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Cell-Free and Cell-Based Biosynthesis of Complex Natural Products: An Evidence-Mapping Review of Pathway Portability, Enzyme Constraints, Cofactor Demand, and Scale Readiness

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ABSTRACT

Complex natural-product pathways are increasingly reconstructed outside their native organisms, yet successful biosynthesis is often interpreted too broadly as evidence of pathway portability or manufacturing readiness. Cell-free and cell-based systems solve different parts of this problem and expose different constraints. We conducted a qualitative evidence-mapping review of peer-reviewed Q1-journal literature published from 2017 through 2026. Searches covered cell-free biosynthesis, heterologous cellular production, pathway reconstruction, enzyme compatibility, energy/cofactor and precursor demand, compartmentation, burden, process control, and readiness. Fifty-five DOI-normalized candidates were screened; 49 full texts were assessed and 39 studies were mapped. Evidence was coded by platform, pathway class, enzyme constraint, resource demand, context, process evidence, portability level, and transferability boundary. Cell-free evidence shows high experimental access to enzyme behavior, energy balance, pathway modularity, extract provenance, maturation requirements, and temporal control. Cell-based evidence demonstrates reconstruction of increasingly complex plant and microbial pathways while revealing constraints from precursor supply, heterologous enzyme function, toxicity, spatial organization, transport, and host fitness. Across both platforms, pathway completion is not equivalent to process robustness or scale readiness. The evidence instead supports a proposed staged interpretation in which pathway knowledge, biochemical function, pathway-level production, and process evidence are assessed separately. Cell-free and cell-based biosynthesis are best interpreted as conditionally complementary rather than universally substitutable production strategies. Comparative inference remains limited by heterogeneous products, reaction formats, hosts, and scarce shared readiness benchmarks.

Keywords: Cell-free biosynthesis, Heterologous biosynthesis, Natural products, Pathway portability, Metabolic engineering, Biomanufacturing

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Introduction

Natural-product engineering has moved from isolated gene transfer toward iterative discover–design–build–test–learn cycles in which pathway discovery, enzyme selection, construction, measurement, and redesign are repeatedly coupled [1]. That shift makes “portability” a multistage engineering problem rather than a binary property of a biosynthetic gene set. Here, portability is used narrowly to mean retained, interpretable function after a pathway, enzyme module, or production architecture is moved into a new biochemical context; it is not synonymous with DNA transfer, detectable product formation, or scalable manufacture. This distinction matters

especially for complex natural products, whose biosynthesis can couple long reaction sequences to redox chemistry, precursor routing, enzyme maturation, and context-dependent organization. Cell-free natural-product systems sharpen this issue by providing an open, modular reaction environment in which pathway components and resources can be manipulated directly, while enzyme expression, resource supply, and pathway-specific compatibility remain recurrent constraints [2].

Living production systems impose a different constraint layer. In addition to catalytic competence, engineered microorganisms must allocate transcriptional, translational, metabolic, and stress-response resources while maintaining growth or production robustness; metabolic burden therefore remains host- and pathway-dependent rather than a universal explanation for low output [3]. Conversely, removing the cell does not remove resource demands. A reconstituted synthetic-biochemistry pathway has converted glucose to monoterpenes, demonstrating that multienzyme natural-product synthesis can be organized outside living cells when energy, cofactors, and enzyme activities are engineered explicitly [4].

Cell-based reconstruction can reach comparable biochemical complexity through different means. Complete cannabinoid biosynthesis in yeast required coordinated precursor provision and heterologous pathway engineering, establishing feasibility without implying facile transfer of other plant pathways [5]. More recently, complete QS-21 biosynthesis in engineered yeast extended the demonstrated complexity frontier but likewise did not establish process or manufacturing readiness [6]. This review therefore proposes three deliberately separate interpretive endpoints: platform feasibility, pathway portability, and scale readiness. Evidence for one may inform another, but none is treated as a surrogate for the others. An evidence map is therefore more appropriate than a simple platform ranking: it can show where evidence accumulates, where constraints change form between environments, and where apparent technological progress still rests on pathway-specific demonstrations.

