I5) and *NapA* (uniprotkb:P33937) *physically interact* (MI:0915) by *two hybrid* (MI:0018)

1. Introduction

A subset of proteins in *Escherichia coli* are synthesized with a SRRxFLK twin-arginine (RR-) motif in their N-terminal signal peptides [1]. They are targeted and translocated post-translationally by the twin-arginine translocation (Tat) system in a folded state [2]. The translocon is formed by the TatABC subunits in the cytoplasmic membrane.

Of the growing list of Tat-substrates predicted and identified, we find that a number of redox enzymes also appear to have their own system specific accessory chaperone protein [3,4]. The chaperones termed REMPs (redox enzyme maturation proteins) are required for assembly, protease protection, maturation, and targeting to the translocon through a complex multi-step process [5,6]. The REMPs are not part of the final active holoenzyme complexes, but instead seem to be important in monitoring assembly processes by mechanisms unknown.

The Tat dependent redox enzymes can be separated into two groups based on their catalytic cofactors [5]. Here we investigate the molybdopterin-containing enzymes dimethyl sulfoxide reductase DmsABC, trimethylamine *N*-oxide reductase TorAC, formate dehydrogenases FdnGHI and FdoGHI, periplasmic nitrate reductase NapABC, and cytoplasmic nitrate reductase NarGHI. The respective REMP chaperones for each of the enzymes are DmsD, TorD, NapD, NarJ, FdhD, and FdhE [5,7]. The other group consists of two hydrogenases, HyaAB and HybOC, which have a Ni–Fe cofactor in their

Abbreviations: REMP, redox enzyme maturation protein; Tat, twin-arginine translocase system; BACTH, bacterial two-hybrid

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catalytic sites, where HyaE and HybE assist their maturation, respectively.

Previously our group has shown that the different RR-containing N-terminal peptides and/or preprotein enzymes subunits indeed interact with specific REMPs [7]. Here using the in vivo adenylate cyclase based two-hybrid (BACTH) system, we present a comparative study of the REMP interactions with different regions of the RR-leader containing subunits. Hybrid recombinants of the full preprotein ($Enzyme_F$, complete sequence including RR-peptide, peptidase cleavage site, and mature region), RR-leader peptides ($Enzyme_P$, RR-peptide region before the peptidase cleavage site), mature proteins ($Enzyme_M$, mature region only), and the peptidase cleavage site deleted ($Enzyme_X$) forms of RR-containing subunit were generated (Fig. 1A). The interaction between these constructs and their cognate REMPs were investigated in both wildtype and Tat subunit(s) deletion strains. We find all REMPs interact with the full preprotein of RR motif-containing subunit. However, not all REMPS interact with the RR-leader peptide or the mature protein alone. Many REMP interactions are also affected by the absence of the Tat system.

2. Materials and methods

2.1. Plasmid constructions and TatABC/E deletion mutant constructions

Strains and recombinants used and produced in this study are described in Supplementary Table S1. Recombinants of REMPs with T25- and enzyme with T18-domains of the adenylate cyclase fused to their C-termini were generated as described in [7] using primers listed in Table S2. The peptidase I cleavage sites were removed from the preprotein sequences by site-directed

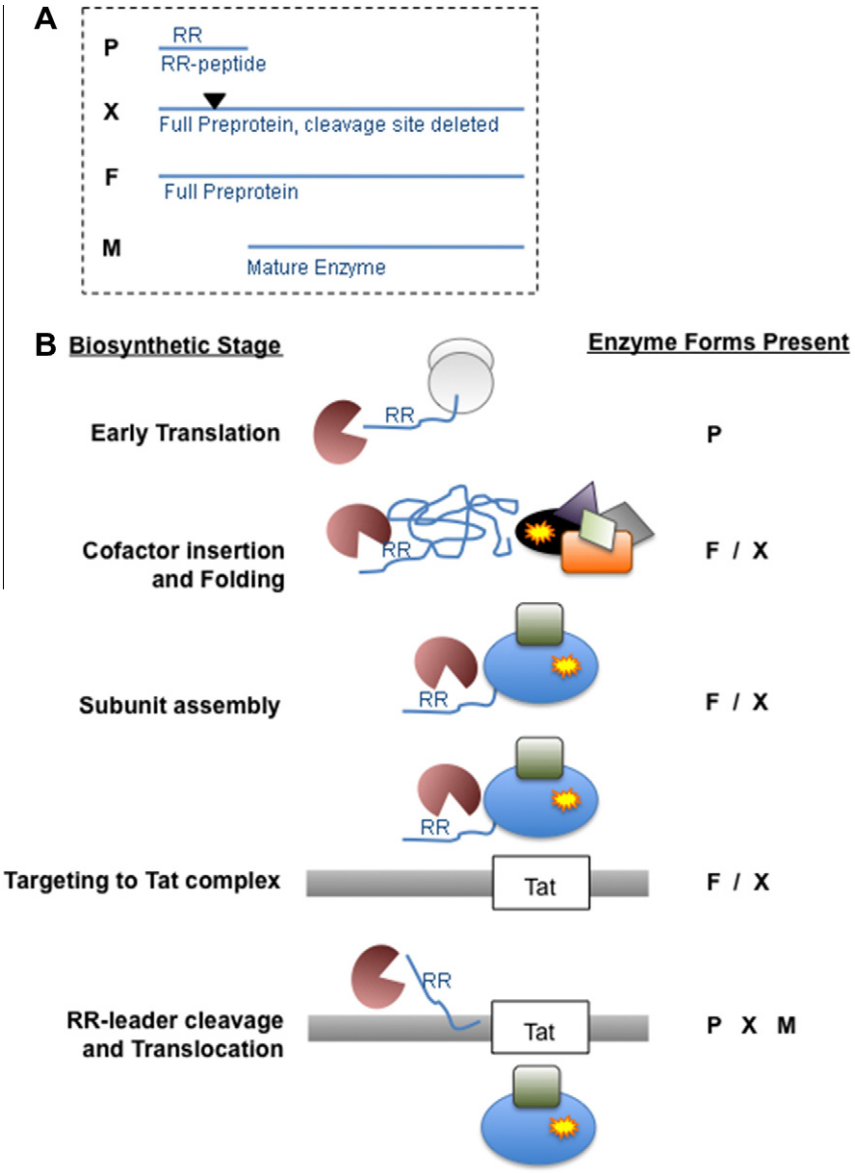

Fig. 1. Regions of RR-leader containing redox enzymes. (A) The different regions of the substrate enzymes studied here. The translated sequence consists of the RR-containing N-terminal leader peptide (P), the leader cleavage site (triangle) within the full preprotein (F), the full preprotein with the cleavage site deleted (X), and the mature region that remains following leader cleavage and translocation (M). (B) An abbreviated model of the proposed Tat-dependent biogenesis of RR-leader containing redox enzymes. The nascent polypeptide chain synthesized with a RR-motif in its amino terminal leader would be present during early translation. The corresponding REMP chaperone recognizes a site(s) within the redox enzyme preprotein. Cofactor biosynthesis and insertion into the mature region of the enzyme as the mature polypeptide folds into its holoenzyme form. Following recruitment and assembly with any other enzyme subunit(s), the enzyme complex is targeted to the membrane and Tat translocon where the enzyme subunits are moved across the membrane with cleavage of the RR-leader peptide.