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EXAMINTION OF THE SIGNIFANCE OF URACIL LEVEL IN DNA AND OF IMPORTANT PROTEINS IN ITS METABOLISM

Thesis booklet

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1 Introduction and background

Maintenance of genome integrity is crucial for every living organism. DNA damages without repair could be source of many diseases (including cancer and neurodegenerative diseases), and can even lead to cell death. Therefore, specific DNA repair mechanisms evolve during evolution. In case of one strand damage the lesion will be done by one of the following excision repair mechanism: BER (base excision repair), MMR (mismatch repair), NER (nucleotide excision repair). In case of double strand break the lesion will be repair via HR (homologous recombination) or NHEJ (non-homologous end joining)[1,2,3,4].

One of the most frequent DNA lesion is the uracil. Uracil is very similar to thymine, therefore DNA polymerases cannot distinguish between them. The cellular dUTP/dTTP level determines which will incorporate into the DNA. Uracil can occur in DNA via cytosine deamination as well. The members of the uracil-DNS glycosylase family are responsible for elimination of the uracil basis from DNA by BER pathway[5].

Nevertheless, the uracil occurrence in DNA is not necessarily a fault. There is some example when uracil in DNA is normal or essential:

i, in case of immune system: B-lymphocytes are responsible for the construction of antibodies. After antigen induced activation, the AID (activation induced deaminase) – one of the APOBEC family member – catalyzes the cytosine oxidative deamination to uracil, thereby contributing to the diversity of antibodies[6]. Other members of the APOBEC family (primarily the APOBEC3s) also have an important role in fighting against viruses. The strict regulation of these enzymes is essential. Elevated APOBEC3B expression correlate with the formation of „kataegic like" mutation pattern. In molecular biology kataegis describes a pattern of localized hypermutation identified in some cancer genomes. The base mutations in these regions were found to be almost exclusively cytosine to thymine. APOBEC3B (A3B) has a constitutive nuclear localization. Significantly elevated A3B level was found in breast-,

[1] Hans E Krokan and Magnar Bjørås, 'Base Excision Repair.', *Cold Spring Harbor Perspectives in Biology*, 5.4 (2013), a012583 <https://doi.org/10.1101/cshperspect.a012583>.

[2] Guo-Min Li, 'Mechanisms and Functions of DNA Mismatch Repair.', *Cell Research*, 18.1 (2008), 85–98 <https://doi.org/10.1038/cr.2007.115>.

[3] Graciela Spivak, 'Nucleotide Excision Repair in Humans.', *DNA Repair*, 36 (2015), 13–18 <https://doi.org/10.1016/j.dnarep.2015.09.003>.

[4] Joonyoung Her and Samuel F Bunting, 'How Cells Ensure Correct Repair of DNA Double-Strand Breaks.', *The Journal of Biological Chemistry*, 293.27 (2018), 10502–11 <https://doi.org/10.1074/jbc.TM118.000371>.

[5] Elisa Fadda and Régis Pomès, 'On the Molecular Basis of Uracil Recognition in DNA: Comparative Study of T-A versus U-A Structure, Dynamics and Open Base Pair Kinetics.', *Nucleic Acids Research*, 39.2 (2011), 767–80 <https://doi.org/10.1093/nar/gkq812>.

[6] M Muramatsu and others, 'Class Switch Recombination and Hypermutation Require Activation-Induced Cytidine Deaminase (AID), a Potential RNA Editing Enzyme.', *Cell*, 102.5 (2000), 553–63 <https://doi.org/10.1016/S0092-8674(00)00078-7>.

uterus-, bladder-, head/neck- and lung cancers[7]. My aim was to examine the effect of the elevated A3B level to the genomic DNA uracil level in case of p53 proficient and deficient 293 (HEK) cells. For the quantitative determination of genomic uracil level I utilized a dot based method (is also the subject of this thesis) which was developed by our research team. We use an inactive mutant of the UNG which is the main uracil DNA repair enzyme as a specific uracil sensor.

