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Combating DNA damage and maintenance of genome integrity in pseudomonads

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

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Author's contribution:

Ref. I   –   designed and performed the assay construction, constructed the strains, performed all the experiments, prepared the tables and figures, wrote the manuscript

Ref. II   –   designed and performed a part of the experiments, contributed to the manuscript editing

Ref. III –   performed most of the experiments, participated in the construction of the strains and the manuscript writing and editing

ABBREVIATIONS

3-meA	3-methyladenine
4-NQO	4-nitroquinoline
8-oxoG	7, 8-dihydro-8-oxoguanine
AP site	apurinic/apyrimidinic site
BER	base excision repair
bp	base pairs
CFU	colony forming units
DSB	double strand break
dsDNA	double strand DNA
DUE	DNA unwinding element
GG-NER	global genomic nucleotide excision repair
HR	homologous recombination
kb	kilo-base pair
kDa	kilo-Dalton
MFP	membrane fusion protein
MMS	methyl methane sulfonate
MNNG	N-methyl-N´-nitro-N-nitrosoguanidine
NAP	nucleoid-associated protein
NER	nucleotide excision repair
NHEJ	nonhomologous end-joining
OM	outer membrane
OMF	outer membrane factor
Phe	phenol
PI	propidium iodide
Pol	DNA polymerase
QS	quorum sensing
Rif	rifampicin resistant
RNAP	RNA polymerase
RND	resistance-nodulation-division
ROS	reactive oxygen species
SAM	S-adenosylmethionine
SNP	single-nucleotide polymorphism
SSB	single-strand DNA binding proteins
ssDNA	single-strand DNA
T3SS	type three secretion system
TC-NER	transcription-coupled nucleotide excision repair
TLS	translesion synthesis
UV	ultraviolet
WT	wild-type

INTRODUCTION

Alternations in the chemical structure of DNA molecule can have mutagenic or even fatal consequences for a cell and need to be promptly repaired. DNA lesions occur spontaneously at a high frequency but can be dramatically enhanced upon encountering environmental stress. An elaborate cooperation of partly redundant DNA repair mechanisms enables the removal of DNA lesions and maintenance of genome integrity even in the presence of DNA damage and involves direct reversal of DNA damage and excision repair pathways. Failure to remove DNA lesions from DNA can result in replication stalling and formation of DNA gaps and breaks. Homologous recombination (HR) is involved in restoration of replication forks, repair of single stand DNA gaps and double strand DNA breaks and thus serves as a powerful back-up DNA repair mechanism. Alternatively, lesions can be tolerated by potentially mutagenic replication across the damage by specialized DNA polymerases.

Nucleotide excision repair (NER) is one of the major DNA repair pathways involved in removal of a broad range of structurally unrelated DNA lesions through a process of excising of a 12–13 nucleotide long lesion-containing DNA stretch which is followed by re-synthesis of the missing nucleotides. A tight interconnection between DNA replication, excision repair and recombination is indicated by high frequencies of strand exchanges and deficiency in resumption of DNA replication after UV-irradiation in the absence of NER in *Escherichia coli* (Ganesan & Smith, 1971; Rupp *et al*, 1971; Courcelle *et al*, 1999). However, NER has been generally studied in bacteria exposed to DNA damaging agents and its role in maintenance of genome integrity under normal growth conditions has attracted less attention. DNA polymerase I (Pol I), involved in filling-in the gap after the lesion excision in NER pathway is also important for DNA repair synthesis in base excision repair pathways, and, most importantly, in processing of Okazaki fragments during the lagging strand replication. The lack of Pol I has been shown to result in hyper-recombination phenotype in *E. coli* and reduces markedly viability of Pol I-deficient mutants on rich growth medium (Joyce & Grindley, 1984; Konrad, 1977). Yet, the ability of such mutants to grow on minimal medium indicates that other systems can substitute for Pol I functions.

Notably, although extensively studied in *E. coli*, many DNA repair and damage response processes differ between bacterial species. The examples include the absence of genes for a DNA damage-inducible error-prone DNA polymerase V in genomes of most bacteria, the differences in the size of the SOS regulons and the presence of non-homologous end joining (NHEJ) pathway in many species. Pseudomonads is a large diverse group of ubiquitous, mostly saprophytic bacteria found widely in the environment, but includes also several pathogenic species like *P. syringae*, a plant pathogen, and an opportunistic human pathogen *P. aeruginosa*, known for its ability to develop multi-drug resistance phenotype due to mutational changes which often include active

extrusion of antibiotics from the cell. In this thesis I have studied the involvement of NER and Pol I for genome integrity in *P. putida* cells and variations of the levels of active efflux systems between wild-type laboratory strains of *P. aeruginosa*. The elevated of HR is a potent indicator of a replication stress and reduced genome integrity and can be used to assess the roles of various factors in maintenance of genome stability. Since the experimental system for detection of HR events in pseudomonads was missing, two assays that enable monitoring HR in *P. putida* cells were constructed.

REVIEW OF LITERATURE

Introduction

It is hard to underestimate the importance of maintenance of the heritable information in the cell. The chromosomal replication is extremely processive and precise process despite being constantly challenged by numerous impediments. DNA damage, active transcription and DNA-associated proteins can prevent the replication machinery from efficiently completing its task which can result in either mutagenesis or lethality for the cell. Still, it takes only about 40 minutes to duplicate a genome of *Escherichia coli* of 4.6×10^6 nucleotide pairs with spontaneous mutation rate only about 0.0033 per DNA replication (Drake, 1991). Such efficiency is achieved by an elaborate interplay of numerous processes, involving the high fidelity of the replicative DNA polymerases and competent DNA damage repair and tolerance mechanisms.

In the literature review section I will describe the process of the replication of the chromosome of the iconic model organism *E. coli* chromosome and give a brief overview of the most abundant endogenous DNA lesions and the repair systems involved in removal of DNA damage. I will also concentrate on the fate of replication upon encountering DNA damage which could not be removed and discuss the ways of possible replication reactivation mechanisms. SOS response and DNA damage tolerance mechanisms will also be discussed. As one possibility of combating DNA damage is its prevention, I will also describe the properties of *Pseudomonas aeruginosa* membrane and efflux-pumps, which enable this organism to efficiently reduce concentration of DNA damaging agents in the cell.

1. DNA replication

1.1. DNA polymerases

Several DNA polymerases are commonly present in the cells of living organisms. For instance, there are eight of them in *Saccharomyces cerevisiae* while human cells possess 14 DNA polymerases. *E. coli* uses five DNA polymerases, three of them, Pol II, Pol IV and Pol V, are non-essential, being activated in case of DNA damage, while Pol III and Pol I are needed to replicate bacterial chromosome (Sutton, 2010).

Pol I was the first DNA polymerase discovered by Arthur Kornberg in 1956 and was assumed to be the major replicative enzyme in the cell (Lehman *et al*, 1958; Kresge *et al*, 2005). However, discovery of the viable mutant with less than one percent of the normal level of DNA polymerase put into doubt its functions as the only polymerase (De Lucia & Cairns, 1969). Moreover, Pol I did not fit into this role due to its low processivity (about 20–100 nucleotides) and a slow synthesis rate (about 20 nucleotides per second). High abundance of the Pol I in the cell (approximately 400 molecules per cell) also was inconsistent

with existence of only two replication forks in the cell. All this indicated the presence of another enzyme, able to rapidly and efficiently replicate bacterial chromosome. In 1970 DNA Pol II was identified, and in 1971 DNA Pol III, which matched the replicative polymerase characteristics, was discovered (Kornberg & Baker, 2005). Despite not being a major replicative polymerase, Pol I functions are important for maturation of Okazaki fragments during replication and DNA repair synthesis.

1.1.1. DNA polymerase I

E. coli Pol I is a 928 amino acid long protein encoded by *polA* gene. Prokaryotic Pol I belongs to the A-family DNA polymerases which involves, for instance, mitochondrial DNA polymerase γ and several bacteriophage (e.g., T7, T5) DNA polymerases (Patel *et al*, 2001). Pol I contains two functional domains and three enzymatic activities. The N-terminal domain is 5´-nuclease, responsible for removal of RNA primers from the Okazaki fragments, and the remaining part, referred to as Klenow domain, possesses 5´-3´ polymerase activity with a 3´-5´ proofreading exonuclease functions (Kornberg & Baker, 2005).

The main function of Pol I in the absence of DNA damage is removing RNA primers from Okazaki fragments and filling in the resulting gaps by the "nick translating" activity. Pol I is suggested to interact with β-clamp left at the site of RNA primer after Pol III core finishes Okazaki fragment synthesis and dissociates (O'Donnell, 2006). β-clamp interacts with Pol I with its hydrophobic cleft between the domains II and III at the C-terminal tail of the β-clamp, which is the site for interaction with all DNA polymerases and a clamp loader, thereby increasing Pol I processivity *in vitro*, and is important for proper Pol I function *in vivo* (López de Saro & O'Donnell, 2001; Maul *et al*, 2007). Pol I initiates DNA synthesis from a ssDNA nick, displacing RNA- or damage-containing strand and creating a substrate for the 5´-nuclease, which cuts specifically between the two paired nucleotides of the forked substrate (Lyamichev *et al*, 1993; Xu *et al*, 2000). Pol I 5´-nuclease homologues are identified in mammals and archaea, whereas in these organisms they are independent proteins (Robins *et al*, 1994; Matsui *et al*, 1999). In *E. coli* DNA synthesis performed by Pol I is relatively error-free due to its associated 3´-5´ exonuclease proofreading activity. However, despite retaining the 3´-5´ exonuclease domain, the proofreading activity is lacking in many bacteria, including species from *Bacillus, Thermus* and *Rickettsiae,* due to missing essential amino acids (Joyce, 2004).

1.1.2. DNA polymerase III

While Pol I constitutes a single polypeptide with three distinct enzymatic activities, Pol III core consists of three subunits: α subunit, which performs actual DNA synthesis (encoded by *dnaE*), ε subunit (*dnaQ*) is a proofreading 3´-5´-exonuclease, and θ subunit (*holE*) which stimulates the ε subunit's proofreading

activity. Pol III core is a part of Pol III holoenzyme, a multiprotein complex which includes three major functional units: 1) Pol III core, 2) the processivity β sliding clamp, and 3) the heteropentameric clamp loader which holds the complex together by interacting with the catalytic α subunits of the Pol III and also assembles circular β-clamps onto primed DNA in an ATP-dependent process. Pol III holoenzyme together with DnaG primase and DnaB helicase constitute a replisome (Fig. 1) (O'Donnell, 2006).

1.1.3. Specialized DNA polymerases

Three specialized DNA polymerases Pol II, Pol IV and Pol V in *E. coli* are encoded by *polB* (*dinA*), *dinB* and *umuDC* genes, respectively. The names of the genes reflect their features: "damage inducible" for *din* genes and "UV-mutagenesis" for *umu* genes. The absence of these polymerases does not influence bacterial viability. Pol II is a B-family polymerase and has high fidelity as it contains the proofreading activity (Qiu & Goodman, 1997). DNA polymerases IV and V are members of the Y family of error-prone DNA polymerases. However, most of bacteria lack chromosomally encoded *umuDC* orthologs (Pol V), while non-chromosomal counterparts, mostly encoded in large conjugative plasmids, are described in various bacterial species (Woodgate & Sedgwick, 1992). Plasmid-encoded *umuDC* orthologs, conferring high UV-mutability and environmental fitness, are widely distributed also in *Pseudomonas* species, including *P. putida* (Tark *et al*, 2005; Zhang & Sundin, 2004). Nevertheless, chromosomes of a wide range of bacterial species lacking Pol V orthologs carry a multiple gene cassette *imuA-imuB-dnaE2,* also referred to as a mutagenesis cassette, where *imuA* has a weak similarity to *E. coli sulA* and *recA*, while *imuB*-encoded ImuB resembles Y-family polymerase, although it lacks active site residues required for DNA polymerase activity (Abella *et al*, 2004; Koorits *et al*, 2007; Warner *et al*, 2010). *dnaE2* (alternatively termed *imuC*) encodes a C-family DNA polymerase, a second copy of catalytic subunit of Pol III (Timinskas *et al*, 2014; Ippoliti *et al*, 2012). While mutagenesis cassettes are widely distributed in bacteria, their organization can vary (Erill *et al*, 2006). For instance, the cassette can be split and *dnaE2* gene can be located separately from *imuA-imuB* cassette (e.g., *Mycobacterium tuberculosis*). Moreover, the expression of the damage-inducible mutagenesis cassette in most bacteria, similarly to *E. coli* specialized polymerases, is under the control of LexA repressor. Still, in several cases (e.g., *P. putida*, *P. fluorescens*, *P. syringae* and *Xanthomonas* species) *lexA2-imuA-imuB-dnaE2* cassettes exist, where *lexA2* encodes a LexA homologue, LexA2, which specifically controls the adjacent *imuA-imuB-dnaE2* genes as a part of RecA-dependent SOS regulon. Interestingly, the *lexA2* gene is absent in *P. aeruginosa* and the expression of the mutagenesis cassette in this organism is regulated by LexA (Abella *et al*, 2004).

1.2. Initiation of replication

Replication of the circular bacterial chromosome initiates at a single site of the chromosome called origin of replication, *oriC* (Leonard & Mechali, 2013). Origins of replication are usually continuous and range from ~250 in *E. coli* to ~950 bps in *Streptomyces*, however longer bipartite origins also exist and contain a spacer gene between the *oriC* sub-regions (e.g., *Bacillus subtilis, Helicobacter pylori*) (Jakimowicz *et al*, 1998; Moriya *et al*, 1999; Donczew *et al*, 2012). The *oriC* of *P. putida* chromosome is 605 bp long (Wolański *et al*, 2014). *oriC* regions comprise three basic functional modules: a set of DnaA-recognition sites (boxes), the AT-rich <u>D</u>NA <u>u</u>nwinding <u>e</u>lement (DUE), the site at which *oriC* melts, and the sequences that are recognized by regulatory proteins. DnaA boxes are highly conserved and in most cases are asymmetrical nine-nucleotide-long motifs. The "perfect" box sequence of *E. coli* is TTATCCACA, which is conserved also in *P. putida*. The number of DnaA boxes varies in different organisms, e.g., there are 5 of them in *P. aeruginosa*, 8 in *P. putida* and 13 in *E. coli,* of which only 3 are high-affinity boxes (Wolański *et al*, 2014). DUE is usually thermodynamically unstable AT-rich region. In *E. coli* and *P. putida* DUE comprise three AT-rich 13-mer repeats. The replication process is initiated when the initiator protein, DnaA, binds to multiple DnaA-boxes within the *oriC* region, which triggers separation of the DNA strands at the DUE, providing a place for a replication fork assembly.

Activity of the *oriC* region is tightly controlled by specific origin-binding proteins which control the binding of DnaA (Wolański *et al*, 2014). The functions of these proteins involve the regulation DnaA protein activity or modulation of the interaction of the DnaA protein with the origin of replication by binding to *oriC*. About 11 *oriC* binding proteins have been identified in *E. coli*. The best characterized examples are SeqA, Fis, IHF, HU, Dps and ArcA proteins. In *E. coli* SeqA protein, which binds to hemimethylated GATC sequences at the *oriC* region shortly after chromosomal replication until the GATC sites are fully methylated by the Dam methylase, strictly prevents the initiation of new rounds of replication by blocking DnaA from binding to its boxes (Campbell & Kleckner, 1990). However, this system is lacking in pseudomonads (Rybenkov, 2014). Nucleiod-assotiated proteins Fis, HU and IHF are also known to bind to the *oriC* region in *E. coli*, whereas Fis inhibits and IHF and HU stimulate the DnaA-induced *oriC* unwinding (Ryan *et al*, 2002, 2004). In addition to general regulators of the chromosome replication initiation, other regulators can repress initiation in response to various stresses, e.g., to oxidative stress and oxygen depletion (Dps and ArcA, respectively) (Chodavarapu *et al*, 2008; Lee *et al*, 2001).

## 1.3. The structure and dynamics of the replisome

The latest vision of the composition and function of the chromosomal DNA replication machinery reveals extraordinary fluidity of the bacterial replisomes, which enables rapid and efficient bypass of blocks and template lesions during highly processive replication (Kurth & O'Donnell, 2013). After separation of the DNA strands at the *oriC*, loading of the replicative helicase DnaB, mediated by the helicase loader DnaC, occurs and a complex replication machinery, replisomes, are assembled to form two replication forks. Replication forks move bidirectionally with the speed up to 1000 nucleotides per second being able to replicate 4,6 Mbp genome of *E. coli* in just 40 minutes.

E. coli replisome comprises the hexameric helicase, the DNA primase, and the Pol III holoenzyme (see Fig. 1 and section 1.1.2). Due to antiparallel structure of the duplex DNA the leading and lagging strands are extended in the opposite directions where the leading strand is synthesized continuously and the lagging strand is synthesized as series of discontinuous 1–3 kb long Okazaki fragments. The DnaB helicase encircles and moves along the lagging strand to unwind the parent strands. The unwinding rate of the helicase depends on the interaction with Pol III holoenzyme and increases about 10-fold after establishing the contact (Kim *et al*, 1996). The DnaG primase interacts transiently with the DnaB helicase and initiates Okazaki fragments by RNA primer synthesis at the forked junction. The processivity clamps (β-clamps) are then loaded onto RNA primers to initiate the DNA strand synthesis by the Pol III core. As the synthesis of the two strands is coupled, the progression of leading strand synthesis results in a growing replication loop and the lagging strand template accumulates single-strand DNA (ssDNA), which is rapidly bound by ssDNA binding proteins. After the Okazaki fragment synthesis is finished, the Pol III core rapidly dissociates from the clamp and the loop dismantles. Dissociation of the Pol III core from the processivity clamps and reloading of the clamp at the next primed site is known as a processivity switch mechanism and enables coordinated synthesis of the lagging strand (Leu *et al*, 2003). β-clamps left at the lagging strand can further interact with DNA polymerase I and ligase to process Okazaki fragments and seal the finished fragments.

Until recently it has been assumed that the holoenzyme contains two copies of the pol III core, which are connected by the attachment to the clamp loader (O'Donnell, 2006). However, recent advances in microscopy and reconstitution studies challenge this historically accepted point of view. First, *E. coli* DNA polymerase III holoenzyme reconstituted from purified proteins is shown contain three active Pol III cores, connected to one of the three identical τ subunits within the clamp loader, where the third Pol core functions on the lagging strand (McInerney *et al*, 2007). Moreover examination the replisome architecture using single molecule fluorescence microscopy, reveals three molecules of the replicative polymerase at active replisomes of *E. coli* (Reyes-Lamothe *et al*, 2010). The assembly of the trimeric replicase was demonstrated *in vivo* in subsequent studies as well (Georgescu *et al*, 2012; Montón Silva *et al*, 2015).

Trimeric replicase appears to be more processive than dipolymerase replisomes and reduce the amount of ssDNA gaps on the lagging strands (Georgescu *et al*, 2012; Montón Silva *et al*, 2015). However, the frequency with which all three polymerases are used simultaneously is not certain.

Another novel insight into replication mechanism suggests that not all Okazaki fragments are extended to completion but leave ssDNA gaps on the lagging strand. The soluble Pol III cores can then be recruited to fill-in the ssDNA gaps left by the replisome (Kurth & O'Donnell, 2013).
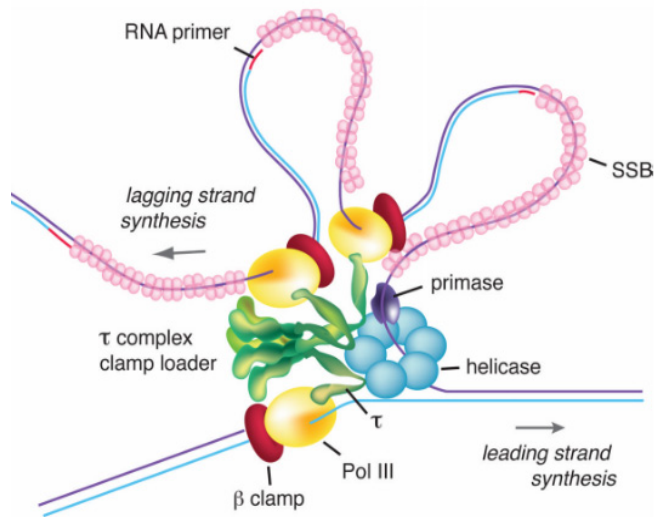
Figure 1. Organization of a moving bacterial replisome containing three Pol III cores (Kurth & O'Donnell, 2013). DnaB helicase (blue) encircles the lagging strand and unwinds the parental DNA ahead of Pol III core (yellow). DnaG primase (purple) synthesizes short RNA primers to initiate the synthesis of the Okazaki fragments on the lagging strand. The lagging strand ssDNA is coated by single-strand DNA binding proteins (SSB) (pink). In the trimeric replicase three Pol III cores are coupled through the three τ subunits of the clamp loader (green), which assembles β-clamps (red) onto primed DNA.

2. DNA damage removal
2.1. Endogenous DNA damage and its repair

DNA of the living cells is constantly damaged both endogenously and exogenously, although sometimes it is hard to draw a line between two sources. The endogenous chemical processes that damage DNA include spontaneous hydrolysis, alkylation, and reactions with radicals. However, these processes can be enhanced by various stress conditions that bacteria encounter. Enormous amounts of spontaneous DNA damage highlight the extreme need for elaborate repair pathways that are evolved in the living cells to combat the damage and maintain genome integrity. Approximate calculations of spontaneous hydrolysis events of DNA in mammalian cells indicate that deamination of DNA bases, where the loss of amine groups from cytosine is most common, yields 100–500 uracil residues, and spontaneous loss of DNA bases, producing apurinic/ apyrimidinic (AP) sites, generates about 10,000 abasic sites per day per cell (Ciccia & Elledge, 2010; Lindahl & Barnes, 2000). Moreover, DNA can be endogenously methylated, using S-adenosylmethionine (SAM) as the reactive methyl donor. The major cytotoxic lesion generated by SAM, 3-methyladenine (3-meA), is estimated to be generated at the amount of 600 lesions per mammalian cell per day (Lindahl & Barnes, 2000). Reactive oxygen species (ROS) is probably one of the most hazard spontaneously formed sources of damage to all cellular components, including DNA, proteins and lipids. Superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are continuously formed in aerobically grown cells through the auto-oxidation of its redox enzymes at the respiratory chain. Further, highly reactive hydroxyl radicals (•OH) are formed due to reactions between H_2O_2 and the intracellular pool of ferrous iron (Fe^{2+}) through Fenton reaction (Imlay & Linn, 1988). Endogenous levels of ROS are kept under control by scavenging enzymes such as catalases, peroxidases, and superoxide dismutases (Imlay, 2008). However, ROS levels can increase dramatically during times of environmental stress. The incomplete list of the factors that trigger ROS stress includes ionizing radiation, hyperoxia, starvation, clinical antibiotics, plant wound response, heat, salinity, near-UV-irradiation, metal ions and many more (Imlay, 2015). ROS can damage DNA directly by attacking the sugar or the base, giving rise to a variety of lesions, such as ssDNA break in case of sugar attack or numerous base modifications (Imlay & Linn, 1988). Guanine is most easily oxidised and is most frequently converted to 7, 8-dihydro-8-oxoguanine (8-oxoG) (Bjelland & Seeberg, 2003). While this lesion was shown not cause a significant block to DNA polymerisation *in vivo*, it is highly mutagenic and induces G-to-T transversions due to 8-oxoG mispairing with adenine during replication (Bjelland & Seeberg, 2003). Moreover, oxidation of the guanine nucleotide pool and subsequent incorporation of the oxidized nucleotides by Y-family polymerases is also implied to be a serious hazard to the cells, and can cause genome instability and even death (Shimizu *et al*, 2003; Foti *et al*, 2012).

DNA containing oxidised or methylated bases is processed predominantly through the base excision repair (BER) pathway, where DNA glycosylases that cleave the N-bond between the 2'-deoxyribose and base, generate AP site. At least 8 different glycosylases with partially overlapping substrate specificities are identified in *E. coli* cells (Krwawicz *et al*, 2007). AP site is further processed by AP-endonucleases to produce ssDNA gap, which will be filled in by the Pol I. Involvement of the Pol I in BER pathway is supported by the evidence that Pol I-deficient strains are extremely sensitive to alkylating agents N-methyl-N´-nitro-N-nitrosoguanidine (MNNG) and methyl methane sulfonate (MMS) (Nowosielska *et al*, 2006), and oxidative DNA damage (Imlay & Linn, 1988).

2.2. Nucleotide excision repair (NER)

Exogenous DNA damaging agents include numerous chemicals and electromagnetic radiation which can produce bulky DNA lesions that are predominantly excised through NER pathway. Nevertheless, some of the oxidized and methylated bases (e.g., 8-oxoG, thymine glycols) also serve as substrate for NER-mediated excision repair (Truglio *et al*, 2006). NER was initially discovered as the major pathway in removing UV-induced thymine dimers from DNA in *E. coli* (Boyce & Howard-Flanders, 1964). According to the lesion recognition mechanism, NER can be divided into global genomic repair (GG-NER, or simply NER) and transcription-coupled repair (TC-NER) pathways (Hanawalt, 2002). In both pathways UvrABC proteins play a central role in recognition, verification and excision of DNA damage. In the GG-NER DNA damage is identified directly by UvrA and UvrB proteins that form UvrA$_2$B$_2$-complex to scan the DNA for potential lesions. After DNA damage has been verified, UvrA dissociates and leaves an UvrB-DNA pre-incision complex, a substrate for UvrC binding. Subsequently UvrC incises the damage-containing DNA strand on both sides of the lesion. The TC-NER constitutes a sub-pathway of NER that acts preferentially on the transcribed DNA strands by recognizing RNA polymerase (RNAP) that has stalled by DNA lesions and thereby couples transcription and DNA repair processes. As a result, a transcribed strand is repaired much faster than the non-transcribed one (Mellon & Hanawalt, 1989). The TC-NER differs from the global repair pathway only in the damage recognition process, which requires participation of Mfd protein to elicit DNA damage repair. Mfd is a large 130-kDa monomeric protein which shares homology with UvrB and DNA helicase DnaG, and possesses an RNAP interaction domain. RNAP stalled at the site of lesion makes it inaccessible to repair proteins. Therefore, Mfd functions to remove RNAP from the damaged DNA. Mfd binds to RNAP and to 25 bp of DNA upstream of the stalled RNAP and pushes it forwards from the site of DNA damage in ATP-dependent process to facilitate its dissociation. By interacting with UvrA, Mfd recruits the NER machinery necessary for damage excision (Deaconescu *et al*, 2007; Savery,

2007). Both the GG-NER and the TC-NER pathways are completed when UvrD (DNA helicase II) is recruited to release the incised 12–13 nucleotide long DNA fragment containing the lesion and DNA polymerase I and DNA ligase complete the repair by filling in the resulting gap and sealing DNA ends (Hanawalt, 2002).

