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# Specificity of DNA import into isolated mitochondria from plants and mammals

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***Aim***. *Investigation of different features of DNA import into plant and human mitochondria, for a better understanding of mitochondrial genetics and generation of biotechnological tools.* ***Methods***. *DNA up-take experiments with isolated plant mitochondria, using as substrates various sequences associated or not with the specific terminal inverted repeats (TIRs) present at each end of the plant mitochondrial linear plasmids.* ***Results***. *It was established that the DNA import efficiency has a non-linear dependence on DNA size. It was shown that import into plant mitochondria of DNA molecules of «medium» sizes, i. e. between 4 and 7 kb, barely has any sequence specificity: neither TIRs from the 11.6 kb Brassica plasmid, nor TIRs from the Zea mays S-plasmids influenced DNA import into Solanum tuberosum mitochondria.* ***Conclusion****s. The data obtained support the hypothesis about species-specific import mechanism operating under the mitochondrial linear plasmids transfer into plant mitochondria.*

*Keywords: mitochondrial DNA import, plant mitochondrial linear plasmids, mitochondrial genome.*

**Introduction**. It is well known that horizontal gene transfer (HGT) is one of the substantial factors, mainly in the evolution of bacteria. Recent studies indicate that plant mitochondria are unusually active in HGT, relative to all other organelles of multicellular eukaryotes [1]. The distinctive feature of plant mitochondria is the size of their genomes, which are much larger than those of other eukaryotes. Moreover, in addition to a large and complex main mitochondrial genome, plant mitochondria contain small circular and linear DNAs regarded as extrachromosomal replicons or plasmids [2]. The linear mitochondrial plasmids are present in many fungi and in some plant species, but they seem to be absent in most animal cells. They usually have in common an «invertron» structure that is characterized by the presence of terminal inverted repeats (TIRs) and proteins covalently attached to their 5' termini [3]. The *Brassica* 11.6 kb plasmid, one of the linear mitochondrial plasmids in plants, shows a non-maternal inheritance, in contrast to mitochondrial genomes [4]. The origin of these plasmids is unknown, but indirect evidence indicates the possibility of horizontal transfer from fungal mitochondria. These pecularities suggest that plant mitochondria might possess a mechanism of natural competence to take up foreign DNA, resembling that of the process in the bacterial cells [5]. The aim of the project is to study different features of DNA import into plant mitochondria and into human mitochondria, for a better understanding of mitochondrial genetics and generation of biotechnological tools.

**Materials and methods**. We developed DNA uptake experiments with isolated plant mitochondria, using as substrates various sequences associated or not with the specific TIRs present at each end of the 11.6 kb linear plasmid from rapeseed (*Brassica napus* L.) [4].

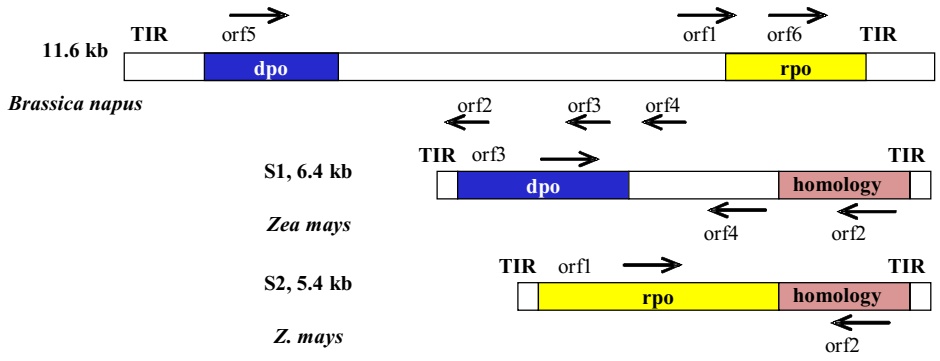


Fig. 1. Organization of the mitochondrial linear plasmids used in the studies: *Brassica napus* 11.6 kb and *Zea mays* 6.4 kb S1 and 5.4 kb S2 plasmids; dpo: DNA polymerase, rpo: RNA polymerase; the region of perfect sequence homology between the S1 and S2 plasmids is designated by pink color

Further substrates for mitochondrial import were the S1 and S2 linear plasmids from maize (*Zea mays*) [6, 7]. Organization of these plasmids is shown in Fig. 1. Isolation of mitochondria and uptake assays were performed as described earlier [5, 8].

