Activation of Microbial Silent Gene Clusters: Genomics Driven Drug Discovery Approaches

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Abstract

Microorganisms have provided mankind with a plethora of small molecule natural products ranging from industrial enzymes to therapeutic agents. Analyses of microbial genome sequences have revealed the presence of numerous ‘silent’ or ‘cryptic’ biosynthetic gene clusters (BGCs). Activation of these cryptic biosynthetic pathways can pave way to the discovery of novel bioactive secondary metabolites (SMs). This article summarizes various approaches employed to unlock the hidden biosynthetic potential of microbes and methods developed to study their silent gene products.

Keywords: Secondary metabolites; Biosynthetic gene clusters; Silent gene activation

Introduction

The hidden microbial secondary metabolomes

Since the discovery and development of antibiotic penicillin, microorganisms have evolved as cornerstones of drug discovery process [1,2]. The end of the 20th century witnessed the upsurge of diverse secondary metabolites as anti-bacterial, anti-fungal, anti-parasitic, immune-suppressive and anti-cancer agents [3-5]. Even with this advanced chemical space, emerging and reemerging infectious diseases and resistant cancer types demand the discovery of novel chemical entities (NCEs). Conventional drug discovery process largely relies on classic bioassay guided approach which often leads to the rediscovery of known compounds. Moreover, laborious nature of the method as well as challenges associated with the purification and characterization of the compounds has kindled a withdrawal of pharmaceutical companies from natural product based drug discovery [6]. However, inefficacy of chemical synthesis to generate anticipated chemical diversity prompts the return of drug discovery to nature.

Secondary metabolites or small molecule natural products (SMNPs) confer adaptive advantages to the producing organisms serving as defense molecules, attractants or signaling agents [7,8]. Hence, secondary metabolite profile of an organism is likely to vary with the complexity of the niche they occupy [9]. In microbes, secondary metabolite biosynthetic genes are organized in contiguous DNA segments known as gene clusters. The rearrangement of modular genes on the course of evolution contribute to the structural diversity of natural compounds [10,11]. Analyses of microbial genome sequences have revealed the presence of several biosynthetic gene clusters (BGCs) that either remain silent or weakly expressed when cultured in laboratory conditions, presumably due to paucity of environmental cues required to trigger their activation [12,13]. Thus the biosynthetic potential of microorganisms is far greater than that was thought from classic bioactivity screens. Genome mining for novel natural compounds and activation of silent gene clusters has become a dynamic and rapidly advancing area of research in past years.

Triggering microbial silent BGCs

Environmental cues and co-cultivation: Microorganisms, in their natural realms form diverse multispecies communities. Secondary metabolites (SMs) play a key role in interspecies communication within the microbial communities. SMs also bolster survival value of microbes mediating stress tolerance in their natural environment and competition with co-existing microbes. In other words, specific environmental factors shape the secondary metabolic profiles of microorganisms in their natural habitats [14-16]. An empirical approach to activate silent BGCs involves cultivation of microbes in conditions mimicking their natural environment. It has been reported that the cultivation of marine bacteria on chitin, a polymer abundant in its native habitat, turned on several cryptic biosynthetic pathways [17]. Co-cultivations of bacteria-bacteria, fungi-fungi, or fungi-bacteria are naturally driven approaches which try to exploit the chemico-ecological relationships existing in microbial communities to activate silent gene clusters [18]. Since the successful production of polyketide, enacycloxin by Gluconobacter sp. W-315, upon co-cultivation with fungi Neurospora crassa or Aspergillus oryzae, in 1982, microbial co-cultivation has turned to be a major approach in natural product discovery [19-21].

