Gene cluster regulators from plants to microbes: Key role in vesicular targeting, transport of intermediate compounds and secondary metabolite biosynthesis

Prasanta Chakraborty, PhD
Indian Institute of Chemical Biology, Kolkata, West Bengal, India
Scopus: https://www.scopus.com/authid/detail.uri?authorId=57200536237

https://doi.org/10.57098/SciRevs.Biology.3.4.2
Received October 10, 2024. Revised October 25, 2024. Accepted November 05, 2024.

Abstract: Plants and microbes (e.g., bacteria, fungi) produces various types of secondary metabolites (SMs) that may be used as nutraceuticals, defense molecules, drugs/antibiotics etc. Efficient biosynthesis of these SMs depend upon the transport and accumulation of biosynthetic intermediates, enzymes and transporters in a specific compartment of cells of the organism. Signals for biosynthesis of these important SMs often comes from the related gene clusters and their regulators situated inside or outside (global) the cluster. In bacteria, cluster situated regulators (CSRs), like SARPs (Streptomyces antibiotic regulatory proteins) and other regulators regulates biosynthesis of many important drugs and antibiotics, in fungi, 40% CSRs and 60% global regulators control the SM biosynthesis, whereas in plants mainly global regulators works. In this review, how these regulators play their part in the transport and efficient biosynthesis of some important compounds in plant and microbes will be discussed.

Keywords : Regulators; Gene cluster; Secondary metabolites; Biosynthesis; Plants; Microbes

Introduction

Secondary metabolites from plant and microbes are considered indispensable for survival, growth, and metabolism, and to perform many other numerous functions. To counter the resistance issue, drug discovery is essential. Hence secondary metabolites are important natural sources which are focused to find new drugs. Many valuable molecules like defense molecules, anti-nutritional compounds, drugs/antibiotics have already been obtained from these metabolites, e.g., cyanogen glycosides, steroidal alkaloids from crop plants, anti-cancer drugs vinblastine/vincristine, noscapine from medicinal plants (1-5), penicillin, tetracycline and many more from microbes (6-9). Efficient production of any valuable molecules/drugs in plants and microbes depends upon the localization of their secondary metabolite biosynthetic intermediates, biosynthetic enzymes and transporters in the specific vesicles of the organism e.g., biosynthetic enzymes for penicillin biosynthesis, isopenicillin N-acyltransferase along with their substrate was localized in peroxisome of fungus *Penicillium chrysogenum* (10,11), biosynthetic intermediates vindolines, catharanthine along with their transporters for anticancer drug vinblastine/vincristine was localized in internal leaf cells and leaf surface of medicinal plant, *Catharanthus roseus* (12-14), and MATE(multidrug and toxin extrusion) transporter, SbMATE2 was localized along with cyanogen glucosides in vacuolar membrane of food plant sorghum (15). Targeting and subcellular localization of the transporters and the biosynthetic enzymes are tightly regulated by the expressions and regulations of their genes. Though the recent literature indicates that the genes of the biosynthetic enzymes of secondary metabolites and many transporters in plant and microbes are organized as gene cluster in the genome, e.g., metabolic gene clusters for terpenoids and alkaloids in plants (16-20), gene clusters for penicillin, tetracyclines in microbes (21-24); there are very scanty reports about their regulations. Many global and cluster situated regulators (CSRs) may be operative in the regulation/ expression of

these genes responsible for secondary metabolite biosynthesis.

The regulators are family of activator-repressor type of transcription factors which plays important role in the regulation of plant and microbe's secondary metabolism. In plants, R3-MYB (MYOBLASTOSIS), bHLH (helix-loop-helix) and Apetala2/ Ethylene Response Factor (AP2/ERF) families known to regulate secondary metabolism, e.g., regulation of anthocyanin biosynthesis and transport of MATE3 transporter in vacuolar membrane in grapevine fruit by MYB-type transcription factors (25), and in microbes, specifically in *Streptomyces*, SARP (Streptomyces antibiotic regulatory proteins) regulators contain HLH motif through which they bind DNA during expression of biosynthetic genes. SARPs are a large family of CSRs and its homologues are encoded by many biosynthetic gene clusters (BGCs) including beta-lactam antibiotic BGCs and works in the expression of genes within the same cluster (26-28). However, the global regulators (GRs) work from a distant site and in addition to biosynthetic gene clusters; they can also induce or repress gene activity of those not belonging to secondary metabolism (29-31). A considerable number of global regulators have been characterized in Streptomyces till 2022 (28,31). These regulators may regulate directly or indirectly in the localization and expression of many transporters and other necessary enzymes in the specific vesicles. In fungus, the global regulator VeA regulates the penicillin biosynthesis in *P.chrysogenum* (32,33); whereas an LaeA-, BrlA-dependent cellular network governs tissue-specific secondary metabolism in the human pathogen *Aspergilus fumigatus* (34,35).

Understanding the regulatory mechanism will eventually help to learn how these pathways contribute to the efficient production of valuable compounds in plant and microbes specially in cases of unexplored ones.

Organelle specific secondary metabolite biosynthesis in plants and microbes

Biosynthetic intermediates along with biosynthetic enzymes and transporter for any SM assemble together in specific organelles of cells of plants and microbes for efficient biosynthesis of valuable molecules including drugs, antibiotics, nutraceuticals and others. Though, the transport mechanism in cases of many SM biosynthesis is still not very clear, the major organelles in plant and microbes and their role and involvement in the efficient biosynthesis of some important molecules will be discussed here.

Vacuoles are the major organelles, known as the final storage point of and sites for biosynthesis of secondary metabolites including many important alkaloids in plant cells (36-40). In medicinal plant, *Catharanthus roseus*, anti-cancer vinblastine and vincristine are important dimeric terpenoid indole alkaloids, biosynthesized from the central intermediate strictosidine. The central intermediate is biosynthesized in the vacuoles of leaf epidermal cells of *C.roseus* from tryptamine and secologanin. Their transport mechanism into the vacuole is not known, but the formation of strictosidine is catalyzed by strictosidine synthase localized in the vacuolar lumen (37). The biosynthetic pathway of vinblastine and vincristine is shown in Fig.1.

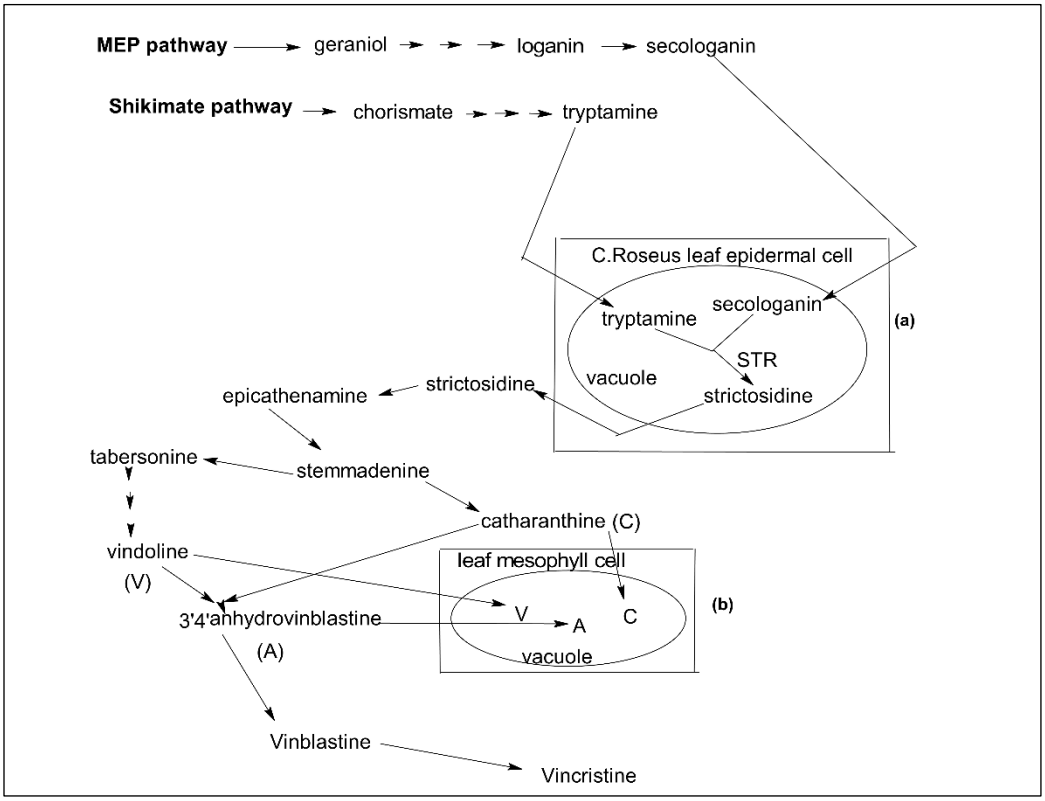

Figure 1: *Role of the vacuole in the biosynthesis of secondary metabolite, vinblastine in medicinal plant C.roseus.*

(a) In C.roseus leaf epidermal cells, two intermediates of vinblastine pathway, tryptamine and secolaganin, are transported into the vacuole, and converted to strictosidine by an enzyme strictosidine synthase (STR), located in the vacuolar lumen. Strictosidine is the central intermediate of vinblastine biosynthetic pathway.

