# Interplay between dNTP metabolism, uracil-DNA repair and horizontal gene transfer in *Staphylococcus aureus*

In the foreword, I wish to say that we have carried out the experiments with publication quality outcomes of AIM1 and AIM2, and most of the planned experiments of AIM3 were also done. We met several difficulties that had to be overcome, however, on the other hand, thanks to the same difficulties, we published 4 papers driven by the need for methodological development (*1–4*). We are now in the process of preparing a manuscript from the results of AIM1 and 2, while the experiments of AIM 3 are in the finishing line (OTKA rules allow the joining of a prospective publication's final report of the grant later on). Therefore, this report is a relatively long one aiming to present the data in a quasi publication-like manner.

## Background, hypothesis and aims -recap:

*Staphylococcus aureus* is a major opportunistic pathogen causing nosocomial and community acquired infections (*5*). Mobile genetic elements, such as *S. aureus* pathogenicity islands (SaPIs), transposons and staphylococcal cassette chromosomes (SCC), account for ~20% of its genome (*5, 6*). These vary a lot between *S. aureus* lineages and contribute largely to pathogenesis by spreading genes of virulence factors and antibiotic resistance (*5–7*). When our project started, **our results had suggested an interplay between dNTP metabolism, uracil DNA-repair and horizontal gene transfer in *S. aureus.***

Uracil is one of the most abundant erroneous bases that may occur in DNA. Heavily uracilated DNA may be degraded by uracil-DNA repair. Nucleotide metabolism and uracil-DNA repair together determine the uracil content of DNA. Two enzymes are primarily involved in keeping DNA uracil-free: dUTPase (DUT) breaks down dUTP, and uracil-DNA glycosylase (UDG) which excizes uracil from DNA and initiates base excision repair (*8*). We found that the core genome of *S. aureus* lacks dUTPase (*8, 9*). This finding is intriguing as knocking down dUTPase activity is generally cytotoxic. The resulting uracil-incorporation events will overload the base excision repair mechanism and transform it into a hyperactive futile cycle potentially leading to increased mutation rates and double strand breaks (*10–13*). The lack of dUTPase activity may be tolerated only in the absence of UDG activity (*13, 14*). However, UDG is present in the core genome of S. aureus. How the *dut- udg+* genotype is paralleled with a normal, viable and infectious phenotype in S. aureus is an intriguing question.

Surprisingly we have found also in our genomic analysis that a conserved staphylococcal UDG inhibitor, SaUGI (*Staphylococcus aureus* Uracil DNA glycosylase inhibitor) (*15*), is strictly encoded on a mobile genetic element, SCCmec (Staphylococcal Cassette Chromosome mec) carrying the mecA gene responsible for methicillin resistance of MRSA strains (Methicillin Resistant *Staphylococcus aureus*).

These findings indicated that although *S. aureus* can maintain its genome integrity in a *dut- udg+* background, mobile genetic elements need dUTPase or SaUGI to avoid uracil-DNA and DNA damage caused by uracil-DNA repair.

**Based on this, the hypotheses of the project were the following:**

1. *S. aureus* strains with *dut- udg+* genotype possess a somewhat elevated dUTP/dTTP ratio and tolerate uracil incorporation into their genomic DNA, probably by down-regulating U-DNA repair
2. Mobile genetic elements integrated into *S. aureus* contribute to the U-DNA tolerance of the *dut- udg+* strains by encoding U-DNA modifying proteins. This contribution may be even more important under U-DNA promoting conditions.
3. DNA uracilation interferes with horizontal gene transfer and therefore, mobile genetic elements carry factors to avoid U-DNA and U-DNA repair.

**To test these hypotheses, we set up the following aims:**
**AIM 1:** We will determine the degree of uracilation in *S. aureus* strains and relate DNA uracilation to the regulation of dUTP/dTTP ratio and the expression levels of U-DNA repair genes.
**AIM 2:** We will investigate the U-DNA tolerance of *S. aureus* strains in the absence, and in the presence of different integrated mobile genetic elements. We will identify uracil promoting stress conditions and include these in the study.
**AIM 3:** We will investigate the influence of DNA uracilation on the efficiency of horizontal gene transfer.

## Results of the project

**AIM 1: We will determine the degree of uracilation in *S. aureus* strains and relate DNA uracilation to the regulation of dUTP/dTTP ratio and the expression levels of U-DNA repair genes.**

To test hypothesis 1 and 2, we have chosen five *S. aureus* strains (Figure 1). To be able to catch differences caused by the presence of dUTPase and SaUGI of mobile genetic elements, we took care of choosing isogeneic strains that only differ in their mobile genetic element content. The most often used *S. aureus* strain is RN4220. This strain is a descendent of the reference *S. aureus* strain NCTC8325 (https://www.ncbi.nlm.nih.gov/genome/154) (Figure 1). RN4220 was created from NCTC8325 by removing prophages and by mutagenesis to create a strain which is capable of the uptake of engineered DNA acquired from *Escherichia coli* (Figure 1). To create such a strain was very important for the genetic manipulation of *S. aureus* as most strains are incompatible even with each other due to restriction-modification system incompatibilities. We planned to investigate extrachromosomal expression of relevant genes in later stages of the research, therefore our first choice was this strain. After this, it was straightforward to investigate the ascendent strains NCTC8325 (carries 3 prophages and thus 3 dUTPase genes), and RN450 which only differs from NCT8325 in that it lacks the 3 phages.
To test also the effect of SAUGI protein encoded on SCCmec chromosomal cassette, we have chosen the JKD6159 MRSA strain, and the WBG10566 strain which only differs from JKD6159 in that it lacks the SCCmec element (Figure 1) (these two strains were a kind gift of our collaborator).

