Retraction

Retraction: Functional Substitution of an Indirect Pathway for tRNA Glutaminylation with a Direct Pathway by \textit{T. thermophiles} GlnRS (\textit{IOP Conf. Ser.: Earth Environ. Sci.} 276 012012)

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Functional Substitution of an Indirect Pathway for tRNA Glutaminylation with a Direct Pathway by *T. thermophiles* GlnRS

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**Abstract.** In protein synthesis mechanism, there is aminoacyl-tRNA synthetase (AaRSs) enzyme which is essential for housekeeping enzymes and could be formed a ternary complex in various kingdoms. The differences of this phenomena, unfortunately because there are some of AaRSs synthesize by an indirect pathway using other AaRSs which has a high similarity of amino acid formula. Aminoacylation of transfer RNA \( \text{Gln} \) (tRNA\text{Gln}) is one of AaRSs carried out by an indirect pathway in most of organism, and even there are following distinctions in various kingdoms and also the mechanism in various kingdoms. ScGlnRS cytoplasm produced by the direct pathway of glutaminylation tRNA\text{Gln}, besides mitochondrial Gln-tRNA\text{Gln} generated a slightly complicated than the cytoplasm, because it must go through the step of amido attachment needed extra enzyme non-discriminating glutamyl-tRNA synthetase by specifically changing Glu-tRNA\text{Gln} to Gln-tRNA\text{Gln}. Genetic engineering has been done by combining enzymes from EcGlnRS bacteria with non-specific binding tRNA cofactors from yeast, known as Arc1p, capable of increasing tRNA binding and activity of enzyme from bacteria to yeast system in vivo both in cytoplasm and mitochondria. By using the same combination of Arc1p to TtGlnRS bacterial enzyme, different mechanisms were found because they were only able to function in the mitochondria as well, not with the cytoplasm. *T. thermophiles* possesses similarity in tRNA identity determinants of *E. Coli* but has a difference in yeast cytoplasm and mitochondrial tRNA\text{Gln} isoaceptors. Arc1p-TtGlnRS fusion significantly enhanced its protein expression. In this finding, we report the trans-kingdom and eukaryotic glutaminylation were able to replace the role of the enzyme from yeast, it can also express equally noteworthy.

**Keywords:** Aminoacyl-tRNA synthetases, GlnRS, Arc1p, Indirect Pathway, Direct Pathway

1. Introduction
The central dogma is a simple process by which the instructions in DNA are converted to mRNA into protein synthesis a functional product. The decomposition of mRNA faithfully transformed into a protein depends on the accuracy of the aminoacyl-tRNA synthetase (AaRSs) enzyme with a specific codon for its anticodon. AaRS Aminoacyl-tRNA synthetases abbreviated with AaRSs are a structurally divide in twenty of diverse group enzymes, each pair between a specific amino acid to its...

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cognate tRNA. AaRSs structurally from amino acids are divided into 20 types which representing each amino acid, LeuRS, IleRS, ValRS, ArgRS, CysRS, MetRS, SerRS, ThrRS, AlaRS, GlyRS, ProRS, HisRS, GluRS, GlnRS, LysRS, AspRS, AsnRS, TyrRS, TrpRS, and PheRS. Mechanically in figure 1A shows that starting from amino acid activation, the binding of amino acids with tRNA then protein synthesis reaction catalyzed with AaRS enzyme.

Naturally, there are 20 types of AaRS which can generally be found in euakaryotes, whereas in most bacteria and archaea only around 18 sets. This is due to the presence of AaRS which is not crucial for the bacteria; it could be the synthesis of two proteins by the same AaRS. AaRS plays a vital role in the process of translating amino acid nucleotides into protein [1-3]. AaRSs possess two specific functions in cytoplasm and mitochondria, each AaRSs form complex with two distinct sets both of tRNA cytoplasm (tRNA_Glu) and tRNA mitochondria (tRNA_Gln). Besides, a set of eukaryotic AaRS contains two types based on the needs of two different cell organelles namely cytoplasm and mitochondria, whereas in prokaryotic only cytoplasm. Moreover, in euakaryotes, there are four genes by determining multiple functions that are driven both mitochondrial and cytoplasmic activity. ALA1, GRS1, HTS1, and VASI [5-9].

