

Association of mitochondrial lysyl-tRNA synthetase with HIV-1 GagPol involves catalytic domain of the synthetase and transframe and integrase domains of Pol

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Aim. Analyze the interaction between Lysyl-tRNA synthetase (LysRS) and HIV-1 GagPol to know whether a particular N-terminal sequence of mitochondrial LysRS triggers a specific recognition with GagPol. **Methods.** Yeast two-hybrid analysis, immunoprecipitation. **Results.** We have shown that LysRS associates with the Pol domain of GagPol. **Conclusions.** A model of the assembly of the LysRS:tRNA₃^{Lys}:GagPol packaging complex is proposed.

Keywords: tRNA₃^{Lys}, lysyl-tRNA synthetase, HIV-1, packaging.

Lysyl-tRNA synthetase (LysRS) is an essential protein involved in translation of genetic information in the cytosol, where it is a component of a supramolecular complex [1], but also in mitochondria of human cells. Beyond its role in translation, this enzyme is also involved in various pathologies. Cytosolic human LysRS triggers proinflammatory response, and serves as a key signaling molecule in the immune response [2, 3]. The mitochondrial species of LysRS interacts with a mutant of Cu, Zn-superoxide dismutase (SOD1) and contributes to mitochondrial dysfunction in amyotrophic lateral sclerosis (ALS) [4]. This enzyme is also hijacked from the host cell following HIV-1 infection to carry the primer tRNA₃^{Lys} into the virions [5]. The regulation of the spatio-temporal organization of this enzyme is a key element to understand the regulation of its activities.

Cytosolic and mitochondrial LysRS are encoded by a single gene [6] and can only be distinguished accor-

ding to their very N-terminal sequences [5]. It was previously believed that cytosolic LysRS is packaged into HIV-1 virions via its association with Gag [7]. Using monospecific antibodies, it was later shown that only the mitochondrial LysRS is taken up in viral particles along with tRNA₃^{Lys}, the primer for reverse transcription of the HIV-1 genome [5]. We re-analyzed the interaction between LysRS and GagPol to know whether the particular N-terminal sequence of mitochondrial LysRS triggers a specific recognition with GagPol, or if differential routing of the two LysRS species *in vivo* could explain specific and exclusive packaging of the mitochondrial species. We showed that LysRS associates with the Pol domain of GagPol. More specifically, the transframe (TF or p6*) and integrase (IN) domain proteins of Pol interact with the catalytic domain of LysRS [8]. A model of the assembly of the LysRS:tRNA₃^{Lys}:GagPol packaging complex is proposed, which is also consistent with the release of its different components after maturation of GagPol in the virions

(Figure, see inset). The cytoplasmic and mitochondrial LysRS species share an identical catalytic domain. Accordingly, we found that both enzymes have the intrinsic capacity to bind to GagPol *in vitro*. In addition, both enzymes interact with p38 *in vitro*, the scaffold protein of the cytoplasmic multi-aminoacyl-tRNA synthetase complex, even though only the cytoplasmic species of LysRS is a bona fide component of this complex. These results suggest that the different LysRS species are strictly targeted *in vivo*. Maturation of the premitochondrial form of LysRS upon its mitochondrial targeting is a prerequisite for its catalytic activation. These data open new perspectives for the search of a new class of inhibitors of the HIV-1 development cycle that would block the packaging of tRNA₃^{Lys} into viral particles.

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Асоціація мітохондріальної лізил-тРНК синтетази з білком GagPol ВІЧ-1 відбувається через каталітичний домен синтетази і трансматричний та інтеграційний домен білка Pol

Резюме

Мета. Встановити, чи може N-кінцева послідовність, яка є специфічною для мітохондріальної форми лізил-тРНК синтетази, забезпечувати взаємодію цього ферменту з білком GagPol ВІЧ-1. **Методи.** Двогібридна дріжджова система, імунопреципітація. **Результати.** Ми показали що лізил-тРНК синтетаза взаємодіє з доменом Pol білка Gag. **Висновки.** Запропоновано модель утворення комплексу ЛізРС:тРНК₃^{Lys}:GagPol.

Ключові слова: тРНК₃^{Lys}, лізил-тРНК синтетаза, ВІЧ-1, збирання віріона.

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Ассоциация митохондриальной лизил-тРНК синтетази с белком GagPol ВИЧ-1 происходит посредством каталитического домена синтетази и трансматричный и интегральный домены белка Pol

Резюме

Цель. Выяснить, может ли N-концевая последовательность, являющаяся специфичной для митохондриальной формы лизил-

тРНК синтетази, обеспечивать взаимодействие этого фермента с белком GagPol ВИЧ-1. **Методы.** Двогибридная дрожжевая система, иммунопреципитация. **Результаты.** Мы показали, что лизил-тРНК синтетаза взаимодействует с доменом Pol белка Gag. **Выводы.** Предложена модель образования комплекса ЛизРС:тРНК₃^{Lys}:GagPol.

Ключевые слова: тРНК₃^{Lys}, лизил-тРНК синтетаза, ВИЧ-1, сборка вириона.