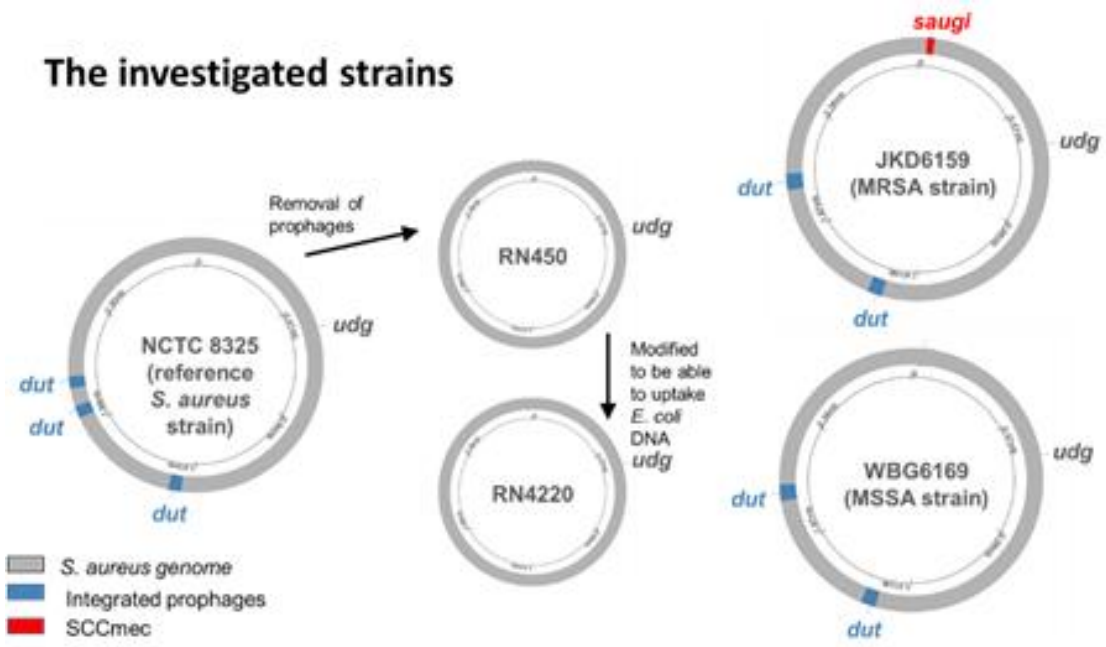



***Figure 1**: Stains investigated in the study*

For the determination of uracil content and dUTP/dTTP ratios, first we had to work out a procedure in which we could investigate parallel isolated DNA and dNTP extracts to be able to relate dUTP levels and uracil DNA levels. Besides, dNTP pools were also determined, because dNTP pools are highly regulated by allosteric mechanisms, and thus change in the level in one dNTP may result in overall dNTP pool changes.

To determine the dNTP concentrations, and the dUTP/dTTP ratio in *S. aureus* was not an easy task. dNTP quantitation method of our choice was a fluorescence-based, TaqMan-like polymerase assay published by Wilson *et al*., NAR 2011.(*16*)

Although this method works well in diluted samples with high dNTP levels, we observed that the sample matrix largely decreases assay performance. *S. aureus* proved to have a relatively low dNTP level; therefore, we could determine the dNTP concentration in this organism only after optimization of the chosen method and after renewing the evaluation method. Although, in our preliminary results we could determine the dUTP/dTTP ratio in NCTC8325 (the concentration of dTTP is relatively high in *S. aureus compared* to the other dNTPs), with dUTP determination we had to face some difficulties too. Cellular dUTP concentrations are measured by extracting the concentration of dTTP measured in dUTPase-treated sample from dTTP measured in the same, non-treated sample. To arrive at a reliable dUTPase treatment and dUTP quantification, we replaced the human dUTPase suggested by the original method with the less sensitive Mycobacterial dUTPase. Determining dNTP pools and dUTP/dTTP ratios *in S. aureus* was a major challenge in the first year of the research period. Our method development was inevitable to arrive at a reliable fluorescent dNTP measurement method, which we could use to determine dNTP pools in *S. aureus* and in other samples. Our method development was published in the Methods section of Nucleic Acid Research (D1, IF 16.97) (*1*). In the manuscript *S. aureus* data are also provided.