For a long time Mfd has been referred to as the only transcription coupling factor in *E. coli*, since *mfd* deletion mutants lacked the strand-specific DNA repair (Selby *et al*, 1991). However, recently UvrD has been identified as a novel player in the TC-NER (Epshtein *et al*, 2014). It has been demonstrated that UvrD binds to RNAP during transcription and can facilitate TC-NER by pulling RNAP backwards, which is in opposite direction of the Mfd-catalyzed translocation, in the case if it stalls, thereby exposing the shielded DNA lesions. Since RNAP back-tracking could be deleterious in the absence of DNA damage, as it could impede transcription, the UvrD-dependent TC-NER is implied to be promoted upon DNA damage, possibly by UvrD dimerization (Epshtein, 2015)

Thus, the UvrD appears to be a really multifunctional enzyme. In addition to its role in unwinding DNA regions in the NER and the DNA mismatch repair pathways (MMR), this helicase is involved in displacement of DNA-bound proteins. In addition to RNAP, the targets identified for the UvrD-mediated displacement include Tus proteins, which UvrD removes from Ter sites (Bidnenko *et al*, 2006), and the RecA proteins being disassembled from RecA nucleoprotein filaments (Veaute *et al*, 2005; Petrova *et al*, 2015). Moreover, of all NER enzymes, UvrD is the only protein (apart from Pol I), the lack of which is shown to activate constant SOS response in the cells and result in a hyper-recombination phenotype in *E. coli* (Arthur & Lloyd, 1980; Bierne *et al*, 1997; SaiSree *et al*, 2000; Veaute *et al*, 2005).

3. When replication forks run into damage

Replication machinery appears to be more flexible than previously imagined, being able to replicate genome with high fidelity and processivity even in the presence of DNA damage due to its ability to hop over DNA lesions, leaving ssDNA regions behind (Indiani & O'Donnell, 2013; Kurth & O'Donnell, 2013) Still, what happens when a replication fork encounters a DNA lesion? The result will substantially depend whether the lesion is located on the leading or the lagging strand. While the lesion located on the leading strand stalls the replication fork progression, the lagging strand DNA lesions does not affect the progression of the replication fork (Higuchi *et al*, 2003; McInerney & O'Donnell, 2007, 2004; Pagès & Fuchs, 2003). Due to discontinuous DNA synthesis mechanism on the lagging strand only the completion of the lesion-containing Okazaki fragment will be blocked and the DNA synthesis will be resumed at the new primed site (Fig. 2A). Recent findings that Okazaki fragments are not always fully extended due to premature dissociation of the

lagging strand Pol III core extends our understanding of the possibilities of lesion tolerance without blocking replication fork progression, as the lesions are left in a ssDNA gap to await repair (Kurth & O'Donnell, 2013).
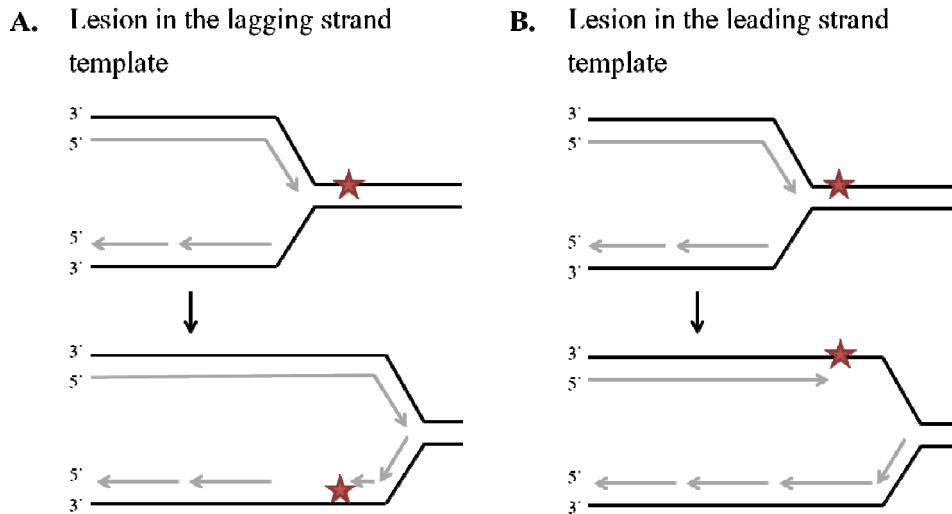
Figure 2. Fate of the replication fork encountering DNA damage on the leading or the lagging strands. (A) The lagging strand lesion does not block the progression of the replication fork and only the completion of the lesion-containing Okazaki fragment is affected. (B) DNA replicase stalls encountering the lesion on the leading strand. Since the synthesis of the leading and the lagging strands can be uncoupled, DnaB helicase will continue unwinding of the parent DNA duplex, producing ssDNA

The situation with the leading strand lesions is more complicated. According to the generally accepted semi-conservative replication model, synthesis of the leading strand is continuous and occurs in the direction of the replication fork movement. When the replicase encounters a block in the leading strand, it stalls, while the DnaB helicase will uncouple and continue unwinding DNA ahead of the stalled Pol III for at least 1 kb, thereby producing ssDNA (Fig. 2B). Such mechanism of producing SOS-response inducing ssDNA is referred to as a "runaway helicase" model (Pagès & Fuchs, 2003; Indiani & O'Donnell, 2013). Several models have been proposed to explain how the replication fork that has stalled at the leading strand block could be restarted in order to complete replication of the chromosome. Recombinational events underlie many of the proposed mechanisms indicating the exceptional importance of homologous recombination (HR) in maintenance of the genome integrity.
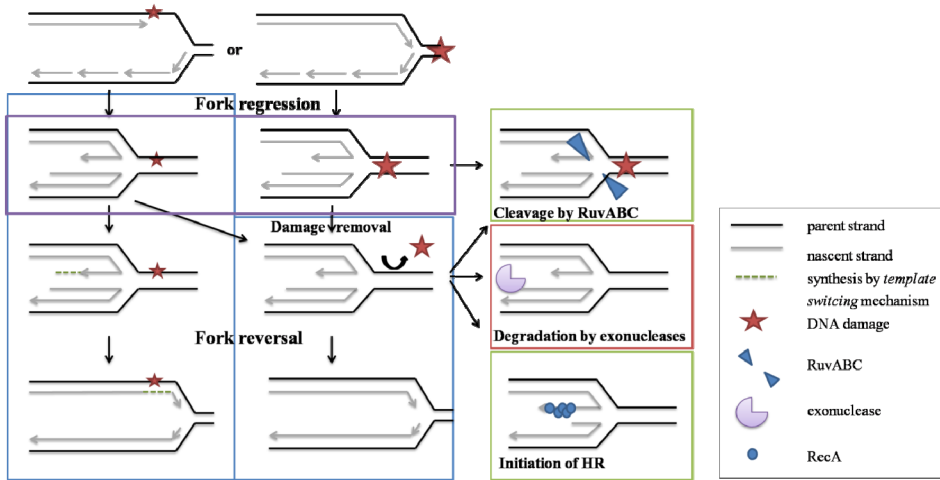
3.1. Replication fork regression

Arrested replication forks can undergo regression where unwinding will facilitate the nascent strands to anneal and form a so called "chicken foot" structure resembling a Holliday junction (Fig. 3., purple box), (Atkinson & McGlynn, 2009). Regression of the forks is suggested to be associated with the positive supercoiling strain but can be also promoted by various DNA helicases/translocases, e.g., RecG (Postow *et al*, 2001; Atkinson & McGlynn, 2009; McGlynn & Lloyd, 2002). Formation of such structure is possible if the replisome is dismantled (Atkinson & McGlynn, 2009). However, it is questionable whether the replisome readily dissociates from the replication fork as it is seen *in vitro* (McGlynn & Guy, 2008) or remains stable as measured by live-cell imaging (Possoz *et al*, 2006). The fork clearing role is proposed for the RecA protein with the help of the RecF, RecO and RecR proteins (McInerney & O'Donnell, 2007). Moreover, regression of arrested replication forks is shown to be promoted by the RecA *in vitro* (Robu *et al*, 2001). Replication fork regression gives multiple possibilities for the replication fork restart and can be processed in several ways as discussed below (Atkinson & McGlynn, 2009).

- Fork regression and reversal (Fig. 3., blue box)
  ssDNA produced by the helicase "runaway" after the replication of leading and lagging strands has been uncoupled may prevent the repair of the lesion by the excision repair systems as the template strand is missing. Duplex formation by the fork regression may thereby enable excision of the damage. Alternatively, the undamaged lagging strand could be used as a template for replication by the mechanism termed *template switching*. In UV-irradiated *E. coli* cells this process depends on the Pol II functions (Rangarajan *et al*, 2002). In both cases the regressed fork would be reversed and followed by reloading of the replisome onto the restored fork, resulting either in lesion repair or bypass. In the latter case DNA will retain the lesion which should be later removed by the excision repair.
- degradation of the duplex (Fig. 3., red box)
  Alternatively, after regression and restoring the duplex DNA at the lesion site, thereby providing possibility for repair of the lesion, the nascent-strand-duplex can be degraded by multiple exonucleases which can rapidly degrade free DNA ends.
- processing involving recombination (Fig. 3., green boxes)
  Restart of regressed replication forks may depend also on the homologous recombination. As the formed structure highly resembles Holliday junction, it can be processed by cleavage by the RuvABC complex. Resolution of the four-stranded structure by the cleavage would produce one intact duplex (the sealing of the nick by DNA ligase is needed) and a duplex with a dsDNA end. Processing of the dsDNA ends trough double strand break repair pathway mediated by the RecBCD recombination proteins would restore the replication fork by reassembly of the replication machinery at a D-loop and

replication can be restarted (Section 3.5.). However, direct processing of the extruding duplex by exonucleases, including RecBCD complex is also possible and will result in generation of an ssDNA tail which could initiate HR to remodel the complex structure into a D-loop.

After processing and remodelling of the regressed fork, reassembly of the replication machinery mediated by restart proteins will occur, generating a functional replisome to continue chromosome replication.

Figure 3. Replication fork blocked by a leading-strand- or a both-strand-lesion can regress forming a "chicken foot" structure (Purple box) and be restored by the following mechanisms (see text for details): Regressed fork reversal, (Blue boxes); Degradation of the extruded duplex by exonucleases, (Red box); Recombinational repair, (Green boxes).

3.2. Replication fork stabilization by HR proteins

The model proposed by Courcelle and Hanawalt, suggests yet another possibility for replication proteins to be involved in replication reactivation where HR proteins are necessary for replication fork stabilization and protection until DNA synthesis resumes, rather than strand exchange (Courcelle & Hanawalt, 2003). As mentioned above, after replicase stalling at the lesion, uncoupling of strands and formation of ssDNA prevents the lesion repair by excision pathway since the complement strand is missing (Fig 3B). The NER-mediated excision is essential to restore arrested replication forks after UV-induced DNA damage and in order to gain access to the lesion for NER enzymes, replication fork is proposed to regress similarly to the previously described model (Courcelle *et al*, 2005). However, there are several important differences. First, the regression depends on recombination proteins of the RecFOR pathway, including RecF, RecO, RecR, RecJ, and RecQ. It has been shown that after the replication fork

arrest by UV-damage, the nascent strand is partially degraded by the concerted actions of the 5′-3′ exonuclease RecJ and the RecQ helicase (Courcelle *et al*, 2006). Thus, instead of forming an extruding duplex, the nascent lagging strand is degraded. After the degradation, RecFOR proteins facilitate the RecA loading to promote strand pairing and to maintain replication fork integrity until the offending lesion is removed or bypassed (Courcelle *et al*, 2004). In the absence of RecFOR or RecA, the nascent strands at inactivated forks are extensively degraded (Chow & Courcelle, 2004). Second, it has been shown that while Pol III cores uncouple and dissociate from the stalled replisome, the helicase–primase (DnaB and DnaG) complex remains critically associated with the fork, while the above-discussed regression model implies the fork clearance (Jeiranian *et al*, 2013; Atkinson & McGlynn, 2009).
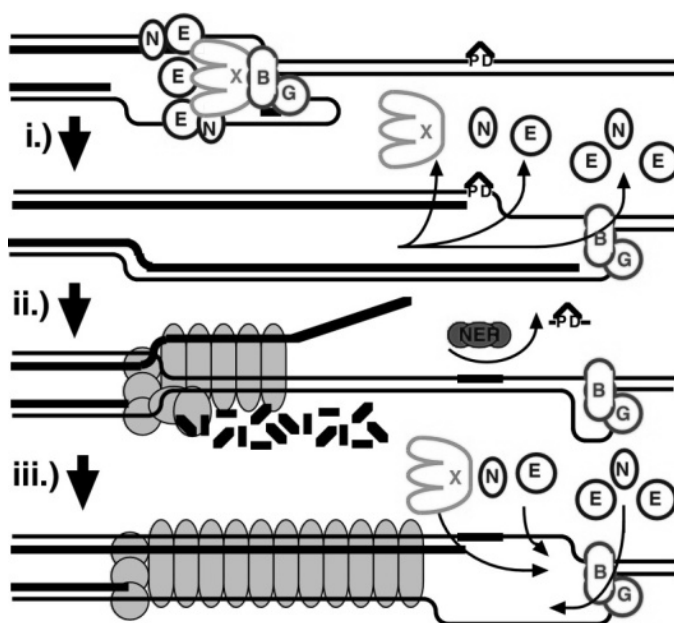
Figure 4. Model of HR proteins' role in stabilization of the replication fork stalled at UV-induced damage. (*i*) DNA synthesis becomes uncoupled and the polymerases transiently dissociate after replication fork encounters an arresting lesion (PD, pyrimidine dimer). (*ii*) DNA from the replication fork is processed and stabilized by the RecF-pathway proteins (gray circles) allowing repair enzymes (NER) or translesion polymerases to access the lesion. (*iii*) The helicase–primase complex remains bound to the template DNA, serves to maintain integrity of the replication fork and directs replisome reassembly once the lesion has been processed. Figure from (Jeiranian *et al*, 2013).

The lesions are predominantly excised by NER after the fork regression, whereas translesion synthesis by Pol V becomes important for replication to resume in the case of extensive DNA damage when repair capacity of the cell

has been exceeded (Courcelle *et al*, 2005). DNA polymerases Pol II and Pol IV have been shown not to contribute detectably to the restoration of DNA synthesis (Courcelle *et al*, 2005). However, another study demonstrates the involvement of Pol II in replication restart process via template switching mechanism. While in the wild-type strain replication is restarted in about 10 minutes, this process is delayed until 50 minutes in Pol II (PolB)-deficient cells (Rangarajan *et al*, 1999). This correlates well with induction timing of the TLS polymerases. Pol II is induced promptly after the SOS induction, as well as UvrAB proteins, whereas Pol V is expressed only after 45 minutes (Sommer *et al*, 1998; Michel, 2005).

3.3. Direct re-priming

Lately the evidence for discontinuous leading strand synthesis has been accumulating (Amado & Kuzminov, 2013; Yeeles & Marians, 2011). While the nature of the lagging strand synthesis allows bypassing of the lesion by simple re-priming of and reinitiating the next Okazaki fragment, the leading strand is synthesized continuously and the lesions that force replicative polymerase to stall and result in replication arrest need to be removed or bypassed by TLS polymerase in order for replication to resume. For example, in the absence of NER, DNA synthesis fails to recover after UV-irradiation at the dose of 27 J/m^2 (Courcelle *et al*, 2005). Since such UV-dose produces extensive DNA damage, estimated about a thousand pyrimidine dimers per chromosome, the removal of the damage appears to be critical for replication to restart (Rudolph *et al*, 2007). However, bacterial replisome seems to be tolerant to single lesions at the leading strand, since it appears that also the leading-strand lesions can be bypassed by re-initiating DNA synthesis downstream (Heller & Marians, 2006; Yeeles & Marians, 2011). *E. coli* primase, DnaG, has been shown to catalyze RNA primer synthesis on the leading-strand template on a variety of model fork structures (Heller & Marians, 2006). Such leading-strand priming can enable the replisome to effectively skip over leading-strand template lesions and resume DNA synthesis without strand regression or fork breakage. The demonstrated re-priming was occurred after PriC-dependent loading of DnaB helicase and implied chromosome disassembly after the block (Heller & Marians, 2006). Yet more elegant way to tolerate DNA damage has been reported recently, where replisome is not dismantled upon encountering a lesion but can hop over it. Yeeles et al., have shown that upon encountering a single, site-specific pyrimidine dimer or abasic site, replisome stops transiently but does not dissociate, and after a short time the leading- and lagging-strand synthesis was initiated downstream from the damage via a *de novo*, DnaG-dependent priming of leading-strand template independently of the replication restart proteins (Yeeles & Marians, 2011). Such replisome hopping over template lesions is proposed as the source of ssDNA gaps on the leading strands (Indiani & O'Donnell, 2013). Moreover, accumulation low-molecular-weight DNA replication intermediates

that come from both the lagging and the leading strands in the absence of induced DNA damage and with inactivated DNA excision repair suggest discontinuous replication mechanism also for the leading DNA strand (Amado & Kuzminov, 2013). Thus, the ability of the replisome to hop over the lesions would enable extreme fluidity and flexibility of the replisome encountering the impediments on its road, ensuring the forward progression while maintaining replication fork integrity (Kurth & O'Donnell, 2013).

3.4. Processing of the gaps

ssDNA gaps left behind opposite the lesion upon the lesion skipping both on the lagging an leading strands can be repaired either by homologous recombination or filled in by specialized translesion DNA polymerases. However, it should be noted that in either case the DNA damage is tolerated, not removed from DNA, and needs to be further processed by postreplication repair pathways.

It is suggested that ssDNA gaps are primarily handled by RecFOR pathway of recombinational repair (Kowalczykowski *et al*, 1994; Morimatsu & Kowalczykowski, 2003; Morimatsu *et al*, 2012). The RecA protein is the central protein for all recombination processes as it promotes the homology search and associated DNA strand exchange (Lusetti & Cox, 2002). The active form of the RecA is the ATP-bound nucleoprotein filament formed on DNA. *In vitro* assembly of such filaments occurs spontaneously, and does not require any accessory proteins (Galletto & Kowalczykowski, 2007). Still, in the context of the cell ssDNA regions of the chromosome are rapidly coated with single strand binding (SSB) proteins, preventing the assembly of RecA-filament. Thus, accessory proteins are needed to load RecA onto DNA since it is not able to outcompete SSB protein by itself. In order to assembly the RecA filament onto SSB-coated ssDNA gap the functions of RecFOR proteins are required. These three proteins specifically recognize a dsDNA-ssDNA junction flanking an ssDNA gap and recruit RecA protein to promote the RecA nucleoprotein filament formation in the 5´-3´ direction by displacing SSB proteins (Morimatsu & Kowalczykowski, 2003). Moreover, RecFOR proteins are shown to recognize RNA-DNA junction within an ssDNA gap and promote the RecA loading (Morimatsu *et al*, 2012). Such ssDNA gaps with RNA primer at its 5´ terminus would form when lagging strand synthesis is impeded and Pol III dissociates, which is compatible the role of RecFOR mediated recombination in the repair of lagging strand gaps.

Homology search by the RecA-coated ssDNA along a dsDNA proceeds by a highly coordinated process. ssDNA in the nucleoprotein filament is stretched and underwound and its structure exhibits three-base periodicity, thus implying that the homology check process occurs in a stepwise fashion involving base triplets (Savir & Tlusty, 2010; Chen *et al*, 2008b). After the homology has been established, a nick has to be introduced in dsDNA molecules to enable strand invasion and exchange. As a result, Holliday junction structure is formed and

subsequent branch migration provides an undamaged template for the filling of the gap.

Alternatively, the lesion on the template strand can be overcome by the gap filling with one of the specialized DNA polymerases which are able to perform synthesis past the lesion and incorporate a random or correct nucleotide opposite the damaged site via process termed translesion DNA synthesis (TLS) (Nohmi, 2006; Waters *et al*, 2009). However, it is not clear whether such synthesis would be accomplished by soluble DNA polymerases or involves some replisome components. Polymerase-switching within the replisome enables the restart of a stalled replication fork (Courcelle *et al*, 2004; Indiani *et al*, 2005; Friedberg *et al*, 2005) and will be discussed below (Section 3. 6.).

3.5. DSB repair

Double strand breaks (DSBs) can be formed by exogenous sources including ionizing radiation. Yet, reactivation of replication forks can also depend on DSB repair. First, persisting ssDNA gaps or nicks on the leading strand template can lead to replication fork collapse when reached by the replisome in the next replication. Such situation can be common when genome integrity is disturbed, e.g., in Pol I or ligase deficient cells, thereby making recombinational repair essential for conferring the viability, but can presumably occur also in normally grown cells (Gross *et al*, 1971; Cao & Kogoma, 1995; Morimyo & Shimazu, 1976; Cox *et al*, 2000). Moreover, cleavage of regressed fork can also generate DSBs (Section 3. 1.). Repair of DSBs involves either homologous recombination or nonhomologous end-joining (NHEJ) pathway, which is missing in *E. coli*, but can be found in many bacteria, including pseudomonads (Pitcher *et al*, 2007; Paris *et al*, 2015). Nevertheless, HR pathway is implied to be the major pathway for repair of DSB. As mentioned before, in order to recombinational processes to occur, RecA must be loaded onto ssDNA. DSB repair begins with by the 5′-3′ degradation of DSB ends to generate 3′ ssDNA tails. dsDNA ends are rapidly recognized by the RecBCD complex, which possesses both helicase and nuclease activities, and unwinds and degrades both strands of the duplex DNA until it encounters a specific site named Chi (for crossover hotspot instigator). Its interaction with the RecBCD complex switches the polarity of the RecBCD nuclease activity to the 5′ strand, leaving the 3′ strand intact and promotes loading of the RecA to the 3′ ssDNA tail to form the RecA nucleoprotein filament which will further initiate homology search and strand invasion to form the D-loop structure where replication restart proteins can bind (Dillingham & Kowalczykowski, 2008). Chi sites are frequent, regularly distributed, and overrepresented in bacterial genomes; however Chi sequences are not identical (El Karoui *et al*). The well characterized Chi site of *E. coli* is an octamer with the sequence 5′-GCTGGTGG-3′ and can be found every 4,5 kb in *E. coli* chromosome. A five-nucleotide Chi sequence in *Bacillus subtilis* is present every 0.35 kb, and its extended version (7 bp), once every

20 kb in its genome (El Karoui *et al*). In pseudomonads Chi sequence has not been yet identified (Smith, 2012).

Recently yet another role for ReBCD enzymes has been demonstrated. It appears that RecBCD is required to complete chromosomal replication by resecting over-replicated regions in a process that does not involve RecA or recombination processes (Courcelle *et al*, 2015).

3.6. SOS response and TLS polymerases

The cell senses the presence of DNA damage as ssDNA regions accumulate and become coated with RecA proteins. As mentioned above, two nonexclusive models exist to describe the source of ssDNA in the cell following DNA damage (Indiani & O'Donnell, 2013). The "helicase runaway" model proposes uncoupling of the leading and lagging strand synthesis while helicase continues unwinding the parent duplex after Pol III is stalled at the site of DNA damage on the leading strand. The alternative model implies that replisome may hop over template lesions, thereby leaving single-strand gaps. Regardless of its origin, RecA-coated nucleoprotein filament catalyzes the auto-proteolysis of the LexA transcriptional repressor which in the absence of DNA damage represses the genes belonging to the SOS regulon by binding to a consensus DNA sequence, termed LexA box, in the promoter regions of SOS-regulated genes. The size and "content" of the SOS-regulon varies between bacterial species, encompassing, for instance, about 40 genes in *E. coli* and 33 in *B. subtilis*, whereas only eight of them are common to both organisms (Courcelle *et al*, 2001; Au *et al*, 2005). Pseudomonads appear to have far fewer genes regulated by LexA: there are only 15 genes that constitute SOS-regulon in *P. aeruginosa* and 18 transcriptional units in *P. putida* (Cirz *et al*, 2006; Abella *et al*, 2007). Noteworthy, the nucleotide excision repair proteins encoding *uvrA*, *uvrB*, and *uvrD* which are among the first genes to be induced upon DNA damage in *E. coli* escape the LexA regulation and are constitutively expressed in *P. aeruginosa* whereas in *P. putida* expression of *uvrB* and *uvrD* is damage-inducible (Rivera *et al*, 1996, 1997; Cirz *et al*, 2006; Abella *et al*, 2007).

When LexA is inactivated, the level of expression of three of five *E. coli* polymerases, Pol II, Pol IV and Pol V, which can perform potentially mutagenic DNA synthesis across template lesions, is dramatically increased (Sutton, 2010; Napolitano *et al*, 2000). Most of UV-induced mutagenesis in *E. coli* depends on Pol V (Kato & Shinoura, 1977; Wrzesiński *et al*, 2005; Elledge & Walker, 1983). Pol V is functional as UmuD´$_2$C complex, where the catalytic activity of the UmuC subunit requires the RecA-mediated autocleavage of UmuD, to form a shorter active UmuD´ (Nohmi *et al*, 1988). The combination of UmuD´ homodimer with UmuC forms the active UmuD′$_2$C complex capable of TLS (Tang *et al*, 1999). The level of Pol V in non-induced cells is almost unde-tectable and reaches about 200 molecules per cell after LexA inactivation (Woodgate & Ennis, 1991). It is interesting to note that full-length UmuD is

involved in prevention of mutagenesis by UmuC or Pol IV, and the mutagenic UmuD′$_2$C complex forms only after 20 to 40 minutes after the initiation of the SOS response, which corresponds to the time of UmuD cleavage. Thus, the cleavage of UmuD acts as a switch in regulating the mutagenic state of a cell (Ollivierre *et al*, 2010). Moreover, all three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are capable of TLS and can be responsible for damage-induced mutagenesis, whereas catalyzing both error-free and mutagenic synthesis, implying that cells appear to use a pool of TLS DNA polymerases in order to bypass various DNA lesions (Napolitano *et al*, 2000).

In bacteria, where *umuDC* orthologs are absent, induced mutagenesis depends on the products of the mutagenesis cassette. DnaE2 is implicated in error-prone TLS in *Mycobacterium tuberculosis*, *Caulobacter crescentus, Deinococcus deserti*, and *P. aeruginosa* where it confers a mutator phenotype upon exposure to UV-irradiation (Boshoff *et al*, 2003; Galhardo *et al*, 2005). Deletion of the *imuA* or *imuB* genes also abolishes induced mutagenesis in *M. tuberculosis* and *C. crescentus,* implying that these proteins act together to perform TLS (Warner *et al*, 2010; Galhardo *et al*, 2005). Moreover, modifying catalytic residues of DnaE2 reproduces the *dnaE2* gene deletion phenotype, strongly implying that DnaE2 is directly involved in the mutagenic lesion bypass (Warner *et al*, 2010). ImuB function in TLS is independent of polymerase activity and it is proposed to mediate access of DnaE2, which itself does not bind β-clamp, to the replisome. Consistently with its role in regulating polymerase traffic in replisome, ImuB is shown to interact both with DnaE and DnaE2, as well as with the β-clamp. Interaction of the ImuB with β-clamp appears to be essential as its disruption significantly reduces induced mutagenesis and damage tolerance in *M. tuberculosis* (Warner *et al*, 2010). In *P. putida* ImuB and DnaE2 appeared to fulfil antagonistic roles in UV-induced mutagenesis, where DnaE2 had anti-mutator effect following UV-irradiation (Koorits *et al*, 2007), however DnaE2 appears to be essential for MMS-induced mutagenesis (Jatsenko *et al*., unpublished data).

