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Review

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Cellular view of metabolism: metabolic biomolecular condensates

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Summary

Recent studies across biological systems highlight an important function of biomolecular condensates (hereafter biocondensates) in regulating physiology. Biocondensates are membraneless organelles that compartmentalize cellular processes and allow regulatory control of key biomolecules through their assembly and disassembly. Biocondensates have been identified in molecular pathways ranging from RNA regulation to metabolism to seed germination in plants. In this review, we focus on biocondensates that control metabolism. Most of this review addresses metabolic biocondensates in bacteria, algae, and animals whose functions are involved in core metabolism relevant to plants, even if their existence as metabolic biocondensates has not yet been described in plants. We hope this review provides useful information for a broad audience and encourages new directions into previously characterized enzymes and pathways to understand how their subcellular localization could impact function.

I. Introduction: metabolic biocondensates and metabolons

Biocondensates are membraneless macromolecular structures that concentrate biomolecules *in vivo* (Banani *et al.*, 2017) into a physical state (solid, liquid droplet, or gel-like) different from dispersed localization (Field *et al.*, 2023). In plants, biocondensates vary in function from driving primary metabolism to regulating abiotic stress responses and development (Field *et al.*, 2023). Some biocondensates function as metabolons, transiently forming complexes bringing pathway enzymes in close proximity to facilitate metabolite channeling between enzymes (Zhang & Fernie, 2020). Metabolons increase pathway efficiency by allowing metabolites to transfer from the active site of one enzyme to the active site of another enzyme without being released into solution (Zhang & Fernie, 2020). In this review, we focus on biocondensates with this function, which we call metabolic biocondensates.

Historically, metabolons and metabolic biocondensates have been discussed separately. However, the distinction between metabolons (defined by enzymatic cooperation and biochemical properties) and biocondensates (defined by biophysical properties) is more blurred than previously considered. The identification of a metabolon, including examples characterized in plants (Laursen *et al.*, 2016; Mucha *et al.*, 2019; Nakayama *et al.*, 2019), requires

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Table 1 Evidence underpinning the discovery of each biocondensate.

Metabolic biocondensate	Evidence for structure or function of biocondensate	References for each stage of discovery
Pdu microcompartment	The pdu microcompartment was observed initially as an electron-dense structure in wild-type bacterial cells.	Shively *et al*. (1998)
	The genome locus encoding the microcompartment was later found to contain enzymes involved in 1,2-propanediol metabolism.	Bobik *et al*. (1999)
Carboxysome	The carboxysome was observed initially as an electron-dense structure in wild-type bacterial cells.	Shively *et al*. (1970)
	The carboxysome was later purified and shown to contain active RuBisCO.	Shively *et al*. (1973)
Pyrenoid	The pyrenoid was initially observed using light microscopy in wild-type algal cells.	Vaucher (1803)
	Purified pyrenoids were shown to have RuBP carboxylase activity *in vitro*.	Holdsworth (1971)
Purinosome	The purinosome was initially hypothesized following copurification of purine salvage enzymes.	Smith *et al*. (1980)
	Later, cytoplasmic speckles were confirmed for some purine salvage enzymes.	An *et al*. (2008)
	Substrate channeling was confirmed using mass spectrometry imaging.	Pareek *et al*. (2020)
G-bodies	G-bodies were initially discovered by tagging the proteins phosphofructokinase and enolase, which were found to form cytoplasmic aggregates *in vivo*.	Miura *et al*. (2013); Jang *et al*. (2016)
	The G-body proteome was uncovered using Co-immunoprecipitation Mass Spectrometry, with phosphofructokinase and enolase as bait proteins.	Miura *et al*. (2013); Jang *et al*. (2016)
Rhamnosome	*Arabidopsis thaliana* RHM1 was shown to localize to cytoplasmic speckles.	Wang *et al*. (2009)
	Formation of rhamnosome was shown to correlate with UDP-rhamnose synthesis based on molecular genetic approaches.	Field *et al*. (2024)

pdu, propanediol utilization; RuBP, ribulose 1,5-bisphosphate; UDP, uridine diphosphate.

first elucidating the enzymes in a pathway and then demonstrating meaningful physical interactions between pathway enzymes (Bassard & Halkier, 2018). Alternatively, for metabolic biocondensates, subcellular compartments have been observed first, then shown to have a metabolic function, and later demonstrated to act as metabolons. Examples of metabolic biocondensates are described in the next section.