After the method optimization we determined the dNTP pools in our five strains, and the dUTP/dTTP ratios in three (Figure 2). The dNTP pool balance was similar in all strains: dTTP and dGTP was roughly similar, and about 2-3X higher than dATP and dCTP. Although, there were some minor differences between the strains, e.g., in NCT8325 dATP was higher than dCTP, while in WBG10566 dCTP concentration exceeded dATP concentration. These differences were unrelated to the number of the dUTPase genes present in the individual strains.

dUTP was measured in 3 strains that differ in their dUTPase content (NCTC8325:3 dUTPases, RN4220: no dUTPase, JKD6159: 2 dUTPases). Normally dUTPase possessing bacteria contain undetectable amounts of dUTP. In our experiments dUTP was detected in all the investigated strains at a significant level. The measured dUTP/dTTP ratio in RN4220 is comparable to the dUTP/dTTP ratio in bacteria that contain a dUTPase that is inactivated by point mutation. (Figure 2B). Interestingly the dUTP/dTTP ratio was increasing with decreasing number of dUTPases. However, this difference was not significant. dUTP is measured as the difference of dUTPase treated and non-treated samples, and usually dUTP concentrations are relatively small (if detected at all), the non-significant difference may be technical. Thus, it cannot be stated for sure that dUTP level changes with the number of prophage dUTPases. However, the tendency observed on Figure 4 may indicate leaky expression of these genes. Alternatively, it may be the sign of prophage induction in a few cells. If the prophages are activated and the cells are lysed, then the genomic DNA of the lysed cell will not be isolated with the cellular DNA during uracil-DNA measurements. Thus, this method can decide whether the increased dUTP/dTTP ratio is due to leaky prophage dUTPase expression or due to low level prophage activation.

In parallel with dNTP pool measurements, we also determined the uracil content of the DNA within these strains in logarithmically growing cell cultures (Figure 3), and to compare exponential and stationary phases, also in stationary phase bacteria.

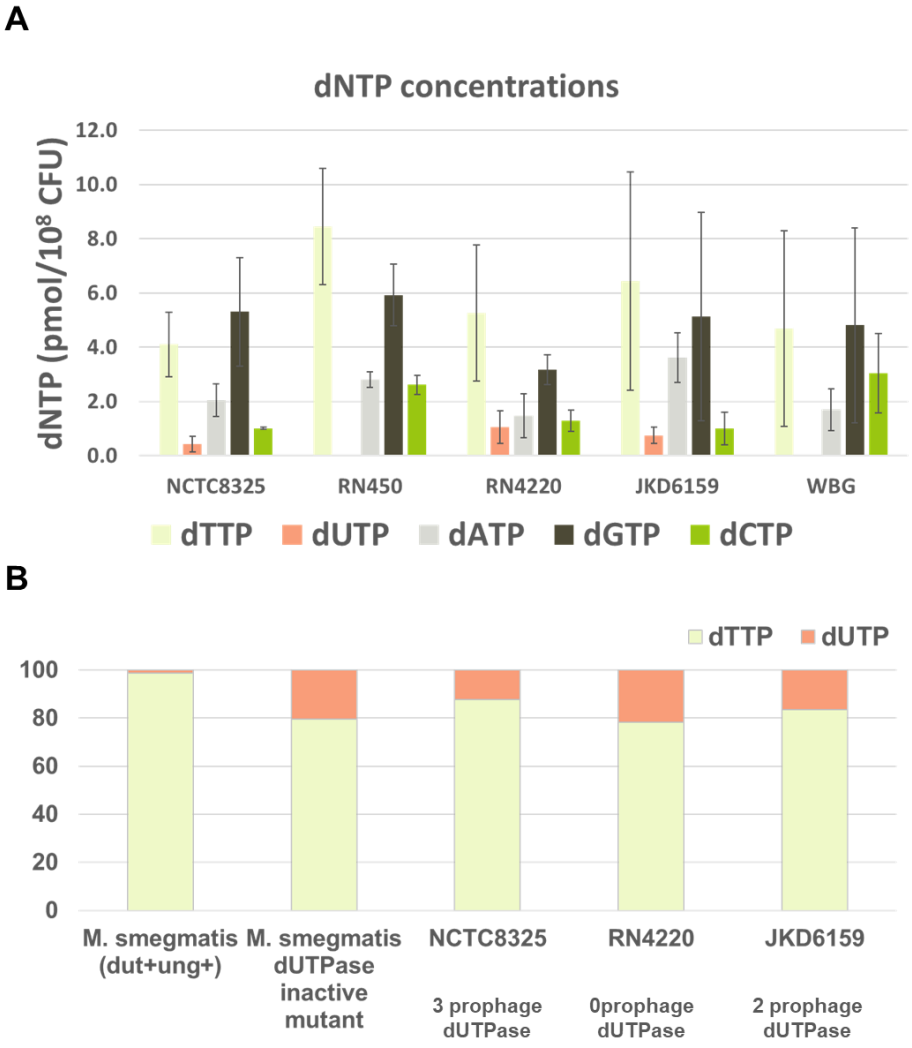



***Figure 2: A)*** *dNTP pools determined in different S. aureus strains. It is important to note that the error of dNTP pool measurement is large because of the difference between biological parallels. The exact size of dNTP pool highly depends on the exact growth phase of the culture, and a few differences in the OD may result in relatively large differences in dNTPs measured in parallel biological samples. The error of dNTP determination on a given sample is usually <10 %.)* ***B)*** *Ratios of dUTP and dTTP in S. aureus strains in comparison with active and inactive dUTPase possessing Mycobacterium smegmatis strains. Mycobacterium smegmatis data are from Hirmondó et al 2018 (17).*