ii, In case of *Drosophila melanogaster* ontogeny: The genome of *Drosophila melanogaster* (fruit fly) doesn't contain the *ung* gene. Moreover, in larval stages the dUTPase, which is responsible for keeping the cellular dUTP/dTTP level low, is not present. Without these enzymes the uracil level of the genomic DNA significantly increases. In the lack of UNG the question arises: is there any other enzyme which can be responsible for the degradation of uracilated DNA?[8]. The UDE (uracil-DNA degrading factor) was identified by our group in 2007 as a potential enzyme which could be responsible for this. The activity of this enzyme is under a strict regulation, it can be detected only before pupation. The elevated uracil levels in larvae, pupae and imago stages can play an important role in cell death processes during metamorphosis. The uracil level is tissue specific as well. The salivary glands which is a representative tissue of degradation contain high uracil level while imaginal discs which do not undergo metamorphosis-coupled cell death contain much less uracil. The amount of uracil is significantly decreases after pupation. UDE represents a new class of proteins, which presumably playing role the process uracil-DNA with potential involvement in metamorphosis. This protein has some sequential homologues in other pupating insects (e.g. honeybee, silkworm and malaria mosquito), but lack any homologues with known structure or function[9]. In case of UDE a nuclear localization can be observed[10]. My aim was the structural analysis of UDE.

[7] Stefan Rebhandl and others, 'AID/APOBEC Deaminases and Cancer.', *Oncoscience*, 2.4 (2015), 320–33 <https://doi.org/10.18632/oncoscience.155>.
[8] Villő Muha and others, 'Uracil-Containing DNA in Drosophila: Stability, Stage-Specific Accumulation, and Developmental Involvement.', *PLoS Genetics*, 8.6 (2012), e1002738 <https://doi.org/10.1371/journal.pgen.1002738>.
[9] Angéla Békési and others, 'A Novel Fruitfly Protein under Developmental Control Degrades Uracil-DNA', *Biochemical and Biophysical Research Communications*, 355.3 (2007), 643–48 <https://doi.org/10.1016/j.bbrc.2007.01.196>.
[10] Gábor Merényi, Emese Kónya, and Beáta G Vértessy, 'Drosophila Proteins Involved in Metabolism of Uracil-DNA Possess Different Types of Nuclear Localization Signals.', *The FEBS Journal*, 277.9 (2010), 2142–56 <https://doi.org/10.1111/j.1742-4658.2010.07630.x>.

iii, In case of viruses with uracil-DNA: Up until now researchers found three viruses with DNA in which the thymine bases are substituted with uracil bases. This three virus is: the *Bacillus subtilis* infecting PBS phage, the a Yersinia species infecting ΦR1-37 and the S6 phage which infect Staphylococcal species[11,12,13]. These phages infect bacteria with several enzymes which is responsible for keeping the genomic uracil level low (e.g. dUTPase and UNG). All known DNA-polymerase use also dTTP and dUTP as substrate, so only their cellular level define which will be built into the DNA. Therefore these phages must have strategies with which can protect their uracilated DNA and can change the intracellular conditions. In case of PBS phage the UGI protein was identified. UGI proved to be a general UNG inhibitor, which can protect the phage DNA[14]. Neither in the case of ΦR1-37 nor of S6 a protein could be identified as a similar protein to UGI. This does not exclude that these phages don't contain UNG inhibiting protein, since the three known UNG inhibitor proteins don't display sequential similarities. They are similar in their surface - all of them have a negatively charged surface. They bind the active site of UNG by mimicking the DNA[15]. I would like to examine the survival strategies of viruses with uracilated DNA. I used the Yersinia species infecting (e.g. the human pathogen *Yersinia enterocolitica*) ϕR1-37 phage genomic DNA library during my work.

I participated in the validation of „The Metagenomic Telescope" method. This „telescope" could help find functions for unknown proteins and new protein functions in well-studied model organisms by using artificial intelligence. As a proof-of-principle demonstration of The Metagenomic Telescope, we considered DNA repair enzymes in the present work. We use metagenomes from extreme environment, because the microorganisms living in these places have specific and extensive DNA repair systems to survive under the extreme environmental conditions.

