Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Compartmentalized biosynthesis of fungal natural products

Lei Du[1] and Shengying Li[1,2]

Fungal natural products (NPs) with diverse chemical structures and biological activities are rich resources of both drugs and toxins, thus causing Janus-like effects on human beings. Significant progress has been made in discovery and mining of novel fungal NPs in the past decades. Unlike prokaryotic organisms, eukaryotic cells of fungi have discrete organelles to form compartmentalized assembly lines for the highly ordered and hence efficient biosynthesis of fungal NPs. In this review, we summarize a limited but growing number of studies on compartmentalized biosynthesis of fungal NPs. The emerging strategies and efforts for engineering of subcellular localization of relevant biosynthetic enzymes are also discussed. We expect to provide some new insights and perspectives on the more complex NP biogenesis in higher microorganisms.

Addresses
[1] State Key Laboratory of Microbial Technology, Shandong University, Qingdao, Shandong 266237, China

[2] Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao, Shandong 266237, China

Corresponding author: Li, Shengying (lishengying@sdu.edu.cn)

Current Opinion in Biotechnology 2021, **69**:128–135

This review comes from a themed issue on **Pharmaceutical biotechnology**

Edited by **Blaine Pfeifer**, **Guojian Zhang** and **Dehai Li**

https://doi.org/10.1016/j.copbio.2020.12.006

Introduction

Fungi, the eukaryotic microorganisms, produce a tremendous number of natural products (NPs) with diverse chemical structures and biological activities, such as the antibiotic penicillin [1], the cholesterol-lowering drug lovastatin [2], the immunosuppressant cyclosporine A [3], and the poisonous carcinogens aflatoxins [4••]. Structurally, the fungal NPs mainly include polyketides, nonribosomal peptides, terpenoids, alkaloids [5] and the hybrids thereof [6]. Due to their bifacial biological activities (e.g. beneficial drugs and harmful toxins) and intriguing chemical structures, the NPs produced by fungi have been drawing significant attention from biologists, chemists and biotechnologists [7,8].

Technical advances in strain and compound isolation and more powerful spectrometric instruments have led to discoveries of much more novel and low abundance NPs from fungi [8]. Moreover, rapid development of computer-assisted bioinformatics technology and multiple genetic strategies including transcriptional activation, heterologous host expression and epigenetic regulation of fungal NP biosynthetic gene clusters (BGCs) have been revolutionizing the conventional NP discoveries. In the synthetic biology era, engineering of the NP structures has become viable given that the biosynthetic enzymes and their catalytic mechanisms are well understood [9].

Comparatively, the NP biosynthetic machineries of eukaryotic cells are more complicated than those of prokaryotic microorganisms since the former can partition small-molecules and macro-molecules by different organelles. Compartmentalization of secondary metabolism pathway enzymes plays important roles in biosynthesis of fungal NPs, such as precursor channeling, concentration of biosynthetic components, prevention of unnecessary metabolic crosstalk, improvement of pathway efficiency, isolation of toxic substances, and trafficking pathway intermediates and products [10]. However, subcellular localization of biosynthetic enzymes and the functionalities as well as mechanisms of compartmentalization have long been underexplored and hence remain largely unknown.

In this review, we focus on a limited but growing number of studies about compartmentalized biosynthesis of some important fungal NPs including mycophenolic acid, β-lactams, aflatoxins and trichothecenes. The molecular basis of compartmentalization in these examples could serve as an excellent model for understanding the biosynthetic mechanisms of other fungal NPs. Moreover, an emerging strategy of compartmentalization engineering for re-localization of the fungal biosynthetic pathways at a cellular biology level is discussed. Taken together, we aim to provide some new insights and perspectives on NP biosynthesis in higher microorganisms.

Characterization of the compartmentalized fungal NP biosynthetic pathways

To date few studies have focused on compartmentalization of fungal NP biosynthetic pathways, and the limited examples include mycophenolic acid [11••], β-lactams [12,13], aflatoxins [14,15], trichothecene mycotoxins

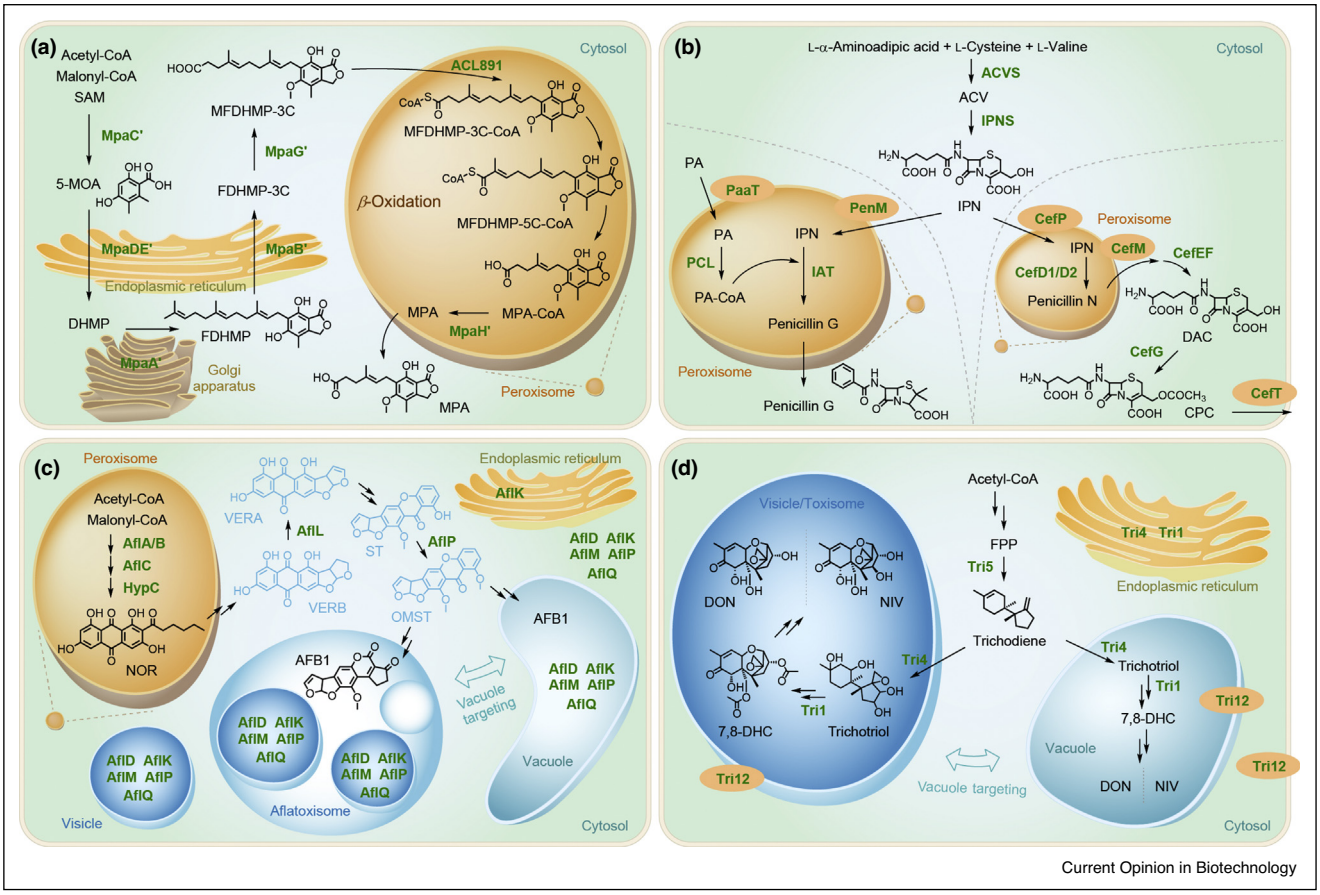
[16–18] (Figure 1), and several others (e.g. viriditoxin [19•] and melanin [20]) (Table 1).