II. Classical examples of metabolic biocondensates

Initially, the discovery of metabolic biocondensates followed observation of organelle-scale structures using microscopy (Table 1). Several of the first discovered biocondensates were bacterial microcompartments, which are conspicuous structures under transmission electron microscopy. Bacterial microcompartments are large (40–600 nm; Kerfeld *et al*., 2018) icosahedral proteinaceous compartments that physically separate pathway enzymes and intermediates from the cytoplasm. Here, the outer protein layer of a microcompartment is termed the shell. Bacterial microcompartments house diverse metabolic pathways, including catabolism of ethanol, ethanolamine, and sugar phosphates (Sutter *et al*., 2021). In the interest of space, we limit discussion of bacterial microcompartments to two well-studied examples: the propanediol utilization (pdu) microcompartment and the carboxysome. The functional diversity of bacterial microcompartments is detailed in Sutter *et al*. (2021). Beyond bacterial microcompartments, we discuss the pyrenoid, a metabolic biocondensate discovered using light microscopy in algae.

Propanediol utilization microcompartment

Some bacteria that colonize mammals can grow on highly reduced carbon sources from the environment, including 1,2-propanediol (Toraya *et al*., 1978; Jeter, 1990). To accomplish this, they contain a microcompartment that houses the pathways required for the conversion of 1,2-propanediol into 1-propanol or propionyl-PO_4^{2-} (Bobik *et al*., 1999). This propanediol utilization (pdu) microcompartment is best described in *Salmonella enterica*, although components of the pdu microcompartment have been identified in a wider range of bacteria (Sutter *et al*., 2021). The pdu microcompartment contains enzymes in three pathways that degrade 1,2-propanediol and facilitates efficient utilization of 1–2 propanediol through its selective permeability (Jakobson *et al*., 2017).

The committed step of 1,2-propanediol utilization converts 1,2-propanediol into propionaldehyde, a toxic intermediate (Sampson & Bobik, 2008). Downstream, propionaldehyde is oxidized to form propionyl-PO_4^{2-} or reduced to form 1-propanol. The oxidation of propionaldehyde reduces NAD^+ to NADH, while reduction of propionaldehyde oxidizes NADH to NAD^+. Thus, NADH and NAD^+ are cycled to sustain propionaldehyde consumption in the pdu microcompartment (Fig. 1). By concentrating enzymes sufficient for metabolism of 1,2-propanediol, the pdu microcompartment facilitates efficient metabolite channeling and avoids introducing toxic intermediates to the cytoplasm.

Carboxysome

Another microcompartment initially found microscopically is the carboxysome, which is a specialized microcompartment found primarily in cyanobacteria and some proteobacteria (Sutter *et al*., 2021). Carboxysomes act in the carbon concentrating mechanism (CCM) to permit efficient photosynthesis. Together with active bicarbonate (HCO_3^-) transport across the cellular membrane, the carboxysome increases efficiency of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) by concentrating CO_2 around its active site (Price & Badger, 1991).

