Investigation of the traditional enzymatic role and the emerging regulatory function of dUTPases

Theses of the Ph.D. dissertation

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1 INTRODUCTION

My thesis focuses on the enzymatic properties of the trimeric dUTP pyrophosphatase and the interconnections of these properties with the traditional and regulatory functions of dUTPase. The dUTPase enzyme belongs to the dUTPase superfamily, which comprises also the dCTP deaminase (DCD) and the bifunctional dCTP deaminase-dUTPase (DCD-DUT) enzymes. The members of this superfamily produce important precursors for dTTP biosynthesis. In addition, dUTPase also has a preventive DNA repair role by the removal of dUTP from the dNTP pool which prevents dUTP incorporation into DNA.

dUTPase, DCDs and DCD-DUTs share a common homotrimeric structural core [1–4]. The three subunits form a central channel and three equivalent active sites at the intersubunit clefts. This intricate quaternary structure intuitively suggests the possibility for allosteric control within the enzyme. In effect, the allosteric communication between the active sites of DCD and DCD-DUT family enzymes has been investigated in several species [3,5–7] and was found to operate through the central channel [3,7]. dUTPases display an even more intricate interaction pattern between their subunits than DCDs and DCD-DUTs do. Their C-terminal part reaches across the trimer to the remote active site and therefore, all three subunits contribute conserved residues to each active site [4]. This structure inspired the proposition that allosteric communication between the active sites of dUTPases should also exist [8–10].

Beside their metabolic and preventive DNA repair role, dUTPases participate in several additional processes, including the activation of the horizontal gene transfer (HGT) of *Staphylococcus aureus* pathogenicity islands (SaPI). SaPIs themselves do not encode any machinery for HGT, they take advantage of phage reproduction instead [11]. In the absence of a helper phage, the expression of SaPI-encoded transfer initiating proteins (integrase and excisionase [12]) is repressed by SaPI-encoded repressor proteins called Stl. Helper phage infection or prophage activation relieves Stl repression and leads to the excision and extensive replication of SaPI DNA that is subsequently packaged into phage capsids [11]. In case of SaPIbov5 and SaPIbov1 it was found that they are de-repressed by dUTPases from phage 80α (for both) and phage Φ11 (for SaPIbov1))[13,14]. Here, the enzymatic cycle of dUTPase was suggested to play a key role in the activation process. Moreover, in this process the dUTPase-regulated gene transfer was proposed to adopt a mechanism highly similar to G protein mediated signaling, where the switching conformational change between the on/off states occurs upon GTP binding to the G protein [13].

2 AIMS

dUTPases play key roles both in the maintenance of the appropriate dUTP/dTTP ratio and in various additional biological processes. No surprise that dUTPases are investigated as potential drug targets. Understanding the exact role and mechanism of this enzyme is essential.

In this study, we had two main goals:

I. We set out to investigate the manifestation of allostery within the superfamily of dUTPases. We attempted to detect allosteric behavior in dUTPases by engineering hybrid enzymes (Figure 1) to restrict putative allosteric transmission between active sites at various stages of the enzymatic cycle. Besides, we also aimed to investigate the characteristics of the central channel of dUTPases to explain the manifestation of allosteric behavior within the dUTPase superfamily.

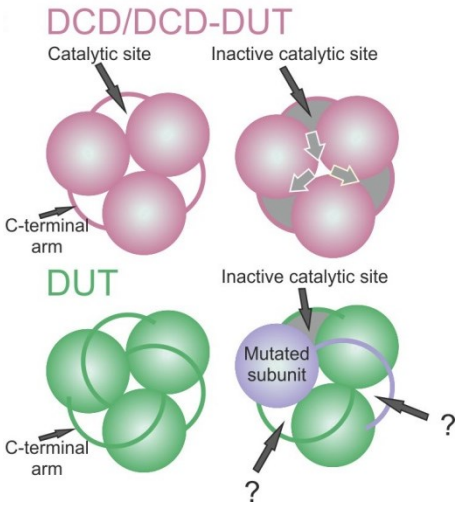

Figure 1
Schematic representation of the potential active site communication within dUTPase, based on the example of DCD-s and DCD-DUTs
Grey color at the active sites represents inactive catalytic site. In case of DCD (-DUT) arrows in the central channel show that the conformational switch is transformed through the central channel in a concerted way. Note that in case of dUTPase the C-terminal arms are reaching out from one subunit to contact the remote active site.