Somehow, there is an exception in asparaginylation by Asp-tRNA synthetase and glutaminyl by Gln-tRNA synthetase, which is synthesized proteins in the cytoplasm directly, but in mitochondria using a unique synthesis pathway [4]. The interesting phenomena of synthesis Gln-tRNA^Gln^ was performed using GluRS besides GlnRS, with addition non-discriminating-GluRS (ND-GluRS) in amidotransferase function. In euakaryotic cytoplasm of organism, Glutaminyl synthetase is synthesized using direct pathway [17] while most prokaryotes, including bacteria and all archaea, were found to have an indirect synthesis pathway, using an additional enzyme ND-GluRS which donated NH_4^+ to convert Glu to Gln in amino acids bound to tRNA^Gln^ which were wrongly loaded and then transfused [18] (Figure 1B). This pathway occurs in most euakaryotes such as yeast even high euakaryote human in mitochondria Gln-tRNA^Gln^ formation, and it shows that a big reason could be due to the evolution of mitochondrial compartments originating from bacteria gene through horizontal gene transfer.

Generally, due to fundamental differences in cell components and their needs, two types of enzymes that function as aminotransferase are also due to the different needs of each organism. Euakaryotes have trimeric in the form GatCAB (heterotrimeric), whereas prokaryotes have amidotransferases in the form of GatDE (heterodimeric). Conceptually, if a bacterium does not have GlnRS, GatCAB/GatDE play function as a Glu/Amidotransferase, while in bacteria absence AsnRS, they function as an Asp-Amidotransferase [19,20]. In most euakaryotic organelles, its rise a big question to find out in depth which mechanism is used between the two mechanisms. In the specific condition of Gat function in low prokaryote, such archaea, if there is a GatCAB homolog in species lacking archaea, then the extra function of this enzyme as AspAdT, on the other hand, the presence of GatDE in archaea act exclusively as a Glu-Amidotransferase. There is no debate about the route which is used to generate yeast mitochondrial Glutaminyl synthetase because there have been many studies that led to conclusions the pathway to produce Glu-tRNA^Gln^ in yeast mitochondria possess indirect pathway [9]. Besides, each yeast has a unique amidotransferase, which is almost different compared to some homolog organisms and there is even a heterotrimeric GatFAB in S. pombe instead GatCAB in S. cerevisiae [4].

There is a fundamental difference in the physiology of the gene that encodes the AaRS enzyme in the length of the gene. In prokaryotic AaRS has a gene that is shorter that euakaryotic with the presence of an extra domain in each subdomain of the gene. This extra domain can be found in N-domain (catalytic domain) or C-domain (binding domain) of the gene which cannot be found in the part of the prokaryotic cell [10]. Physically, the schematic of the extra domain is rich in lysine amino acid residues driven positive charge to play involved and enhance in tRNA binding activity or might participate in protein-ligand interaction and protein-protein. As an example, a ternary complex is formed between Methionyl-tRNA synthetase, Glutamyl-tRNA synthetase with Arc1p a cofactor through N-terminal extra domains [14]. Furthermore, some extra domains having a particular signal functions as a nucleus signal which is believed to directly synthetase to nucleic. S. cerevisiae GlnRS
extra domain in N-terminal (ScAdGlnRS) possess similar manner as non-specific tRNA binding as Arc1p instead of protein-protein interaction [13,14].