Review scope and evidence-mapping questions

The review maps experimental and substantive review evidence for cell-free and cell-based biosynthesis of complex natural products, with emphasis on where pathway transfer succeeds, where it becomes conditional, and what kinds of evidence remain absent. Cell-free evidence includes extract-based expression, reconstituted synthetic biochemistry, purified multienzyme cascades, and related open systems used to produce or interrogate specialized metabolites. These systems are mapped for enzyme function, pathway assembly, ATP/cofactor or substrate requirements, extract provenance, maturation, and process-control constraints rather than ranked by yield [2].

Cell-based evidence includes engineered microbial hosts and distributed cellular systems reconstructing non-native natural-product pathways. Host burden is coded as a cellular constraint because living hosts couple pathway operation to resource competition, growth, stress responses, and robustness; it is not assumed to explain every cell-based failure, nor is it assumed absent from extract-derived systems [3]. Across both platform families, the map asks five linked questions: What level of pathway knowledge is available? Which enzymes remain context-sensitive? How are cofactors, precursors, and energy supplied? Which spatial, host, extract, or temporal variables condition production? What evidence exists beyond pathway completion for reproducibility, process robustness, or scale? Because reaction formats, products, hosts, extracts, and measurement objectives differ substantially, the map emphasizes evidence type and boundary conditions rather than treating reported titers or yields as directly comparable platform scores.

The organizing synthesis is proposed rather than a validated readiness framework. It treats genetic or pathway access, biochemical function, pathway-level production, and process/scale evidence as separable states, then asks where cell-free testing, cellular reconstruction, or hybrid handoffs can reduce uncertainty. **Figure 1** summarizes this mapping logic while preserving the boundary between evidence-supported platform characteristics and proposed cross-platform interpretation.

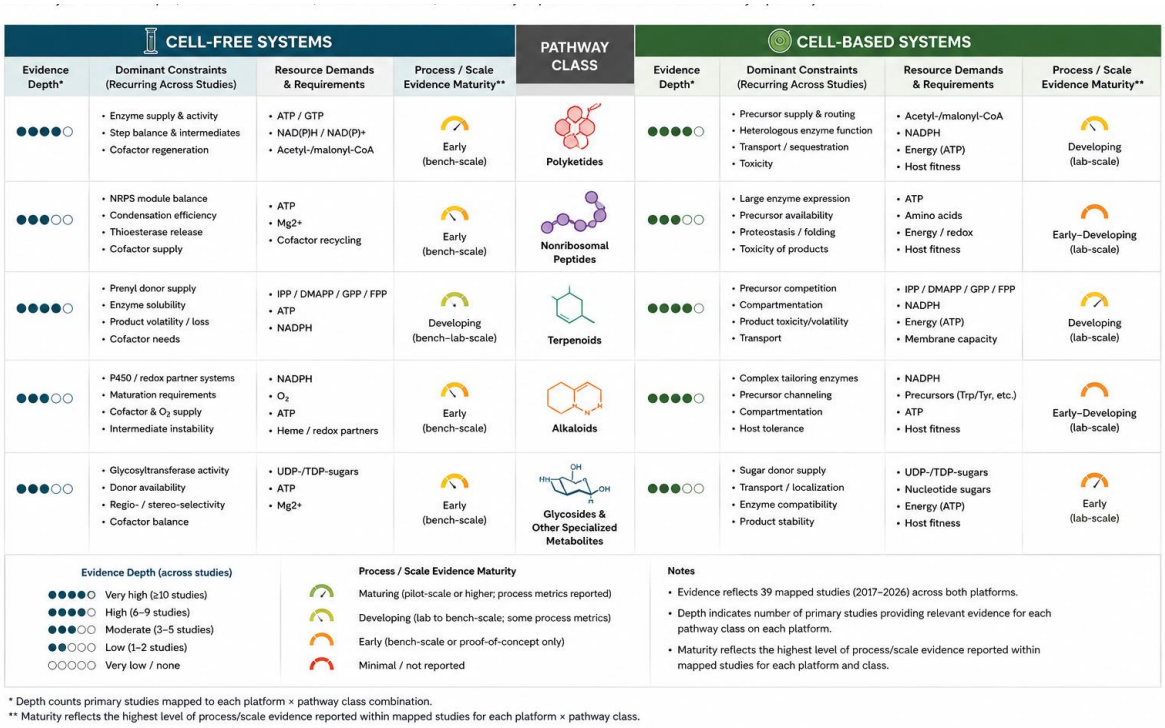

Figure 1. From platform-specific biosynthesis evidence to a staged interpretation of pathway portability and readiness