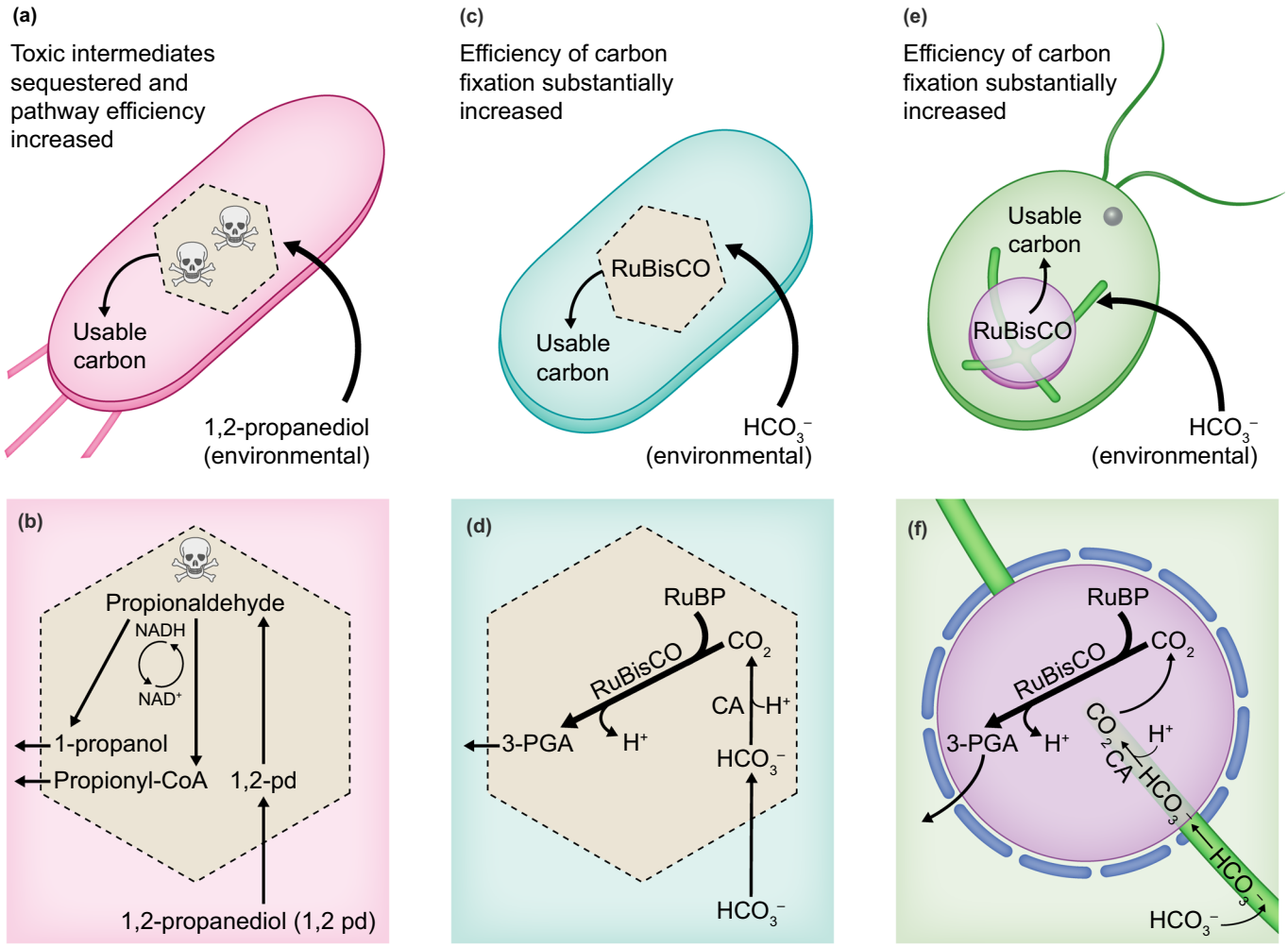

Fig. 1 Physiological and cellular view of three classically studied metabolic biocondensates. An illustration of the function of the 1,2-propanediol utilization (pdu) microcompartment (a, b), the carboxysome (c, d), and pyrenoid (e, f) at the whole-cell physiological level (a, c, e) and metabolic pathway level (b, d, f). The pdu microcompartment permits the efficient use of 1,2-propanediol without releasing the toxic intermediate, propionaldehyde, into the cytoplasm. The carboxysome permits efficient carbon fixation at low carbon dioxide (CO_2) by converting bicarbonate (HCO_3^-) into CO_2 in a biocondensate that contains ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). It has been proposed that the carboxysome shell serves as a diffusion barrier to gaseous CO_2 and O_2. By rapidly converting HCO_3^- into CO_2, cells maintain a strong gradient of HCO_3^- ions across the carboxysome shell, facilitating diffusion. The pyrenoid, in conjunction with the thylakoid tubules (shown in green), delivers CO_2 to RuBisCO using a luminal carbonic anhydrase (CA). The carbonic anhydrase is active in the lumen of the thylakoid. Additional metabolite abbreviations include oxygen (O_2), proton (H+), oxidized nicotinamide adenine dinucleotide (NAD+), reduced nicotinamide adenine dinucleotide (NADH), 3-phosphoglycerate (3-PGA), 2-phosphoglycolate (2-PG), and ribulose 1,5-bisphosphate (RuBP). Arrows in (a), (c), and (e) represent the transport of metabolites into and out of the metabolic biocondensates. Arrows in (b), (d), and (e) represent enzymatic reactions.

