Acta Microbiologica et Immunologica Hungarica, 49 (2–3), pp. 331–335 (2002)

ROLE OF MOBILE INTRONS IN MITOCHONDRIAL GENOME DIVERSITY OF FUNGI

(A MINI REVIEW)

ZSUZSANNA HAMARI, Á. JUHÁSZ, F. KEVEI

Department of Microbiology, Faculty of Sciences, University of Szeged, P.O. Box 533, H-6701 Szeged, Hungary

(Received: 4 January 2002; accepted: 4 March 2002)

Keywords: *Aspergillus* spp., mobile introns, mitochondrial genome diversity

Our previous studies on intraspecific mitochondrial DNA (mtDNA) polymorphism among black Aspergilli, carried out using restriction length polymorphisms (RFLP) of mtDNAs of different isolates of the *Aspergillus niger, A. tubingensis, A. japonicus* and *A. carbonarius* species, revealed a high level of intraspecific mtDNA variability [1, 2, 3].

Strains of the *A. niger* species exhibit a high degree of molecular variability. They can be divided into three main groups based on the *Hae*III-*Bgl*II digested mtDNA banding patterns of the various strains [1]. MtDNA RFLP types 1, 2 and 3 exhibiting nuclear rDNA types I, II and III correspond to three different species, *A. niger, A. tubingensis* and *A. brasiliensis*, respectively [1, 4, 5, 6]. MtDNA groups 1 and 2 consist of several subgroups (1a-1e, and 2a-2f) [1]. Among the examined 80 individual field isolates and collection strains of *A. japonicus*, eight different mtDNA RFLP groups (mtDNA types 1-8) were distinguished [3]. When thirteen individual isolates of *A. carbonarius* were analysed, three different mtDNA RFLP groups (mtDNA types 1a, 1b and 2) were detected [2]. The observed variety of mtDNA indicated by the various RFLP profiles is the main basis for this classification. We attempted to determine the reason for the detected mtDNA polymorphism. Restriction maps of isolates representing different mtDNA RFLP types were constructed [7, 8, 9]. These revealed that the presence of introns at different positions and their appearance in altered

numbers in the mtDNAs are responsible for the mitochondrial genome diversity. Apart from the observed intron variability, sequence analysis of mtDNAs showed that single nucleotide changes also play a role in the emergence of polymorphism if the change generates a new recognition motif for the restriction endonuclease used in RFLP analysis. The reasons for variability between 1a and 1b mtDNA types of *A. carbonarius* can be attributed to a 1.1 kb group I intron, which is present in *cox*2 of 1b and is absent in the same region of 1a mtDNA [7]. Structural and sequence comparison of two mtDNA RFLP types (1 and 4) of *A. japonicus* revealed that they differed from each other in at least two group I introns in *cox*1 gene and one group I intron in *cob* [8]. Structural organisation of *A. niger* mtDNA types 1a, 1b, 1c and 1e proved to be very similar; these mtDNAs differed, however, from each other in three variable regions [9]. These regions included a group I intron in the *cox*1 gene that was present in three different forms (A, B or B') and two intergenic regions in which mini insertions or deletions and nucleotide changes were identified (the intergenic region between tRNA-Met and tRNA-His and the intergenic region after tRNA-Gly) [9]. Nucleotide changes that were observed between intron types B and B' and in the intergenic region after tRNA-Gly gene resulted in the appearance or disappearance of *Bgl*II restriction sites [9].

Our results indicate that group I introns in mtDNAs play a significant role in generating mtDNA diversity. These mitochondrial introns containing ORFs are known to be mobile [10, 11]. Earlier studies have shown that the mobile introns play the most significant role in the interaction among mitochondrial genomes, with their homing process generating recombinant mtDNAs. The process is initiated by the homing endonuclease activated double strand break that is repaired by a common mechanism known as the double strand break repair [10, 11]. MtDNA rearrangements caused by intron movements were reported earlier by Earl et al. [12], Turner et al. [13] and Croft and Dales [14, 15] when mitochondrial transmissions between the closely related species *A. nidulans, A. nidulans* var. *echinulatus* and *A. quadrilineatus* were detected. The interspecific recombinants differed from the parental strains only in the presence or absence of introns located mainly in the *cox*1 gene. Different studies on *Podospora anserina* and closely related species have shown that the ORFs themselves, either standing free or being inserted in group I introns are mobile due to their endonuclease products [16, 17].

Spreading of introns between distantly related organisms may occur via horizontal transfer [18, 19, 20]. However this phenomenon can also explain differences in introns among mitochondrial genomes of closely related species. Generally, during mating, extranuclear genomes, including mtDNA, do not recombine, because of their strict uniparental (in most cases maternal) inheritance [21, 22]. Fungal species that

enter sexual cycle frequently do not usually exhibit intron polymorphisms, e.g. there were no intraspecific mtDNA polymorphisms observed within *A. nidulans* species even between their heterokaryon incompatible strains [23]. Closely related species of section *Nidulantes* have shown slight alterations based on intronal differences [12]; but these introns might have moved among isolates of these species *in vitro*, as mentioned above. Length variability of mtDNAs of *Podospora anserina*, *Neurospora crassa* and their relatives was found to be mainly due to the presence and/or absence of optional introns and intronic ORFs [16, 24, 25, 26] suggesting successful intron movements in nature.

To reveal that mobile introns were causing mtDNA RFLP variability among isolates of the imperfect species of *Aspergillus* that we studied, experimental systems for mitochondrial transmission were worked out. Two transmission systems were developed, one for mitochondrial transfer among *A. niger* strains and between isolates of *A. niger* and the closely related *A. tubingensis* [27] and a second system for the mitochondrial transfers between isolates of *A. japonicus* [8]. Since the mitochondrial transfer was attempted between vegetative-incompatible strains, the transmission experiments were performed by protoplast fusion. The various mtDNA RFLP profiles served as molecular markers and the mitochondrial oligomycin resistance (*oliR*) of certain isolates provided a suitable tool for selection [8, 9, 27]. Mitochondrial oligomycin resistance occurs rarely and only one such *A. niger* strain (mtDNA type 1a) and one *A. japonicus* strain (mtDNA type 1) were available for these experiments. These strains were marked using conidia color and auxotrophy. After the fusion, protoplasts were recovered in minimal medium in the presence of oligomycin, and the resulting progeny were found to have rearranged mtDNA. In these experimental systems the progeny inherited the mtDNA of the *oliR* strain, which has been modified by the mobile introns of the other fusion partner. Transmission of mitochondria from the *A. niger oliR* strain with mtDNA type 1a to the *oliS* (oligomycin sensitive) strains with mtDNA types 1b and 1e confirmed the movement of the intron situated in *cox*1, with all of the progeny gaining the *B* or *B'* type intron [9]. Transfer of mtDNA from the *A. japonicus oliR* strain with mtDNA type 1 to the *oliS* strain with mtDNA type 4 resulted in movement of the above-mentioned two *cox*1 introns and the one *cob* intron in all the recovered progeny [8].

Despite the high level of intraspecific vegetative incompatibility among isolates of the imperfect species, intron movements occurred frequently probably via horizontal transfer among the individual strains thus generating this extended mtDNA polymorphism that we have reported.

Acknowledgements. This work was supported by grant #T025849 and #F032704 of the Hungarian Scientific Research Fund (OTKA) and a grant (FKFP-0218/97) of the Hungarian Ministry of Education.

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