Materials and Methods

We searched on 16 August 2026 using PubMed/MEDLINE record pages, publisher-site searches, DOI/Crossref metadata confirmation, and backward citation chaining. Search families combined terms for cell-free or in-vitro biosynthesis, engineered yeast or microbial cell factories, natural products and major compound classes, heterologous expression, pathway reconstitution, cofactors, ATP, precursor supply, compartmentation, transport, toxicity, burden, robustness, process control, and scale-up. Eligible records were peer-reviewed journal articles from 2017–2026 with a verified DOI, Q1 status in a relevant category where verifiable, and an exact claim-level role in the evidence map. Screening proceeded from scope relevance at title/abstract level to full-text assessment of whether each article could support a defined mapping claim without extrapolation beyond its system.

DOIs were normalized before registration. The candidate register contained 55 unique records: 24 assigned to PubMed/MEDLINE, 25 to publisher-site discovery, and six to backward citation chaining. All 55 were screened; six were excluded at title/abstract stage as adjacent or insufficiently relevant. Forty-nine full texts were sought and available, and 49 were assessed. Ten were excluded after full-text review because they were review-redundant or superseded, superseded by newer direct evidence, or chemically redundant for the planned claim. Thirty-nine studies entered the final map. Identification, screening, eligibility, and inclusion were reported as an auditable flow, using PRISMA 2020 only as a transparency anchor rather than as an assertion of meta-analytic design [7].

Included studies were coded for platform mode, product/pathway class, pathway completeness, enzyme/function constraint, cofactor/energy or precursor demand, host or extract context, compartmentation, transport, toxicity, burden, process control, validation level, portability level, readiness evidence, and transferability boundary. Cross-study titers, yields, and fold changes were not pooled because products, platforms, operating conditions, and experimental objectives were heterogeneous. **Figure 2** reports the verified selection ledger.

