Review

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Designing biological compartmentalization

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Intracellular organization is a key factor in cell metabolism. Cells have evolved various organizational systems to solve the challenges of toxic pathway intermediates, competing metabolic reactions, and slow turnover rates. Inspired by nature, synthetic biologists have utilized proteins, nucleic acids, and lipids to construct synthetic organizational systems that mimic natural systems. Many of these systems have been applied to metabolic pathways and shown to significantly increase the production of industrially and commercially important chemicals. Further engineering and characterization of synthetic organizational systems will allow us to better understand native cellular strategies of spatial organization. Here, we discuss recent advances and ongoing efforts in designing and characterizing synthetic compartmentalization systems to mimic natural strategies and increase metabolic yields of engineered pathways.

Compartmentalization benefits natural and engineered systems

Biological complexity requires varying degrees of organization. Cells require spatial organization to perform the various enzymatic reactions and processes necessary to sustain life [1]. This is achieved through compartmentalization, the physical separation of biological reactions. Examples of compartmentalization include membrane-bound organelles, bacterial microcompartments [2,3], multienzyme complexes, and others [4,5].

Inspired by nature, synthetic biologists have recently devised strategies to mimic cellular organizational systems. These synthetic systems have been predominantly designed toward metabolic engineering of pathways, harnessing the capability of cells to produce industrially [6,7] or pharmaceutically useful [8,9] compounds.

In this review, we describe the various difficulties faced by the cell when performing metabolic reactions and natural compartmentalization systems that solve these problems. We review recent advances in designing synthetic compartments that provide modular solutions to overcome these same challenges. These systems capture the benefits of spatial organization and apply them to engineered pathways. This has also recently been reviewed in [10–12]. Our review will discuss the latest progress and challenges in designing compartmentalization, especially in building bacterial microcompartments and RNA scaffolds. We also analyze the degree to which these mimic natural systems and discuss how they aid in our understanding of the biological organization of the cell.

The need for intracellular organization

Cells face many challenges that benefit from compartmentalization (Figure 1a). First, some enzymes, such as ribulose 1,5-bisphosphate carboxylase oxygenase (RuBisCO) [13], suffer from slow turnover, which results in flux imbalances or bottlenecks in pathways. Reliance on such enzymes may require establishing local concentration gradients of substrates. This would increase reaction rates to support adequate pathway flux [14]. Second, diffusion of volatile intermediates through the cell membrane results in their loss from the cell [15]. Third, biosynthetic pathways can generate toxic intermediates that inhibit growth, such as hydrogen sulfide accumulated during bacterial sulfur metabolism [16]. Finally, metabolites can participate in multiple competing reactions, reducing their availability for any single pathway. An example of this is malonyl-CoA, an intermediate that is consumed in fatty acid and phospholipid production but is also used in the biosynthesis of polyketides and flavonoids [17].

Nature's solutions

To deal with these challenges, nature has evolved compartmentalization strategies (Figure 1b), such as large enzyme complexes [10,18,19] and organelles [2,20], to spatially organize metabolism. In eukaryotes, compartmentalization in the form of membrane-bound organelles is common. The peroxisome, for example, encapsulates reactions that generate or consume hydrogen peroxide, a toxic intermediate from the breakdown of organic substrates in oxidative reactions [21].

Until recently, prokaryotes were generally thought to lack internal organization [22]. However, researchers have recently discovered different types of bacterial microcompartments that partition the internal space of the bacterial cell for specialized functions [2,23]. In cyanobacteria and other autotrophic prokaryotes, carboxysomes encapsulate RuBisCO and carbonic anhydrase, enzymes involved in the rate-limiting step of the Calvin cycle [24,25] (Figure 2a). These proteinaceous microcompartments are the primary 'carbon-concentrating mechanism' in these bacteria. They

