

## The Rise of Epigenetics in Microbial Eukaryotes

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Rec date: July 28, 2014, Acc date: August 20, 2014, Pub date: August 22, 2014

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### Abstract

The 12th European Conference on Fungal Genetics (ECFG) was held in Seville, Spain on March 23-27, 2014. The meeting covered topics as diverse as fungal genetics, genomics, molecular cell biology, evolution, pathogenesis, and biotechnology. Despite such diversity, as the meeting progressed, it became evident that epigenetics are now an important part of genomic studies on fungal biology and evolution. In the meeting, regulation of genome integrity, secondary metabolism gene cluster and light responses by histone modifications and also small RNA-mediated antifungal drug resistance were reported. In this report, we highlight those epigenetics-related talks presented in the 12th ECFG that give new insights into the biology and evolution of microbial eukaryotes.

**Keywords:** Fungi; Genetics; Fungal biology; Epigenetics; Epigenomics; Non-coding RNA introduction

### Standing on the Shoulders of Giants

The 12<sup>th</sup> European Conference on Fungal Genetics was held in the warm and sunny city, Seville, Spain, from the 23<sup>rd</sup> to the 27<sup>th</sup> of March this year. More than 700 attendees joined the meeting from all around the globe. There were three plenary sessions, nine concurrent sessions and two poster sessions covering topics ranging from genetics and genomics to molecular cell biology, evolution, pathogenesis, and biotechnology. The three morning sessions were devoted to plenary sessions that consist of fifteen lectures. Each plenary session was dedicated to Drs. Charles Yanofsky, John Clutterbuck, and Claudio Scazzocchio, respectively, who have made lasting impact on the molecular genetics of fungi. Some plenary session speakers reminisced about their lives and legacies, reminding young scientists like us of the fact that we all are standing on the shoulders of the 'giants'. Recently, high throughput sequencing technologies have enabled complete genome sequencing of diverse fungal species and the analysis of genome evolution and epigenetic attributes. This advancement has helped scientists address both old and new questions about fungal biology and evolution from genomic and epigenomic perspectives. In particular, recent epigenetic studies generated novel insights into the molecular basis of fungal lifestyles. The scope of the meeting was far beyond what can be covered in a short report, so I will highlight some of the exciting presentations about fungal epigenetics and epigenomics.

### Histone Modifications and Fungal Biology

*Neurospora crassa* has been a model for studying DNA methylation and other epigenetic factors such as histone modifications in filamentous fungi. The first plenary speaker, Eric Selker (University of Oregon, USA) has made major contributions to understanding fungal DNA methylation and histone methylation associated with DNA methylation (H3K9me3, a heterochromatic mark). Selker first described how he identified and characterized genes encoding proteins

that directly or indirectly influence DNA methylation in *N. crassa* by combining genetic and biochemical approaches in his earlier studies [1-3]. He then summarized his recent works showing that distinct DNA methylation and histone deacetylation complexes are required for heterochromatin formation and gene silencing [4]. He also discussed the presence and the role of H3K27me3 in *N. crassa* [5]. Considering that H3K27me3 is absent in the chromatin of *Saccharomyces cerevisiae* and many filamentous fungi, its evolution and function in fungal species will be of great interest.

Zachary Lewis (University of Georgia, USA) gave a concurrent session talk about  $\gamma$ H2A and heterochromatin in *N. crassa*.  $\gamma$ H2A is a phosphorylated form of H2A and recruits chromatin-binding proteins that stabilize stalled replication forks or promote DNA repair [6]. Through a ChIP-seq experiment, he showed that  $\gamma$ H2A is enriched in heterochromatin domains in a DIM-5 (a histone methyltransferase for H3K9me3) dependent manner (unpublished). However,  $\gamma$ H2A is required neither for H3K9 methylation nor DNA methylation. Given that  $\gamma$ H2A is a biomarker for double strand break induced by DNA damage, his data suggest that proper heterochromatin formation is important for DNA repair and replication.

In fungi, it was known that secondary metabolite (SM) gene cluster are silenced by the formation of facultative heterochromatin [7]. In another concurrent session, Joseph Strauss (BOKU University, Austria) reported a genetic and biochemical investigation of two histone demethylases that are involved in the regulation of SM clusters in *Aspergillus nidulans*: KdmA and KdmB (unpublished). KdmA acts as a repressor of SM clusters by binding to H3K9me3 and demethylating H3K36me3, whereas KdmB acts as an activator by binding to H3K4me3 and demethylating H3K9me3. Deletion of the genes followed by transcriptome and ChIP analysis showed that the enzymes really regulate transcription of genes at the tested SM clusters.

