HOKKAIDO UNIVERSITY

Title	Molecular basis of transsulfursome for Cys-tRNACys synthesis in indirect pathway [an abstract of dissertation and a summary of dissertation review]
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Degree Grantor	北海道大学
Degree Name	博士(生命科学)
Dissertation Number	甲第12443号
Issue Date	2016-09-26
Doc URL	https://hdl.handle.net/2115/63253
Rights(URL)	https://creativecommons.org/licenses/by-nc-sa/2.1/jp/
Type	doctoral thesis
File Information	CHEN_MEIRONG_abstract.pdf, 論文内容の要旨

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学 位 論 文 内 容 の 要 旨
Abstract of Doctoral Dissertation

博士の専攻分野の名称　博士（生命科学）　氏　名　陳 美容
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学 位 論 文 題 名
Title of Doctoral Dissertation

Molecular basis of transsulfursome for Cys-tRNA^{Cys} synthesis in indirect pathway
(間接経路を用いた Cys-tRNA^{Cys} 合成複合体 transsulfursome の分子基盤)

Aminoacylation of tRNA is an essential stage during translation process. In this stage, a set of enzymes designated as aminoacyl-tRNA synthetases (aaRS) specifically match amino acids to their cognate tRNAs. In majority of organisms, CysRS catalyzes attachment of cysteine to tRNA^{Cys} to synthesize Cys-tRNA^{Cys}. However, CysRS and/or cysteine biosynthesis enzymes are absent in many methanogenic archaea, Cys-tRNA^{Cys} is formed by a two-step indirect pathway in such organisms[1]. Recent research of our group discovered that a large complex named transsulfursome takes charge of Cys-tRNA^{Cys} synthesis in indirect pathway[2]. Transsulfursome (540 kDa) consists of three protein components in a ratio of 4: 4: 4, O-phosphoseryl-tRNA synthetase (SepRS), Sep-tRNA:Cys-tRNA synthase (SepCysS), and SepRS/SepCysS pathway enhancer, SepCysE which is known as a bridge to connect SepRS and SepCysS. Synthesis of Cys-tRNA^{Cys} is performed on transsulfursome by two steps: firstly O-phosphoserine (Sep) is ligated to tRNA^{Cys} by SepRS; and then the mischarged intermediate Sep-tRNA^{Cys} is transferred to SepCysS where it is converted into Cys-tRNA^{Cys}. This indirect pathway is the sole source of cysteine biosynthesis in methanogenic archaea, and may be a ancestor pathway that the first time introduced cysteine into the genetic code.

Previous research revealed interaction between SepRS and tRNA^{Cys} in the first step of indirect pathway; in contrast, interaction between SepCysS and tRNA^{Cys} in the second step remains unclear. In this study, we reconstructed a sub-complex of transsulfursome (SepCysS-SepCycE) by co-expression of SepCysS and SepCysE in *Escherichia coli*, and determined structure of SepCysS-SepCycE with tRNA^{Cys}, which represents a binding manner of tRNA^{Cys} in the second step. The structure of SepCysS-SepCysE-tRNA^{Cys} consists of a SepCysS dimer, a SepCysE dimer and only one tRNA^{Cys}, leaving one active site empty. Interestingly, in this complex, tRNA-bound SepCysE contains two domain; SepCysS-binding domain (RSBD), and C-terminal domain (CTD), while CTP disordered in tRNA-free one. Three regions of SepCysS-SepCysE interact with tRNA^{Cys}. The N-teminal loop (L16-L30) of SepCysS crosses over the TΨC loop of tRNA^{Cys}, and this weak interaction is considered important to direct acceptor arm into active site. In addition, a helix-loop-helix-loop region (P333-E367) of SepCysS at the entrance of active site compactly interacts with acceptor arm of tRNA, in which four residues (R345, G346, F347, G364) specifically recognize the discriminator base U73. Binding assay showed that mutant of tRNA^{Cys} U73A lost binding ability to SepCysS-SepCysE, while substitution of G73 with uracil in tRNA^{Phe} acquired binding ability to the complex. As previous research already revealed the recognition of

$tRNA^{Cys}$ at anticodon and U73 by SepRS in the first step, recognition of $tRNA^{Cys}$ at U73 by SepCysS suggests that the two-step process is rigorously scheduled on transsulfursome to guarantee fidelity of the reactions. Moreover, the third tRNA interaction region is the C-terminal domain of SepCysE (SepCysE(CTD)). The protein-tRNA interface revealed a positive charged region of SepCysE(CTD) to interact with the elbow of $tRNA^{Cys}$ via electrostatic interaction. Further investigation on binding affinity of SepCysE(CTD) and tRNAs by EMSA indicates that SepCysE(CTD) can be characterized as an non-specific tRNA binding domain, as it can bind different tRNAs with affinity almost at the same level as $tRNA^{Cys}$.

In order to understand how transsulfursome assembles and tRNA channeling on it, we successfully reconstructed transsulfursome-tRNA complex using purified three proteins and *in vitro* transcribed $tRNA^{Cys}$, and tried to determine the structure of the transsulfursome-tRNA. However, only structure of SepRS and N-terminal helix of SepCysE (SepRS-SepCysE(Nhelix)) was obtained although the full transsulfursome mixed with $tRNA^{Cys}$ was used to crystallize, as the rest parts of transsulfursome disorder in the structure. The packing of structure shows there are enough spaces at the two sides (the side of C- terminus of N-helix) of SepRS-SepCysE(Nhelix) to accommodate the other parts of SepCysS-SepCysE. In this structure, two dimers of SepCysE(Nhelix) insert deeply into clefts at interfaces of anticodon binding domains of SepRS tetramer (dimer of dimer). Three loops of SepRS at the interface are mainly responsible for binding SepCysE(Nhelix). These interactions were confirmed by binding assay of SepRS and SepCysE mutants. Truncation of each loop of SepRS separately showed no significant decrease on binding affinity, while complex formation of SepRS and SepCysE cannot be observed when deleting SepCyE(Nhelix) or introducing double-loop truncations of SepRS. This tight binding manner facilitates the formation of transsulfursome. Furthermore, prediction of secondary structure of SepCysE suggests that SepCysE(Nhelix) is followed by a long loop, which is invisible in current structures and explains the flexibility of SepCysS-SepCysE. Taken current structures and assay data together, the architecture of SepCysE can be concluded: SepCysE consists of three domains, the SepRS binding domain (RSBD: M1-I25; N-terminal helix), the SepCysS binding domain (CSBD: P41-F98; two helices), and the tRNA binding domain (tRBD: L112-D213; C-terminal domain), connected by two loops, linker1 (R26-K40) and linker2 (G99-A111). Linker1 may contribute to movement of SepCysS-SepCysE on transsulfursome, while linker2 may be essential for the movement of SepCysE(tRBD) with tRNA during transferring.

By superposing 2-fold axes of SepRS-SepCysE(RSBD) and SepCysS-SepCysE(CSBD) structures, we built a model of transsulfursome without SepCysE(tRBD), which displays a rectangular shape with dimensions of approximate 315 x 124 x 94 Å (L*W*H). This transsulfursome model was further supported by SEC-SAXS and TEM data, which also indicated the flexibility of two flanking SepCyS-SepCysE(without RSBD). Finally, models of transsulfursome-tRNA in the first and the second step were also built by superposing tRNAs of SepCysE(tRBD)-tRNA and SepRS-tRNA, and we proposed the tRNA channeling on transsulfursome via cooperation work of two linkers of SepCysE.

Reference:

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