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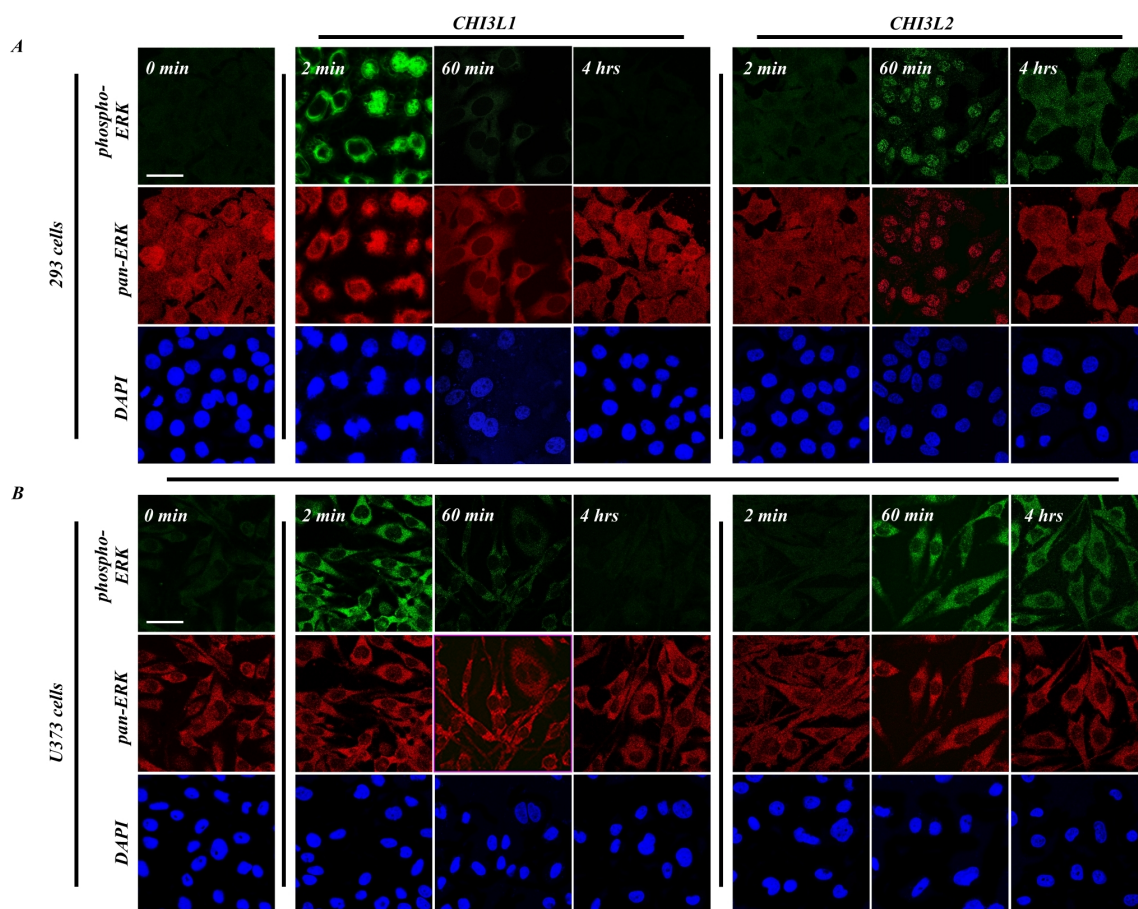
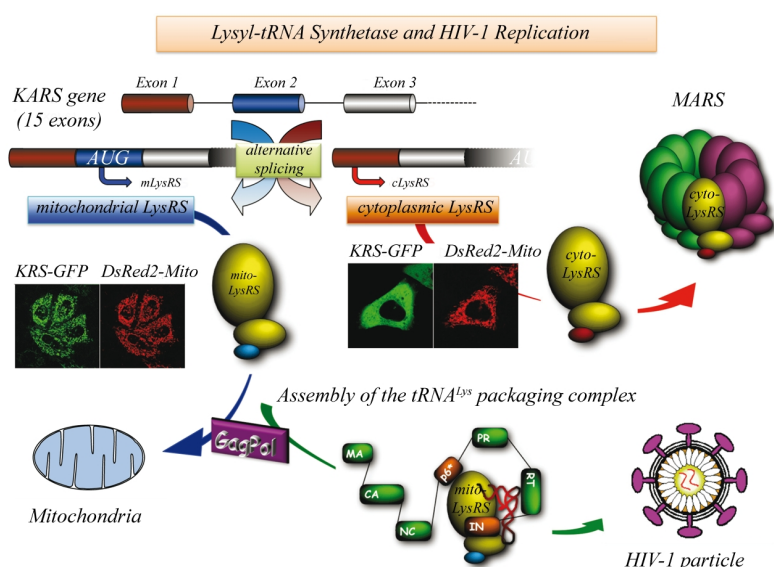


Fig. 3. Time course of ERK1/2 phosphorylation and localization in activated and inactivated cells. Immunofluorescence and confocal microscopy analysis of CHI3L1 or CHI3L2 treated 293 cells (A) and U373 cells (B) with phospho-ERK (green) and pan-ERK (red) antibodies. (Scale bar: 25µm)

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In human, a single gene encodes the cytoplasmic and mitochondrial forms of lysyl-tRNA synthetase by means of alternative splicing. The cytoplasmic isoform is produced from the mRNA lacking exon 2. This isoform is targeted to the MARS (Multi Aminoacyl-tRNA Synthetase) complex. The mitochondrial species is produced by translation initiation at the level of exon 2. Mitochondrial LysRS is specifically hijacked by the GagPol precursor protein during the process of packaging of HIV-1 particles. Association of mLysRS with GagPol involves catalytic domain of the synthetase and transframe (p6*) and integrase domains of the Pol region of GagPol. The formation of the tRNA(Lys, 3) packaging complex is an essential process of the HIV-1 life cycle, since this tRNA is required for initiation of reverse transcription of the RNA genome of HIV-1. The tRNA(Lys, 3) packaging complex is a potential therapeutic target