Bacterial replisomes move with a speed of up to 1000 bp per second and thus, due to the low replication rate of the TLS polymerases, it is unlikely that they would replace Pol III in the context of a moving replisome. This is consistent with the observation that under conditions when SOS is not induced, TLS over a chromosomally introduced lesion accounts only for a minor fraction (0.5–3%) of events in comparison to damage avoidance (recombinational gap filling or template switching) events (Pagès *et al*, 2012). Notably, the study was performed in UvrA-deficient background to prevent the central pathway of the blocking lesion repair. However in case when repair capacity of the cell is overwhelmed due to severe DNA damage and replication is stalled, specialized polymerases could gain access to the replication fork and enable replication reactivation by the polymerase switching mechanism. All the five *E. coli* DNA polymerases, including translesion DNA polymerases Pol II, Pol IV and Pol V, are described to interact with replisome β-clamp (Vivona & Kelman, 2003).

Moreover, this interaction plays a major role during the TLS process since disruption of the interaction by modifying beta-clamp binding motif of polymerases strongly affects both error-free and mutagenic bypass activities of all three TLS polymerases (Becherel *et al*, 2002). One of the hypotheses to explain how TLS polymerases switch on a clamp of the replisome is regulated is the sliding-clamp toolbelt hypothesis. This proposes that β-clamp binds two different DNA polymerases, α subunit of replicative Pol III and Pol IV, simultaneously and Pol IV dynamically switches with stalled Pol III while Pol III can regain the control after the stall is relieved (Indiani *et al*, 2005). In a complex network of mechanisms, involved in regulation of the access of TLS polymerases to the replication, also Pol-Pol and β-clamp-DNA interactions contribute to polymerase switching. Such tight control reduces the probability of unwanted Pol at the replisome, which can be deleterious (Sutton, 2010). Several examples of the harm of Pol include lethal effect of Pol IV overexpression on the *E. coli* cell and suppression of conditional lethality of Pol III by inactivation of one or more specialized polymerases (Sutton, 2010; Uchida *et al*, 2008). One more interesting way of how the specialized polymerases could aid the cell to tolerate excessive DNA damage involves their ability to act as a molecular brake of replication fork progression when they gain access to the replisome, since both Pol II and Pol IV slow down the rate of DnaB helicase unwinding to 1 bp/s *in vitro* (Indiani *et al*, 2009). Such slow replication fork progression can give repair enzymes additional time to fix the damaged DNA which could lower further the frequency of replisome encounters with DNA lesions.

3.7. SOS response and antibiotic resistance

The levels of TLS polymerases are tightly controlled by SOS response due to their mutagenic potential of turning lesions into mutations. Still, mutagenesis can be beneficial to bacteria to adapt to the hostile environment and to survive under conditions of stress. One of the associated problems with hyper-mutability or induced SOS response of bacteria is associated with the development of resistance to various antimicrobials. For example in *E. coli*, the development of ciprofloxacin resistance in the presence of the drug requires LexA cleavage and all three TLS polymerases (Cirz *et al*, 2005). Moreover, ciprofloxacin-mediated DSB formation activates recombinational repair which could promote genome rearrangements. Such ROS-induced DSB repair was shown to be required for biofilm-mediated diversity and emergence of the anti-biotic-resistant *P. aeruginosa* (Boles & Singh, 2008). Noteworthy, antibiotics at the subinhibitory concentrations itself provide a source of stress for bacteria, thereby inducing SOS-response (Ysern *et al*, 1990; Yim *et al*, 2011; Gutierrez *et al*, 2013; López *et al*, 2007). Antibiotics may induce SOS response through different pathways such as blocking DNA replication by fluoroquinolones and trimetoprim, cell wall damage by β-lactams (in *E. coli* this stress is sensed through two-component system DpiBA), and targeting translation mechanisms,

e.g., by aminoglycosides, chloramphenicol, and tetracycline (Baharoglu & Mazel, 2014). In addition to its primary target, antibiotics also induce acute oxidative stress that can induce mutagenesis or even kill bacteria (Kohanski *et al*, 2010a, 2010b). Still, other research groups provide evidence that ROS do not play a role in killing of bacteria by antibiotics and its effect is rather bacteriostatic than bacteriocidal (Imlay, 2015; Keren *et al*, 2013; Liu & Imlay, 2013)

The outcome of the treatment of bacterial infections can thus depend on the pre-existing spontaneous mutations in the population, which could give a rise to antibiotic resistance, or be directly induced by antibiotic treatment or environmental conditions (e.g., elevated levels of ROS at the place of infection) and depend on the ability of cells to induce SOS response and acquire mutations. Understanding the process of induced mutagenesis has led to development of several strategies to combat SOS response and inhibit bacterial resistance (Baharoglu & Mazel, 2014). For instance, an engineered bacteriophage that suppresses the SOS response by over-expressing a non-cleavable LexA has been reported to enhance killing by quinolones, aminoglycosides and β-lactams in *E. coli* and could be used in combination with antibiotics to prevent SOS-induced mutagenesis (Lu & Collins, 2009). Another strategy to suppress SOS response is to inhibit RecA expression, which can be achieved by artificial small RNAs complementary to the *recA* mRNA (Sharma *et al*, 2013). Interestingly, opposite to ciprofloxacin, a DNA gyrase inhibitor, novobiocin, which also inhibits DNA gyrase, is involved in repression of the *recA* and *umuC* genes and inhibits the frequency of recombination and mutation in *Staphylococcus aureus*. Moreover, the combination of novobiocin with ciprofloxacin suppresses the ciprofloxacin-mediated induction of the *recA* gene expression (Schröder *et al*, 2013). Taken together, SOS response is one of the bacterial adaptation mechanisms which can increase fitness of the population in hostile conditions, but needs to be tightly controlled to prevent accumulation of deleterious mutations, as intermediate mutation frequencies can be most favourable for bacteria.

4. Reduced membrane permeability and efflux pumps as the damage protection mechanism

4.1. *Pseudomonas aeruginosa* as a model organism

While multiple ways exist to fight with the consequences of the DNA damage caused by various chemicals, there is an alternative strategy that cells can benefit from. This is to diminish the harmful effect of the damaging agents by reducing their cellular concentration. In this aspect, *P. aeruginosa* is an invaluable model organism for studying mechanisms that allow the cells to escape the damage. *P. aeruginosa* is a versatile organism, able to adapt to various environments and is well-known as a hospital-acquired opportunistic human pathogen

responsible for both acute and chronic infections with a high excess mortality rate (Sadikot *et al*, 2005; Lambert *et al*, 2011). Characterized by the high intrinsic resistance to antimicrobials, including antibiotics and disinfectants, the treatment of *P. aeruginosa* infections is further complicated by the ability of the organism to develop even higher level resistance to multiple classes of antibacterial agents and developing a multidrug resistance phenotype.

The intrinsic resistance of *P. aeruginosa* is mediated by a low membrane permeability that ensures the restricted passage of the drugs through the outer membrane and the efficient energy-dependent efflux to extrude the drug molecules and reduce the cellular drug concentration (Poole, 2011; Strateva & Yordanov, 2009). Additionally, *P. aeruginoga* possesses a chromosomally encoded β-lactamase AmpC (cephalosporinase) (Lodge *et al*, 1990), a hydrolytic enzyme that disrupts β-lactam ring of β-lactam antibiotics and renders them ineffective (Jacoby, 2009), thus contributing to intrinsic resistance to a number of β-lactam antibiotics (e.g., benzylpenicillin (penicillin G) and narrow-spectrum cephalosporins. Activity of AmpC is additionally induced by these β-lactams and this enzyme is resistant to β-lactamase inhibitors used in clinical practice (Nordmann & Guibert, 1998; Poole, 2011)

The acquired high level antimicrobial/multidrug resistance involves two major strategies: (i) acquisition of additional enzymes to efficiently degrade antimicrobial molecules (plasmid- or integron-encoded extended spectrum-β-lactamases and aminoglycoside modifying enzymes) (ii) mutation of endogenous genes. Overexpression of the chromosomal efflux pumps and OprD porin downregulation can reduce the cellular concentration of the drugs (Strateva & Yordanov, 2009; Aghazadeh *et al*, 2014). Modification of the antibiotic target (e.g., DNA gyrase or DNA topoisomerase IV for fluoroquinolones or RNA polymerase for rifampicin) renders the cells unresponsive to certain antibiotics (Lister *et al*, 2009). Mutational modification and evolvement of enzymes, like extended-spectrum β-lactamases AmpC, and their overexpression has been also identified as one of the resistance mechanisms (Rodríguez-Martínez *et al*, 2009a; Juan *et al*, 2005; Tam *et al*, 2007)

4.2. OprF and OprD porins and membrane permeability

The outer membrane (OM) of Gram-negative bacteria comprises the first line of defence against toxic compounds. Semipermeable outer membrane allows the influx of nutrient molecules such as sugars, ions, and amino acids to support growth; it also serves as a selective barrier to prevent the entry of noxious compounds. Semipermeability of outer membranes depends and is controlled by the presence of porins, the proteins that form water-filled channels across the outer membrane lipid bi-layer to allow the passive penetration of different types of hydrophilic molecules (Nikaido, 2003). According to their activity, porins can be classified into general/non-specific porins, substrate-specific porins, ligand-gated porins, and efflux porins (Hancock & Brinkman, 2002), while the

first two can be considered as the main porins, attributed to uptake of the antibiotics and other chemicals. General diffusion porins own poor substrate selectivity and allow the nonspecific diffusion of small hydrophilic molecules. Specific porins differ from general porins by possessing a substrate-specific binding site that facilitates the diffusion of bound molecules at a higher rate than other molecules of comparable size (Nikaido, 2003).

OmpF, OmpC and PhoE are classical non-specific porins, studied in detail in *E. coli* (Nikaido, 2003). Classical porins exist as transmembrane trimer β-barrels, and form highly permeable, mostly open porin channels. However, many environmental bacteria which are constantly exposed to antimicrobials have evolved lower OM permeability by using alternative less permeable porins as major nonspecific porins (Sugawara *et al*, 2012).

P. aeruginosa outer membrane is poorly permeable, challenging the efficient diffusion of the drug molecules into the cells. It has been shown that OM of *P. aeruginosa* is up to 100 fold less permeable than that of *E. coli*, resulting in a slow uptake and a lower antibiotic susceptibility (Yoshimura & Nikaido, 1982; Angus *et al*, 1982; Hancock & Brinkman, 2002). Such limited permeability is associated with the distinct physico-chemical properties of the porins found in the outer membrane of pseudomonads compared with the porins of the *Enterobacteriaceae* (Pagès *et al*, 2008). *P. aeruginosa*, as well as other bacteria from genus *Pseudomonas*, does not produce high-permeability classical porins. Instead, the organism utilizes the major nonspecific porin OprF, which allows very slow diffusion of solutes (Hancock *et al*, 1979; Nikaido *et al*, 1991; Bellido *et al*, 1992; Sugawara *et al*, 2012). The distinct characteristic of the OprF is the ability to form channels that are wider than the channels of *E. coli* porins to allow the diffusion of large molecules. Nevertheless, the diffusion rates of small molecule are much slower than in *E. coli*. Such controversy is attributed to the fact that OprF can fold into two distinct conformers, existing mostly in weakly conductive conformation with only small fraction (about 5%) of molecules forming fully open channels (Sugawara *et al*, 2006; Nestorovich *et al*, 2006). Additionally, the pore size of OprF has been demonstrated to be dependent on growth temperature in *P. aeruginosa*, *P. putida* and *P. fluorescens* (Jaouen *et al*, 2004).

Deficiency in OprF results in growth problems at low-osmolarity conditions and a significantly shortened cell length, reflecting its importance in maintaining the structural integrity of the outer membrane (Gotoh *et al*, 1989). OprF is associated with the low intrinsic membrane permeability but is rarely involved in the acquired drug resistance. Still, the loss of OprF in the multiple antibiotic resistant strain of *P. aeruginosa* has been reported (Pumbwe *et al*, 1996; Chamberland *et al*, 1990).

OprD is another porin found in the outer membrane of *P. aeruginosa* which is important for the drug resistance. Unlike OprF, the loss of OprD is dispensable for growth of the organism, and on the contrary can result in the better fitness of bacteria (Skurnik *et al*, 2013). OprD is a specific porin that

possesses a binding site for basic amino acids and facilitates their diffusion but also promotes the entry of antibiotics from carbapenem family, because of their structural resemblance (Trias & Nikaido, 1990; Huang & Hancock, 1993). The loss or alteration of the porin OprD is the major mechanism for development of acquired resistance to carbapenems, notably the broad-spectrum β-lactam imipenem (Lynch *et al*, 1987; Rodríguez-Martínez *et al*, 2009b; Lee & Ko, 2012; Giske *et al*, 2008; Wang *et al*, 2010). The loss of OprD and the emergence of antibiotic resistance are mostly attributed to inactivating mutations of the *oprD* gene and the upstream promoter region (Rodríguez-Martínez *et al*, 2009b). However, multiple alternative ways exist to regulate the expression of OprD. The channel can be both induced and repressed. Basic amino acids, such as arginine, glutamate, histidine, and alanine can induce *oprD* expression (Ochs *et al*, 1999a). The presence of heavy metals, such as zinc and copper, however, reduces the expression of the *oprD*, thereby increasing the resistance to carbapenems (Perron *et al*, 2004; Conejo *et al*, 2003; Caille *et al*, 2007).

OprD expression can also be negatively regulated in a common pathway with the induction of the multidrug efflux system MexEF-OprN (Köhler *et al*, 1999; Ochs *et al*, 1999b). Being a positive regulator of the *mexEF-oprN* operon, MexT is also involved in the repression of OprD (Köhler *et al*, 1999). As will be discussed later, MexEF-OprN efflux system is overexpressed in *nfxC*-type mutants of *P. aeruginosa* and confers resistance to quinolones and some other antibiotics (Section 4.2.3.), (Köhler *et al*, 1997). The co-regulation of MexEF-OprN and OprD results additionally in cross-resistance of *nfxC*-type mutants to the carbapenems (Köhler *et al*, 1997).

4.3. Energy-dependent efflux

Reduction of the accumulation of the drugs and other toxic compounds in bacterial cells can also be achieved through the active export of the chemicals by the efflux pumps. The most important systems in the efflux of toxic compounds in *P. aeruginosa* are members of the resistance-nodulation-division (RND) family (Poole, 2004). RND efflux systems function as secondary transporters, utilizing proton or sodium gradient as a source of energy to extrude the noxious compounds. The best-characterized RND pump in *E. coli* is AcrAB-TolC (Nikaido, 2009). In *P. aeruginosa* there are 12 RND efflux systems, while four of them MexAB–OprM, MexCD–OprJ, MexEF–OprN and MexXY–OprM have been linked to clinically relevant resistance (Lister *et al*, 2009). The name Mex stands for the multiple efflux. The pumps exist as three-component systems, consisting of a cytoplasmic membrane (RND) transporter (MexB, MexD, MexF and MexY) that operates as an energy-dependent pump, a periplasmastic membrane fusion protein (MFP) (MexA, MexC, MexE and MexX) and an outer membrane efflux porin (outer membrane factor (OMF)) (OprM, OprJ, OprN) (Fig. 5). The genes encoding efflux system components

are organized into operons with the similar organization *mfp-rnd-(omf)*. One of the exceptions is the MexXY–OprM efflux pump, which utilizes OprM, encoded in the *mexAB–oprM* operon as OMF since the gene encoding OMF is missing from the *mexX–mexY* operon. OprM, thus, functions in both MexAB–OprM and MexXY–OprM complexes. Efflux systems are regulated by the regulator proteins and mutations in regulator genes allow to overexpress the efflux-pumps and mediate multi-drug-resistance (Guénard *et al*, 2014; Adewoye *et al*, 2002; Cao *et al*, 2004; Köhler *et al*, 1999; Poole *et al*, 1996a).
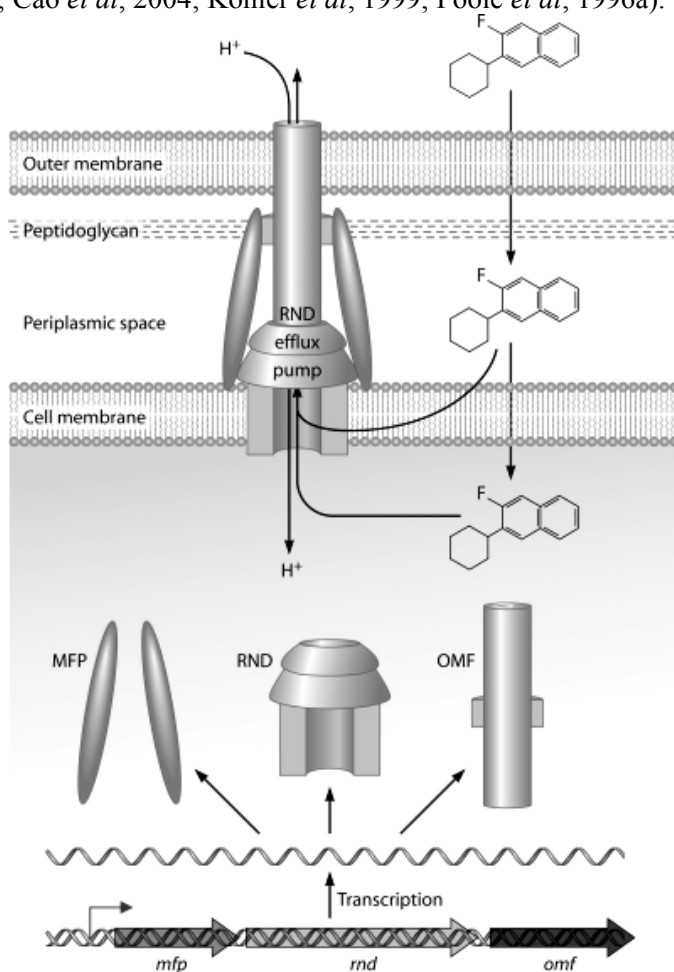
Figure 5. Structure and organization of tripartite RND efflux systems in *P. aeruginosa* RND pumps typically consist of an RND cytoplasmic membrane transporter (RND), a membrane fusion protein (MFP), and an outer membrane factor (OMF), usually encoded in one operon. The complex forms a channel spanning through membrane, allowing the active efflux of lipophilic and amphiphilic drugs from the cell. Efflux of the fluoroquinolone is shown. Adapted from Lister, Wolter and Hanson (2009)

4.3.1. MexAB-OprM efflux system

MexAB-OprM was the first multidrug efflux system identified in *P. aeruginosa* (Poole *et al*, 1993). The *mexAB-oprM* operon is expressed constitutively and contributes to the intrinsic resistance of bacterium to a variety of toxic substances (Li *et al*, 1995). Substrates for the MexAB-OprM system include fluoroquinolones, β-lactams, β-lactamase inhibitors, tetracyclines, chloramphenicol, aromatic hydrocarbons and, additionally, homoserine lactones associated with quorum sensing (Lister *et al*, 2009). Expression of the *mexAB-oprM* genes can be regulated in several ways. Mutational events within regulatory genes can drive the overproduction of the MexAB–OprM efflux system, being one of the major problems in development of multidrug resistance in clinical isolates (Ziha-Zarifi *et al*, 1999; Aghazadeh *et al*, 2014; Terzi *et al*, 2014; Kiser *et al*, 2010).

MexR protein is a major negative transcriptional regulator of the *mexAB-oprM* operon. Expression of the MexR represses *mexAB–oprM* and *mexR* itself, which is located directly upstream of the *mexAB-oprM* genes but is transcribed in opposite direction (Poole *et al*, 1996b). *nalB*-, *nalC*- and *nalD*-type multidrug-resistant mutants overexpressing *mexAB-oprM* have been described (Lister *et al*, 2009). *nalB*-type *P. aeruginosa* strains, highly resistant to fluoroquinolones, chloramphenicol, and most classical β-lactam antibiotics, carry mutations within the *mexR* gene which compromises the MexR repressor activity and enhances MexAB-OprM levels (Adewoye *et al*, 2002; Kiser *et al*, 2010; Saito *et al*, 1999). Apart from mutations, activity of the MexR and the levels of *mexAB-oprM* transcription can be modulated by oxidative stress conditions (Chen *et al*, 2008a, 2010). MexR has been described to serve as a sensor of oxidative stress. Oxidation of the MexR triggers dissociation of oxidized repressor from the *mexAB-OprN* promoter and derepression of the drug efflux operon to extrude the chemicals potentially inducing the oxidative stress. *nalC*-type mutants overexpress the MexAB-OprM pump less extensively than the *nalB*-type strains (Srikumar *et al*, 2000). *nalC*-type phenotype involves a secondary regulation and is associated with mutation in the *nalC* gene, coding for a TetR family repressor. Inactivation of NalC allows the expression of ArmR, a protein that interacts and inhibits the MexR repressor (Cao *et al*, 2004; Daigle *et al*, 2007). Finally, overexpression of *mexAB-oprM* in *nalD*-type mutants is due to disruption another regulator gene, *nalD*, encoding a second repressor of the *mexAB-oprM* genes (Sobel *et al*, 2005a; Morita *et al*, 2006a).

Moreover, expression of the *mexAB-oprM* cluster has been demonstrated to be growth-phase-dependent, regulated independently of the MexR repressor (Evans & Poole, 1999) and suggested to involve a quorum sensing signal (Sawada *et al*, 2004). Consistently, it has been demonstrated that addition of quorum-sensing autoinducer N-butyryl-L-homoserine lactone (C4-HSL), indeed, enhances the expression of the MexAB-OprM efflux system (Maseda *et al*, 2004). Moreover, β-lactam hypersusceptibility in *nfxC*-type mutants is associated with the repression of the quorum sensing-mediated enhancement of

the *mexAB-oprM* operon expression via efflux of cell-signaling intermediates (Maseda *et al*, 2004; Tian *et al*, 2009a).

Orthologs of the *mexAB-oprM* genes in *P. putida* are *ttgAB-ttgC*. These genes code for the efflux system which is involved in toluene tolerance and has a broad substrate specificity, including antibiotics ampicillin, carbenicillin, tetracycline, nalidixic acid and chloramphenicol (Ramos *et al*, 1998; Terán *et al*, 2003)

4.3.2. MexCD–OprJ efflux system

The MexCD–OprJ pump is an envelope stress-inducible multidrug efflux system and is generally repressed in wild-type cells under normal growth conditions. Although fluoroquinolones, tetracycline, chloramphenicol, streptomycin, can be efficiently extruded by this efflux pump, it does not contribute to intrinsic antimicrobial resistance in *P. aeruginosa* and is not induced in their presence (Lister *et al*, 2009; Morita *et al*, 2001). Consistently, deletion of the *mexCD-oprJ* genes does not affect its susceptibility to antimicrobials (Srikumar *et al*, 1997; Morita *et al*, 2001).

Transcription of the *mexCD-oprJ* genes is strictly repressed by a negative regulator encoded by the *nfxB* gene and the overexpression of the *mexCD-oprJ* operon occurs in *nfxB*-type mutants (Poole *et al*, 1996a; Shiba *et al*, 1995). The name Nfx refers to the characterization of the mutations producing resistance to norfloxacin (nfx) (Hirai *et al*, 1987). Various types of mutations in the *nfxB* gene render both laboratory strains and clinical isolates of *P. aeruginosa* resistant to multiple antibiotics, including quinolones while often being associated with hypersusceptibility to β-lactams and aminoglycosides (Hirai *et al*, 1987; Masuda *et al*, 1995; Jalal *et al*, 2000). The characteristic of the isolated flouro-quinolone-resistant *nfxB*-type mutants is the appearance of a 54-kilodalton outer membrane protein, which has been determined to be outer membrane protein OprJ (Hirai *et al*, 1987; Hosaka *et al*, 1995). The increasing levels of OprJ in various *nfxB*-type mutants have been shown to correlate with the susceptibility to certain antibiotics (Hirai *et al*, 1987; Masuda *et al*, 1996). Similarly to *mexR*, encoding a negative regulator of the *mexAB-oprM* operon, *nfxB* is located upstream the efflux genes and is transcribed in opposite direction (Shiba *et al*, 1995; Purssell & Poole, 2013). NfxB is also shown to functions as a repressor of a recently identified second negative regulator of the *mexCD-oprJ* operon, EscR, encoded by PA4596 and located downstream of *oprJ* (Purssell *et al*, 2015). EscR is induced under envelope stress conditions and is suggested to moderate envelope stress-inducible expression of *mexCD-oprJ* (Purssell *et al*, 2015). Induction of the MexCD–OprJ system can occur also in wild-type strains following the exposure to membrane-damaging agents such as clinically important disinfectants (e.g., chlorhexidine), implying that the use of disinfectants in hospitals can contribute to development a higher intrinsic drug resistance even without additional mutations (Morita et al., 2003).

4.3.3. MexEF-OprN efflux system

Similarly to the MexCD-OprJ system, the MexEF-OprN efflux pump contributes to acquired, not intrinsic resistance to antimicrobials. The MexEF-OprN efflux system differs from the other efflux systems in that unlike the majority of RND-type efflux systems, which are negatively regulated, the expression of the *mexEF-oprN* genes is positively regulated by a LysR family transcriptional activator MexT. The *mexT* gene is located right upstream of the efflux operon and is transcribed in the same direction (Fig. 6) (Köhler *et al*, 1997, 1999). Cells overexpressing the *mexEF-oprN* genes are referred to as *nfxC*-type mutants, where overproduction of the MexEF-OprN efflux pump renders *P. aeruginosa* resistant to fluoroquinolones and carbenicillin (Masuda *et al*, 1995; Fukuda *et al*, 1990). Moreover, MexT represses the OprD porin-encoding *oprD* gene causing a cross-resistance to β-lactam imipenem (Ochs *et al*, 1999b). *nfxC*-type mutants, similarly to *nalB*-and *nfxB*-type mutants, were isolated as spontaneous quinolone-resistant strains (Fukuda *et al*, 1990; Hirai *et al*, 1987). Although all three phenotypes provide *P. aeruginosa* resistance to quinolones, their resistance patterns and the profiles of outer membrane proteins are different.

Intact MexT is needed for the expression of the *mexEF-OprN* genes (Köhler *et al*, 1999; Sobel *et al*, 2005b). However, some strains that possess intact *mexT* sequence do not overproduce the MexEF-OprN efflux pump and need additional mutation to express *nfxC*-phenotype, which implies that MexT is additionally regulated (Maseda *et al*, 2000; Tian *et al*, 2009a; Sobel *et al*, 2005b; Köhler *et al*, 1999). Since overexpression of the MexEF-OprN efflux pump involves also mutations which are not necessarily linked to *mexT, nfxC* is therefore considered as a phenotype (Fig. 6).

Variation of the *mexT* gene occurs in different *P. aeruginosa* strains used as wild-type strains in laboratories worldwide (Maseda *et al*, 2000). Most of the laboratory *P. aeruginosa* strains as well as the reference strain PAO1 sequenced in the Genome Project (designated as PAO1-GP or PAO1-UW (University of Washington)) possess a non-functional *mexT* gene and thus do not express MexEF-OprN (Köhler *et al*, 1999; Maseda *et al*, 2000). The sequence of the PAO-UW *mexT* gene reveals an 8-bp nucleotide insert resulting in an out-of-frame sequence and inactive MexT (Maseda *et al*, 2000). Differences in the *mexT* sequences and additional regulatory genes account for different mechanisms involved in development of the *mexEF-oprN* genes-overexpressing *nfxC*-type mutants.