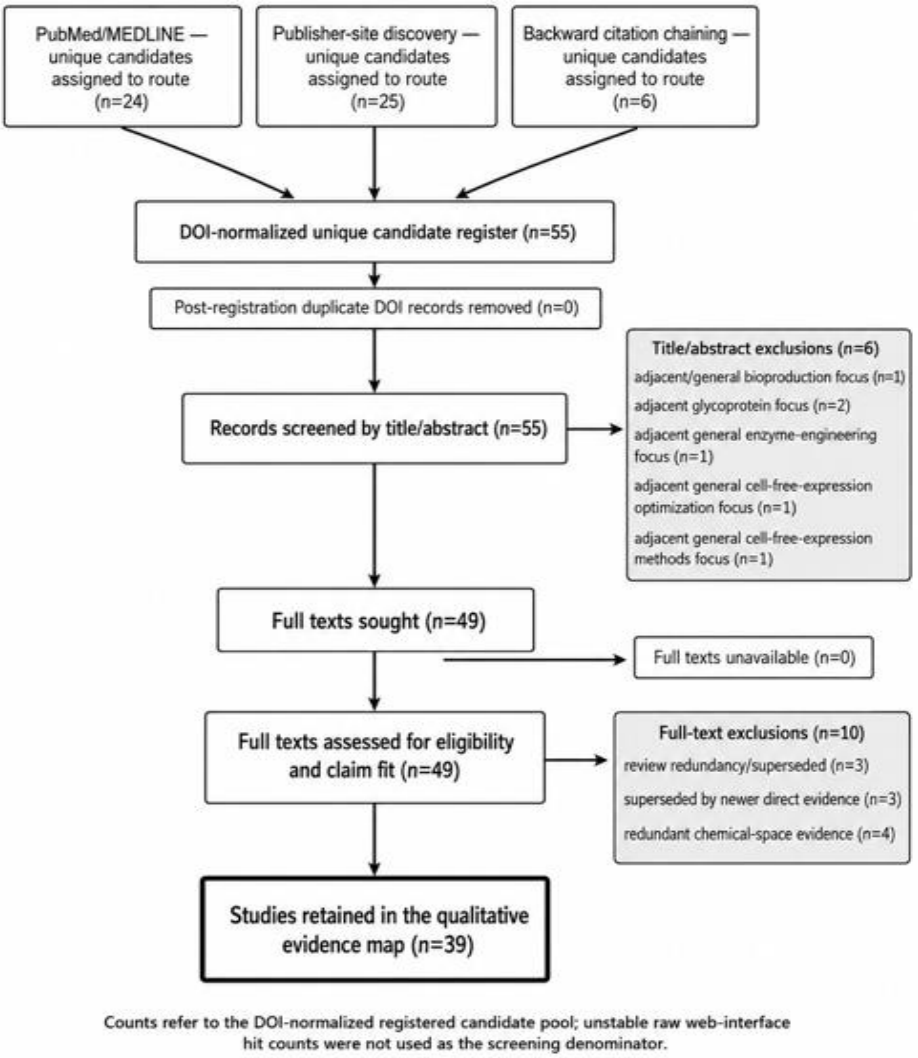

Figure 2. Verified evidence-selection flow for the cell-free and cell-based natural-product biosynthesis map

Landscape of cell-free biosynthetic evidence

The mapped cell-free literature shows that openness converts several hidden cellular dependencies into explicit engineering variables. ATP availability can become a system-level control parameter requiring active balancing in synthetic biochemistry [8]. Cell-free prenylation demonstrates how a difficult natural-product transformation can be isolated and optimized without simultaneously rebuilding an entire production host [9]. In-vitro prototyping can compare biosynthetic enzymes and pathway configurations before cellular construction, although such rankings are not guaranteed to survive the added constraints of a living cell [10]. Hybrid designs further blur a strict platform divide: metabolic rewiring of a source organism can improve the composition and performance of downstream extracts [11], while a *Streptomyces venezuelae* toolkit shows that extract provenance itself can condition compatibility with specialized-metabolism machinery [12].

Recent studies broaden the accessible chemistry. Cell-free work now encompasses lanthipeptide maturation and engineering, computationally guided peptide-natural-product exploration, and reconstitution of multi-enzyme precursor pathways for secondary metabolites [13–15]. Purified one-pot cascades for phenylethanoid glycosides additionally show that enzyme compatibility must be evaluated at the reaction-network level rather than assumed from single-enzyme activity [16]. High-throughput cell-free expression can isolate post-translational-modification requirements relevant to enzyme maturation [17], and a 2026 secondary-metabolite workflow indicates increasing practical breadth without establishing universal pathway coverage [18].

Process control emerges as a separate portability layer. Timed reagent inputs can materially change enzymatic-cascade performance, suggesting that static reaction composition may conceal temporal resource constraints; transfer of that result to natural-product manufacturing requires direct validation [19]. The proposed synthesis is therefore not that cell-free systems remove complexity, but that they relocate complexity toward explicit

orchestration of energy, cofactors, enzyme stability, reaction compatibility, extract composition, and time. This relocation is analytically valuable because failure variables can be perturbed directly, but it also means that favorable small-volume performance cannot be treated as self-evident evidence of robust operation at larger scale. **Table 1** organizes the evidence characteristics that support this distinction.