II. dUTPase and its enzymatic function were shown to play a key role in SaPI HGT activation [13,14]. A G protein-like mechanism was proposed including a dUTP induced conformational change as key switch between the active and inactive states of dUTPase. However, the interconnection of the enzymatic cycle and the de-repressing function was not studied.

   a. We aimed to characterize the enzymatic properties of Φ11 dUTPase and its interaction with the Stl protein.
   b. We also wished to explore how the dUTP substrate and the Stl protein influence the binding of each other to dUTPase.

3 METHODS

For Aim I, we designed mutations to access inactive conformational states in dUTPase potentially mimicking the ones observed in DCD and DCD-DUT. To this end, we created covalently linked human dUTPase pseudoheterotrimers (called hybrids henceforth) in which the active sites could be turned off selectively. We studied how the interruption of different steps of the enzyme cycle in one active site of the hybrid affects the non-mutated active sites using various enzymatic and structural biology approaches, such as steady-state and transient kinetics, circular dichroism spectroscopy, ThermoFluor assay and limited trypsinolysis. Our most powerful tool for the kinetic characterization was a tryptophan sensor engineered to the active site of the investigated dUTPases [15,16]. With this active site sensor we could investigate the detailed kinetic properties of the different dUTPase variants using fluorescence stopped-flow [17].

For Aim II, we used similar enzymatic and structural biology approaches. Luckily, the same active site tryptophan could also sense the binding of the Stl protein to phage dUTPase, which allowed us to perform the kinetic characterization of the complex formation of dUTPase with dUTP and Stl. Besides, quartz crystal microbalance method and used electrophoretic shift assay were also used. To check whether *S. aureus* strains contain dUTPases, we used bioinformatic tools.

4 RESULTS AND CONCLUSIONS

RESULT AND CONCLUSIONS FOR AIM 1

We created hybrid human dUTPases to study possible other means of cooperativity in detail. In these hybrid enzymes one or two monomers ***a,*** were catalytically inactive ***b,*** had low activity and reduced substrate affinity due the lack of the C-terminal swapping arm ***c,*** or did not bind substrate at all. Using these asymmetric hybrids, we investigated whether the interruption of different steps of the enzyme cycle – and thereby the interruption of the possible conformational changes occurring during these steps – affects the functioning of the non-mutated active sites. We also investigated the central channel.

We determined the followings:

(i) The active sites work independently from each other.

(ii) Mg^{2+} binding in the central channel reduces the flexibility and increases the thermal stability of the quaternary structure.

(iii) The allosteric loop that connects the active site to the central channel is conformationally restricted in dUTPases compared to DCD family enzymes. This phenomenon is interrelated with structural solutions for increased dUTP specificity in every case.

We propose that the trade-off between cooperativity and specificity in the dUTPase superfamily represents instances of adaptation to the distinct roles of dUMP production for dTTP synthesis and dUTP elimination for uracil-DNA avoidance, respectively.

Investigating the Φ11 phage dUTPase driven SaPI HGT we have found the following:

(i) Φ11 phage dUTPase is a highly efficient dUTPase which hydrolyzes dUTP with a kinetic mechanism fundamentally similar to the kinetic mechanism described earlier for other dUTPases.

(ii) Stl is a slow and tight binding, competitive inhibitor of the Φ11 dUTPase, which contradicts the earlier proposed G protein-like mechanism of this molecular switch.

(iii) The sequenced *S. aureus* strains contain dUTPases only as part of integrated prophages, which are not expected to be expressed in lysogenic phase, therefore the dUTP level is probably high within the bacterium.

Our results allowed setting up a molecular model for the activation of SaPI HGT by dUTPases. In this model, the phage dUTPase is able to interact with Stl only after sanitizing the dNTP pool from dUTP thereby promoting the uracil free replication of the excised SaPI DNA.