[11] I TAKAHASHI and J MARMUR, 'Replacement of Thymidylic Acid by Deoxyuridylic Acid in the Deoxyribonucleic Acid of a Transducing Phage for Bacillus Subtilis.', *Nature*, 197 (1963), 794–95 <http://www.ncbi.nlm.nih.gov/pubmed/13980287>.

[12] Saija Kiljunen and others, 'Yersiniophage PhiR1-37 Is a Tailed Bacteriophage Having a 270 Kb DNA Genome with Thymidine Replaced by Deoxyuridine.', *Microbiology (Reading, England)*, 151.Pt 12 (2005), 4093–4102 <https://doi.org/10.1099/mic.0.28265-0>.

[13] Jumpei Uchiyama and others, 'Intragenus Generalized Transduction in Staphylococcus Spp. by a Novel Giant Phage.', *The ISME Journal*, 8.9 (2014), 1949–52 <https://doi.org/10.1038/ismej.2014.29>.

[14] Zhigang Wang and Dale W Mosbaughs, 'Uracil-DNA Glycosylase Inhibitor Gene of Bacteriophage PBSB Encodes a Binding Protein Specific for Uracil-DNA Glycosylase "', 264.2 (1989), 1163–71.

[15] Hao-Ching Wang and others, 'Staphylococcus Aureus Protein SAUGI Acts as a Uracil-DNA Glycosylase Inhibitor.', *Nucleic Acids Research*, 42.2 (2014), 1354–64 <https://doi.org/10.1093/nar/gkt964>.

2 Methods

In order to search for possible UNG inhibitors in the genome of bacteriophage ΦR1-37, I developed a high-throughput method. For this experiment I used *E. coli* CJ236 (*dut- ung-*) cells. I cotransformed them with the plasmid DNA containing the genomic library of the phage and the UNG gene. Phage genomic pieces in the growing colonies were identified by colony PCR followed by sequencing and analyzed by bioinformatics methods.

We have used an inactive form of the human UNG enzyme as a specific uracil sensor to quantify the genomic uracil level with a dot blot based assay which was developed and validated by our group. The success of inactivation was verified with agarose-assay, the specific uracil-DNA binding of this UNG construct was tested with EMSA (electrophoretic mobility shift assay). The DNA samples were immobilized to a positively charged nylon membrane and then the inactive UNG was added, from which the first 84 amino acids were removed to avoid non-specific interactions. We detected the dots through the Flag epitope tag which was fused to the UNG constructs. The genomic DNA isolated from *E. coli* CJ236 (*dut-ung-*) was used as standard, because its genomic uracil level is known from literature.

We have participated in many collaborations with connection to this method. The experiment presented in this thesis was a collaboration with Professor Chris Lord group (The CRUK Gene Function Laboratory and The Breast Cancer Now Toby Robins Breast Cancer Research Centre, The Institute of Cancer Research, London). We examine the effect of APOBEC3B expression on genomic uracil level of 293 (HEK) cells in the presence or absence of p53 protein. After genomic DNA isolation from these cells we measured those uracil level with the above mentioned dot blot based method.

For the investigation of the structure of UDE, the 9 protein constructs created by ESPRIT technique were produced in *E. coli* BL21 (DE3) *ung*-151 cells and then purified by Ni-affinity chromatography. The oligomerization status of these proteins were analyzed by analytical gel filtration, thermostability and folding by differential scanning fluorimetry. To measure the secondary structure composition of these constructs I applied circular dichroism spectroscopy.

The “Metagenomic Telescope” method was developed in collaboration with the ELTE PIT bioinformatics research group and tested on DNA repair proteins. HMM (Hidden Markov Model) and metagenomics data from extreme environmental samples were used to identify unknown DNA repair proteins in well-known model organisms.

3 Results and Discussion

Results from survival experiments with ΦR1-37 phage genomic library indicate that *E. coli* CJ236 (*dut- ung-*) cells are not viable in the presence of active UNG due to their high genomic uracil levels, and the presence of genomic pieces of the phage library are able to rescue double mutant cells. To find out that which phage genomic regions are responsible for rescuing the cells, I used colony PCR followed by Sanger sequencing. To identify these sequences I used NCBI blastx database and analyze with bioinformatics methods. I classified the identified proteins according to their size and isoelectric point, taking into account when they are expressed during the infection - based on the "[16]" publication - and how many times that sequence was returned during the sequencing.