Mycophenolic acid (MPA)

MPA is the first antibiotic to be isolated and crystallized in human history. This meroterpenoid is produced by many *Penicillium* species. MPA has been developed into an immunosuppressive drug for organ transplantations and autoimmune diseases [21]. For such an old antibiotic, the biosynthetic pathway featuring intriguing compartmentalization (Figure 1a) has recently been elucidated by our laboratory [11••,22].

The biogenesis of MPA starts from 5-methylorsellinic acid (5-MOA), which is assembled by the cytosolic polyketide synthase MpaC' from one molecule of acetyl-coenzyme A (acetyl-CoA), three malonyl-CoA units, and one *S*-adenosyl-L-methionine (SAM) molecule. The endoplasmic reticulum (ER) membrane-bound MpaDE' comprising a cytochrome P450 domain (MpaD') and a hydrolase domain (MpaE') converts 5-MOA into 3,5-dihydroxy-7-(hydroxymethyl)-6-methylbenzoic acid (DHMB) via the C8 hydroxylation activity of MpaD' and the subsequent intramolecular lactonization by MpaE' to generate 3,5-dihydroxy-6-methylphthalide (DHMP). Next, the Golgi apparatus-associated farnesyltransferase MpaA' farnesylates DHMP to yield 4-farnesyl-3,5-dihydroxy-6-methylphthalide (FDHMP). FDHMP undergoes the first oxidative cleavage step of the C19=C20 double bond by a globin-like ER-bound oxygenase MpaB' to form the chain-shortening intermediate FDHMP-3C. After the cytosolic methylation mediated by methyltransferase MpaG' and the peroxisomal CoA activation by an acyl-CoA ligase PbACL891, the

Figure 1



Compartmentalized biosynthesis of representative fungal NPs. **(a)** Mycophenolic acid, **(b)** β-Lactams, **(c)** Aflatoxins, and **(d)** Trichothecenes. Notes: Penicillin G, CPC, DON and NIV are biosynthesized in different fungi. The subcellular localization of the compounds in light blue remains unclear. Parallel double arrows indicate multiple biosynthetic steps. Abbreviations: SAM, *S*-adenosyl-L-methionine; 5-MOA, 5-methylorsellinic acid; DHMP, 3,5-dihydroxy-6-methylphthalide; FDHMP, 4-farnesyl-3,5-dihydroxy-6-methylphthalide; MFDHMP, 5-*O*-methyl-FDHMP; MPA, mycophenolic acid; ACV, δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine; IPN, isopenicillin N; PA, phenylacetic acid; DAC, deacetylcephalosporin C; CPC, cephalosporin C; Nor, norsolorinic acid; VERA, versicolorin A; VERB, versicolorin B; ST, sterigmatocystin; OMST, *O*-methylsterigmatocystin; AFB1, aflatoxin B1; FPP, farnesyl pyrophosphate; 7,8-DHC, 7,8-dihydroxycalonectrin; DON, deoxynivalenol; NIV, nivalenol.

Table 1

Subcellular localization of biosynthetic enzymes of the representative fungal natural products