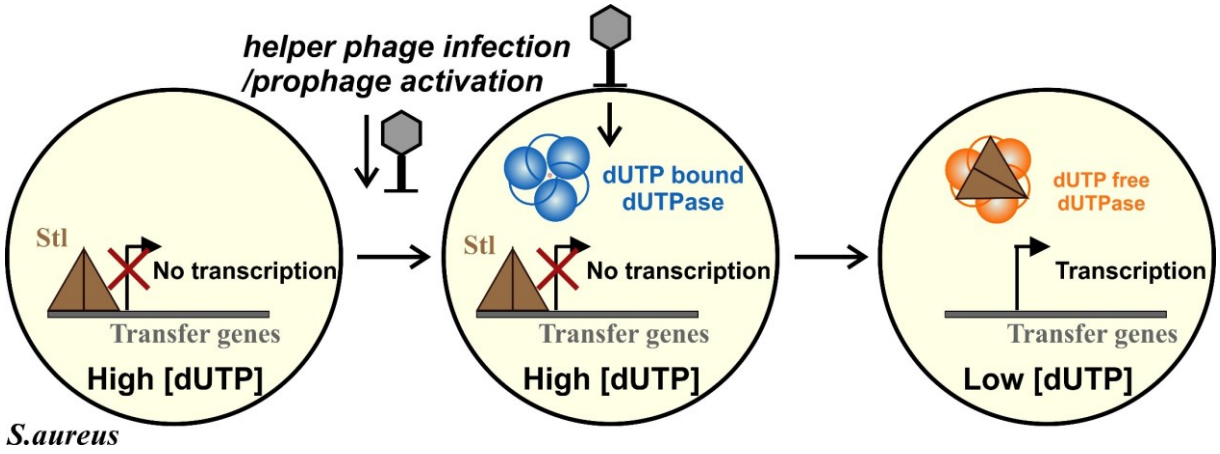

Figure 2
Model of the mechanism of dUTPase-controlled SaPI activation

Besides the understanding of such an important mechanism like the HGT of pathogenicity islands, it is also important that we identified Stl as the first, highly efficient protein inhibitor of dUTPase. If Stl proves to be a general dUTPase inhibitor – which it seems to be [18] – it can ease the clarification of the various roles of dUTPases in different species *in vivo*.

5 REFERENCES

1. Johansson, E., Bjornberg, O., Nyman, P. O. & Larsen, S. Structure of the bifunctional dCTP deaminase-dUTPase from Methanocaldococcus jannaschii and its relation to other homotrimeric dUTPases. *J. Biol. Chem.* **278,** 27916–22 (2003).
2. Johansson, E. *et al.* Structures of dCTP deaminase from Escherichia coli with bound substrate and product: reaction mechanism and determinants of mono- and bifunctionality for a family of enzymes. *J. Biol. Chem.* **280,** 3051–9 (2005).
3. Helt, S. S. *et al.* Mechanism of dTTP inhibition of the bifunctional dCTP deaminase:dUTPase encoded by Mycobacterium tuberculosis. *J. Mol. Biol.* **376,** 554–69 (2008).
4. Vértessy, B. G. & Tóth, J. Keeping uracil out of DNA: physiological role, structure and catalytic mechanism of dUTPases. *Acc. Chem. Res.* **42,** 97–106 (2009).
5. Price, a R. Bacteriophage PBS2-induced deoxycytidine triphosphate deaminase in Bacillus subtilis. *J. Virol.* **14,** 1314–7 (1974).
6. Björnberg, O., Neuhard, J. & Nyman, P. O. A bifunctional dCTP deaminase-dUTP nucleotidohydrolase from the hyperthermophilic archaeon Methanocaldococcus jannaschii. *J. Biol. Chem.* **278,** 20667–72 (2003).
7. Johansson, E. *et al.* Regulation of dCTP deaminase from Escherichia coli by nonallosteric dTTP binding to an inactive form of the enzyme. *FEBS J.* **274,** 4188–98 (2007).
8. Mol, C. D., Harris, J. M., McIntosh, E. M. & Tainer, J. a. Human dUTP pyrophosphatase: uracil recognition by a beta hairpin and active sites formed by three separate subunits. *Structure* **4,** 1077–92 (1996).
9. Fiser, a & Vértessy, B. G. Altered subunit communication in subfamilies of trimeric dUTPases. *Biochem. Biophys. Res. Commun.* **279,** 534–42 (2000).
10. Dubrovay, Z. *et al.* Multidimensional NMR identifies the conformational shift essential for catalytic competence in the 60-kDa Drosophila melanogaster dUTPase trimer. *J. Biol. Chem.* **279,** 17945–50 (2004).
11. Novick, R. P., Christie, G. E. & Penadés, J. R. The phage-related chromosomal islands of Gram-positive bacteria. *Nat. Rev. Microbiol.* **8,** 541–51 (2010).
12. Mir-Sanchis, I. *et al.* Control of Staphylococcus aureus pathogenicity island excision. *Mol. Microbiol.* **85,** 833–45 (2012).
13. Tormo-Más, M. Á. *et al.* Phage dUTPases control transfer of virulence genes by a proto-oncogenic G protein-like mechanism. *Mol. Cell* **49,** 947–58 (2013).
14. Tormo-Más, M. A. *et al.* Moonlighting bacteriophage proteins derepress staphylococcal pathogenicity islands. *Nature* **465,** 779–82 (2010).
15. Varga, B. *et al.* Active site closure facilitates juxtaposition of reactant atoms for initiation of catalysis by human dUTPase. *FEBS Lett.* **581,** 4783–8 (2007).
16. Varga, B. *et al.* Active site of mycobacterial dUTPase: structural characteristics and a built-in sensor. *Biochem. Biophys. Res. Commun.* **373,** 8–13 (2008).
17. Tóth, J., Varga, B., Kovács, M., Málnási-Csizmadia, A. & Vértessy, B. G. Kinetic mechanism of human dUTPase, an essential nucleotide pyrophosphatase enzyme. *J. Biol. Chem.* **282,** 33572–82 (2007).
18. Hirmondó, R. *et al.* Cross-species inhibition of dUTPase via the Staphylococcal Stl protein perturbs dNTP pool and colony formation in Mycobacterium. *DNA Repair (Amst).* **30,** 21–7 (2015).

