ENZYMOLOGY

A complex struggle for direction

To avoid strife at the interface of basic carbon and nitrogen metabolism, *Bacillus subtilis* has developed a rather combative solution. If needed, its glutamate synthase suppresses conflicting glutamate breakdown by directly binding and immobilizing its metabolic opponent, glutamate dehydrogenase.

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Changes in the environment, such as in nutrient availability, necessitate adaptations of cellular metabolism. The available regulatory modalities are manifold, including the differential transcription of metabolic genes and the post-translational modification or allosteric regulation of already present metabolic enzymes. Allosteric regulation can be mediated by many types of (bio-)molecules, including other enzymes. Here, of special note are 'metabolons', a term coined by Paul Srere for transient complexes of enzymes that form to optimize metabolic throughput[1]. While several such synergistic complexes have been studied functionally and structurally, in this issue Jayaraman et al.[2] describe a metabolic complex in *B. subtilis* that could be regarded as the exact opposite of a classical metabolon. When necessary, six protomers of the heterodimeric glutamate synthase GltAB smother the homohexameric glutamate dehydrogenase (GDH) GudB from all sides, suppressing its activity in a 1.6-megadalton 'counter-enzyme' complex, and thereby switching the metabolic program from glutamate breakdown to its synthesis[2] (Fig. 1).

Glutamate is a key metabolite that is generally maintained at high concentrations and serves as a cellular nitrogen reservoir. When glutamate or other amino acids that can be catabolized via glutamate (such as histidine or arginine) are available as nutrients, GDH enzymes are utilized to access them as carbon and nitrogen sources, via conversion to α-ketoglutarate and ammonia. However, in glutamate-limited conditions, the glutamate level has to be actively maintained via glutamate synthesis, using α-ketoglutarate as a substrate. In this scenario, GDH activity is redundant, if not counterproductive. Dan Tawfik's investigations into the regulatory mechanisms of two *B. subtilis* GDH paralogs, RocG and GudB[3,4], led to the discovery that the latter can be inactivated in a direct interaction with its counter-enzyme GltAB. Together with collaborator James Fraser, the team obtained a cryo-EM structure of the whole complex, which constitutes one of Tawfik's last pieces of published work[5].

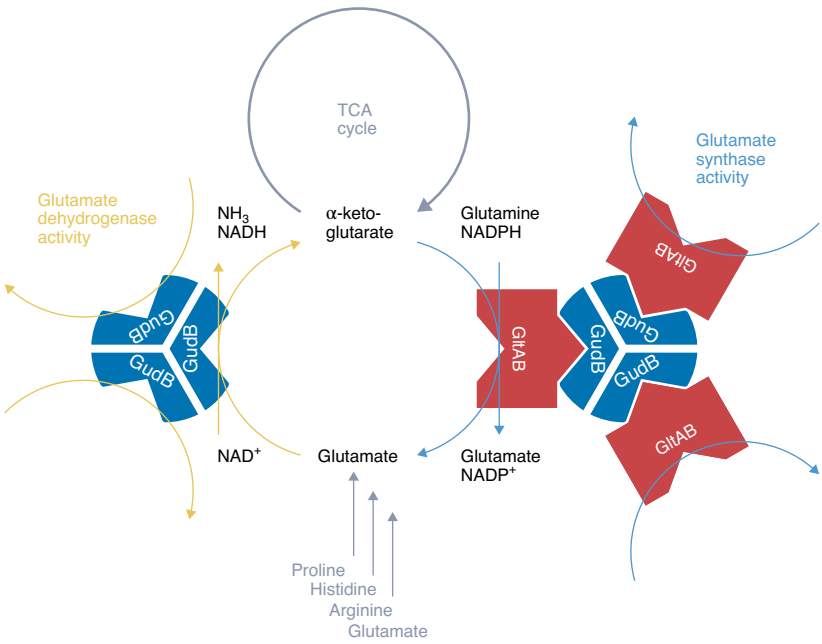

Fig. 1 | The GudB–GltAB counter-enzyme system at the intersection of carbon and nitrogen metabolism. The GudB homo- and the GudB–GltAB heterocomplex are shown schematically in a top view, with the bottom half of the complex omitted. On the left, GudB is working on glutamate breakdown, while on the right, GltAB is working toward glutamate synthesis, overruling GudB activity by blocking its active site.

Unlike its paralog RocG, which is tightly regulated at the transcriptional level, GudB is constitutively expressed. Although its activity is less required in glutamate-limited conditions, its protein levels turned out to be essentially the same in bacilli grown in either glutamate-poor or glutamate-rich medium. However, under glutamate-poor conditions, in vivo crosslinking experiments revealed that GudB forms a specific complex with the two GltAB components GltA and GltB. The latter were found to be expressed only in glutamate-poor conditions, being regulated at the transcriptional level in a GDH-dependent manner[6]. Kinetic analysis suggested that GudB activity is strongly suppressed within the GudB–GltAB complex, which is stabilized by the GltAB substrate and cofactor α-ketoglutarate and NADPH. The mechanism of this suppression becomes obvious in the complex structure: each of the six active-site clefts of the GudB hexamer is completely blocked by the specific binding of a single GltAB heterodimer (Fig. 1). Key to this interaction is a regulatory loop in GltA that discriminates between RocG and GudB and binds and blocks only the latter. The stability of the complex is compromised when α-ketoglutarate is depleted, releasing GudB and enabling it to restore the α-ketoglutarate level.

Despite its central importance and remarkable size, the GudB–GltAB complex was essentially identified by serendipity in classical in vivo experiments. The study of complexes is typically driven by expectations, often aimed at deciphering interactions within a given set of biomolecules and at obtaining a high-resolution snapshot of their architecture. However, as GudB and GltAB are catalyzing opposing reactions and are under the control of different promotors in different areas of the genome, the likelihood of a direct interaction between these gene products was far from obvious. Within the past decade, we have just experienced two revolutions in structural biology: the resolution revolution in cryo-EM, providing experimental high-resolution access to many previously intractable molecular assemblies[7], and the breakthrough in protein structure prediction suddenly providing genome-wide protein structure prediction at astounding accuracy[8]. Though the former was instrumental in revealing the GudB–GltAB structure, neither provides the cue to seek the interaction partner in the first place. For now, it seems that the identification of transient regulatory interactions will remain mostly an art of the biochemical wet lab. Consequently, similar discoveries remain spectacular, such as the recognition of a previously overlooked, weakly associated regulatory subunit of the yeast fatty acid synthase through careful extraction of the complex from its host organism[9].

We have to admit that the scarcity of mechanistic studies on isolated transient or weakly associated metabolic complexes highlights gaps in our understanding of the organization and regulation of cellular metabolism. Transient regulatory interactions remain largely elusive, and in a several cases in which stable metabolon-like complexes have been characterized, their functional relevance is debatable[10]. The study by Jayaraman et al. serves as a valuable inspiration for a new approach: specifically probing counter-enzyme pairs as possible regulatory hotspots, for which functional relevance would be clearly warranted. ❐

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Competing interests

The author declares no competing interests.