Natural products	Enzymes	Localization	Function	Reference
Mycophenolic acid	MpaC'	Cytosol	Polyketide synthase	[11••]
	MpaDE'	Endoplasmic reticulum	P450 and hydrolase	[11••]
	MpaA'	Golgi apparatus	Farnesyltransferase	[11••]
	MpaB'	Endoplasmic reticulum	Oxygenase	[11••,22]
	MpaG'	Cytosol	Methyltransferase	[11••]
	MpaH'	Peroxisome	Hydrolase	[11••]
Penicillin/cephalosporin	ACVS/PcbAB/AcvA	Cytosol/vacuole	NRPS	[13,25]
	IPNS/PcbC/IpnA	Cytosol	Dioxygenase	[13,25]
	IAT/PenDE/AatA	Peroxisome	Acyltransferase	[13,25]
	PCL	Peroxisome	CoA ligase	[10]
	CefD1-CefD2	Peroxisome	Epimerase	[12,23]
	CefEF	Cytosol	Oxygenase	[23]
	CefG	Cytosol	Acetyltransferase	[23]
	PenM	Peroxisome	Transporter	[24•,25]
	PaaT	Peroxisome	Transporter	[24•]
	CefP	Peroxisome	Transporter	[24•]
	CefM	Peroxisome	Transporter	[24•]
	CefT	Cell membrane	Putitve transporter	[24•]
Aflatoxins	AflA/B	Peroxisome	Fatty acid synthase	[41]
	AflD/Nor-1	Cytosol/vesicle/vacuole	Oxidoreductase	[27,32]
	AflK/Vbs	Cytosol/endoplasmic reticulum/vesicle/vacuole	Cyclase	[27,39]
	AflM/Ver-1	Cytosol/vesicle/vacuole	Ketoreductase	[27,33]
	AflP/OmtA	Cytosol/vesicle/vacuole	Methyltransferase	[27,34]
	AflQ/ArdA	Cytosol/vesicle/vacuole	P450	[15]
Trichothecenes	Tri5	Cytosol	Terpene cyclase	[17]
	Tri4	Endoplasmic reticulum/vesicle	P450	[16–18]
	Tri1	Endoplasmic reticulum/vesicle	P450	[16–18]
	Tri12	Vesicle/vacuole/vell membrane	Transporter	[38]
Viriditoxin	VdtA	Peroxisome-like structure	Polyketide synthase	[19•]
	VdtG	Endoplasmic reticulum/versicle	Transporter	[19•]
Melanin	Alb1	Endosome	Polyketide synthase	[20]
	Arp1	Endosome	Dehydratase	[20]
	Arp2	Endosome	Reductase	[20]
	Abr1	Cell wall	Oxidase	[20]
	Abr2	Cell wall	Laccase	[20]

resulting product MFDHMP-3C-CoA is successively chain-shortened in peroxisomes by the β-oxidation machinery to produce MPA-CoA. Finally, the peroxisomal acyl-CoA hydrolase MpaH' hydrolyzes MPA-CoA, giving rise to MPA (Figure 1a).

In this compartmentalized pathway, the inner membrane-integrated nature of MpaA' and MpaB' is compatible with the lipophilicity of their substrates. In particular, the CoA ligation step mediated by perxisomal PbACL891 is essential to imprison the charged intermediate MFDHMP-3C-CoA (molecular weight: 1136 Da, which is higher than the 400 Da cutoff for crossing the single membrane of peroxisome by free diffusion) within peroxisomes for the chain-shortening process by the co-localized β-oxidation machinery until MPA-CoA is hydrolyzed by MpaH' with a high substrate specificity.

To elucidate the relationship between the peroxisomal residence and catalytic function of the α/β hydrolase MpaH' in MPA biosynthesis, subcellular co-localization and biochemical feeding experiments were carried out [11••]. Heterologous co-expression of the peroxisome-targeting red fluorescent protein RFP^{SKL} reporter and the green fluorescent protein (GFP) fused to the full length MpaH' in *Aspergillus oryzae* M-2-3 confirmed the peroxisomal localization of $MpaH'^{full\text{-}length}$ that carries a type 1 peroxisomal targeting sequence-like (PTS1-like) glycine-lysine-leucine (GKL) tripeptide at the *C* terminus. The results that MpaH' is a dedicated acyl-CoA hydrolase with strict substrate specificity toward MPA-CoA rather than other acyl-CoA substrates, and that a significantly higher amount of demethylmycophenolic acid (DMMPA) accumulated in the $MpaH'^{full\text{-}length}$-expressed hosts than in those expressing the PTS1-like signal truncated $MpaH'^{\triangle GKL}$, indicated that MpaH' should exert a 'valve-like' function to prevent MPA-CoA from further β-oxidation. Thus, peroxisomal compartmentalization of MpaH' greatly enhances the specific production of MPA.

β-Lactams

The most widely used β-lactam antibiotics include penicillins and cephalosporins with broader spectra [1]. The biosynthetic pathways [12,13,23] (Figure 1b) of common β-lactam producers share the two early enzymatic steps: (1) the condensation of the three activated precursor amino acids including L-α-aminoadipic acid, L-cysteine and L-valine, which is catalyzed by the cytosolic/vacuolar non-ribosomal peptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV) synthetase (ACVS, encoded by the gene *pcbAB*) to form the tripeptide ACV; and (2) the oxidative cyclization of ACV mediated by the cytosolic isopenicillin N (IPN) synthase (IPNS), a non-heme iron-dependent enzyme encoded by *pcbC*, resulting in the formation of IPN with the β-lactam core. Of note, IPN is the branch point of the biosynthetic pathways of penicillins and cephalosporins.

In the penicillin branch [13,23], the hetero-dimeric acyl-coenzyme A (CoA):6-aminopenicillanic acid (6-APA) acyltransferase (IAT) encoded by *penDE* and the phenylacetyl-CoA ligase (PCL), both of which are located in peroxisomes, co-mediate the assembly of penicillin G. At this peroxisomal stage, the α-aminoadipyl group of IPN is replaced by a phenylacetyl side chain. By contrast, in the cephalosporin branch [12,23], IPN is converted into penicillin N, the D-isomer of IPN, by an epimerization system of CefD1/D2, which also resides in peroxisomes. The rest of biosynthetic steps all occur in cytoplasm, including the ring-expansion and hydroxylation reactions catalyzed by the α-ketoglutarate dependent oxygenase encoded by *cefEF* to generate deacetylcephalosporin C (DAC), and the last acetylation by DAC-acetyltransferase CefG to form cephalosporin C (CPC). Two transporters PenM/PaaT are involved in the translocation of IPN and phenylacetic acid (PA) from cytosol to peroxisomes [24•,25]. The other three transporters including the peroxisome-associated CefP/CefM and the cell membrane-bound CefT are likely responsible for importing the cephalosporin precursors and excreting cephalosporin C [24•,26].

