METABOLIC BIOCHEMISTRY

The *Bacillus subtilis* glutamate anti-metabolon

Enzyme–enzyme interactions are largely assumed to act co-operatively to render biochemical pathways more efficient. However, under stress the glutamate synthase of *B. subtilis* does the exact opposite: it inhibits glutamate degradation by sterically hindering the activity of glutamate dehydrogenase.

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Although transcriptional and translational control mechanisms ensure efficient use of cellular resources, various methods of post-translational regulation are used to facilitate rapid shifts in metabolism that are required under specific cellular circumstances. An array of regulatory mechanisms including allosteric regulation and covalent modifications of proteins are well characterized[1], whilst mechanisms underlying the dynamic assembly and disassembly of enzyme–enzyme complexes have slowly been unraveled over the last five decades[2–4] (Fig. 1a). The canonical form of the enzyme–enzyme complex is the assembly of consecutive enzymes of a pathway that interact in such a way as to channel the metabolic intermediate they share between their active sites. In such a conformation, metabolic intermediates are sterically constrained to be efficiently passed between the active sites of the consecutive enzymes (Fig. 1a, bottom) rather than encountering the correct active site via diffusion (Fig. 1a, top). The term metabolon to define such assemblies was first coined almost 50 years ago by Paul Srere in a series of seminal papers that aimed to explain why the behavior of several core biochemical pathways differed from that which would have been anticipated from the action of their constitutive enzymes in isolation: the pathways were considerably more efficient when enzymes were arranged in such a complex. Jayaraman et al.[5] describe in exquisite detail the exact opposite situation: six protomers of the glutamate synthase GltAB assemble with the glutamate dehydrogenase GudB and, in doing so, sterically block access to the GudB active site, thereby inhibiting its activity and favoring the synthesis of glutamate over its degradation (Fig. 1b). As in the metabolon, enzyme activity is sterically regulated; however, in this instance the active site of one enzyme is actively blocked on enzyme–enzyme assembly (Fig. 1b, bottom), rather than being physically constrained to be adjacent to the active site of the subsequent enzyme in the pathway.