It was already established earlier that RN450 has a somewhat higher uracil-DNA level than other bacteria that possess a dUTPase (previously published results (*18*)). In exponentially growing cells the uracil DNA level of NCT8325, RN450 and RN4220 did not differ (Figure 3A). While RN450 and RN4220 completely lacks dUTPase, NCTC8325 possesses 3 prophage dUTPase. Thus, even if the presence of prophage dUTPases slightly decreases dUTP/dTTP level (Figure 2B) it does not manifest in the uracil DNA level.

The uracil DNA level of JKD6159 was like the uracil DNA level of the first three strains, while the uracil DNA level of theWBG10566 was somewhat lower (Figure 3A). The latter two strains differ only in the presence of the SCCmec element and thus in the presence of the SaUGI gene. SaUGI inhibits uracil base excision repair thus the difference may have originated from this inhibition.

In stationary phase bacteria the uracil level of DNA was 23 % lower than the uracil level in logarithmically growing bacteria (Figure 3B), indicating that during extensive DNA replication in logarithmical phase not all of the incorporated dUTP can be removed by UDG, but these uracils are later repaired.

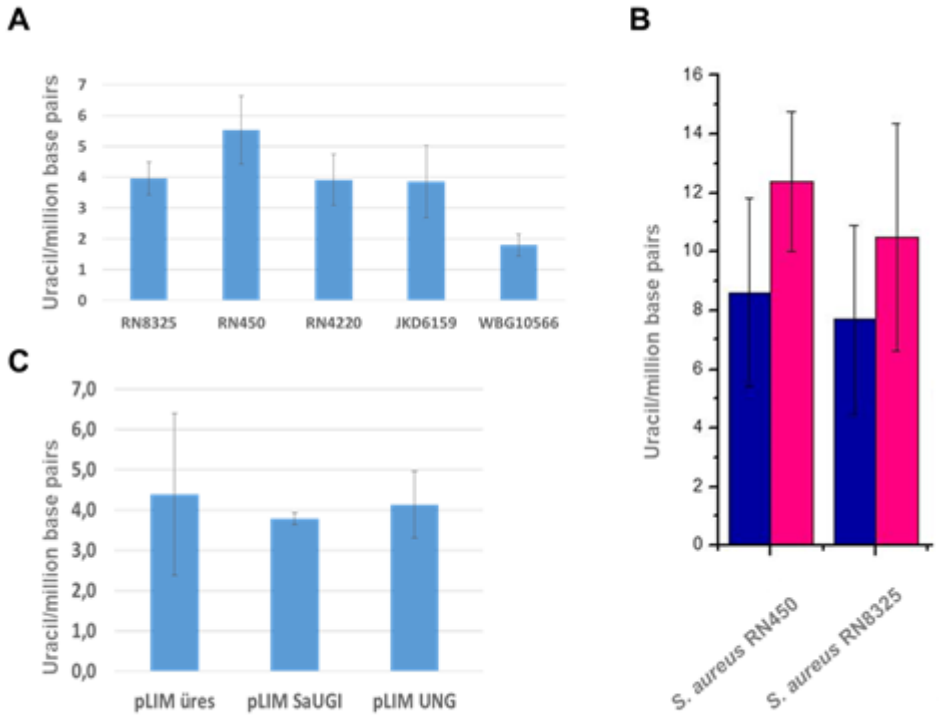



**Figure 3:** ***A)*** *Uracil DNA levels in different S. aureus strains* ***B)*** *Uracil DNA levels in stationary phase (blue) vultures and in logarithmically growing cultures (pink)* ***C)*** *Uracil DNA level in S. aureus strains expressing SaUGI or UDG extrachromosomally.*

To further study the effect of SaUGI, and the uracil DNA tolerance of *S. aureus*, we investigated the effect of *E. coli-S. aureus* plasmids carrying SaUGI and UDG genes. The expression pattern of SaUGI is not known in Staphylococcus strains. UDG is a housekeeping enzyme and in the bacterium, and it is expressed constantly at a roughly similar level. Our plasmids, created in collaboration, carried SaUGI and UDG with their own promoters allowing 2X expression of the investigated genes compared to the base expression level. Neither the SaUGI nor the UDG plasmid affected uracil DNA level compared to the controls in logarithmically growing RN4220 culture (Figure 3C).