RuBisCO can catalyze the carboxylation or oxygenation of ribulose-1,5-bisphosphate (RuBP). The latter reaction produces 2-phosphoglycolate, which is converted back into RuBP through an energetically costly process termed photorespiration. To limit photorespiration, carboxysomes condense RuBisCO and carbonic anhydrase in a proteinaceous shell. The carboxysome increases the relative ratio of CO_2 to O_2, around Rubisco's active site, favoring carbon fixation (Price & Badger, 1991). The increased local concentration of RuBisCO and carbonic anhydrase promotes synergy between the two enzymes. Recent modeling suggests that the RuBisCO reactions, when performed in a concentrated organelle, produce protons at a sufficient rate to serve as a substrate for the conversion of HCO_3^- to CO_2 by carbonic anhydrase (Long *et al.*, 2021; Fig. 1). Production of protons by RuBisCO occurs within the carboxysome and is a distinct phenomenon from the production of protons by the photosynthetic electron transport.

Carboxysomes are considered the key innovation that allowed cyanobacteria to survive the global depletion of CO_2 over geological time (Rae *et al.*, 2013). Supporting this notion, genetic ablation of the carboxysome is lethal at ambient CO_2 (Cameron *et al.*, 2013). Carboxysomes have been maintained in all tested extant lineages of cyanobacteria and multiple lineages of proteobacteria, further highlighting the importance of this metabolic biocondensate (Axen *et al.*, 2014; Sutter *et al.*, 2021).

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Pyrenoids

The CCM in many unicellular eukaryotic algae relies on the pyrenoid – a membraneless organelle formed via liquid–liquid phase separation (Barrett *et al.*, 2021). First observed in the 19th century microscopically, the pyrenoid is located within the chloroplasts of algae and boosts CO_2 levels around RuBisCO to promote efficient carbon fixation under low CO_2 conditions (Wang *et al.*, 2015). Pyrenoids, found across eukaryotic algae and diatoms, are highly diverse in structure (reviewed in He *et al.*, 2023). Here, we focus our discussion on the best-studied pyrenoid, found in *Chlamydomonas reinhardtii*. Pyrenoids in *Chlamydomonas reinhardtii* are surrounded by starch plates that are essential for the proper localization of the CCM protein, low-CO_2-inducible protein B (LCIB; Toyokawa *et al.*, 2020). The pyrenoid is traversed by thylakoid tubules that allow production of CO_2 in the pyrenoid (Mackinder *et al.*, 2017; Burlacot *et al.*, 2022; Mukherjee *et al.*, 2019; Fig. 1). The production of protons by photosynthetic electron transport lowers the pH in the thylakoid tubules to produce CO_2 (Raven, 1997; Burlacot & Peltier, 2023).

Pyrenoids are dynamic structures, transiently adjusting their physical properties in response to carbon availability. When CO_2 is plentiful, the pyrenoid becomes smaller, maintaining a small core structure of tubules containing RuBisCO and pyrenoid structural proteins (Barrett *et al.*, 2021). Pyrenoid assembly is facilitated by the phase-separating protein ESSENTIAL PYRENOID COMPONENT 1 (EPYC1), which mediates RuBisCO condensation through physical interaction mediated by two alpha helices in the small subunit of RuBisCO (Atkinson *et al.*, 2019).

These examples of discovering functions of metabolic biocondensates started with direct observation of the biocondensate in unlabeled cells. This approach cannot identify all metabolic biocondensates as it requires the biocondensate to be a large, electron-dense cellular feature. More recently described metabolic biocondensates result from examining enzymes that are already biochemically characterized using cell biology techniques.

III. Recently described metabolic biocondensates

Some enzymes in biochemically well-studied pathways have been evaluated for their localization *in vivo* with a surprising result: several well-known enzymes assemble into biocondensates. The biocondensates discussed below contain enzymes whose functions have been known for 25–100 years, including enzymes in purine biosynthesis (purinosome), glycolysis (glycolytic bodies or g-bodies), and rhamnose biosynthesis (rhamnosome). Recent technological developments enabling direct visualization of enzyme localization through live imaging allowed researchers to classify these pathways as metabolic biocondensates (Table 1).