SM production by microbes is observed to vary with composition of culture media and culture conditions [22]. Hence culturing of microbes in diverse media is a classic approach [often termed as OSMAC (one strain many compounds)] to activate silent BGCs [23,24]. Alteration of other cultivation parameters, such as temperature, salinity, flask shapes and aeration have proved effective in triggering cryptic biosynthetic pathways in A. ochraceus [22]. It has been reported that, the presence of various stress inducing chemicals such as menadione and hydrogen peroxide (oxidative stress inducers), and sodium chloride and sorbitol (osmotic stress inducers), significantly modulate the SM production in microbial cultures [25]. A high-throughput screening approach for activating silent gene clusters using small molecule elicitors demonstrated obvious activation of two cryptic gene clusters in Burkholderia thailandensis cultures [26]. This method facilitates the read out of target gene cluster activation by means of a genetic reporter construct. Isolation of novel compounds such as lunalides A and B, oxylipins, cladochromes F and G, nigerone A, chaetoglobosin-542, 540 and 510, sphaerolone, dihydrospachrolone, mutolide and pestalon, unveil the hidden biosynthetic potential of microbes and methods developed to study their silent gene products.

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Received: January 25, 2016; Accepted: May 26, 2016; Published May 30, 2016

Citation: Valayil JM (2016) Activation of Microbial Silent Gene Clusters: Genomics Driven Drug Discovery Approaches. Biochem Anal Biochem 5: 276. doi:10.4172/2161-1009.1000276

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within cryptic coelichelin biosynthetic cluster [28]. This guided the selection of iron deficient culture conditions for the identification of coelichelins.

**Mutagenic and epigenetic activation of silent gene clusters**

Several studies have demonstrated the importance of global as well as epigenetic regulatory mechanisms in activation of silent gene clusters. 'Ribosome engineering' has emerged as a propitious avenue for silent gene cluster activation based on the observation of dramatic increases in antibiotic biosynthesis in ribosomal mutant bacterial strains. A streptomycin resistant strain of *S. lividans* was found to produce abundant quantities of a blue pigmented antibiotic, actinorhodin, by a BGC that is customarily silent in *S. lividans* strains. It was found that this enhanced antibiotic production by *S. lividans* strain attributed to certain mutation in a gene coding for ribosomal protein S12 (rpsL gene), a component of 30 S subunit [29]. The mutation in ribosomal protein S12 enhances the stability of 70 S complexes, thereby augmenting the bacterial biosynthetic synthesis [30]. Bacterial gene expression is regulated by a bacterial alarmone, ppGpp, synthesized by ribosome. ppGpp positively regulates bacterial secondary metabolism when cells enter stationary phase. In response to nutrient limitation, stringent response is turned on following the binding of uncharged tRNA to ribosomal A site. This results in a transient increase of hyperphosphorylated guanosine nucleotide ppGpp, synthesized from GDP and ATP by relA gene product (ppGpp synthetase) [31].

In addition to the ribosomal mutations, certain mutations in RNA polymerase (RNAP) have been proven to play impressive roles in antibiotic overproduction. It was found that in *S. coelicolor* A3(2) and *S. lividans*, the impaired ability to produce antibiotic due to relA gene deletion could be circumvented by introducing certain rifampicin resistant mutations in RNAP β-subunit [32]. This impels RNAP to behave as stringent RNAP even in the absence of ppGpp [32,33]. Ribosome engineering to waken the silent BGCs target ribosomal proteins, translation factors or RNAP, assuming that the alteration in transcription and translation pathways can enhance biosynthetic gene expression. Ribosome and RNAP mutant strains may be obtained by applying selective pressure using antibiotics that target ribosome and RNAP. This approach successfully enhanced the yields of different classes of secondary metabolites including polyketides, macrolides, aminoglycosides, and nucleosides. In addition to its direct application in silent gene activation, ribosome engineering also enables construction of amenable hosts for heterologous expression [34].

Microbial genome sequence analyses reveal the presence of genes encoding transcription regulatory proteins within individual BGCs. Identifying these activator signals opens up a platform to activate BGC encoding transcription regulatory proteins within individual BGCs.

