# 博士論文の要約

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論文題目　The evolutionary study of genomic changes
associated with morphological evolution of septal pore cap in Agaricomycotina

**Abstract**

Morphological characters change in various directions during species divergence. During this evolutionary process, similar phenotypes appear independently, called convergent evolution. Since these differences have often played ecological roles, e.g., performing a physiological function, the molecular basis of these evolutionary events is important to understand phenotype-genotype relationships, adaptive evolution and measuring evolutionary repeatability.

In the major groups of filamentous fungi Agaricomycotina, one such example for typical morphological evolution occurs in the septal pore cap (SPC), which is involved in a plugging process of cell-like compartments. SPC is located around the hole of cell-like compartments (pore), and was derived from the endoplasmic reticulum. SPC is classified into three morphological types: perforate, imperforate, and vesiculate types. Perforate SPC has many small holes (perforations) on their SPC. Imperforate SPC has a slightly flattened closed membranous structure. The vesiculate SPC consists of vesicles or tubules arranged in a hemisphere and surrounding the pore. Since perforations of perforate SPC allows passing mitochondria and actin filament, this fact suggests that the difference of SPC types contributes to the difference of the functional performance of SPC. Current integrated results of the species phylogeny in fungi and morphological characterization of SPC showed that vesiculate SPC is the most ancestral trait, and perforate SPC is the most neomorphic trait. Interestingly, perforate SPC emerged from imperforate SPC multiple times independently. This fact indicates that

morphological convergent evolution had occurred on the perforate type lineages.

Despite the interesting evolutionary history of the variable morphological characters of SPC as described above, the genetic background of these evolutionary events remains unknown. In this doctoral thesis, I therefore aim to clarify genomic changes associated with the morphological evolution of SPC using comparative genomics. I conducted three analyses; candidate gene extraction, gene search for understanding sequence evolution correlated with the morphological difference between vesiculate SPC and imperforate SPC, and sequence comparison for understanding morphological convergence from imperforate SPC to perforate SPC. More details about the background and aim of this study are described in Chapter 1.

In chapter 2, to detect candidate genes that correlate with the morphological convergent evolution of Perforate SPC, I conducted genome-wide surveys. Using the 12 fungal genomes, I created an orthologous gene dataset and extracted the candidate gene from the dataset based on the results of the phylogenetic analysis of each ortholog. I found the SPC-related gene *spc33* showed different branching pattern than the well-supported species tree. In particular, amino acid sequences of *spc33* showed convergent substitutions at the same site between diverged species with perforate SPC. These results suggest the possibility of the involvement of *spc33* to morphological convergence of perforate SPC. In chapter 3, in order to understand sequence evolution related to morphological evolution from imperforate SPC to perforate SPC, I verified how convergent amino acid substitutions occurred in the *spc33* of *Rhizoctonia solani* (Rhiso). The possible hypotheses are: 1, Rhiso and other perforate type species acquired the convergent substitutions independently, 2, the same substitutions had gained by transferring homologous region from other perforate type species. To clarify which hypothesis is more possible, I conducted synteny analysis, determination of exon/intron structure and phylogenetic analysis of *spc33*. The results provided no supporting evidence of hypothesis 2 from the genome sequences of Rhiso. Thus, I concluded that the hypothesis 1 is more possible for the cause of convergent substitutions in amino acid sequences of *spc33*. In Chapter 4, I presume the origin of *spc33* in vesiculate type species since the result of sequence similarity searches showed that vesiculate type species do not have *spc33* in their genomes. The *spc33* was also not detected from the other eukaryotes and all prokaryotes. Thus, I searched a weak sequence homology to assess the ancestral gene of *spc33* by using sequence motifs. To see

the track of *spc33* from the genome of outgroup species, synteny analysis was conducted. Based on these results, I discussed the origin of *spc33* and its evolution.