The identified phage proteins are listed in the Table 1.

protein	Mw (kDa)	pI	expression	number of hits
g084	13,4	9,12	constitutive	3
g085	50,5	5,16	constitutive	4
g089	40,2	4,56	constitutive	4
g119	12,99	5,39	early	3
g120	14,6	8,73	constitutive	3
g133	17,2	6,29	constitutive	5
g185	14,6	9,60	constitutive	7
g186	14,6	5,87	constitutive	6
g191	49,9	9,01	constitutive	4
g295	151,9	5,36	constitutive	3

Table 1. Phage proteins identified and selected during survival experiments.

From the proteins listed in Table 1, g119, g185 and g186 were further investigated by bioinformatics methods. g119 is similar to the three known UNG inhibitor both its size and isoelectric point. g185 and g186 proteins were recovered most times during sequencing, i.e., the majority of surviving cells contained a vector containing the gene coding these phage proteins. To analyze the order of these proteins I used the IUPred program and the JPred program to examine the secondary structural elements. Based on the results, it can be stated that these proteins do not have known sequential or structural homologs, so the reliability of tertiary structural models is limited, but presumably all of them are ordered proteins[17]. In order to verify that these proteins are capable of inhibiting UNG, *in vitro* experiments are planned.

[16] Katarzyna Leskinen and others, 'RNA-Sequencing Reveals the Progression of Phage-Host Interactions between ΦR1-37 and Yersinia Enterocolitica.', *Viruses*, 8.4 (2016), 111 <https://doi.org/10.3390/v8040111>.
[17] Nagy Kinga and Vértessy G. Beáta, 'URACIL-DNS ALAPÚ VÍRUSOK:MI LEHET A TÚLÉLÉSI STRATÉGIA?', *BIOKÉMIA*, XVIII.1 (2019).

We have developed a method for determining uracil levels in DNA using the UNG enzyme - which is specific for uracil bases in DNA - as a uracil sensor (Figure 1). The N-terminal first 84 amino acids responsible for interactions with other DNA binding proteins was removed from the wild-type enzyme (ΔUNG). This plays an important role in *in situ* studies. The protein was inactivated by two point mutations (D154N and H277N). Labeling with antibodies is possible through the 1x- or 3xFlag-tag.