Jessica Soyer (National Institute for Agricultural Research (INRA), France) showed in her concurrent session talk that epigenetic control of gene expression could be extended to the regulation of genes encoding putative secreted proteins (possibly effectors) [8]. In many

plant pathogenic fungi, putative effector genes are often found in the regions that are rich in repeat sequences and transposable elements (TE) [9]. The genome of *Leptosphaeria maculans*, a pathogen of Brassica crops, is compartmentalized into gene-rich GC-isochores and gene-poor AT-isochores. The latter is enriched in both TEs and putative effector genes. Genetic analyses of genes (orthologs of DIM-5 and HP1 in *N. crassa*) involved in heterochromatin formation using RNAi and microarray analyses revealed that at least a part of the effector genes located in AT-isochores is under regulation at the chromatin-level. However, the impact of lifting such chromatin-mediated repression on fungal virulence is not clear, since knockdown strains of DIM-5 and HP-1 were not defective in virulence.

Unlike other talks that focused on histone methylation, Reinhard Fisher (Karlsruhe Institute of Technology, Germany) presented evidence that histone acetylation is involved in light regulation in *A. nidulans* (unpublished). He showed that in the mutant of FphA, the red-light sensor phytochrome, H3K9 acetylation (H3K9ac) is not induced in the promoter regions to which FphA is recruited for transcriptional activation. This suggests that H3K9ac is important for light-induced changes in gene expression of *A. nidulans*.

### Non-Coding RNAs in Fungi

Non-coding RNA is another important factor in epigenetic regulation of gene expression. Among diverse non-coding RNAs, small interfering RNAs (siRNA) have been well studied for its functions and biogenesis pathways in diverse fungal species. Silvia Calo Varela (Duke University Medical Center, USA) provided in her concurrent session talk an interesting case of siRNA-based epimutation conferring antifungal drug resistance in a human pathogenic fungus, *Mucor circinelloides* (unpublished). She showed that the fungus repeatedly challenged with antifungal drug FK506 yielded resistant isolates that harbor no mutations in the target genes. Northern and Western blot analyses showed that the expression of target genes is repressed in the resistant isolates. Furthermore, she showed using high throughput sequencing that small RNAs accumulated in the target genes from these isolates. The data presented in her talk point out small RNA-based epimutation as a novel mechanism of developing drug resistance. Recent work published in *Nature Communications* described small RNA-based silencing of Avr genes (Avr3a) in interaction of *Phytophthora sojae* with its host plant [10]. Such naturally occurring silencing of Avr gene expression allows the pathogen to escape detection by cognate R gene, resulting in gain of virulence. Although *P. sojae* is not a fungus, the observed parallels between the two suggest that small RNA-based epimutations may be a common mechanism of generating phenotypic diversity for adaptation. Epimutation in fungal pathogens certainly deserves further investigation in the future, since it has significant clinical and agricultural ramifications.

In contrast to siRNAs, long non-coding RNAs (lncRNA) in fungi are just beginning to be appreciated. Yi Liu (Southwestern Medical Center, USA) presented a good example of such lncRNA in *N. crassa* in his concurrent session talk. He elegantly showed that light-inducible production of the antisense transcript, *qrf* from the *frq* locus, one of the circadian genes, is required for the resetting of clock property by forming a negative feedback loop [11]. He speculated that the observed negative regulation of *frq* by *qrf* could be due to transcriptional interference in which two RNA polymerases collide during convergent

transcription on the same locus. It would be interesting to see in the coming years how prevalent lncRNAs and natural antisense transcripts are and their implications in fungal biology.

### Conclusions

The 12th European Conference on Fungal Genetics was a great success in inspiring people with thought-provoking talks given by great speakers and also in paving the path to future investigations by touching on a broad range of topics. There were many more exciting talks including ones about endosome trafficking, fungal effectors and evolution of fungal sexual reproduction that we unfortunately do not have enough space to discuss here. Approaching the end of the meeting, it was obvious that epigenetic factors such as histone modifications and non-coding RNAs have become an important part of many genomic studies, especially in filamentous fungi. Considering that the number of sequenced fungal genomes represents the widest sampling of genomes from any eukaryotic kingdom, the coming years would be exciting and mind-blowing with a better understanding of life and its evolution.

### Acknowledgements

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013-056580).

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