**Identifying cryptic gene products**

The identification of cryptic gene products from secondary metabolite pool is as challenging as the activation of BGCs. Bioinformatic analyses enables the prediction of putative functions of target gene clusters. Modular arrangement of biosynthetic genes allows prediction of metabolic building blocks incorporated into final product. Moreover, the presence or absence of domains with tailoring activities within individual modules enable prediction of modifications underwent by the building blocks [39-41]. Putative physicochemical properties of final product such as molecular masses or UV-visible absorbance, as predicted by bioinformatic tools can be employed for the detection of the compound in the fermentation broth [42]. 'Genomisotopic approach' or 'in vitro reconstitution approach' has been proven useful in cases of cryptic biosynthetic pathways in which substrates of biosynthetic enzymes are known. In genomisotopic approach, the organism is fed with stable isotope labelled precursor and NMR detection of labelled metabolites is used to guide their fractionation and purification. Orfamides, the novel macrocyclic lipopeptides produced by *Pseudomonas fluorescens* Pf-5 was identified by this approach [43]. In *in vitro* reconstitution approach, the predicted substrates are incubated with recombinant biosynthetic enzyme and the final products are characterized. This approach directed the identification of epi-isoziaene, the product of a cryptic sesquiterpene synthase gene cluster in *S. coelicolor* genome [44]. Another approach employed for the identification of cryptic gene products involve the inactivation or deletion of one or two genes predicted essential for metabolite biosynthesis. The comparison of metabolites in fermentation broths or extracts of wild type and mutant strains using analytical techniques such as liquid chromatography-mass spectrometry (LC-MS) allow the identification of cryptic gene products. Aspyridones, the metabolic products of cryptic gene clusters in *A. nidulans*, were identified by comparative metabolic profiling of wild-type and mutant strains [45]. A second comparative metabolic profiling approach involves the cloning of entire BGC into a single cosmid or BAC vector and its expression in a heterologous host. The metabolite profile of the heterologous host containing and lacking the cloned cryptic BGC are compared using LC-MS or other analytical techniques [46]. The strategies discussed have their limitations, depending on the expression of cryptic gene clusters, the structural information of the metabolite that can be deduced from bioinformatics analyses, and the size of target gene clusters. These points have to be considered for the successful selection of the best approach to identify the cryptic gene products.

**Conclusion**

Genomics driven searches for novel natural compounds have made remarkable progress in the past years (Figure 1). Genome mining approach sets the cutting edge of a second golden era of novel natural compound discovery. However, more advancement has to be made in...
developing and refining the general methods for silent gene activation as well as for the discovery of cryptic gene products. Collaborative approaches aligning metabolomics and metagenome based analyses can augment our grasping regarding the physiological role of secondary metabolites as well as the bio-discovery hit rates.