Purinosome

Purinosomes are biocondensates critical for purine synthesis in eukaryotes. Although purine biosynthesis has been studied since the 1950s, researchers only validated the compartmentalization of purine biosynthesis in 2008 (An *et al.*, 2008). Fluorescent tagging revealed that several purine biosynthetic enzymes formed cytoplasmic speckles, with strong interactions among the enzyme complex among bifunctional phosphoribosylaminoimidazole carboxylase and phosphoribosyl aminoimidazole succinocarboxamide synthase (PAICS), phosphoribosyl formylglycinamide synthase (FGAMS), amidophosphoribosyl transferase (PPAT), and trifunctional glycinamide ribonucleotide transformylase complex (TGART; Pedley *et al.*, 2022; An *et al.*, 2008; Fig. 2a). Mass spectrometry imaging showed metabolite channeling in purinosomes adjacent to mitochondria, providing evidence for their function as metabolic biocondensates whose activity can be regulated (Pareek *et al.*, 2020). By clustering enzymes in the 10-step pathway, purinosomes enhance substrate channeling, improving the efficiency of ATP and GTP synthesis, especially under stress or high-energy demand (Doigneaux *et al.*, 2020; Fig. 2b). Their assembly is dynamic and regulated by cellular cues, such as nutrient status and AMP-activated protein kinase (AMPK) signaling (Schmitt *et al.*, 2016). Once purine levels are restored, the purinosome disassembles. Purine biosynthesis is essential for life, and enzymes that synthesize purine are widely conserved, although purinosome formation and regulation remain unexplored in many organisms, including plants.

Glycolytic bodies

G-bodies are dynamic, membraneless structures that concentrate glycolytic enzymes to optimize ATP production, especially under stress (Jin *et al.*, 2017). Although glycolysis has been studied for over a century, g-bodies were only identified in 2017 as phase-separated assemblies proposed to improve metabolic efficiency by minimizing diffusion and promoting substrate channeling (Jin *et al.*, 2017). G-bodies form in response to conditions that require fast ATP synthesis, such as hypoxia, nutrient scarcity, or high-energy demand. G-body assembly is mediated by protein–protein and protein-RNA interactions that bring phosphofructokinase and enolase into proximity (Miura *et al.*, 2013; Jin *et al.*, 2017; Fuller *et al.*, 2020; Fig. 2c, d). This organization allows cells to quickly modulate glycolysis as conditions change. G-bodies are typically absent when energy is plentiful, with enzymes dispersed throughout the cytoplasm. However, in low-oxygen or nutrient-scarce environments, g-bodies assemble rapidly (Miura *et al.*, 2013; Jang *et al.*, 2016; Jin *et al.*, 2017; Fuller *et al.*, 2020). Additionally, the enzymes within g-bodies often form transient interactions that enable quick adjustment of glycolytic activity according to energy needs (Jang *et al.*, 2016). G-bodies may even alter their physical states, becoming more gel-like or fluid-like depending on cellular conditions to tune pathway activity (Fuller *et al.*, 2020). As phosphofructokinase and enolase are broadly conserved enzymes, g-body-like structures might be conserved in diverse eukaryotes, but this has not been demonstrated to-date. Whether the g-body can functionally regulate metabolism in plants, for example, remains undemonstrated, and further study of g-bodies holds promise to clarify how plants regulate energy production.