Summary and conclusions

1. Logarithmically growing *Staphylococcus aureus* strains that lack genomic dUTPase have an elevated dUTP and uracil DNA level. Uracil DNA level is lower in stationary phase culture, thus the uracil bases in DNA – probably accumulated by increased dUTP incorporation – are repaired later.
2. The dUTPase genes carried by prophages do not influence significantly the dUTP concentration of the cell, and the uracil level of DNA. However, dUTP/dTTP ratio seems to be decreasing with the increasing number of prophage dUTPases, this difference is not manifested in uracil DNA level. The tendency in dUTP/dTTP ratio may be rather the consequence of low-level prophage induction, then leaky expression of non-induced prophage dUTPases.
3. In JKD6159 MRSA strain that carry the UDG inhibitor SaUGI gene the uracil DNA level was higher than in its isogenic pair lacking the SCCmec chromosomal cassette together with the SaUGI gene (WBG10566). Extrachromosomal expression of SaUGI with its own promoter did not result in the uracil DNA level in RN4220. These results indicate that SaUGI may be expressed in JKD6159 but other factors of SCCmec are necessary for its expression.

Together these results suggest that under non-stress conditions the genes of dUTPase and SaUGI proteins are not useful for the bacterium. These genes may be advantageous either for the bacterium under stress conditions or for the mobile genetic elements that carry them.

**AIM 2: We will investigate the U-DNA tolerance of *S. aureus* strains in the absence, and in the presence of different integrated mobile genetic elements. We will identify uracil promoting stress conditions and include these in the study.**

To test whether the dUTPase and SauGI encoded on mobile genetic elements are advantageous for the bacterium under uracil promoting stress conditions we aimed to identify uracil promoting conditions and apply them as a stress on the different *S. aureus* strains with different mobile genetic elements.

We set up a dNTP metabolic model to identify enzyme reactions that upon inhibition may result in elevated dUTP level in the absence of dUTPase activity. The model was needed, as dNTP metabolic pathways are highly regulated by allosteric mechanisms and feedback inhibition. dUTPase inhibition for example in different model organisms results in various outcomes.

We set up a kinetic model containing the dNTP synthesis pathways in *Mycobacterium smegmatis* and in *E. coli* as a part of a different project. The dNTP synthetic pathways of these to bacteria cover all the dNTP synthetic pathways present in living organisms, thus they also overlap with the dNTP synthetic pathways of *S. aureus*. Thus, from the original model it was easy to set up the *S. aureus* model. From these models it was obvious that the dNTP balance, and the production of dUTP largely depends on the enzyme set available for the organism and on the concentrations of the enzymes involved in the different pathways.

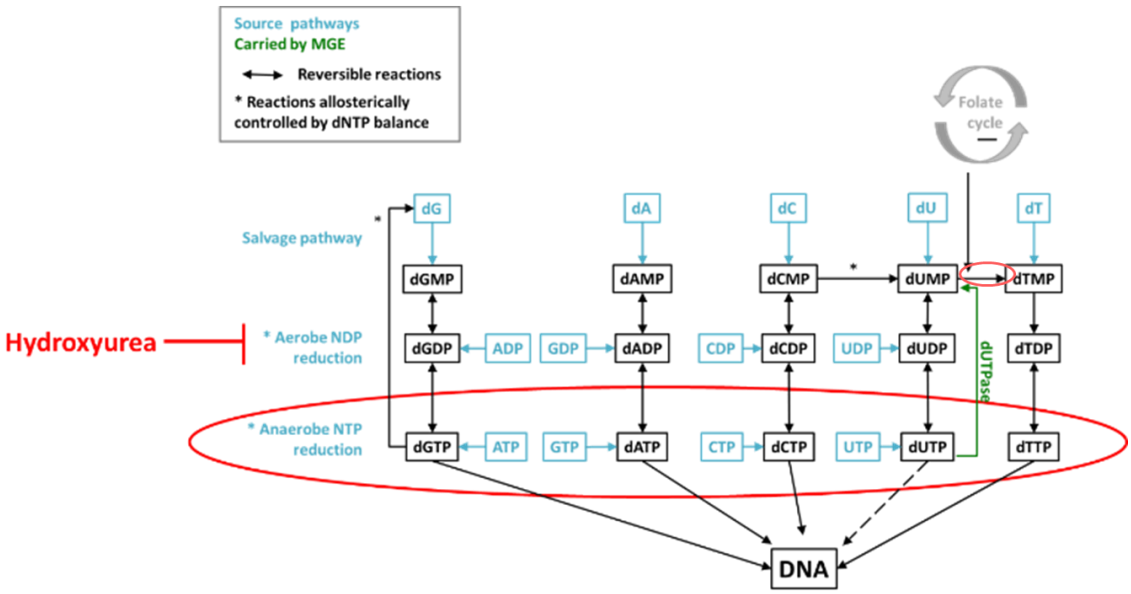
Taking into consideration the enzyme set of *S. aureus*, we identified 2 intervention points based on the model in *S. aureus*:

