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UNIVERSITY OF TARTU
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Institute of Molecular and Cell Biology, University of Tartu, Estonia

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III. **Ukkivi K, Kivisaar M.** Involvement of transcription-coupled repair factor Mfd and DNA helicase UvrD in mutational processes in *Pseudomonas putida*. DNA Repair (Amst.). DNA Repair (Amst). 2018 Dec; 72:18–27.

My contribution to the publications is following:

Ref I – I participated in the construction of plasmids and strains and performed some of the experiments

Ref II – I participated in the construction of plasmids and strains and contributed to planning some of the experiments

Ref III – I participated in planning the experiments, construction of plasmids and strains, conducted the experiments and wrote the manuscript

ABBREVIATIONS

DNAP	DNA polymerase
GG-NER	global genomic NER
HR	homologous repair
IPTG	isopropyl ß-D-1-thiogalactopyranoside
MFD	mutation frequency decline
MMC	mitomycin C (DNA inter- and intrastrand cross-links inducing chemical)
MMR	DNA mismatch repair
MNNG	1-methyl-3-nitro-1-nitrosoguanidine (alkylating agent)
NER	nucleotide excision repair
NQO	4-nitroquinoline 1-oxide (bulky DNA damage inducing chemical)
ppGpp	guanosine tetraphosphate
RNAP	RNA polymerase
TAM	transcription-associated mutagenesis
TC-NER	transcription-coupled nucleotide excision repair
TCR	transcription-coupled repair
UV	ultraviolet
XR-seq	excision repair sequencing

INTRODUCTION

In nature bacteria rarely meet conditions of unlimited growth and most of their lives are exposed to variating and harsh environmental conditions. Numerous strategies to overcome or cope with these stressful circumstances have been developed, one of which is rapid evolution by incorporation of foreign DNA or through mutations caused by exo- or endogenous factors.

The essential process of transcribing DNA into RNA has also been proposed to be one of the factors contributing to the generation of mutations (reviewed in (Kim and Jinks-Robertson, 2012; Jinks-Robertson and Bhagwat, 2014)). Since in bacteria RNA and DNA synthesis occur at the same time, collisions between transcription and replication complexes are unavoidable and may lead to genetic instability (Paul *et al.*, 2013; Sankar *et al.*, 2016). While transcribing, RNA polymerase also encounters DNA lesions that block elongation of RNA, increasing the risk of collisions and leaving DNA open to damage. For removal of such lesions and stalled RNA polymerases organisms have transcription-coupled repair (TCR), mediated by TCR factors Mfd and helicase UvrD in bacteria (Monnet *et al.*, 2013; Epshtein *et al.*, 2014). Although both factors participate in maintaining the genome integrity, a growing body of evidence suggests that TCR, more specifically the Mfd-directed pathway, might also be a mechanism of how transcription induces genetic diversity. While the lesion removing mechanism of TCR has been extensively studied, the role of TCR in mutational processes is still unclear.

As mutations enable the bacteria to overcome stressful conditions, which also include providing resistance to antibiotics and inhabiting new hosts, it is necessary to understand the mechanisms that promote genetic changes. In this thesis the role of transcription and TCR factors Mfd and UvrD in inducing mutations was studied in a cosmopolitan soil bacterium *Pseudomonas putida*. This bacterium is a member of a large genus, which comprises a diverse group of bacteria that are metabolically diverse and have a broad potential for adaptation to changing environmental conditions. Several members are also of great interest because of their prospects in biotechnological applications or their ability to cause disease in plants and animals.

I REVIEW OF LITERATURE

1. Overview of mutational processes

Mutation is an alteration in the nucleotide composition of a DNA molecule, that can occur as a result of DNA damage and via errors made during DNA replication. These changes in DNA can range from insertion, deletion or substitution of only a single base pair to mega base pair alterations. Importantly, changes in the DNA composition are essential for genetic variation on which evolutionary process are based on.

Depending on the mechanism of formation, mutations can be divided into spontaneous mutations, that occur as a result of endogenous metabolic processes, and induced mutations, that occur as a result of exogenous factors. One of the mayor factors leading to formation of mutations in the cells is DNA damage and the resulting erroneous DNA replication or damage repair. Exogenous damage occurs when a physical or chemical agent from the environment damages the DNA. Examples include ultraviolet light (UV), ionizing radiation and various genotoxic chemicals. Intrinsic mechanisms causing endogenous damage are still poorly understood. A recent study in *Escherichia coli* revealed 208 proteins that increase DNA damage when overproduced, however this number is presumably even higher (Xia *et al.*, 2019). These proteins were seen to promote DNA loss, reduce DNA-repair capacity and cause replication stalling, which could lead to mutagenesis. Various by-products of cellular metabolism have also been shown to mediate formation of spontaneous mutations by interacting with DNA and causing lesions. These include reactive oxygen species and nitrogen oxides, which are formed as a product of aerobic metabolism and during nitrate or nitrite respiration, respectively (Sakai *et al.*, 2006; Weiss, 2006). Other intrinsic mechanisms can also promote mutagenesis, for example processes involved in the DNA metabolism. An example of such a process is DNA replication. In *E. coli* misinsertion rate of the DNA polymerase III holoenzyme complex, which replicates genomic DNA, is ~10^{-5} errors per base per round of replication and proofreading activity of the enzyme reduces the error rate to 10^{-7} (Drake, 1991; Schaaper, 1993). Additionally, faulty DNA repair may be activated as a part of global response to DNA damage, i.e. SOS-response, which enables the bacteria to survive genomic damage but increases mutagenesis (for overview see (Baharoglu and Mazel, 2014)). Additionally, faulty DNA repair may be activated as a part of global response to DNA damage, i.e. SOS-response, which enables the bacteria to survive genomic damage but increases mutagenesis (for overview see (Baharoglu and Mazel, 2014))

Mutations can have a neutral, deleterious or beneficial influence on the fitness of the organism. Majority of the occurred mutations are neutral or deleterious and advantageous mutations are rare (Eyre-walker and Keightley, 2007). In *E. coli* the mutation rate per bacterium per generation for deleterious mutations is about $2–8 \times 10^{-4}$ (Kibota and Lynch, 1996; Boe *et al.*, 2000), while the rate of beneficial

mutations is much lower, ~4 × 10^{-9} (Imhof and Schlötterer, 2001). However, increasing the mutation rate can improve the chances of acquiring a beneficial mutation that enables the cells to gain new or improved traits and survive environmental changes. The phenomenon of growth-restricted cells to acquire genetic changes in response to environmental pressure, e.g. exposure to antibiotics, DNA damage and nutrient starvation, enabling them to overcome growth-limiting conditions is called adaptive, stress-induced or stationary-phase mutagenesis. During stationary-phase mutagenesis mutation rates are temporarily upregulated by various stress responses, for instance up-regulation of error-prone DNA polymerases and down-regulation of error-correcting enzymes (for overview see (Kivisaar, 2003; Foster, 2007)), which in turn can lead to accelerated adaptation of the cell to novel growth conditions.

2. Transcription as a source of DNA instability

Transcribing DNA into RNA is an essential part of DNA metabolism. However, accumulating body of evidence suggests that besides just passively copying the DNA template, transcription also contributes to genetic instability and leads to alterations in the template DNA. This phenomenon is known as transcription-associated mutagenesis (TAM), which is not to be confused with transcriptional mutagenesis, a process whereby RNA polymerase (RNAP) bypasses a lesion in DNA and consequently produces a mutated transcript. Transcription was suggested to facilitate formation of mutations in DNA already in the early 1970s. One of the first studies to report TAM was conducted by Herman and Dworkin, who investigated the effect of gene induction on the rate of mutagenesis caused by ICR-191 (Herman and Dworkin, 1971), a chemical that induces frameshift mutations. They observed about a twofold rise in the rate of occurrence of Lac^- to Lac^+ revertants induced by ICR–191 in *E. coli* in the presence of a transcriptional inducer. After further studies it was concluded that this increase was attributable to the elevated transcription of the *lacZ* gene, that is needed for metabolising lactose. Several other studies conducted in bacteria (Beletskii and Bhagwat, 1996; Klapacz and Bhagwat, 2002; Pybus *et al.*, 2010; Chen and Zhang, 2013; Wang *et al.*, 2016), yeast (Datta and Jinks-Robertson, 1995; Park *et al.*, 2012) and humans (Da Sylva *et al.*, 2009; Park *et al.*, 2012) have also reached to the conclusion that transcription is one of the factors contributing to genetic diversity.

During transcription the double-stranded DNA is unwound. While the template ssDNA passes through the RNA polymerase and is protected by the transcriptional machinery, the non-template strand is exposed and more susceptible to damage. In addition, transcription complexes can also physically block the access of DNA repair enzymes to the underlying damaged area and interfere with lesion repair (Selby and Sancar, 1990). As a result, the replicative polymerase may insert a wrong nucleotide into the new DNA strand creating a nonconventional

base pair, which consequently induces mutations. If the replicative DNA polymerase is unable to copy past a lesion, it might be replaced by a specialised translesion polymerase, i.e. polymerase switching, which is able to synthesize past the lesion (for overview see (Friedberg *et al.*, 2005)). However, these specialised polymerases lack proof-reading activity and can therefore insert a wrong nucleotide into the newly synthesized DNA strand promoting occurrence of mutations (Fuchs and Fujii, 2013; Goodman and Woodgate, 2013).

Genomic stability in bacteria is also threatened by the fact that transcription shares the same DNA template with replication, due to which these two processes inevitably interfere with each other. Since RNA polymerase moves on DNA slower than the replicating DNA polymerase (DNAP) (Pham *et al.*, 2013; Großmann *et al.*, 2017) and RNAP also stalls or backtracks on DNA during elongation, e.g. because of a lesion or regulatory elements in DNA (Nudler, 2012; Howan *et al.*, 2014), transcription complexes pose an obstacle to the replication machinery. A collision between the transcription and replication machineries can lead to premature transcription termination, replication fork arrest or fork collapse that in turn may result in increased mutagenesis (Pomerantz and O'Donnell, 2010; Srivatsan *et al.*, 2010; Sankar *et al.*, 2016).