Currently, two pathway of MexT-mediated regulation of the MexEF-OprN efflux pump expression has been suggested (Uwate *et al*, 2013; Sobel *et al*, 2005b). First, the *nfxC*-type mutants evolved from parent strains carrying the intact *mexT* allele is often associated with additional mutation in the nearby located *mexS* gene in several clinical isolates (Fig. 6A) (Sobel *et al*, 2005b; Uwate *et al*, 2013). MexS is a putative oxidoreductase/dehydrogenase homo-logue and alterations in the cellular redox state associated with a *mexS* mutation

may induce activity of MexT, implying MexT functions as a redox-responsive regulator (Fargier *et al*, 2012). Consistently, it was reported that introduction of an intact MexS into *nfxC*-type clinical isolates to reverse both the enhanced *mexE* expression and multidrug resistance of the *nfxC*-type cells to the wild-type level (Sobel *et al*, 2005b). However, introduction of the intact MexS was not able to restore wild-type phenotype of three laboratory-derived *nfxC*-type mutant, implicating a second, MexS-independent pathway of the MexT-mediated regulation of the *mexEF-oprN* expression (Fig. 6B).

Moreover, MvaT-dependent negative regulation of *mexEF-oprN* expression has been shown not to involve MexT or MexS, indicating several levels of regulation of this efflux system (Westfall *et al*, 2006).

A. MexS-mediated pathway
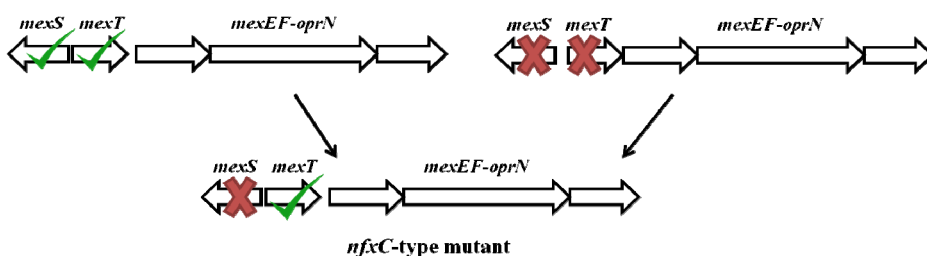
B. MexS-bypassed pathway
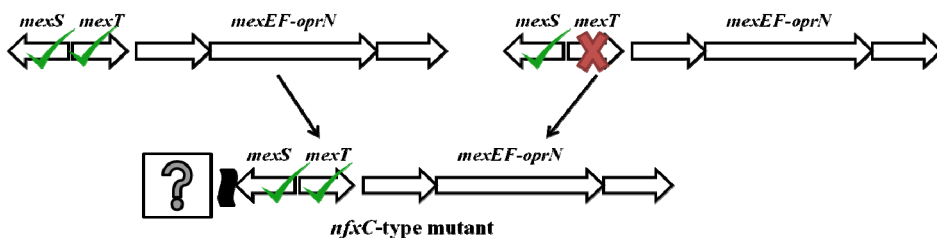
Figure 6. Two routes of MexT-mediated expression of the *mexEF-OprN* operon. In both pathways the intact *mexT* sequence is essential. The unimpaired *mexT* gene can pre-exist (the left part of the pictures) or be produced mutationally (the right part of the pictures). Additional mutation in *mexS* (panel A) or yet unidentified mutation (panel B) is needed to produce *nfxC*-type phenotype in *P. aeruginosa*

Understanding the regulation of efflux systems is complicated by the elaborate interplay and co-regulation processes. Besides being a major regulator of the *mexEF-OprN* operon expression, MexT is involved in the regulation of several other genes and associated phenotypes, being regarded as a global regulator

(Fig. 7) (Lister *et al*, 2009; Tian *et al*, 2009a, 2009b). First, as mentioned above, MexT is shown to downregulate the expression of OprD at the transcriptional and posttranscriptional levels, causing a significant reduction in the amount of OprD in *nfxC*-type mutants. The decrease of the porin OprD in the outer membrane is associated with the cross-resistance of the *nfxC*-type mutants to carbapenems (e.g., imipenem) (Ochs *et al*, 1999b; Köhler *et al*, 1999).

Additionally, the levels of extracellular quorum sensing (QS)-dependent virulence factors such as elastase and rhamnolipids along with motility deficiency are affected by the MexT-dependent overexpression of the MexEF-OprN (Köhler et al., 2001). Active extrusion of precursor of signal molecules by this efflux system, indeed, has been shown to be responsible for the downregulation of QS-regulated genes (Olivares et al., 2012). On the other hand, MexT regulates genes associated with type III secretion system (T3SS) and pyocyanin production independently of the MexEF-OprN system (Tian et al., 2009b; Jin et al., 2011; Olivares et al., 2012). The role of MexT as a global regulator was also predicted from the fact that *mexT* gene is not linked to the *mexEF-oprN* orthologs in other pseudomonads. For instance, the *mexT* (PP2826) and *mexEF-oprN* (PP3425-PP3427) genes can be found in different loci in the genome of *P. putida* KT2440 (Tian et al., 2009b). Transcriptome profiling has further demonstrated that *mexS*, and 12 other genes were upregulated by overexpressing *mexT* independently from the MexEF-OprN system, confirming its role as a global regulator (Tian et al., 2009b).

Interestingly, although the *nfxC*-type mutants overexpressing *mexEF-oprN* are resistant to chloramphenicol and fluoroquinolones, they are hypersusceptible to certain β-lactams and aminoglycosides (Fukuda *et al*, 1990; Sawada *et al*, 2004; Köhler *et al*, 1997). β-lactam hypersusceptibility is proposed to be associated with the repression of the QS-mediated enhancement of the MexAB-OprM system in the *nfxC*-type mutants (Fig. 7) (Maseda *et al*, 2004).
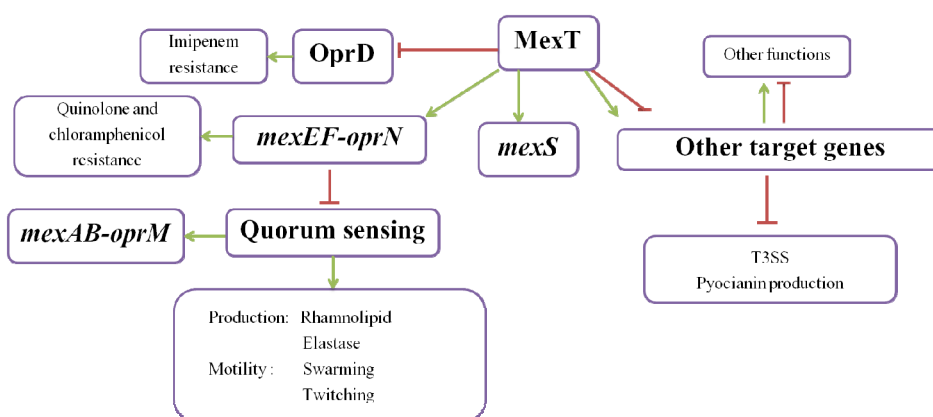
Figure 7. The global regulatory network of MexT. Green arrows indicate a positive effect, and red bars a negative effect (modified from (Tian *et al*, 2009a)).

4.3.4. MexXY-OprM efflux system

The operon encoding the MexXY-OprM efflux system lacks a gene coding for an outer membrane protein, but the efflux system is able to associate with OprM which is encoded by the constitutively expressed operon *mexAB-oprM* (Mine *et al*, 1999). Interestingly, the multidrug resistant *P. aeruginosa* PA7 possesses a unique gene (*oprA*) encoding an outer membrane channel downstream of *mexXY*, which is absent in most *P. aeruginosa* strains, and the MexXY component in this strain utilizes either the OprA or OprM outer membrane channel (Morita *et al*, 2012).

The MexXY efflux system substrates include antibiotics such as macrolides, fluoroquinolones and tetracyclines (Morita *et al*, 2012). In addition, the MexXY-OprM system is the only pump to mediate aminoglycoside resistance in *P. aeruginosa* (Poole, 2011; Aires *et al*, 1999). The MexXY efflux system is inducible with antimicrobials that target the ribosome and cause ribosome disruption or defects in translation (e.g., chloramphenicol, tetracycline, erythromycin, and kanamycin), and those antimicrobials that do not affect ribosomes fail to induce this efflux pump (fluoroquinolones and β-lactams) (Morita *et al*, 2006b).

Multidrug resistant *P. aeruginosa* clinical isolates have often been reported to be MexXY overproducers (Llanes *et al*, 2004; Xavier *et al*, 2010; Aghazadeh *et al*, 2014; Terzi *et al*, 2014; Pasca *et al*, 2012; Guénard *et al*, 2014). The pathways that lead to the overexpression of the efflux system MexXY involve the TetR family transcriptional repressor MexZ, the MexZ anti-repressor ArmZ which is induced upon exposure of the cells to subinhibitory concentrations of ribosome targeting antibiotics and reactive oxygen species (ROS), and ArmZ-independent two-component regulatory system ParRS. However, most of the clinical MexXY-overproducing strains harbor mutations that inactivate the *mexZ* repressor gene (Fraud & Poole, 2011; Guénard *et al*, 2014; Morita *et al*, 2006b; Matsuo *et al*, 2004)

RESULTS AND DISCUSSION

AIMS OF THE STUDY

A complex network of DNA damage repair and tolerance mechanisms operates in bacterial cells to ensure prominent maintenance of genome integrity. Highly efficient, this process begins with faithful DNA replication and proceeds with repair of DNA damage. Certainly, this is not an easy task, given the high amount of DNA damage which bacteria are confronted with in natural environment and plenty of endogenous DNA lesions that emerge during the normal metabolism. Homologous recombination (HR) is one of the fundamental DNA repair strategies and is an important back-up mechanism to tolerate and repair DNA damage. The fact that the genome integrity needs to be constantly maintained by recombinational repair is supported by the evidence that the recombination-deficient cells display substantial lethality (*recA*, *recBC*), accumulation of dsDNA breaks (DSBs) (*recBC*) and degradation of chromosomes (*recA*) even in the absence of exogenous damage (Michel *et al*, 1997; Capaldo-Kimball & Barbour, 1971; Skarstad & Boye, 1993). Consequently, mutations and recombinational events arise even during normal growth but can be enhanced upon certain stress conditions.

Here, to study genome maintenance mechanisms, we used as model organisms *Pseudomonas putida* and *Pseudomonas aeruginosa*, two representatives of genus *Pseudomonas*, which constitutes a large diverse group of ubiquitous, mostly saprophytic bacteria found widely in the environment. Notably, although characterized in detail in *E. coli*, many DNA repair and damage response processes can differ between bacterial species.

As the availability of valid assays often limits the research opportunities, we started from creating a novel assay for studying recombinational processes in *P. putida* chromosome and investigated the factors that influence the frequency of HR between different chromosomal loci in *P. putida* genome. Then we assessed the importance of nucleotide excision repair (NER) and DNA polymerase I (Pol I) for maintenance of genome integrity and avoidance of mutations in *P. putida*. Additionally, the reduction of the amounts of damaging agents in the cell as a damage prevention mechanism was studied in *P. aeruginosa*.

I. ASSAYS TO MONITOR HR IN *P. PUTIDA* (REFERENCE I AND II)

Generally regarded as an error-free DNA repair mechanism, HR acts together with other DNA damage repair and tolerance systems to ensure cell survival in the presence of DNA damage. HR is used by cells to reactivate collapsed replication forks, repair ssDNA gaps and dsDNA breaks. Thus, alteration in HR frequency can be used as an indicator of perturbed genome integrity and the reduced ability to cope with DNA lesions, unravelling the importance of various proteins and systems for efficient genome maintenance.

Several assays developed to study HR and associated conditions in *E. coli* make use of the ability of this bacterium to utilize lactose as a carbon source. Recombinational events in this case can be detected by the ability of bacteria to use lactose after HR between two non-functional *lacZ* alleles, which produce a functional *lacZ* gene (Konrad, 1977; Veaute *et al*, 2005; Elez *et al*, 2007; López *et al*, 2007; López & Blázquez, 2009). However, an experimental system to monitor HR in bacteria not able to metabolize lactose had been missing for a long time. We have introduced experimental assays to study HR in *P. putida*, where we have employed the potential of *P. putida* to utilize aromatic compounds as a carbon source (Ref. I and Ref. II). The first assay was designed to assess the frequency of HR between a chromosome and a plasmid and enabled us to study HR in both growing and stationary-phase *P. putida* cells (Ref. II). Briefly, two non-functional phenol monooxygenase-encoding *pheA* alleles were introduced into a chromosome and a plasmid in *P. putida* cells. HR events between these alleles which restored the functionality of the *pheA* gene could be detected, i. e. the recombinants expressing the active phenol monooxy-genase were able to use phenol as a sole carbon source and could be selected for the ability to grow on phenol minimal plates. Using this assay we have demon-strated that HR between a chromosome and a plasmid is increased during pro-longed starvation of the cells in the presence of phenol and that reduction of reactive oxygen species (ROS) during starvation period could suppress HR frequency, which implied that ROS is an important factor inducing HR in resting cells. Additionally, we have determined that the frequency of HR varied depending on the location of HR target across chromosome, being lower in the strains where the cassette containing the *pheA* allele was flanked by DNA regions with AT content higher than average in the genome. These regions were enriched in binding sites for a subset of nucleoid-associated proteins (NAPs). From these results we concluded that binding of these proteins could influence the local structural organization of the genome, affect the accessibility of the chromosomal DNA to HR and thereby the frequency of HR events (Ref. II).

However, replication mechanism, size and structural organization of a plasmid are different from that of the chromosome which imposes possible differences regarding regulation of HR process between an extrachromosomal DNA and a chromosome and intrachromosomal recombination. To investigate

HR between chromosomal loci we have adapted the previous assay to monitor HR between two non-functional *pheA* alleles located in various positions in the *P. putida* chromosome (Ref. I). The assay comprised two gene cassettes randomly inserted into *P. putida* chromosome within mini-Tn*5* transposon (Fig. 8A, 8B, 8C). The first cassette, named PaWterpheBA, is identical to the one used in the plasmidial assay, and contains the functional *pheBA* operon encoding phenol degradation genes but these genes are not transcribed due to the lack of an active promoter (Fig. 8A). In this study the location of the promotorless *pheBA* genes was fixed as we used the strain PaWTerphe2 that bears the PaWTerpheBA cassette in the locus PP4519 (Ref. I) to further introduce the other *pheA* allele into various locations of the chromosome (Fig. 8C). The second cassette, TacprpheA+2, contains the *pheA* actively transcribed from the strong constitutive P$_{tac}$ promoter, however, this *pheA* allele is inactivated by the +2 frameshift mutation in the coding sequence of *pheA* (Fig. 8B). Likewise in the previous assay, HR between two non-functional *pheA* alleles restores the functionality of the latter allele and enables *P. putida* cells to form colonies on phenol minimal plates where phenol is present as a sole source of carbon and energy.

In addition to HR, mutational events such as a -2 frameshift mutation in TacprpheA+2 or mutational activation of a promoter region of PaWterpheBA could also generate phenol-degrading Phe[+] revertants. To ensure that the emerging Phe[+] colonies represent recombinational events we measured the appearance of such revertants in cells containing each of two non-functional *pheA* alleles alone (PaWTerpheBA or TacprpheA+2). For the experiment about 1×10^9 tester cells were plated on a phenol minimal plate. As anticipated, spontaneous restoration of the *pheA* alleles by mutation appeared almost undetectable in both cases and a significant amount of Phe[+] colonies emerged only in case when both alleles were present in the chromosome (Fig. 9). Additionally, the frequency of HR events in a RecA-defective derivative of the tester strain with both *pheA* alleles remained comparable to that of PaWTerphe2 (data not shown), implying that the emergence of Phe[+] revertants in this assay is fully dependent on functional RecA and that our assay detects HR events.
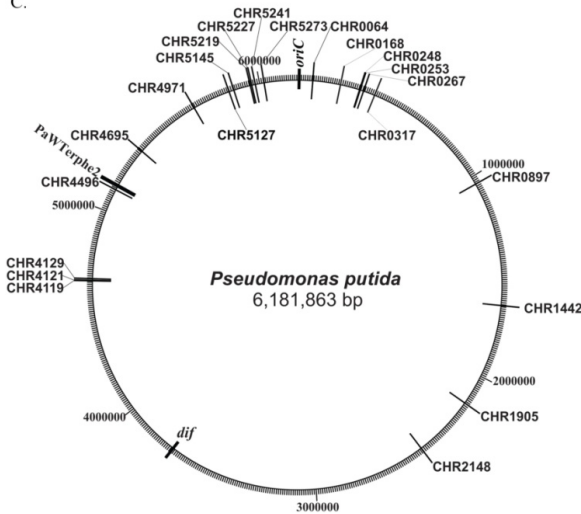
Figure 8. Two parts of the assay for the detection of the recombinational events between various chromosomal regions and locations of the insertions of the TacprpheA+2 cassette in the *P. putida* chromosome.

A. PaWTerpheBA cassette within the context of the mini-Tn*5*. The cassette carries a promoterless *pheBA* operon. *pheB* gene is the ortholog of the chromosomal *catA* gene and encodes catechol 1,2-dioxygenase, *pheA* encodes phenol monooxygenase. The cassette is inserted into the locus PP4519 of the *P. putida* PaW85 chromosome (strain PaWterphe2)

B. TacprpheA+2 cassette carries the *pheA* gene inactivated by +2 frameshift mutation under the control of the constitutive P*tac* promoter. The cassette is inserted randomly into different locations of the PaWterphe2 chromosome

C. Positions of the chromosomal insertions of the TacprpheA+2 cassette in the *P. putida* chromosome for 22 independent strains. The name of the strain derives from the insertion site (the locus number) of the cassette. The insertion position of the PaWTerpheBA is shown as PaWTerphe and is the same for all the strains

1.1. Insertion position of the HR target in the chromosome affects the frequency of HR

Analyzing the frequency of HR between various chromosomal loci can shed light on the factors that influence this process. Enhanced or reduced frequency HR can be caused by the physical location of the recombining alleles along the chromosome, which includes the distance between the alleles, their orientation, proximity to the origin of replication and characteristics of the flanking DNA region. Additionally, introduction of the assay cassettes into the chromosome can disrupt the function of specific genes and therefore enhanced frequency of HR could indicate the importance of such genes for a competent maintenance of genome integrity.

In our study we analyzed HR frequencies in 22 independent strains bearing the frameshift-containing HR target, TacprpheA+2, in different positions in *P. putida* chromosome, while the location of the other non-functional *pheA* allele remained fixed in the locus PP4519 (Fig. 8C). The total number of Phe⁺ recombinants appeared onto phenol plates in the studied strains is demonstrated in Fig. 9. We found that in most of the cases (16 out 22 strains) the frequency of HR was similar, with an average 157 ± 11 recombinants accumulated onto selective plates per 10^9 cells during 10 days of incubation. Significantly higher HR frequency was observed in six of the studied strains (CHR4496, CHR2148, CHR1905, CHR4119, CHR4121 and CHR4129) and was 3–10 higher than the average (Fig 9). The analysis of chromosomal insertion sites of HR target in these strains revealed that in the strain with the highest HR frequency (CHR4496) the two recombining alleles were located in very close proximity, only 20 kb apart, indicating that the vicinity of the recombining sequences strongly facilitates HR events. Further, strains CHR4119, CHR4121 and CHR4129, all displaying moderately higher recombination frequency, were bearing the transposon insertions in the *nuoA-N* operon that encodes the subunits of NADH dehydrogenase I. The *nuoA-N* operon is located relatively close to HR target allele, 445 kbp downstream the PaWTerpheBA cassette, which could promote recombination. However, for example, in the strain CHR4695 where the recombining alleles were located 222 kbp apart, the HR frequency remained average. This implies that solely the distance between the recombining alleles cannot determine the recombination frequency and there are usually several factors involved. Notably, NADH dehydrogenase I is the first enzyme complex in the respiratory chain and is a significant source of cellular reactive oxygen species (ROS) even under normal conditions (Esterházy *et al*, 2008) and its dysfunction may result in elevated amount of ROS that damage DNA and thereby be a source of recombination-initiating lesions.

Loss of function of the gene PP2148 due to transposon insertion in the strain CHR2148 was another case where disruption of the gene functions lead to hyper-recombination phenotype and helped to establish the effect of this gene on the recombination process. Locus PP2148 encodes a transcription-repair-

coupling factor Mfd, a key protein in transcription-coupled nucleotide excision repair (TC-NER), an important branch of the NER pathway (Savery, 2007). Mfd couples transcription and DNA repair by recruiting NER enzymes to the site of the DNA lesions encountered by RNA polymerase and thereby facilitates repair on actively transcribed regions. Therefore, loss of Mfd functions in the strain CHR2148 would abolish TC-NER process and the lesions left unrepaired could promote HR in such cells. We have further confirmed the involvement of Mfd in suppressing HR by constructing the Mfd-defective derivate of the tester strain CHR5127 and evaluating the HR frequencies in both strains (the results are discussed below in the section 2.5).

Altogether, our results imply that the frequency of recombinational events is influenced by chromosomal insertion position of the homologous sequences and can depend on both physical position of the cassette and the functions of the interrupted genes.
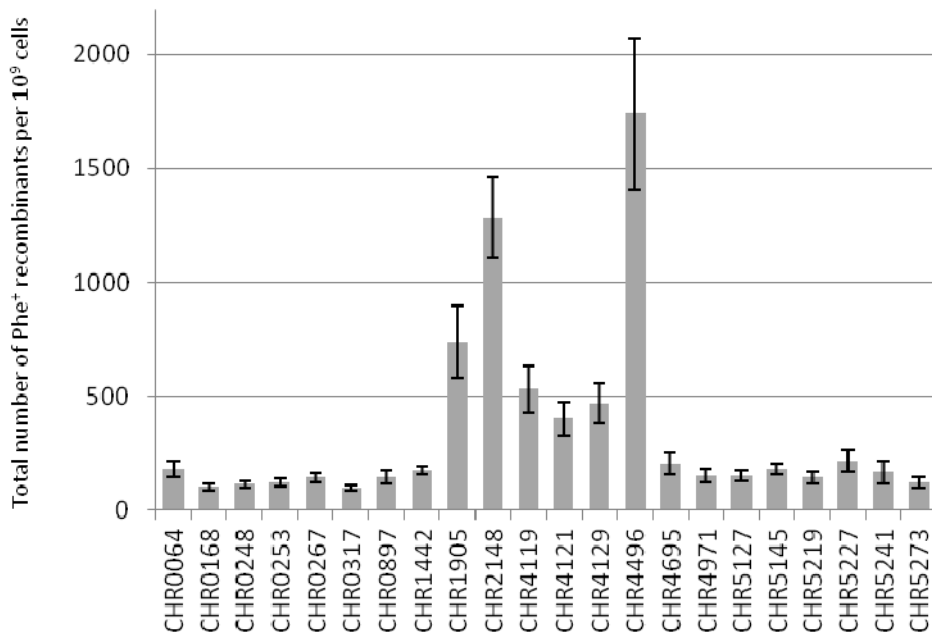

Figure 9. The frequencies of HR in 22 independent strains with a different location of the HR target in *P. putida* chromosome. The total number of Phe[+] recombinants appeared onto phenol minimal plates per 1×10^9 cells for day 10 is shown. The mean values with 95% confidence levels are presented

1.2. HR between chromosomal loci occurs in growing cells

The assays based on non-lethal selection of Phe$^+$ recombinants allows monitoring recombination events taking place both in growing cultures and in starving populations, as the cells survive in the absence of carbon source for at least 2 weeks (Ref. II and (Saumaa *et al*, 2006)). Our previous study has shown that the frequency of HR events between the chromosome and a plasmid was enhanced during the carbon starvation of bacteria in the presence of phenol. In that case the first recombinants appeared on day 3 and the number of Phe$^+$ recombinants increased subsequently or remained high during the studied 10-day period, indicating that HR occurred preferentially in resting, non-growing cells (Ref. II).

Distinctly from that, we have observed that HR between chromosomal loci was taking place in actively replicating cells and was restricted during the starvation (Fig. 10.). In all of the studied strains 70–90% of the total number of recombinants appeared onto phenol plates at once and then the emergence of Phe$^+$ colonies declined sharply. Figure 10 shows the dynamics of the emergence of Phe$^+$ recombinants for the strain CHR5127, which was chosen as a tester strain for all further experiments. We observed that in most of the strains the majority of Phe$^+$ colonies emerged on day 4 after plating but in some cases (e. g. strains CHR5219 and CHR2148) the emergence of recombinants was delayed until day 6. By plating Phe$^+$ recombinants of the corresponding strains with scavenger cells onto phenol plates, we assessed the growth rate of the colonies expressing the functional *pheA* allele. It appeared that the pre-existing Phe$^+$ recombinants formed colonies similarly to their parental Phe$^-$ strains only after 4 to 6 days of incubation. These results indicated that HR occurred in actively replicating bacteria, before or shortly after the plating and the time of the appearance of the recombinants depended on the growth rate of the strains on phenol plates.
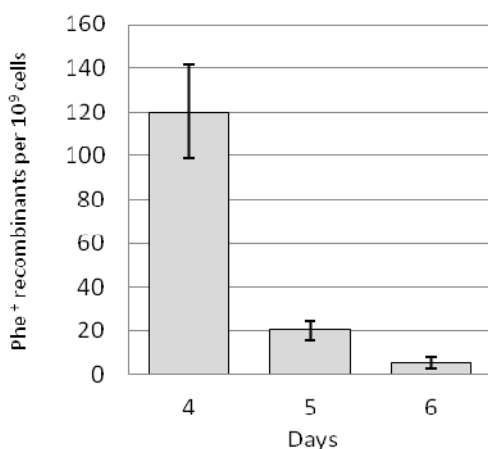
Figure 10. The dynamics of appearance of Phe$^+$ recombinants onto phenol minimal plates in the tester strain CHR5127. The number of Phe$^+$ recombinants per 1×10^9 cells emerged per day is shown. The mean values with 95% confidence levels are presented.

The difference in the dynamics of HR events between chromosomal loci and between a chromosome and plasmid is intriguing and could be attributed to different size and structure of the plasmid and a chromosome. Most of the recombination events in the plasmidial assay were detected on the plasmid, indicating that restoration of the functional *pheA* allele occurred by replacing the frameshift-containing *pheA* coding sequence on the plasmid with the wild-type *pheA* allele from the chromosome (the other option was to replace the non-functional promoter of the chromosomal *pheA* allele with the functional promoter sequence from the plasmid). These results implied that HR in stationary-phase cells was initiated by plasmid replication or repair. It is notable that the similar decline in the emergence of Phe[+] revertants appeared also in case when the mutagenesis assay that detects 1-bp frameshift mutations in *pheA* gene was introduced into chromosome of *P. putida* (Juurik *et al*, 2012) compared to that when the allele was located on the plasmid (Tegova *et al*, 2004), indicating that also the mutational processes are suppressed in chromosomes of the resting cells while proceed on the plasmids. During cell growth the structure of the bacterial chromosome undergoes dynamic changes: while in actively dividing cells the nucleoid is loosely packed, it becomes tightly condensed in stationary-phase cells (Kim *et al*, 2004) and replication of the chromosome is diminished. Additionally, the copy number of the chromosome in the cells declines in bacteria during a passage into stationary phase (Lewis *et al*, 2002). This all can significantly affect the frequency of HR initiation events and reduce the probability of a homology encounter within a chromosome. Distinctly, due to a small size, replication of plasmids in resting cells could still occur, enabling recombination and mutation processes that would create a potential for adaptation and gaining an advantage in natural environment.