**Results and discussion**. It has been shown previously [8] that (i) the efficiency of the import of large DNA molecules into plant mitochondria depends on the sequence and (ii) the specificity of DNA import can be mediated by the presence of certain elements in their sequence, especially TIRs at the ends of the molecules. Conversely, the efficiency of DNA import into mammalian mitochondria seemed to depend neither on the DNA sequence, nor on its size. Until now the role of the sequence and structure (in particular of the TIRs, which are different in linear plasmids from mitochondria of various plant species) in both mitochondrial DNA translocation and mitochondrial genetic processes (autonomous replication, integration into the genome, *etc*.) is unknown.

We addressed the possible universal role of the TIRs of mitochondrial linear plasmids from *Z. mays* and *Brassica* in the mechanism of DNA import into plant mitochondria. Using a vector containing the TIRs (327 bp) from the 11.6 kb *B. napus* plasmid [4], we obtained several DNA constructs and tested the importance of the size and DNA structure in the efficiency of the import in-

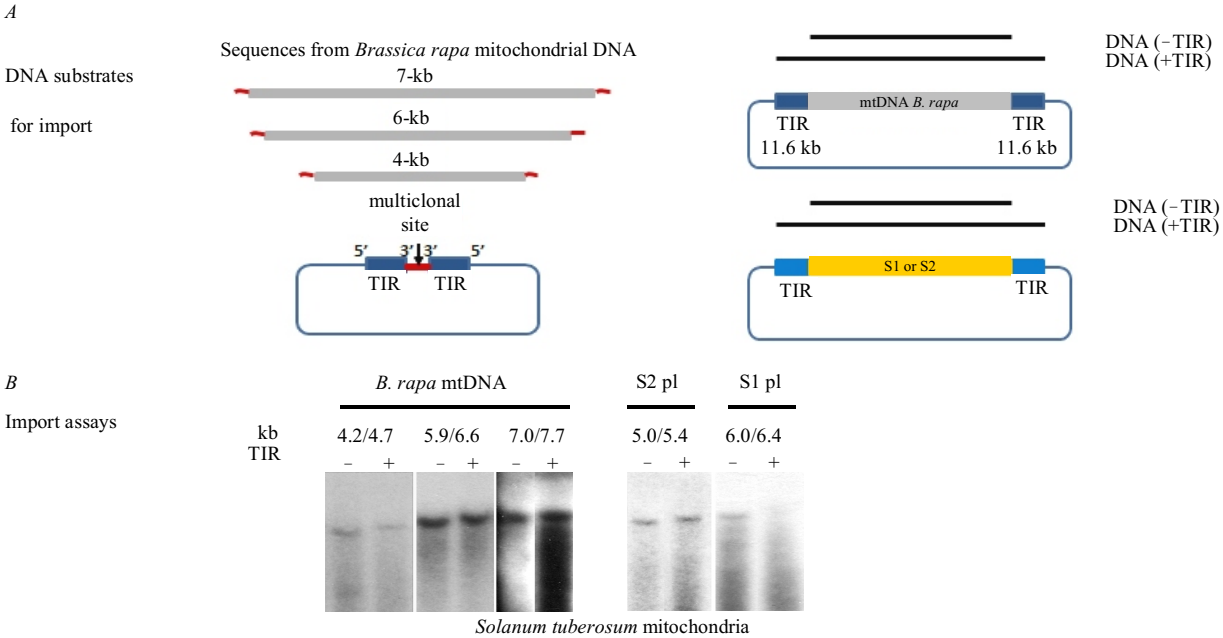


Fig. 2. Import of «medium» size DNA into *Solanum tuberosum* mitochondria does not depend on the presence of TIRs from mitochondrial linear plasmids: *A* – different sequences from the *Brasica rapa* mitochondrial genome were cloned between the TIRs from the *B. napus* 11.6 kb plasmid (the *Zea mays* S1 and S2 plasmids were cloned in parallel; all sequences were subsequently amplified by PCR with or without the TIRs); *B* – the amplified PCR products were radioactively labeled and used as substrates for import into isolated *S. tuberosum* mitochondria. Nucleic acids subsequently recovered in the mitochondrial fraction were analyzed by agarose gel electrophoresis, Southern blotting and autoradiography