(b) (b) Carqueijeiro et al. have also shown that in C.roseus mesophyll cells, vacuolar accumulation of vindoline, catharanthine and anhydrovinblastine prior to final step of vinblastine biosynthesis (Carqueijeiro et al., 2013).

As shown in Fig.1, final step of biosynthesis of vinblastine takes place from two biosynthetic intermediates, the monomeric monoterpenoid indole alkaloids catharanthine and vindoline (38). Before the vacuolar accumulation of the mesophyll cells of the main leaf of *C.roseus*, the biosynthetic intermediates travel through different cells during biosynthesis of vinblastine and vincristine. A transporter known as ABC (ATP-binding cassette) transporter, CrTPT2 which localizes in plasma-membrane helps in the efflux of catharanthine to the leaf surface. This phenomenon helps in plant protection against herbivores, the accumulation of catharanthine at leaf surface inhibits the production of vinblastine, vincristine as vindoline stays in internal leaf cells. Down regulation of the expression of the transporter, CrTPT2, inhibits the targeting of catharanthine at the leaf surface and increases the availability of catharanthine for the synthesis of dimeric alkaloids in internal leaf cells. This indicates how organelle specificity and transport engineering dictates the production of valuable compounds at the high-level (41,42).

Vacuoles in plant cells are also the site where many important flavonoids are transported/ accumulated. Flavonoids are accumulated in vacuoles/cell wall probably to attract different pollinators through influencing their colors, and to protect cells from different stresses (43,44). The coloration of flowers and fruits specially different fruit berries and their transition from growth to ripening, all these depends upon the flavonoids, they, therefore, should be properly transported and stored in distinct organelles, e.g., vacuoles, cell wall (43-45). However, till now, the knowledge about the transport and accumulation of the flavonoids is very limited (46). Anthocyanins, one major class of flavonoids, found in grapevine tissues and other

plants, whose transport and accumulation in vacuoles have been well studied (47,48). Various transporters, e.g., ABC transporters, MATE transporters have been involved in the transport and accumulation process of anthocyanins. Glycosidic form of anthocyanins and their further modified forms e.g. hydroxylated, methylated and esterified forms increases their stability, colour variation of the pigments, as well as they facilitate the binding of the transporters. Anthocyanin glucosides when in malonylated forms binds strongly with MATE transporters, and thus transportation of the pigment into vacuoles of leaf cells in *Medicago truncatula* (49) is fecilitated, and consequently this helps in pigmentation of leaves and in flower coloration. ABC transporters are also involved in anthocyanin transport. This transporter prefers the glutathione conjugated anthocyanin and transport them into vacuoles through the hydrolysis of ATP. This is based by the finding that a mutant defective in GST (glutathione S- transferase) unable to accumulate anthocyanin into vacuoles of maize (50). These studies indicate that specific localization of MATE and ABC- transporters on the vacuolar membranes facilitates the transport of this flavonoid in the very organelle. How this transport is regulated by various regulators/transcription factors that will be discussed in the next section.

Peroxisome is a spherical intracellular organelle found in all eukaryotes from microbes to plants and animals and are the sites for accumulation and biosynthesis of many important molecules. These organelles contain at least one H_2O_2-producing oxidase and H_2O_2-decomposing catalase and thus protect cells via generation of toxic metabolites. The organelles are also the sites for acetyl CoA generation through β-oxidation of fatty acids. The valuable and important β-lactam antibiotic penicillin in it's final step is synthesized in this compartment of fungus, *P.chrysogenum* (51). The enzymes isopenicillin N-acyltransferase (IAT) and phenylacetyl-CoA ligase (PCL) responsible for conversion and ligation of isopenicillin N (IPN) to penicillin G are localized in this compartment (Fig.2).

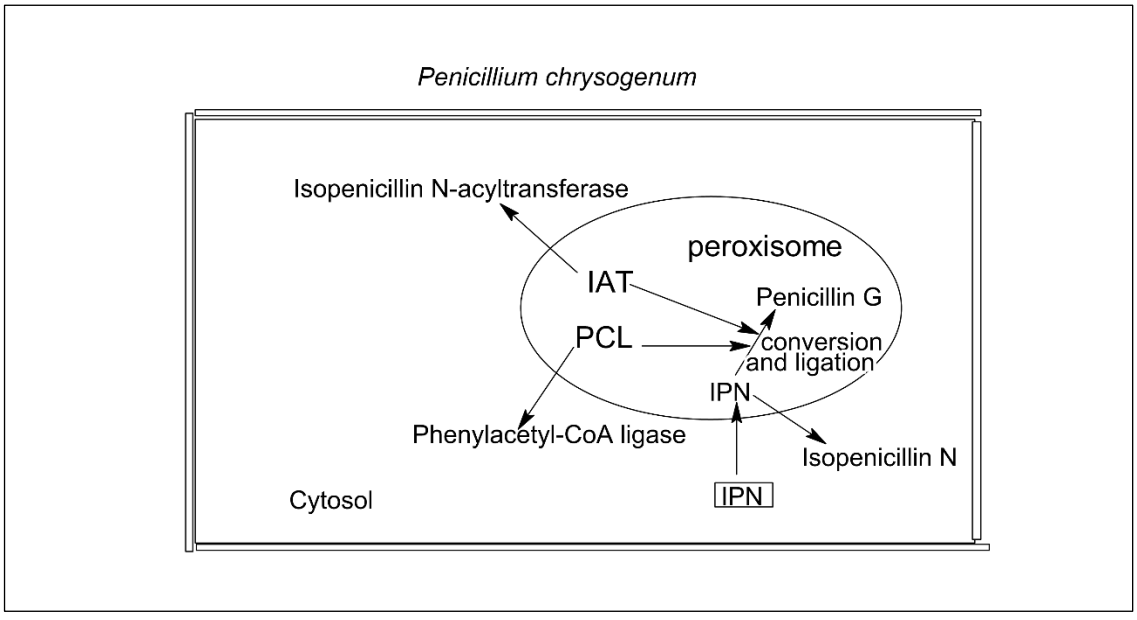

Figure 2: *Beta-lactam antibiotic penicillin synthesis in peroxisome of the fungus P. Chrysogenum*

In it's final step of biosynthesis, the enzymes isopenicillin N-acyltransferase (IAT) and phenylacetyl-CoA ligase (PCL) are involved in conversion and ligation of isopenicillin N (IPN) to penicillin G are localized in peroxisomes. IPN (the beta-lactam nucleus) is formed into the cytosol and then transported to peroxisome.

IPN (the β-lactam nucleus) is formed into the cytosol and then transported to peroxisome. The enzyme IAT helps in the exchange of α-amino adipyl side chain of IPN with CoA-activated phenylacetic acid and the enzyme PCL acts in the activation of side chain precursor during formation of penicillin G (52). These enzymes also help in the formation of other forms of penicillin through the exchange of other carboxylic acid substrates. These results indicate peroxisomes play important role for efficient production of penicillin. High penicillin producing strains expresses a greater number of peroxisomes (53). Another important antibiotic, tetracycline, the most used molecule in livestock and human worldwide, is produced by the Streptomyces genus of Actinobacteria. Despite having importance of these bacteria in the production of the antibiotic, the organelles of the bacterial cells for accumulation and biosynthesis of this antibiotic is not yet known. In fact, the cellular features of Streptomyces and their industrial strains are very poorly understood. However, in some industrial strain of *Streptomyces vinaceus* L-6 which produces viomycin, a large number of organelles with electron-dense dark contents were found. Viomycin, a nonribosomal peptide antibiotic having affinity for heavy metals strongly visible as dark material under electron microscope (54), and these types of organelles also been observed in other *Streptomyces* strains, e.g., *S.erythreus*, *S.melanochromogenes* (54,55). Finding these types of organelles in bacterial cells will really help in identifying the sites of antibiotic accumulation and biosynthesis. The biosynthesis of tetracyclines in *Streptomyces aureofaciens* was studied using its mutant culture and substrate feeding experiments. Feeding of pretetramid and 6-methylpretetramid in mutant culture could restore the biosynthesis of tetracycline, however, feeding of C4-dimethylamino-pretetramid did not restore tetracycline biosynthesis indicating pretetramid and 6-methylpretetramids are key intermediates in the biosynthesis (56). Finally, as we know, for it's action as antibiotic in target cells, tetracycline has to move to the cell cytoplasm, where it binds to 30S ribosomal subunit and blocks the protein synthesis of the target cell. For this, tetracycline penetrates the cell walls of organism e.g., gram-negative bacteria with the formation of positively charged magnesium-tetracycline complexes and use the OmpF and OmpC porin channels to cross the outer membrane (57,58). Then, they enter the periplasmic space and tetracycline dissociates and accumulates as uncharged tetracycline. This in short covers the transport mechanism of the antibiotic tetracycline around the cell.