Aflatoxins

Aflatoxins are extremely toxic fungal NPs that are predominantly produced by *Aspergillus* species. Because of the importance of food contaminations caused by aflatoxins, their biosynthetic details have been well studied (Figure 1c) [4••,27–30]. Aflatoxin B1 (AFB1) is known as the most potent naturally occurring carcinogen and immunosuppressant [31]. The biogenesis of AFB1 depends on a complex biosynthetic pathway comprised of at least 27 enzymatic steps. Peroxisomal fatty acid synthases AflA/B catalyze the first step to condense acetyl-CoA and malonyl-CoA into the triketide intermediate hexanoate, which is next processed by polyketide synthase AflC to produce norsolorinic acid anthrone (NAA), and further oxidized into the first stable metabolite norsolorinic acid (NOR) by a small enzyme HypC.

Then, NOR is sequentially transformed by oxidoreductase AflD, cytochrome P450 monooxygenase AflG, dehydrogenase AflH, cyclase AflK, P450 enzyme AflV, monooxygenase AflW, P450 monooxygenase AflI with uncertain functionality, and esterase AflJ to generate the common precursor versicolorin B (VERB). VERB is subsequently converted by P450 monooxygenase AflL into versicolorin A (VERA). Five enzymes including ketoreductase AflM, P450 enzyme AflN, oxidoreductases AflY/AflX, and *O*-methyltransferase AflO are involved in the transformation of VERA into sterigmatocystin (ST) and further into *O*-methylsterigmatocystin (OMST) by *O*-methyltransferase AflP. The late conversion from OMST to AFB1 involves the precisely identified P450 monooxygenase AflQ and oxidase HypB, and two functionally uncertain enzymes including dehydrogenase AflE and the ethD-domain-containing protein HypE. Two additional proteins including the fungal transporter AflT and the membrane-bound protein HypD may also be involved in AFB1 biosynthesis [4••,27,28].

Regarding the localization of AFB1 biosynthetic enzymes, AflA/B, AflC and HypC are believed to be peroxisomal enzymes since their product NOR has been found to be localized within peroxisomes [10]. More subcellular localization studies [32–34] have shown that AflD, AflK, AflM, AflP and AflQ initially reside in cytoplasm and then move to vesicles (forming special organelles named aflatoxisomes) and vacuoles when aflatoxin production reaches a high rate. For young colonies, AflK could be observed in ER around nuclei. These studies have depicted a dynamic cytoplasm-to-vacuole targeting mechanism.

Trichothecenes

Deoxynivalenol (DON) and nivalenol (NIV) belong to the common family of trichothecene mycotoxins that are frequently detected in agricultural products [35]. Significant advances have been achieved in establishing the biosynthetic pathway involving at least 16 genes at four loci (two gene clusters and two isolated genes) on different chromosomes of *Fusarium* spp. (Figure 1d) [16,36–38]. The cytosolic trichodiene synthase Tri5 catalyzes the first step, cyclization of farnesyl pyrophosphate (FPP) to produce the non-toxic trichodiene, which is subsequently oxidized by P450 monooxygenase Tri4 at multiple positions to yield isotrichotriol. After two spontaneous steps, the formed isotrichodermol is acetylated by acetyltransferase Tri101 to yield isotrichodermin, which is then hydroxylated by P450 enzyme Tri11 to form 15-decalonectrin. Acetyltransferase Tri3 is responsible for generating calonectrin, which is next tailored by P450 monooxygenase Tri1 giving rise to 7,8-dihydroxycalonectrin (7,8-DHC). The following transformations to 3,15-

acetyldeoxynivalenol (3,15-ADON), the branching compound for DON and NIV biosynthesis, remain unknown. The final steps of DON biosynthesis are catalyzed by two esterases (Tri8 and an unknown one) via the intermediate 3-ADON or 15-ADON. As for NIV biosynthesis, 3,15-ADON is hydroxylated and acetylated by P450 monooxygenase Tri13 and acetyltransferase Tri7, respectively, to form 3,4,15-triacetylnivalenol (3,4,15-ANIV). Similar to DON, the final three deacetylation steps are mediated by Tri8 and other unknown esterase(s).

A few of DON/NIV biosynthetic enzymes have been studied for their subcellular localization. Specifically, Tri5 is a cytosolic enzyme. P450 enzymes Tri4 and Tri1 co-localize at ER and vesicles (also called toxisomes). Transporter Tri12 is found to be localized at multiple sites including vesicles, vacuoles and plasma membrane.