to plant mitochondria (Fig. 2, *A*). The DNA sequences of *Z. mays* linear plasmids, S1 [6] and S2 [7] with or without TIRS (208 bp) were also cloned and used as substrates for import assays (Fig. 2, *B*). Using radioactively labeled DNA subst rates and the in organello potato (*S. tuberosum*) mitochondrial import system [5], it was established that the import efficiency has a non-linear dependence on DNA size: DNA fragments of 6–7 kb in size can be imported into *S. tuberosum* mitochondria more effectively than molecules with a 4 kb size. It was also shown (Fig. 2) that import into plant mitochondria of DNA molecules of «medium» sizes, *i. e*. between 4 and 7 kb, barely has any sequence specificity: neither TIRs from the 11.6 kb *Brassica* plasmid, nor TIRs from the *Z. mays* S-plasmids influenced DNA import into *S. tuberosum* mitochondria. Conversely, the role of the TIRs from the 11.6 kb linear plasmid in the import of large DNA molecules was established earlier for the *B. rapa* mitochondria [8].

**Conclusions**. The data obtained support a species-specific import mechanism of the mitochondrial linear plasmids, and more generally of large DNA molecules, into plant mitochondria, which needs further investigation.

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Специфіка імпорту ДНК в ізольованих мітохондріях з рослин і ссавців

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Резюме

***Мета***. *Вивчення відмінностей в імпорті ДНК у мітохондрії рослин і людини для кращого розуміння генетики мітохондрій та розробки біотехнологічних підходів до її дослідження.* ***Методи***. *Експерименти з імпорту ДНК в ізольоовані мітохондрії рослин з використанням як субстратів послідовностей, які містять або не містять специфічних кінцевих інвертованих повторів (КІП), характерних для лінійних плазмід рослинних мітохондрій.* ***Результати***. *Встановлено, що ефективність імпорту ДНК у мітохондрії нелінійно залежить від розміру ДНК. Показано, що імпорт у рослинні мітохондрії молекул ДНК «середніх» розімрів (4–7 тис. п. н.) має незначну структурну специфічність: ані КІП плазміди 11,6 тис. п. н. з Brassica napus, ані КІП S-плазмід із Zea mays не чинять впливу на імпорт ДНК у мітохондрії Solanum tuberosum.* ***Висновки***. *Отримані дані свідчать на користь гіпотези існування видоспецифічного механізму перенесення мітохондріальних лінійних плазмід у рослинні мітохондрії.*

*Ключові слова: імпорт ДНК у мітохондрії, мітохондріальні лінійні плазміди рослин, мітохондріальний геном.*

Специфика импорта ДНК в изолированных митохондриях растений и млекопитающих

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Резюме

***Цель***. *Изучение различных свойств импорта ДНК в митохондрии растений и человека для лучшего понимания генетики митохондрий и разработки биотехнологических подходов для ее исследования.* ***Методы***. *Эксперименты по импорту ДНК в изолированные митохондрии растений с использованием в качестве субстратов последовательностей, содержащих или не содержащих специфических концевых инвертированных повторов (КИП), характерных для линейных плазмид растительных митохондрий.* ***Результаты***. *Установлено, что эффективность импорта ДНК в митохондрии имеет нелинейную зависимость от размера ДНК. Показано, что импорт в растительные митохондрии молекул ДНК «средних» размеров (4–7 тыс. п. н.) имеет незначительную структурную специфичность: ни КИП плазмиды 11,6 тыс. п. н. из Brassica napus, ни КИП S-плазмиды из Zea mays не оказывают влияния на импорт ДНК в митохондрии Solanum tuberosum.* ***Выводы***. *Полученные данные свидетельствуют в пользу гипотезы существования видоспецифического механизма переноса митохондриальных линейных плазмид в растительные митохондрии.*

*Ключевые слова: импорт ДНК в митохондрии, митохондриальные линейные плазмиды растений, митохондриальный геном.*

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