In summary, I identified each genetic difference correlated with the morphological difference between vesiculate SPC and imperforate SPC, and morphological convergence from imperforate SPC to perforate SPC. Also, I clarified the process of these genetic changes. These results provide important advances for understanding the genetic basis of morphological evolution of SPC. These achievements contribute to uncovering the molecular mechanisms of convergent evolution and to finding a missing link between morphological evolution and sequence evolution in SPC. My results present the hints for deep understanding about genetic basis of morphological evolution and convergent evolution. In addition, this study provides an impetus for developments in fungal evolutionary biology and genomics.

**Introduction**

An important problem in evolutionary biology is what changes occurred at the genome sequence level when deriving morphological diversity. The study of convergent evolution, i.e., the independent evolution of similar phenotypic changes in different lineages, has been said to provide insight into hotspots and key mutations for phenotypic differentiation (Stern and Orgogozo, 2009). In addition, focusing on morphological convergence provides clues for understanding the relationship between phenotypic evolution affected by natural selection and sequence evolution driven by the protein adaptive landscape (Castoe et al., 2010; Stern 2013; Almén et al., 2016). However, genome-wide surveys of convergent evolution are relatively rare and more empirical genome-scale studies are needed to elucidate the genetic basis of morphological convergent evolution.

One such example of traits representing typical morphological convergence is the traits of septal pore caps (SPC) in fungi, which are involved in the construction of mycelia's complex multicellularity (Jedd, 2011; Nguyen et al., 2017). It has been suggested that SPC was newly derived from the endoplasmic reticulum (ER) because SPC was stained by some ER-targeting markers (van Driel et al., 2008; Müller et al., 1999) and SPCs are connected at their base to the ER (Moore, 1975; Müller et al., 1998a, 2000). I focused on a couple of morphological

types of SPC: vesiculate, imperforate, and perforate types. Vesiculate SPC is the most ancestral type of SPC, imperforate SPC had emerged from vesiculate SPC only once, and perforate SPC is remarkable because it independently emerged from imperforate SPC multiple times (van Driel et al., 2009). The difference between SPC types leads to the derivation of different functions for cytoplasmic transport inside the mycelium (Bracker and Butler, 1964; Moore and Marchant, 1972), which implies that SPC types had evolved through adaptation for transportation of various substances. These SPC types are highly conserved at the order level (Hibbett, 2006; van Driel et al., 2009; Oberwinkler et al., 2013; Hibbett et al., 2014) and the understanding genetic background of SPCs' morphological differentiation can substantially contribute to understanding the evolution of higher taxa in Basidiomycota.

Previous SPC studies reported on its functions, fine features, and applicability to fungal classification (Bracker and Butler, 1964; Moore and Marchant, 1972; Lisker et al., 1975; Hibbett, 2006; van Driel et al., 2009); however, despite the long history of the morphological studies of SPC, the genetic basis driving the morphological differentiation of SPC remains unclear. In animals, studies have reported mutations associated with morphological convergent evolution by whole-genome analysis. For example, Hu et al. (2017) found a candidate gene for the evolution of a pseudo-thumb in pandas using positive selection as an indicator. Using positional cloning, Colosimo et al. (2005) found that the Eda pathway is involved in the phenotypic evolution of armor plate in sticklebacks. The genomic background associated with SPCs' morphological convergence should also be elucidated using a similar genomic approach.

In this study, I aimed to clarify the genomic changes associated with the morphological evolution of SPC in terms of comparative genomics. I used publicly available whole-genome sequences from the 1000 Fungal Genomes Project (http://1000.fungalgenomes.org/) to identify evolutionary events at the sequence level. Data for morphological types of SPC were also accumulated because of their importance for taxonomic classification (Wells, 1994; Müller et al., 1998b, 2000; Lutzoni et al., 2004; Hibbett et al., 2014). By combining these genomic and morphological data, I obtained evidence from 13 fungal genomes for evolutionary events of SPC at the amino acid sequence level.