1.3. Exogenous DNA damage stimulates homologous recombination

Recombination stimulating lesions can arise both endogenously or can be induced by DNA damaging agents. We have previously demonstrated that elevated levels of ROS, which accumulate during starvation of bacteria in the presence of phenol, could stimulate HR between a plasmid and the chromosome and that decreasing the levels of ROS by addition of a potent hydroxyl radical scavenger thiourea into selective media suppressed the frequency of HR events in starving cells (Ref. I). To test whether addition of an antioxidant into growing cultures could suppress HR between chromosomal loci due to decreased endogenous oxidative DNA damage in the growing cells we measured HR frequency in the tester strain CHR5127 grown overnight in the presence of 50 mM thiourea. As seen in Figure 11, in this case the frequency of HR remained comparable to that when cells were grown without addition of the antioxidant. This implied that in the wild-type cells growing under normal conditions the levels of ROS are not critical for stimulation of HR between chromosomal loci.

ssDNA regions and dsDNA breaks provide sites for the initiation of recombinational repair in bacterial cells. Such sites can be induced by exogenous DNA damaging chemicals that directly damage DNA but also by some antibiotics. Fluoroquinolones, like ciprofloxacin, target DNA gyrase and DNA topoisomerase IV and trapping these enzymes releases dsDNA ends (Drlica & Zhao, 1997). In fact, sub-lethal concentrations of ciprofloxacin were shown to stimulate intrachromosomal recombination in *E. coli* cells (López & Blázquez, 2009). Therefore, as the next step, we monitored whether the DNA damaging agents 4-nitroquinoline 1-oxide (4-NQO), the chemical producing bulky DNA adducts, an alkylating agent methyl methanesulfonate (MMS) and a fluoroquinolone antibiotic ciprofloxacin would induce HR between the chromo-somal *pheA* alleles in *P. putida*. The presence of low concentrations (10 μM 4-NQO, 0.1 mM MMS and 0.01 μg/ml ciprofloxacin) of all the three chemicals into growing medium of the tester strain CHR5127 markedly induced the HR frequency in treated cells. The total number of Phe$^+$ recombinants per 10^9 cells increased about 10-, 15- and 31-fold respectively (Fig. 11). These results reflected the importance of HR in coping with DNA damage and confirmed the ability of the constructed assay to detect the conditions when an enhanced recombinational repair is required to sustain genome stability. Notably, addition of the chemicals affected the frequency, not the dynamics of emergence of Phe$^+$ recombinants, about 90% of the Phe$^+$ colonies emerged onto phenol plate at once both when the cells were pre-grown with or without DNA-damaging agents, confirming that HR between chromosomal loci events both under normal and damage-induced conditions occur in actively replicating cells (data not shown).
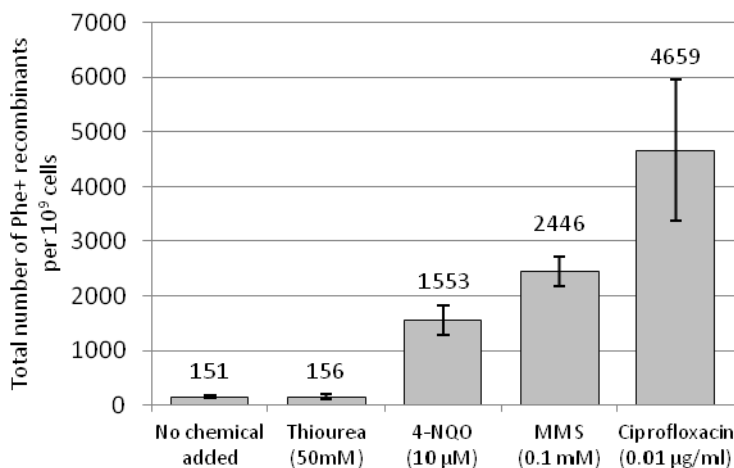
Figure 11. Effect of the different chemicals on the HR frequency in the tester strain CHR5127. The chemicals were added into growing cultures of the bacteria and bacteria were incubated overnight. The total number of Phe$^+$ recombinants per 1×10^9 cells is shown. The mean values with 95% confidence levels are presented.

II. NER IS INVOLVED IN MAINTENANCE OF GENOME INTEGRITY IN *P. PUTIDA* EVEN IN THE ABSENCE OF EXOGENOUS DNA DAMAGE (REFERENCE I)

Initially discovered as an important pathway for repair of UV-induced DNA damage (Boyce & Howard-Flanders, 1964), NER has now been shown to recognize and excise a broad range of structurally unrelated DNA lesions including even misincorporated ribonucleotides in the DNA molecule (Vaisman *et al*, 2013; Truglio *et al*, 2006). NER is generally assumed to be important for DNA repair in the cells exposed to exogenous DNA damage and its role in maintenance of genome integrity under normal growth conditions has attracted little attention. Genes encoding NER proteins UvrA, UvrB and UvrC that are needed for damage recognition and excision of DNA damage are universally present in genomes of bacteria including *P. putida* (PP0483, PP1974 and PP4098, respectively). Additionally, *P. putida* possesses a second copy of UvrA protein, a class II UvrA homologue UvrA2 (encoded by the locus PP3087), the functions of which in NER remain unclear. Here, we constructed a set of derivatives of the tester strain CHR5127, which are deficient in NER pathway proteins UvrA, UvrB, UvrC, and UvrA2 and assessed the importance of these proteins for maintaining genome stability by measuring HR frequency in corresponding strains. We also investigated the effects of Mfd (encoded by PP2148), a key protein for initiation of transcription-coupled nucleotide excision repair (TC-NER) and UvrD helicase, the protein involved in both in TC-NER and post-incision events of NER, on the frequency and dynamics of intra-chromosomal HR events in *P. putida.* DNA polymerase I, involved in re-synthesis step of the NER pathway, is an essential enzyme in bacterial chromosome replication, being involved in maturation of Okazaki fragments during lagging strand synthesis. The role of Pol I will be discussed in detail below (Section 3.1).

2.1 NER proteins UvrA, UvrB and UvrC are important for *P. putida* growth under normal growth conditions

Though NER pathway has been extensively studied, especially in *E. coli*, no effect on cell growth or viability in the absence of induced DNA damage has been previously reported in other bacteria. Surprisingly, deletion of *uvrA*, *uvrB* or *uvrC* genes in *P. putida* resulted in significant instability of the corresponding strains. The cells lacking UvrA, UvrB or UvrC experienced growth difficulties and formed filaments when grown in liquid medium (Fig. 12, CHR5127UvrA and CHR5127UvrB are shown). Also, the number of viable cells in overnight

cultures of UvrA- and UvrB-deficient strains was reduced about 2-fold if compared to the parent strain. The loss of UvrC protein resulted even in more severe growth defects with the number of CFU (colony forming units) from overnight cultures decreased up to 15-fold in comparison to that of the NER-proficient bacteria. Unexpected growth deficiency of NER mutants, however, was quickly lost due to rapid adaptation of the NER-deficient bacteria. The initial mutants displayed distinctive colony morphology on LB plates, being more translucent than wild-type bacteria, with visible differentiating cell populations. Similar differentiation occurred also in liquid medium: 10–20% of single colonies from the overnight cultures of the UvrA- and UvrB-deficient bacteria and about 80% of the UvrC-deficient bacteria exhibited a phenotype similar to that of the wild-type bacteria. The emerging variants lacked the growth defects and did not form filaments (Fig. 12, compare CHR5127UvrA and CHR5127UvrA+; CHR5127UvrB and CHR5127UvrB+); nevertheless, the adapted populations remained deficient in NER genes. Notably, while re-streaking of translucent colonies onto LB plates gave both normal and translucent variants, which showed the ongoing differentiation, the acquired phenotype of adapted strains remained stable, indicating genetic adaptation by suppressor mutations (Fig. 13). We further designated the adapted variant of NER deficient derivatives of the tester strain CHR5127 as CHR5127UvrA+, CHR5127UvrB+ and CHR5127UvrC+ and the initial variants are referred as CHR5127UvrA, CHR5127UvrB and CHR5127UvrC. It should be noted that the described characteristics of NER-deficiency is not attributed to the presence of the assay cassettes in the chromosome of *P. putida* as the deletion of *uvrA*, *uvrB* or *uvrC* genes in the wild-type strain PaW85 produces the same phenotype.

Distinctly from deleterious effects of UvrA, UvrB and UvrC deficiency, the loss of Mfd, UvrA2 and UvrD did not affect the viability or colony morphology in *P. putida*. The microscopic examination of the cells revealed normal cell morphology in the case when Mfd and UvrA2 were absent (Fig. 12). However, UvrD-deficient cells displayed a minor fraction of filaments (Fig. 12) when grown overnight in liquid medium, indicating the importance of UvrD for genome integrity in *P putida*. The effects of Pol I deficiency on the *P. putida* cells will be discussed in detail below (Section 3.1.).
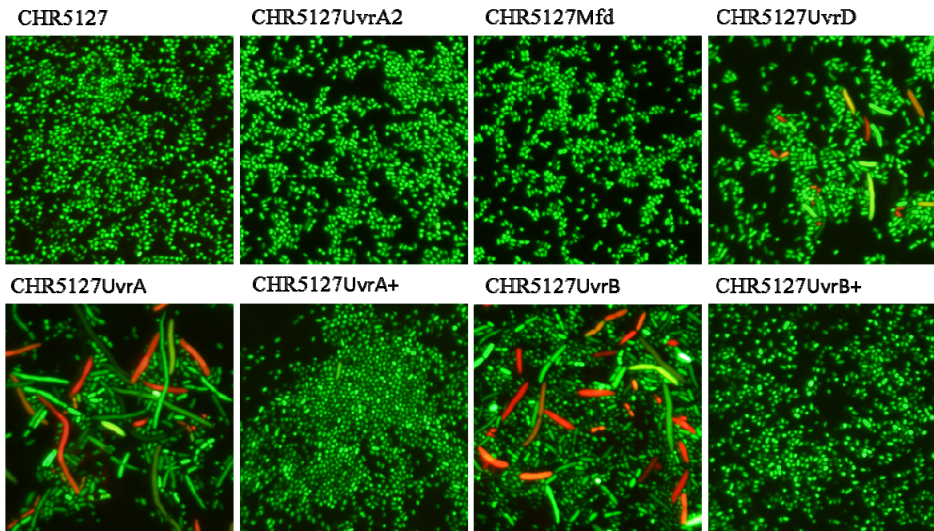
Figure 12. Effects of the absence of NER enzymes on *P. putida* cell morphology. Bacteria were stained with SYTO-9 and propidium iodide (PI) and visualized by epifuorescent microscopy using the 1000-fold magnification. Cells were grown overnight in glucose minimal medium supplemented with CAA. "+" indicates the adapted variants of NER-deficient strains.
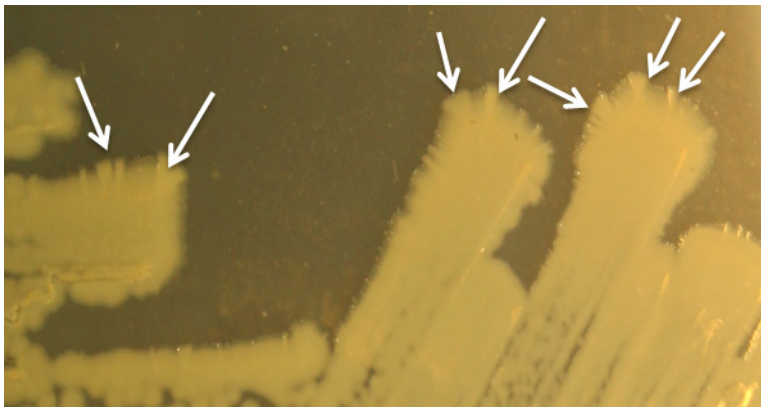
Figure 13. Differentiation in the population of the non-adapted CHR5127UvrB strain. Restreaking of the non-adapted cells onto LB medium yields adapted variants (indicated by arrows)

2.2 NER proteins UvrA and UvrB are important for suppressing intrachromosomal HR both in growing and stationary phase cells

As the next step of our research, we investigated the frequency of HR in the strains deficient in NER proteins UvrA, UvrB and UvrC. In the case of UvrA and UvrB the HR frequency was assessed in both initial and adapted variants of UvrA- and UvrB-deficient strains. However, due to low viability of the initial UvrC-deficient cells, only the adapted variant CHR5127UvrC+ was used in our study.

In comparison to the tester strain CHR5127, the frequency of HR events was enhanced in all NER-deficient variants tested (Fig. 14A). Remarkably, the total number of Phe$^+$ recombinants, accumulated onto phenol plates by day 10 of incubation differed significantly in the initial UvrA- and UvrB-deficient bacteria and their adapted derivatives, being 7–11- fold higher in non-adapted strains if compared to the strains CHR5127UvrA+ and CHR5127UvrB+ and about 30 times higher than that in the parent strain CHR5127 (Fig. 14A). Noteworthy, we have observed that, in the wild-type background the emergence of Phe$^+$ colonies ceased in 2–3 days after the initial appearance of recombinants in 22 independent strains with various location of the HR target in the chromosome of *P. putida*, including the tester strain CHR5127 (Fig. 9). Strikingly, the Phe$^+$ recombinants continued to emerge throughout the starvation period in the initial UvrA- and UvrB-deficient strains, indicating the ongoing recombination events (Fig. 14B).

To exclude the possibility that the recombinants which emerged onto the plates on days 5 and later were slowly growing variants we conducted the reconstruction experiments as described before: the cells harvested from the Phe$^+$ colonies which appeared on different days were re-plated onto selective medium with the scavenger cells. As expected, all the Phe$^+$ recombinants formed colonies onto the phenol plates on a day 4 independently from their initial appearance moment in the recombination assay, confirming that HR events detected in the initial UvrA- and UvrB-deficient cells proceeded in stationary-phase.

The high HR frequency in resting cells in the absence of UvrA and UvrB demonstrated the importance of these proteins for maintenance of genome integrity and suppressing intrachromosomal HR not only in growing cells but also under limited growth conditions. Notably, the observed frequencies and dynamics of HR events in the strains CHR5127UvrA and CHR5127UvrB were very similar which indicated that both UvrA and UvrB are equally important in this process. The adapted strains CHR5127UvrA+, CHR5127UvrB+ and CHR5127UvrC+ still had an elevated frequency of HR in comparison to the parent strain. However, the dynamics of the emergence of Phe$^+$ recombinants was similar to that observed in NER-proficient strains (Fig. 14B), implying that the need of NER proteins in stationary-phase cells was abolished as a result of genetic adaptation.

A.

B.
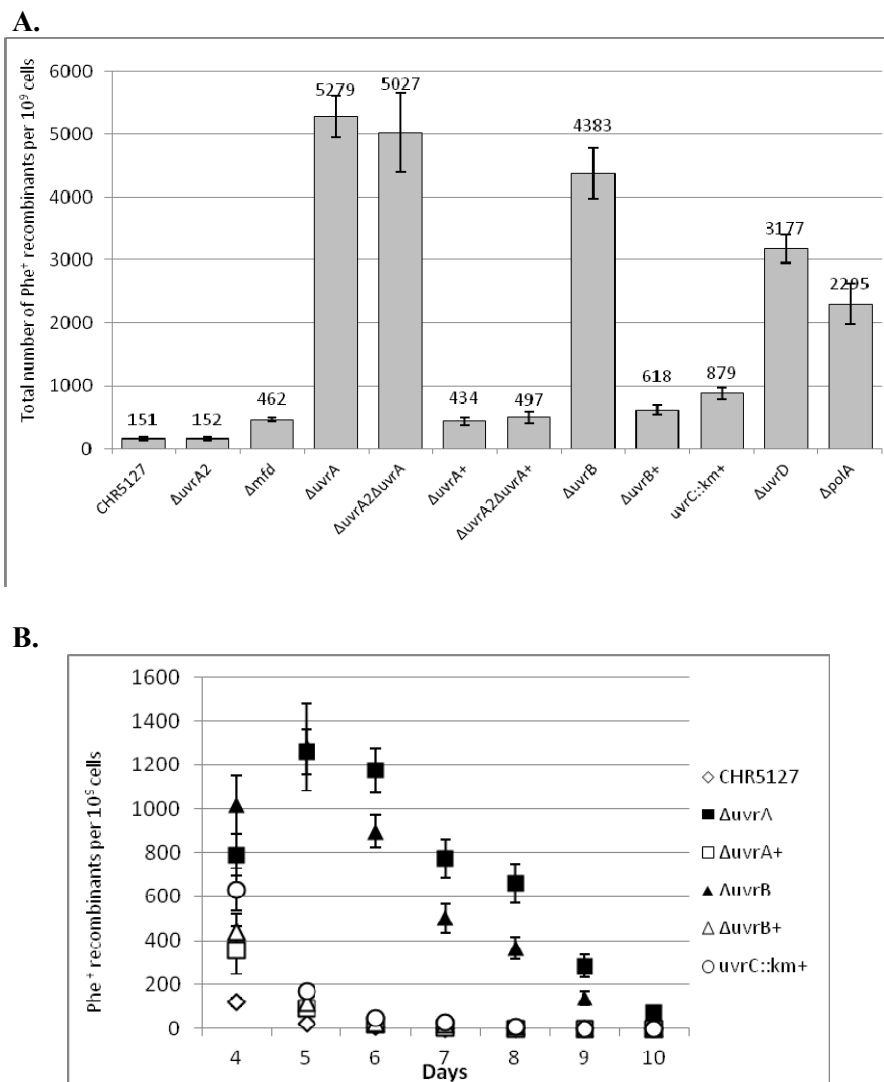
Figure 14. Effect of NER proteins on the frequency of HR in the tester strain CHR5127. "+" indicates the adapted variants of NER-deficient strains.

(A) The total number of Phe$^+$ recombinants per 1×10^9 cells in the tester strain and its NER-deficient derivatives.

(B) The frequency of emergence of Phe$^+$ recombinants per day in the tester strain and its NER-deficient derivatives. The mean values with 95% confidence levels are presented.

2.3 The adaptation mechanism of NER-deficient strain does not involve improvements in damage-specific repair

The observed instability of UvrABC-deficient strains demonstrates the importance of NER for genome maintenance in *P. putida* even in the absence of exogenous DNA damage. However, the ability to abolish the growth defects and to eliminate the need for UvrABC proteins in stationary-phase cells due to suppressor mutations raised the question about the mechanism of such adaptation which could shed some light on the functions of NER beyond the repair of induced DNA damage.

Filamentous growth of the NER-deficient bacteria indicates the replication stress and the presence of ssDNA which triggers SOS response and subsequent inhibition of cell division (Sassanfar & Roberts, 1990). Such ssDNA regions may arise as a result of replication fork stalling when the replicative DNA polymerase is blocked upon encountering a DNA lesion and DNA helicase continues to unwind DNA. The stalled or collapsed replication forks, in turn, activate recombinational repair thereby enhancing the frequency of HR. A broad range of DNA lesions can arise during bacterial growth in the absence of exogenous DNA damage, e.g. as a result of spontaneous depurination, oxidation and alkylation of DNA (Lindahl & Barnes, 2000). Such damage is caused by reactive by-products of normal cellular metabolism and could be recognized and repaired by NER. Thus, the need for NER proteins for normal growth of *P. putida* could be associated with the deficiency to repair endogenous DNA lesions in the absence of functional NER pathway.

We anticipated that if that was the case, the adaptation of the NER-deficient strains could involve an enhanced ability of coping with DNA lesions through the alternative pathways. For example, the mechanism of adaptation might include efficient repair of the lesions by up-regulated BER pathway or enhanced translesion synthesis by specialized or a replicative DNA polymerases. To test this hypothesis, we compared the sensitivity of both adapted and initial NER mutants to UV-irradiation and 4-NQO, known to induce the DNA lesions repaired by NER (Ikenaga *et al*, 1975; Howard-Flanders *et al*, 1966). Nevertheless, the adapted variants experienced extreme sensitivity to UV-light and 4-NQO, comparable to initial strains (Fig. 15), indicating that the adapted cells were still deficient in damage-specific repair and that the adaption mechanism did not involve the improved tolerance of DNA damage. Taken together, these results show that the instability of the NER-deficient *P. putida* cells and elevated frequency of HR both in growing and stationary-phase might be associated with NER functions beyond the repair of canonical replication-blocking lesions.
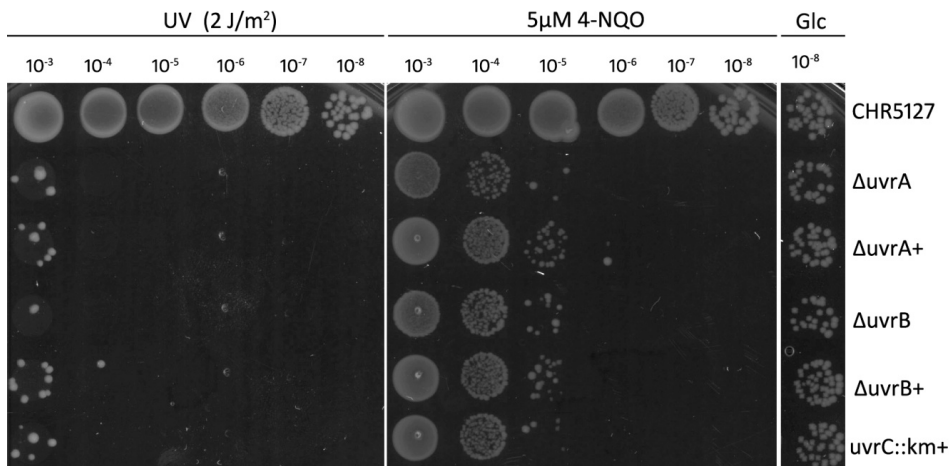
Figure 15. Sensitivity of initial and adapted *P. putida* NER-deficient derivatives of the strain CHR5127 to UV-irradiation and exposure to 4-nitroquinoline (4-NQO). Serial dilutions from overnight cultures were spotted onto glucose minimal plates containing 5 μM 4-NQO or minimal plates, which were then exposed to UV-irradiation (2 J/m2). "+" indicates the adapted variants of NER-deficient strains. Only the 10^{-8} dilution of the untreated cells is shown.

2.4. UvrA2 functions do not affect HR process in *P. putida*

As mentioned above, *P. putida* harbours a second copy of UvrA protein, UvrA2. The proteins share only 44% identity in amino acid sequence but UvrA2 is closely related to UvrA2 homologues in *Xanthomonas*, *Streptomyces* species and *Deinococcus radiodurans* which also encode a duplicate of *uvrA* gene (Tark *et al*, 2008; Martins-Pinheiro *et al*, 2004; Tanaka *et al*, 2005). UvrA2 lacks the UvrB binding domain (Timmins *et al*, 2009) and is shown to have only a minor role in DNA repair and tolerance to DNA-damaging agents, such as UV-irradiation or chemical treatment (Tark *et al*, 2008; Tanaka *et al*, 2005; Shen *et al*, 2007). Nevertheless, the involvement of UvrA2 in the generation of base substitution mutations and –1 frameshift deletions was observed in *P. putida* stationary-phase cells (Tark *et al*, 2008). However, the exact functions UvrA2 remain obscure.

The deletion of *uvrA2* in the tester strain CHR5127 did not result in any visible phenotype of *P. putida* colony or in cell morphology in contrast to the deleterious effects of *uvrA*, *uvrB* or *uvrC* genes (Fig. 12). We further assessed the frequency of HR between chromosomal loci to see whether UvrA2 may be involved in suppression of HR in *P. putida* chromosome. Figure 14A demonstrates that the lack of UvrA2 did not influence HR frequency in the tester strain and indicated no importance of UvrA2 for genome maintenance

under normal growth conditions. Still, it was possible that UvrA2 functions may become evident only in the absence of UvrA due to redundancy of the protein. Therefore, we also monitored the frequency of HR in strains lacking both UvrA homologues. Since in the case of UvrA-deficiency the frequency and dynamic of recombination events depended on the adaptation state of the cells, we used the strain CHR5127UvrA2 to construct the UvrA-deficient derivative CHR5127UvrA2UvrA. The deletion of the *uvrA* gene in the *uvrA2*-deficient background resulted in the same unstable phenotype of the double mutant CHR5127UvrA2UvrA as described for the UvrA single mutant. Similarly, the adapted variants emerged rapidly. Neither the initial nor the adapted double mutants showed significant change in the HR frequency or dynamics if compared to that in CHR5127UvrA and CHR5127UvrA+ strains, lacking only UvrA (Fig. 14A). Taken together, our results indicate that UvrA2 has no evident role in suppression of HR in *P. putida* in both growing and starving cells even in the absence of the primary UvrA.

2.5. TC-NER is important for suppressing intrachromosomal HR

As described previously, the frequency of HR depended on the insertion position of the assay cassettes in the chromosome. This enabled us to identify the new genes important for genome stability. Among the constructed strains with enhanced recombination frequency we have identified the strain with the insertion into the locus PP2148, which encodes the transcription repair coupling factor Mfd (Fig. 8C and Fig. 9). The role of Mfd in transcription-coupled repair, a sub-pathway of NER, where Mfd facilitates the repair of DNA damage encountered by transcribing RNAP, is well established in *E. coli* and *B. subtilis* (Ross *et al*, 2006; Savery, 2007) and is likely to be similar also in *P. putida*. *P. putida* and *E. coli* Mfd proteins share 54.6% identity (www.uniprot.org). Since perturbation of DNA repair pathways affects the genome integrity, the enhanced frequency of HR in the absence of functional Mfd in the cells could be attributed to Mfd functions in DNA damage repair. To verify the effect of the loss of Mfd functions on HR frequency in the strain CHR2148 we constructed an Mfd-deficient derivate of the tester strain CHR5127 and compared the frequency of HR in both strains. As observed for the strain CHR2148, the deletion of the *mfd* gene in the tester strain also resulted in higher frequency of emergence of Phe$^+$ recombinants (Fig. 14A). The mean frequency of HR measured in the Mfd-deficient derivative of CHR5127 was enhanced about 3-fold if compared to the parent strain (462±33 Phe$^+$ recombinants vs. 151±23 re-combinants per 10^9 cells). It should be noted that similarly to the strain CHR2148 with the transposon insertion into *mfd* gene, the appearance of the first Phe$^+$ colonies in Mfd-deficient strain CHR5127Mfd was delayed until day 6 of incubation, indicating that Mfd-deficiency affected the growth rate of the Phe$^+$

cells under the experiment conditions. Interestingly, in the reconstruction experiments *mfd*-deficient Phe$^+$ cells were able to form colonies comparatively with the tester strain if the scavenger cells were not present, while in the presence of the scavenger cells the appearance of Phe$^+$ colonies was delayed. This implied that the growth suppression of the Mfd-deficient cells was attributed to enhanced sensitivity to the inhibitory effect of the scavenger cells. Otherwise, the dynamics of HR events remained the same as in the Mfd-proficient tester strain with about 85% of the total number of recombinants emerged at once at the beginning of the experiment (data not shown), implying that Mfd functions are important to suppress HR in growing cells, not during starvation.