In the mechanism of action, AaRSs polymerase of amino acid through peptide bond then produced by a much more sophisticated process in ribosomes [15]. Recently, scientists found that four of aa-tRNAs species using indirect lines to be synthesized, among them Gln-tRNA\textsuperscript{Gln} has attracted the attention of many people, mainly because of its kingdoms-specifics pathway or enzymes [16]. We found in the previous study of protein engineering by combining Arc1p cofactors with specific genes from bacteria capable of increasing their activity, both in complementing or expressing proteins [11,12]. The combination of Arc1p to EcGlnRS allows or functionally bacterial enzymes to replace yeast homologs in vivo activity by increasing the recognition of tRNA. This increased gene activity applies not only to the cytoplasm, but also to the mitochondria after the gene imported into mitochondria using a mitochondrial signal. Herein, we used the same strategies fusion Arc1p enzyme to TiGlNRS, and then tested in cytoplasmic activity. The fusion gene then inserted mitochondrial signaling to import the fusion into mitochondria to analyze complementation activity.

**Figure 1.** A schematic reaction of aminoacylation-tRNA synthetases. The first step activation of amino acid, following tRNA attachment, and polymerase protein synthesis (A). A yeast Glutaminyllation pathway, the direct pathway form Gln-tRNA\textsuperscript{Gln} in the cytoplasm, while the indirect pathway using ND-GluRS introduce amidotransferase form Gln-tRNA\textsuperscript{Gln} in mitochondria (B).

2. **Methods**

2.1. **Construct of Plasmid (Gene engineering)**

The plasmid construct begins with bacterial DNA isolation using the previous procedure (12,13) then design a set of RNA primer Primer CC25 3’ GAG TAG ACT AGT CTT ATG GGC CTT GTC CCC GAG and Primer CC26 5’ GGG TCA GAC CAT ATG CCC CTC TTT TAG GGG CAC for PCR amplification the gene target. PCR product was cloned into pYY1 pADH yeast shuttle vector by Spel in 3’ site and Ndel in 5’site. [13]. TiGlNRS then modified by fusing with MTS a mitochondrial signal, which is containing a signal for import gene to mitochondria to the N-terminus of TiGlNRS or its derivates. The Mitochondria targeting sequences of DNA sequence was provided from a small plasmid.
construct contain in plasmid vector yeast, was cloned from VaRS in previous construct pWYH8 (AAGCTT-ADH-prom-AGCTATACCAAGCATACAATCTGCACGCTGCGCAGAAA-(MTS)-ACTAGTCATATGCTCGAG-(6xHis)-GAATTC). pWYH8 contain an MTS and the construct then fused with Sph/Nde1 cutting site by subclone which modified previous protocol (Stratagene, La Jolla, CA, USA).

2.2. Gene localization and Protein Expression Assay.

To verify the gene localization after cloned in several domain derivates, we used GFP assay green fluorescence microscopy modified previous protocol (14), that exhibited bright green fluorescence, when we exposed to light in the blue to ultraviolet range. Fluorescence microscopy used yeast cells, which were grown to OD600 reaching 0.6 in selective medium (SD without Leu), cells were pretreated with MitoTracker (300nM) for 30 minutes. After incubation, the sample then analyzes by microscopy using fluorescence dye a 100 objective at room temperature (25 °C) (Axio observer, Al; Carl Zeiss, Inc) and images were captured with a CCD camera. We merge it and tracked the mitochondrial localization using Axio Vision Rel.4.8 software and subjected to 2D deconvolution with AutoQuantant X2. This research was carried out to make sure that the genes have been imported to specific cells target. Moreover, the further analysis we carried out by Western blot assay using His6-tag antibody to characterize our several genes cloning/construct expression in yeast system cloning. The Western blot assay has been modified from a protocol described in the previous paper [22].