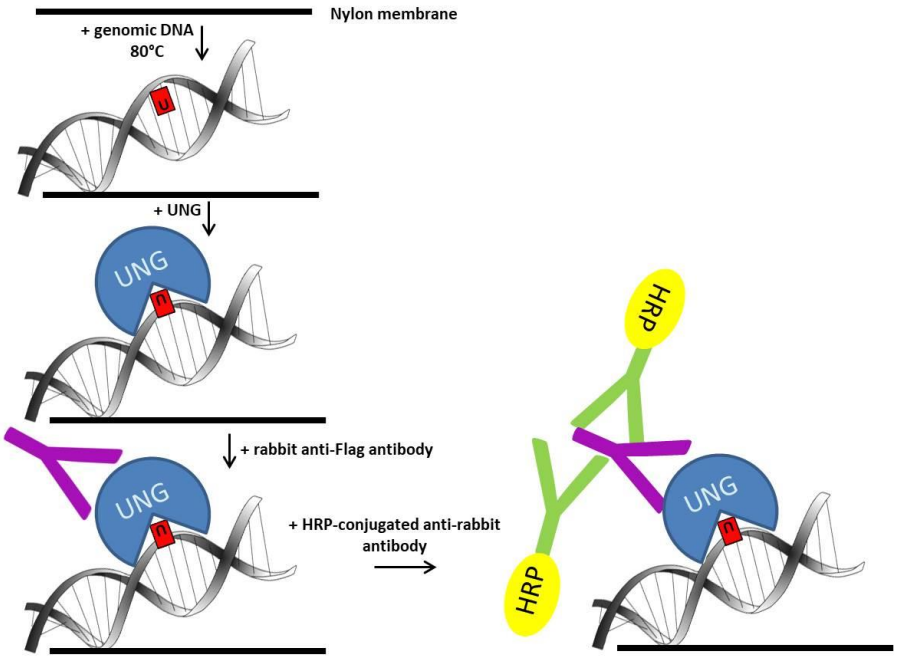

Figure 1. The schematic illustration of the dot blot method. The DNA samples are fixed in a known amount to a positively charged membrane and then added to the inactive UNG construct responsible for DNA recognition and labeling. We use the Flag epitope tag to label the UNG with antibodies.

The uracil binding ability of the UNG constructs produced by us was tested by electrophoretic mobility shift assay, its activity (or its absence) was checked by agarose assay. The results show that ΔUNG constructs containing both 1x- and 3xFlag-tag are inactive and specifically bind uracil-containing DNA[18].

[18] Gergely Róna and others, 'Detection of Uracil within DNA Using a Sensitive Labeling Method for in Vitro and Cellular Applications.', *Nucleic Acids Research*, 44.3 (2016), e28 <https://doi.org/10.1093/nar/gkv977>.

The 293 (HEK) cells - from Professor Chris Lord's Laboratory (Toby Robins Breast Cancer Research Centre, London) - genomic uracil level was measured using the dot blot method developed by us. The results are shown in Figure 2.

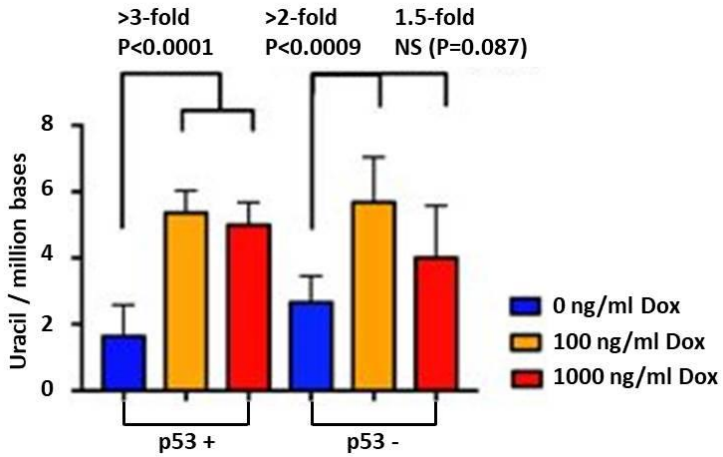

Figure 2. Effect of APOBEC3B expression on the genomic uracil levels. The expression of APOBEC3B can be induced by doxycycline (Dox). As a result of A3B expression, genomic uracil levels of 293 (HEK) cells are significantly elevated also in case of p53 wild-type (p53 +) and of p53 (p53) cells. Each data point represents an average of at least six repetitions. Two-sample T-test was used for statistical analysis (NS: not significant).

As a result of the present collaborative work, we found that the expression of APOBEC3B results elevated genomic uracil levels in both p53-expressing and p53-silenced 293 (HEK) cells. DNA damage triggers a cellular response, and stops cell division in the G2 / M phase in p53 wild-type cells, but not in silenced ones. These results suggest that losing the p53 function allows cells to tolerate elevated APOBEC3B expression in an ongoing DDR (DNA damage response) and tumor cells remain able to continue divide[19].

My colleague, Dr. Mária Pukáncsik has created several truncated versions with the ESPRIT technique for the structure analysis of the UDE protein[20]. We selected the 9 most promising constructs (Figure 3), which were analyzed by analytical gel filtration, differential scanning fluorimetry (Thermofluor) and circular dichroism (CD) spectroscopy. The results are summarized in Table 2.

[19] Jenni Nikkilä and others, 'Elevated APOBEC3B Expression Drives a Kataegic-like Mutation Signature and Replication Stress-Related Therapeutic Vulnerabilities in P53-Defective Cells.', *British Journal of Cancer*, 117.1 (2017), 113–23 <https://doi.org/10.1038/bjc.2017.133>.