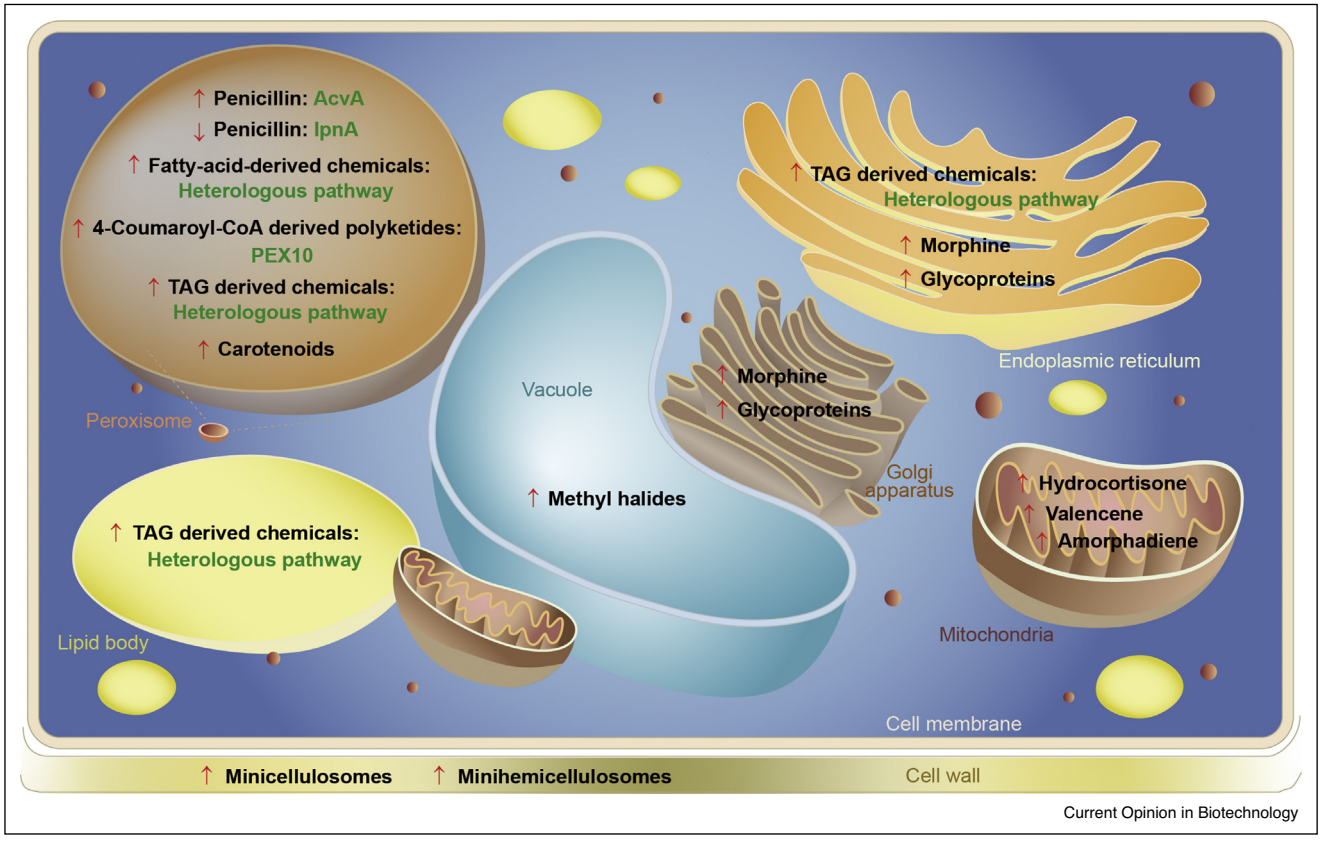
Others fungal NPs

Other characterized biosynthetic pathways with compartmentalization include viriditoxin [19•] and melanin [20]. In the biosynthesis of viriditoxin, polyketide synthase VdtA resides within a specialized peroxisome-like structure [19•]. The transporter VdtG is localized within an internal organelle similar to the aflatoxisomes of *Aspergillus* species [39] and toxisomes of *Fusarium* spp. [18]. Subcellular compartmentalization of fungal melanin biosynthetic enzymes has also been found to occur in both *Aspergillus fumigatus* and *Aspergillus nidulans* [20]. Early melanin biosynthetic enzymes (Alb1/Arp1/Arp2) are recruited to endosomes through a non-conventional secretory pathway, while late enzymes (Abr1/Abr2) accumulate in cell wall. More examples of less-characterized compartmentalized biosynthesis of fungal NPs can be found in some earlier reviews [10,40,41].

Compartmentalization engineering in fungal cells

Growing understandings of compartmentalized biosynthesis have been spurring the development of compartmentalization engineering to synergize metabolic engineering and enzyme engineering. Compartmentalization engineering of fungal NP biosynthetic pathways by changing the subcellular localization of involved enzymes has been proven as an effective strategy for both

Figure 2



Compartmentalization engineering in different organelles. Up and down arrows indicate increased and decreased titers, respectively. Subcellularly engineered enzymes or pathways are shown in green. 'Heterologous pathway' means the introduction of non-native biosynthetic pathway enzymes for titer improvements.

elucidation of biosynthetic mechanisms and titer improvements of the target products (Figure 2). Single cell yeasts including *Saccharomyces cerevisiae* and *Yarrowia lipolytica* have become model systems for practicing compartmentalization engineering due to the abundant knowledge and genetic tools for manipulating the subcellular localization of proteins/enzymes. Now, more NP-related compartmentalization engineering work can be conducted in filamentous fungi, either the native producer strain or a heterologous host, both of which are more evolutionarily relevant than the single cell yeasts.

Compartmentalization engineering in filamentous fungi

A. nidulans has been used as a model system to study the improvement of penicillin production [42]. Assembly of penicillins only require three functional genes (i.e. *pcbAB*, *pcbC* and *penDE* in *Penicillium* spp., and the counterparts *acvA*, *ipnA* and *aatA* in *A. nidulans*). AcvA and IpnA are localized in cytoplasm, and AatA resides in peroxisomes. It was revealed that the production of penicillin G could be altered by targeting these enzymes to different sites [42]. Specifically, targeting AcvA to peroxisomes increased the production by 3.2-fold. By contrast, peroxisomal targeting of IpnA led to a dramatic decrease in penicillin production. A fivefold increase was achieved by combination of AcvA peroxisomal targeting and overexpression of IpnA and AatA. Interestingly, the higher-producer strains showed significantly increased levels in both penicillin biosynthetic enzymes and in the number and volume fraction of peroxisomes [43]. Moreover, the engineered strain with the doubled number of peroxisomes through overexpression of PexK (a peroxin that is involved in microbody abundance) produced several times more penicillins [42,44].

As described above, the three key enzymes AflD, AflM and AflP involved in multiple stages of aflatoxin biosynthesis are initially localized in cytoplasm and subsequently to vesicles and vacuoles when aflatoxin biosynthesis reaches a high rate [32–34]. Two independent approaches were applied to investigate the function of vesicles for aflatoxin biosynthesis [39]: (1) chemical reagent Sortin3 was used to disrupt endomembrane trafficking; and (2) *avaA*, whose gene product belongs to the homotypic fusion and protein sorting (HOPS) tethering complex that mediates the fusion of prevacuolar compartments to vacuoles, was knocked out. The resulting increased number of small vesicles and accumulation of aflatoxin demonstrated that vesicles play an essential role in biosynthesis and secretion of this toxin. Under aflatoxin-inducing conditions, development of vesicles/aflatoxisomes and expression of biosynthetic enzymes were revealed to be significantly improved [39].

Compartmentalization engineering in yeast

Besides a few examples of compartmentalization engineering in the filamentous fungal hosts, there are much more reports of compartmentalization engineering in yeasts in the current synthetic biology era [45].