References

1. Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Metabolites 2: 303-336.
2. Crigg GM, Newman DJ (2013) Natural products: a continuing source of novel drug leads. Biochimica et Biophysica Acta (BBA)-General Subjects 1830:3670-3695.
3. Bentley R (1997) Microbial secondary metabolites play important roles in medicine; prospects for discovery of new drugs. Perspect Biol Med 40: 364-394.
4. Demain AL (2000) Microbial biotechnology. Trends Biotechnol 18: 26-31.
5. Bérdy J (2005) Bioactive microbial metabolites. J Antibiot (Tokyo) 58: 1-26.
6. Li JW, Vederas JC (2009) Drug discovery and natural products: end of an era or an endless frontier? Science 325: 161-165.
7. Vining LC (1990) Functions of secondary metabolites. Annu Rev Microbiol 44: 395-427.
8. Chadwick DJ, Whelan J (1992) Secondary metabolites: their function and evolution. John Wiley & Sons Ltd.
9. Luckner M (2013) Secondary metabolism in microorganisms, plants and animals. Springer Science & Business Media.
10. Cimermancic P, Medema MH, Claessen J, Kurita K, Wieland Brown LC, et al. (2015) Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. Cell 158: 412-421.
11. Osbourn A (2010) Secondary metabolic gene clusters: evolutionary toolkits for chemical innovation. Trends Genet 26: 449-457.
12. Hertweck C (2009) Hidden biosynthetic treasures brought to light. Nat Chem Biol 5: 450-452.
13. Chiang YM, Chang SL, Oakley BR, Wang CC (2011) Recent advances in awakening silent biosynthetic gene clusters and linking orphan clusters to natural products in microorganisms. Curr Opin Chem Biol 15: 137-143.
14. Yu JH, Keller N (2005) Regulation of secondary metabolism in filamentous fungi. Annu Rev Phytopathol 43: 437-458.
15. Fox EM, Howlett BJ (2008) Secondary metabolism: regulation and role in fungal biology. Curr Opin Microbiol 11: 481-487.
16. Bibb MJ (2005) Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol 8: 208-215.
17. Wietz M, Mansson M, Gram L (2011) Chitin stimulates production of the antibiotic antrimid in a Vibriocorallilicuicis strain. Environmental microbiology reports 3: 559-564.
18. Netzer T, Fischer J, Weber J, Mattern DJ, König CC, et al. (2015) Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol 6: 299.
19. Watanebe T, Izaki K, Takahashi H (1982) New polyenic antibiotics active against gram-positive and -negative bacteria. I. Isolation and purification of antibiotics produced by Gluconobacter sp. W-315. J Antibiot (Tokyo) 35: 1141-1147.
20. Schneekloth V, Soherlach K, Nützmann HW, Shelest, E., Schmidt-Heck W, et al. (2009) Intimate bacterial- fungal interaction triggers biosynthesis of archetypal polyketides in Aspergillus nidulans. Proceedings of the National Academy of Sciences 106: 14558-14563.
21. Moody SC (2014) Microbial co-culture: harnessing intermicrobial signaling for the production of novel antimicrobials. Future Microbiol 9: 575-578.
22. Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: possible ways to explore nature's chemical diversity. Chembiochem 3: 619-627.
23. Chiang YM, Lee KH, Sanchez JF, Keller NP, WC CC (2009) Unlocking fungal cryptic natural products. Nat Prod Commun 4: 1505-1510.
24. Takahashi JA, Teles APC, Bracarense ADAP, Gomes DC (2013) Classical and epigenetic approaches to metabolite diversification in filamentous fungi. Phytochemistry reviews 12: 773-789.
25. Valayil JM, Kuriakose GC, Jayabaskaran C (2015) Modulating the biosynthesis of a bioactive steroidal saponin, cholestanol glucose by Lassiodiplodia theobromae using abiotic stress factors. International Journal of Pharmacy and Pharmaceutical Sciences 7: 114-117.
26. Seyed Sayamdost MR (2014) High-throughput platform for the discovery of elicitors of silent bacterial gene clusters. Proc Natl Acad Sci USA 111: 7266-7271.
27. Pettit RK (2011) Small-molecule elicitation of microbial secondary metabolites. Micro Biotechnol 4: 471-478.
28. Challis GL, Ravel J (2000) Coelichelin, a new peptide siderophore encoded by the Streptomyces coelicolor genome: structure prediction from the sequence of its non-ribosomal peptide synthetase. FEMS Microbiol Lett 187: 395-427.
29. Komatsu M, Komatsu K, Koiwai H, Yamada Y, Kozone I, et al. (2013) Engineered communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol 6: 299.
30. Takahashi JA, Teles APC, Bracarense ADAP, Gomes DC (2013) Classical and epigenetic approaches to metabolite diversification in filamentous fungi. Phytochemistry reviews 12: 773-789.
31. Valayil JM, Kuriakose GC, Jayabaskaran C (2015) Modulating the biosynthesis of a bioactive steroidal saponin, cholestanol glucose by Lassiodiplodia theobromae using abiotic stress factors. International Journal of Pharmacy and Pharmaceutical Sciences 7: 114-117.
32. Chiang YM, Lee KH, Sanchez JF, Keller NP, WC CC (2009) Unlocking fungal cryptic natural products. Nat Prod Commun 4: 1505-1510.
33. Yokoyama Y, Takahashi J, Ohtani R, Inoue M, et al. (2004) Ribosome engineering and secondary metabolite production. Adv Appl Microbiol 56: 155-184.
34. Chatterji D, Ojha AK (2001) Revisiting the stringent response, ppGpp and transcriptional regulation. Curr Opin Microbiol 4: 160-165.
35. Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: possible ways to explore nature's chemical diversity. Chembiochem 3: 619-627.
36. Seyed Sayamdost MR (2014) High-throughput platform for the discovery of elicitors of silent bacterial gene clusters. Proc Natl Acad Sci USA 111: 7266-7271.
37. Pettit RK (2011) Small-molecule elicitation of microbial secondary metabolites. Micro Biotechnol 4: 471-478.
38. Challis GL, Ravel J (2000) Coelichelin, a new peptide siderophore encoded by the Streptomyces coelicolor genome: structure prediction from the sequence of its non-ribosomal peptide synthetase. FEMS Microbiol Lett 187: 395-427.
39. Komatsu M, Komatsu K, Koiwai H, Yamada Y, Kozone I, et al. (2013) Engineered communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol 6: 299.
40. Watanebe T, Izaki K, Takahashi H (1982) New polyenic antibiotics active against gram-positive and -negative bacteria. I. Isolation and purification of antibiotics produced by Gluconobacter sp. W-315. J Antibiot (Tokyo) 35: 1141-1147.
41. Schneekloth V, Soherlach K, Nützmann HW, Shelest, E., Schmidt-Heck W, et al. (2009) Intimate bacterial- fungal interaction triggers biosynthesis of archetypal polyketides in Aspergillus nidulans. Proceedings of the National Academy of Sciences 106: 14558-14563.
42. Moody SC (2014) Microbial co-culture: harnessing intermicrobial signaling for the production of novel antimicrobials. Future Microbiol 9: 575-578.
43. Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: possible ways to explore nature's chemical diversity. Chembiochem 3: 619-627.
35. Colombo V, Fernández-de-Heredia M, Malpartida F (2001) A polyketide biosynthetic gene cluster from Streptomyces antibioticus includes a LysR-type transcriptional regulator. Microbiology 147: 3083-3092.