Depending on the movement direction, replication and transcription complexes can collide either in a co-directional or head-on manner (Figure 1). When transcription occurs on the leading strand of replication, then RNA and DNA polymerases move in the same direction and collide in a co-directional manner. However, when lagging strand is the template for RNA synthesis, the replication complex and RNA polymerase move towards each other and collide head-on. Both types of collisions affect replication, but it is generally thought that head-on collisions are more detrimental than co-directional collisions. For example, several studies have indicated that co-directional collisions have a moderate or no effect on replication, while head-on collisions severely impede replication fork progression and elevate mutation frequency of the lagging strand (Mirkin and Mirkin, 2005; Wang *et al.*, 2007a; Srivatsan *et al.*, 2010; Paul *et al.*, 2013). Consequently, it has been hypothesised that the deleterious effect of replication-transcription head-on collisions has shaped the composition of genomes. Indeed, in most bacteria the majority of genes, especially essential genes that are needed for the survival of cells, are coded on the leading strand of replication (Guy and Roten, 2004; Paul *et al.*, 2013; Zheng *et al.*, 2015). In *B. subtilis*, for example, 83% of core genes are located on the leading strand and out of 148 core genes that were determined to be essential only 6 are located on the lagging strand (Paul *et al.*, 2013). Gene orientation-dependent collisions of transcription and replication and the resulting mutagenesis have also been proposed to be a mechanism of how cells can target rapid evolution of specific genes and thereby accelerate evolution (for review see (Merrikh, 2017)).

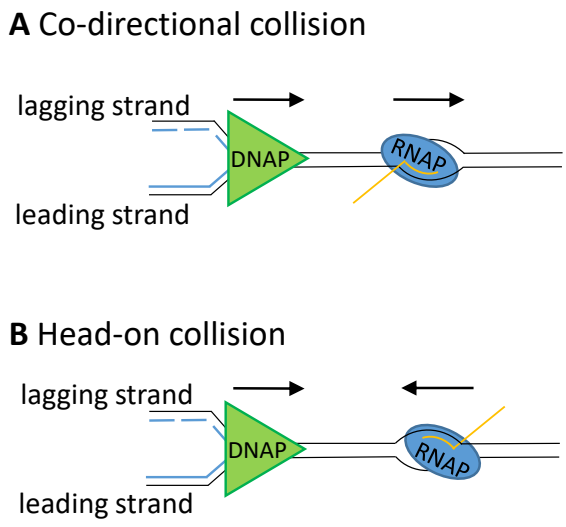

Figure 1. **Collisions between transcription and replication machineries.** (A) RNA and DNA polymerases collide co-directionally when transcription occurs on the leading strand of replication and both polymerases move in the same direction. (B) A head-on collision occurs when RNA polymerase transcribes the lagging strand of replication and both machineries move towards each other.

Notably, transcription-associated mutagenesis may be relevant for the bacteria to cope with stress and to adapt to novel or hostile environments, i.e. adaptive or stress-induced mutagenesis. In human pathogen *Pseudomonas aeruginosa* the *lasR* gene, coding for a transcriptional regulator activating expression of acute virulence factors, is often mutated during infection (Smith *et al.*, 2006). This benefits the long-term bacterial survival by diversifying metabolic profiles and developing antibiotic resistance (D'Argenio *et al.*, 2007). Wang et al. found out that mutagenesis of the *lasR* gene is associated with the transcription level of this gene – reduced transcription level resulted in lower mutation rate, while enhanced transcription level promoted the occurrence of mutations (Wang *et al.*, 2016). In nutrient-starved *Bacillus subtilis* cells transcription also promotes the occurrence of mutations, however, it does not seem to have a similar mutagenic effect in conditions of exponential growth (Pybus *et al.*, 2010). This indicates to the potential of transcription promoting stationary-phase mutagenesis and enabling the stressed bacteria to acquire genetic alterations to overcome growth-limiting conditions.

3. Transcription-coupled repair in bacteria

In addition to causing DNA instability transcription also contributes to DNA integrity. It was first reported in mammalian cells that DNA lesions are removed from actively transcribed genes more efficiently than from the genome overall

(Bohr *et al.*, 1985; Mellon *et al.*, 1987). This phenomenon, known as transcription-coupled repair (TCR) or transcription-coupled nucleotide excision repair (TC-NER), was later described in a variety of other organisms, including bacteria (Mellon and Hanawalt, 1989), yeasts (Sweder and Hanawalt, 1992) and archaea (Stantial *et al.*, 2016). In this sub-pathway of nucleotide excision repair (NER) the RNA polymerase, an important partner of DNA repair, acts as a damage sensor. When transcribing RNAP may encounter roadblocks, for example a damaged DNA base that cannot serve as a template for RNA synthesis, that cause the elongating complex to stall and subsequently conceal the lesion from repair proteins (Selby and Sancar, 1990; Zhou and Doetsch, 1993). Transcription-coupled repair is activated when the stalled RNAP is recognised by one of the two known TCR factors in bacteria, Mfd translocase or DNA helicase UvrD, which remove the stopped RNAP from the lesion and thereafter direct NER proteins to the damaged area (Park *et al.*, 2002; Monnet *et al.*, 2013; Epshtein *et al.*, 2014; Hawkins *et al.*, 2019). Lesion-containing DNA fragment is then removed by the NER proteins and resynthesized.

3.1 Overview of the prokaryotic nucleotide excision repair

Nucleotide excision repair is one of the major cellular DNA repair mechanisms. It is highly conserved and found in all domains of life. Depending on how the lesion is recognised, NER can be divided into two distinct pathways: (1) TC-NER that preferentially detects and repairs lesions in the transcribed DNA strand and (2) global genomic NER (GG-NER) that detects and removes damage in the whole genome. The global genomic NER pathway is the primary mechanism removing bulky, helix distorting DNA lesions such as UV-induced cyclobutane-pyrimidine dimers and 6–4 photoproducts. However, a feature that makes this repair mechanism unique among DNA repair pathways is its ability to repair a variety of structurally unrelated lesions (for overview see (Truglio *et al.*, 2006)). For example, NER has been reported to process DNA-protein cross-links (Minko *et al.*, 2002), synthetically modified single-strand nicks (Truglio *et al.*, 2006) and ribonucleotides falsely inserted into the DNA (Vaisman *et al.*, 2013).

In bacteria nucleotide excision repair has been most studied in *E. coli*. The basic mechanism of NER involves: (1) damage verification; (2) dual incisions on either side of the lesion; (3) removal of the resulting DNA strand; (4) DNA repair synthesis to fill the gap and (5) ligation of the newly synthesised strand (Figure 2). The exact timing and mechanistic details of NER damage verification step are still under debate. However, in the widely accepted model NER begins with lesion recognition by $UvrA_2$-UvrB heterotrimer or $UvrA_2$-$UvrB_2$ heterotetramer (Orren and Sancar, 1989; Verhoeven *et al.*, 2002; Malta *et al.*, 2007). A homodimer of UvrA in the $UvrA_2B_{(2)}$ complex scans the genome and it is the first NER component that detects the lesion presumably by damage-induced deformation of the DNA (Zou *et al.*, 2001; Jaciuk *et al.*, 2011; Stracy *et al.*, 2016). Although UvrA is able to bind to both damaged and undamaged DNA, its affinity to

damaged DNA is stronger (Mazur and Grossman, 1991; Barnett and Kad, 2019). After the lesion is localized, the DNA is transferred from UvrA to UvrB, which thereafter searches for the exact location of the damage and forms a UvrB-DNA pre-incision complex, resulting in dissociation of the UvrA proteins (Zou *et al.*, 2001; Malta *et al.*, 2007; Stracy *et al.*, 2016). Next, the pre-incision complex is bound by endonuclease UvrC that generates a cut on either side of the damaged area (Moolenaar *et al.*, 1995; Verhoeven *et al.*, 2000). The resulting 12–13 nt oligonucleotide containing the lesion is removed by the UvrD helicase and resynthesized by DNA polymerase I (Caron *et al.*, 1985; Adebali *et al.*, 2017a). These two proteins are also necessary to dissociate the post-incision UvrBC-DNA complex and turnover the Uvr proteins (Caron *et al.*, 1985; Husain *et al.*, 1985; Adebali *et al.*, 2017a). NER is finished when the newly synthesised DNA patch is sealed by DNA ligase.

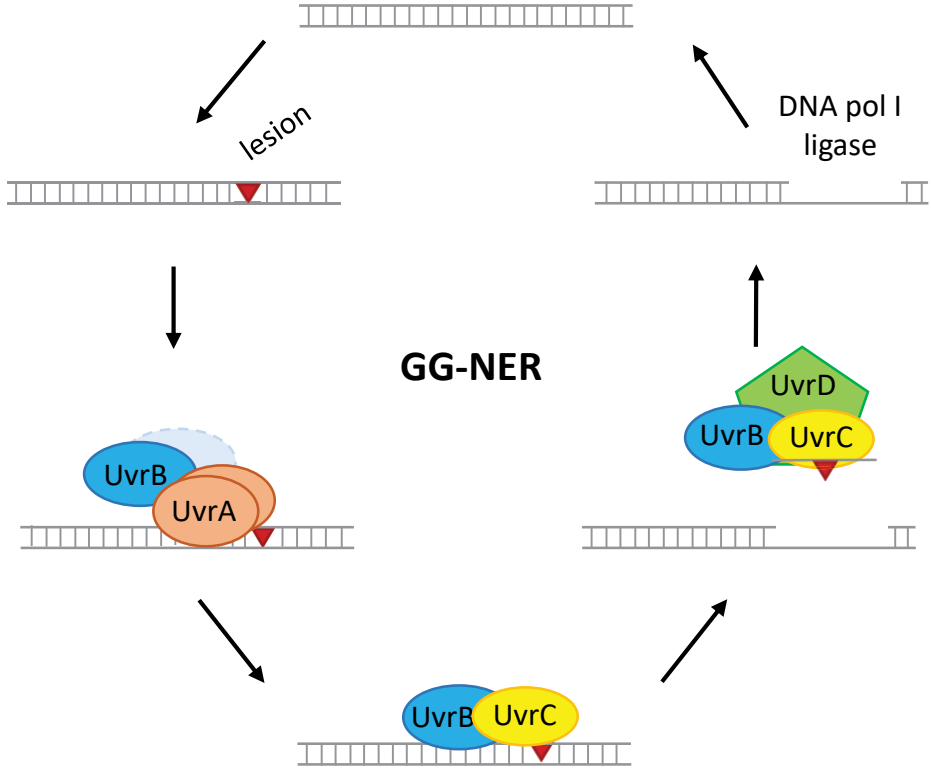

Figure 2. **Overview of the prokaryotic global genomic NER.** A complex of $UvrA_2B_{(2)}$ proteins scans the DNA and locates a lesion. UvrA loads UvrB onto the damaged site and dissociates. Thereafter, UvrC binds to the UvrB-DNA pre-incision complex and makes a cut in the 5′and 3′direcetion of the lesion. UvrD displaces the disjoint oligonucleotide and turns over the UvrBC proteins. The resulting gap is filled by DNA polymerase I and sealed by ligase.