1) Inhibition of thymidylate synthesis (dUMP→dTMP; Figure 4A, which may result in the elevation of dUMP concentration and consequently in the elevation of dUTP level – this intervention point is relatively well described in the literature, and in most organism inhibition of thymidylate synthesis result in the increase of dUTP level in the absence of dUTPase
2) Anaerobic condition that result in the working of the anaerobic ribonucleotide reductase enzyme, which in contrast to the aerobic enzyme acts on the triphosphate level and thus may result in direct dUTP production (Figure 4A)

Besides, in a collaborative project we measured dNTP levels in human cells, partly in order to optimize the dNTP measurement method described earlier. In these measurements I found that hydroxyurea treatment increases dUTP levels in human cells (these experiments were published in Cancer Research (3)). Hydroxyurea inhibits the working of the aerobic ribonucleotide reductase and was not expected to elevate dUTP level, especially not in the presence of dUTPase activity (present in human cells). Probably dUTP level is increased through the salvage pathway (Figure 4A) by taking up uridine and deoxyuridine from the environment. As hydroxyurea increased the dUTP level in human cells, it was also included in the study.

As for thymidylate synthesis inhibition it is well described that it results in the elevation of dUTP level in the absence of dUTPase, the effect of thymidylate synthase inhibitors on dUTP level and uracil DNA was not checked. Thus, we started with testing whether anaerobic growth and hydroxyurea affect uracil DNA level. These experiments could be performed parallel, especially because hydroxyurea is also a control for checking anaerobiosis (hydroxyurea inhibits aerobic growth but not the anaerobic growth).

**A**




**B**

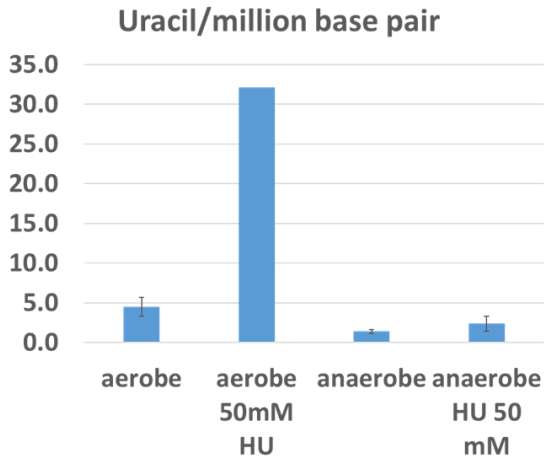



***Figure 4: A)*** *The dNTP synthetic pathways of Staphylococcus aureus. The investigated intervention points for elevating uracil DNA level are highlighted by red circles.* ***B)*** *Uracil DNA levels measured in anaerobiosis and with hydroxyurea (HU) treatment.*

The anaerobic lifestyle would have been expected to result in an increased uracil content of the genomic DNA, since dNTPs are formed directly during anaerobic metabolism by reduction of the nucleotides, thus providing a good chance of incorporation of dUTP in the absence of dUTPase. Surprisingly, uracil levels of genomic DNA decreased under this condition (Figure 4B). According to literature and structural analysis, this may be because ribonucleotide reductase only process ATP, CTP and GTP, but not UTP, thus bypasses the directly dUTP generating pathway (observed for some viruses, but not described for the staphylococcal enzyme). In case of hydroxyurea the uracil DNA level was highly elevated, reinforcing the prediction that inhibiting the aerobe ribonucleotide reductase results in elevated dUTP concentration. Thus, this drug was included in further studies. To see whether the presence of prophage dUTPase and SCCmec SaUGI contibuts to uracil stress tolerance of the bacterium, we determined the different minimal inhibitory concentrations (MIC) of drugs that cause the

inhibition of thymidylate synthesis (trimethoprim, sulfamethoxazole, trimethoprim / sulfamethoxazole combination, methotrexate, 5-fluorouridine) and of hydroxyurea. Two strains of *S. aureus* with *dut-udg* + genotype (RN4220, and RN450) and three strains containing SCCmec and / or prophages (NCTC8325, JKD6159 and WBG10566) were investigated in this experiment. Besides, we also investigated RN4420 strains carrying the pLIM plasmids with UDG and SaUGI.
For the 5-fluorouridine all strains were extremely sensitive, but there was no difference in sensitivity between the different strains, neither for 5-fluorouridine nor for other drugs (data not shown).

Summary and conclusions
Presence of mobile genetic elements, or presence of UDG or SaUGI expressed from plasmids did not influence the sensitivity of different *S. aureus* strains under uracil DNA generating conditions. Thus, the uracil DNA repair influencing genes of mobile genetic elements does not contribute the uracil toleration of the bacterium, and probably they are needed for mobile genetic elements (different lifestyle/replication circumstances

