IV. Evolution of Gene Clusters: The Mating Type Locus Paradigm

In contrast to operons that are prominent in the bacterial kingdom, most eukaryotic genes are not organized in any recognizable fashion. But prominent exceptions are fungal gene clusters that play roles in the production of secondary metabolites, in some metabolic cascades, and in defining mating-type. Our studies focus on the evolution and function of the mating-type locus (*MAT*) as a paradigm to understand how gene clusters are formed and function, with implications for the evolution of clustered gene classes. Recombination plays a central role in reshaping the genome, fashioning genes into clusters, and enabling higher order regulation of gene expression and function in eukaryotes .

We study the human fungal pathogen *Cryptococcus neoformans*, which causes life-threatening infections of the central nervous system, most commonly in immunocompromised hosts. This organism is a basidiomycete and therefore divergent from other common human fungal pathogens. The organism has a defined sexual cycle involving haploid α and **a** cells, genes can be readily disrupted following transformation and homologous recombination, and animal virulence models are well established. Our studies focus on signaling cascades that govern virulence, an unusual mating type locus linked to differentiation and virulence, and the role of sexual reproduction in the evolution of microbial pathogens. Signaling cascades that control virulence and mating have been defined, and the **a** and α alleles of the mating type locus have been cloned and sequenced from all three varieties. The *MAT* locus spans over 100 kb and contains more than 20 genes, several of which function in differentiation and virulence. The *MAT* alleles are composed of divergent sets of the same genes that evolved by extensive remodeling from common ancestral DNA regions. The only *MAT* allele specific genes encode two homeodomain proteins, Sxi1α and Sxi2**a**, which physically interact and are necessary and sufficient to govern post-fusion events enabling completion of the sexual cycle.

A detailed model has been developed for the evolution of *MAT* from an ancestral tetrapolar mating system, involving a series of gene acquisitions into two unlinked sex-determining genomic regions, a chromosomal translocation leading to an unstable tripolar intermediate state, collapse of the tripolar to a true bipolar state via gene conversion linking to the two unlinked sex determinants in the opposite mating type, and a series of ongoing rearrangements, gene conversions, and gene loss or eviction events, likely driven by the abundant presence of transposons and their remnants in this unique genomic region. These studies reveal marked parallels with the evolution and features of sex chromosomes of plants and animals.

In a series of recent studies, recombinational enhancers have been discovered that flank and lie within *MAT*, and which may have contributed to its evolutionary trajectory. The *MAT* locus has been found to function as a quantitative trait locus, and

the α allele of *MAT* promotes both hyphal differentiation and virulence in concert with other unlinked genomic loci. A mating type locus specific microarray has been developed, enabling both high-resolution analysis of transcriptional programs enforced by *MAT*, and studies of *MAT* allelic configurations and naturally occurring variants based on comparative genome hybridization (CGH). Finally, recent studies focus on the structure, function, and evolution of *MAT* via studies of species closely aligned with the pathogenic *Cryptococcus* species cluster, including *Cryptococcus amylolentus*/*Tsuchiyaea wingfieldii*, *Filobasidiella depauperata*, *Cryptococcus heveanensis*, and *Tremella mesenterica*, representing homothallic and heterothallic fungi that are either tetrapolar, bipolar, or currently asexual. These studies are validating central tenets for the proposed evolution of *MAT*, and also revealing novel insights.