3.2 Overview of the Mfd protein and Mfd-directed TCR

Mutation frequency decline (MFD) as a phenomena was discovered by Evelyn M. Witkin in the course of her early work studying UV mutagenesis in bacteria (Witkin, 1956; Witkin, 1966). MFD was defined as the rapid and irreversible decrease in the frequency of certain UV-induced mutations upon transient inhibition of protein synthesis immediately after UV irradiation, i.e. UV-induced damage was repaired before replication continued and was not fixed as a mutation during DNA synthesis. The gene responsible was named *mfd*. Loss of MFD did not affect the overall survival, however, Mfd^- strain was more sensitive to UV light and produced about 5 times more UV-induced mutants compared to the Mfd^+ parent strain (Witkin, 1966). Witkin also showed that MFD is a special case of nucleotide excision repair (Witkin, 1966; Witkin, 1994). Years later, the studies of transcription-coupled repair lead to the conclusion that preferential repair of a lesion on the transcribed strand is inhibited by stalled RNAPs (Selby and Sancar, 1990). It was proposed that a factor coupling transcription with repair overcomes this inhibitory effect and recruits NER enzymes to the damage site. Selby and colleagues showed that the mfd^- mutants were incapable of strand-specific repair and demonstrated that Mfd protein was the "missing" factor mediating TCR (Selby *et al.*, 1991; Selby and Sancar, 1993).

The *E. coli* Mfd protein is a big 130 kDa monomeric DNA translocase made up of 8 domains that are arranged in functional modules needed to perform TCR (Selby and Sancar, 1993; Deaconescu *et al.*, 2006). First, Mfd contains a RNAP interaction domain, which mediates its binding to RNA polymerase. This interaction activates the ATP-dependent translocase activity of Mfd needed to displace a stalled elongation complex (Smith *et al.*, 2007). Second, Mfd harbours an area structurally homologous to UvrB protein, the UvrB homology module, that recruits NER machinery to the damaged area by interacting with the UvrA protein (Selby and Sancar, 1993; Deaconescu *et al.*, 2006; Manelyte *et al.*, 2010). Third, Mfd contains a region of motives characteristic to superfamily 2 helicases. This region is responsible for the Mfd translocation activity along DNA. Depending on the elongation efficiency of the stalled RNA polymerase Mfd can use its translocation activity to function as either a transcription elongation factor or as a transcription terminator (Park *et al.*, 2002; Ho *et al.*, 2018; Le *et al.*, 2018). In the absence of a physical blockage, but in the presence of nucleotides, Mfd promotes forward translocation of arrested or backtracked RNAP and rescues the complex into productive elongation. However, if the complex is unable to continue elongation or transcribes slowly due to nucleotide deprivation, Mfd terminates transcription by releasing the RNA polymerase from DNA and thereafter initiates TCR (Figure 3A).

In addition to its role in TCR Mfd has been reported to participate in other cellular processes. For example, in *Bacillus subtilis* (Ayora *et al.*, 1996), *Helicobacter pylori* (Lee *et al.*, 2009) and *Bacillus cereus* (Darrigo *et al.*, 2016) Mfd is involved in DNA recombination. In *B. subtilis* Mfd also protects the cells against oxidation damage to proteins after treatment with diamide and reduces

expression of the transcription factor OhrR, which represses the cellular response to organic peroxide exposure (Martin *et al.*, 2019). Additionally, Mfd plays a vital role in *B. subtilis* spore morphogenesis by processing genetic damage during sporulation (Ramírez-Guadiana *et al.*, 2013; Valenzuela-García *et al.*, 2018). The *mfd* mutant of human pathogen *Clostridium difficile* displays increased toxin expression and unusual branched colony morphology, which indicates that Mfd regulates toxin expression and affects phenotypic features in this bacterium (Willing *et al.*, 2015). In another human pathogen, *Staphylococcus aureus*, Mfd positively influences biofilm formation through a yet unknown mechanism (Tu Quoc *et al.*, 2007).

3.2.1 Mfd-directed transcription-coupled repair

Mfd-directed TCR begins with Mfd recognising a stalled RNA polymerase (Figure 3A). How Mfd discriminates between an elongating and a stalled polymerase is still unclear, however, Le and colleagues have proposed a "catch-up mechanism" (Le *et al.*, 2018). Using novel real-time translocase tracking assays they discovered that Mfd can translocate on naked DNA on its own, but it is not as fast and processive as the elongating RNAP. When translocating Mfd reaches a stalled RNAP, it pushes the polymerase forward. If the polymerase continues to elongate, Mfd releases from the polymerase, continues independent translocation and eventually dissociates from the DNA. However, if the polymerase is unable to continue elongation, Mfd remains associated with the RNAP and terminates transcription by collapsing the transcription bubble (Howan *et al.*, 2012; Le *et al.*, 2018). Stalled RNAP and transcript are both displaced from the DNA, but the polymerase remains bound to Mfd (Selby and Sancar, 1993; Roberts and Park, 2004; Howan *et al.*, 2014; Fan *et al.*, 2016). Presumably, this DNA-Mfd-RNAP intermediate enables binding of NER proteins to the Mfd UvrB homology module (Deaconescu *et al.*, 2012; Howan *et al.*, 2014). UvrAB proteins bind to Mfd-RNAP complex with 20–200 times higher affinity than to damaged DNA (Fan *et al.*, 2016) and therefore in the Mfd-directed repair pathway the damage recognition function by UvrAB proteins may be different form the one they have in the GG-NER (Manelyte *et al.*, 2010). After DNA is handed over to the NER proteins, Mfd and RNA polymerase dissociate (Fan *et al.*, 2016), and the subsequent incision, unwinding of the lesion-containing oligonucleotide and resynthesis steps are the same as in global genomic repair.

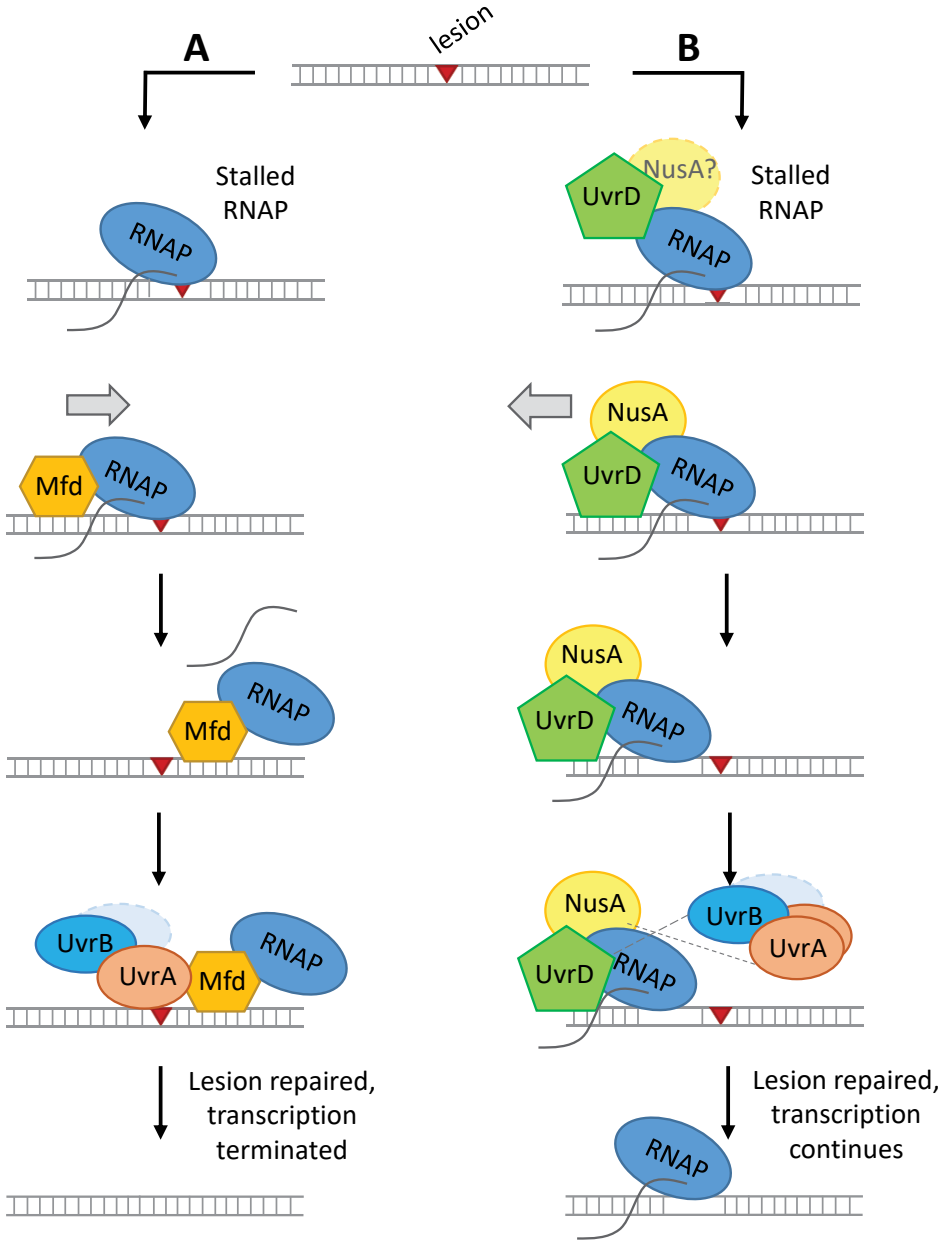

Figure 3. Mfd- and UvrD directed NER. A stalled RNA polymerase (RNAP) triggers Mfd- or UvrD-mediated TCR pathway. (A) Mfd recognises a stalled polymerase, attaches and pushes it forward. Transcription is terminated by collapsing the transcription bubble and detaching RNAP from DNA, nascent transcript is also lost. Thereafter, Mfd directs NER protein to repair the lesion. (B) UvrD (and NusA?) moves along with elongating RNAP. When RNAP stalls at a lesion, UvrD and NusA move the stalled RNAP backwards, revealing the lesion, and direct NER proteins to repair the damaged area. RNAP continues transcription after the lesion is repaired.

In addition to promoting repair of the lesion causing polymerase stalling, stopped RNAPs might also promote repair of lesions located close to the stalling site. Haines and colleagues reported that the Mfd-dependent pathway promotes repair of lesions downstream of the stalled RNAP (Haines *et al.*, 2014). Only lesions located in the transcribed strand are subject to faster repair. However, polymerase pause sites or lesions that do not stall RNAP but that are located on the template strand downstream of the stalled RNAP are also targeted for Mfd-directed accelerated repair. Therefore, it has been proposed that transcriptional pausing and pause sites might play an important role in the genome maintenance by promoting repair of specific genome regions (Haines *et al.*, 2014).