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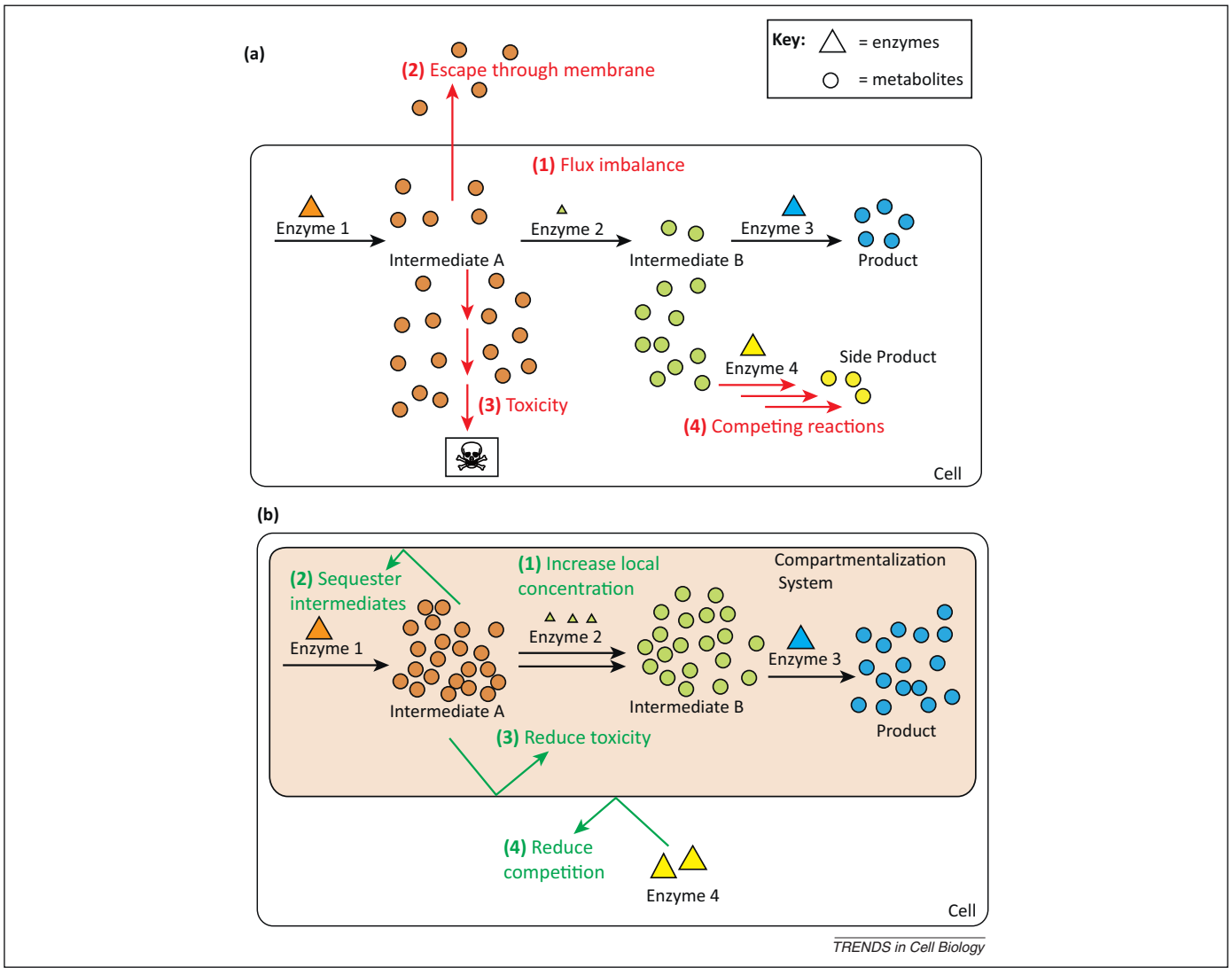

Figure 1. Compartmentalization: nature's solution to various challenges. **(a)** Nature faces many challenges when conducting the chemical reactions of the cell. (1) Differing enzyme kinetics may result in flux imbalances. (2) Intermediates may be lost through the cell membrane. (3) Toxic intermediates can result in growth inhibition. (4) Competing reactions can divert flux through undesired pathways. **(b)** Compartmentalization systems specifically solve challenges 1–4, respectively, by: (1) creating areas of local concentrations to favor reaction kinetics; (2) sequestering intermediates; (3) reducing toxicity; and (4) reducing competition. (Adapted from [11].)

are proposed to help overcome the slow turnover rate of RuBisCO by providing a high local concentration of carbon dioxide to the enzyme [26,27].

Two other bacterial proteinaceous microcompartments protect the cell from toxic aldehyde intermediates. The ethanolamine utilization (Eut) microcompartment sequesters acetaldehyde, a volatile and toxic intermediate of the ethanolamine utilization pathway [28]. Likewise, the 1,2-propanediol utilization (Pdu) microcompartment encapsulates propionaldehyde, minimizing its toxicity [29]. These and numerous other bacterial microcompartments have been found in approximately 400 microbial genomes [2].

Another method of compartmentalization found in nature is multienzyme complexes, which directly link enzymes involved in a given pathway. Ideally, this results in substrate channeling, the process by which intermediates are directly transferred between the active sites of two enzymes that catalyze sequential reactions in the pathway [30]. Substrate channeling prevents the loss of intermediates and minimizes competing cross-reactions. A classic example is tryptophan synthase, a multienzyme complex that catalyzes the last two reactions in the biosynthesis of L-tryptophan [18,31]. The intermediate, indole, is channeled from one active site to the next without being released into the surrounding environment. This is advantageous for the cell not only because indole is reactive and easily lost through the cell membrane, but also because, in the absence of indole, tryptophan synthase catalyzes dehydration of serine to pyruvate at 5% the rate of tryptophan formation [32]. Other multi-enzyme complexes found in nature include polyketide synthase [33], carbamoyl phosphate synthetase [34], and cellulosomes [35], which may function similarly by increasing reaction kinetics and reducing the loss of intermediates.

Synthetic compartmentalization

The goal of metabolic engineering is to optimize a given biosynthetic pathway to increase production of a particular substance [7,36]. Many of these pathways present the same challenges of toxic intermediates, competing reactions, and flux imbalances found in nature [10,14]. Therefore,