2.3. Rescue activity by Cytoplasmic complementation assay

The cloning construct have to verify the rescue activity by complementation assay of TtGlnRS and derives for cytoplasmic function of the yeast GLN4 (encoded GlnRS) knock out strains, YR2 (MATa, his3-D1, leu2-D0, ura3-D0, lys2-D0, gln4, kanMX4), was contain a maintained plasmid carrying the wild type GLN4 gene and a URA3 as a marker. Rescue activity of TtGlnRS and derives for cytoplasmic carried out by complementation assays were performed by introducing a test plasmid carrying the gene of interest and a LEU2 marker into YR2, and the ability of the transformants to grow in the presence of 5-fluoroorotic acid (5-FOA) was determined. Starting from a cell density of 4.0 A600, cell cultures were 5-fold serially diluted, and 10 ml aliquots of each dilution were spotted onto the designated plates containing 5-FOA. Then the plates were an incubated in an incubator at 30 °C for 3-5 days and the transformants monitored by maintenance plasmid with URA3 and the presence of 5-FOA markers and thus could not grow on selection media except functional cytoplasmic GlnRS encoded by plasmid test.

2.4. Rescue activity by Mitochondria complementation assay

The cloning construct has to verify, TtGlnRS was fusion to Mitochondrial targeting sequence (MTS) then we tested to completed our research by complementation assays for mitochondrial of GatCAB (heterotrimer Gat A, Gat B, Gat C) activities were performed by introducing a test plasmid (carrying a LEU2 marker and the gene of interest) into yeast knockout strain, and then the resultant of transformants were selected on 5-FOA lacking leucine. In the presence of 5-FOA, the maintenance plasmid (carrying a URA3 marker) was evicted from the transformants. The transformants that survived in 5-FOA selection were further we tested in yeast extract peptone glycerol/ mitochondria function tested plates and incubator at 30 °C for 3-5 days, with results were documented on day 3 following plating. As a yeast cell cannot survive on glycerol, a non-fermentable carbohydrate if there is unfuctional of mitochondria, then transformant cell growth on the YPG plats unless has function GatCAG was generated from plasmid construction. Rescue activity of GatCAB carried out by complementation assays for Gat B (encoded by pET112) rescue GatF (encoded by Ygr102) knockout strain followed a similar protocol.
3. Results and Discussion

3.1. Protein sequence comparison

Aminoacyl-tRNA synthetases (AaRSs) belong to enzymes that play an essential role in the translation of proteins from mRNA and ancient families of enzymes that establish their genetic codes by attaching specific amino acids to their tRNAs cognate to form aminoacyl-tRNAs. Then, it is sent and delivered to the ribosome to decompose mRNA codons through base pairing with the anticodon of the aminoacyl-tRNAs (Figure 1A). Because the process of protein translation occurs in two different places, both cytoplasm, and mitochondria in eukaryotes, it takes two sets of each AaRS. Two sets of AaRS needs can come from the same gene but using different signals and translation initiation codon, there was an extra sequence in the N-terminal domain or two sets or AaRS needed from two different genes.

Bacteria generally possess one aminoacyl-tRNA synthetase enzyme that functions specifically on each of 20 natural amino acids. Somehow, the bacterial enzymes which were produced from the cell nucleus possess high similarities with mitochondria in eukaryotes, yeast counterpart. This is allegedly due to gene evolution, through the horizontal gene transfer process. In general, one bacterium with each other has proximity to a single sequence of the same gene. To find out the percent identity and percent similarity among genes, we used the NCBI gene database to calculate more accurately between two bacteria E. coli and T. thermophilus, which belong to gram-negative bacteria groups. Our data showed that there are similarities and differences between E. coli and T. thermophilus GlnRS (Figure 2). These data we used as preliminary data, to analyze the similarity and identity for function prediction of GlnRS family EcGlnRS and TtGlnRS heterologues. The results revealed that the sequences of these have 39% identity and 69% similarity. This case was similar to both E. coli and T. thermophilus which came from different gene evolutionary following by gene duplication and raised our question whether T. thermophilus GlnRS possess a similar mechanism for rescue GlnRS as E. coli GlnRS.