Although the Mfd-directed TCR has been extensively studied, there are still several aspects of the pathway that remain elusive. Normal growth (Witkin, 1966) and mild UV-sensitivity of the *mfd*⁻ strains (Witkin, 1966; Selby and Sancar, 1993) have raised questions about the physiological role of the Mfd-directed repair. Additionally, some studies have found that TCR still occurs in Mfd deficient cells (George and Witkin, 1974; Kunala and Brash, 1995) and termination of stalled polymerases functions relatively slow to account for the rapid repair *in vitro* (Howan *et al.*, 2012; Howan *et al.*, 2014), suggesting that other factors besides Mfd also mediate TCR. For example, it was recently suggested that transcription terminator Rho can compete with or augment the Mfd function (Jain *et al.*, 2019). *In vitro* Rho was seen to remove RNA polymerases stopped at a DNA lesion and thereof this enzyme might facilitate DNA repair by making the lesion available for repair proteins (Jain *et al.*, 2019). Another transcription regulator, NusA, has also been implicated to participate in a Mfd-independent but NER-dependent TCR (Cohen *et al.*, 2010). The mechanism of NusA-dependent repair has not yet been established, however, recent findings suggest that it promotes DNA repair as a part of the UvrD-directed TCR pathway (Epshtein *et al.*, 2014).

3.3 DNA helicase UvrD and TCR

UvrD is a superfamily 1 helicase/translocase, which has a role in several aspects of DNA metabolism, for example DNA replication (Carter *et al.*, 2012; Hawkins *et al.*, 2019), recombination (Lestini and Michel, 2007; Carter *et al.*, 2012) and protein displacement (Veaute *et al.*, 2005; Bidnenko *et al.*, 2006). It also contributes to genome maintenance by participating in the mismatch repair (MMR) (Hall *et al.*, 1998) and nucleotide excision repair pathways (Caron *et al.*, 1985). In MMR UvrD removes a 1000–2000 nucleotides long DNA region containing an incorrectly incorporated base (for overview see (Matson and Robertson, 2006)), whereas in NER the function of UvrD is to remove a short 12–13 nt long damage-containing oligonucleotide and to release Uvr proteins from the UvrBC-DNA complex (Caron *et al.*, 1985; Adebali *et al.*, 2017a). *uvrD* is also upregulated during SOS-response as a consequence of DNA damage (Arthur and Eastlake, 1983; Siegel, 1983).

Whether UvrD functions as a helicase or translocase depends on its assembly state and interactions with other proteins. *In vitro E. coli* UvrD monomers have translocase activity and are able to move along ssDNA with a 3′to 5′directionality, but are either unable to unwind DNA or have a low unwinding processivity (Maluf *et al.*, 2003; Fischer *et al.*, 2004; Comstock *et al.*, 2015). Active form of UvrD helicase is a dimer (Maluf *et al.*, 2003; Fischer *et al.*, 2004). In MMR and NER the UvrD-catalyzed unwinding is further stimulated by the MMR protein MutL or NER proteins UvrA and UvrB (Matson and Robertson, 2006; Atkinson *et al.*, 2009). Interestingly, UvrB protein is bound by the N-terminal and by the unstructured C-terminal domains of UvrD; however, the interaction between UvrB and the UvrD C-terminal domain is dispensable for NER (Manelyte *et al.*, 2009), suggesting to another function of this interaction. Recently it was proposed, that in addition to participating in global genomic NER, UvrD also contributes to transcription-coupled repair (Epshtein *et al.*, 2014; Epshtein, 2015).

3.3.1 UvrD-directed TCR

In 2014 an alternative Mfd-independent TCR pathway was proposed by Epshtein and colleagues (Epshtein *et al.*, 2014; Epshtein, 2015). While searching for proteins that interact with *E. coli* RNAP they discovered something unexpected – UvrD appeared in RNAP cross-linked complexes at an abundancy level comparable to common transcription factors (Epshtein *et al.*, 2014). Furthermore, it was discovered that UvrD can pull backtracked or paused RNAP backward and enable NER proteins to gain access to the underlying lesion. UvrD-dependent RNAP backtracking was found to be supported by the transcription elongation/ termination factor NusA, a protein that has also been reported to participate in Mfd-independent TCR pathway and interact with the NER protein UvrA (Cohen *et al.*, 2010). Consequently, a UvrD/NusA assisted TCR pathway was proposed in which a monomer of UvrD likely moves with RNAP throughout elongation (Epshtein *et al.*, 2014; Epshtein, 2015) (Figure 3B). When elongating RNAP encounters a lesion that causes the complex to stop, UvrD forms a dimer gaining helicase/translocase activity (Maluf *et al.*, 2003; Fischer *et al.*, 2004). UvrD then unwinds the upstream fork of the elongation bubble and, with the help of NusA, forces the RNAP to move backwards on DNA. Thereafter, UvrD and NusA interact with UvrAB proteins and recruit the NER system to the exposed lesion (Manelyte *et al.*, 2009; Cohen *et al.*, 2010). After the damage is repaired, RNA polymerase can promptly resume transcription without losing the nascent transcript. It was later suggested that alarmone ppGpp, a central regulator of bacterial stress response under starvation conditions, is also an important component of this TCR pathway (Kamarthapu *et al.*, 2016). Kamarthapu and colleagues reported a loss of transcription-coupled repair in $ppGpp^0$ cells which means that transcribed DNA strand is repaired as fast as the non-transcribed strand (Kamarthapu *et al.*, 2016). It was also determined that ppGpp is required for the UvrD-directed

TCR pathway, where it probably assists UvrD in the backtracking process by binding to RNAP and rendering it prone to moving backwards.

The proposed model states that UvrD-assisted pathway is the primary TCR pathway when there is massive DNA damage in the cells (Epshtein *et al.*, 2014; Epshtein, 2015). In non-stress conditions UvrD concentration is low and monomeric UvrD is bound to elongating RNAPs. However, in stress conditions SOS-response is activated and the expression of UvrD is up-regulated (Arthur and Eastlake, 1983; Siegel, 1983). Consequently, UvrD can form dimers and is able to backtrack stopped RNA polymerases. Mfd-directed TCR pathway was suggested to mainly work in non-stress conditions, where it removes damage occurred during normal cell growth, and help clean out irreversibly backtracked RNAPs (Epshtein *et al.*, 2014; Epshtein, 2015).

Although the UvrD-dependent TCR model is promising, there are still several aspects that need clarification. For example, the ability of UvrD and NusA to direct Uvr proteins to a lesion during TCR is hypothetical and based on previous reports of their interactions with Uvr proteins (Atkinson *et al.*, 2009; Manelyte *et al.*, 2009; Cohen *et al.*, 2010). Furthermore, recent excision repair-sequencing (XR-seq) studies by Adebali and colleagues in *E. coli* suggested that UvrD plays no role in TCR and the genome-wide TCR is mediated by the Mfd translocase (Adebali *et al.*, 2017b; Adebali *et al.*, 2017a). Deletion of *mfd* drastically reduced the ratio of transcribed/non-transcribed strand repair while *uvrD* deficiency had no effect or this rather slightly elevated the TCR ratio. Similarly, normal TCR was carried out in the ppGpp mutant (Adebali *et al.*, 2017b). The obtained results also confirmed that the role of UvrD in excision repair is not to couple repair with transcription, but to unwind the lesion-containing oligonucleotide in NER and turnover Uvr proteins (Adebali *et al.*, 2017a). Further studies are needed to elucidate the physiological role and specific mechanism of the UvrD-directed TCR pathway.

4. Controversial role of TCR in mutagenesis

Although transcription coupled repair is important to maintain genome integrity by removing DNA lesions and lowering the possibility of transcription-replication collisions, it has also been reported to have an opposite effect and stimulate genome instability. More specifically, several studies have shown that under some conditions the TCR factor Mfd promotes formation of mutations. This unexpected role of Mfd has been widely reported in *B. subtilis*, where disruption of *mfd* leads to decreased mutagenesis of the transcribed lagging-strand genes and reduces the generation of mutations in stress or non-growing conditions i.e. stationary-phase mutagenesis (Ross *et al.*, 2006; Pybus *et al.*, 2010; Martin *et al.*, 2012; Million-Weaver *et al.*, 2015; Gómez-Marroquín *et al.*, 2016; Villegas-Negrete *et al.*, 2017). Controversially, in normally growing *B. subtilis* cells Mfd has been seen either to elevate (Ross *et al.*, 2006) or to have no effect on the frequency of occurrence of spontaneous rifampicin resistant mutants (Pybus

et al., 2010; Martin *et al.*, 2012). In outgrowing spores of *B. subtilis* Mfd, however, plays an antimutagenic role and greatly decreases the level of mutation frequency to rifampicin resistance (Valenzuela-García *et al.*, 2018). In *Bacillus*-related non-pathogenic thermophile *Geobacillus kaustophilus* Mfd has also an antimutagenic role suppressing the emergence of both exponential- and stationary-phase spontaneous mutagenesis (Suzuki *et al.*, 2018). Interestingly, the function of Mfd might be depressed in this bacterium to induce genetic changes enabling the development of resistance to rifampicin and streptomycin (Suzuki *et al.*, 2018).

Mfd-dependent mutagenesis can be beneficial to human pathogenic bacteria in the context of host infection. In several human pathogens, including *Pseudomonas aeruginosa*, Mfd has been identified as an evolvability factor promoting development of antibiotic resistance by inducing mutations (Han *et al.*, 2008; Ragheb *et al.*, 2019). In the study performed by Ragheb and colleagues using divergent bacterial species a 2–5 fold decline in the amount of rifampicin resistant mutants, depending on the species, was seen in Mfd deficient strains compared to the wild type strains (Ragheb *et al.*, 2019). Additionally, when a copy of *Mycobacterium tuberculosis mfd* gene was introduced into *Salmonella typhimurium* strains lacking *mfd*, the reduced rifampicin mutation rate of *S. typhimurium mfd* mutants was increased to the mutation rate of the wildtype, suggesting that the mechanism facilitating the evolvability function of Mfd might be conserved across bacterial species. On the other hand, in pathogenic bacteria *Bacillus cereus* and *Shigella flexneri* Mfd gives advantage to the bacteria over the hosts immune system by removing DNA damage induced by reactive nitrogen species and decreasing the frequency of point mutations (Guillemet *et al.*, 2016).