For example, peroxisomal targeting of various enzymes has been performed for production of fatty-acid-derived chemicals including alcohols, alkanes and olefins in *S. cerevisiae* [46], by which the titers of target chemicals were improved to different extents and the accumulation of byproducts was attenuated. By increasing the peroxisome population, the production of these target compounds were enhanced up to threefold. Of note, the combination of metabolic engineering efforts with the compartmentalization engineering by overexpressing the peroxisome biogenesis factor PEX10 (a structural protein associated with peroxisome formation) in *Y. lipolytica* resulted in significant titer improvements for the 4-coumaroyl-CoA derived type III polyketide metabolites [47••]. Furthermore, different subcellular localization strategies in yeasts were examined in order to enhance the production of triacylglycerol (TAG) derived chemicals [48••]. Remarkably, the change of the localization of TAG biosynthetic enzymes from cytoplasm to lipid body (LB) resulted in a 10-fold higher titer of fatty acid methyl esters, fatty alkenes and alkanes. Other significant progresses on compartmentalization engineering in yeasts include the enzyme targeting to mitochondria for hydrocortisone, valencene and amorphadiene; to peroxisomes for penicillins and carotenoids; to ER/Golgiosome for morphine and glycoproteins; to vacuoles for methyl halides, and to cell wall for minicellulosomes and minihemicellulosomes [45].

Conclusions and perspectives

NP biosynthesis on the basis of secondary metabolism relies on the supply of energy, precursors, cofactors and even some enzymes from primary metabolism under suitable environments [49]. One can imagine that subcellular compartmentalization would provide an isolated reaction space, by which a better physiochemical environment for optimal enzyme catalysis can be created. Moreover, the substrates are highly concentrated within an organelle; the efficiency of precursor supply and intermediate channeling is improved; the hijacking of intermediates by off-target pathways is prevented; and the toxic intermediates/products are isolated to avoid the negative effects on other cellular networks [10,40]. Although subcellular compartmentalization plays critical roles in fungal NPs biosynthesis, most previous studies focused on the catalytic mechanisms at biochemical levels rather than from a cell biology perspective. In the future studies, we suggest that more attention should be paid to compartmentalization of biosynthetic steps in order to better understand the physiological and biochemical mechanisms of fungal NP biosynthesis.

With respect to compartmentalization engineering, subcellular localization screening using different signal

peptides seems to be a useful strategy [45,48••]. Peroxisome, mitochondria, ER, Golgiosome, and vacuole could be hot spots for enzyme targeting. Physicochemical properties and physiological conditions of these organelles could provide differential advantages for biosynthetic enzymes dealing with certain types of NPs. For examples, peroxisomes are involved in the fungal β-oxidation process of long-chain fatty acids to supply acetyl-CoA, thereby benefiting the biosynthesis of fatty acid or polyketide related chemicals.

Multi-locations of biosynthetic enzymes of aflatoxins/trichothecenes highlight the continuity and dynamics of the endomembrane system of fungi. Thus, the timing of subcellular compartmentalization is another important issue worth more attention. There is correlation between the change of subcellular location of NP biosynthetic enzymes and the growth/biosynthesis stages, which reflects elaborate regulations of NPs production in fungi [40]. To achieve more efficient and specific production of fungal NPs, fine regulation of enzyme localization based on metabolic flow changes should be more practiced in the future. One pioneering study has shown that the light-switchable assembly and disassembly of deoxyviolacein biosynthetic cluster in yeast cells dramatically decreased the concentrations of intermediates and inhibited the competing side pathways, thus resulting in significant production improvement [50••].

Synthetic artificial organelles provide new opportunities for compartmentalization engineering [51]. For instance, the artificial liposomes 'capsosomes', which carry a biosynthetic cascade consisting of glucose oxidase and horseradish peroxidase could be introduced into eukaryotic cells by macrophages to synthesize a non-natural fluorescent compound resorufin [52]. It is anticipated that these new strategies of compartmentalization engineering will be applied for more fungal NP biosynthesis work on titer improvement, new derivative generation, and other synthetic biology purposes.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported the National Key Research and Development Program of China (2019YFA0706900 and 2019YFA0905100), the National Natural Science Foundation of China (31800041 to L.D. and 32025001, 31872729 and 21472204 to S.L.), the Shandong Provincial Natural Science Foundation (ZR201807060986 to L.D. and ZR2019ZD20 to S.L.).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Campos C, Lázaro-Rodríguez TG, Hernández-Pérez E, Rincón-Heredia R, Fernández FJ: **Pexophagy modes during penicillin biosynthesis in *Penicillium rubens* P2-32-T**. *Arch Microbiol* 2020, **202**:2337-2341.

2. Xiong Z, Cao X, Wen Q, Chen Z, Cheng Z, Huang X, Zhang Y, Long C, Zhang Y, Huang Z: **An overview of the bioactivity of monacolin K/lovastatin**. *Food Chem Toxicol* 2019, **131**:110585.

3. Wu Q, Kuca K: **Metabolic pathway of cyclosporine A and its correlation with nephrotoxicity**. *Curr Drug Metab* 2019, **20**:84-90.

4. •• Caceres I, Khoury AA, Khoury RE, Lorber S, Oswald IP, Khoury AE, Atoui A, Puel O, Bailly J-D: **Aflatoxin biosynthesis and genetic regulation: a review**. *Toxins (Basel)* 2020, **12**

This article reviewed biochemical functions of the enzymes involved in biosynthesis of aflatoxin B1, thus providing an overview of aflatoxin B1 biosynthetic pathway. This review also highlighted the effects of extracellular environmental factors on intracellular regulatory genes and aflatoxin production.

5. Quin MB, Flynn CM, Schmidt-Dannert C: **Traversing the fungal terpenome**. *Nat Prod Rep* 2014, **31**:1449-1473.

6. Reverberi M, Ricelli A, Zjalic S, Fabbri AA, Fanelli C: **Natural functions of mycotoxins and control of their biosynthesis in fungi**. *Appl Microbiol Biotechnol* 2010, **87**:899-911.

7. Hyde KD, Xu J, Rapior S, Jeewon R, Lumyong S, Niego AGT, Abeywickrama PD, Aluthmuhandiram JVS, Brahamanage RS, Brooks S *et al.*: **The amazing potential of fungi: 50 ways we can exploit fungi industrially**. *Fungal Divers* 2019, **97**:1-136.

