Tight coevolution of proliferating cell nuclear antigen (PCNA)-partner interaction networks in fungi leads to interspecies network incompatibility

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AUTHOR SUMMARY

All biological processes are mediated by proteins working together through highly specific interactions. To maintain the specificity and strength of these interactions through the course of evolution, proteins often coevolve by accumulating mutations in a coordinated manner to preserve the integrity of protein-protein interfaces. Here, we examined the consequences of this coevolution on the molecular recognition and function of one model system containing several interacting proteins. We showed that the coevolution of these proteins leads to increased specificity in recognizing cognate or closely related partners but eliminated binding to distantly related partners, resulting in loss of function of such mixed networks. Our results demonstrate that the coevolution of proteins can form functional barriers for gene transfer between organisms, and can thus accelerate the generation of new species.

One mechanism for conserving the integrity and function of protein interaction networks over evolution involves coordinated coevolution in protein-protein interfaces (1). Because of experimental challenges, little is currently known about the characteristics of this coevolution. Our model system to examine the coevolution of proteins is the proliferating cell nuclear antigen (PCNA)-partner interaction network, which plays a key role in promoting DNA replication and repair processes in all eukaryotes (2). PCNA forms a sliding platform on DNA, interacting with various partners to enhance their accessibility to the DNA template. Interestingly, many PCNA partners compete for the same binding site on PCNA, and can thus accelerate the generation of new species.

Here, we used bioinformatics and experimental tools to examine the coevolution of PCNA networks in fungal species spanning ~300 million years of evolution. We first analyzed the amino acid sequences at the binding sites of PCNA and six different partners in different fungal species to identify coordinated sequence changes. These can indicate coevolution (Fig. P1, step 1). Guided by this analysis, we generated PCNA variants containing different, natural, short IDCL sequences grafted onto three PCNA backbones from three different species (Saccharomyces cerevisiae, Yarrowia lipolytica, and Schizosaccharomyces pombe) (Fig. P1, step 2). Thus, we could examine the evolution of IDCL-mediated PCNA-partner interactions while minimizing extraneous alterations. Next, these interspecies (or “chimeric”) variants were analyzed for binding to several PCNA partners from the three species. In parallel, the chimeras were examined for their in vivo activity in S. cerevisiae (Fig. P1, step 3). Overall, this approach enables the examination of both the molecular basis and the functional implications of the coevolution of PCNA-partner interactions.

Using bioinformatics, we found significant divergence in the binding-site sequences of PCNA and six of its different partners. We identified changes in four amino acids of the PCNA-binding site that are partially conserved over evolution within one part of the fungal ancestral tree but are significantly different in other parts of the tree. We found that the sequences of the binding sites of the different PCNA partners divide into the same two groups as observed in the case of PCNA’s sequences. This bioinformatics analysis indicated that PCNA-partner interactions diverged into two groups with coevolving sites, both in PCNA and in its partners.

Experimental analysis showed that the divergence of the IDCL sequence to form two distinct groups leads to a dramatic decrease in PCNA-partner interactions and to a loss of function in S. cerevisiae. Analyzing PCNA-partner interactions in yeast, we observed that chimeric PCNA containing IDCL from one of the groups...
groups interacts very weakly with a partner from the opposite group. We also found that *S. cerevisiae* containing chimeric PCNA with IDCL from the second group cannot efficiently support in vivo DNA replication and repair. These PCNA mutant strains were highly sensitive to DNA-damaging agents, and were nonviable in some cases. These results show that the coordinated sequence changes in PCNA and its different partners lead to dramatic changes in PCNA-partner recognition and loss of function of networks containing PCNA from one species and partners from distantly related species. Moreover, the groups of species diverged according to their distances in the ancestral tree, indicating a common ancestor. Our results show that the coevolution of PCNA-partner interactions can form functional barriers for gene transfer between fungi species, and thus accelerate fungi speciation.

In this work, we have developed an integrated approach based on bioinformatics, as well as genetics and protein engineering, to study the coevolution of protein-protein interactions. This approach, especially the combination of bioinformatics for the identification of coordinated changes with experimental assays to examine the impact of these changes on function, can provide an in-depth view of protein coevolution. This approach was successfully demonstrated on a PCNA-partner interaction network and can easily be generalized to illuminate the coevolution of a range of other protein-protein interaction networks promoting various processes, such as signal transduction and transcription regulation.