The exact mechanism underlying the Mfd pro-mutagenic function is still unclear. Reports in *B. subtilis* suggest that Mfd may promote mutagenesis by coordinating error-prone repair mediated by additional factors. One of these factors is base excision glycosylase MutY, which has been reported to conduct faulty DNA repair in cooperation with Mfd under conditions of nutritional and oxidative stress (Gómez-Marroquín *et al.*, 2016; Martin *et al.*, 2019). Error-prone DNA polymerase PolY1 and DNA polymerase Pol I have also been implicated to generate mutations and participate in the same mutagenic pathway as Mfd in *B. subtilis* (Million-Weaver *et al.*, 2015; Gómez-Marroquín *et al.*, 2016). Gómez-Marroquín and colleagues showed that the NER enzymes in the TCR pathway can also promote genetic change – UvrA protein was found to promote accumulation of Leu^+ revertants in stationary-phase bacteria and this mutagenic pathway required the participation of Mfd (Gómez-Marroquín *et al.*, 2016). In addition, another study demonstrated that deletion of *uvrA*, *uvrB* or *uvrC* diminishes the transcription-dependent asymmetry in mutation rates of the leading and lagging strand genes also seen in the *mfd* deficient strains (Million-Weaver *et al.*, 2015). Further insight into Mfd-promoted mutagenesis will allow for a more comprehensive understanding of the mechanism underlying this mutagenic pathway and its role in evolution.

II THE AIM OF THE THESIS

Mutations are permanent changes in the genome, which drive the evolutionary processes. These genetic changes can occur as a result of broad variety of exo- and endogenous processes and, depending on their context or location, have a widely different impact on the organism. For example, mutations can have a harmful effect on the fitness of the organism. However, under some conditions they can also be beneficial. For instance, elevated mutation rates can help the bacteria to overcome adverse circumstances and adapt to ever-changing environmental conditions (Kivisaar, 2003; Foster, 2007), which include e.g., attack by the host immune system and treatment with antibiotics (D'Argenio *et al.*, 2007; Han *et al.*, 2008; Suzuki *et al.*, 2018). Therefore, it is highly important to understand the mechanisms underlying mutagenic processes.

Transcription has also been proposed to be one of the factors generating genetic diversity (Datta and Jinks-Robertson, 1995; Klapacz and Bhagwat, 2002; Pybus *et al.*, 2010; Park *et al.*, 2012), but its exact mutagenic mechanism and role in inducing mutations is still under debate. Additionally, recent findings have raised a question about the role of transcription coupled repair (TCR) in mutagenesis – while TCR helps to maintain genome integrity (Selby *et al.*, 1991; Epshtein *et al.*, 2014; Haines *et al.*, 2014; Stantial *et al.*, 2016), accumulating body of evidence suggest that it also functions as an evolvability mechanism (Han *et al.*, 2008; Martin *et al.*, 2012; Million-Weaver *et al.*, 2015; Ragheb *et al.*, 2019).

To shed light to the role of transcription in the mutagenic processes the first aim of this thesis was to find out whether transcription causes mutations in soil bacterium *Pseudomonas putida* – a representative of the *Pseudomonas* genus, which members are metabolically very diverse and able to rapidly adapt to changing environmental conditions. The second aim of this thesis was to elucidate the role of TCR in mutagenesis by assessing the influence of TCR factors Mfd and UvrD on mutation frequency in *P. putida*.

III RESULTS AND DISCUSSION

1. The effect of transcription level on the mutation frequency in growing culture of *P. putida* (Ref I)

The first step in studying the influence of transcription on mutagenesis in *P. putida* was to assess whether elevated transcription level affects mutation frequency. For this we created a Ptac-pheA+C test system which enabled us to artificially increase the level of transcription of the mutational target gene and detect mutations in the chromosome of *P. putida* wild type strain PaW85. The test system was based on the activation of phenol monooxygenase gene *pheA*, which enables the bacteria to use phenol as a sole source of carbon (Nurk *et al.*, 1991). In the test system the *pheA* gene with an additional C nucleotide (+1 frameshift) in a six C-nucleotides repeat at position 221 relative to the translation initiation codon of the gene was transcribed from the constitutively expressed P_{gc} promoter and was also put under the control of LacI repressor and IPTG-inducible P_{tac} promoter. In the absence of IPTG the *pheA* allele was transcribed from the P_{gc} promoter, while in the presence of IPTG the *pheA* allele was transcribed also from the P_{tac} promoter and the *pheA* transcription level was increased. We inserted the test system into the chromosome of *P. putida* with mini-transposon Tn5 and selected two strains with co-oriented transcription and replication of the *pheA* allele (strains Ptac-pheA+C_2 and 13) and two with head-on orientation (strains Ptac-pheA+C_3 and 4) for further experiments. Occurrence of 1-bp deletion mutation in the phenol growing revertants was confirmed by sequencing of the *pheA* gene.

To examine the effect of IPTG on the level of *pheA* transcription, we conducted Western blot analysis with the phenol growing revertant (Phe^+) cells of the strain Ptac-pheA+C_2 cultivated in the presence or absence of IPTG. Addition of IPTG elevated the expression of the *pheA* gene in the exponentially growing bacteria, however, IPTG did not have any effect on the *pheA* expression level in the stationary-phase cells (Ref I, Fig. S2), demonstrating that the constructed test system enabled to study the influence of transcription level on mutagenesis, but only in exponential-phase cells.

Thereafter, we conducted the mutagenesis assay by cultivating tester cells in the presence or absence of IPTG and plating them onto phenol-containing minimal medium. Comparison of the number of accumulated Phe^+ mutants of the tester cells grown with and without IPTG revealed that despite the nature of transcription and replication collision, all strains with elevated transcription of the *pheA* allele formed more mutants in comparison with conditions when transcription level of the *pheA* allele was not artificially increased (Ref I, Fig. 2). From these results we can conclude that transcription induces mutagenesis in growing cells of *P. putida*, and changes in the level of transcription of the mutational target gene may affect mutagenic processes – elevated transcription of the mutational target gene results in elevated mutation frequency.

Although the first reports of transcription-induced mutations were already published about half a century ago (Herman and Dworkin, 1971; Savić and Kanazir, 1972), the potential of transcription to implicate evolutionary processes has been acknowledged only recently. This is understandable, as contradictory results about the participation of transcription in mutagenesis have been published. For example, in bacteria several studies have found no evidence that more mutations occur in highly transcribed genes or that the occurrence of mutations is affected by the nature of transcription-replication collisions (Lee *et al.*, 2012; Foster *et al.*, 2015). Several other studies, however, have reported the opposite (e.g. (Pybus *et al.*, 2010; Srivatsan *et al.*, 2010; Paul *et al.*, 2013)). Our results support the opinion that in bacteria transcription is a mechanism contributing to genetic variety which constitutes the basis of evolution.

2. Deficiency of NER proteins UvrA, UvrB or UvrC causes instability and genetic adaptation (Ref II and III)

To study and distinguish the role of transcription-coupled NER from the role of the global genomic NER in mutational processes in *P. putida*, we created NER-deficient strains by deleting *uvrA*, *uvrB* or *uvrC* gene from *P. putida* wild type strain PaW85. Strains lacking UvrA, UvrB or UvrC enzyme experienced growth problems and cells formed filaments when grown in liquid medium (Ref II, Fig. S1). Additionally, the number of viable cells in the overnight cultures of UvrA- or UvrB-deficient strains and of UvrC-deficient strain was approximately 2-fold and 15-fold lower, respectively, if compared to the wild type *P. putida*. To our surprise, a rapid differentiation of the cell populations occurred in all NER-defective strains – while the initial NER-defective bacteria formed translucent colonies on LB plates, the emerging variants formed colonies similar to the wild type. Subsequent replating of the emerged variants confirmed that their phenotype remained stable, indicating to a genetic adaptation (Figure 4). Additionally, these cells had overcome growth defects and did not form filaments in liquid medium (Ref II, Fig. S1). Replating of the initial translucent colonies, however, resulted in colonies displaying translucent or adapted colony morphology, implying that adaptation was still continual.

When one DNA repair pathway is non-functional, other repair pathways might be up-regulated and replace the defective pathway. For example, falsely inserted ribonucleotides can be removed from DNA by ribonucleotide excision repair, by NER and by MMR pathways (Vaisman *et al.*, 2013; Vaisman and Woodgate, 2015). In our studies the frequency of homologous recombination (HR) in *P. putida* was enhanced in all the NER-deficient strains, however, HR frequency in the initial UvrA- and UvrB-deficient strains was significantly higher compared to the adapted strains (Ref. II, Fig. 4). Consequently, we questioned whether the adaptation phenotype could be a result of increased efficiency of DNA damage repair. We therefore tested the survival of NER-deficient strains under conditions

when cells were exposed to various exogenous DNA damaging agents (Ref II, Fig 5 and Ref III, Fig. 4 and Table 1). All tested NER-defective strains (regardless of whether they were adapted or not) were similarly more sensitive to UV-light, 4-nitroquinoline 1-oxide (NQO) and Mitomycin C (MMC) when compared to the wild type *P. putida*. This was expected since these agents cause bulky DNA lesions and DNA inter- or intrastrand cross-links repaired by the NER pathway (Ikenaga *et al.*, 1975; Kohn *et al.*, 1992; Weng *et al.*, 2010). This also suggests that adaptation of the NER-deficient strains is not a result of an alternative way of coping with DNA lesions. However, there was a small difference in the tolerance of DNA damage induced by an alkylating agent 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) (Ref III, Table 1). In fact, there were no statistically significant differences in the survival of adapted and non-adapted NER-deficient Δ*uvrA* cells in the presence of MNNG. However, while the adapted strain tolerated the MNNG treatment similarly to the wild type, the non-adapted cells were more sensitive to the MNNG exposure than the wild type cells. Therefore, we cannot completely exclude the possibility that adaptation of the NER-deficient strains could be a result of mutation(s) leading to improved DNA repair efficiency.

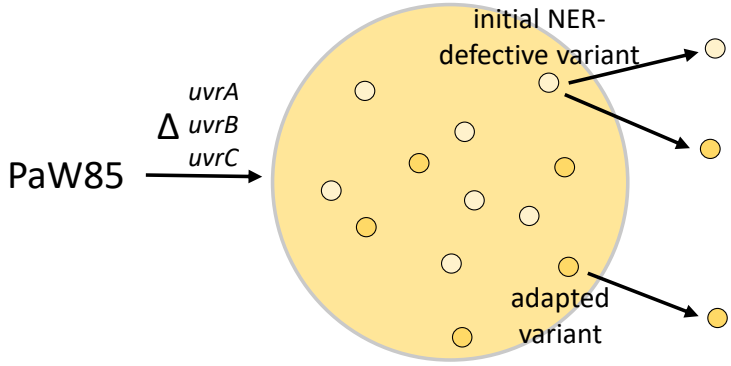

Figure 4. Adaptation of the NER-deficient populations. Initial NER-deficient bacteria formed translucent colonies on LB plates (beige circles), however, wild-type-like adapted variants emerged (yellow circles). Replating of initial variant resulted in initial and adapted colonies, replating of the adapted variant resulted in only adapted colonies.