Aflatoxisomes are another important organelles responsible for aflatoxin biosynthesis and its transport to the cell exterior. Aflatoxins are synthesized by several fungal species in the genus Aspergillus. The biosynthetic pathway for this mycotoxin has been studied very thoroughly and this pathway has now become model system to study secondary metabolism in eukaryotes (59,60). Using purified aflatoxisome vesicles and analysing proteomic profiles of the vesicles, it has been shown that various aflatoxin pathway enzymes are present in the organelles (61). In addition, in the past few years, the genes involved and the entire aflatoxin gene cluster of the aflatoxin biosynthetic pathway have been characterized (62,63).

In the following section, the regulatory role of various regulators in the regulation of transport process of the intermediates/biosynthetic enzymes and subsequently biosynthesis of the SMs in different organelles of the cells will be discussed.

Role of regulators (cluster situated and global) in SM-specific gene cluster regulation, vesicle targeting of biosynthetic enzymes, intermediates and transporters

The drugs/antibiotics and other important SMs discussed above are all derived from specific gene clusters. How different regulators regulate these clustered genes which directly or indirectly affects in the localization of the biosynthetic enzymes, intermediates, transporters of the secondary metabolites to the specific vesicles will be discussed here.

In plant specialized metabolism, biosynthetic gene clusters for several secondary metabolites including nutraceuticals, glycoalkaloids, drugs, e.g., noscapine, vinblastine have been reported (18,19, 64-66). From the genomic research of medicinal plant, *C. roseus*, the important anticancer drug vinblastine was discovered. of this plant Surprisingly, gene cluster in the genome encoding vinblastine pathway biosynthetic enzymes and the MATE transporter genes were found to be coexpressed (67). MATE was also found to be coexpressed as SbMATE2 in gene clusters encoding cyanogenic

glucoside biosynthetic enzymes in the *Sorghum bicolor* genome (68). MATEs, a class of transporters involved in transport of natural product biosynthetic intermediates whose coexpression is quite common in bacterial and fungal biosynthetic gene clusters (69-71). The coexpression of these transporter genes in plant biosynthetic gene clusters might also have significance in transport of specific biosynthetic enzymes and intermediates in specific organelles for specific secondary metabolite biosynthesis. In C.roseus genomes, partial clustering of genes of biosynthetic enzymes TDC/STR of vinblastine biosynthetic pathway occurs. TDC is a Tryptophan decarboxylase that generates tryptamine from tryptophan, and STR is strictosidine synthase responsible in the generation of strictosidine, the central intermediate in vinblastine biosynthetic pathway (37).

There are now plenty of evidences that vinblastine pathway gene clusters/genes are strictly regulated by various regulators/transcription factors. ORCA2 and ORCA3 are two transcription factors of large family AP2/ERF transcription factors characterized as critical regulators in vinblastine biosynthesis (72-74). In *C.roseus*, ORCA2 to ORCA6 forms a cluster (75,76) that regulates the biosynthesis of important terpenoid indole alkaloids (TIA). Though, ORCAs are key regulators in TIA biosynthetic pathways, there are some TFs, e.g., BIS2, ZCT2 and WRKY2 act as negative regulators in the pathway. The ORCA regulators are highly expressed in flowers, roots and stems and induced by different inducers. These regulators bind specifically to the elicitor-responsive element in STR promoter region and interferes in changes of the expressions of STR and modulates in the expression of other biosynthetic genes of vinblastine pathway (Fig. 3).

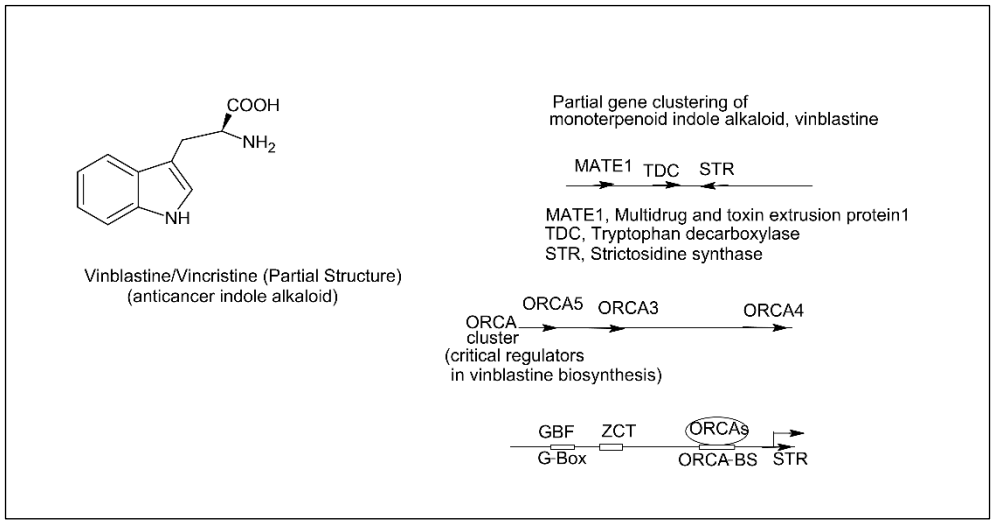

Figure 3: *Critical regulators of vinblastine biosynthetic pathway.*

The regulators ORCA of AP2/ERF transcription factor family form cluster and regulate biosynthesis of terpenoid indole alkaloids in medicinal plant C. roseus. In the vinblastine biosynthetic pathway genes, TDC/STR are partially clustered in the genome where MATE1 transporter genes are also co-expressed. Co-expression may facilitate specific localization of biosynthetic enzymes and intermediates to specific organelles. Now, ORCA regulators bind in the promoter region and interfere in the expression of STR and modulate in the expression of other biosynthetic genes of the vinblastine pathway. Upregulation of these regulators also helps in the transport and accumulation of many intermediates of the vinblastine pathway, e.g., catharanthine, tabersonine, to the specific organelle of biosynthesis.

The upregulation of ORCA2 in the cell also plays an important role in the transport and the accumulation of various biosynthetic intermediates e.g., catharanthine and tabersonine of vinblastine pathway in the specific organelles of the cell via the regulation of vinblastine/vincristine metabolism (77,78). ORCA2, ORCA3 and other signaling cascades/mechanism induced biosynthesis of vinblastine/vincristine and vacuolar localization of intermediates in *C.roseus* cells requires lots of energy. These might require the induction of MATEs which

could be used as secondary transporters (79). However, further studies will be required to support this hypothesis. In addition, many other transporters which play important role in plant and microbe secondary metabolism and whose expressions are regulated by various regulators are shown in Table 1.

Table 1. Transporters in plant and microbes involved in the transport of biosynthetic enzymes, intermediates to a specific organelle in the biosynthesis of secondary metabolites.