UvrD helicase is an important enzyme in the post-incision events of the NER pathway, where it removes the damage-containing DNA oligonucleotide. However, UvrD has wider functionality in the cells, including the recently established role of UvrD in TC-NER, where it is believed to move together with RNAP throughout elongation and to actively pull RNAP backward when it stalls due to the encountered DNA damage in an Mfd-independent way (Epshtein *et al*, 2014). Of all NER enzymes, UvrD is the only protein (apart from Pol I), the lack of which is shown to activate constant SOS response and result in a hyper-recombination phenotype in *E. coli* (Arthur & Lloyd, 1980; Bierne *et al*, 1997; SaiSree *et al*, 2000; Veaute *et al*, 2005). Here we have shown that the absence of UvrD causes a mild filamentous phenotype also in *P. putida* cells (Fig. 12), which is consistent with the reported induction of SOS response in UvrD-deficient *E. coli* cells (Bierne *et al*, 1997; SaiSree *et al*, 2000). Similarly to a hyper-recombination phenotype in *E. coli* UvrD-deficient strains, deletion of *uvrD* enhanced the frequency of HR in *P. putida* about 20 fold (Fig. 14A). However, no effect of UvrD on the HR in stationary phase was detected, which implies that UvrD functions are not essential in UvrABC-mediated process to suppress HR in starving cells.

Taken together, our data show that HR is enhanced the most in the absence of NER enzymes UvrABCD, slightly induced by the lack of Mfd, whereas UvrA2 has no effect on HR frequency in *P. putida*. The noteworthy importance of the UvrAB(C) proteins for the cells growth in the absence of any induced DNA damage and preventing of HR the stationary phase *P. putida* cells remains obscure. We have demonstrated that the compensation mechanism which abolished the need of UvrABC enzymes in stationary phase cells and restored the normal growth of the in *P. putida* cells is not associated with improved ability to repair the DNA lesions, suggesting the possible role of these enzymes outside the repair process.

It is possible that a kind of substrate exists which is recognized preferentially by NER and processed in a different way than the canonical lesions. Specifically, the possible candidates for excision by NER enzymes are ribonucleotides, synthesized as primers during lagging strand synthesis or, alternatively, misincorporated by DNA polymerase during replication. Recently, it has been

demonstrated that the NER machinery is involved in recognizing and removing ribonucleotides from DNA strands (Vaisman *et al*, 2013; Cai *et al*, 2014). In fact, the idea that NER enzymes could function outside DNA repair in DNA replication is not new (Moolenaar *et al*, 2000). This hypothesis has been based on the fact that the viability of Pol I-deficient cells is dependent on the presence of functional UvrA, UvrB, and UvrD proteins. While Pol I is the key enzyme involved in maturation of Okazaki fragments during lagging strand synthesis, NER enzymes have been proposed to be essential to substitute for Pol I functions in removing RNA primers from the lagging strand (Moolenaar *et al*, 2000). In this model UvrD helicase could unwind the DNA-RNA hybrids starting from the nick in the Okazaki fragments and thereby facilitate the removal of the RNA primers by exonucleases or RNAses. The role of UvrA and UvrB proteins in this process has been proposed to orient UvrD helicase. Consistently, stimulation of UvrD helicase by UvrAB proteins has been demonstrated later (Atkinson *et al*, 2009). The resulting gaps in the absence of Pol I could be then filled in by one of the DNA polymerases including the replicative DNA polymerase III. Unwinding of RNA-DNA duplex by UvrD could leave a larger gap, which could facilitate the entry of polymerase. Yet, it is possible that in *P. putida*, the removal of RNA-DNA hybrids of the Okazaki fragments by NER could be also essential in the presence of Pol I and UvrABC could act cooperatively with Pol I. Still, the effect of the absence of UvrD in *P. putida* appears to be less deleterious, e.g. the cells do not exhibit distinct morphology and extended accumulation of Phe$^+$ recombinants, compared to the loss of UvrA, UvrB or UvrC. Thus, the function of UvrD can be redundant in this process and after the excision of the RNA primer by UvrABC-excinuclease the further unwinding could be performed also by to some other helicase in the absence of UvrD. Notably, the mechanism of UvrA and UvrB action beyond traditional NER pathway could be distinct, since the N-terminal zinc-binding domain of UvrA, which was shown to be not critical for DNA repair of bulky lesions (Visse *et al*, 1993) is vital for substitution of Pol I functions in DNA replication (Moolenaar *et al*, 2000). This strongly suggests additional role of NER enzymes in the cells and could explain the deficiency in damage-specific repair in adapted variants of NER-deficient strains while the cells were able to avoid replication stress.

Another interesting fact about NER is that in *E. coli* UvrA together with 16 other proteins, including UvrC, subunits of RNA polymerase, DNA topo-isomerase I, DNA gyrase and Pol I are relocated to the inner membrane following UV-induced DNA damage, indicating that the repair and transcription processes are tightly connected and may associate with the inner membrane. Notably, this recruitment was dependent on UvrA, UvrC and RecA functions and about 40% of the UvrC protein was found to be associated with the inner membrane even in the absence of UV-damage (Lin *et al*, 1997). Thus, drawing the parallels with the results described above, NER enzymes might be important for association of certain proteins to the inner membrane in *P. putida* and be

involved in its functions. The fact that *P. putida* UvrABC mutants exhibited translucent colony morphology suggests that the membrane properties were altered in comparison to wild-type or adapted variants of NER-deficient strains. Since proper functioning of membrane in essential for all processes in the cells, its perturbed function in the absence of NER enzymes could account for the observed deleterious effects.

III. DNA POLYMERASE I IS ESSENTIAL BOTH FOR DNA REPLICATION AND REPAIR (REFERENCE III)

DNA polymerase I is involved in a re-synthesis step in NER and BER pathways. However, the principal role of Pol I is proposed to be the processing of the lagging strand during DNA replication. According to the database search (www.uniprot.org), *P. putida* Pol I is encoded by 2748 bp long *polA* gene (PP0123) and shares 62% identity at amino acid sequence with *E. coli* DNA polymerase I. Similarly to *E. coli* Pol I, Pol I of *P. putida* is predicted to possess three distinct enzymatic activities and is organized into two domains: the Klenow domain with DNA polymerase activity and 3'→5' exonuclease proofreading activity, and the 5'-nuclease domain that enables removal of RNA primers during DNA lagging strand synthesis. In contrast to UvrABC enzymes the importance of Pol I under normal growth conditions is well established. In *E. coli* Pol I is essential for growth on rich but not on minimal medium whereas providing either of domains *in trans* is sufficient to restore the viability (Joyce & Grindley, 1984). Pol I deficiency is described by enhanced recombination frequency and a moderate mutator phenotype (Konrad, 1977; Tago *et al*, 2005; Bates *et al*, 1989; Jankovic *et al*, 1990). Here we have examined phenotypic effects, the involvement of Pol I in suppression of HR and mutations in *P. putida* cells. We also have assessed the role of specialized DNA polymerases in mutagenic processes in *P. putida* lacking Pol I functions.

3.1 Deficiency in Pol I functions impairs growth and causes filamentation of *P. putida* cells

To investigate the importance of Pol I functions on the growth of *P. putida* we constructed strains deficient either in the Klenow domain only (strain PaWPolA1) or lacking all Pol I functions (strains PaWPolAdel and PaWPolAdel2). In contrast to PaWPolAdel, which completely lacked the *polA* gene, strain PaWPolAdel2 contained 662 nucleotides from the 5′ end of the coding sequence of *polA*, but still was deficient in 5'-nuclease functions as determined by the growth deficiency on LB medium: similarly to the growth defects demonstrated in *E. coli* Pol-deficient cells both PaWPolAdel and PaWPolAdel2 displayed significantly impaired growth on rich growth medium and formed up to 1000 times less colonies on LB agar plates compared to that on glucose minimal plates, which is consistent with the loss of all Pol I functions (Fig. 16., PaWPolAdel is shown as an example). The ability to grow on minimal medium in the absence of Pol I, however, implies that alternative pathways are able to substitute for its functions to support DNA replication. Distinctly, the survival of PaWPolA1 strain, which retained 5'-nuclease

domain, was not reduced on LB plates in comparison to minimal plates, while the amount of CFU in both cases was slightly lower than in the wild-type strain (Fig. 16). Similarly to NER-deficient bacteria, the Pol I deficient strains quickly acquired suppressor mutations, which allowed them to grow normally on LB plates. During 2–3 rounds of inoculation of stationary-phase LB-sensitive Pol I mutants into fresh LB medium mutants with restored growth on rich medium emerged and took over the population. Such bacteria had comparable plating efficiency on LB and minimal plates.
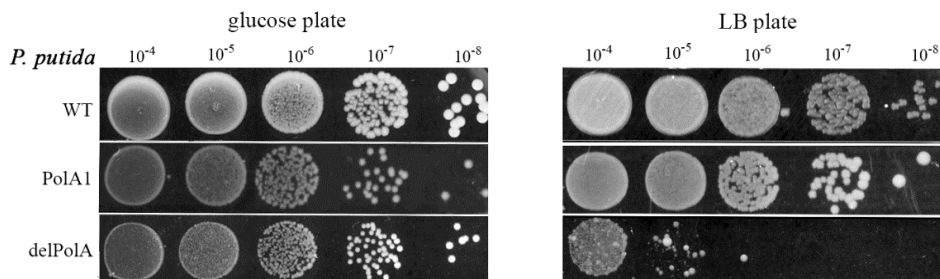
Figure 16. Colony formation efficiency of the *P. putida* for wild-type strain PaW85 (WT) and Pol I-deficient strains PaWPolA1 (PolA1) and PaWPolAdel (delPolA) on glucose minimal LB agar plates. Serial dilutions of bacterial cultures grown overnight in glucose minimal medium spotted onto agar plates are shown.

As the next step, we investigated the cell morphology of the PaWPolA1 and PaWPolAdel strains grown in liquid minimal or rich liquid medium. The cells lacking both Pol I domains formed long filaments when grown in LB medium (Fig. 17, PolAdel, LB). At the same time, when grown in glucose minimal medium the PaWPolAdel cells did not reveal any significant differences in cell morphology compared to the wild-type cells (Fig. 17, PolAdel, Glc), confirming that Pol I functions can be efficiently substituted when grown on minimal medium. The cells retaining 5'-nuclease domain of Pol I displayed only minor filamentation in LB medium (Fig. 17, PolA1, LB).

Interestingly, when we compared the growth characteristics of the populations derived from separate colonies of PaWPolAdel, we observed that growth rate as assessed by increase in optical density of the cultures and maximum cell density of these populations differed from each other both in LB and minimal medium, indicating that these cultures were adapted differently for the growth in liquid medium (Ref. III). Moreover, the cells derived from these cultures were still LB-sensitive despite of temporary growth advantage, which suggested physiological, not genetic adaptation. Thus, both stable genetic and a variety of transient physiological adaptations allow *P. putida* to grow in the absence of both Pol I domains.
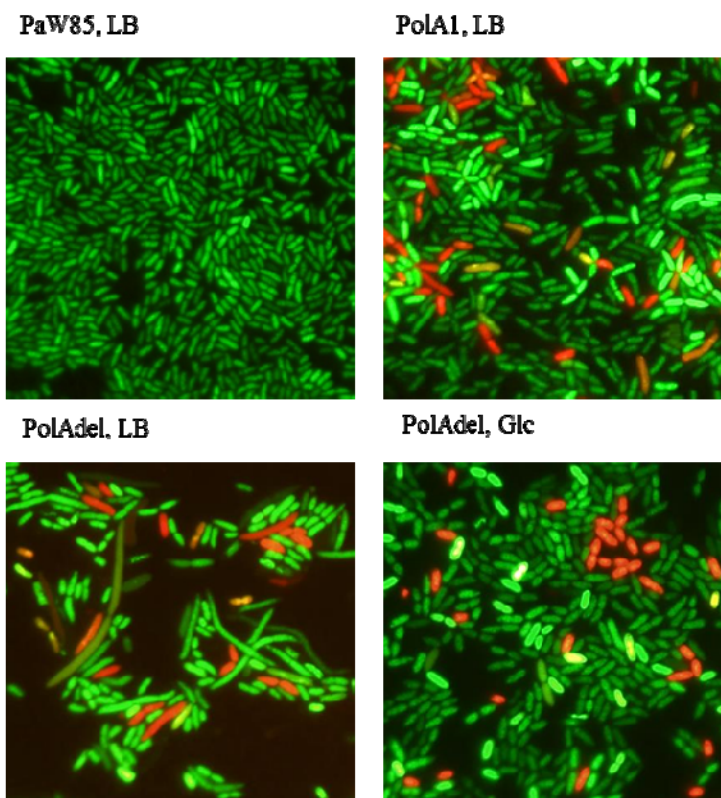
Figure 17. Effects of the absence of Pol I functions on cell morphology of *P. putida* under different growth conditions. Bacteria were grown exponentially in LB or glucose minimal medium. PaW85 (WT) grown in LB medium is shown as a comparison. The cells were stained either with SYTO-9 and propidium iodide (PI) and visualized by epifluorescent microscopy using the 1000-fold magnification.

3.2. Reduction of reactive oxygen species restores the viability of Pol I-deficient bacteria on LB plates

The reduced viability of Pol I deficient bacteria in rich medium is generally assumed to be associated with Pol I role in DNA replication process due to inability efficiently process Okazaki fragments during fast growth conditions (Joyce & Grindley, 1984). However, since the Pol I-deficient cells grown overnight in rich medium form only 5–10-fold less colonies on minimal plates than the wild type strain (compared to about 1000 fold decrease of CFU on LB plates), it shows that a Pol I-deficient cells survive must be specifically sensitive to the rich medium agar plates. It is known that inactivation of Pol I dramatically sensitizes the cells to killing by H_2O_2 (Imlay & Linn, 1988). Hydrogen peroxide molecules can be reduced to hydroxyl radicals (OH•) through Fenton

reaction and can directly damage DNA. H_2O_2 is also produced in sterile medium through photochemical mechanisms under room lighting (Imlay, 2008) and can be a source of oxidative stress under experimental conditions. Moreover, elimination of oxygen from the growth medium by the incubation of *E. coli* cells in the presence of cytochrome-containing membrane fraction restored the CFU forming ability of *polA* and *polArecB* deficient cells (Boling *et al*, 1984; Morimyo, 1982). The sensitivity to H_2O_2 together with the restoration of the growth under anaerobic conditions made us hypothesize that poor viability of Pol I-deficient cells on LB plates is associated with the inability to cope with DNA damage induced by ROS.

We used the iron chelator 2, 2'-dipyridyl, which blocks Fenton reaction-mediated hydroxyl radical formation by sequestering unbound iron, and the hydroxyl-radical scavenger thiourea to reduce the amount of ROS and compared the efficiency of colony formation of Pol I null mutant PaWPolAdel on LB plates in the presence of these agents. Figure 18 clearly demonstrates that the presence of 50 mM thiourea or 0.5 mM 2, 2'-dipyridyl in LB agar plates restored the CFU forming ability of Pol I-deficient *P. putida* strain PaWPolAdel. We also observed that the viability of this strain increased about 5–10-fold when 5000 units of beef liver catalase were top-spread onto LB plates. Since the presence of thiourea and 2, 2'-dipyridyl also reduced the growth rate of both wild type and Pol I deficient bacteria, it was possible that improved survival Pol I null mutants on LB agar plates could be also facilitated by the slower growth of cells in the presence of these compounds to allow more time to substitute Pol I functions. However, incubation of the plates at lower temperatures which significantly reduced the growth rate of bacteria (at temperatures 24℃, 20℃ and 15℃) Pol I-deficient cells were still extremely sensitive to the growth on LB plates and showed the comparable survival as the cells incubated at 30℃
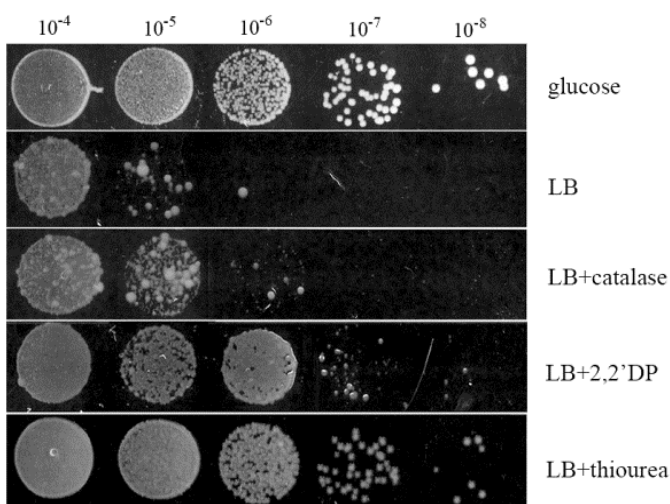
Figure 18. Effects of addition of thiourea, 2,2'-dipyridyl (2,2'-DP) and catalase on the survival of Pol I-deficient strain PaWPolAdel on LB plates. Serial dilutions of PaWPolAdel culture grown overnight in glucose minimal medium were spotted onto glucose minimal, LB, and LB agar plates containing 50 mM thiourea, 0.5 mM 2, 2'or 5000 units of beef liver catalase.

These results altogether indicate that solely the reduction of growth is not enough to sustain the viability of Pol I-deficient bacteria on LB plates and supports the idea that the poor viability of the cells on LB plates is due to increased sensitivity to ROS. The deleterious effect of the LB plates could also be enhanced by the fact that only certain amount of cells is spotted on the plate and if unable to efficiently maintain genome integrity in the absence of Pol I the cell dies and no colony is formed. In liquid medium the cell physiologically more adapted might replicate with more efficiency and contribute to the growth of population.

Notably, while reduction of ROS was sufficient to restore the growth of Pol I null mutants on the LB plates (Fig. 18), addition of thiourea into liquid growth medium did not abolish the filamentation of Pol I-deficient bacteria. The presence of thiourea reduced growth rate of bacteria also in the liquid medium. Nevertheless, there was no obvious difference in the length of filaments when the cultures of Pol I-deficient bacteria reached the same optical density as those grown without thiourea (data not shown). These results imply that the filamentation of the cells in rich liquid medium is likely to be a result of unprocessed Okazaki fragments which are shown to accumulate in the cells lacking Pol I functions (Okazaki *et al*, 1971; Konrad & Lehman, 1974). Formation of the filaments is associated with inhibition of the cell division as a result of activation of SOS response in the presence of ssDNA regions (Sassanfar & Roberts, 1990). Moreover, during the fast growth the next round of replication would reach the unprocessed nick or gap of the Okazaki fragment and the replication fork could collapse. The increased number of collapsed replication forks and ssDNA regions would account for filamentation and lower viability of Pol I-deficient bacteria in rich liquid medium. Consistently, the deficiency in recombinational repair, which is essential to restore collapsed replication forks and ssDNA gaps, leads to lethality of *recApolA* cells (Cao & Kogoma, 1995).

3.3. Pol I functions are important to suppress HR and mutagenesis in growing cells

Increased accumulation of nicks and gaps in the chromosome due to impaired joining of the Okazaki fragments and also dsDNA breaks resulting from replication fork collapse in the absence of Pol I provide sites for initiation of recombinational repair and elevated HR frequency can be anticipated. A hyper-recombination phenotype in Pol I-deficient *E. coli* cells was demonstrated by (Konrad, 1977) years ago. Yet, later, Pol I was shown to be required for spontaneous and cisplatin-induced recombination process (Nowosielska *et al*, 2004). We used the derivative of the strain CHR5127, CHR5127PolAdel, lacking the *polA* gene, to monitor the effect of Pol I deficiency on the HR process between chromosomal loci in *P. putida* cells. Notably, the strain

CHR5127PolAdel was not adapted for the growth on rich medium. As can be seen on Figure 14A, the deletion of the *polA* gene resulted in about 15-fold increase of the HR frequency. Noteworthy, Pol I deficiency did not cause the prolonged accumulation of Phe$^+$ recombinants throughout starvation period (data not shown), indicating that in contrast to UvrA and UvrB, the functions of Pol I are not critical for suppression of intrachromosomal HR in the starving cells.

The loss of Pol I in *P. putida* also resulted in a moderate mutator phenotype of bacteria (Table 1). As indicated above, the cells lacking all Pol I functions were sensitive to growth on LB agar plates but the suppressor mutants with improved tolerance to rich medium emerged easily. Notably, the frequency of spontaneous Rif^r mutations in the initial derivative was elevated 10–20 fold when assessed on Rif-glucose minimal plates (data not shown) but due to instability of growth phenotype of initial Pol I mutants the studies of the mutagenic processes were performed with the PaWPolAdel2 derivative adapted for the growth on LB. The mutation frequency in the adapted Pol I-deficient bacteria was increased about 8-fold in comparison to wild-type cells (Table 1, strains PaW85 and PaWPolAdel2), indicating that replication of the chromosome is erroneous in the absence of Pol I in both adapted and initial strains.

Moreover, the absence of Pol I significantly influenced the spectrum of spontaneous Rif^r mutations in *rpoB* gene (Table 1). Most importantly, the cells deficient in Pol I showed a marked increase in the frequency of deletions in comparison to *P. putida* wild-type strain. While being a rare event in the wild-type *P. putida* cells (the frequency 0.041×10^{-9} ($6.97 \times 10^{-9} \times 1/167$)), deletions occurred with more than 200 times higher frequency in the strain PaWPolAdel2 ($59.21 \times 10^{-9} \times 15/92 = 9.65 \times 10^{-9}$). The identified deletions in the Pol I-deficient bacteria encompassed 6, 9 or 12 nucleotides and started at nucleotide position 1611 of the *rpoB* gene, which is different from the position of the deletions detected in the wild-type *P. putida*. Importantly, due to limitations of Rif assay, only deletions of a certain length and at certain positions that do not disrupt the functionality of RNA polymerase could be detected.

The spectrum of base substitution was also affected by the absence of Pol I. For example, the frequency of A-to-G transitions at the position 1553 was increased about 40-times, from 0.58×10^{-9} in the wild-type strain to 22.53×10^{-9}, making this position a mutational hot-spot in Pol I-deficient cells. Yet, the mutational hot-spot of the wild-type cells (A-to-G transitions at the position 1562) disappeared in Pol I-deficient background. Whereas A-to-G base substitutions at this position occurred at the comparable frequency in both strains (2.63×10^{-9} in PaW85 and 2.57×10^{-9} in PaWPolAdel2) this mutation was only moderately present among other mutations in PaWPolAdel2 due to overall increased mutation frequency. Additionally, some novel base substitutions (e.g. A-to-T transversions at the position 1562) were identified in the spectrum of Rif^r mutations in the Pol I-deficient bacteria (Table 1). Altogether, our data

indicate that both the frequency of deletions and base substitutions in certain positions is increased in the absence of Pol I.

Given the distinct enzymatic activities of Pol I, two possibilities for the elevated mutation frequency in *E. coli* Pol I deficient mutants were proposed by (Tago *et al*, 2005). First, Pol I may be involved in removal of replication errors generated by the replicative DNA polymerase Pol III using its 5´ nuclease or Klenow 3´- 5´ exonuclease activities cooperatively with mismatch repair system (MMR). Alternatively, in the absence of Pol I processing of Okazaki RNA primers can be erroneous. Notably, deficiency in 5´ nuclease was shown to be associated with increased number of duplications and plus frameshifts, while Klenow domain is necessary to suppress formation of deletions and minus frameshift mutations (Nagata *et al*, 2002). Both domains are involved in preventing base substitutions (Imai *et al*, 2007). In our study we have also observed increased number of base substitutions and deletions in the *rpoB* gene in the absence of Pol I. However, due to limitations of Rif assay, mutations which disrupt the functions of RNA polymerase e.g. frameshifts or long deletions or insertions could not be detected. The generation of deletions and frameshifts identified by Nagata *et. al*., (2002) in the *tonB* assay was strongly associated with GC-clusters, which form direct or inverted repeats. This favours formation of the secondary structures that can be misaligned during replication. Similar association of deletions with GC-clusters in absence of Pol I was demonstrated analyzing spontaneous histidine mutations (Jankovic *et al*, 1990). Deletions in the *rpoB* gene which were detected in *P. putida* Pol I deficient strain also occurred in GC-rich DNA. However, no clear sequence repeats were found.

Table 1. Characterization of spontaneous Rif mutations in *P. putida* wild-type, NER-deficient and polymerase-deficient strains.

Position	aa change	nt change	Rif^R mutation frequency per 10^9 cells									
			PaW-85	PaW-PolB	PaW-DinB	PaW-DnaE2	PaW-UvrB	PaW-PolAdel2	PaW-PolAPolB	PaW-PolADinB	PaW-PolADnaE2	PaW-PolAUvrB
1546	L516V	C → G	0.04									
1547	L516P	T → C						1.29	1.25	3.78	4.74	2.70
1549	S517P	T → C								2.84		
1550	S517Y	C → A			0.13							
1550	S517F	C → T	0.08	0.21	0.88[a] (0.006)	0.32	0.11	3.22[a] (0.04)	3.12		0.95	2.70
1552	Q518K	C → A	0.08	0.11		0.11	0.11					
1553	Q518R	A → G	0.58	1.58[a] (0.029)	2.63[a] (0.001)	2.02[a] (0.0036)	1.07	22.53[a] (<10^{-4})	6.87[b] (<10^{-4})	11.35[b] (0.0027)	21.78	20.22
1553	Q518L	A → T	0.79	1.27	1.50	0.75	0.86	7.08	6.25	6.62	8.52	5.39
1553	Q518P	A → C	0.08	0.42	0.25							
1561	D521Y	G → T	0.08					1.29		0.95		
1561	D521N	G → A	0.04	0.21			0.11	0.64	3.75	2.84		1.35
1562	D521V	A → T		0.11		0.11	0.21	5.15[a] (<10^{-4})	1.25[b] (0.049)	–[b] (0.01)	0.95	–[c] (0.01)
1562	D521G	A → G	2.63	1.06	0.13[a] (<10^{-4})	1.28	3.32	2.57	20.62[b] (<10^{-4})	16.08[b] (<10^{-4})	3.79	2.7
1563	Del D521	Del CCA					0.11					
1567	Ins 4 aa	Ins 12 nt	0.04					0.64				1.35
1569[a]	Del N524	Del CAA										
1580	S527F	C → T	0.21	0.74[a] (0.0047)	1.88[a] (<10^{-4})	1.17[a] (0.0035)	0.64					

Table 1. (Continued)

Position	aa change	nt change	Rif^R mutation frequency per 10^9 cells									
			PaW-85	PaW-PolB	PaW-DinB	PaW-DnaE2	PaW-UvrB	PaW-PolAdel2	PaW-PolAPolB	PaW-PolADinB	PaW-PolADnaE2	PaW-PolAUvrB
1591	H531Y	C → T	0.25	0.42	0.25	0.75	0.75	0.64		0.95	1.89	
1591	H531D	C → G	0.04	0.11								
1592	H531L	A → T	0.25	0.21	0.38	0.21	0.11	1.93	6.87[b] (0.027)	6.62	2.84	
1592	H531	A → C			0.13	0.21						
1592	H531R	A → G	0.75	0.95	2.00	1.39	0.11			0.95	1.89	
1607	S536Y	C → A			0.13							
1607	S536F	C → T	0.67	1.27	0.63	1.07	0.54	1.29	2.50	1.89	3.79	2.70
1611	Del 2-4 aa	Del 6-12 nt						9.65[a] (<10⁻⁴)	5.62	13.24	9.47	8.09
1612	L538I	C → A	0.33									
1613	L538P	T → C			0.13	0.11		1.29				14.83[c] (0.0001)
1706	P569L	C → T		0.32	0.38	0.11	0.21					1.35
Un-known							0.21					1.35
Overall mutation frequency			**6.97**	**8.98**	**11.4**	**9.59**	**8.47**	**59.21**	**58.11**	**68.09**	**60.61**	**64.71**
Total number analyzed			**167**	**85**	**91**	**90**	**79**	**92**	**93**	**72**	**64**	**48**

[a] Denotes statistically significant differences ($P < 0.05$) between wild-type strain and strain lacking one of the DNA polymerases.
[b] Denotes statistically significant differences ($P < 0.05$) between Pol I-deficient strain and its derivative lacking additionally one of the specialized DNA polymerases.
[c] Denotes statistically significant differences ($P < 0.05$) between Pol I-deficient strain and its derivative lacking additionally UvrB.
P-values are shown in parentheses.

