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Review

Regulation of secondary metabolism by chromatin structure and epigenetic codes

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ABSTRACT

Chromatin, composed of DNA wrapped around an octamer of histones, is the relevant substrate for all genetic processes in eukaryotic nuclei. Changes in chromatin structure are associated with the activation and silencing of gene transcription and reversible post-translational modifications of histones are now known to direct chromatin structure transitions. Recent studies in several fungal species have identified a chromatin-based regulation of secondary metabolism (SM) gene clusters representing an upper-hierarchical level for the coordinated control of large chromosomal elements. Regulation by chromatin transition processes provides a mechanistic model to explain how different SM clusters located at dispersed genomic regions can be simultaneously silenced during primary metabolism. Activation of SM clusters has been shown to be associated with increased acetylation of histones H3 and H4 and, consequently, inhibition of histone de-acetylase activities also leads to increased production of secondary metabolites. New findings suggest that SM clusters are silenced by heterochromatic histone marks and that the "closed" heterochromatic structures are reversed during SM activation. This process is mediated by the conserved activator of SM, LaeA. Despite the increase in knowledge about these processes, much remains to be learned from chromatin-level regulation of SM. For example, which proteins "position" the chromatin restructuring signal onto SM clusters or how exactly LaeA works to mediate the low level of heterochromatic marks inside different clusters remain open questions. Answers to these and other chromatin-related questions would certainly complete our understanding of SM gene regulation and signaling and, because for many predicted SM clusters corresponding products have not been identified so far, anti-silencing strategies would open new ways for the identification of novel bioactive substances.

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1. Introduction

Production of secondary metabolites by filamentous asco- and basidiomycetes is a specialized process that occurs only under specific environmental conditions or at specific points in their life cycle (Bennett, 1987; Hoffmeister and Keller, 2007). The term "secondary metabolism" (SM) is not well defined but it is generally accepted that it denotes a stage in the fungal life history at which "primary metabolism", i.e. metabolic processes connected with active growth (cell expansion and cell division), is phased out. It has been shown that the transition from primary to secondary

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metabolism is often correlated with depletion of nutrients, absence of light, development of reproductive structures, or changes in ambient pH (Calvo et al., 2002; Yu and Keller, 2005). Secondary metabolism is intimately connected to sexual fruiting body formation in *Aspergillus nidulans* or the production of sclerotia in *Aspergillus flavus* (Calvo et al., 2002; Chang et al., 2002). Also, conidiation (asexual spore formation) is coordinated with SM-gene expression through the production of spore-related products such as spore pigments by polyketide synthetases (Adams and Yu, 1998). Despite the enormous progress made in understanding the regulation and signal transduction of secondary metabolite gene expression, the plethora of these diverse environmental signals until now results in a quite fuzzy picture of regulatory keypoints in gene regulation of secondary metabolite genes.

Aspergilli represent a highly diverse group of filamentous fungi with a high capacity to produce secondary metabolites and the aflatoxin, sterigmatocystin (ST) and penicillin (PN) production pathways have served as instructive models to understand, at the molecular level, the regulation of the involved genes. Studies in *A. nidulans*, mainly on PN and ST gene clusters, have paved the way for a general understanding of secondary metabolic gene regulation. Results obtained from this model provided a constructive guidance for similar studies in economically important species such as *A. flavus*, *Aspergillus parasiticus*, *Penicillium chrysogenum* or *Fusarium graminearum* (Goswami et al., 2006; Kosalkova et al., 2009; Payne and Yu, 2010). Secondary metabolite (SM) signaling and regulation in *A. nidulans* has been dealt with in detail in a large number of reviews (Brakhage et al., 2004; Calvo, 2008; Hoffmeister and Keller, 2007; Keller et al., 2005). Recent results from a number of laboratories added a novel aspect of a higher-hierarchical level of regulation in this model fungus, i.e. the role of chromatin structure and nucleosome modifications in expression of secondary metabolite genes. Chromatin structure and function is currently one of the most actively studied topics in molecular biology and progress in the field is constantly reviewed from different perspectives. Numerous reviews have extensively dealt with the function and modifications of chromatin for basic cellular processes such as transcription, DNA replication and repair, RNA interference, and the role chromatin plays in mitotic and meiotic chromosome separation (Holbert and Marmorstein, 2005 and references therein).

Here we focus our view on chromatin processes related to fungal secondary metabolism and refer the reader to recent overview articles for more detailed information on mechanisms regulating chromatin-related processes.

