Feature Review

CellPress

Plasticity of specialized metabolism as mediated by dynamic metabolons

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The formation of specialized metabolites enables plants to respond to biotic and abiotic stresses, but requires the sequential action of multiple enzymes. To facilitate swift production and to avoid leakage of potentially toxic and labile intermediates, many of the biosynthetic pathways are thought to organize in multienzyme clusters termed metabolons. Dynamic assembly and disassembly enable the plant to rapidly switch the product profile and thereby prioritize its resources. The lifetime of metabolons is largely unknown mainly due to technological limitations. This review focuses on the factors that facilitate and stimulate the dynamic assembly of metabolons, including microenvironments, noncatalytic proteins, and allosteric regulation. Understanding how plants organize carbon fluxes within their metabolic grids would enable targeted bioengineering of high-value specialized metabolites.

Specialized metabolites and their infrastructure

Plants are sessile organisms and their growth and development is totally dependent on highly specialized biosynthetic capacities, such as photosynthesis and cell wall formation, and on the intimate interplay of multiple genetic and metabolic networks, which require a high degree of functional organization and infrastructure. Compartmentalization is an efficient way to separate cellular processes in eukaryotic cells. Within each compartment, proteins may be organized into functional units (modules), which, like electronic circuits, provide structured responses. Each biosynthetic pathway includes multiple modules, where the output of one serves as the input to the next [1–4]. Organization in functional units offers the opportunity to redirect the circuit by combining modules. Likewise, the plethora of plant specialized metabolites requires a sophisticated metabolic infrastructure. These metabolites are derived from primary metabolism with intermediates in the pathways for the synthesis of carbohydrate, amino acid, and lipid as precursor molecules. Specialized metabolites include a plethora of compounds with highly diverse functional properties and offer a way for the plant to communicate with other plants, deter herbivores, attract pollinators, and facilitate adaptation to climatic change.

The cytochrome P450 (CYP) enzyme superfamily plays a crucial role in catalyzing the formation of a diversity of specialized metabolites, including terpenoids, phenylpropanoids, and alkaloids [5,6]. The classic CYP-catalyzed reaction involves substrate hydroxylation and uses molecular O_2 and two electrons [7]. Besides hydroxylation, CYPs may catalyze diverse reactions such as dehydration, isomerization, dimerization, decarboxylation, and reduction [8]. As catalysts of reactions that are often difficult to carry out by organic chemical synthesis, CYPs have attracted the attention of synthetic biologists. The rationale for using biological systems for the production of high value compounds versus classical organic chemical synthesis is a matter for debate between synthetic biologists and synthetic chemists [9]. The type II CYPs are anchored to the endoplasmic reticulum (ER) membrane with the globular domain facing the cytosol, and require electrons donated by the NADPH-dependent cytochrome P450 oxidoreductase (POR) [10]. The many biosynthetic pathways that include CYPs require fine organization. Furthermore, the biosynthesis of specialized metabolites typically requires the coordinated action of multiple enzymes, which would require the presence and availability of a highly complex intracellular mixture of substrates, intermediates, and products. To optimize metabolism, some biosynthetic pathways are organized as multienzyme complexes, metabolons, to facilitate swift processing and to avoid undesired metabolic crosstalk [4,11].

This review focuses on the factors stimulating metabolon formation. A detailed understanding of the mechanisms governing optimized metabolic flux is likely to play a crucial role in the engineering of biosynthetic pathways of lucrative compounds in the future to satisfy the desire to move towards a bio-based society. We provide an overview of the complexity of cellular metabolic highways facilitated by dynamic metabolons. Furthermore, we highlight factors that are likely to play essential roles in the regulation of metabolon assembly.

Organization of pathways generates metabolic highways

The organization of enzymes in modules or metabolons is an efficient way to direct metabolic processes. The metabolon concept was initially defined as complexes of sequential

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1360-1385/
 http://dx.doi.org/10.1016/j.tplants.2014.11.002

metabolic enzymes and structural elements [12–14]. According to this definition, sequential enzymes occur in close proximity, creating a microenvironment where substrates are successively transferred between enzymes, which results in high local concentrations or in direct transfer to the adjacent enzyme [12,14,15]. This provides intracellular metabolic highways where the production of compound D from substrate A circumvents free diffusion and equilibrium of the intermediates B and C (Figure 1). Hereby, the total flux increases significantly and simultaneously prevents leakage of labile and potentially toxic intermediates [4].

Metabolon assembly may directly regulate the flux and allosterically alter the efficiency of individual enzymes [16]. Protein interactions are thought to play a crucial role in metabolon formation and may be differentiated into obligate and non-obligate interactions [17]. Obligate interactions include complexes of homo- and hetero-oligomeric complexes where each individual protein is not able to catalyze a reaction. By contrast, non-obligate complexes involve interactions between proteins that may function independently. Non-obligate or transient complexes with dissociation constants in the micromolar range play a crucial role in cellular networks [18]. In the biosynthesis of specialized metabolites, metabolons are assumed to be dynamic, allowing regulated assembly and disassembly. This enables different combinatorial uses of the individual proteins, thereby expanding the product output profile [19]. Although the stability of such complexes may be functioning dependent [20,21], the regulation of assembly is most likely achieved through complex mechanisms, including protein–protein interactions and changes in microenvironments.