![Figure 2. Protein Sequence comparison between TtGlnRS and EcGlnRS. (A) Percent Identity and Similarity between bacterial GlnRS (Ec: Escherichia coli, Tt: Thermus thermophilus, Bs: Bacillus subtilis, Sc: Saccharomyces cerevisiae) (B) Sequence protein amino acids composition between TtGlnRS and EcGlnRS.](image)

3.2. T. thermophilus and Escherichia coli isoacceptors are highly similar

In the process of protein synthesis, amino acid chains are carried by tRNA into the ribosome, according to the appropriate codon and anticodon (cognate tRNA). Evolutionary shows that tRNA\(^\text{Gln}\) have unique features as interdependent tRNA. The nature of amino acids can be analyzed and compared with the linear combination of digital information in the identity of tRNA elements to reveal the coding relationships in each molecular region. The correlation with the identity of tRNA elements can be established by using regression analysis. This accessories, non-enzymatic proteins are responsible for complex transient interaction with eEF1A (p18), complex assembly (p38) and increase affinity for tRNA (p43). Our previous studies on tRNA\(^\text{Gln}\) recognition of E. coli GlnRS and S.
cerevisiae GlnRS seems that the identity of an element which is specific between one tRNA and another. Mainly affected only one or two identity elements but the tRNA\(^{\text{Gln}}\) possess a interdependent tRNA mechanism in the base discriminator, acceptor stem and three anticodon loops (Figure 3) [33,34]. Particularly, there are several nucleotides that significantly contribute to the aminoacylation performance of tRNA\(^{\text{Gln}}\) by GlnRS: U1:A72, G2:71, G3:70 in the first of three base pairs the acceptor stem, following the differentiating base of tRNA G73, then the D-stem in position G10, and U35, U36 of the anticodon position.

The resultant of tRNA sequence, then we analyze sequences and secondary structure of which tRNA\(^{\text{Gln}}\), also mitochondria showed that all identities of tRNA elements have diverged (Figure 3). Somehow, the discriminator base is different which is there is a U in S. cerevisiae cytoplasm, an A in S. cerevisiae mitochondria, and G base in E. coli of tRNA\(^{\text{Gln}}\). From this result of the profoundly different of tRNA discriminators, S. cerevisiae tRNA\(^{\text{Gln}}\), and tRNA\(^{\text{Gln}}\) are expected to be low-affinity substrates for T. thermophiles GlnRS as an E. coli Gln-tRNA synthetase recognition to yeast tRNA\(^{\text{Gln}}\).

![Figure 3. Comparison secondary structure of yeast S. cerevisiae tRNA\(^{\text{Gln}}\), E. coli tRNA\(^{\text{Gln}}\), T. thermophiles tRNA\(^{\text{Gln}}\) isoacceptors.](image)

3.3. TtGlnRS unable rescue yeast Cytoplasmic GlnRS knockout strain

Our previous data was published in NAR journal, shown that genetic engineering of EcGlnRS fusion Arc1p in N-terminal then successful function both of yeast organelles cytoplasmic and mitochondrial form of GlnRS using direct pathway [3]. Therefore, we were wondering whether T. thermophiles which closely related bacteria family to E. coli. We used BLAST search and analyzed for levels of similarity between several types of bacteria and non-bacteria regarding GlnRS gene. The comparison of E. coli and T. thermophiles showed that not significantly homologous to each other (~52% identical), but profoundly different with the yeast enzyme has an extension domain ~39-42% identical. Construct cloning of T. thermophiles GlnRS encoded by GLN4 gene into yeast strong and shuttle vector of pADH promoter, and the functionally into rescue activity the growth defect yeast GLN4 knockout strain on 5-Fluoroorotic acid (5-FOA) to select for the absence of the URA3 gene by several mechanism and a toxic metabolite was tested (Figure 5). As shown in Figure 5, TtGlnRS of plasmid construction failed to complement ScGLN4 knockout strain (Figure 5 number 5 and 6). This data suggested that the cytoplasmic activity of yeast can’t be replaced by TtGlnRS, due to the level of gene similarity profoundly different each other (39% identity), also, the evolution of yeast cytoplasmic organelles are derived from different origins to bacteria as mitochondria.
Figure 4. Summary of the diagram the construction of E. coli and T. thermophiles Gln-tRNA synthetase and various fusions with Arc1p and MTS.