Table 1. Mapped characteristics of the cell-free evidence landscape and the constraints each mode exposes

Cell-free evidence mode	Primary analytical value	Constraint made visible	Interpretation boundary
Reconstituted synthetic biochemistry	Multienzyme pathway operation	ATP and cofactor balance	Configuration-specific; not scale evidence
Transformation modules	Isolate difficult catalytic steps	Enzyme–substrate and reaction compatibility	Class- and substrate-bounded
Extract-based prototyping	Rapid enzyme/configuration comparison	Expression and extract context	In-vitro ranking need not transfer in vivo
Host-conditioned or tailored extracts	Couple chassis rewiring to open reactions	Extract provenance and inherited metabolism	Pathway- and preparation-specific
Peptide/secondary-metabolite reconstitution	Test maturation and pathway coupling	PTM completeness and multi-enzyme compatibility	Breadth does not establish universality
One-pot and time-managed cascades	Direct sequence and input control	Condition compatibility and temporal demand	Scale transfer requires validation

Landscape of cell-based biosynthetic evidence

Cell-based studies demonstrate how living hosts can supply regeneration, membrane architecture, organelles, and sustained biosynthesis while simultaneously imposing context. Complete noscapine biosynthesis in yeast required extensive pathway assembly, showing that long heterologous routes can function without making pathway length a readiness metric [20]. Medicinal tropane-alkaloid biosynthesis further illustrates that plant enzymes may require host-compatible expression and organization in a non-native cellular environment [21]. When a single host becomes impractical, pathway labor can be partitioned: a microbial supply chain enabled vinblastine production, but distributed production adds coordination and metabolite-transfer requirements [22]. Once established, some pathways can also be extended into analogue space, as shown for natural and halogenated monoterpene indole alkaloids in yeast [23].

Recent reconstructions expand this chemical breadth while exposing distinct bottlenecks rather than one generic "cellular" limitation. De novo yeast production now spans ajmaline, protoberberine and benzophenanthridine alkaloids, and sanguinarine, with pathway-specific solutions involving heterologous enzyme optimization, precursor control, and, for sanguinarine, dynamic regulation to separate growth from biosynthetic demand [24–26]. Independent *Taxus* reconstructions likewise show that baccatin III production depended on resolving missing or uncertain pathway functions before host optimization could even be interpreted meaningfully [27, 28]. Pathway elucidation and heterologous construction were similarly coupled for echinacoside-related phenylethanoid glycosides [29].

Cell-based portability can therefore be engineered at multiple organizational levels. Yeast block-building can test artificial pathway combinations rather than only transplanting native sequences [30]. Peroxisome engineering shows that organelle architecture and precursor localization can alter production for selected acetyl-CoA-derived metabolites [31]. Engineered *Escherichia coli* consortia demonstrate another architecture in which pathway load is distributed across strains, trading some single-host constraints for inter-strain coordination [32]. Collectively, these studies support a bounded conclusion: living systems can host exceptionally complex chemistry, but successful reconstruction remains contingent on pathway knowledge, enzyme biogenesis, precursor supply, spatial organization, toxicity management, and host-specific physiology; none of those demonstrations alone establishes process or scale readiness.

Comparative constraints across production systems

Comparing cell-free with cell-based biosynthesis is most informative when constraints are matched by function rather than by platform label. Resource supply illustrates this point. In a living host, precursor availability is embedded in central metabolism, growth, and competing sinks; Li and colleagues showed that redesigning malonyl-CoA formation through a non-carboxylative route can alter this resource architecture for malonyl-CoA-

derived products [33]. In an open reaction, by contrast, precursor and cofactor provision can be adjusted directly but must still be supplied, regenerated, or scheduled. Thus, the shared problem is resource sufficiency, whereas the failure mode differs: metabolic competition and stoichiometric coupling in cells versus explicit depletion or imbalance outside them.

Enzyme function is similarly multilevel. Catalytic competence cannot be inferred from an annotated sequence if the enzyme is not produced in a functional form. Rescue of truncated transcripts into functional polyketide-synthase subunits improved polyketide biosynthesis in a tested cellular system, demonstrating that transcript integrity and translation can constrain large biosynthetic enzymes independently of their catalytic chemistry [34]. Cell-free systems can expose expression and maturation failures directly, but they do not automatically reproduce membrane insertion, organelle localization, partner availability, or the intracellular physicochemical context on which some enzymes depend.

