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CHROMOSOMES, KARYOTYPE ANALYSIS, CHROMOSOME REARRANGEMENTS IN FUNGI

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In this review the organization of fungal chromosomes and the methods used for karyotype analysis are briefly summarized. The role of chromosome rearrangement, supernumerary chromosomes and repeated DNA sequences in the genetic change of fungi is evaluated.

Fungal chromosomes

The genome organization of fungi is typical of eukaryotic systems. The chromosomes are composed of DNA, as well as histone and non-histone proteins. The size of the haploid nuclear genome of fungi ranges between 15–60 Mb (megabase pairs), the number of chromosomes varies from three to a few dozens depending on species. The chromosomes are organized into nucleosomes and contain a localized centromere-kinetochore complex (which is the nucleating site for the spindle microtubules), as well as telomeres (that maintain the integrity of the chromosomes and help in directing chromosome pairings). A typical spindle mechanism is the driving force of mitosis divided into a normal prophase, metaphase and a non-synchronous anaphase-telophase taking together 6 min. under optimal conditions. The meiotic process – like in other eukaryotes – includes DNA replication (pre-meiotic S phase), as well as one reductional (meiosis I) and one equational (meiosis II) division. In the majority of fungi the meiotic S phase precedes karyogamy.

Karyotype analysis

Chromosome numbers and sizes (the karyotype) can be determined by light and electron microscopy as well as by pulsed-field gel electrophoresis. The latter technique – used for the first time in 1984 to separate yeast chromosome-sized DNAs [1] – raised a

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revolution in fungal genetics. Since then, numerous electrophoretic karyotypes for a wide range of fungi have been published. Hungarian workers provided the first comparative karyotype analysis for selected species of several economically important genera, like *Fusarium, Mucor, Phaffia* and *Trichoderma* [2–5]. Cloned DNA fragments may be assigned to electrophoretically separated chromosomes by Southern hybridization, therefore molecular karyotyping allows direct gene mapping and identification of linkage relationships. Chromosome-specific libraries may also be prepared after separation of chromosome-sized DNAs. Chromosome-specific probes may be used to recognize homologous chromosomes within polymorphic chromosome patterns. Karyotype analysis may provide additional information for the appropriate taxonomic affiliation of controversial species.

Chromosome polymorphisms

Genomic rearrangements play an important role in the evolution of fungi [6]. Such rearrangements may result in chromosome polymorphisms found to be accompanied by phenotype differences including morphology [7], antibiotic production [8] or host range [9].

Karyotype analysis revealed high levels of variation in chromosome sizes and/or numbers within most species of fungi. According to a persuasive hypothesis proposed by Kistler and Miao [10] the extent of intraspecific karyotype polymorphism is inversely correlated to the frequency of meiosis. Asexual fungi have more extensive polymorphisms than sexual ones, which frequently undergo meiosis, an efficient process selecting against detectable chromosome aberrations. Both meiotic and mitotic processes may lead to chromosome polymorphisms and genomic stress can also account for karyotype variability. Repeated DNA sequences may also generate chromosome polymorphisms (see below).

For asexual plant pathogenic fungi, variation in chromosome sizes and numbers is an efficient non-Mendelian mechanism of genetic change resulting in the emergence of virulent races or pathotypes.

Supernumerary chromosomes

The occurrence of ‘extra’ chromosomes is very common in fungi. Various terms (B chromosomes, dispensable chromosomes, mini-chromosomes and supernumerary chromosomes) have been used for naming these structures. According to a recent review of this topic [11] the term ‘supernumerary chromosomes’ was suggested as a preferred name. A chromosome could be qualified as a supernumerary one, if it is not present in one or other viable individual of a given species. In sexual fungi the supernumerary chromosomes are inherited by non-Mendelian transmission, their absence is non-lethal. In spite of this seemingly dispensable nature of these elements there are examples for the long-term mitotic stability of supernumerary chromosomes [12, 13].

The movement of supernumerary chromosomes in pulse-field gels, their structural stability during mitosis and their positive hybridization to telomeric DNA probes indicate strongly that these elements contain centromeres and telomeres. Information on DNA composition of these chromosomes is limited to a few species. A mixture of repeated as well as low- and single-copy DNA sequences have been identified in supernumerary chromosomes of *Colletotrichum gloeosporioides* and *Nectria haematococca* [14, 15]. In our experiments mini-chromosomes in *Fusarium sporotrichioides* (some of them were undoubtedly supernumerary ones) were found to constitute a mosaic composed of repeated DNAs dispersed throughout the genome and unique sequences. These chromosomes were most probably formed by major genomic rearrangements and were preserved during the evolution of the species. The mosaic structure was identified in all genetically isolated strains of the fungus indicating that the mini-chromosomes – although lacking any coding region – could be useful components of the genome [12].