[20] Hayretin Yumerefendi and others, 'ESPRIT: An Automated, Library-Based Method for Mapping and Soluble Expression of Protein Domains from Challenging Targets.', *Journal of Structural Biology*, 172.1 (2010), 66–74 <https://doi.org/10.1016/j.jsb.2010.02.021>.

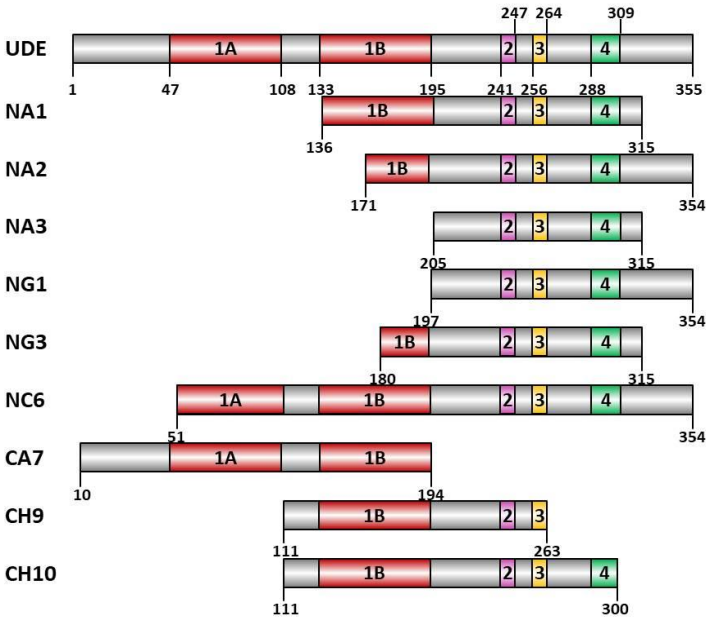

Figure 3. A schematic illustration of UDE and its nine truncated constructs created with ESPRIT technique. 1A, 1B, 2, 3, 4: conserved motifs identified in the protein sequence.

UDE constructs	T_m (°C)	Analytical gel filtration			Oligomer status
		V_{el} (ml)	M_{app} (kDa)	Mc_{alk} (kDa)	
NA1	51.3	62.4	42.1	25.7	dimer
NA2	53.9	56.6	57.0	26.3	dimer
NA3	57.1	69.6	28.9	18.0	dimer
NC6	53.7	55.7 65.6	59.7 35.6	34.7	monomer
NG1	56.4	60.0	47.7	23.3	dimer
NG3	51.5	64.6	37.5	20.9	dimer
CA7	+	44.3	-	25.4	-
CH9	+	58.3	52.1	22.1	dimer
CH10	-	56.0	58.8	32.1	dimer
UDE	-	-	-	-	monomer

Table 2. Summary table of the results of measurements performed with UDE constructs. The "+" signs indicate that the measurement has been performed on the construct, but they do not have a well-defined melting temperature. V_{el}: elution volume; M_{app}: apparent molecular weight (based on column calibration); M_{calk}: molecular weight (based on the amino acid sequence); T_m: melting temperature (determined by thermofluor measurements). As a criterion for monomer / dimer classification, a maximum of 20% deviation between the M_{calc} and the M_{app} was allowed. In case of NC6 construct we observed 2 peaks on the chromatogram. One corresponds to the same molecular weight as the monomer, while the other is between the elution volumes of the monomer and the dimer.

The CD spectra of the nine fragments of UDE indicate an exclusive α-helical secondary structural elements content (62-92%). One of the main advantages of using ESPRIT technology and CD spectroscopy is that it can be applied to dilute solutions of high molar mass proteins, where the X-ray- and magnetic nuclear resonance spectroscopy techniques are not applicable[21].

[21] Mária Pukáncsik and others, 'Secondary Structure Prediction of Protein Constructs Using Random Incremental Truncation and Vacuum-Ultraviolet CD Spectroscopy.', *PloS One*, 11.6 (2016), e0156238 <https://doi.org/10.1371/journal.pone.0156238>.