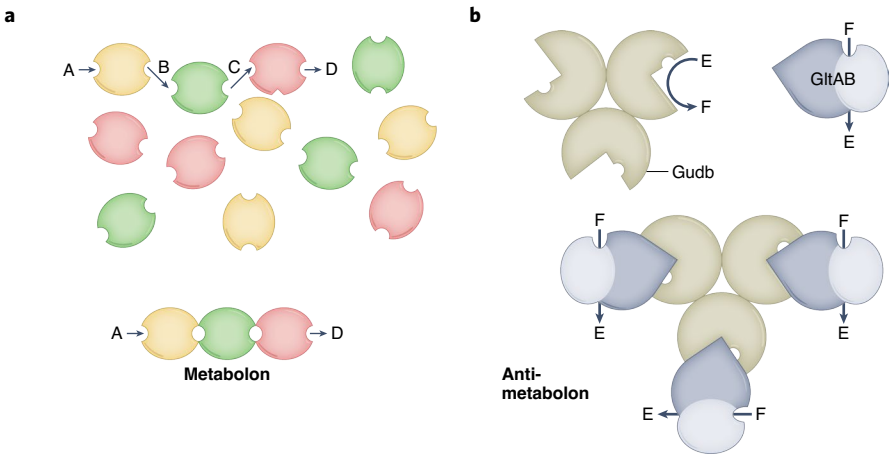

Fig. 1 | Metabolic regulation by enzyme–enzyme assembly. a, Illustration of the mode of action of the metabolon using the glycolytic metabolon as a canonical example[11]. Top: the enzymes phosphoglycerate mutase (yellow), enolase (green) and pyruvate kinase (red) do not form associations with other enzymes, and the reactions of glycolysis occur independently, with the intermediates (B and C; in the case of glycolysis, 2-phosphoglyceric acid and phosphoenolpyruvate) traveling from active site to active site by diffusion. Bottom: the enzymes form a complex in which the active sites are aligned in a hollow tube, and thus the metabolic intermediates (B and C; in the case of glycolysis, 2-phosphoglyceric acid and phosphoenolpyruvate) are channeled from active site to active site, thereby both being highly efficient and preventing competing side reactions. In all enzyme cartoons, the active site is represented by the invaginations on the circle, indicating that when the proteins are correctly folded the active sites are located internally in the hollow tube structures they form. **b**, Illustration of the mode of action of the metabolon using the GudB–GltAB complex as a newly identified example[5]. GudB is an isoform of glutamate dehydrogenase, and when not complexed with the glutamate synthase, GltAB can catalyze the conversion of E (glutamate) to F (α-ketoglutarate). Conversely, GltAB can catalyze the conversion of F to E, in both the free and bound form. The inhibition of GudB in the bound form occurs due to the occlusion of the active site of GudB on the binding of GltAB. The access to the active site of GudB is sterically hindered on binding, whilst that of GltAB is not.

Glutamate plays multiple roles in cellular metabolism, including representing the main nitrogen reserve, being involved in pH buffering and osmolarity regulation, and acting as a counter-ion to potassium[5]. α-Ketoglutarate, by contrast, is present at much lower levels and displays rapid turnover[6]. These metabolite pools are linked by both glutamate synthase, which converts ketoglutarate to glutamate, and glutamate dehydrogenase, which catalyzes the reverse reaction. Jayaraman et al. found that under conditions in which glutamate needs to be synthesized, GuDB binds to its counter enzyme GltAB with cryo-EM structures revealing an unusually large 1.6 MDa complex in which the active site of GuDB is occluded by the regulatory loop of GltA. A range of subsequent in vitro and in vivo experiments established that the primary role of this interaction was the inhibition of GuDB by GltA, suggesting that for complete inhibition, six GltA molecules are required for the GuDB hexamer. This observation is the first of which we are aware in which a catabolic enzyme is inhibited by

an anabolic one; in the arginase–ornithine transcarbamylase complex, the anabolic enzyme is inhibited by the catabolic one[7]. Because many enzymes have evolved toward unidirectional catalysis, the existence of a mechanism to stop or at least minimize the simultaneous operations of counter enzymes is logical since this prevents the establishment of futile cycles.

The study of Jayaraman et al. is not only fascinating in its own right, but also opens the possibility that such inhibitory enzyme–enzyme interactions represent a hitherto overlooked mechanism of regulation. Surveys across the kingdoms of life have revealed that a staggering number of proteins interact with one another[2], and despite the obvious attraction of efficiency it is equally obvious that most of these thousands of interactions do not represent consecutive enzymes, let alone ones that channel metabolites. Taking a fresh look into these interactomics datasets in light of the findings concerning the GltAB–GudB complex will be a good starting point to assess the generality of this finding. Irrespective of whether it is a rare or common regulatory mechanism, the strategy adopted by *B. subtilis* is fascinating.

When surveying the vast lists of proteins that interact with one another[3], most metabolic biologists have been interested in interactions of consecutive enzymes in biosynthetic pathways and in particular those that may mediate metabolic channeling. In light of the study by Jayaraman et al.[5], and indeed of the earlier studies on the arginase–ornithine transcarbamylase complex[7], these lists, alongside targeted enzymology and structural biochemistry, will likely reveal whether the operations of such anti-metabolons are truly rare events or whether they represent the first examples of a more widespread mechanism of metabolic regulation. Recent advances in microscopy, in particular in cryo-EM[8] and in bioinformatic prediction of protein structure[9], will likely aid future studies, although the final proof of their biological relevance will need to be provided by wet lab enzymology and physiology[10]. The discovery of this mechanism in *B. subtilis* by Dan Tawfik's group is perhaps fittingly reminiscent of the meticulous experiment, driven by theoretical logic, by which Paul Srere arrived at the definition of the metabolon nearly 50 years ago[2]. It is a great shame that this work will be one of the last papers of the late Dan Tawfik. However, he leaves behind a truly wonderful scientific cannon and, with this work, the tantalizing suggestion that a widespread novel mechanism of enzyme regulation may await to be uncovered. ❐

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Competing interests

The authors declare no competing interests.