## Materials and Methods

The genome sequence data for 13 species of Basidiomycota, 11 representatives of Agaricomycetes (6 perforate-type species and 5 imperforate-type species), one representative of Dacrymycetes (imperforate type), and one representative of Wallemiomycetes (vesiculate type) were used for this study. These species are representative of major Basidiomycota species. All sequence data were obtained from the Joint Genome Institute portal (JGI, http://www.genome.jgi.doe.gov) of the United States Department of Energy. The species name and analysis methods are described in the main body of the doctoral thesis.

## Results and Conclusions

In my doctoral thesis, I have aimed to clarify genomic changes associated with the morphological evolution of SPC in terms of comparative genomics. As a result of investigating the amino acid substitution pattern of orthologous genes among 12 fungal species, SPC-forming gene *spc33* was identified as the gene that most reflects the evolutionary history of SPC (Chapter 2). Gene present/absent pattern, amino acid changes and the molecular background of its genetic changes of *spc33* connected to both two patterns of evolution; the morphological difference between vesiculate SPC and imperforate SPC (Chapter 4), and the morphological convergence from imperforate SPC to perforate SPC in multiple lineages (Chapter 3). Below, I will summarize detected genomic changes and possible evolutionary history in each morphological evolution.

In the morphological difference between vesiculate SPC and imperforate SPC, I clarified that the gene gain had occurred with the morphological differentiation. I found vesiculate type species do not have *spc33* that is essential to form SPC in perforate type species. Furthermore, by analyzing synteny around *spc33* and site-specific similarity of *spc33*, MRCK Ser/Thr protein kinase in vesiculate type species was successfully detected as an ancestral gene of *spc33*. These findings indicate that vesiculate SPC has a different formation process. Since this kinase has a function related to actin-myosin regulation, and one of the remarkable differences between imperforate SPC and perforate SPC is the existence of perforation that allows actin

filaments to pass through the SPC, it is possible that *spc33* interacts with actin filament located in the vicinity of SPC.

In the evolution from imperforate SPC to multiple lineages of perforate SPC, I discussed what kind of mutation occurred in the amino acid sequences of *spc33* and how *spc33* functions to cause the morphological convergence. From the sequences of *spc33*, five substitutions that correlated to the morphological parallel evolution of perforate SPC were detected. Among these sites, three key sites showed convergent changes that are related to the morphological convergence. In these substitutions, the possibility of "Common origin" e.g. horizontal gene transfer was denied by conducting synteny analysis, exon/intron pattern inference and constructing gene trees with and without key substitutions. Therefore, I conclude that perforate SPC was independently acquired in each lineage from common traits imperforate SPC without a lateral genetic transfer event. The amount of these changes was significantly higher than other orthologs. Therefore, it is possible that parallel substitutions in *spc33* occurred not randomly but accordingly occurred with morphological evolution. Moreover, three key sites were localized within 17 amino acid residues that are the homologous region with the actin-myosin regulatory region of MRCK Ser/Thr protein kinase. This finding suggests the new hypothesis that the key sites-containing region was derived from the actin-myosin regulatory region of MRCK Ser/Thr protein kinase and be involved in the important function of *spc33*.

In summary, I identified direct genetic changes correlated with both morphological differences between vesiculate SPC and imperforate SPC, and morphological convergence of from imperforate SPC to perforate SPC. Based on my genome-wide survey, SPC-related gene *spc33* was detected as the candidate gene of the morphological evolution of SPC. The findings of the origin and SPC type-specific amino acid substitutions of *spc33* provide huge clues for understanding the genetic basis of morphological evolution of SPC. These achievements contribute to uncover the molecular mechanisms of convergent evolution and to find a missing link between morphological evolution and sequence evolution. In addition, this study is a leading-edge study in the early days of fungal evolutionary genomics, and will contribute greatly to future fungal evolutionary biology.