Organisms living in extreme environments are reasonably expected to have a more complex set of DNA repair mechanisms to protect their genome from drastic damaging effects. This hypothesis is confirmed in the literature.

Metagenomic sequences from extreme environmental samples were used to identify new proteins involved in DNA repair in well-known model organisms, or to assign new features to already known proteins. We have developed the "Metagenomic Telescope" method in collaboration with the PIT bioinformatics research team, which applies artificial intelligence offers powerful methods for distilling relevant information from large sets of data. In this work, HMM has been used in a novel way to find unknown protein functions in known model organisms. Based on the sequence alignment of proteins involved in DNA repair, we have created hidden Markov models, and these models were used to search for similar genes in metagenomic samples from different environments. Combining the original HMM with the genes found in the metagenomes, we created a second, more trained HMM that we used to interrogate proteomes of higher order model organisms (Figure 4). We have found well known DNA repair proteins, numerous proteins with unknown functions, and also proteins with known, but different functions in the model organisms. These results show that Metagenomic Telescope can be an effective method for identifying new proteins in higher model organisms[22].

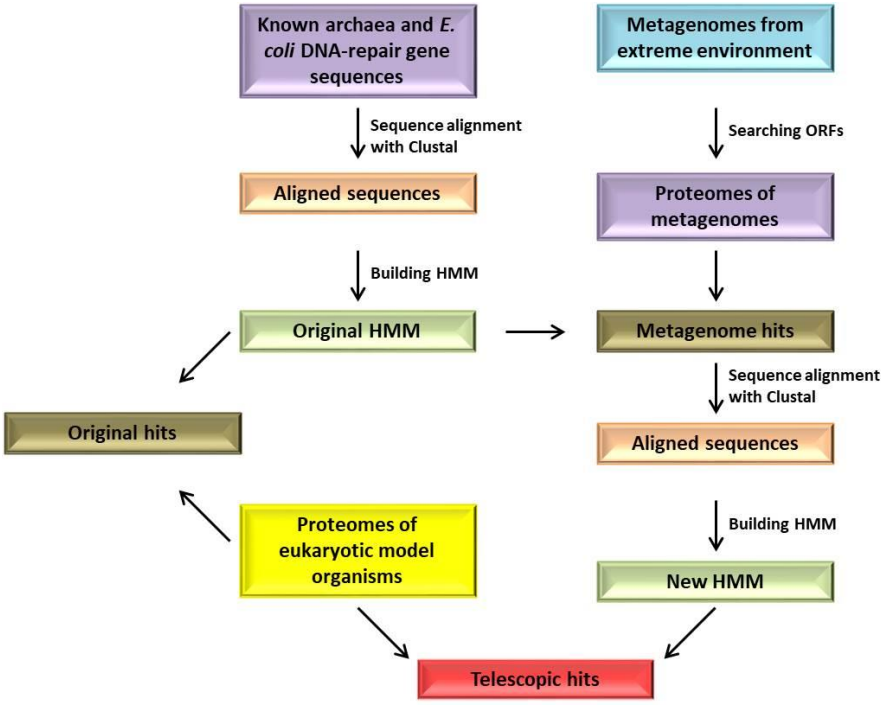

Figure 4. A flow diagram of metagenomic telescope.

[22] Balázs Szalkai and others, 'The Metagenomic Telescope.', *PloS One*, 9.7 (2014), e101605 <https://doi.org/10.1371/journal.pone.0101605>.

4 Thesis

1) I have shown that the expression of APOBEC3B protein results a significantly elevated genomic uracil levels in both p53 wild-type and silenced cells. (1)
2) I examined the thermostability of UDE constructs by differential scanning fluorimetry. I have found that the CA7 and CH9 constructs lack a cooperative unfolding transition, so they probably do not have a well-defined tertiary (and quaternary) structure. (2)
3) I analyzed the oligomerization state of the UDE constructs by analytical gel filtration. According to the gel filtration data, the CH9 construct is presumably less disordered than CA7. (2)
4) I confirmed the high α-helical secondary structural element content of the UDE constructs by CD spectroscopy measurements. (2)
5) I demonstrated by EMSA method, that both 1x and 3xFlagΔUNG specifically bind to uracil-containing DNA by EMSA. (3)
6) I found several DNA repair functions among the protein identified by the Metagenomic Telescope method. (4)
7) I have demonstrated that UNG expression in *E. coli* CJ236 (*dut- ung-*) cells with high genomic uracil content is lethal. (5)
8) I established a high-throughput method suitable for searching in genome libraries for proteins that protect uracil DNA against UNG activity. Using this method I have identified several potential UNG inhibitors in the genome of bacteriophage ΦR1-37. (5)