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3.4. Specialized DNA polymerases Pol II and Pol IV are involved in DNA synthesis in the absence of Pol I

P. putida harbours a set of specialized DNA polymerases, Pol II and Pol IV (encoded by PP2393 and PP1203, respectively), the homologues of the same DNA polymerases in *E. coli* encoded by the *polB* and *dinB* genes, and DnaE2 (encoded by PP3119), a paralogue of DnaE, the alpha subunit of DNA polymerase III (Abella *et al*, 2004; Tegova *et al*, 2004; Koorits *et al*, 2007). All these polymerases could potentially carry out DNA synthesis in the absence of Pol I. We compared the frequency of spontaneous Rif^r mutations in Pol-I deficient bacteria lacking additionally Pol II, Pol IV or DnaE2 to elucidate the possible role of these polymerases in mutational processes in the cells lacking Pol I. However, the frequency of Rif^r mutations was comparable in all cases, indicating that none of the specialized polymerases alone in responsible for the mutagenesis and the replication errors observed in Pol-I deficient cells were caused either by the replicative DNA polymerase III or concerted actions of several DNA polymerases.

Still, despite the fact that the absence of specialized polymerases did not affect the overall mutation frequency in the Pol I-deficient background, the differences of mutational spectra in *rpoB* gene in the Pol I mutant and its Pol II- and Pol IV-deficient derivatives indicated the involvement of these polymerases in DNA synthesis in the absence of Pol I, whereas the effect of the loss of DnaE2 was not significant (Table 1 and 2). For instance, the A-to-G transitions at the position 1553 present as a mutational hot-spot in the strain PaWPolAdel2 were significantly suppressed in the absence of both Pol II and Pol IV from 22.53×10^{-9} to 6.87×10^{-9} and 11.35×10^{-9}, respectively. On the contrary, the frequency of A-to-G transitions at the position 1562 was increased about 8- and 6-fold, respectively, in the strain PaWPolAPolB and PaWPolADinB in comparison to the single Pol I mutant. Such increase in A-to-G transitions in PaWPolAPolB and PaWPolADinB was also accompanied with decrease of the A-to-T transversions at the same position, which were present with the frequency 5.15×10^{-9} in Pol I-deficient bacteria. We have also observed that these transversions were abolished in the strain PaWPolAUvrB, suggesting that in the absence of Pol I the mutations could be introduced by Pol II and Pol IV during re-synthesis step of the NER pathway. These data altogether indicated that Pol II and Pol IV do participate in DNA synthesis in the absence of Pol I and that the ability to promote or suppress certain type of mutations of these polymerases depends on genetic context.

Table 2. Comparisons of mutational spectra

Comparisons	P-values
Spontaneous mutations	
Wild-type PaW85 ($n = 167$)	
versus PaWPolB ($n = 85$)	$P = 2.0 \times 10^{-4}$ ($4.0 \times 10^{-5} - 3.6 \times 10^{-4}$)
versus PaWDinB ($n = 91$)	$P < 4.0 \times 10^{-7}$
versus PaWDnaE2 ($n = 90$)	$P < 4.0 \times 10^{-7}$
versus PaWUvrB ($n = 79$)	$P = 4.7 \times 10^{-3}$ ($3.9 \times 10^{-3} - 5.5 \times 10^{-3}$)
versus PaWPolAdel2 ($n = 79$)	$P < 4.0 \times 10^{-7}$
PaWPolAdel2 ($n = 79$)	
versus PaWPolAPolB ($n = 93$)	$P < 4.0 \times 10^{-7}$
versus PaWPolADinB ($n = 72$)	$P < 4.0 \times 10^{-7}$
versus PaWPolADnaE2 ($n = 64$)	$P = 1.97 \times 10^{-1}$ ($1.9 \times 10^{-1} - 2.0 \times 10^{-1}$)
versus PaWPolAUvrB ($n = 48$)	$P = 8.1 \times 10^{-3}$ ($7.1 \times 10^{-3} - 9.1 \times 10^{-3}$)

UV-induced mutations	
Wild-type PaW85 spontaneous ($n = 167$)	
versus wild-type PaW85 UV ($n = 81$)	$P < 4.0 \times 10^{-7}$
PaWPolAdel2 spontaneous ($n = 79$)	
versus PaWPolAdel2 UV ($n = 78$)	$P < 4.0 \times 10^{-7}$
PaWPolAdel2 UV ($n = 78$)	
versus PaWPolADnaE2 UV ($n = 48$)	$P < 4.0 \times 10^{-7}$

Values in parentheses indicate the 95% confidence limits on the P-value. P-value of <0.05 means that the spectra are different in a pairwise comparison, but since we compared 13 mutational spectra, a Bonferroni correction for multiple comparisons with a corrected significance level of 6.4×10^{-4} ($0.05/12 \times 13 \times 0.5$) should be used. n in parentheses shows the number of mutants analysed.

As a rule, specialized DNA polymerases are damage inducible and their expression under normal growth conditions is repressed (Courcelle *et al*, 2001; Abella *et al*, 2004). The involvement of DNA polymerases Pol II and Pol IV in DNA replication is demonstrated in *E. coli* cells in the case when the SOS-induced polymerases are fully derepressed (in the strain *recA730 lexA*(Def)) (Curti *et al*, 2009) or overexpressed (Wolff *et al*, 2004; Kuban *et al*, 2005). The basal levels of specialized DNA polymerase do not contribute significantly to spontaneous mutational processes in *E. coli* (Kuban *et al*, 2005; Wolff *et al*, 2004; Tago *et al*, 2005).

Nevertheless, our results demonstrated that in *P. putida* specialized DNA polymerases participate in DNA replication also in the wild-type background in the absence of exogenous DNA damage, as the absence of all three polymerases (Pol II, Pol IV and also DnaE2) substantially affected the mutational spectra of spontaneous Rif^r mutations in the strains PaWPolB, PaWDinB and PaWDnaE2 if compared to the spectrum characterized in the wild-type strain PaW85 (Table 1). Interestingly, the absence of DNA polymerases Pol II and Pol IV affected the frequency of certain mutations in the opposite way in Pol I-proficient and deficient cells. For example, while A-to-G transitions at the position 1553 were significantly suppressed by the absence of Pol II and Pol IV in Pol-I-deficient cells, the occurrence of the same mutations was promoted in wild-type background in PaWPolB and PaWDinB strains (Table 1). Also the lack of DnaE2 enhanced the frequency of these base substitutions. Another example is the mutational hot-spot of the wild-type strain at the position 1562, where A-to-G transitions were significantly suppressed (20-fold) in the Pol-I proficient strain PaWDinB (and about two-fold in the strain PaWPolB) while being highly enhanced by the absence of Pol II and Pol IV in the cells lacking Pol I functions (PaWPolAPolB and PaWPolADinB). Lack of specialized polymerases also influenced the frequency of other base substitutions. For instance, the loss of all three polymerases promoted the frequency of C-to-T transitions at the positions 1580, and the absence of Pol II also increased the frequency of these transitions at the position 1550. The frequency of the deletions was not affected by any of the polymerases, indicating that deletions are most likely to be generated during DNA synthesis carried out by the replicative DNA polymerase III.

Differently from *E. coli*, the SOS regulon of pseudomonads does not include Pol II (Abella *et al*, 2007) and expression of Pol IV is only slightly enhanced upon exposure to DNA damaging agent in *P. putida* (Tegova *et al*, 2004). Thus, it is plausible that the basal level of expression of specialized DNA polymerases in *P. putida* is significantly higher than in *E. coli* and allows participation of DNA polymerases Pol II, Pol IV and DnaE2 in DNA replication under the normal growth conditions in wild-type background.

3.5. UV-irradiation affects the spectrum of Rif^r mutations in wild-type and Pol I-deficient *P. putida*

DNA-damaging agents and UV-irradiation is a source of genotoxic and mutagenic lesions. When exposed to UV-light, the primary damage to DNA is made by formation of covalent linkages between adjacent pyrimidines. Dimers are mostly formed in a thymine pair or between adjacent thymine and cytosine and if not repaired block the progression of replication fork. UV-induced mutagenesis in such cells is associated with error-prone translesion synthesis (TLS) past replication blocking lesions and requires participation of DNA polymerase V in *E. coli* while being mostly independent of DNA polymerases

Pol II and Pol IV (Wrzesiński *et al*, 2005; Napolitano *et al*, 2000). We have previously shown that *P. putida* expresses only a weak UV-mutagenesis phenotype with not more than two-fold difference in mutation frequency between non-induced and UV-C-irradiated wild-type *P. putida* cells (Tark *et al*, 2005).

Here, we further investigated the effects of UV-induced DNA damage on mutagenesis in *P. putida* wild-type and also in cells lacking Pol I functions. While the frequency of Rif^r mutations in both Pol I-proficient and -deficient bacteria exposed to UV-irradiation remained comparable to that of the strains without UV-treatment, indicating the absence of UV-induced mutagenesis, the spectra of Rif^r mutations in both cases clearly depended on the exposure of the cells to UV-light (Table 4,5 and 6). Consistent with the fact that UV-induced lesions involve pyrimidine dimers, most of the discussed mutations occurred at the sites with adjacent pyrimidines either in the *rpoB* coding sequence or in the complementary DNA strand. For example, a novel C-to-T transition at the position 1706 (frequency 0.61×10^{-9}) was observed in UV-irradiated wild-type cells while being undetected in the spectrum characterized in non-treated bacteria. Another significant increase was in the frequency of 1553 A-to-G transitions from 0.58×10^{-9} in non-induced to 1.74×10^{-9} in induced wild-type cells. The similar increase in A-to-G transitions was detected in spontaneous spectra of the cells lacking Pol I functions in comparison to wild-type strain and was dependent on the presence of Pol II and Pol IV. It is proposed that UV-induced DNA lesions are initially processed with high fidelity by NER (Courcelle *et al*, 2005). If the repair capacity is exceeded and the damage is not removed, the replication complex can resume movement, leaving an ssDNA gap in the daughter strand DNA. This gap can be restored either by recombinational repair or processed by translesion synthesis. In the absence of Pol I replication is also accompanied with increased amounts of ssDNA as gaps may be left due to impaired filling of the NER-generated gap or inefficient maturation of Okazaki fragments. Since the similar of distribution of A-to-G transitions in the case of increased amounts of DNA damage and ssDNA regions exists, we can hypothesize that it is associated with recombinational repair or translesion synthesis.

In Pol-I deficient background the most significantly was influenced the frequency of 1562 A-to-G transitions, being increased more than 10-fold upon UV-irradiation, while the frequency of 1553 A-to-G transitions, which were a mutational hot-spot in Pol I-deficient cells not exposed to UV-light, declined about 3-fold. Interestingly, this mutational fingerprint is similar to non-UV treated wild-type, PaWPolApolB and PaWPolADinB cells. Also, the number of deletions was significantly decreased as a result of UV-induced treatment of Pol I-deficient bacteria. The described differences in mutational spectra between wild-type and cells lacking Pol I functions imply the different mechanism of formation of the mutations in UV-induced cells. While in wild-type bacteria, as hypothesized before, mutations would be introduced during recombinational

repair of ssDNA gaps or translesion synthesis by Pol II and Pol IV, the absence of Pol I in the case of UV-induced damage would mostly be deleterious for NER functions, and the occurrence of mutations would be attributable to filling the ssDNA gaps, generated by excision during the NER pathway or recombinational repair of the collapsed replication forks.

3.6. Involvement of DnaE2 in DNA synthesis is stimulated by UV-damage

Although the lack of DnaE2 did not significantly affect the spectrum of spontaneous Rif^r mutations in the absence of Pol I functions (Table 1), considerable differences in mutational spectra appeared in the presence of UV-irradiation (Table 3). While the overall frequency of mutations remained significantly unaffected, the frequency of various base substitutions was affected in opposite ways. For example, the frequency of 1562 A-to-G transitions declined about 9-fold when DnaE2 was absent in Pol I-deficient cells. Also A-to-T transversions at the position 1592 occurring with the frequency 9×10^{-9} in the UV-irradiated cells lacking Pol I were not found in the mutational spectra when DnaE2 was additionally missing. Decline in the frequency of the mutations indicated an involvement of DnaE2 in generation of these base substations. However, the frequency of 1553 A-to-G transitions was significantly higher and a novel 1547 T-to-C transitions appeared with high frequency in the spectrum of UV-irradiated cells deficient in Pol I and DnaE2, indicating a suppressing role of DnaE2 on these mutations. Altogether, these results suggest that in contrast to negligible role of DnaE2 in DNA synthesis in the absence of Pol I under normal growth conditions, DnaE2 functions become important in the case of UV-induced DNA damage. As discussed before, in the absence of Pol I DnaE2 can be involved in filling in the gaps left after UvrABC-mediated incisions or be involved in translesion synthesis to resume DNA synthesis at arrested replication forks due to increased amounts of DNA damage in UV-irradiated cell.

In conclusion, Pol I functions are important for both chromosome replication and DNA repair of both endogenous and exogenous damage. The functions of Pol I can be substituted by various mechanisms including participation of specialized DNA polymerases in DNA synthesis in the absence of Pol I. The roles of Pol II and Pol IV appear also in the normally grown cells, whereas participation of DnaE2 is important when bacteria are exposed to UV-irradiation and DNA damage is induced

Table 3. Characterization of UV-induced Rif[r] mutations in *P. putida* wild-type and polymerase-deficient strains.

Position	aa change	nt change	Rif[r] mutation frequency per 10^9 cells[a]		
			PaW85	PaWPolAdel2	PaWPolADnaE2
1547	L516P	T→C			**14.36[c]**($<10^{-4}$)
1549	S517P	T→C			1.03
1550	S517L	C→T	0.17		
1553	Q518R	A→G	**1.74[a]**(0.0005)	**6.75[b]**(0.0001)	**22.56[c]**($<10^{-4}$)
1553	Q518L	A→T	0.52	6.75	−[c](0.015)
1553	Q518P	A→C	0.26		
1561	D521Y	G→T			
1561	D521N	G→A			
1562	D521V	A→T		1.50	1.03
1562	D521G	A→G	1.91	**27.77[b]**($<10^{-4}$)	**3.08[c]**($<10^{-4}$)
1580	S527F	C→T	0.26		
1591	H531Y	C→T	0.61	0.75	
1592	H531L	A→T	0.43	**9.01[b]**(0.006)	−[c](0.0045)
1592	H531R	A→G	**0.17[a]**(0.013)		
1607	S536F	C→T	0.35	3.75	2.05
1611	DEL			**1.50[b]**(0.0027)	5.13
1706	P569L	C→T	**0.61[a]**($<10^{-4}$)		
Unknown				0.75	
Total number of mutants analyzed			81	78	48
Overall mutation frequency (median value)			7.0	58.5	49.2

P–values are shown in parentheses

[a] Denotes statistically significant differences (P < 0.05) between irradiated and non-irradiated (see Table 1) wild-type strain.

[b] Denotes statistically significant differences (P < 0.05) between irradiated and non-irradiated (see Table 1) Pol I-deficient strain.

[c] Denotes statistically significant differences (P < 0.05) between UV-irradiated Pol I-deficient strain and its derivative lacking DnaE2.

IV. REDUCTION OF THE CONCENTRATION OF DNA DAMAGING AGENTS AS THE DAMAGE PROTECTION MECHANISM

Here we have demonstrated that NER enzymes UvrABC are important for genome maintenance in *P. putida* and the loss of their functions results in growth defects and filamentation of the cells even in the absence of exogenous DNA damage. Although functions of NER have been thoroughly studied in *E. coli,* the UvrABC enzymes have not been described to affect the viability or growth characteristics under normal growth conditions in this organism. Therefore, we were interested whether the observed effect was specific to *P. putida* and turned to another member of the genus *Pseudomonas*, *P. aeruginosa,* an opportunistic human pathogen. During this study we have encountered the variance of *P. aeruginosa* sublines of the laboratory strain PAO1 which implied the importance of the genetic background in the cells used for a research.

Rapid adaptation and emergence of multidrug resistance phenotype due to mutations in *P. aeruginosa* genome is a well-known mechanism that ensures the advantage of this organism during host infection and complicates its treatment. However, the diversification of the genomic sequences and differences in antimicrobial susceptibility of the laboratory strains has also been reported as a common problem. PAO1, the major laboratory strain used for the research on *P. aeruginosa*, is a spontaneous chloramphenicol-resistant mutant that had been isolated by Bruce Holloway from a wound in Melbourne, Australia (Holloway, 1955). The reference genome of *P. aeruginosa* strain PAO1 has been sequenced in 2000 and received a name PAO-UW, after the University of Washington, which led the sequencing project (Stover *et al*, 2000). While being the major source of genomic sequence of the PAO1 sublines available for researchers, it possesses a major genomic difference in comparison to other strains, which is a large 2.2 Mb inversion between two ribosomal operons *rrnA* and *rrnB* (Stover *et al*, 2000). Such inversion results in an asymmetric localization of the terminus of replication and is lacking in all other sublines (Stover *et al*, 2000; Klockgether *et al*, 2010). Additionally, the sublines of this strain maintained worldwide in laboratories and strain collections have been shown to have other variations both in their genotypes and phenotypes, including multiple single-nucleotide polymorphisms (SNPs), small deletions and the differences in their virulence, antimicrobial susceptibility and their ability to cope with nutrient limitation (Klockgether *et al*, 2010; Maseda *et al*, 2000). Mutations in the *lasR* gene, affecting quorum-sensing signalling have also been documented in some strains (Heurlier *et al*, 2005). Such variation of reference strains considerably complicates the comparability of the results and is a challenge for researchers.

4.1. The effect of UvrB on DNA-damage tolerance varies in PAO1 sublines

P. aeruginosa and *P. putida* are closely related and share about 85% of the predicted coding regions (Nelson *et al*, 2002). In order to investigate the effect of the absence of NER enzymes on genome maintenance and DNA damage tolerance in *P. aeruginosa*, we constructed UvrB-deficient derivative of the *P. aeruginosa* strain MPAO1, MPAOUvrB. MPAO1 subline was obtained from the collection of the University of Washington, Seattle. This subline has been used for the construction of the PAO1 nearly saturated two-allele mutant library, available for order online (Jacobs *et al*, 2003).

Distinctly from *P. putida*, *P. aeruginosa* MPAO1 cells lacking UvrB did not display any colony heterogeneity or significant filamentation when grown in a rich liquid medium (data not shown). To further assess the role of UvrB in DNA-damage tolerance in *P. aeruginosa*, we exposed UvrB-deficient *P. aeruginosa* cells to UV-irradiation and DNA-damaging agent 4-nitroquinoline 1-oxide (4-NQO). Similarly to the result observed in *P. putida* (Fig. 15), the deletion of the *uvrB* gene extremely sensitized *P. aeruginosa* to UV-irradiation, with the survival reduced to zero after exposure to a dose of 3 J/m^2 (Fig. 19). Interestingly, MPAOUvrB was only slightly more sensitive to 5 µM 4-NQO than the parent strain, while the exposure of *P. putida* UvrB-deficient cells to the same 4-NQO concentration resulted in a drastic (3–4 orders of magnitude) decrease in the survival of bacteria (Fig. 15). Additionally, while the deficiency in TLS DNA polymerase DinB has been shown to sensitize both *E. coli* and *P. aeruginosa* to 4-NQO induced damage (Sanders *et al*, 2006; Jarosz *et al*, 2006), we were unable to detect any effect of the lack of DinB on the viability of *P. aeruginosa* MPAO1 in the presence of 4-NQO (data not shown). Such inconsistency prompted us to use and analyze other sublines of PAO1 strain obtained from different strain collections: PAO-UT (the stock in our laboratory, University of Tartu, obtained more than 10 years ago from the Pseudomonas Genetic Stock Centre, the East Carolina University), PAO1-L (from Switzerland) and PAO1-DSM (from Germany). In all these sublines the deficiency in UvrB resulted in hypersensitivity to UV-damage and significantly reduced the tolerance to 4-NQO (Fig. 19, the subline PAO1-DSM is shown as an example), confirming that the absence of the effect of UvrB-deficiency on 4-NQO induced DNA damage tolerance was specific to the MPAO1 strain (Fig. 19, the subline PAO1-DSM is shown as example)
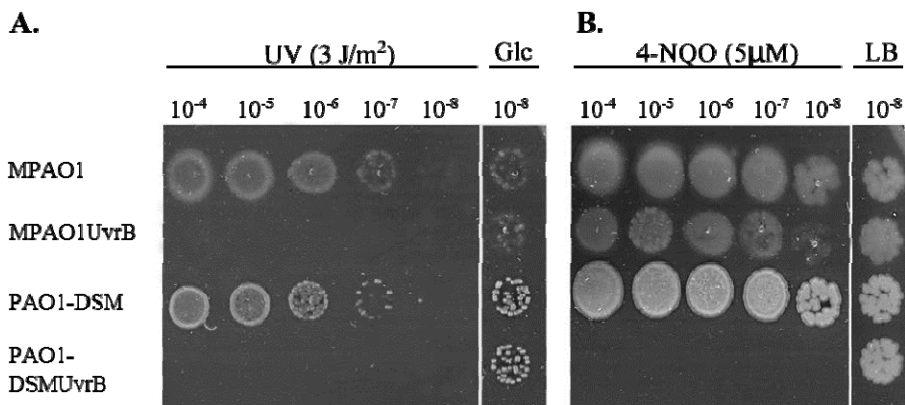
Figure 19. Sensitivity of *P. aeruginosa* PAO1 sublines MPAO1 and PAO1-DSM and their UvrB-deficient derivatives to UV-irradiation and exposure to 4-nitroquinoline (4-NQO). Serial dilutions from overnight cultures were spotted onto LB plates containing 5 μM 4-NQO or glucose minimal plates, which were then exposed to UV-irradiation (3 J/m2). Only 10^{-8} dilution for non-treated cells is shown.

4.2. The *mexEF-oprN* operon is overexpressed in sublines MPAO1 and PAO1-UT

MPAO1 is described to be 4-fold more resistant to β-lactam antibiotic imipenem, up to 4-fold more resistant to fluoroquinolones, and 16-fold more resistant to chloramphenicol than the PAO1-UW and PAO1-DSM sublines (Klockgether *et al*, 2010). Such phenotype strongly resembles *nfxC*-phenotype, caused by the up-regulation of the normally quiescent MexEF-OprN multidrug efflux system. Intact MexT protein is required for activation of the expression of the *mexEF-oprN* operon (Köhler *et al*, 1999). Variations in the *mexT* sequence have been described in different sublines of PAO1, whereas many sublines carry inactive *mexT* gene due to mutations in it (Köhler *et al*, 2001; Maseda *et al*, 2000). However, although MPAO1 subline has been shown to have higher resistance to antimicrobials, no differences from the reference genome PAO1-UW *mexT* gene sequence has been detected to account for such phenotype (Klockgether *et al*, 2010). We have analyzed the sequence of the *mexT* gene in four PAO1 sublines: MPAO1, PAO-UT, PAO1-L and PAO1-DSM and in two independent *dinB*-deficient transposon mutants obtained from the two-allele transposon mutant library (PW2074 and PW2075). The *mexT* allele of the reference genome PAO1-UW (obtained from www.pseudomonas.com) contains a 8-bp insert (5'-CGGCCAGC-3') at the 240th position of the gene, rendering the MexT inactive (Maseda *et al*, 2000). Analysis of the nucleotide sequence of the *mexT* gene in four PAO1 sublines and PW2074 and PW2075 transposon mutants showed that the 8-bp insertion was present only in PAO1-L

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subline and was missing in MPAO1, PAO1-UT, PAO1-DSM, PW2074 and PW2075. The strain PAO1-DSM, however, had a 4-bp insertion (5'-CTAT-3') at the position 548, which should also result in a frameshift and a truncated MexT. Additionally, a SNP was detected at the position 514, with T for the reference genome PAO1-UW and PAO1-DSM and A for MPAO1, PAO1-UT, PAO1-L, PW2074 and PW2075. This SNP, however, was shown to be dispensable for the MexT functionality (Maseda *et al*, 2000). Thus, according to the gene sequence, MPAO1, PAO1-UT, PW2074 and PW2075 appeared to have intact *mexT* genes that would allow expression of the *mexEF-oprN* genes (Table 4).

To test whether the intact MexT activates the expression of the *mexEF-oprN* genes in the studied strains, we quantified the levels of the *mexF*-specific mRNA in the cells grown overnight in LB medium by a real-time qRT-PCR. The sublines MPAO and PAO1-UT displayed an approximately 150-fold higher level of *mexF* mRNA than the LPAO and PAO1-DSM sublines (Table 4). These data implied that the operon is fully expressed in the sublines MPAO1 and PAO1-UT and confirmed the restoration of the functionality of MexT predicted by the *mexT* sequence in these sublines. Nevertheless, despite of the intact *mexT* sequence such overexpression was absent in the strains PW2074 and PW2075 (Table 4). Notably, these strains are the transposon mutants of the MPAO1 subline and thus should have the similar high expression of this multidrug efflux system. The described discrepancy implies that the parent strain MPAO1 and its transposon derivatives PW2074 and PW2075 do not have an identical genetic background and thus cannot be used in comparative assays.

4.3. MPAO1 is an *nfxC*-type mutant

We further investigated the susceptibility of the PAO1 sublines and MPAO1 transposon mutants to antibiotics ciprofloxacin, chloramphenicol, ampicillin and kanamycin (Table 4). Consistently with the previously published results (Klockgether *et al*, 2010), MPAO1 showed the increased resistance to fluoroquinolone ciprofloxacin and to chloramphenicol and in accordance with the *nfxC*-phenotype, was slightly more susceptible to ampicillin and kanamycin (Table 4). These results together with the real-time qRT-PCR results and the observation of the absence of the 8-bp insertion in the *mexT* gene clearly indicate that the subline MPAO1 is an *nfxC*-type mutant. Thus, the increased tolerance of MPAO UvrB mutant to 4-NQO is most likely attributed to the *nfxC*-phenotype and effective extrusion of DNA-damaging molecules.