**AIM 3: We will investigate the influence of DNA uracilation on the efficiency of horizontal gene transfer.**

To investigate whether uracilation of mobile genetic element DNA influences the horizontal gene transfer we aimed to 1) test the natural uracil DNA content of mobile genetic elements 2) create mobile genetic elements with uracilated DNA and test their efficiency. AIM 3 was the most complex and most complicated part of the project. First, we had to induce mobile genetic elements and isolate their DNA; second, we had to manipulate *S. aureus* genetically to create uracilated mobile genetic elements. As our laboratory had just a few experience in these techniques, we expected the help of our more experienced collaborators. Originally, we aimed to test both SSCmec and phages. In the first year of the project, it turned out that SCCmec in the investigated MRSA strain has lost its mobility, therefore SCCmec DNA could not be isolated in collaboration from MRSA strains. The activation mechanism of SCCmec is not fully understood, and it seems that the mobilization of different SCCmec types in different MRSA strains may vary. The questions of AIM 3 could be answered based on the investigation of prophages, therefore we decided that we will focus on this, until the mechanism of SCCmec activation will be discovered in a strain which is available for us.
In order to generate uracil DNA phages and test their horizontal gene transfer efficiency, we had to validate the null hypothesis that phage dUTPase protects phage DNA from uracilization and therefore contains less uracil than bacterial genomic DNA. First, we had to set up the experiments, which took about a year. Finally, we managed to achieve induction of prophages in NCTC8325 and we could also isolate a large amount of phages, and to set up a protocol which made possible also the quantification of the produced phages. The next challenge was to purify phage DNA and genomic DNA separately from the same culture. This problem was also solved, but it also took a relatively long time. After we managed to activate prophages from the NCTC8325 strain, and purify their DNA, we had to find a condition which elevates uracil in DNA and also induces the prophages (in order to treat the sample only with one stress condition). Our choice was the hydroxyurea. We already knew that this drug elevates uracil in DNA and that it causes DNA breaks, thus it induces SOS response, and as a consequence also the prophages. Indeed, hydroxyurea induced phages, and we were able to isolate DNA both from the bacteria and the phages of the treated culture. We found, as we expected, that the uracil level of the phage DNA is lower than the uracil level of the genomic DNA. Thus, phage dUTPases indeed protect the phage DNA from uracilization (Figure 5).

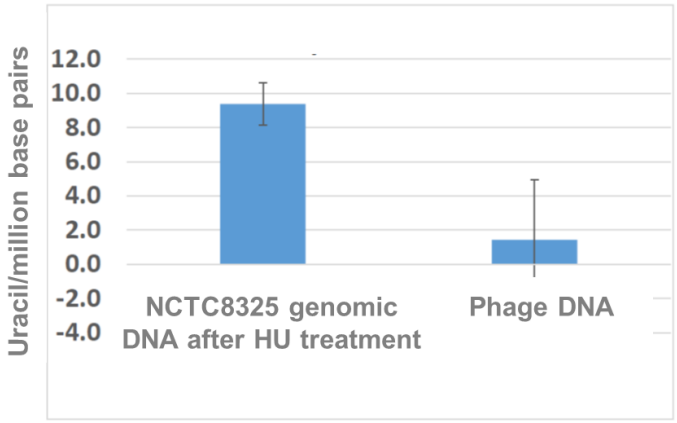



***Figure 5:*** *Uracil DNA level in the genomic DNA of NCTC8325 treated with hydroxyurea and in the DNA of activated and isolated phages of the same bacterium*

Next, we aimed to create uracilated phages. Since phage dUTPase protects phage DNA from uracilization, we can artificially generate uracil phages only by inhibiting both phage dUTPase activity. To protect the uracilated phage DNA from degradation we also needed to inhibit the uracil DNA error repair, UDG. One of the possibilities of inhibiting phage dUTPase is Stl protein. Stl is a staphylococcal transcription factor (present only in a few strains, on another mobile genetic element SaPIbov1 and 5) which interacts with phage dUTPases. Stl not just interacts with dUTPases but also inhibits a variety of dUTPases from different sources as described by our laboratory. Unfortunately, Stl does not inhibit all dUTPases with the same efficiency. We knew that the *S. aureus* ϕ11 phage dUTPase is inhibited by high efficiency. However, most *S. aureus* strains carry more than one phage with different dUTPases. Therefore, we had to make sure that all dUTPase is inhibited in our model system. In our laboratory we also studied what is the structural base of the differential dUTPase inhibition of Stl. We wished to use the knowledge acquired from these studies to choose the model system for uracil phage generation Although key amino acids of the interaction have been identified in the structural studies, and two papers were published from this, we have come to the conclusion, that it would be difficult to predict from a sequence alone whether all phage dUTPases of a given *S. aureus* strain are inhibited by Stl. Based on this, it was decided that we will isolate a strain that contains only one Stl affected dUTPase carrying prophage. For this purpose, the ϕ11 lysogenic phage was selected, which has a known trimeric dUTPase that is affected by the inhibition of the Stl protein. We had no access to an exclusively ϕ11 lysogenic *S. aureus* strain that is also able to uptake *E. coli* DNA (we wished to express the inhibitor proteins from an expression vector), therefore we had to create this strain and a model system which is suitable for the production of uracilated phages (Figure 6).
To this end, we activated the prophages in NCTC8325, then infected the RN4220 strain with the phage suspension acquired from NCTC8325 culture. Then we attempted to isolate lysogenic *S. aureus* strains containing ϕ11 prophage exclusively. We tried more prophage activation agents (mitomycin C, ciprofloxacin, hydroxyurea), and altogether tested ~ 80 lysogenic strains in PCR reactions for all the three prophages of NCTC8325 (primers used were planned for the integration site, thus only the integrated phages were detected. Interestingly, with mitomycin C and ciprofloxacin we obtained mostly ϕ12 prophage containing strains, and we could not isolate a lysogen exclusively containing the ϕ11 prophage. However, with hydroxyurea we could obtain 5 different exclusively ϕ11 lísogenic strains. The generated lysogens were also tested by next-generation sequencing. We also tested whether the lysogen strain we isolated was capable of uptaking *S. aureus-E. coli* shuttle expression vectors (pLIM, and pRMC2 plasmids).