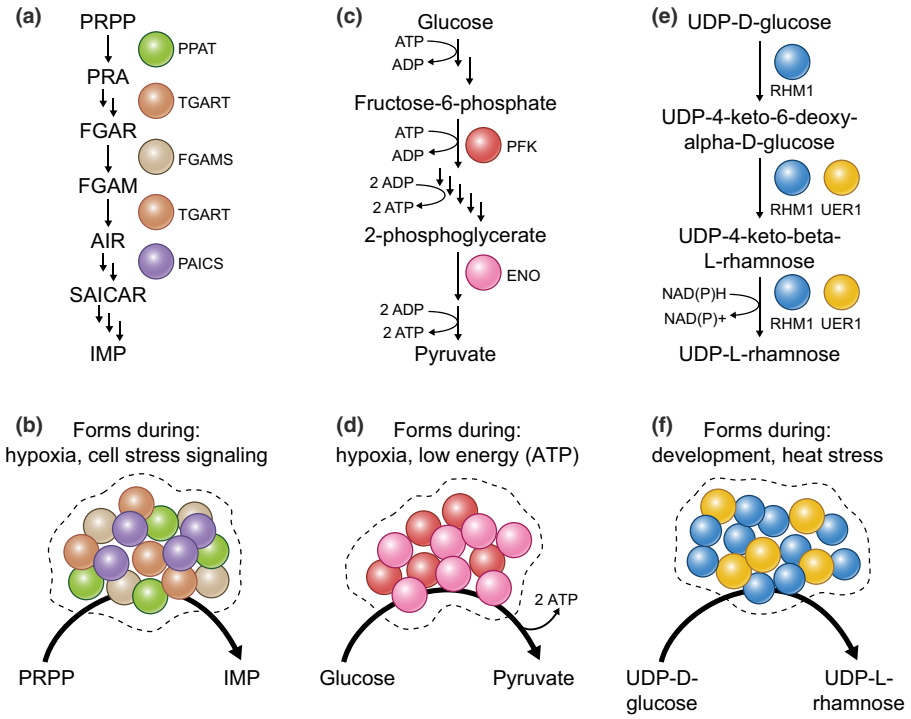

Fig. 2 Function and regulation of recently found metabolic biocondensates. Illustrations representing the purinosome from mammalian systems (a, b), g-bodies from yeast (*Saccharomyces cerevisiae*) and *Caenorhabditis elegans* (c, d), and rhamnosome from *Arabidopsis thaliana* (e, f) at the pathway (a, c, e) and cellular (b, d, f) levels. Pathway diagrams are simplified to only contain names for the starting and ending metabolites, as well as intermediates that are directly consumed or produced by enzymes reviewed here. Single reactions that produce intermediates are represented by a straight arrow. Abbreviations of metabolite names and enzymes: phosphoribosylpyrophosphate (PRPP), phosphoribosylpyrophosphate amidotransferase (PPAT), phosphoribosylamine (PRA), N-formylglycinamide ribonucleotide (FGAR and FGAM), aminoimidazole ribonucleotide (AIR), N-succinocarboxyamide-5-aminoimidazole ribonucleotide (SAICAR), inosine monophosphate (IMP), phosphofructokinase (PFK), enolase (ENO), RHAMNOSE BIOSYNTHESIS 1 (RHM1), uridine diphosphate-4-keto-6-deoxy-D-glucose-3,5-epimerase-4-reductase 1 (UER1), adenosine diphosphate (ADP), adenosine triphosphate (ATP), oxidized nicotinamide adenine dinucleotide phosphate (NADP+), and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

Rhamnosome

Recent research examined the role of *Arabidopsis thaliana* RHAMNOSE BIOSYNTHESIS 1 (RHM1) condensation in uridine diphosphate (UDP)-rhamnose synthesis (Field *et al.*, 2024). UDP rhamnose is a building block of the cell wall and is involved in rhamnosylation of proteins and specialized metabolites (Mistou *et al.*, 2016; Jiang *et al.*, 2021). RHM1 enzymatic function and involvement in organismal development have been studied in plants for decades. RHM1 condensation was first described in 2009 when RHM1-GFP was expressed in *Arabidopsis thaliana* and localized to small bodies in response to stress (Wang *et al.*, 2009). RHM1 bodies were further identified in developing petals (Saffer *et al.*, 2017) and were suggested to be localized to stress granules (Kosmacz *et al.*, 2019). Recent work identified RHM1 bodies forming in leaves and the developing seed coat cells, which are strongly associated with an increased demand for UDP rhamnose (Field *et al.*, 2024). Mutants in RHM1 that prevent body formation also prevent UDP-rhamnose synthesis. Another component of the UDP-rhamnose synthesis pathway, UER1, requires RHM1 body formation to function in UDP-rhamnose synthesis, further suggesting that rhamnosome formation is strongly tied to UDP-rhamnose synthesis (Field *et al.*, 2024).