Transporter Family	Function	Source	Concerned secondary Metabolite	References
MATE	vacuolar targeting of alkaloids	*N.tabacum*	Alkaloid	(Shitan et al.2015)
MATE	transport catechin and anthocyanin	*A.thaliana*	Phenolic polyketides	(Marinova et al.2007)
MATE	vacuolar accumulation of nicotine	*N.tabacum*	Alkaloid	(Shoji et al. 2008)
ABC	transport of mono-terpenes, indolealkaloids	*C.roseus*, *H.irregulare*	Vinca alkaloids, terpenoids	(Shitan,N. 2016, Baral et al.2016)
ABC	Drug efflux	*S.rimosus*	Phenolic/polyketides	(Petkovic et al.2006)
PUP	Purine, nicotine uptake	*N.tabacum*	Nicotine/alkaloids	(Kato et al. 2014)
MFS	Cephalosporin transport	*A.chrysogenum*	NRPs	(Ullan et al.2010)
MFS	transporter in antibiotic biosynthesis	*P.chrysogenum*	NRPs	(Juan 2020)

Table 1a. Co-expressed transporters with biosynthetic gene clusters of secondary metabolites

Transporters	Biosynthetic gene clusters	Plant/microbes	Secondary Metabolite
MATE	TDC/STR, partial clustering vinblastine biosynthetic pathway	*C.Roseus*	Vinblastine
MATE/SbMATE2	Cyanogenic glucoside Gene cluster	*Sorghum bicolor*	Cyanogenic glycosides
MFS/cefM, cefP	Cephalosporin gene cluster	*A.chrysogenum*	Cephalosporin

Another important secondary metabolite, anthocyanin, is responsible for skin color of various fruits/crops, whose regulatory system of biosynthesis is controlled by MYB-type transcription factors (80-82). In purple color rice plants, OsC1, a gene encoding R2R3-MYB transcriptional factor specifically binds to a pigmentation gene OsPa to activate OsDFR encoding dihydroflavonol 4-reductase, and other anthocyanin biosynthesis genes (83). All these proteins are localized in the nucleus or cytoplasm and plays important role in the regulatory mechanism of anthocyanin biosynthesis in rice (84). In pears, the expression of anthocyanin biosynthetic genes and R2R3 MYB transcription factor, PcMYB10 found to be strongly correlated with anthocyanin accumulation during developmental stages of fruits. The expression patterns during clustering analysis showed that most of the anthocyanin biosynthetic genes and PcMYB10 genes are related to the same cluster (85). In grapevine (*V.vinifera*) fruit, the dark skin color of the fruit is due to the accumulation of anthocyanins. Accumulation of anthocyanins and their transport in cell organelles via MATE transporters, is regulated by MYB-type transcription factors (86). In *V.vinifera,* MATE transporters are directly or indirectly transcriptionally regulated by MYB-transcription factors. The MYB-transcription factor, VvMYBA1, transcriptionally regulate the enzyme, *V.vinifera* vacuolar H^+ PPase 1;2, *Vv*VHP1;2. The overexpression of the enzyme led to the increased expression of VvMATE3. In *V.vinifera*, anthoMATE1 and anthoMATE3 were found to be tonoplast-localized MATE transporters that transport acylated anthocyanins in the vacuole (80,87). These results collectively show that regulators-biosyn-

thetic genes/clusters-transporters network functions altogether for transport, accumulation and biosynthesis of secondary metabolites.

The regulation mechanism of important antibiotic tetracycline from Streptomyces recently got importance due to having not only it's enormous potential against various bacteria but also for it's anticancerous activity (88). Therefore, tetracycline molecules and their derivativesall are significantly important from pharmaceutical point of view. Oxytetracycline and chlorotetracycline are two such notable molecules in tetracycline family and both are derived from their respective gene clusters. Now regarding regulation of these gene clusters, a gene known as otcR (oxytetracycline regulator) encoding a SARP protein, was discovered in *Streptomyces rimosus* (89) which is located immediately adjacent to otrB gene of oxy cluster (90). Later, the SARP-binding sequences of the promoter regions of oxyclusters were characterized and it has been shown that otcR directly activated the transcription of oxy promoters (91). Interestingly, during working with chlorotetracycline (CTC), Wang et al. (92) found that the SARP regulator, encoded by ctcB from CTC gene cluster was able to activate the transcription of oxy cluster in heterologous host. It was proposed that since chloro and oxy-tetracyclines are structurally similar antibiotics, they might share the similar regulatory mechanisms.

The regulation of biosynthesis of another important β-lactam antibiotic, penicillin in filamentous fungi is controlled by global regulators (93,94). The penicillin pathway biosynthetic enzymes encoded by genes, penDE, pcbC and pcbAB are clustered in the genome of *Penicillium chrysogenum*. No regulators of penicillin biosynthesis are found in this gene clusters; however, global regulators, e.g., LaeA , PacC etc. have been identified (Fig.4).

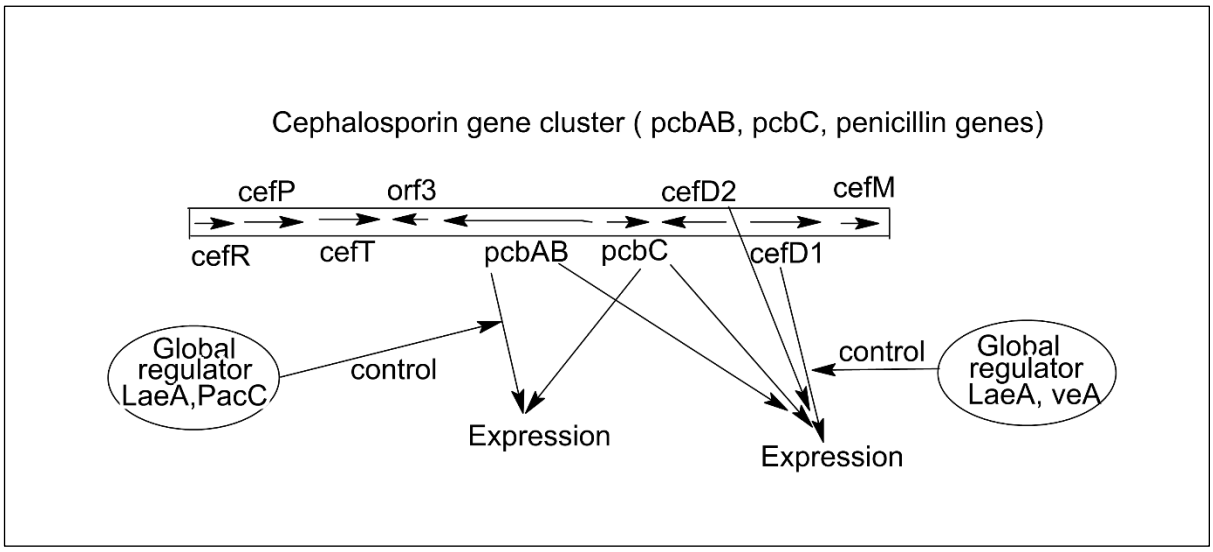

Figure 4: *Global regulators in the regulation of penicillin, cephalosporin gene clusters in P.chrysogenum, Acremonium and Streptomycetes.*

Global regulators LaeA and PacC control the expression of penicillin genes pcbAB, pcbC, and penDE, whereas LaeA and VeA control the cephalosporin genes pcbAB, pcbC, cefD1, and cefD2 expression. Genes pcbAB and pcbC encoding the first two enzymes valine synthetase and isopenicillin N synthase of the cephalosporin pathway, are very similar to those involved in penicillin biosynthesis. Two genes, cefT and cefM, of transporter MFS family, are involved in the transport of intermediates and the secretion of cephalosporins are also co-expressed in the cluster. The regulator LaeA regulates the synthesis through a SAM binding site, unique for methyltransferases. Thus it regulates the gene clusters through chromatin modification and heterochromatin repression.

Global regulators LaeA and PacC control the expression of penicillin genes pcbAB, pcbC, and penDE, whereas LaeA and VeA control the cephalosporin genes pcbAB, pcbC, cefD1, and cefD2 expression. Genes pcbAB and pcbC encoding the first

two enzymes valine synthetase and isopenicillin N synthase of the cephalosporin pathway, are very similar to those involved in penicillin biosynthesis. Two genes, cefT and cefM, of transporter MFS family, are involved in the transport of intermediates and the secretion of cephalosporins are also co-expressed in the cluster. The regulator LaeA regulates the synthesis through a SAM binding site, unique for methyltransferases. Thus it regulates the gene clusters through chromatin modification and heterochromatin repression.