6 PUBLICATIONS RELATED TO THE DOCTORAL THESIS

PUBLICATIONS CLOSELY RELATED TO THE THESIS

PEER-REVIEWED PUBLICATIONS RELATED TO THE THESIS

2014 **Szabó, J. E.**, Németh, V., Papp-Kádár, V., Nyíri, K., Leveles, I., Bendes, A. Á., Zagyva, I., Róna, G., Pálinkás, H. L., Besztercei, B., Ozohanics, O., Vékey, K., Liliom, K., Tóth, J., and Vértessy, B. G. (2014) Highly potent dUTPase inhibition by a bacterial repressor protein reveals a novel mechanism for gene expression control. *Nucleic Acids Res. 42*, 11912–20.

2013 Leveles, I., Németh, V., **Szabó, J. E.**, Harmat, V., Nyíri, K., Bendes, Á. Á., Papp-Kádár, V., Zagyva, I., Róna, G., Ozohanics, O., Vékey, K., Tóth, J., and Vértessy, B. G. (2013) Structure and enzymatic mechanism of a moonlighting dUTPase. *Acta Crystallogr. D. Biol. Crystallogr. 69*, 2298–308.

SUBMITTED PUBLICATIONS RELATED TO THE THESIS

2015 **Szabó, J.E.**, Takács, E., Merényi, G., Vértessy, B.; Tóth, J. Trading in cooperativity for specificity to maintain uracil-free DNA. *Submitted to eLife.*

OTHER PEER-REVIEWED PUBLICATIONS

2015 Nyíri, K., Papp-Kádár, V., **Szabó, J. E.**, Németh, V., and Vértessy, B. G. (2015) Exploring the role of the phage-specific insert of bacteriophage Φ11 dUTPase. *Struct. Chem. DOI: 10.1007/s11224-015-0652-2*

2015 Hirmondó, R., **Szabó, J. E.**, Nyíri, K., Tarjányi, S., Dobrotka, P., Tóth, J., and Vértessy, B. G. (2015) Cross-species inhibition of dUTPase via the Staphylococcal Stl protein perturbs dNTP pool and colony formation in Mycobacterium. *DNA Repair (Amst). 30*, 21–7.

2013 Sebesta, M., Burkovics, P., Juhasz, S., Zhang, S., **Szabo, J. E.**, Lee, M. Y. W. T., Haracska, L., and Krejci, L. (2013) Role of PCNA and TLS polymerases in D-loop extension during homologous recombination in humans. *DNA Repair (Amst). 12*, 691–698.

2011 Pécsi, I.*, **Szabó, J. E.***, Adams, S. D., Simon, I., Sellers, J. R., Vértessy, B. G., and Tóth, J. (2011) Nucleotide pyrophosphatase employs a P-loop-like motif to enhance catalytic power and NDP/NTP discrimination. *Proc. Natl. Acad. Sci. U. S. A. 108*, 14437–42. (* equal contribution)

CONFERENCE PROCEEDINGS RELATED TO THE DOCTORAL THESIS

ORAL PRESENTATIONS – PRESENTING AUTHOR UNDERLINED

2015 **Szabó, J.E.**, Hirmondó, R., Dobrotka, P., Tarjányi, Sz., Nyíri, K., Tóth, J., Vértessy, B.G., ***Characterization of species-specific inhibitory effect of a Staphylococcal repressor protein on dUTPases.*** FEBS3+ meeting; Portorož, Slovenia