To examine whether TtGlnRS by fusion Arc1p following the same manner with Arc1p-EcGlnRS can express in the yeast system and confirm that all of the constructs were cloned well we examine the protein expression levels which contain His6x-tagging then identified by tagging antibody of Western blotting. Eight plasmid constructions were transformed into yeast wild-type INVSC1 overnight and protein extracted using buffer solution, then loading into the SDS-PAGE gel and express by X-rays film Western blotting. As shown in Figure 6, T. thermophiles GlnRS and derivates were all properly express in yeast but not for TtGlnRS single gene without fusion in cytoplasmic. This result reinforced the idea that the negative phenotype activity of TtGlnRS in the rescue activity assay was not caused by gene expression, a lower level of protein, and somehow even not express a protein in cytoplasmic but it was expressed in mitochondrial somehow could not rescue particularly.

3.4. Imported TtGlnRSs to function in mitochondria of Yeast S.cerevisiae

It was recently established that the mitochondria yeast Gln-tRNA\textsuperscript{Glu} were known to be produced by indirect pathways. It was supported by previous research in an in vivo assay of yeast GlnRS is missing in mitochondria and supported by \textit{in vitro} assay ScGlnRS imported into mitochondria as a substrate to tRNA\textsuperscript{Glu} and Km/Kcat of is lower than normal that indicated aminoacylate is poor in mitochondria \cite{9}. Somehow imported fusion of Arc1p and EcGlnRS (Arc1p-EcGlnRS) into cytoplasmic and mitochondria yeast knock-out system enables to replace the function of a yeast gene. The question arose as for whether by imported Arc1p-EcGlnRS with MTS to test functionally of the enzyme can substitute for the indirect pathway by gene homologs bacterial family instead of E. coli. Moreover, it was supported that the yeast mitochondrial driven from bacterium gene by horizontal gene transfer, then affect and modified during evolution, from a close origin and sequence homology, so this is an alternative that we guest can be applied to all bacteria, including TtGlnRS. Therefore, to answer our hypothesis, whether the mitochondria of S. cerevisiae GlnRS able to functionally, and activity if we using TtGlnRS instead of EcGlnRS, we fused a DNA small fragment contain mitochondrial signal (MTS) of yeast and inserted in-frame at the 5’end of TtGlnRS and Arc1p-TtGlnRS to facilitate the gene imported into mitochondrial and elucidated the bacterial protein performance and biological activity. All of the constructs transform to the yeast system then determine protein expression and rescue ability (Figure 5A and 5B).

Our results (Figure 5A and 5B) show that the TtGlnRSs enzyme \textit{per se} completely fails to replace the yeast gene function that has been knockout or deleted from S. cerevisiae in both mitochondria and cytoplasm, fusion MTS events can only replace the yeast knockout gene (loss of function). It seems like divergence of evolutionary E. coli, T. thermophiles, and yeast mitochondrial. Sc\textit{GlnA}\textsubscript{m} failed
attached bacterial GlnRS alone, while the fusion of an MTS and Arc1p become MTS-Arc1p-TtGlnRS successfully operated as a key of glutaminylation in yeast mitochondria (Figure 5B, number 8). All of these results also confirm that the uniqueness is in Glutaminylation synthesis in various organisms. Moreover, the tRNA independent have significant influences in the process of forming aminoacylate complexes into proteins synthesis; most of the species have a specific tRNA identity element for attaching specific AaRS.