PacC activates penicillin biosynthesis at alkaline pH (93), while LaeA was found in light-dependent fungal morphology and development to secondary metabolism as in *Aspergillus nidulans* (94). About 50% of BGCs in fungi was shown to be affected by LaeA and reduced secondary metabolite formation including penicillin was observed with the loss of LaeA (95). Deletion of these regulators blocks the expression of penicillin and other gene clusters and conversely overexpression of this regulator induces penicillin and other gene transcription and corresponding product formation (95, 96). Penicillin biosynthesis also requires specific localization of biosynthetic enzymes and intermediates to specific organelles and several transporters play important roles in the process. In biosynthetic process, phenylacetyl-CoA ligase and isopenicillin N acyl transferase, the last two enzymes of penicillin pathway along with the intermediates isopenicillin N and phenylacetic acid were located in peroxisomes(97). Two MFS (major facilitator superfamily) transporters PenM and PaaT were shown to be involved in the import of intermediates into peroxisomes (98). Similar vesicle localization of the intermediates was also seen in *Acremonium chrysogenum* during cephalosporins (CPC), another class of β-lactam antibiotic biosynthesis. Isopenicillin N is being converted through a long pathway in *A.chrysogenum* into penicillin N. In the conversion process, Isopenicillin N is epimerized to penicillin N by the action isopenicillin N-CoA ligase and the isopenicillin N-CoA epimerase encoded by genes cefD1 and cefD2 (99,100). These enzymes along with MFS transporters were shown to be localized in peroxisomes. In the early cephalosporin gene cluster, pcbC, pcbAB, cefD1, cefD2 genes along with two genes encoding MFS transporters, cefM and cefP were found to be present (Fig.4). Generally, these MFS superfamily transporters are regulated by Yap1 transcription factors, however, recently, Perez-Perez et al. (101) reports that Yap1 protein, PcYap1 binds to the regulatory sequence TTAG-TAA in the pcbAB gene promoter of *P.chrysogenum*. This is also a first report showing a Yap1 protein in addition to its other role regulating transcription of a secondary metabolism gene. No doubt, these finding indicates how the regulators of gene clusters and transporters coordinately acts in the expression of genes and localization of biosynthetic enzymes, intermediates and transporters to the specific organelles. Consequently, these processes help in the efficient biosynthesis of antibiotics.

Do cluster situate regulators (CSR) and global regulators (GR) act differently in the secondary metabolite biosynthetic pathway of plants and microbes?

In the previous section it has been discussed how various important secondary metabolites, drugs/antibiotics derived from different gene clusters are regulated by variety of CSRs and GRs. It was also discussed how these regulators play important role in various transport process responsible for efficient biosynthesis of these important molecules. Now it will be discussed whether at all and if yes, how these regulators work differently in plant and microbes.

In bacteria, the cluster situated regulator is more common; SARP (*Streptomyces* antibiotic regulatory protein) is one of the largest families of CSRs present in BGCs of Streptomyces. This bacteria is used to produce most of the today's antibiotics in use. The BGCs coding for many antibiotics from *Streptomyces* contain SARP family regulators. As discussed in the previous section that in oxytetracycline cluster, a gene known as otcR (oxytetracycline regulator) encoding a SARP protein was found immediately adjacent to otrB gene of antibiotic cluster in *Streptomyces rimosus* (89). Oxytetracycline regulators thereby help in the activation of the transcription of oxy promoters through the interaction of SARP binding sequences and the promoter regions of the clusters. The BGCs coding for another antibiotic actinorhodin in *Streptomyces coelicolor* (102) contain SARP family regulators that activate the transcription of biosynthetic genes within the cluster. In addition, there are many other SARP family regulators as CSRs found for many other antibiotics in *Streptomyces* species (103-105), and most importantly, beside SARPs there are other class of CSRs found not only in *Streptomyces* but also in

Gram-negative proteobacteria (106, 107). Nonetheless, in some cases, the cluster situated regulators may be controlled by global regulatory systems.

In fungi, approximately 60% of secondary metabolite gene cluster do not contain genes for regulatory proteins, but they are controlled by global regulators. That means for 40% gene clusters there are cluster situated regulators. As described in the previous section, global regulators, PacC, LaeA regulates penicillin biosynthesis in *Penicillium chrysogenum* (93,94). PacC activates penicillin biosynthesis at alkaline pH, whereas LaeA controls secondary metabolism in *A.nidulans* and other aspergilli in light dependent manner. Lae A is a universal regulator protein in several aspergilli and control the synthesis of penicillin and other antibiotics e.g., lovastatin, sterigmatocystin (108,109). This regulator as PcLaeA also regulates the secondary metabolism of *P.chrysogenm* (110). LaeA protein has methyl transferase activity and is thought to function at the level of chromatin modification (111). It was also proposed that LaeA regulates several gene clusters through repression of heterochromatin as suggested for sterigmatocystin gene cluster in *Aspergillus* (109,112), and in regulation of several genes in gilitoxin gene cluster of *Trichoderma reesei* (113). And for cluster specific regulators in fungi, they often make a direct connection with secondary metabolite formation network. A typical feature of cluster specific regulators is that they are mostly involved in the transcriptional activation as in AflR of the aflatoxin cluster in *A.flavus* (114) and in ApdR of aspyridone biosynthesis cluster in *A.nidulans* (115). For detailed actions of regulatory proteins in fungal secondary metabolism, an article by Knox et al. (116) may be consulted.

In plants, several cluster-specific transcription factors (TF) are found, though the genes of these transcription factors are not located within the metabolic gene clusters they control. These TFs remotely control the genes of metabolic gene clusters. In some cases these TFs forms clusters, however these clusters are not thoroughly identified and characterized (117). In the previous section, TF clusters of ORCA was discussed in the regulation of terpenoid indole alkaloid biosynthetic pathway (75,76). These ORCA regulators were also shown in the accumulation and transport of many biosynthetic intermediates of the pathway. In the control of cucurbitacin gene cluster in cucumber, melon, and watermelon (118), a novel basic helix-loop-helix, bHLH TF cluster consisting of two genes were found. These regulators strongly binds to the promoter region of the biosynthetic genes of cucurbitacin and helps in the biosynthesis of bitter substances cucurbitacins C (CuC), B (CuB), and E (CuE) of the fruits. Three clustered bHLH genes (BIS1, BIS2, and BIS3) were also found in the regulation of iridoid biosynthesis in terpenoid indole alkaloid biosynthetic pathway in *Catharanthus roseus* (119). Furthermore, other transcription factors have been found in the regulation of many clusters, e.g., GAME9 regulates the expression of steroidal glycoalkaloid gene clusters in potato and tomato (120) and basic leucine zipper domain,bZIP TF OsTGAP1 in momilactone gene cluster in rice and oat (121).

The above findings clearly indicate that cluster situated regulators over global regulators plays an important role in the production of huge number of valuable compounds as in bacteria. It may happen that for CSRs, due to the closer proximity of the regulators to the genes of the metabolic gene cluster, bacteria are able to regulate production of specific valuable compounds/ secondary metabolites at a much faster pace compared to plants and fungi. To date, almost all antibiotics globally used in the clinics are derived from bacteria and the contribution of plants and fungi in that respect still remains a few.

Concluding remarks:

Plants and microbes are tremendous source of natural products that may give rise to many valuable compounds including new target drug molecules. In the era of drug-resistance, continuous efforts are necessary for achieving new drugs and antibiotics. In fact, the gene clusters of plant and microbes may help develop new molecules, help explore the pathways wherein drugs can be made fit. Drugs can be developed particularly keeping in mind the specific pathway and/or specific reaction regulated by gene clusters.

The efficient biosynthesis of any valuable molecule/secondary metabolite(SM) in the specific vesicles of cells of plant or microbe depend upon the transport of all the ingredients like biosynthetic intermediates, enzymes, transporters into the specific vesicles. Though it is known that the genes of the biosynthetic enzymes, transporters in many cases are clustered within BGC of the genomes of the organisms, there are scanty of reports regarding

the regulation/activation of the gene clusters. The gene clusters and their regulators would definitely play important role in the transport and efficient biosynthesis of any valuable molecule/SM in specific vesicles of the cells of the organism. Various regulators like, cluster situated regulators and global regulators would be the main players in the discovery process. Cluster situated regulators are more common in bacteria, like SARP-activated gene clusters in Streptomyces, several thousands of antibiotics and secondary metabolites have been identified from these gene clusters. Still, researchers around the globe are struggling to develop methods for the activation of many silent or poorly expressed gene clusters in various plants and microbes.

Elucidation of the regulatory mechanisms and finding new regulators in the secondary metabolism specific gene clusters will provide deeper insight into SM biosynthesis and ensure identification of novel SMs.

Declaration of competing interest

The author does not have any conflict of interest.

Acknowledgments

The author gratefully acknowledges the overwhelming support from American Center Library and National Library, Kolkata.

References

1. A.M. Takos, C. Knudsen, D. Lai, et al., Genomic clustering of cyanogenic glucoside biosynthetic genes aids their identification in *Lotus japonicas* and suggests the repeated evolution of this chemical defense pathway, Plant J. 68 (2011) 273-286.