2015 **Szabó, J.E.**, Nyíri, K., Hirmondó, R., Papp-Kádár, V., Dobay, O., Szabó, D., Tóth, J., Vértessy, B. G. ***A Novel Mechanism Provides Genome Quality Control For Pathogenicity Island Transfer In Staphylococcus.*** 17th International Congress of the Hungarian Society for Microbiology, Budapest, Hungary

2015 **J. E. Szabó**, Dóra Szabó, Orsolya Dobay, Beáta G. Vértessy. ***Genome integrity and pathogenicity island transfer in staphylococcus*** Hungarian Molecular Life Sciences; Eger, Hungary

2014 **J. E. Szabó**, V. Németh, V. Papp-Kádár, K. Nyíri, I. Leveles, Á. Á. Bendes, I. Zagyva, G. Róna, H. Pálinkás, B. Besztercei, O. Ozahonics, K. Vékey, K. Liliom, J.Tóth and B.G. Vértessy. ***The role of a DNA repair enzyme in the spread of staphylococcal virulence factors.*** RCNS HAS Doctoral Conference; Budapest, Hungary

2013 **Szabó ,J.E.**, Papp-Kádár, V., Németh-Pongrácz, V., Róna, G., Nyíri, K., Besztercei, B., Nyíri, K., Leveles, I., Bendes, Á. Á., Révész, Á., Róna, G., Pálinkás, H., Vékey, K., Liliom, K., Tóth, J., Vértessy, B. G. ***Genomic integrity of virulence genes is preserved by a dUTPase-based molecular switch***. 9th EBSA. Lisbon, Portugal

2013 **J. E. Szabó,** V. Németh-Pongrácz, I. Leveles, V. Papp-Kádár, B. Besztercei, K. Liliom, I. Zagyva, Á. Á. Bendes, G. Róna, H. Pálinkás, K. Nyíri, J. Tóth, B. G. Vértessy. ***dUTP level controls transfer of virulence genes in order to preserve***

integrity of the transferred mobile genetic elements. Hungarian Molecular Life Sciences; Siófok, Hungary

2012 **J. E. Szabó**, E. Takács, G. Merényi, B. G. Vértessy and J.Tóth. ***Trade - off between cooperativity and specificity in the dUTPase superfamily*.** Kálmán Erika Doctoral Conference, Mátraháza, Hungary

POSTER PRESENTATIONS – PRESENTING AUTHOR UNDERLINED

2015 **J. E. Szabó,** P. Dobrotka, J. Tóth, B. G. Vértessy ***Characterization of species-specific inhibitory effect on dUTPases by a transcription factor encoded by Staphylococcus aureus.*** Hungarian Molecular Life Sciences; Eger, Hungary.

2013 **J. E. Szabó,** V. Németh-Pongrácz, V. Papp-Kádár, K. Nyíri, I. Leveles, Á. Á. Bendes, Á. Révész, G. Róna, H. Pálinkás,B. Besztercei, K. Vékey, K. Liliom, J. Tóth, B. G. Vértessy. ***Genomic integrity of virulence genes is preserved by a dUTPase-based molecular switch.*** FEBS Congress; St. Petersburg, Russia.

2013 **J. E. Szabó**, V. Németh-Pongrácz, V. Papp-Kádár, K. Nyíri, I. Leveles, Á. Á. Bendes, Á. Révész, G. Róna, H. Pálinkás,B. Besztercei, K. Vékey, K. Liliom, J. Tóth, B. G. Vértessy. ***Novel dUTPase based switch controls the horizontal transfer of pathogenicity island in Staphylococcus aureus.*** The 5th EMBO Meeting. Amsterdam, Netherlands.

2013 **J. E. Szabó**, E. Takács, G. Merényi, B. G. Vértessy, J. Tóth. ***Trade-off between cooperativity and specificity to ensure uracil free DNA***. EMBO Conference on Allosteric interactions in cell signaling and regulation. Paris, France

2012 **J. E. Szabó**, E. Takács, G. Merényi, B. G. Vértessy and J.Tóth. ***Trade-off between cooperativity and specicity in the dUTPase superfamily*.** FEBS3+ Meeting: "From molecules to life and back", Opatija, Croatia

2012 **J. E. Szabó**, G. Merényi, B. G. Vértessy and J.Tóth. ***Functional adaptation and allosteric regulation of the dUTPase superfamily***.75th Anniversary of Albert Szentgyörgyi's Nobel Prize Award, Szeged, Hungary

2010 **J. E. Szabó**, G. Merényi, B. G. Vértessy and J.Tóth. ***Jósolható-e a kooperativitás a fehérje szerkezet alapján?*** Hungarian Biochemical Society Annual Meeting, Budapest, Hungary