8. Keller NP: **Fungal secondary metabolism: regulation, function and drug discovery**. *Nat Rev Microbiol* 2019, **17**:167-180.

9. Skellam E: **Strategies for engineering natural product biosynthesis in fungi**. *Trends Biotechnol* 2019, **37**:416-427.

10. Kistler HC, Broz K: **Cellular compartmentalization of secondary metabolism**. *Front Microbiol* 2015, **6**:68.

11. •• Zhang W, Du L, Qu Z, Zhang X, Li F, Li Z, Qi F, Wang X, Jiang Y, Men P *et al.*: **Compartmentalized biosynthesis of mycophenolic acid**. *Proc Natl Acad Sci U S A* 2019, **116**:13305-13310

This study completely elucidated the biosynthetic pathway of mycophenolic acid in *Penicillium brevicompactum*. Using gene inactivation, heterologous gene expression, *in vitro* enzymatic assays and protein subcellular localization, the authors showed the compartmentalized biosynthetic steps in detail and the unique linkage between primary peroxisomal β-oxidation metabolism and secondary metabolic steps distributed in different organelles.

12. Kurzatkowski Wieslaw, Gebska-Kuczerowska Anita: **Compartmentalization in cephalosporin C biosynthesis by industrial strains of *Acremonium chrysogenum***. *Postep Mikrobiol* 2015, **54**:374-379.

13. Kurza?tkowski W, Staniszewska M, Bondaryk M, Ge?bska-Kuczerowska A: **Compartmentalization in penicillin G biosynthesis by *Penicillium chrysogenum* PQ-96**. *Pol J Microbiol* 2014, **63**:399-408.

14. Linz JE, Wee JM, Roze LV: **Aflatoxin biosynthesis: regulation and subcellular localization**. In *Biosynthesis and Molecular Genetics of Fungal Secondary Metabolites.* Edited by Martín JF, García-Estrada C, Zeilinger S. Springer; 2014:89-110.

15. Chanda A, Roze LV, Pastor A, Frame MK, Linz JE: **Purification of a vesicle-vacuole fraction functionally linked to aflatoxin synthesis in *Aspergillus parasiticus***. *J Microbiol Methods* 2009, **78**:28-33.

16. Tang G, Chen Y, Xu J-R, Kistler HC, Ma Z: **The fungal myosin I is essential for *Fusarium* toxisome formation**. *PLoS Pathog* 2018, **14**:e1006827.

17. Boenisch MJ, Broz KL, Purvine SO, Chrisler WB, Nicora CD, Connolly LR, Freitag M, Baker SE, Kistler HC: **Structural reorganization of the fungal endoplasmic reticulum upon induction of mycotoxin biosynthesis**. *Sci Rep* 2017, **7**:44296.

18. Menke J, Weber J, Broz K, Kistler HC: **Cellular development associated with induced mycotoxin synthesis in the filamentous fungus *Fusarium graminearum***. *PLoS One* 2013, **8**:e63077.

19. • Urquhart AS, Hu J, Chooi Y-H, Idnurm A: **The fungal gene cluster for biosynthesis of the antibacterial agent viriditoxin**. *Fungal Biol Biotechnol* 2019, **6**:2

This research identified the gene cluster responsible for viriditoxin biosynthesis and elucidated the functions of related genes. Moreover, subcellular localizations of VdtA and VdtG demonstrated the pathway compartmentalization during the biosynthetic process.

20. Upadhyay S, Xu X, Lowry D, Jackson JC, Roberson RW, Lin X: **Subcellular compartmentalization and trafficking of the biosynthetic machinery for fungal melanin**. *Cell Rep* 2016, **14**:2511-2518.

21. Kiang Tony KL, Ensom Mary HH: **Population pharmacokinetics of mycophenolic acid: an update**. *Clin Pharmacokinet* 2018, **57**:547-558.

22. Chen X, Wang L, Zhang J, Jiang T, Hu C, Li D, Zou Y: **Immunosuppressant mycophenolic acid biosynthesis employs a new globin-like enzyme for prenyl side chain cleavage**. *Acta Pharm Sin B* 2019, **9**:1253-1258.

23. Martín JF, Ullán RV, García-Estrada C: **Regulation and compartmentalization of β-lactam biosynthesis**. *Microb Biotechnol* 2010, **3**:285-299.

24. • Martín JF: **Transport systems, intracellular traffic of intermediates and secretion of β-lactam antibiotics in fungi**. *Fungal Biol Biotechnol* 2020, **7**:6

This article reviewed transportation of β-lactam antibiotics in fungi. Transporters, PenM and PaaT in penicillin biosynthesis, and CefP and CefM in cephalosporin C biosynthesis, were found to be involved in the import of precursors and intermediates. Transporter CefT located in cell membrane is related to the secretion of cephalosporin C.

25. Fernández-Aguado M, Martín JF, Rodríguez-Castro R, García-Estrada C, Albillos SM, Teijeira F, Ullán RV: **New insights into the isopenicillin N transport in *Penicillium chrysogenum***. *Metab Eng* 2014, **22**:89-103.

26. Ullán RV, Teijeira F, Guerra SM, Vaca I, Martín JF: **Characterization of a novel peroxisome membrane protein essential for conversion of isopenicillin N into cephalosporin C**. *Biochem J* 2010, **432**:227-236.

27. Roze LV, Hong S-Y, Linz JE: **Aflatoxin biosynthesis: current frontiers**. *Annu Rev Food Sci Technol* 2013, **4**:293-311.

28. Yu J: **Current understanding on aflatoxin biosynthesis and future perspective in reducing aflatoxin contamination**. *Toxins (Basel)* 2012, **4**:1024-1057.

29. Huffman J, Gerber R, Du L: **Recent advancements in the biosynthetic mechanisms for polyketide-derived mycotoxins**. *Biopolymers* 2010, **93**:764-776.

30. Yabe K, Nakajima H: **Enzyme reactions and genes in aflatoxin biosynthesis**. *Appl Microbiol Biotechnol* 2004, **64**:745-755.

31. Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG: **Aflatoxins: a global concern for food safety, human health and their management**. *Front Microbiol* 2016, **7**:2170.

32. Hong S-Y, Linz JE: **Functional expression and sub-cellular localization of the early aflatoxin pathway enzyme Nor-1 in *Aspergillus parasiticus***. *Mycol Res* 2009, **113**:591-601.

33. Hong S-Y, Linz JE: **Functional expression and subcellular localization of the aflatoxin pathway enzyme Ver-1 fused to enhanced green fluorescent protein**. *Appl Environ Microbiol* 2008, **74**:6385-6396.

34. Lee L-W, Chiou C-H, Klomparens KL, Cary JW, Linz JE: **Subcellular localization of aflatoxin biosynthetic enzymes Nor-1, Ver-1, and OmtA in time-dependent fractionated colonies of *Aspergillus parasiticus***. *Arch Microbiol* 2004, **181**:204-214.

35. Audenaert K, Vanheule A, Höfte M, Haesaert G: **Deoxynivalenol: a major player in the multifaceted response of *Fusarium* to its environment**. *Toxins (Basel)* 2013, **6**:1-19.

36. Amarasinghe CC, Fernando WGD: **Comparative analysis of deoxynivalenol biosynthesis related gene expression among different chemotypes of *Fusarium graminearum* in spring wheat**. *Front Microbiol* 2016, **7**:1229.

37. Seong K-Y, Pasquali M, Zhou X, Song J, Hilburn K, McCormick S, Dong Y, Xu J-R, Kistler HC: **Global gene regulation by *Fusarium* transcription factors Tri6 and Tri10 reveals adaptations for toxin biosynthesis**. *Mol Microbiol* 2009, **72**:354-367.

38. Kimura M, Tokai T, Takahashi-Ando N, Ohsato S, Fujimura M: **Molecular and genetic studies of *Fusarium* trichothecene biosynthesis: pathways, genes, and evolution**. *Biosci Biotechnol Biochem* 2007, **71**:2105-2123.

39. Chanda A, Roze LV, Kang S, Artymovich KA, Hicks GR, Raikhel NV, Calvo AM, Linz JE: **A key role for vesicles in fungal secondary metabolism**. *Proc Natl Acad Sci U S A* 2009, **106**:19533-19538.

40. Lim FY, Keller NP: **Spatial and temporal control of fungal natural product synthesis**. *Nat Prod Rep* 2014, **31**:1277-1286.

41. Roze LV, Chanda A, Linz JE: **Compartmentalization and molecular traffic in secondary metabolism: a new understanding of established cellular processes**. *Fungal Genet Biol* 2011, **48**:35-48.

42. Herr A, Fischer R: **Improvement of *Aspergillus nidulans* penicillin production by targeting AcvA to peroxisomes**. *Metab Eng* 2014, **25**:131-139.

43. Valenciano S, de Lucas JR, van der Klei I, Veenhuis M, Laborda F: **Characterization of *Aspergillus nidulans* peroxisomes by immunoelectron microscopy**. *Arch Microbiol* 1998, **170**:370-376.

44. Kiel JAKW, van der Klei IJ, van den Berg MA, Bovenberg RAL, Veenhuis M: **Overproduction of a single protein, Pc-Pex11p, results in 2-fold enhanced penicillin production by *Penicillium chrysogenum***. *Fungal Genet Biol* 2005, **42**:154-164.

45. Hammer SK, Avalos JL: **Harnessing yeast organelles for metabolic engineering**. *Nat Chem Biol* 2017, **13**:823-832.

46. Zhou YJ, Buijs NA, Zhu Z, Gómez DO, Boonsombuti A, Siewers V, Nielsen J: **Harnessing yeast peroxisomes for biosynthesis of fatty-acid-derived biofuels and chemicals with relieved side-pathway competition**. *J Am Chem Soc* 2016, **138**:15368-15377.

47. •• Palmer CM, Miller KK, Nguyen A, Alper HS: **Engineering 4-coumaroyl-CoA derived polyketide production in *Yarrowia lipolytica* through a β-oxidation mediated strategy**. *Metab Eng* 2020, **57**:174-181

In this research, impressive titer improvements of the 4-coumaroyl-CoA derived type III polyketide metabolites were achieved through combination of metabolic engineering efforts with the compartmentalization engineering by overexpressing the peroxisome biogenesis factor PEX10 in *Y. lipolytica*.

48. •• Yang K, Qiao Y, Li F, Xu Y, Yan Y, Madzak C, Yan J: **Subcellular engineering of lipase dependent pathways directed towards lipid related organelles for highly effectively compartmentalized biosynthesis of triacylglycerol derived products in *Yarrowia lipolytica***. *Metab Eng* 2019, **55**:231-238

This work showed great potentials of compartmentalization engineering in lipid body, endoplasmic reticulum and peroxisome of yeast for production of triacylglycerol derived chemicals, as well as fatty alkenes and alkanes.

49. Thirumurugan D, Cholarajan A, Raja SSS, Vijayakumar R: **An introductory chapter: secondary metabolites**. In *Secondary Metabolites: Sources and Applications.* Edited by Vijayakumar R, Raja SSS. IntechOpen; 2018.

50. •• Zhao EM, Suek N, Wilson MZ, Dine E, Pannucci NL, Gitai Z, Avalos JL, Toettcher JE: **Light-based control of metabolic flux through assembly of synthetic organelles**. *Nat Chem Biol* 2019, **15**:589-597

This study designed a suite of light-switchable genetic tools to trigger assembly and disassembly of a biosynthetic enzymes cascade that greatly enhanced product formation and reduced the competing side metabolic fluxes.

51. Simmel FC: **Synthetic organelles**. *Emerg Top Life Sci* 2019, **3**:587-595.

52. Godoy-Gallardo M, Labay C, Trikalitis VD, Kempen PJ, Larsen JB, Andresen TL, Hosta-Rigau L: **Multicompartment artificial organelles conducting enzymatic cascade reactions inside cells**. *ACS Appl Mater Interfaces* 2017, **9**:15907-15921.