Chassis–pathway fit therefore remains an empirical property. Strategic engineering improved oviedomycin production in *Escherichia coli* by coordinating pathway and host-level interventions [35]. A subsequently developed chassis supported multiple type II polyketide pathways, showing that reusable host engineering can broaden portability within a related biosynthetic class [36]. These studies justify neither a universal chassis nor a universal cell-free formulation. They instead support a proposed comparative principle: common biosynthetic demands—resources, enzyme biogenesis, spatial organization, and temporal control—should be mapped separately from the platform-specific mechanisms through which those demands fail.

A production increase after precursor engineering, localization, extract modification, or timed feeding does not establish a sole bottleneck because expression, pathway balance, toxicity, and process variables can interact. The map therefore treats such constraints as demonstrated leverage points within specified systems, not universal determinants.

Pathway portability and technology readiness

Pathway portability should not be collapsed into the ability to move DNA. Multiplexed mobilization and heterologous expression of biosynthetic gene clusters demonstrates that genetic material can be transferred efficiently across discovery workflows, but successful mobilization does not guarantee that all encoded enzymes function, that the intended product is formed, or that the resulting process is robust [37]. Autologous DNA mobilization and multiplication likewise accelerates access to bacterial natural-product pathways for discovery, yet its direct evidence concerns pathway access and expression rather than manufacturing performance [38]. Genetic portability is therefore an upstream enabling state, not a proxy for downstream biochemical or process readiness.

On the basis of the mapped literature, we propose four evidence states that should remain explicitly separate. Pathway-access evidence establishes that the relevant genetic or biochemical components can be obtained and assembled. Functional-portability evidence establishes that required enzymes or modules operate in the recipient biochemical context. Pathway-production evidence establishes formation of the intended product or defined intermediate through the reconstructed route. Process-readiness evidence requires evidence that operation remains reproducible and controllable under conditions relevant to process development, including resource management, stability, and scale-dependent behavior. These states are not a validated maturity scale, and progression through them need not be linear.

This framework clarifies why chemically impressive pathway reconstructions can coexist with unresolved readiness. A long pathway that produces a target molecule may still contain fragile enzymes, expensive or poorly regenerated cofactors, toxic intermediates, transport dependencies, or host-specific spatial requirements. Conversely, an open system may provide exceptional mechanistic control while lacking evidence that enzyme lifetime, reagent economics, volumetric productivity, or mixing and feeding strategies remain favorable as reaction volume increases. Readiness therefore depends less on whether a pathway is “cell-free” or “cell-based” than on whether the relevant failure modes have been tested at the evidence state being claimed.

Technology readiness should therefore be described claim by claim. Genetic access supports discovery; product formation supports biochemical feasibility; repeated controlled production can support process robustness; scale readiness requires additional evidence that cannot be inferred from earlier states. This separation prevents proof-of-concept completion from being upgraded into platform maturity.

Areas of complementarity between platforms

The strongest relationship between the two platform families is conditional handoff rather than substitution. Cell-free prototyping can compare enzyme variants and pathway configurations before cellular construction, thereby reducing uncertainty about catalytic choices while leaving intact-cell transfer to be tested explicitly [10]. This role is especially useful when poor cellular performance could arise from either enzyme chemistry or host context: an open reaction can isolate the former, whereas subsequent cellular implementation tests the latter.

Complementarity can also be engineered in the opposite direction. Metabolically rewired yeast can generate extracts whose inherited biochemical state improves downstream cell-free biosynthesis, demonstrating that cellular design and open-reaction design can form a deliberately hybrid workflow [11]. Information can therefore flow in both directions: cells can supply optimized machinery to extracts, while cell-free experiments can identify configurations worth retesting in cells. Such transfer remains conditional because extracts and intracellular physiology differ.

Some pathway functions may remain better served by intact or distributed cellular organization. The microbial supply-chain strategy used for vinblastine shows that a complex route can be partitioned across engineered cellular modules when a single-host implementation is impractical [22]. Organelle engineering provides another spatial solution: modifying yeast peroxisome assembly altered precursor availability and production of selected acetyl-CoA-derived 5-deoxyflavonoids [31]. Open systems can replace these functions only through different physical arrangements, staged reactions, membrane-containing preparations, or externally imposed transport and feeding logic.