We also studied the influence of adaptation of NER-deficient bacteria on mutagenic processes. The employed test systems and experimental principles will be further discussed in the upcoming mutagenesis chapters. Our results indicated that the loss of NER functions and the adaptation of NER-deficient bacteria do not affect the frequency of occurrence of frameshift mutations in the exponential-phase cells and base substitution mutations in the starving cells of *P. putida*. In fact, the mutation frequencies of the wild type and both Δ*uvrA* strains (the original and the adapted strain) were all comparable (Ref III, Fig. 1 and 2A, Tables S3 and S5). However, the frequency of base substitution mutants in exponential-phase cultures of the original Δ*uvrA* strain was elevated about 6 times compared

to the wild type and the adapted Δ*uvrA* strain (Ref III, Fig. 2B and Table S6). These results indicate that the mechanism underlying adaptation reduces the occurrence of base substitution mutations to the level seen in the wild type *P. putida* strain.

To our knowledge similar adaptation phenomenon of NER-defective strains has not been reported previously and the mechanism underlying the adaptation is not known. In *P. putida* rapid adaptation has been previously reported also in the case of DNA polymerase I deficient strains (Sidorenko *et al.*, 2011). Initial Pol I-deficient strains had severe growth problems in LB liquid medium and cells formed filaments. Additionally, these strains grew very poorly on LB solid medium. However, after 2–3 rounds of passages adapted mutants with improved growth appeared and growth on LB solid medium was restored. Genetic basis of adaptation of Pol I-deficient strains is also yet unknown. Notably, Pol I fills in the gap made during NER and this enzyme participates in the processing of Okazaki fragments by removing RNA primers and resynthesizing the missing DNA (Okazaki *et al.*, 1971; Caron *et al.*, 1985). NER is also able to excise ribonucleotides from the DNA template and UvrA, UvrB and UvrD proteins have been proposed to participate in alternative replication pathways that can substitute for the functions of Pol I enzyme (Caron *et al.*, 1985; Moolenaar *et al.*, 2000). This raises a possibility that the functions of NER and Pol I are interconnected and NER enzymes could also function beyond DNA repair. This in turn allows to speculate that adaptation of NER-deficient strains is not to overcome the loss of repair function but the loss of another NER function, for instance, Okazaki fragments processing. Further studies are needed to elucidate the mechanism and role of adaptation and its influence on mutagenic processes.

3. Influence of Mfd and UvrD on mutation frequency in *P. putida* (Ref III)

Although one could assume that the frequency of mutations is reduced as a result of DNA repair processes, contradictory reports have been published about transcription-coupled repair, in particular the Mfd-directed repair pathway, suggesting that this pathway may instead increase the occurrence of mutations (see Review of literature, chapter 4). As we confirmed that transcription promotes mutagenesis in *P. putida*, the next question we asked was whether DNA repair accompanying transcription could also play a role in transcription-associated mutagenesis in this bacterium.

3.1 Role of UvrD and Mfd in stationary-phase mutagenesis

The role of Mfd and TCR in stationary-phase mutagenesis is unclear. It is well documented that Mfd facilitates the occurrence of mutations in stressed *B. subtilis* cells (Ross *et al.*, 2006; Pybus *et al.*, 2010; Martin *et al.*, 2012; Gómez-Marroquín *et al.*, 2016; Villegas-Negrete *et al.*, 2017; Martin *et al.*, 2019). However, in a *Bacillus*-related thermophilic bacterium *G. kaustophilus* Mfd has an antimutagenic role and Mfd instead reduces the occurrence of stress-induced mutations (Suzuki *et al.*, 2018). In *E. coli* SMR4562 strain Mfd has been shown to induce accumulation of stationary-phase mutations and potentially promote R-loop instigated genomic changes (Wimberly *et al.*, 2013). However, in *E. coli* WU3610 strains starvation-associated mutagenesis was reported to be independent of Mfd (Bridges, 1995). To elucidate whether Mfd and TCR do have a more general role in promoting mutagenesis in bacteria, we first decided to study the influence of Mfd and UvrD on stationary-phase mutagenesis in *P. putida*. For this we created *mfd* and *uvrD* single- and double-deficient strains. It is important to keep in mind that UvrD is a very versatile protein participating in other cellular processes in addition to TCR (see Review of literature, chapter 3.3). Therefore, it is impossible to differentiate *in vivo* the effect of UvrD-mediated TCR from the effect of other UvrD-mediated pathways in *uvrD* deletion strains, and the overall effect of UvrD in mutagenesis was examined.

To study how the loss of Mfd or UvrD influences the occurrence of stationary-phase mutagenesis, we employed a previously developed plasmidial pKTpheA22TAG test system (Tegova *et al.*, 2004). Similarly to the test system used to study the influence of transcription level on mutagenesis in *P. putida* chromosome (Results and discussion, chapter 1), the pKTpheA22TAG system was based on the activation of the *pheA* gene. This test system enabled us to monitor accumulation of phenol-degrading mutants on phenol minimal plates emerged due to base substitutions which eliminated the premature TAG stop codon introduced into the *pheA* tester gene instead of the original 22nd codon (Leu).

From the attained results we can conclude that the UvrD helicase participates in suppression of stationary-phase mutations in *P. putida* – the *uvrD* deletion strain accumulated about 2-fold more Phe^+ mutants than did the wild type (Ref III, Fig. 1 and Table S3 and S4). This is in accordance with the results of several other studies demonstrating that *uvrD* is an antimutator gene and its inactivation results in elevated mutation frequency in various bacteria (for overview see (Horst *et al.*, 1999)), most likely caused by the lack of UvrD function in NER and MMR pathways. In *P. putida*, the Phe^+ mutant frequency of neither the original nor the adapted NER-deficient Δ*uvrA* strain was different from that of the wild type mutant frequency, suggesting that the elevated mutagenesis of the Δ*uvrD* strain was probably not caused by the loss of NER but by the loss of MMR function. Interestingly, the MutS-defective strain, where MMR function is absent, formed significantly more (~ 27-fold) Phe^+ mutants than the Δ*uvrD* strain, suggesting that MMR is only slightly abolished in the *P. putida* UvrD-deficient strain. UvrD is considered to play a central role in MMR, however, our results

suggest that in stationary-phase *P. putida* cells another enzyme (e.g. helicase) may also take part in the MMR pathway.

Similarly to the results attained with *E. coli* SMR4562 strain (Wimberly *et al.*, 2013) the deletion of *mfd* from *P. putida* resulted in ~2-fold lower mutant frequency in comparison with the wild type (Ref III, Fig. 1 and Table S3 and S4), indicating that in *P. putida* Mfd participates in the occurrence of stationary-phase mutagenesis. The potential of Mfd to induce mutations in stationary-phase *P. putida* cells was further supported by the results demonstrating that the deletion of *mfd* in the Δ*uvrD* strain suppressed the elevated mutant frequency observed in the Δ*uvrD* single mutant.

3.2 Role of Mfd and UvrD in mutagenesis in growing bacteria

Since plasmid and chromosome can differ in several features, for example in size, replication mechanism and copy number, they could also differ in mutagenesis mechanism. To investigate whether in *P. putida* mutagenic processes occurring in the plasmid are similar to the ones taking place in the chromosome, we constructed two novel chromosomal test systems. The first test system, the Tn5TAG system, was derived from the plasmidial pKTpheA22TAG test system (Tegova *et al.*, 2004) and therefore enabled to monitor base substitutions. In the second test system, the Tn5pheA-C system, a –1 frame shift was introduced into the *pheA* gene enabling to monitor 1-bp insertions restoring the reading frame of the phenol monooxygenase gene. Both test systems were inserted into the chromosome of *P. putida* in the composition of Tn5 mini-transposon. Notably, we confirmed that the spontaneous Rif^r mutant frequency (Jatsenko *et al.*, 2010) of the tester strains was similar to the Rif^R frequency measured in *P. putida* wild type, indicating that the mini-Tn5 insertions do not affect general mutation frequency in the tester strains.

Monitoring of the Phe^+ mutant frequency confirmed that both new test systems enable to detect mutations in the chromosome. Phe^+ revertants of the tested strains harbouring the Tn5pheA-C test system appeared onto phenol minimal plates on days 2–5 after plating (Ref III, Table S5) and revertants harbouring the Tn5TAG test system appeared onto the selective plates on days 3–7 after plating (Ref III, Table S6). In order to distinguish mutations occurred in stationary-phase cells from the ones occurred in actively growing cells, we carried out reconstruction experiments and observed how long it would take for a Phe^+ cell to form a colony. About 100–200 cells of an individual Phe^+ mutant were mixed with scavenger cells (wild type cells not able to grow on phenol) to mimic the cell density of the mutagenesis assays, and plated onto phenol containing plates. Phe^+ mutants harbouring the Tn5pheA-C or Tn5TAG test systems that had emerged onto phenol plates on days 2–5 and 3–7 in the mutagenesis assays, respectively, formed colonies onto phenol plates in the reconstruction experiment also on the same days. Therefore, although we initially constructed the new chromosomal test systems to monitor mutations occurred in stationary-phase cells, the reconstruction

experiment indicated that both test systems enable to monitor mutations mainly emerged during exponential-phase.

It has been demonstrated that *P. putida* is a polyploid species, containing on average 20 origins and 14 termini in exponential-phase (Pecoraro *et al.*, 2011). However, DNA content in *P. putida* cells is dependent on the growth phase of bacteria – the amount of chromosomal DNA decreases during transition to stationary-phase (Lewis *et al.*, 2002). It was also demonstrated that cells grown in L-broth contained more DNA than cells grown in M9 medium (Lewis *et al.*, 2002). As we conducted a stationary-phase mutagenesis assay and used minimal medium, which is similar to the M9 medium used in the mentioned study, we hypothesized that the newly constructed test systems did not enable us to monitor mutations occurred in stationary-phase cells because of a decrease in the content of genomic DNA. Indeed, while we did not see any growth phase-dependent change in the copy number of the plasmidial pKTpheA22TAG test system, we did confirm that the amount of chromosomal DNA was significantly decreased in the carbon starved cells compared to the exponential-phase cells (Ref. III, Fig. 3). Additionally, we have previously observed that accumulation of stationary-phase mutants is negatively affected by the amount of plated bacteria and the presence of earlier appeared Phe^+ mutant colonies (Saumaa *et al.*, 2002). Therefore, in addition to the changes in the chromosome copy numbers, the constructed test systems could not enable to study stationary-phase mutagenesis because of an inhibitory effect of the early arising Phe^+ mutant colonies on the growth of the late-appearing Phe revertants.