### 3.5. Protein Expression

To verify the TtGlnRS gene and its derivatives construct results, we carried out a protein level analysis performed using Western blotting. This experiment used the anti-body His6x-tag and expressed using X-ray film. The expression of cloning genes was performed while constructing TtGlnRS low expression. TtGlnRS gene alone is not expressed in the yeast system, somehow that might because the bacterial gene has a toxic in yeast cytoplasmic plasmid gene system beside mitochondria (Figure 6), and to verify the internal control is used from within the system using PGK (phosphoglycerate kinase-1). Relatively they have different protein level, showing by 1.5-fold between MTS fusion TtGlnRS than Fusion protein by Arc1p.

![Figure 5. Rescue activity complementation assays for yeast GLN4 gene (cytoplasm) and GatCAF (mitochondria) by TtGlnRS and their derivatives construct of the yeast deleted glutaminylation activities strain was tested. (A) A Table constructs name and the summary of their ability in complementation activities (+; positive complementation and -; negative complementation. (B) Cell growth spot on a 5-FOA plate for cytoplasmic activity assay and cell growth spot on a YPG plate for mitochondrial activity assay.](image-url)
Figure 6. Analysis of protein expression from several gene constructs by Western blotting (number 1-8) in A-B denotes shown in figure 5. Upper line blotting, TtGlnRS, and its derivates; lower line blotting, PGK (as a control).

3.6. Cellular Localization of TtGlnRs and derivates

To explore and understand more of the construct gene localization of both construct Arc1p-TtGlnGlnRS and MTS-TtGlnRS, we carried out by fluorescence microscopy using the green fluorescence protein (GFP) from a DNA sequence encoding was inserted right after the of construct gene (30 ends), resulting in Arc1p-TtGlnRS and MTS-Arc1p-TtGlnRS, respectively. The construct was overexpressed in a yeast constitutive strong promoter ADH. The fusion GFP construct form Arc1p-TtGlnRS-GFP and MTS-Arc1p-TtGlnRS-GFP were transformed into yeast wild-type strain INVSc1 and cultured for reached OD600 0.6 then treated with DAPI for cytoplasm localization check or mitochondrial tracker dye for mitochondria localization check. The resultant of localization shown in figure 7, which fusion of Arc1p-TtGlnRS was exclusively localized in the cytoplasm which is missing a Mitochondrial targeting sequence (MTS), while MTS-Arc1p-TtGlnRS was colocalized in mitochondria. The gene expressed was existed in the cytoplasm and could be targeted to mitochondria by fusion to an MTS. The constructs can be localized in both cytoplasm and mitochondria; therefore, the ability or inability of the gene to rescue the yeast GLN4 knock-out strain is not due to the absence the genes in the cells but might be another reason in the incompatibility of gene functions (Figure 5A and 5B). The constructs are colocalized both in cytoplasmic and mitochondrial, unfortunately, unable to complement a yeast knock out strain (Figure 5B) in mitochondrial counterpart.

Figure 7. The cellular localization of two genes plasmid cloning construct. We focus on construct number 7 (Arc1p-TtGlnRS) and number 8 (MTS-Arc1p-TtGlnRS) fusion with GFP and visualize under fluorescence microscopy using dye for a mitochondrial tracker or cytoplasm DAPI.

4. Conclusion

The results from this research, we attempted to construct by genetic engineering for substituting the indirect trans-amidation pathway from Glu-tRNA\textsubscript{Gln} become Gln-tRNA\textsubscript{Gln} which is a synthesis in \textit{S. cerevisiae} mitochondrial counterpart using a direct pathway. Our results showed that Ec-tRNA\textsubscript{Gln} and Tt-tRNA\textsubscript{Gln} carried identical identity elements, however Arc1p-EcGlnRS was able to be recognized by rescue \textit{S. cerevisiae} glutaminylation activity both Sc-tRNA\textsubscript{Gln} (cytoplasm) and Sc-tRNA\textsubscript{m}Gln (mitochondria), but Arc1p-TtGlnRS was specific for Sc-tRNA\textsubscript{m}Gln.
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