The proposed synthesis is therefore a task-allocation model. Cell-free systems are particularly informative for rapid enzyme interrogation, explicit resource manipulation, pathway debugging, and temporal control; intact hosts provide sustained metabolism, membrane and organelle context, growth-linked regeneration, and architectures for intracellular or distributed pathway organization. Hybrid workflows become attractive when uncertainty can be isolated in one environment and the resulting design transferred conditionally to the other. **Table 2** summarizes these complementary operating roles without ranking one platform as universally superior.

Table 2. Conditional operating roles of biosynthesis platforms across portability-relevant tasks

Task or constraint	Cell-free strategy	Cell-based strategy	Proposed interpretation boundary
Enzyme selection	Direct expression or purified-module testing	Test function within host physiology	In-vitro ranking requires cellular confirmation
Resource supply	Explicit precursor/cofactor addition or regeneration	Metabolic-network engineering	Different mechanisms can produce the same resource deficit
Spatial organization	Reaction partitioning or staged modules	Membranes, organelles, transport, consortia	Architecture should match pathway dependency
Temporal control	Programmed additions and reaction staging	Dynamic regulation and physiological control	Timing effects are system-specific
Pathway debugging	Isolate modules and interactions	Reveal toxicity, burden, transport and fitness effects	Failure localization may require both environments
Handoff	Prototype before host construction	Engineer source host or extract provenance	Transfer is conditional, not automatically predictive

Note: The comparison is a proposed synthesis of the mapped evidence. It does not constitute a quantitative performance ranking or readiness score.

Evidence gaps and underdeveloped applications

The map reveals a mismatch between increasingly sophisticated pathway reconstructions and comparatively sparse evidence on process behavior. Temporal scheduling is one example. Timed reagent inputs can improve the performance of enzymatic cascades, but comparable investigation remains underdeveloped for complex natural-product cell-free systems at process-relevant scales [19]. Static endpoint experiments may therefore miss time-dependent depletion, inhibition, or intermediate accumulation, while direct transfer of timed-feeding results from other cascades remains unproven.

A second gap occurs before production begins: pathway definition itself can remain uncertain. Resolution of additional taxane-pathway functions was required before baccatin III reconstruction could be interpreted as a complete biosynthetic achievement [28]. Similar uncertainty is likely to be important wherever annotations, accessory proteins, enzyme order, or transport steps remain incomplete, but the present map cannot quantify its prevalence beyond the represented pathways.

Third, pathway modularization is advancing faster than shared benchmarking. Yeast block-building can identify workable artificial routes for products such as glabridin [30], yet the literature offers little basis for comparing portability across unrelated product classes using common definitions of pathway completeness, resource burden, reproducibility, or process robustness. Negative results and failed transfers are also less visible than successful reconstructions, limiting inference about the denominator of pathway portability.

Finally, large multidomain enzymes remain a hard case. Translation-related rescue of polyketide synthase function shows that enzyme size and biogenesis can create failures distinct from catalytic incompatibility [34]. Comparable diagnostics across cell-free and cell-based environments would help distinguish whether a pathway fails because an enzyme is not made correctly, is catalytically unsuitable, lacks partners or cofactors, or is incompatible with the surrounding process.

Review limitations

This review is an evidence map rather than an effect-size meta-analysis. The selection process was reported transparently using a structured flow, but the reporting framework does not convert heterogeneous biosynthetic studies into directly comparable experiments [7]. Products range from peptides and terpenoids to alkaloids, polyketides, cannabinoids, taxanes, and glycosides; experimental units range from purified enzymes and extracts to single hosts, organelles, consortia, and distributed supply chains. Consequently, the density of publications within a category should not be interpreted as evidence of comparative platform superiority.

Cell-free natural-product literature itself spans substantially different implementations, including crude extracts, reconstituted enzyme systems, pathway-prototyping formats, and discovery-oriented expression platforms [2]. Differences in extract composition, enzyme preparation, reaction scale, substrate loading, cofactor regeneration, analytical endpoints, and optimization objectives restrict quantitative comparison. The same limitation applies to cell-based studies, where host genotype, cultivation regime, pathway copy number, regulation, precursor engineering, and product toxicity vary.