When measuring mutant frequency with the chromosomal test systems, we observed that the deletion of *uvrD* resulted in higher amount of insertion and base substitution mutants compared to the wild type strain (Ref. III, Fig. 2 and Table S5 and S6). These results support the antimutagenic role of UvrD already observed in the stationary-phase mutagenesis assay (Ref. III, Fig. 1) and highlight the importance of UvrD in maintaining the genome integrity in both stationary- and exponential-phase *P. putida* cells.

In the case of TCR factor Mfd, our results revealed that the presence or absence of this enzyme has no influence on the frequency of Phe^+ mutants in the growing cultures of *P. putida* (Ref. III, Fig. 2 and Table S5 and S6). The finding that Mfd does not influence exponential-phase mutagenesis is further supported by the results demonstrating that the spontaneous Rif^R frequency is also the same in the Mfd-deficient and -proficient cells (Ref. 3, Fig.5A and Table S8). These results are in accordance with studies conducted with *B. subtilis* (Pybus *et al.*, 2010; Martin *et al.*, 2012; Martin *et al.*, 2019) and *N. gonorrhoeae* (LeCuyer *et al.*, 2010). However, we noticed that the appearance of base substitution mutants was delayed for one day in the Δ*mfd* strains of *P. putida* in comparison with the Mfd-proficient strains, with an only exception of the original Δ*uvrA* strain (Ref III, Table S6). This could mean that under some circumstances Mfd and Mfd-directed TC-NER could improve the cells growth and thereby promote their adaptation to novel and changing environmental conditions. Additionally, as the above discussed results indicate, test systems can have limitations. Therefore, we cannot completely

exclude the possibility that in some other conditions Mfd could have an influence on mutagenesis also in growing cells of *P. putida*.

3.3 Involvement of Mfd and UvrD in UV-induced mutagenesis

As several studies conducted in *E. coli* have shown that Mfd-directed TCR removes UV-induced DNA damage and thereby reduces UV mutability (Witkin, 1966; George and Witkin, 1975; Oller *et al.*, 1992), we wanted to find out whether Mfd and UvrD are also involved in reducing mutations in UV-irradiated cells of *P. putida*. For this we treated the tester strains with UV-C light and determined their Rif^R mutant frequency.

Since UvrD has an important role in the NER and MMR pathways and our previous mutagenesis experiments confirmed that UvrD is needed to reduce occurrence of mutations in *P. putida*, we were not surprised to find out that the UvrD-deficient strains exhibited considerably higher UV-mutability compared to the UvrD-proficient cells (Ref. III, Fig. 5 and Table S9). At the same time, the Mfd deficient strain of *P. putida* produced less UV-induced mutants than the wild type strain (Ref. III, Fig. 5 and Table S9), which is the opposite of the results obtained withe *E. coli* (Witkin, 1966; George and Witkin, 1975; Oller *et al.*, 1992) and suggests that in *P. putida* UV-mutagenesis is operated through a yet unknown Mfd-dependent pathway. It should also be noted that the elevated UV-mutagenesis in the Mfd-deficient strains of *E. coli* can be explained by activation of error-prone translesion DNA synthesis, conducted mostly by DNA polymerase V (encoded by the *umuDC* locus), as TC-NER is unable to repair the damaged area (Kato and Shinoura, 1977; Sommer *et al.*, 1993). *P. putida* lacks the homologues of *umuDC* and instead harbours damage-inducible "mutagenesis cassette", which encodes the ImuB protein and the DnaE2 polymerase (Erill *et al.*, 2006), of which the first facilitates and the second impedes the occurrence of UV-mutagenesis in *P. putida* (Koorits *et al.*, 2007). This further supports the possibility that UV-induced mutagenesis in *P. putida* could occur through a different pathway than the one(s) known to be operating in *E. coli*.

4. Involvement of Mfd and UvrD in DNA damage repair (Ref III)

As the occurrence of mutations is influenced by DNA lesions, we decided to evaluate the role of Mfd and UvrD in DNA damage repair by assessing the tolerance of Δ*uvrD* and Δ*mfd* strains to various DNA damaging agents. For this we either irradiated the cultures of tester cells with UV-C light or cultivated tester cells on solid medium that contained various DNA damaging chemicals (NQO or MMC or MNNG) and assessed the survival of the treated cells.

The conducted experiments demonstrated very well the importance of UvrD in maintaining the genome integrity, as we observed that *uvrD* deletion strains

were more sensitive to the treatment with UV-light and the above mentioned DNA damage-inflicting chemicals (Ref. III, Fig. 4 and Table 1 and S7). Since MMR deficiency did not affect the cells survival, but both NER deficient *uvrA* deletion strains were very sensitive to all the treatments, we can presume that poor survival of the Δ*uvrD* strains was caused by the loss of NER function in these strains. However, Δ*uvrD* strains tolerated the treatments with UV and NQO considerably better than the Δ*uvrA* strains indicating that NER could still be partially functional in the UvrD-deficient strains. Similar observation has been made in *E. coli*, where it was reported that the better UV tolerance of the Δ*uvrD* strain is caused by maintenance of limited amount of UvrABC-dependent repair in this strain (Kuemmerle and Masker, 1980; Crowley and Hanawalt, 2001; Newton *et al.*, 2012). The improved UV tolerance could be a result of other cellular factors providing alternative or backup mechanisms to substitute for the loss of UvrD in the post-incision steps of NER (Crowley and Hanawalt, 2001).

Similarly to other bacteria (Witkin, 1966; LeCuyer *et al.*, 2010; Martin *et al.*, 2019), in *P. putida* Mfd plays a modest role in protecting the cells against UV-induced damage. At lower UV-C dose (5 J/m^2) the loss of Mfd had no influence on the survival of bacteria and at higher dose (20 J/m^2) the Δ*mfd* strain was only about 10-times less viable than the wild type strain (Ref. III, Fig.4). Additionally, Mfd did not have a noticeable role in tolerating damage caused by MNNG or NQO (Ref. III, Table 1 and S7). Similar result about the tolerance of NQO treatment has also been reported in *E. coli* (Kamarthapu *et al.*, 2016). Notably, the Mfd-deficient strains of *P. putida* formed smaller colonies on NQO-containing medium (Ref III. Fig. S1) suggesting that under certain conditions the presence of Mfd might be beneficial for the growth of bacteria. The higher tolerance to UV and NQO treatment of the Δ*mfd* strain in comparison to the Δ*urvA* strains indicates that GG-NER is still active in the Mfd-deficient cells. Therefore, it was surprising that the Δ*mfd* strain was as sensitive to the MMC treatment as were the NER-deficient Δ*uvrA* strains. This observation indicates that, in addition to the TC-NER, Mfd might also participate in some other repair pathway which removes damage induced by MMC. For example, in *H. pylori* (Lee *et al.*, 2009), *B. subtilis* (Ayora *et al.*, 1996) and *B. cereus* (Darrigo *et al.*, 2016) Mfd has been shown to participate in DNA repair connected with homologous repair (HR). However, this is probably not the case in *P. putida* as we have observed that HR is elevated when Mfd is absent (Ref. II, Fig. 4A).

5. Concluding remarks on the role of Mfd and UvrD on mutagenesis in *P. putida*

Our results of the mutagenesis and DNA damage tolerance experiments revealed that UvrD is essential in *P. putida* to maintain genetic stability both in growing and non-growing cells. Since UvrD has central and well established roles in NER and MMR pathways (Caron *et al.*, 1985; Brosh and Matson, 1997; Hall *et al.*,

1998), it is unlikely that this enzyme or its mediated TCR participates in mutagenic processes in any other way than reducing the occurrence of mutations.

Similarly to several other bacteria (LeCuyer *et al.*, 2010; Pybus *et al.*, 2010; Foster *et al.*, 2015), in *P. putida* Mfd does not seem to have a significant effect on the occurrence of mutations in growing cells. Interestingly, in a clinical isolate of *P. aeurginosa* a 2-fold decrease in spontaneous Rif^R mutant frequency was observed when Mfd was absent (Ragheb *et al.*, 2019). In the same study it was also discovered that in another pathogenic bacterium, *S. typhimurium*, Mfd promotes mutagenesis under laboratory growth conditions and upon host cell infection. It was proposed that Mfd could play a critical role in inducing mutagenesis in pathogenic bacteria during infection and thereby enabling to overcome the new harsh condition met in the host. As the environmental conditions that bacteria, e.g. pathogenic bacteria and soil bacterium *P. putida*, are exposed to, are quite different, it is possible that Mfd contributes to mutagenesis differently in various organisms. Different role of Mfd in mutagenic processes in various bacteria is also supported by our finding demonstrating that Mfd promotes UV-mutagenesis in *P. putida*, whereas the opposite has been observed in *E. coli* (Witkin, 1966; George and Witkin, 1975; Oller *et al.*, 1992).

Although we have observed no influence of Mfd on mutagenesis in exponentially growing bacteria, our results indicate that Mfd has a role in inducing genetic change in stationary-phase *P. putida* cells (Ref III, Fig. 1). In *B. subtilis* the estimated Mfd mRNA amount in stationary-phase cells is about 4 times higher than that measured in exponential-phase cells (Gómez-Marroquín *et al.*, 2016), which indicates to its importance under conditions restricting growth of bacteria. While in growing cells most of the mutations arise as a result of DNA replication, in stressed or non-growing conditions genome replication is reduced (Wang *et al.*, 2007b; Denapoli *et al.*, 2013) and the local DNA synthesis and malfunctioning of certain DNA repair pathways could contribute to the occurrence of mutations. As transcription and TCR are still active in these conditions, Mfd could therefore have an important role in *P. putida* and other bacteria to overcome growth limitations by contributing to the emergence of better adapted mutants. How exactly Mfd induces the occurrence of mutations is still unclear. In *B. subtilis* Mfd was seen to carry out faulty DNA repair together with glycosylase MutY (Gómez-Marroquín *et al.*, 2016; Martin *et al.*, 2019). However the same is unlikely to occur in *P. putida* as the deletion of *mutY* causes a mutator phenotype in *P. putida* (Saumaa *et al.*, 2002). Several studies have also shown that the Mfd-dependent mutagenic events require the NER proteins or DNA polymerase I (Million-Weaver *et al.*, 2015; Gómez-Marroquín *et al.*, 2016; Ragheb *et al.*, 2019). These finding indicate that Mfd mediates faulty DNA repair by directing NER proteins to remove a lesion, which is then followed by an error-prone gap filling. This mechanism is also supported by the observations that NER can cause mutations and carries out gratuitous repair on undamaged DNA (Branum *et al.*, 2001; Hasegawa *et al.*, 2008; Janel-Bintz *et al.*, 2017). However, this does not seem to be the case in *P. putida* as we observed that UvrA-deficiency had no influence on stationary-phase mutation frequency (Ref III, Fig. 1). Additionally, *P. putida* Pol

I-deficient strains express only a moderate mutator phenotype (Sidorenko *et al.*, 2011). Therefore, in *P. putida* the Mfd-directed stationary-phase mutagenesis is probably operated by another pathway than the canonical NER pathway. It should be mentioned that *P. putida* harbours another putative UvrA, the UvrA2 enzyme (PP_3087), which has been demonstrated to facilitate the occurrence of mutations in *P. putida* (Tark *et al.*, 2008). However, other and yet to be discovered Mfd-associated pathways cannot be excluded.