2. M. Itkin, U. Heinig, O. Tzfadia, et al. , Biosynthesis of antinutritional alkaloids in solanaceous crops is mediated by clustered genes, Science 341 (2013) 175-179.

3. P. Chakraborty, Herbal genomics as tools for dissecting new metabolic pathway of unexplored medicinal plants and drug discovery, Biochimie Open. 6 (2018) 9-16..

4. F. Kellner, J. Kim, B.J. Clavijo, et al., Genome-guided investigation of plant natural product biosynthesis, Plant J. 82 (2015) 680-692

5. T. Winzer, V. Gazda, Z. He, et al., A *Papaver somniferum* 10-gene cluster for the synthesis of the anticancer alkaloid noscapine, Science 336 (2012) 1704-1708.

6. S. Ziemons, K. Koutsantas, U. Kuck, et al., Penicillin production in industrial strain *Penicillium chrysogenum* P2niaD18 is not dependent on the copy number of biosynthesis genes, BMC Biotechnol. 17 (2017) 16

7. U. Lesnik, A. Gormand, V. Magdevska, et al., Regulatory elements in tetracycline-encoding gene clusters : the otc gene positively regulates the production of oxytetracycline *in Streptomyces rimosus,* Food Technol. Biotechnol. 47 (2009) 323-330.

8. P. Chakraborty, Gene cluster from plant to microbes : their role in genome architecture, organism's development, specialized metabolism and drug discovery, Biochimie 193 (2022) 1-15

9. A. Becernl, I. Perez-Victoria, S. Ye, et al., Discovery of cryptic largimycins in *Streptomyces* reveals novel biosynthetic avenues enriching the structural diversity of the leinamycin family, ACS Chem. Biol. 15 (2020) 1541-1553.

10. J.F. Martin, Transport systems, intracellular traffic of intermediates and secretion of β-lactam antibiotics in fungi, Fungal Biol. & Biotechnol. 7 (2020) 6

11. W.H. Meijer, L. Gidijala, S. Fekken, et al., Peroxisomes are required for efficient penicillin biosynthesis in *Penicillium chrysogenum*, Appl Environ Microbiol. 76 (2010) 5702-5709.

12. N. Shitan, Secondary metabolites in plants : transport and self-tolerance mechanisms, Biosci. Biotechnol. & Biochem. 80 (2016) 1283-1293.

13. V. De Luca, V. Salim, A. Thamm , et al., Making iridoids/secoiridoids and monoterpenoid indole alkaloids : progress on pathway elucidation, Curr. Opin. Plant Biol. 19 (2014) 35-42.

14. P. Verma, A.K. Mathur, A. Srivastava et al. , Emerging trends in research on spatial and temporal organization of terpenoid indole alkaloid pathway in *Catharanthus roseus* : a literature update, Protoplasma 249 (2012) 255-268.

15. B. Darbani, M.S. Motawia, C.E. Olsen, et al., The biosynthetic gene cluster for the cyanogenic glucoside dhurrin in *Sorghum bicolor* contains its co-expressed vacuolar MATE transporter, Sci. Rep. 6 (2016) 37079.

16. H.W. Nutzmann, A. Huang, A. Osbourn, Plant metabolic clusters-from genetics to genomics, New Phytol. 211 (2016) 771-789.

17. J.A. Boutanev, J. Zi, D.R. Nelson, et al., Investigation of terpene diversification across multiple sequenced plant genomes, Proc. Natl. Acad. Sci. USA, 112 (2015) E81-E88.

18. Y. Shang, Y.S. Ma, Y. Zhou, et al., Biosynthesis, regulation, and domestication of bitterness in cucumber, Science 346 (2014) 1084-1088.

19. S. Boycheva, I. Daviet, J.L. Wolfender et al., The rise of operon-like gene cluster in plants, Trends Plant Sci. 19 (2014) 447-459.

20. S. Swaminathan, D. Morrone, Q. Wang, et al., CYP76M7 phytoalexin/allelochemical biosynthesis is an ent-cassadiene C11alpha-hydroxylase defining a second multifunctional diterpenoid biosynthetic gene cluster in rice, Plant Cell 21 (2009) 3315-3325.

21. B. Diez, S. Gutierrez, J.L. Barredo et al., The cluster of penicillin biosynthetic genes : identification and characterization of the pcbAB gene encoding the alpha-aminoadipyl-cysteinyl-valine synthetase and linkage to the pcbC and penDE genes, J.Biol.Chem. 265 (1990) 16358-16365.

22. J.F. Martin, Molecular expression of penicillin biosynthesis genes in fungi: regulatory proteins interact with a bidirectional promoter region, J Bacteriol. 182 (2000) 2355-2362.

23. L.B. Pickens, Y.Tang, Decoding and engineering tetracycline biosynthesis, Metab Eng. 11 (2009) 69-75.

24. W. Zhang, K. Watanabe, C.C.C. Wang et al., Heterologus biosynthesis of amidated polyketides with novel cyclization regioselectivity from oxytetracycline polyketide synthase, J Nat Prod. 69 (2006) 1633- 1636.

25. A. Fournier-Level, T. Lacombe, L. Le Cunff et al., Evolution of the VvMybA gene family, the major determinant of berry colour in cultivated grapevine (Vitis vinifera L.), Heredity 104 (2010) 351-362.

26. B. Aigle, C. Corre, Waking up *Streptomyces* secondary metabolism by constitutive expression of activators or genetic disruption of repressors, Methods Enzymol. 517 (2012) 343-366

27. T.C. McLean, B. Wilkinson, M.I. Hutchings et al., Dissolution of the disparate: co-ordinate regulation in antibiotic biosynthesis, MDPI, antibiotics 8 (2019) 83-100.

28. S.J.W. Busby, Transcription activation in bacteria: Ancient and modern, Microbiology 165 (2019) 386-395.

29. A.A. Brakhage, Regulation of fungal secondary metabolism, Nat Rev Microbiol. 11 (2013) 21-32.

30. R. Makitrynskyy, B. Ostash, O. Tsypik , et al., Pleiotropic regulatory genes bldA, adpA, and absB are implicated in production of phosphoglycolipid antibiotic moenomycin, Open Biol. 3 (2013) 130121.

31. S. Rigali, F. Titgemeyer, S. Barends, et al., Feast or famine : the global regulator DasR links nutrient stress to antibiotic production by *Streptomyces*, EMBO Rep. 9 (2008) 670-675.

32. A.M. Calvo, The VeA regulatory system and its role in morphological and chemical development in fungi, Fungal Genet Biol. 45 (2008) 1053-1061.

33. J. Zhang, H. Chen, M.W. Sumarah , et al., VeA gene acts as a positive regulator of conidia production, ochratoxin A biosynthesis, and oxidative stress tolerance in *Aspergillus niger*, J. Agric Food Chem. 66 (2018) 13199-13208.

34. J.W. Bok, N.P. Keller, LaeA , a regulator of secondary metabolism in *Aspergillus spp*., Eukaryot Cell. 3 (2004) 527-535.

35. K. Kosalkova, C. Garcia-Estrada, R.P. Godio, et al., The global regulator LaeA controls penicillin biosynthesis, pigmentation and sporulation, but not roquefortine C synthesis in *Penicillium chrysogenum,* Biochimie 91 (2009) 214-225.0

36. R. Croteau, T.M. Kutchan, N.G. Lewis, et al., Natural products (secondary metabolites) , biochemistry & molecular biology of plants, Am. Soc. Plant Physiol. (2000) 1250-1318.

37. N. Shitan, K. Yazaki, Dynamism of vacuoles toward survival strategy in plants, Biochimica et Biophysica Acta 1862 (2020) 183127

38. M. Wink, The plant vacuole: a multifunctional compartment, J.Exp.Bot. 44 (1993) 231-246.

39. I. Carqueijeiro, H. Noronha, P. Duarte, et al., Vacuolar transport of the medicinal alkaloid from *C.Roseus* is mediated by a proton–driven antiport, Plant Physiol. 162 (2013) 1486-1496.

40. B. Deus-Neumann, M.H. Zenk, A highly selective alkaloid uptake system in vacuoles of higher plants, Planta 162 (1984) 250-260,

41. F. Yu, V. De Luca, ATP-binding cassette transporter controls leaf surface secretion of anticancer drug components in *Catharanthus roseus*, Proc. Natl. Acad. Sci. USA 110 (2013) 15830-15835.