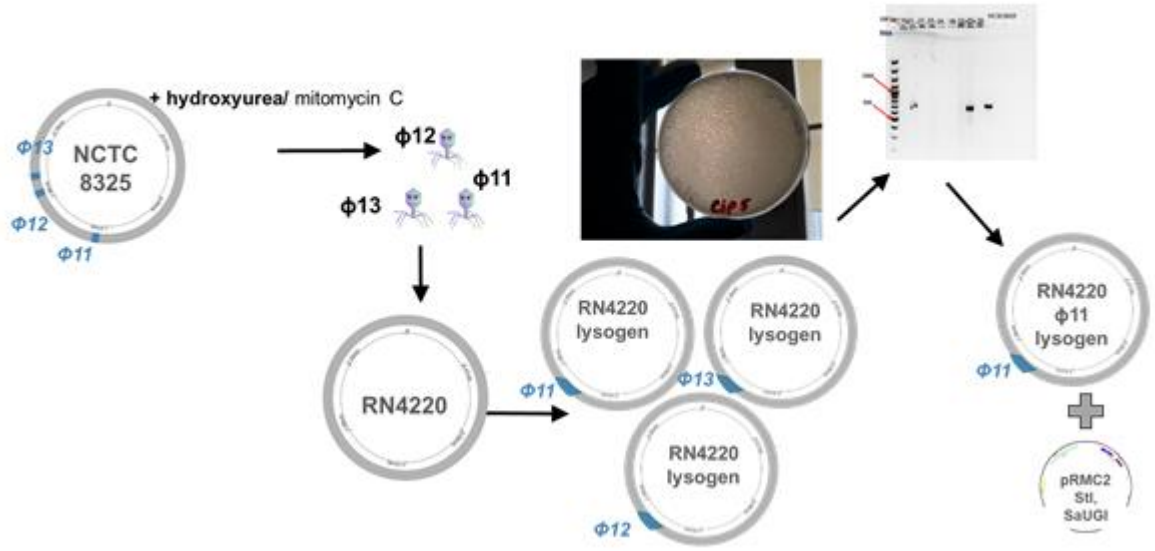



***Figure 6:*** *Schematic representation of creating our model system to produce uracilated phages.*

Thereafter, the Stl and SaUGI protein were cloned in a *S. aureus-E. coli* shuttle vector to express the inhibitor protein in the strain to be tested. As a vector we chose the pRMC2 plasmid which enables the tetracycline and anhydrotetracycline inducible expression of the inserted gene. We tested whether anhydrotetracycline inhibited the growth of the plasmid electroporated strains, and also whether it influences the uracil DNA level of *S. aureus.* Based on the results, the vector was found to be suitable. Unfortunately, this vector was placed in a plasmid bank of Addgene as a version in which the multicloning site is present twice and contains only a few (6) restriction sites. However, we decided to go forward with this vector, as in case of *S. aureus* much less inducible expression vectors are available than in case of. *E. coli*. This made the cloning process more difficult, but finally we managed to clone both genes into the plasmid. Next, the expression of SaUGl and Stl was checked, and the proteins were found to be expressed. With this our model system is ready to generate uracilated phages and to test their efficiency in transduction and horizontal gene transfer.

Summary and conclusions

We investigated successfully the uracil DNA content of mobile genetic elements (phages) and found that the uracil content of these elements is lower than the uracil content of the bacterial genomic DNA. This reinforces the finding of AIM 1 and 2, namely that dUTPases carried by the temperate phages of S. aureus are rather advantageous for the mobile genetic elements than for the bacterium. Besides, we set up a model system in which we will be able to create uracilated phages. With these uracilated phages we will be able to test the influence of uracil DNA level on horizontal gene transfer.

It is worth mentioning that AIM 3 was the most complex and complicated part of this project, especially because it also involved the genetic manipulation of *S. aureus.* As far as we know, our laboratory is the first one which has done experiments involving genetic manipulation of *S. aureus* in Hungary.

## Publication summary

We have published a methodological paper connected to the project (*1*), and 3 research papers loosely connected to the project (*2–4*). One future publication is also in preparation. Besides, in connection with the project 2 BSc and 2 MSc thesis work was submitted and defended. Our results were also published in conferences (5 poster presentations) and we also wrote an article in "Élet és Tudomány" educational journal.

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