The map also inherits publication and visibility biases: successful reconstructions are more visible than failed transfers, nonproductive combinations, instability, or scale-dependent losses. Without denominators for attempted pathways, the review cannot estimate a probability of successful transfer to either platform.

Finally, “scale readiness” is necessarily evidence-bounded. Laboratory production, pathway completion, or improved titer can inform process development but does not by itself establish reproducibility, economics, environmental advantage, regulatory suitability, or manufacturing implementation. The proposed evidence-state framework is therefore an organizing interpretation intended to sharpen claims and identify experiments; it is not a validated technology-readiness scale.

Development priorities

A reusable natural-product manufacturing platform will require coordinated pathway discovery, enzyme adaptation, host engineering, standardization, and process development rather than repeated accumulation of isolated pathway demonstrations; a yeast-focused roadmap has articulated this broader platform-building requirement [39]. The evidence map extends that argument across cell-free and cell-based systems by proposing that future work should test transitions between evidence states instead of assuming them.

First, cell-free workflows should be used prospectively to discriminate enzyme, pathway-order, and resource hypotheses before costly host reconstruction, while recognizing that such experiments are not guaranteed predictors of cellular behavior. Recent versatile cell-free production of secondary metabolites makes this a technically credible direction for selected pathways [18]. The useful benchmark is not whether an enzyme can be expressed, but whether the experiment resolves a specific uncertainty that would otherwise remain confounded inside a host.

Second, spatial engineering should become an explicit design variable when precursor routing, membrane dependence, or compartmental isolation is mechanistically relevant. Peroxisome engineering in yeast provides direct evidence that intracellular architecture can alter precursor availability and product formation in a defined context [31]. Parallel cell-free strategies should test whether comparable functions require membranes, physical partitioning, sequential addition, or simply adjusted concentrations rather than assuming spatial equivalence.

Third, resource accounting should be made explicit. Redesign of malonyl-CoA generation demonstrates that precursor architecture can itself be engineered [33]. Future comparative studies should therefore record major

precursor, redox, ATP, and regeneration assumptions sufficiently to distinguish pathway chemistry from resource limitation, while avoiding a universal bookkeeping scheme that ignores pathway-specific demands.

Fourth, genetic mobilization should remain upstream of functional and process validation. Multiplexed biosynthetic-gene-cluster transfer provides powerful pathway access [37], but transfer should be followed by staged tests of enzyme function, product identity, reproducibility, resource demand, and robustness. Paired-platform studies should use defined modules, comparable endpoints where feasible, report failures, and deliberately perturb suspected bottlenecks. Such experiments could test—and potentially reject—the proposed portability framework.

Conclusion

Cell-free and cell-based biosynthesis have expanded the experimentally accessible space of complex natural products, but they do so through different biochemical and engineering architectures. Cell-free systems expose enzyme behavior, resource requirements, pathway interactions, and temporal control with unusual experimental accessibility. Living systems provide sustained metabolic regeneration, membrane and organelle context, transport, and the capacity to distribute pathway labor, while introducing metabolic burden, toxicity, competition, and host-specific regulation.

The principal contribution of this evidence map is a bounded interpretation of portability. Genetic access, functional enzyme operation, reconstructed pathway production, process robustness, and scale readiness should not be treated as interchangeable evidence. Successful production establishes what was demonstrated in the tested system; it does not automatically establish transferability, comparative superiority, or manufacturing readiness.

The mapped literature also suggests a productive complementarity: open systems can isolate uncertainties that are difficult to diagnose in cells, whereas intact hosts can test dependencies that open reactions cannot faithfully reproduce. Hybrid workflows are therefore plausible but remain conditional. Progress toward reusable biomanufacturing platforms will require more paired-platform experiments, explicit resource accounting, failure reporting, pathway-completeness checks, and staged process validation. The proposed evidence-state framework is intended to organize those tests, not to predetermine their outcomes.

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