42. N.Shitan, F. Dalmas, K. Dan, et al., Characterization of *Coptis Japonica* CjABCB2, an ATP-binding cassette protein involved in alkaloid transport, Phytochemistry 91 (2013) 109-116.

43. I.M. Van Der Meer, M.E. Stam, A.J. Van Tunen, et al., Antisense inhibition of flavonoid biosynthesis in petunia anthers results in male sterility, Plant Cell 4 (1992) 253-262.

44. E. Petrussa, E. Braidot, M. Zancani, et al., Plant flavonoids-biosynthesis, transport and involvement in stress responses, Int. J. Mol. Sci. 14 (2013) 14950-14973

45. K. Yazaki, Transporter of secondary metabolites, Curr. Opin. Plant Biol. 8 (2005) 301-307.

46. K. Springob, J. Nakajima, M. Yamazaki, et al., Recent advances in the biosynthesis and accumulation of anthocyanins, Nat. Prod. Rep. 20 (2003) 288-303.

47. S. Conn, C. Franco, W. Zhang, Characterization of anthocyanic vacuolar inclusions in *Vitis vinifera* L. cell suspension cultures, Planta 231 (2010) 1343-1360.

48. F. He, L. Mu, G.L. Yan, et al., Biosynthesis of anthocyanins and their regulations in colored grapes, Molecules 15 (2010) 9057-9091.

49. J. Zhao, D. Huhman, G. Shadle, et al., MATE2 mediates vacuolar sequestration of flavonoid glycosides and glycoside malonates in *Medicago truncatula*, Plant Cell 23 (2011) 1536-1555.

50. K.A. Marrs, M.R. Alfenito, A.M. Lloyd, et al., A glutathione-S-transferase involved in vacuolar transfer encoded by the maize gene Bronze-2, Nature 375 (1995) 397-400.

51. C. Garcia-Estrada, I. Vaca, F. Fierro, et al., The unprocessable isopenicillin N acyltransferase (IATC103S) of *Penicillium chrysogenum* is located into peroxisomes and regulates the processing of the wild –type preprotein, Fungal Genet Biol. 45 (2008) 1043-1052.

52. C. Garcia-Estrada, I. Vaca, M. Lamas-Maceiras, et al., In vivo transport of the intermediates of the penicillin biosynthetic pathway in tailored strains of *Penicillium chrysogenum*, Appl Microbiol Biotechnol. 76 (2007) 169-182.

53. A. Herr, R. Fischer, Improvement of *Aspergillus nidulans* penicillin production by targeting AcvA to peroxisomes, Metabolic Engg. 25 (2014) 131-139.

54. W. Kurzatkwoski, J. Kuczerowska, Antibiotic biosynthesis and secondary metabolism in high-yielding strains of *Streptomyces, Penicillium chrysogenum* and *Acremonium* chrysogenum, Postepy Mikrobiologii 56 (2017) 422-428.

55. J.M. Weber, C.K. Wierman, C.R. Hutchinson, Genetic analysis of erythromycin production in *Streptomyces erythreus*, J Bacteriol. 164 (1985) 425-433.

56. S. Mahalingam, P.C. Kuzma, Y.C. John, et al., Synthesis of 6-methylpretetramid, the fully aromatic precursor of tetracycline, J.Am. Chem. Soc. 107 (1985) 7760-7761.

57. T.H. Grossman, Tetracycline antibiotics and resistance, Cold Spring Harb.Perspect.Med. 6 (2016) a025387.

58. G. Zhou, Q. Wang, Y. Wang, et al., Outer membrane porins contribute to antimicrobial resistance in Gram-negative bacteria, Microorganisms 11 (2023) 1690.

59. M.F. Dutton, Enzymes and aflatoxin biosynthesis, Microbiol. Rev. 52 (1988) 274-295.

60. J. Yu., P.K. Chang, J.W. Cary, et al., Comparative mapping of aflatoxin pathway gene clusters in *Aspergillus parasiticus* and *Aspergillus flavus*, Appl. Environ. Microbiol. 61 (1995) 2365-2371.

61. I. Caceres, A.A. Khoury, R.E. Khoury, et al., Aflatoxin biosynthesis and genetic regulation : a review, MDPI Toxins 12 (2020) 150.

62. M.S. Price, J. Yu, W.C. Nierman, et al., The aflatoxin pathway regulator AflR induces gene transcription inside and outside of the aflatoxin biosynthetic cluster, FEMS Microbiol. Lett. 255 (2006) 275-279.

63. P. Chang, The *Aspergillus parasiticus* protein AFLJ interacts with the aflatoxin pathway-specific regulator AFLR, Mol. Genet. Genom. 268 (2003) 711-719.

64. K. Shimura, A. Okada, K. Okada, etal., Identification of a biosynthetic gene cluster in rice for momilactones, J. Biol. Chem. 282 (2007) 34013-34018.

65. P. Chakraborty, Medicinal plant genome: a source of finding new enzymes, metabolic pathways and drug discovery, Int. J. Curr. Med. Pharm. Res. 4 (2018) 3318-3326.

66. X. Qi, S. Bakht, M. Leggett, et al., A gene cluster for secondary metabolism in oat : implications for the evolution of metabolic diversity in plants, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 8233-8238.

67. Y.-S. Ku, S.-S. Cheng, M.-Y. Cheung, The roles of multidrug and toxic compound extrusion (MATE) transporters in regulating agronomic traits, MDPI agronomy 12 (2022) 878.

68. B. Darbani, M.S. Motawia, C.E. Olsen, et al., The biosynthetic gene cluster for the cyanogenic glucoside dhurrin in *Sorghum bicolor* contains its co-expressed vacuolar MATE transporter, Sci.Rep. 6 (2016) 37079.

69. H. Omote, M. Hiasa, T. Matsumoto, et al., The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations, Trends Pharmacol Sci. 27 (2006) 587-593.

70. S. Miyamae, O. Ueda, F. Yoshimura, et al., A MATE family multidrug efflux transporter pumps out fluoroquinolones in Bacteriodes thetaiotaomicron, Antimicrob Agents Chemother. 45 (2001) 3341-3346.

71. G.W. kaatz, F. McAleese, S.M. Seo, Multidrug resistance in *Staphylococcus aureus* due to overexpression of a novel multidrug and toxin extrusion (MATE) transport protein, Antimicrob Agents Chemther. 49 (2005) 1857-1864.

72. C.Y. Li, A.L. Leopoid, G.W. Sander, et al., The ORCA2 transcription factor plays a key role in regulation of the terpenoid indole alkaloid pathway, BMC Plant Biol. 13 (2013) 155.

73. H. Zhang, S. Hedhili, G. Montiel, et al., The basic helix-loop-helix transcription factor CrMYC2 controls the jasmonate-responsive expression of the ORCA genes that regulate alkaloid biosynthesis in *Catharanthus roseus*, Plant J. 67 (2011) 61-71.

74. L. van der Fits, J. Memelink, The jasmonate-inducible AP2/ERF- domain transcription factor ORCA3 activates gene expression via interaction with a jasmonate-responsive promoter element, Plant J. 25 (2001) 43-53.

75. P. Paul, S.K. Singh, B. Patra, et al., Mutually regulated AP2/ERF gene clusters modulate biosynthesis of specialized metabolites in plants, Plant Physiol. 182 (2020) 840-856.

76. T. Shoji, L. Yuan, ERF gene clusters : working together to regulate metabolism, Trends Plant Sci., 26 (2021) 23-32.

77. D.H. Liu, W.W. Ren, L.J. Cui, et al., Enhanced accumulation of catharanthine and vindoline in *Catharanthus roseus* hairy roots by overexpression of transcriptional factor ORCA2, African J Biotech. 10 (2011) 3260-3268.

78. J. Memelink, P. Gantet, Transcription factors involved in terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*, Phytochem Rev. 6 (2007) 353-362.

79. T. Han, G. Miao, Strategies, achievements, and potential challenges of plant and microbial chassis in the biosynthesis of plant secondary metabolites, MDPI, Molecules, 29 (2024) 2106.

80. T. Sun, L. Xu, H. Sun, et al., VvVHP1;2 is transcriptionally activated by VvMYBA1 and promotes anthocyanin accumulation of grape berry skins via glucose signal, Front. Plant Sci. 8 (2017) 1811.

81. V. Ferreira, O. Pinto-Carnide, R. Arroyo-Garcia, et al., Berry color variation in grapevine as a source of diversity, Plant Physiol. Biochem. 132 (2018) 696-707.