The organization of multiprotein complexes can be achieved by anchoring to small noncatalytic scaffold proteins with the sole purpose of bringing enzymes together [11,22,23], and allosterically stimulate the individual components [24]. In the synapse, post-synaptic density protein (PSD95)–*Drosophila* disc large tumor suppressor (Dlg1)–zonula occludens-1 protein (zo-1) (PDZ) domain-containing scaffold proteins are involved in organizing signaling complexes, binding of downstream effectors, and coordination with the cytoskeleton [25]. Owing to relatively weak binding affinities, the association and dissociation is dynamic [26,27]. Scaffold proteins composed of multiple consecutive PDZ domains, supramodules, are capable of guiding the assembly of multiprotein complexes by bringing them together or by stabilizing already established interactions [25]. In plants, only a few studies have focused on PDZ domain scaffold proteins and the identification of motifs [28]. The chloroplast Deg proteases involved in protein quality control, specifically of photodamaged photosynthetic subunits [29], are known to organize in hexamers stabilized by PDZ domains [30]. The existence of synthetic scaffold proteins in plants may be used by synthetic biologists to force enzymes together *in vivo* using bioengineering, as well as *in vitro* as experimental tools to gain insight about the consequences of organizing enzymes in a sequential manner. In glycolysis and gluconeogenesis, three enzymes, triosephosphate isomerase, aldolase, and fructose 1,6-bisphosphatase, sequentially convert glyceraldehyde 3-phosphate into fructose 6-phosphate. The aldolase and fructose 1,6-bisphosphatase has been shown to form a dynamic metabolon *in vivo* [31]. A synthetic scaffold protein binding all three enzymes based on high-affinity interactions between dockerin and cohesins resulted in almost 50 times increased reaction rates compared with their individual turnover rates [32]. Similar mechanisms are likely to be involved in controlling cell metabolism.

Allosteric regulation: it's all about protein chemistry

Enzymes are dynamic chemical catalysts constantly undergoing local folding or unfolding events [33]. Structural disorder at one site may affect the properties of regions distal to the effector site via amino acid energy networks [34]. Amino acid and domain flexibility gives rise to an ensemble of conformations that oscillates at multiple timescales associated with functional properties [35]. Single molecule enzyme activity studies have revealed the existence of multiple catalytic states, indicating that enzymes behave as individuals [36,37]. These catalytic states may be linked to the conformational ensemble of enzymes. Single molecule resolution is thus required to understand the detailed function and dynamics of enzymatic processes, which remain masked in bulk studies. The behavior of

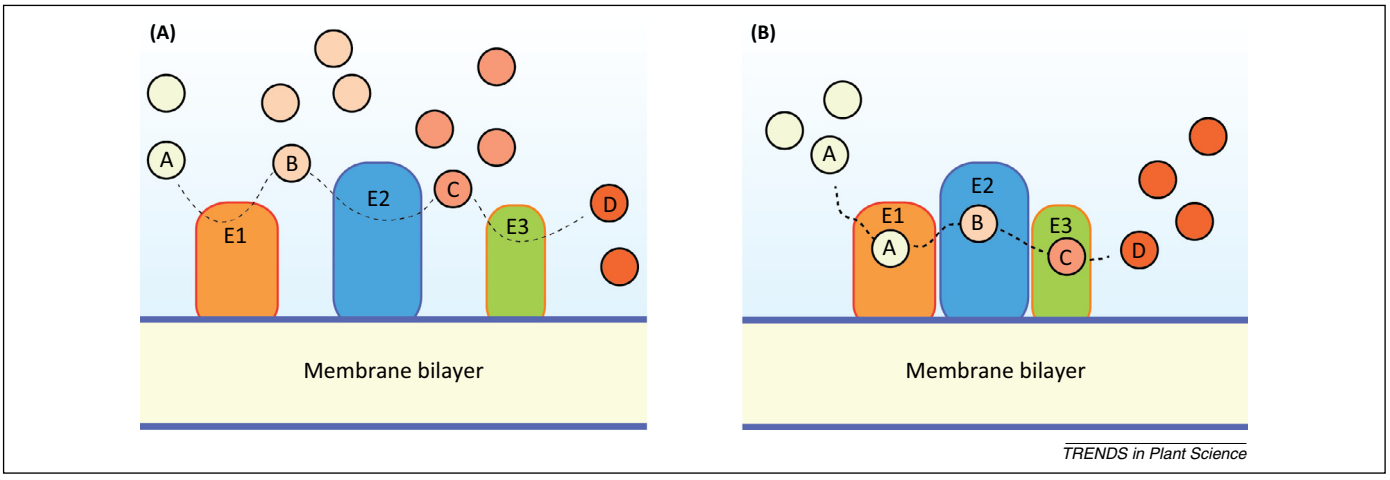

Figure 1. Illustration of the conversion of substrate A to product D with intermediates B and C. The membrane serves as a scaffold for organizing the enzymes E1, E2, and E3. The sequential conversion is speculated to follow independently with the intermediates freely equilibrating with the bulk **(A)**, or through the formation of metabolons channeling substrate A towards product D without free equilibration of the intermediates **(B)**.