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学位論文題目 Genome evolution after WGD in the baker's yeast.

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論文内容の要旨

My research interest is to understand the mechanisms of evolution on a genomic scale. Recent advances of genome sequencing technology and genome-wide experimental technology provide an excellent opportunity of studies of genome evolution. In my PhD work, using the bakers' yeast *Saccharomyces cerevisiae* as a model, I studied genome evolution after a whole genome duplication (WGD) event. All genes were doubled at the WGD event, but only ~ 10 % of them remain as duplicates (called ohnologs) at present and other genes have lost one of the duplicated pairs. In addition, massive genome rearrangement have occurred in this gene deletion process.

This thesis was constructed as three parts. In the first part, I studied the evolution of ohnologs. Interlocus gene conversion is a unique recombinational mechanism to duplicated genes. Because it retards the nucleotide divergence of duplicates, the standard molecular clock model cannot be directly applied to infer the history of duplicates. In this chapter, a maximum likelihood method to estimate the time of the WGD was developed incorporating the effect of gene conversion. It was estimated that the WGD is almost as old as the speciation event with pre-WGD species. It is suggested that the WGD might have caused the speciation.

In the next part, I examined the role of natural selection to the duration of concerted evolution. It was found that duplicated with higher expression (especially ribosome and histone genes) prefers long-term concerted evolution, indicating gene conversion may be favored for such high-demand genes. By genome-wide data analysis with various kinds of experimental data, I found this hypothesis is a likely explanation of the observation.

In the third part, I studied the evolution of gene order in the genome rearrangement process after the WGD. In the analysis I focused on adjacent gene pairs. Comparative genome analysis indicated that newly generated adjacent gene pairs in divergent orientation are relatively rare and they have on average long intergenic distances and low co-expression. I considered that the locations of nucleosome free regions (NFRs) would explain this. It is known that transcription start in both directions when RNA polymerase II (Pol II) binds to a NFR. It is predicted that such coexpression would be deleterious for a random pair of genes that happened to be adjacent to each other. If so, selection should have worked against deletion between newly created divergent gene pairs, thereby keeping them physically away so that their coexpression might be avoided. I verified this hypothesis by comparative genomic analysis of the locations of NFRs and evolutionary simulations.

Through these works, I conclude that the genome of *S. cerevisiae* undergo various types of genome-wide natural selection through the process after the WGD. This study also shows that the post-genomic biological data are useful to determine the target of natural selection.

博士論文の審査結果の要旨

All committee members agreed that this is a strong thesis that made a significant contribution to the field. The major goal of Ryuichi Sugino's PhD work is to understand the mechanisms behind genome evolution. As a model, he used an interesting species, baker's yeast, which has a unique evolutionary history; the entire genome was duplicated about 100-200 million years ago and experienced a number of drastic genomic changes after the whole genome duplication (WGD) event. The availability of genome sequences of other yeast species, including those experienced the same WGD (referred to as post-WGD species) and those diverged before the WGD (referred to as pre-WGD species), made Ryuichi possible to perform nice comparative genomic analyses. Another advantage of using baker's yeast is that it has been one of the model species in molecular biology and tremendous amounts of biological data are accumulating. Ryuichi's evolutionary analyses incorporated these data (e.g., expression and gene-regulation data), which made his conclusions strong.

The thesis consists of three major parts. The first part focuses on duplicated genes created by the WGD. Since the WGD that occurred more than 100 million years, roughly 90% duplicated genes were lost and only 10% still remain as duplicates at present. It has been known that interlocus gene conversion is heavily involved in the evolution of duplicates especially in yeasts. Because this recombination mechanism homogenizes the nucleotide divergence between duplicates and causes concerted evolution, it is difficult to correctly infer the history of duplicated genes by applying a molecular clock, a basic concept of molecular evolution. In this chapter, a maximum likelihood method to estimate the time of the WGD was developed incorporating the effect of gene conversion. It was estimated that the WGD is almost as old as the speciation event with pre-WGD species.

In the second part, which again focuses on duplicated genes, the role of selection on gene conversion is explored. Ryuichi found that duplicated gene with higher expression (especially ribosome and histon genes) prefers long-term concerted evolution, indicating that genetic homogenization by gene conversion may be An oral presentation of the thesis to open audience was held at the Hayama campus of Sokendai on August the 19th. Ryuichi gave a one-hour talk on his entire research with brief descriptions on the background behind his work. Although the talk was in English, it was quite understandable. The following question-session was also in English. Ryuichi provided reasonable answers to all questions from the committee members and the audience, indicating that he has strong understanding of his research area. As one of the committee member, Prof. Hiroshi Akashi at the National Institute of Genetics, was not able to attend, the presentation was taped and sent to him. In the next week, on the 25th, the four committee members and Ryuichi got together again, and we had a closed meeting. In the beginning, Hiroshi asked a number of broad and technical questions and Ryuichi's answers demonstrated his depth of knowledge in population genetics as well as genomics. Hiroshi also provided important comments, which will be fully incorporated in the revised version of the thesis. Then, after a short discussion, the committee made a unanimous decision that Ryuichi deserves a PhD. favored for such high-demand genes. After examining other confounding factors, this chapter concludes that selection for higher dosage would prefer concerted evolution of duplicates.

The third chapter focuses genes that were lost after the WGD. As mentioned earlier, most duplicates created by the WGD lost one copy. Simultaneously, the DNA sequences of these lost genes were eliminated by deletions. This process produced a number of new gene order in the yeast genome, providing an exciting opportunity for exploring the role of selection on gene order. It was found that the proportion of adjacent gene pairs in 'divergent' orientation created after the WGD is significantly low.

Furthermore, these gene pairs have exceptionally long intergenic regions. This strange observation was nicely explained by taking the function of nucleosome free regions (NFRs) into account. It is known that transcriptional factors bind to NFRs, from which transcription of mRNA is initiated in both directions in yeast. Therefore, two adjacent genes in divergent orientation with a single NFR between them are likely coexpressed. As coexpression of two independent genes with different functions should be deleterious, this chapter concludes that selection has worked against deletion between newly created divergent gene pairs. Through these three parts of the thesis, it is demonstrated that a various kinds of selection was involved in shaping the current genome of yeast, which contributes to deep understanding of the mechanisms of genome evolution.

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