The biological function of supernumerary chromosomes is cryptic except for two notable cases. The 1.6 Mb dispensable chromosome of *N. haematococca,* a facultative plant pathogen carries a gene *(PDA1-1)* encoding the enzyme, pisatin demethylase, which catalyses the detoxification of pisatin, an antifungal phytoalexin of pea. Strains that retained this supernumerary chromosome show a high level of pisatin detoxification activity and are highly virulent on *Pisum sativum* [16]. A supernumerary chromosome of *Cochliobolus carbonum* race 1 isolates carries genes responsible for the synthesis of HC-toxin, a host specific cyclic tetrapeptide which causes disease symptoms on maize [17]; strains lacking this chromosome (and the genetic determinants of HC-toxin production) are avirulent on maize.

Repeated DNA sequences

The fungal genome harbors interspersed repetitive elements, that may be mobile or stably integrated elements. The mobile elements are classified as (i) transposons, which transpose through DNA copies and have an open reading frame encoding transposase activity, (ii) retrotransposons, which duplicate via RNA intermediates and have retrovirus-like structures and (iii) retroposons, which also use RNA intermediates during replication but lack the structural features of retroviruses. The fungal retroposons are further sub-grouped into either as short interspersed nuclear elements (SINEs) or long interspersed nuclear elements (LINEs). The first fungal transposon, Fotl discovered in the plant pathogen *Fusarium oxysporum* was found to share structural similarities with the class of Tcl-like elements [18]. Of the retrotransposons, Ty4 present in *Saccharomyces cerevisiae* was the first element subjected to complete molecular analysis [19]. *CfT-1* isolated from *Cladosporium fulvum* was a pioneer example of the LTR-type (long terminal repeat) retrotransposons in filamentous fungi [20]. A short interspersed nuclear element (Mg-SINE) was isolated from the rice blast fungus, *Magnaporthe grisea* [21], whereas LINE-like elements were for the first time detected in *Neurospora crassa* [22]. Numerous other examples of the mobile repetitive sequences have subsequently been reported and some of these sequences proved to be functional transposable elements.

Of the stably integrated (non-rDNA) repeated DNA sequences in fungi one class is characterized as telomere-associated elements, present on all or almost all chromosomes. Such telomeric sequences, like Ca7 [23] or the Rel-2 element [24] of *Candida albicans* are considered essential for proper chromosome maintenance. The second class of the stably integrated non-rDNA repeats is represented by the *CARE-1* element [25] and the RPS sequences cloned from *C. albicans* [26]. These elements are not present on all resolvable chromosomes. *CARE-1* was identified as a non-telomeric, probably non-essential sequence with a copy number ranging between two and twelve per haploid genome, depending on strain, whereas the RPS 1 element, represented by some 80 copies was found to be present on all but one chromosome of *C. albicans*.

In our laboratory a moderately repetitive DNA element has been cloned from *Fusarium poae a* strictly asexual fungus, which is a secondary invader of small-grain cereals. The element, named ZIT1 selectively hybridized to the polymorphic, 1.0–3.7 Mb chromosomal region of the fungus, but no hybridization signal was observed on any of the large chromosomes. ZIT1 shares only a moderate level of similarity to *gag* genes of fungal retrotransposons, which was mainly due to a small cysteine-reach motif, known as zinc-finger DNA binding domain. No significant homology was found with any other published nucleotide sequence. The novelty of this element is that its distribution is restricted to the polymorphic chromosome region of *F. poae*. ZIT1 could be a remnant of a formerly active foreign DNA that had been eliminated from the large chromosomes by a defensive mechanism suitable to prevent the disruption of genes encoding vital functions [27].

Repetitive DNA sequences are regarded to be important determinants of genetic variability in clonal eukaryotic organisms, like most hyphomycete fungi in which recombination through sexual or parasexual cycles is rare or, in fact absent. Transposable elements have been shown to cause chromosome rearrangement and various aberrations like deletions, inversions and translocations. Repeated DNA sequences, even if they are unable to transpose may generate DNA rearrangements through recombination among their homologous sequences scattered throughout the genome.

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*Due to spatial limitations of this review the coverage of literature is only partial. Non-intentional omissions could not be avoided.