CONCLUSIONS

It is widely accepted that endogenous factors have a significant role in inducing genetic change that enables bacteria to adapt to constantly changing surrounding conditions and thrive in the new environment. Transcription is an essential endogenous process of copying DNA into RNA. However, in recent years transcription has been reported to also be an important factor generating genetic diversity. There are several aspects of transcription that may promote the occurrence of mutations, for example better availability of DNA to damaging agents during transcription or collisions between transcription and replication machineries. An unexpected mutagenic aspect of transcription may also be the DNA repair that is coupled with transcription. Transcription coupled repair (TCR) is a sub-pathway of nucleotide excision repair, which preferentially removes lesions from the transcribed DNA strand and reduces the occurrence of transcription-replication collisions. This repair pathway is activated when a stopped RNA polymerase is recognised by the TCR factor Mfd or DNA helicase UvrD. While TCR maintains the genome integrity, accumulating body of evidence suggests that in some circumstances TCR may instead induce the occurrence of mutations through the Mfd-dependent pathway.

In this thesis the role of transcription and TCR factors Mfd and UvrD in mutational processes was studied in soil bacterium *P. putida*.

The main conclusions of this thesis can be summarized as follows:

1. Occurrence of transcription induces mutagenesis in growing cells of *P. putida* – elevated transcription of the mutational target gene results in elevated mutation frequency.
2. Deficiency of NER proteins UvrA, UvrB or UvrC in *P. putida* causes severe growth defects and significant instability of the mutant strains – adapted mutants that have overcome the deleterious phenotype rapidly appear. Adaptation does not improve tolerance to DNA damaging agents, suggesting that the unknown mechanism underlying adaptation is probably not a result of increased efficiency of DNA damage repair. However, adaptation may influence frequency of occurrence of some of the mutation types.
3. Mfd and UvrD both participate in the repair of DNA damage in *P. putida*, however, UvrD has a predominant role in the repair processes.
4. UvrD is essential for preventing occurrence of mutations in both exponential- and stationary phase *P. putida* cells.
5. In *P. putida* TCR factor Mfd has a role in inducing stationary-phase mutagenesis and Mfd also participates in UV-induced mutagenic pathway which is different from those operating in *E. coli*. In the growing cells of *P. putida* Mfd does not promote the occurrence of mutations.

Thus, the results of this thesis support the opinion that transcription elevates mutation frequency and Mfd-directed repair is involved in transcription associated mutagenesis in bacteria. However, the role of Mfd-directed TCR in mutagenesis may differ in various bacteria.

SUMMARY IN ESTONIAN

Transkriptsiooni ja transkriptsiooniseoselist reparatsiooni vahendavate faktorite mutageenne mõju bakteris *Pseudomonas putida*

Enamuse oma elust looduslikes tingimustes on bakterid toitainete näljas ja stressis, mis tuleneb pidevalt muutuvatest keskkonnatingimustest. Selleks, et uute tingimustega kohaneda, on bakteritel välja kujunenud mitmed strateegiad. Üheks selliseks on rakuväliste või rakusiseste tegurite poolt soodustatud mutatsioonide teke, mis võimaldab bakterite kiiret evolutsioneerumist. Elutegevuseks hädavajalik DNA ainevahetuses osalev protsess, transkriptsioon, võib olla üks mehhanismidest, mis geneetiliste muutuste teket soodustab. Kuigi esimesed viited transkriptsiooni osalusele mutatsiooniprotsessides ilmusid juba pool sajandi tagasi (Herman and Dworkin, 1971; Savić and Kanazir, 1972), on alles hiljuti kaaluma hakatud transkriptsiooni võimalikku rolli evolutsioneerumises.

On mitu võimalust kuidas transkriptsioon võib mutatsioonide teket soodustada. Näiteks, toimuvad bakterites transkriptsioon ja replikatsioon ühel ajal ning samal substraadil, mistõttu on kokkupõrked kahe protsessi vahel vältimatud. See aga võib omakorda põhjustada geneetilist ebastabiilsust (Paul *et al.*, 2013; Sankar *et al.*, 2016). Lisaks kohtab transkriptsiooni läbiviiv RNA polümeraas DNA-l ka vigu, mis takistavad transkriptsiooni jätkumist ja suurendavad transkriptsioon-replikatsiooni kokkupõrke võimalust ning DNA paremat kättesaadavust seda kahjustavatele teguritele. Selliste vigade parandamiseks ja peatunud RNA polümeraaside eemaldamiseks on rakkudes transkriptsiooniseoseline reparatsioon (ingl. k. *transcription-coupled repair*, TCR), mida bakterites vahendavad DNA reparatsiooni valk Mfd ja DNA helikaas UvrD (Monnet *et al.*, 2013; Epshtein *et al.*, 2014). Need ensüümid tunnevad ära DNA-l peatunud RNA polümeraasi, eemaldavad selle kahjustatud kohalt ja seejärel suunavad nukleotiidide väljalõike reparatsiooni (ingl. k. *nucleotide excision repair*, NER) viga parandama. Kuigi transkriptsiooniseoseline reparatsioon eemaldab DNA-lt vigu ja peaks seetõttu vähendama mutatsioonide tekkesagedust, on mitmed uurimustööd hiljuti näidanud, et Mfd-st sõltuv rada osaleb teatud tingimustel ka mutatsioonide tekitamises (Han *et al.*, 2008; Million-Weaver *et al.*, 2015; Gómez-Marroquín *et al.*, 2016; Ragheb *et al.*, 2019). Kuna mutatsioonid võimaldavad bakteritel stressi tekitavate tingimustega kohaneda, näiteks saavutada resistentsuse antibiootikumide ravile või olla kaitstud peremeesorganismis immuunsüsteemi rünnaku eest, on oluline mõista mehhanisme, mis soodustavad geneetiliste muutuste teket.

Käesoleva töö eesmärgiks oli välja selgitada transkriptsiooni ja sellega kaasnevas reparatsioonis osalevate valkude Mfd ja UvrD mõju mutatsioonide tekkele mullabakteris *Pseudomonas putida*. See bakter kuulub suurde pseudomonaadide perekonda, mille liikmed on metaboolselt väga mitmekülgsed ja võimelised kiiresti kohanema muutuvate keskkonnatingimustega. Mitmed selle perekonna liikmed on huvi pakkuvad ka oma biotehnoloogilise rakendatavuse või patogeensuse tõttu.

Käesoleva töö peamised tulemused saab kokku võtta järgnevalt:

1. Transkriptsioon soodustab mutatsioonide teket *P. putida* logaritmiliselt kasvavates rakkudes – testgeeni transkriptsioonitaseme tõstmisel suureneb ka mutatsioonide tekkesagedus selles geenis
2. Nukleotiidide väljalõike reparatsiooni valkude UvrA, UvrB või UvrC puudumine bakteris *P. putida* põhjustab rakkudele kasvuraskusi, kuid kiiresti tekivad kohanenud mutandid, millel enam kasvuraskusi ei esine. Kohanemine ei suurenda DNA-d kahjustavate ühendite talumist, mistõttu võib arvata, et kohanemine ei tulene DNA kahjustuste parandamise efektiivsemaks muutumisest. Samas võib kohanemine teatud mutatsioonide puhul nende tekkesagedust mõjutada.
3. Nii Mfd kui UvrD osalevad DNA kahjustuste parandamises, kuid UvrD-l on kahjustuste eemaldamises peamine roll.
4. UvrD on hädavajalik mutatsioonide tekke ärahoidmiseks *P. putida* kasvavates ja statsionaarses faasis olevates rakkudes.
5. Bakteris *P. putida* ei paista Mfd omavat mõju mutatsioonide tekkele kasvavates rakkudes, kuid see ensüüm soodustab statsionaarse faasi mutageneesi ja UV-indutseeritud mutageneesi, mille mehhanism on erinev bakteris *Escherichia coli* kirjeldatust.

Käesoleva töö tulemused näitavad, et transkriptsioon ja Mfd-st sõltuv transkriptsiooniseoseline reparatsioon soodustavad mutatsioonide teket bakterites. Samas tasub silmas pidada, et mehhanismid, mille kaudu Mfd soodustab mutatsioonide teket, võivad erinevates bakteriliikides olla erinevad.

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2. Sidorenko J, Ukkivi K, Kivisaar M. NER enzymes maintain genome integrity and suppress homologous recombination in the absence of exogenously induced DNA damage in Pseudomonas putida. DNA Repair (Amst). 2015 Jan; 25:15–26.
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4. Mikkel K, Tagel M, Ukkivi K, Ilves H, Kivisaar M. Integration Host Factor IHF facilitates homologous recombination and mutagenic processes in Pseudomonas putida. DNA Repair (Amst). 2020 Jan; 85:102745.

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1. Juurik T, Ilves H, Teras R, Ilmjärv T, Tavita K, Ukkivi K, Teppo A, Mikkel K, Kivisaar M. Mutation frequency and spectrum of mutations vary at different chromosomal positions of Pseudomonas putida. PLoS One. 2012 Oct; 7: e48511.
2. Sidorenko J, Ukkivi K, Kivisaar M. NER enzymes maintain genome integrity and suppress homologous recombination in the absence of exogenously induced DNA damage in Pseudomonas putida. DNA Repair (Amst). 2015 Jan; 25:15–26.
3. Ukkivi K, Kivisaar M. Involvement of transcription-coupled repair factor Mfd and DNA helicase UvrD in mutational processes in Pseudomonas putida. DNA Repair (Amst). 2018 Sep; 72:18–27.
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Saadud uurimistoetused ja stipendiumid:
1. Kristjan Jaagu välissõidu stipendium, 2017
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