36. Rodríguez M, Núñez LE, Brana AF, Méndez C, Salas JA, et al. (2008) Identification of transcriptional activators for thienamycin and cephamycin C biosynthetic genes within the thienamycin gene cluster from Streptomyces cattleya. Mol Microbiol 69: 633-645.

37. Gottelt M, Kol S, Gomez-Escribano JP, Bibb M, Takano E (2010) Deletion of a regulatory gene within the cpk gene cluster reveals novel antibacterial activity in Streptomyces coelicolor A3(2). Microbiology 156: 2343-2353.

38. Shwab EK, Bok JW, Tribus M, Galehr J, Graessle S, et al. (2007) Histone deacetylase activity regulates chemical diversity in Aspergillus. Eukaryot Cell 6: 1656-1664.

39. Challis GL, Ravel J, Townsend CA (2000) Predictive, structure-based model of amino acid recognition by nonribosomal peptide synthetase adenylation domains. Chem Biol 7: 211-224.

40. Rausch C, Weber T, Kohlbacher O, Wohlfleben W, Husson DH (2005) Specificity prediction of adenylation domains in nonribosomal peptide synthetases (NRPS) using transductive support vector machines (TSVMs). Nucleic Acids Res 33: 5799-5808.

41. Minowa Y, Araki M, Kanehisa M (2007) Comprehensive analysis of distinctive polyketide and nonribosomal peptide structural motifs encoded in microbial genomes. J Mol Biol 368: 1500-1517.

42. Lautru S, Deeth RJ, Bailey LM, Challis GL (2005) Discovery of a new peptide natural product by Streptomyces coelicolor genome mining. Nat Chem Biol 1: 265-269.

43. Gross H, Stockwell VO, Henkels MD, Nowak-Thompson B, Loper JE, et al. (2007) The genomisotopic approach: a systematic method to isolate products of orphan biosynthetic gene clusters. Chemistry & biology 14: 53-63.

44. Challis GL (2008) Mining microbial genomes for new natural products and biosynthetic pathways. Microbiology 154: 1555-1569.

45. Bergmann S, Schuermann J, Scherlach K, Lange C, Brakhage AA, et al. (2007) Genomics-driven discovery of PKS-NRPS hybrid metabolites from Aspergillus nidulans. Nat Chem Biol 3: 213-217.

46. Winter JM, Behnken S, Hertweck C (2011) Genomics-inspired discovery of natural products. Curr Opin Chem Biol 15: 22-31.