IV. Emerging technologies for discovering metabolic biocondensates

To efficiently discover metabolic biocondensates, a cross-disciplinary approach incorporating diverse technologies is needed (Table 2). Microscopic techniques, such as bimolecular fluorescence complementation (BiFC) and Förster (fluorescence) resonance energy transfer (FRET), are useful for demonstrating protein–protein interactions (Sekar & Periasamy, 2003; Kerppola, 2008). Cryo-electron tomography (CryoET) imaging is a promising approach for determining the physical properties of biocondensates in cells (Doerr, 2017). For higher throughput protein–protein interaction-based discovery, biochemical techniques, such as co-fractionation mass spectrometry, cross-linking mass spectrometry, and proximity labeling, have promise to demonstrate protein–protein interactions across diverse plant species (Zhu *et al.*, 2016; Mair *et al.*, 2019; McWhite *et al.*, 2020; Liu *et al.*, 2023, 2024; Luzarowski *et al.*, 2023). Finally, fluorescently tagging enzymes at the genome level to

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Table 2 Promising new technologies for biocondensate discovery in plants.

Technology	Review of technology	Plant reference	Throughput	Key features
CryoET	Doerr (2017); Hong *et al*. (2023)	Otegui & Pennington (2019)	Low – Limited by sample preparation	Allows for high resolution imaging of unlabeled biocondensates
Proximity labeling	Qin *et al*. (2021)	Mair *et al*. (2019)	Low – Limited by requirement for compartment-specific negative controls	Can detect weak or transient interactions
Co-fractionation mass spectrometry	Luzarowski *et al*. (2023)	McWhite *et al*. (2020)	High	Labor-intensive
Cross-linking mass spectrometry	O'Reilly & Rappsilber (2018)	Zhu *et al*. (2016)	Medium – Limited by sample processing	Can be performed on untagged plants
BiFC	Kerppola (2008)	Schütze *et al*. (2009)	Low – Limited by time-intensive imaging	Can only validate pairwise interactions
FRET	Sekar & Periasamy (2003)	Bücherl *et al*. (2014)	Low – Limited by time-intensive imaging	Can only validate pairwise interactions
Yeast two-hybrid	Brückner *et al*. (2009)	Ferro & Trabalzini (2013)	Medium/High – Limited by requirement to test bait and target proteins in pairs	Can only validate pairwise interactions
Genome-scale enzyme tagging	Plant Cell Atlas Consortium *et al*. (2021)	Wang *et al*. (2023)	Medium/High – Limited by transformability of the plants or their cells	Labor-intensive

BiFC, Bimolecular fluorescence complementation; FRET, Förster (fluorescence) resonance energy transfer.

produce enzyme localization atlases can accelerate the discovery of metabolic biocondensates (Wang *et al*., 2023).

V. Considerations for engineering metabolic biocondensates

Recent efforts to engineer synthetic metabolic biocondensates in plants have been promising. A recent study improved pathway efficiency *in planta* through the formation of synthetic biocondensates (Lindstrom Battle *et al*., 2025). While localizing enzymes into metabolic biocondensates can improve pathway efficiency, it is not sufficient to optimize metabolic rates. Models exploring the relationship between biocondensate structure and efficiency suggest that an optimal ratio between enzymes in a compartmentalized pathway is required to maximize metabolic rates (Hinzpeter *et al*., 2017). Biocondensate size is another important determinant of efficiency, with a trade-off existing between surface area and internal enzyme density (Hinzpeter *et al*., 2017). Finally, metabolic biocondensate efficiency can be limited when the substrate turnover rate exceeds the rate of substrate diffusion into the biocondensate (Hinzpeter *et al*., 2017). Efforts to engineer synthetic biocondensates should account for these limitations to meet the theoretical limit of pathway improvement via synthetic biocondensates.

VI. Conclusion

Metabolic biocondensates play a direct role in regulating organismal physiology by enhancing metabolism in response to environmental and developmental cues. Applying cell biology approaches to studying metabolic enzymes and pathways is a promising avenue for discovering metabolic biocondensates and their roles in regulating metabolism (Plant Cell Atlas Consortium *et al*., 2021). Plants remain a promising system for studying metabolic biocondensates with clear physiological impacts.

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Competing interests

None declared.

Author contributions

EVS and SF contributed to the conceptualization, investigation, writing – original draft, writing – review and editing. EVS also

contributed to visualization. SYR contributed to the conceptualization, funding acquisition, project administration, supervision, and writing – review and editing.

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