5 Potential applications

Investigating viruses with uracil genome may be of particular interest as they are unique on Earth and can help understand the formation of life. In addition, bacteriophages may encode proteins in their genome that can inhibit essential enzymes, and may also be useful against pathogenic bacteria. Our genomic uracil quantification method was used for numerous biological problems - many of them in international collaboration. One of these was to investigate the effect of APOBEC3B to the genomic uracil level, which could help to understand of the mechanism of some cancer types and may be useful in developing specific therapies. The work in this collaboration also revealed that high levels of APOBEC3B in p53-defective cells cause hypersensitivity to small molecule inhibitors targeting DDR, suggesting that APOBEC3B expression makes cells vulnerable to targeted therapies. The combination of ESPRIT technique and CD spectroscopy (and with other analytical methods) together could help to get closer to the knowledge of the structure of proteins that cannot be analyzed by either NMR or X-ray crystallography, and the reliability of homology models is limited in the absence of sequential homologs of known structure. The "Metagenomic Telescope" method seems to be capable of identifying previously unknown proteins.

6 Publications list

Original publications related to the thesis:

J. Nikkilä, R. Kumar, J. Campbell, I. Brandsma, H. N. Pemberton, F. Wallberg, **K. Nagy**, I. Scheer, B. G. Vértessy, A. A. Serebrenik, V. Monni, R. S. Harris, S. J. Pettitt, A. Ashworth, C. J. Lord, „Elevated APOBEC3B expression drives a kataegic-like mutation signature and replication stress-related therapeutic vulnerabilities in p53-defective cells." *British Journal of Cancer*, *117*(1), 113–123, 2017.

IF: 5,922 I: 18

M. Pukáncsik, Á. Orbán, **K. Nagy**, K. Matsuo, K. Gekko, D. Maurin, D. Hart, I. Kézsmárki, B. G. Vertessy, „Secondary Structure Prediction of Protein Constructs Using Random Incremental Truncation and Vacuum-Ultraviolet CD Spectroscopy.", *PloS One*, *11*(6), e0156238, 2016.

IF: 2,806 I: 5

G. Róna, I. Scheer, **K. Nagy**, H. L. Pálinkás, G. Tihanyi, M. Borsos, A. Békési, B. G. Vértessy, „Detection of uracil within DNA using a sensitive labeling method for in vitro and cellular applications.", *Nucleic Acids Research*, 44(3), e28, 2016.

IF: 10,162 I: 14

B. Szalkai, I. Scheer, **K. Nagy**, B. G. Vértessy, V. Grolmusz, „The Metagenomic Telescope.", *PLoS One*, 9(7), e101605, 2014.

IF: 3,234 I: 7

Kinga Nagy, Beáta G. Vértessy, „Uracil-DNA-based viruses: what can be the survival strategy?" *Biokémia folyóirat*, 2019.

List of conference presentations related to the thesis:

Kinga Nagy, Viktória Herczeg, Bianka Balogh, Beáta G. Vértessy *Survival With Uracil Genome: How Is It Possible?* XIV. PEME PhD Conference; Budapest, 2017

Kinga Nagy, Viktória Herczeg, Bianka Balogh, Beáta G. Vértessy *Viruses with uracil-DNA: How can they survive?* XIV. George Oláh PhD School Conference; Budapest, 2017

Kinga Nagy, Éva Bertalan, Gergely Róna, Mikael Skurnik, Beáta G. Vértessy *Uracil or thymine? Minor difference in structure - Major key difference in the physiological role* Athen's Chemistry, Budapest, 2015 (poster presentation)