82. L. Chen, B. Hu, Y. Qin, et al., Advance of the negative regulation of anthocyanin biosynthesis by MYB transcription factors, Plant Physiol. Biochem. 136 (2019) 178-187.

83. L. Meng, C. Qi, C. Wang, et al., Determinant factors and regulatory systems for anthocyanin biosynthesis in rice apiculi and stigmas, Rice 14 (2021) 37.

84. J. Zheng, H. Wu, H. Zhu, et al., Determining factors, regulation system, and domestication of anthocyanin biosynthesis in rice leaves, New Phytol. 223 (2019) 705-721.

85. L. Li, B, Zhao, L. Xi-Hong, et al., Differential expression of anthocyanin biosynthetic genes and transcription factor PcMYB10 in Pears (*Pyrus communis* L.), PLoS ONE 7 (2012) e 46070.

86. C. Gomez, N. Terrier, I. Torregrosa, et al., Grapevine MATE-type proteins act as vacuolar H+-dependent acylated anthocyanin transporter, Plant Physiol. 150 (2009) 402-415.

87. R. Perez-Diaz, M. Ryngajllo, j. Perez-Diaz, et al., VvMATE1 and VvMATE2 encode putative proanthocyanidin transporters expressed during berry development in *Vitis vinifera* L. Plant Cell Rep. 33 (2014) 1147=1159

88. J. Li, Y. Qin, C. Zhao, et al., Tetracycline antibiotics : potential anticancer drugs, Eur. J. Pharmacol. 956 (2023) 175949.

89. S. Yin, W. Wang, X. Wang, et al., Identification of a cluster-situated activator of oxytetracycline biosynthesis and manipulation of its expression for improved oxytetracycline production in *Streptomyces rimosus*, Microb Cell Fact. 14 (2015) 46.

90. J. F. Martin, P. Liras, Engineering of regulatory cascades and networks controlling antibiotic biosynthesis in *Streptomyces*, Curr Opin Microbiol. 13 (2010) 263-273.

91. M.J. Ryan, J.A. Lotvin, N. Strathy, et al., Clone of the biosynthetic pathway for chlorotetracycline and tetracycline formation and cosmids useful therein, US Patent, 5 (1999) 866,410.

92. P. Wang, W. Kim, L.B. Pickens, et al., Heterologus expression and manipulation of three tetracycline biosynthetic pathways, Angew Chem Int Ed Engl. 51 (2012) 11136- 11140.

93. J.W. Bok, N.P. Keller, LaeA , a regulator of secondary metabolism in *Aspergillus spp*, Eukaryotic Cell., 3 (2004) 527-535.

94. O. Bayram, S. Krappmann, M. Ni, et al., VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism, Science 320 (2008) 1504-1506.

95. K. Kosalkova, C. Garcia-Estrada, R.V. Ullan, et al., The global regulator LaeA controls penicillin biosynthesis, pigmentation and sporulation but not roquefortine C synthesis in *Penicillium chrysogenum*, Biochimie 91 (2009) 214-225.

96. J.W. Bok, D. Noodermeer, S.P. Kale, et al., Secondary metabolic gene cluster silencing in *Aspergillus nidulans*, Mol.Microbiol. 61 (2006) 1636-1645.

97. W. Kurzatkowski, A.G. Kuczerowska, Compartmentalization in cephalosporin C biosynthesis by industrial strains of *Acremonium Chrysogenum,* Postepy Mikrobiologii 54 (2015) 374-379.

98. J.F. Martin, Transport systems, intracellular traffic of intermediates and secretion of beta-lactam antibiotics in fungi, Fungal Biol Biotechnol. 7 (2020) 6.

99. J.F. Martin, R.V. Ullan, J. Casqueiro, Novel genes involved in cephalosporin biosynthesis: the three-component isopenicillin N epimerase system, Adv Biochem Eng Biotechnol. 88 (2004) 91-109.

100. R.V. Ullan, J. Casqueiro, O. Banuelos, et al., A novel epimerization system in fungal secondary metabolism involved in the conversion of isopenicillin N into penicillin N in *Acremonium chrysogenum*, J Biol Chem. 277 (2002) 46216-46225.

101. W.D. Perez-Perez, U. Carrasco-Navarro, C. Garcia-Estrada et al., bZIP transcription factors PcYap1 and PcRsmA link oxidative stress response to secondary metabolism and development in *Penicillium chrysogenum*, Microbial Cell Fctories 21 (2022) 50.

102. Y. Xu, A. Willems, C. Auyeung, et al., A two-step mechanism for the activation of actinorhodin export and resistance in *Streptomyces coelicolor,* mBio 3 (2012) 00191.

103. Y.S. Yan, Q.Y. Yang, L.S. Zhou, et al., MilR3, a unique SARP family pleiotropic regulator in *Streptomyces bingchenggensis*, Arch. Microbiol. 204 (2022) 631.

104. J. Li, Y. Li, G. Niu, et al., NosP-regulated nosiheptide production responds to both peptidyl and small-molecule ligands derived from the precursor peptide, Cell. Chem. Biol. 25 (2018) 143-153.

105. Y. Yan, H. Xia, The roles of SARP family regulators involved in secondary metabolism in *Streptomyces*, Front. Microbiol. 15 (2024) 1368809.

106. R. Devine, H.P. McDonald, Z. Qin, et al., Re-wiring the regulation of formicamycin biosynthetic gene cluster to enable the development of promising antibacterial compounds, Cell Chem. Biol. 28 (2021) 515-523.e5.

107. A.W. Puri, Specialized metabolites from methylotropic proteobacteria, Curr Issues Mol Biol. 33 (2019) 211-224.

108. J.K. Kennedy, S.G. Auclair, C. Kendrew, et al., Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis, Science 284 (1999) 1368-1372.

109. J.K. Hicks, K. Shimizu, N.P. Keller, Genetics and biosynthesis of aflatoxins and sterigmatocystin, In F. Kempken and J.W. Bennett (ed.), The mycota, vol. XI. Springer-Verlag, Berlin, Germany, (2002) 55-69.

110. C.E.H. Assaf, C. Zetina-Serrano, N Tahtah et al., Regulation of secondary metabolism in the *Penicillium* genus, Int J Mol.Sci. 21 (2020) 9462.

111. Z. Zhao, S. Gu, D. Liu, et al., The putative methyltransferase LaeA regulates mycelium growth and cellulase production in *Myceliophthora thermophila*, Biotechnol Biofuels Bioprod. 16 (2023) 58.

112. N. Kato, W. Brooks, A.M . Calvo, The expression of sterigmatocystin and penicillin genes in *Aspergillus nidulans* is controlled by veA, a gene required for sexual development, Eukaryot Cell 2 (2003) 1178-1186.

113. P.A. Castro, A. C. Colabardini, M. Moraes, et al., Regulation of gilotoxin biosynthesis and protection in Aspergillus species, PLOS Genetics 18 (2022) e1009965.

114. K.C. Ehrlich, J. Yu, P. J. Cotty, Aflatoxin biosynthesis gene clusters and flanking regions, J Appl Microbiol. 99 (2005) 518-527.

115. Z. Wasil, A.K. Khomaizon, C. Butts, et al., One pathway, many compounds: heterologous expression of a fungal biosynthetic pathway reveals its intrinsic potential for diversity, Chem.Sci. 4 (2013) 3845-3856.

116. B.P. Knox, N.P. Keller, Key players in the regulation of fungal secondary metabolism, Biosynthesis and Molecular Genetics of Fungal Sec.Metabolites, 2 (2015) 13-28.

117. Y. Zhang, Y. Lu, H.E. Sayyed, et al., Transcription factor dynamics in plants : Insights and technologies for in vivo imaging, Plant Physiol., 189 (2022) 23-36.

118. P. Paul, S.K. Singh, B. patra, et al., Mutually regulated AP2/ERF gene cluster modulate biosynthesis of specialized metabolites in plants, Plant Physiol. 182 (2020) 840-856.

119. S.K. Singh, B. Patra, P. Paul, et al., Revisiting the ORCA gene cluster that regulates terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*, Plant Sci. 293 (2020) 110408.

120. P. Cardenas, P. Sonawane, J. Pollier, et al., GAME9 regulates the biosynthesis of steroidal alkaloids and upstream isoprenoids in the plant mevalonate pathway, Nat Commun 7 (2016) 10654.

121. A. Okada, K. Okada, K. Miyamoto, et al., OsTGAP1, a bZIP transcription factor, coordinately regulates the inductive production of diterpenoid phytoalexins in rice, J Biol Chem. 284 (2009) 26510-26518.