2. Chromatin modifications and transcription

Chromatin is the complex of nuclear DNA with proteins in which the genetic material of eukaryotes is packed. The fundamental subunit of chromatin is the nucleosome. It consists of roughly 165 bp of DNA wrapped in two superhelical turns around an octamer of four different core histone proteins (two each of H2A, H2B, H3 and H4). This physical arrangement of DNA on nucleosomes results in a roughly 10-fold compaction which is additionally increased by interactions of neighboring nucleosomes to form a 30 nm chromatin fiber (Tremethick, 2007). Additional interactions with non-histone chromatin proteins and RNAs are believed to mediate higher-order chromatin structures, such as condensed mitotic or meiotic chromosomes (Felsenfeld and Groudine, 2003; Luger and Hansen, 2005; Woodcock and Dimitrov, 2001).

However, chromatin is both the natural substrate and a barrier for the molecular machinery that needs to contact and move along DNA during transcription or DNA replication. Thus, chromatin structure needs to be dynamic in order to adapt to different cellular requirements. Flexibility of the chromatin structure is achieved by ATP-dependent chromatin remodeling (Cairns, 2009) and by decorating specific residues of histone proteins with an array of different post-translational modifications (PTMs). This reversible marking includes positioning or removal of acetyl and methyl groups on lysines or arginines, but also phosphorylation of serines or threonines and ubiquitination of lysines (Kouzarides, 2007). An additional level of complexity is introduced by "*trans*-histone" regulation in which a given set of histone modifications regulates the positioning or removal of other modifications on the same histone or on neighboring histones in the nucleosome (Fischle et al., 2003; Suganuma and Workman, 2008; Wang et al., 2001).

The specific pattern of covalent modifications on a given genomic region is proposed to generate a "histone code" (Jenuwein and Allis, 2001) where the modified histone residues serve as docking stations for proteins which promote either an open (euchromatic) or a closed (heterochromatic) chromatin conformation. Euchromatic regions are rich in coding sequences, and their high transcriptional activity correlates with hyper-acetylated nucleosomal histones. Methylated lysines in H3 and H4 can have different impacts on chromatin structure. Whereas euchromatin is usually enriched in methylated H3K4, typical heterochromatic marks are characterized by H3K9, H3K27 and H4K20 methlyation. Pericentromeric and (sub)telomeric segments represent typical gene-poor "heterochromatic" regions which exhibit low levels of transcription (Grewal and Jia, 2007). Essential to the understanding of heterochromatin formation was the identification of the direct link between tri-methylation of H3K9 and binding of HP-1, a central protein functioning in the formation of repressive, highly condensed heterochromatin (Bannister et al., 2001; Horn and Peterson, 2006; Jacobs and Khorasanizadeh, 2002). Surprisingly, certain histone modifications can display dual functions as they can have different effects depending on the physical location of the chromatin stretch. For example, in *Saccharomyces cerevisiae*, H3K4 tri-methylation is a permissive mark for gene transcription in euchromatic regions but it is also required for gene silencing at mating type loci and sub-telomeric regions (Bryk et al., 2002; Mueller et al., 2006).

In filamentous fungi chromatin structure and function has only been studied in a few model systems (summarized in Fig. 1). Pioneering work has revealed an essential function of heterochromatic marks and HP-1 for DNA methylation and telomere silencing in *Neurospora crassa* (Honda and Selker, 2008; Kouzminova and Selker, 2001; Lewis et al., 2009; Selker et al., 2002, 2003; Smith et al., 2008; Tamaru and Selker, 2001; Tamaru et al., 2003). In the same organism histone acetylation and chromation remodeling has been studied in relation to transcriptional activation and repression processes during light regulation (Belden et al., 2007; Grimaldi et al., 2006). Experimental chromatin work in *A. nidulans* initially focussed on nucleosomal organization (Morris, 1976) and the connection of chromatin to regulation of the mitotic cell cycle (Osmani et al., 1988). The genomic inventory of the basic chromatin components in different filamentous fungi has been recently reviewed and chromatin regulation of several catabolic genes was summarized (Brosch et al., 2008; Scazzocchio and Ramon, 2008).

3. Regulation of fungal secondary metabolism by histone acetylation

The biosynthesis of secondary metabolites is a multi-enzyme process, and in most of the cases, the genes coding for the enzymes in a given biosynthetic pathway are clustered in the fungal genome (Keller and Hohn, 1997; Khaldi et al., 2010). In several pathways the specific transcription factor is also embedded within the