The fact that the strains PW2074 and PW2075 with the intact *mexT* sequence do not overexpress the *mexEF-oprN* operon and lack the *nfxC*-phenotype can be attributed to the fact that MexT is additionally regulated. Both clinical isolates and laboratory strains with the intact *mexT* sequences that do not produce *nfxC*-type phenotype are described. Such stains need an additional mutation to

express the *nfxC*-type phenotype (Fig. 6.), (Köhler *et al*, 1999; Uwate *et al*, 2013; Maseda *et al*, 2000). MexS-mediated and yet unidentified MexS-independent mechanisms for the MexT-mediated regulation of the *mexEF-OprN* expression has been described (Fig. 6), (Uwate *et al*, 2013; Sobel *et al*, 2005b). In the MexS-mediated pathway, MexT is activated by mutational disruption of the *mexS* gene, encoding putative oxidoreductase, which is suggested to alter the redox state of the cell (Fargier *et al*, 2012; Sobel *et al*, 2005b).

In order to determine the possible differences in MexT regulation between the *mexEF-oprN*-expressing strains MPAO1 and its transposon-derivatives PW2074 and PW2075, which all have predictably intact MexT, we examined the *mexS* sequences in the four PAO1 sublines and PW2074 and PW2075. However, none of the strains displayed any *mexS* sequence alternations in comparison to the *mexS* allele of the reference genome PAO1-UW (Table 4). This suggests that MexS is unimpaired in all these strains and another mechanism is responsible for the MexT activation in MPAO1. Consistently, MexS-dependent regulation has been confirmed only for clinical isolates and introduction of the intact MexS into *nfxC*-type mutants derived from the laboratory strains failed to abolish MexT-dependent regulation and revert the cells to a wild type-phenotype (Uwate *et al*, 2013).

We additionally tested susceptibility of the PAO1 sublines to a DNA-damaging agent MMS and to an aromatic compound phenol. MPAO1 and PAO1-UT were more tolerant to MMS than sublines PAO1-L and PAO1-DSM, which did not express *mexEF-oprN* (Table 4). Thus, the tolerance to MMS appears to be in accordance with MexEF-OprN expression. However, like being the only strain resistant to quinolones and chloramphenicol, MPAO1 was the only subline sensitive to phenol. Sensitivity of *nfxC*-type mutants to β-lactam antibiotics, observed here also for MPAO1, has been described to be associated with the reduced expression of MexAB-OprM (Maseda *et al*, 2004). Thus, sensitivity to phenol in MPAO1 could be attributed elaborate interplay and co-regulation of various pumps that leads to reduction of expression of one of the efflux pumps, including MexAB-OprM.

Collectively, an effective reduction of the cellular concentration of the drugs can prevent them from imposing significant DNA damage, being a forceful DNA damage protection mechanism. However, increased sensitivity of MPAO1 strain to some β-lactams and aminoglycosides (e.g., ampicillin and kanamycin) and also phenol shows intriguing interplay between different tolerance mechanisms to various compounds.

Table 4. Characterization of the *P. aeruginosa* PAO1 sublines and two *dinB*-deficient transposon-mutants derived from MPAO1.

Strain	*mexS* intact[b]	*mexT* intact[b]	*mexEF-oprN* expression (fold)[c]	MIC[a]					
				Cipro-floxacin (μg/ml)[d]	Chlor-amphenicol (μg/ml)[d]	Ampicillin (μg/ml)[d]	Kanamycin (μg/ml)[d]	MMS (mM)[e]	Phenol sensitive[g]
MPAO1	Yes	Yes	Yes (150.0)	0.5	1024	256–512	32	6	Yes
PAO1-L	Yes	No	No (1.0)	0.06	32–64	512–1024	64	4	No
PAO1-DSM	Yes	No	No (1.2)	0.06	32	512–1024	32	4	No
PAO1-UT	Yes	Yes	Yes (157.4)	0.06	64	512–1024	32–64	5	No
PW2074	Yes	Yes	No (0.5)	0.06	32–64	1024	32–64	ND[f]	No
PW2075	Yes	Yes	No (0.8)	0.06	32–64	1024	32–64	ND[f]	No

[a] MIC, minimum inhibitory concentration
[b] Functionality is predicted from the DNA sequencing data
[c] Levels of the *mexF* mRNA relative to the level of the *mexF* mRNA in the strain PAO1-L in the cells grown overnight in LB medium. PA2875 was used as a reference gene
[d] MIC was determined by a serial two-fold microdilution method in LB medium
[e] MIC was accessed by a step of 1mM
[f] ND, not determined
[g] Phenol sensitivity was determined as the inability to grow on LB agar plates containing 5 mM phenol

4.4. PAO1-UT has a large deletion encompassing *imuA-imuB-dnaE2* and *cat* genes

Surprisingly, despite the actively expressing *mexEF-oprN*, PAO1-UT subline lacked the enhanced resistance to ciprofloxacin and chloramphenicol. It has been demonstrated that the strain PAO1-N, maintained in the laboratory of University of Nottingham has a unique 52-kb deletion between the gene PA0669 and PA0707 (Hardeep Naghara, personal communication). This region was intriguing to us, as it contains a DNA damage inducible *imuA-imuB-dnaE2* cassette (PA0669-PA0671). Additionally, chloramphenicol acetyltransferase gene *cat* (PA0706) is found in this region. We have confirmed the absence of both the *imuA-imuB-dnaE2* operon and the *cat* gene in the PAO1-UT subline using a comparative PCR with the other sublines (Table 4). The absence of chloramphenicol acetyltransferase in PAO1-UT subline can thus be associated with its sensitivity to chloramphenicol. However the absence of other *nfxC*-phenotype features, such as reduced tolerance to ciprofloxacin, remains obscure.
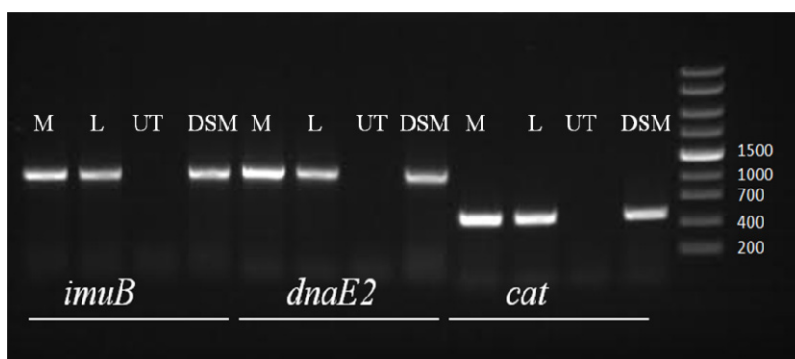
Figure 20. Presence of the *imuB-dnaE2* and *cat* genes in genomes of PAO1 sublines MPAO (M), PAO1-L (L), PAO1-UT (UT), PAO1-DSM (DSM)

The deleted region is located adjacent to the ribosomal operon *rrnB*. Keeping in mind that the reference genome contains a large inversion that have resulted from homologous recombination between *rrnA* and *rrnB* loci, it is possible that an independent similar inversion event could have occurred also in the strain PAO-UT, and this recombination event has entailed the deletion of the adjacent genes.

To conclude, in addition to enormous variation of clinical isolates of *P. aeruginosa*, variation between laboratory strains of *P. aeruginosa* also exists. In our hands the wild-type MPAO1 subline displayed a clear *nfxC*-type phenotype, while the transposon mutants derived from this strain did not. Additionally, PAO-UT subline lacked a set of genes, including damage-inducible operon *imuA-imuB-dnaE2* which is involved in damage-induced mutagenesis. All these differences pose a serious challenge for comparison of the results from different laboratories.

CONCLUSIONS

Efficient error-free replication despite of multiple impediments on the road is a challenge that living cells are confronted during their life cycle. Spontaneous damage to DNA molecules includes deamination, oxidation and methylation of DNA bases and can be mutagenic or even impede the replication machinery. Nucleotide excision repair (NER) is one of the major DNA repair pathways involved in repair of a broad range of DNA lesions generally induced by exogenous chemicals or UV-irradiation but its functions in the cells not exposed to DNA-damaging agents have attracted less attention. Moreover, DNA polymerase I (Pol I), an essential multifunctional enzyme involved in processing of Okazaki fragments during lagging strand synthesis, also acts in the re-synthesis step of the NER and base excision repair (BER) pathways.

Here we have addressed the roles of NER enzymes and Pol I in maintenance of genome integrity in *Pseudomonas putida*. Since homologous recombination (HR) is a key back-up player in DNA repair and damage tolerance, being involved in recovery of replication forks perturbed by DNA damage, we have constructed a novel assay to monitor the frequency of HR between two chromosomal loci and accessed the impact of various NER pathway enzymes and Pol I on genome stability by measuring the frequencies of HR events. We also investigated the phenotypic effects of the absence of Pol I and NER proteins and studied the involvement of specialized DNA polymerases in mutagenesis observed in the absence of Pol I. Additionally, the cells can benefit from extruding a variety of noxious compounds from the cell, thereby reducing potential to DNA damage. Using various sublines of the *P. aeuginosa* strain PAO1 we demonstrated the dynamic evolution and variability in the efflux ability even between the laboratory sublines.

The main conclusions of this thesis are as follows:

1. HR between chromosomal loci occurs with high frequency during the active growth of bacteria in the absence of exogenous DNA damage and, unlike the recombinational events between a plasmid and the chromosome, is suppressed during starvation of wild-type cells. This is consistent with the assumption that HR-mediated restoration of DNA replication or recombinational repair is important under normal growth conditions due to constant DNA damage

2. NER enzymes UvrABC are important for maintenance of genome integrity in the absence of exogenously induced DNA damage in *P. putida*. The cells lacking UvrABC enzymes experience severe growth problems and enhanced recombination frequencies. Moreover, UvrA and UvrB, in contrast to UvrA2, UvrD, Mfd or Pol I, are needed to prevent HR events in starving bacteria. Rapid adaptation of UvrABC-deficient cells abrogates the deleterious phenotype and prolonged accumulation of recombinants, but does not increase the tolerance of the cells to DNA damaging agents and UV-

irradiation, which implies a role of UvrABC complex outside the repair of canonical bulky lesions.

3. Transcription coupled NER sub-pathway (TC-NER) key enzyme Mfd is also needed for suppression of HR in growing bacteria, which indicates the importance of this pathway in DNA damage repair in the normally grown cells. Moreover, the absence of UvrD helicase, which has been recently implied in TC-NER process also significantly, affects the frequency of HR. However, due to multiple roles of UvrD in the cell it is difficult to assign the pathway, where it is needed the most. In contrast, UvrA2, an UvrA homologue lacks any obvious effects on HR process in *P. putida* even in the absence of the primary UvrA protein, indicating its minor role, at least in the absence of exogenous DNA damage.

4. Deficiency in Pol I functions results in extensive filamentation of the cells in nutritionally rich medium (LB medium) and severe growth defects on LB agar plates while only minor growth problems appeared in minimal medium. Restoration of the growth of Pol I-deficient bacteria on LB plates in the presence of chemicals reducing the amounts of reactive oxygen species suggests that the inability to efficiently form colonies on LB plates is attributed mostly to defects of excision repair in the absence of Pol I, not only inability to efficiently process Okazaki fragments under fast growth conditions.

5. Moderate mutator phenotype observed in the absence of Pol I does not depend on any of the specialized DNA polymerases and most likely involves errors produced by the replicative DNA polymerase Pol III. However, involvement of Pol II, Pol IV and DnaE2 in DNA replication in the absence of Pol I is evident by the altered spectra of Rif^r mutations in the *rpoB* gene. Pol II and Pol IV influence the spectrum of mutations both in normally grown and UV-irradiated cells, while the role of DnaE2 appears under conditions of UV-induced DNA damage, which is consistent with DNA damage inducible nature of DnaE2 expression.

6. Diversification of *P. aeruginosa* genomes occurs in PAO1 sublines distributed in laboratories worldwide. Of four different PAO1 sublines, MPAO1 has been shown to be *nfxC*-type quinolone-resistant mutant that actively expresses MexEF-OprN efflux system due to restoration of intact sequence of the regulatory gene *mexT*. The MexEF-OprN efflux pump is quiescent in the PAO1 sublines PAO1-L and PAO1-DSM. Deletion of the chromosomal region in the PAO-UT subline can be partly attributed to the absence *nfxC*-type phenotype in this strain despite the expression of the *mexEF-oprN* genes. Such ongoing microevolution and discordant pheno-types of the laboratory strains including the differences in antimicrobial susceptibility challenge the reproducibility and comparability of research.

REFERENCES

Abella M, Campoy S, Erill I, Rojo F & Barbé J (2007) Cohabitation of two different *lexA* regulons in *Pseudomonas putida*. *J. Bacteriol.* **189:** 8855–62

Abella M, Erill I, Jara M, Mazón G, Campoy S & Barbé J (2004) Widespread distribution of a *lexA*-regulated DNA damage-inducible multiple gene cassette in the Proteobacteria phylum. *Mol. Microbiol.* **54:** 212–22

Adewoye L, Sutherland A, Srikumar R & Poole K (2002) The MexR repressor of the *mexAB-oprM* multidrug efflux operon in *Pseudomonas aeruginosa*: characterization of mutations compromising activity. *J. Bacteriol.* **184:** 4308–12

Aghazadeh M, Hojabri Z, Mahdian R, Nahaei MR, Rahmati M, Hojabri T, Pirzadeh T & Pajand O (2014) Role of efflux pumps: MexAB-OprM and MexXY(-OprA), AmpC cephalosporinase and OprD porin in non-metallo-β-lactamase producing *Pseudomonas aeruginosa* isolated from cystic fibrosis and burn patients. *Infect. Genet. Evol.* **24:** 187–92

Aires JR, Köhler T, Nikaido H & Plésiat P (1999) Involvement of an active efflux system in the natural resistance of *Pseudomonas aeruginosa* to aminoglycosides. *Antimicrob. Agents Chemother.* **43:** 2624–8

Amado L & Kuzminov A (2013) Low-molecular-weight DNA replication intermediates in *Escherichia coli*: mechanism of formation and strand specificity. *J. Mol. Biol.* **425:** 4177–91

Angus BL, Carey AM, Caron DA, Kropinski AM & Hancock RE (1982) Outer membrane permeability in *Pseudomonas aeruginosa*: comparison of a wild-type with an antibiotic-supersusceptible mutant. *Antimicrob. Agents Chemother.* **21:** 299–309

Arthur HM & Lloyd RG (1980) Hyper-recombination in *uvrD* mutants of *Escherichia coli* K-12. *Mol. Gen. Genet.* **180:** 185–191

Atkinson J, Guy CP, Cadman CJ, Moolenaar GF, Goosen N & McGlynn P (2009) Stimulation of UvrD helicase by UvrAB. *J. Biol. Chem.* **284:** 9612–23

Atkinson J & McGlynn P (2009) Replication fork reversal and the maintenance of genome stability. *Nucleic Acids Res.* **37:** 3475–92

Au N, Kuester-Schoeck E, Mandava V, Bothwell LE, Canny SP, Chachu K, Colavito SA, Fuller SN, Groban ES, Hensley LA, O'Brien TC, Shah A, Tierney JT, Tomm LL, O'Gara TM, Goranov AI, Grossman AD & Lovett CM (2005) Genetic composition of the *Bacillus subtilis* SOS system. *J. Bacteriol.* **187:** 7655–66

Baharoglu Z & Mazel D (2014) SOS, the formidable strategy of bacteria against aggressions. *FEMS Microbiol. Rev.* **38:** 1126–45

Bates H, Randall SK, Rayssiguier C, Bridges BA, Goodman MF & Radman M (1989) Spontaneous and UV-induced mutations in *Escherichia coli* K-12 strains with altered or absent DNA polymerase I. *J. Bacteriol.* **171:** 2480–4

Becherel OJ, Fuchs RPP & Wagner J (2002) Pivotal role of the beta-clamp in translesion DNA synthesis and mutagenesis in *E. coli* cells. *DNA Repair (Amst).* **1:** 703–8

Bellido F, Martin NL, Siehnel RJ & Hancock RE (1992) Reevaluation, using intact cells, of the exclusion limit and role of porin OprF in *Pseudomonas aeruginosa* outer membrane permeability. *J. Bacteriol.* **174:** 5196–203

Bidnenko V, Lestini R & Michel B (2006) The *Escherichia coli* UvrD helicase is essential for Tus removal during recombination-dependent replication restart from Ter sites. *Mol. Microbiol.* **62:** 382–96

Bierne H, Seigneur M, Ehrlich SD & Michel B (1997) *uvrD* mutations enhance tandem repeat deletion in the *Escherichia coli* chromosome via SOS induction of the RecF recombination pathway. *Mol. Microbiol.* **26:** 557–67

Bjelland S & Seeberg E (2003) Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. *Mutat. Res.* **531:** 37–80

Boles BR & Singh PK (2008) Endogenous oxidative stress produces diversity and adaptability in biofilm communities. *Proc. Natl. Acad. Sci. U. S. A.* **105:** 12503–8

Boling M, Adler H & Masker W (1984) Restoration of viability to an *Escherichia coli* mutant deficient in the 5'-3' exonuclease of DNA polymerase I. *J. Bacteriol.* **160:** 706–10

Boshoff HIM, Reed MB, Barry CE & Mizrahi V (2003) DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in *Mycobacterium tuberculosis*. *Cell* **113:** 183–93

Boyce RP & Howard-Flanders P (1964) Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K-12. *Proc. Natl. Acad. Sci. U. S. A.* **51:** 293–300

Cai Y, Geacintov NE & Broyde S (2014) Ribonucleotides as nucleotide excision repair substrates. *DNA Repair (Amst).* **13:** 55–60

Caille O, Rossier C & Perron K (2007) A copper-activated two-component system interacts with zinc and imipenem resistance in *Pseudomonas aeruginosa*. *J. Bacteriol.* **189:** 4561–8

Campbell JL & Kleckner N (1990) *E. coli oriC* and the *dnaA* gene promoter are sequestered from dam methyltransferase following the passage of the chromosomal replication fork. *Cell* **62:** 967–79

Cao L, Srikumar R & Poole K (2004) MexAB-OprM hyperexpression in NalC-type multidrug-resistant *Pseudomonas aeruginosa*: identification and characterization of the *nalC* gene encoding a repressor of PA3720-PA3719. *Mol. Microbiol.* **53:** 1423–36

Cao Y & Kogoma T (1995) The mechanism of *recA polA* lethality: suppression by RecA-independent recombination repair activated by the *lexA*(Def) mutation in *Escherichia coli*. *Genetics* **139:** 1483–94

Capaldo-Kimball F & Barbour SD (1971) Involvement of recombination genes in growth and viability of *Escherichia coli* K-12. *J. Bacteriol.* **106:** 204–12

Chamberland S, Malouin F, Rabin HR, Schollaardt T, Parr TR & Bryan LE (1990) Persistence of *Pseudomonas aeruginosa* during ciprofloxacin therapy of a cystic fibrosis patient: transient resistance to quinolones and protein F-deficiency. *J. Antimicrob. Chemother.* **25:** 995–1010

Chen H, Hu J, Chen PR, Lan L, Li Z, Hicks LM, Dinner AR & He C (2008a) The *Pseudomonas aeruginosa* multidrug efflux regulator MexR uses an oxidation-sensing mechanism. *Proc. Natl. Acad. Sci. U. S. A.* **105:** 13586–91

Chen H, Yi C, Zhang J, Zhang W, Ge Z, Yang C-G & He C (2010) Structural insight into the oxidation-sensing mechanism of the antibiotic resistance of regulator MexR. *EMBO Rep.* **11:** 685–90

Chen Z, Yang H & Pavletich NP (2008b) Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures. *Nature* **453:** 489–4

Chodavarapu S, Gomez R, Vicente M & Kaguni JM (2008) *Escherichia coli* Dps interacts with DnaA protein to impede initiation: a model of adaptive mutation. *Mol. Microbiol.* **67:** 1331–46

Chow K-H & Courcelle J (2004) RecO acts with RecF and RecR to protect and maintain replication forks blocked by UV-induced DNA damage in *Escherichia coli*. *J. Biol. Chem.* **279:** 3492–6

Ciccia A & Elledge SJ (2010) The DNA damage response: making it safe to play with knives. *Mol. Cell* **40:** 179–204

Cirz RT, Chin JK, Andes DR, de Crécy-Lagard V, Craig WA & Romesberg FE (2005) Inhibition of mutation and combating the evolution of antibiotic resistance. *PLoS Biol.* **3:** e176

Cirz RT, O'Neill BM, Hammond J a, Head SR & Romesberg FE (2006) Defining the *Pseudomonas aeruginosa* SOS response and its role in the global response to the antibiotic ciprofloxacin. *J. Bacteriol.* **188:** 7101–10

Conejo MC, García I, Martínez-Martínez L, Picabea L & Pascual A (2003) Zinc eluted from siliconized latex urinary catheters decreases OprD expression, causing carbapenem resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **47:** 2313–5

Courcelle CT, Belle JJ & Courcelle J (2005) Nucleotide excision repair or polymerase V-mediated lesion bypass can act to restore UV-arrested peplication forks in *Escherichia coli*. **187:** 6953–6961

Courcelle CT, Chow K-H, Casey A & Courcelle J (2006) Nascent DNA processing by RecJ favors lesion repair over translesion synthesis at arrested replication forks in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **103:** 9154–9

Courcelle J, Belle JJ & Courcelle CT (2004) When replication travels on damaged templates: bumps and blocks in the road. *Res. Microbiol.* **155:** 231–237

Courcelle J, Crowley DJ & Hanawalt PC (1999) Recovery of DNA replication in UV-irradiated *Escherichia coli* requires both excision repair and RecF protein function. *J. Bacteriol.* **181:** 916–22

Courcelle J & Hanawalt PC (2003) RecA-dependent recovery of arrested DNA replication forks. *Annu. Rev. Genet.* **37:** 611–646

Courcelle J, Khodursky A, Peter B, Brown PO & Hanawalt PC (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli. Genetics* **158:** 41–64

Courcelle J, Wendel BM, Livingstone DD & Courcelle CT (2015) RecBCD is required to Complete Chromosomal Replication: Implications for Double-Strand Break Frequencies and Repair Mechanisms. *DNA Repair (Amst).*

Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ & Marians KJ (2000) The importance of repairing stalled replication forks. *Nature* **404:** 37–41

Curti E, McDonald JP, Mead S & Woodgate R (2009) DNA polymerase switching: effects on spontaneous mutagenesis in *Escherichia coli. Mol. Microbiol.* **71:** 315–31

Daigle DM, Cao L, Fraud S, Wilke MS, Pacey A, Klinoski R, Strynadka NC, Dean CR & Poole K (2007) Protein modulator of multidrug efflux gene expression in *Pseudomonas aeruginosa*. *J. Bacteriol.* **189:** 5441–51

Deaconescu AM, Savery N & Darst SA (2007) The bacterial transcription repair coupling factor. *Curr. Opin. Struct. Biol.* **17:** 96–102

Dillingham MS & Kowalczykowski SC (2008) RecBCD enzyme and the repair of double-stranded DNA breaks. *Microbiol. Mol. Biol. Rev.* **72:** 642–71

Donczew R, Weigel C, Lurz R, Zakrzewska-Czerwinska J & Zawilak-Pawlik A (2012) *Helicobacter pylori oriC*-the first bipartite origin of chromosome replication in Gram-negative bacteria. *Nucleic Acids Res.* **40:** 9647–60

Drake JW (1991) A constant rate of spontaneous mutation in DNA-based microbes. *Proc. Natl. Acad. Sci. U. S. A.* **88:** 7160–4

Drlica K & Zhao X (1997) DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol. Mol. Biol. Rev.* **61:** 377–92

Elez M, Radman M & Matic I (2007) The frequency and structure of recombinant products is determined by the cellular level of MutL. *Proc. Natl. Acad. Sci. U. S. A.* **104:** 8935–40

Elledge SJ & Walker GC (1983) Proteins required for ultraviolet light and chemical mutagenesis. Identification of the products of the *umuC* locus of *Escherichia coli*. *J. Mol. Biol.* **164:** 175–92

Epshtein V (2015) UvrD helicase: an old dog with a new trick: how one step backward leads to many steps forward. *Bioessays* **37:** 12–9

Epshtein V, Kamarthapu V, McGary K, Svetlov V, Ueberheide B, Proshkin S, Mironov A & Nudler E (2014) UvrD facilitates DNA repair by pulling RNA polymerase backwards. *Nature* **505:** 372–7

Erill I, Campoy S, Mazon G & Barbé J (2006) Dispersal and regulation of an adaptive mutagenesis cassette in the bacteria domain. *Nucleic Acids Res.* **34:** 66–77

Esterházy D, King MS, Yakovlev G & Hirst J (2008) Production of reactive oxygen species by complex I (NADH:ubiquinone oxidoreductase) from *Escherichia coli* and comparison to the enzyme from mitochondria. *Biochemistry* **47:** 3964–3971

Evans K & Poole K (1999) The MexA-MexB-OprM multidrug efflux system of *Pseudomonas aeruginosa* is growth-phase regulated. *FEMS Microbiol. Lett.* **173:** 35–9

Fargier E, Mac Aogáin M, Mooij MJ, Woods DF, Morrissey JP, Dobson ADW, Adams C & O'Gara F (2012) MexT functions as a redox-responsive regulator modulating disulfide stress resistance in *Pseudomonas aeruginosa*. *J. Bacteriol.* **194:** 3502–11

Foti JJ, Devadoss B, Winkler JA, Collins JJ & Walker GC (2012) Oxidation of the guanine nucleotide pool underlies cell death by bactericidal antibiotics. *Science* **336:** 315–9

Fraud S & Poole K (2011) Oxidative stress induction of the MexXY multidrug efflux genes and promotion of aminoglycoside resistance development in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **55:** 1068–74

Friedberg EC, Lehmann AR & Fuchs RPP (2005) Trading places: how do DNA polymerases switch during translesion DNA synthesis? *Mol. Cell* **18:** 499–505

Fukuda H, Hosaka M, Hirai K & Iyobe S (1990) New norfloxacin resistance gene in *Pseudomonas aeruginosa* PAO. *Antimicrob. Agents Chemother.* **34:** 1757–61

Galhardo RS, Rocha RP, Marques M V & Menck CFM (2005) An SOS-regulated operon involved in damage-inducible mutagenesis in *Caulobacter crescentus*. *Nucleic Acids Res.* **33:** 2603–14

Galletto R & Kowalczykowski SC (2007) RecA. *Curr. Biol.* **17:** R395–7

Ganesan AK & Smith KC (1971) The duration of recovery and DNA repair in excision deficient derivatives of *Escherichia coli* K-12 after ultraviolet irradiation. *Mol. Gen. Genet.* **113:** 285–296

Georgescu RE, Kurth I & O'Donnell ME (2012) Single-molecule studies reveal the function of a third polymerase in the replisome. *Nat. Struct. Mol. Biol.* **19:** 113–6

Giske CG, Buarø L, Sundsfjord A & Wretlind B (2008) Alterations of porin, pumps, and penicillin-binding proteins in carbapenem resistant clinical isolates of *Pseudomonas aeruginosa*. *Microb. Drug Resist.* **14:** 23–30

Gotoh N, Wakebe H, Yoshihara E, Nakae T & Nishino T (1989) Role of protein F in maintaining structural integrity of the *Pseudomonas aeruginosa* outer membrane. *J. Bacteriol.* **171:** 983–90

Gross JD, Grunstein J & Witkin EM (1971) Inviability of *recA*- derivatives of the DNA polymerase mutant of De Lucia and Cairns. *J. Mol. Biol.* **58:** 631–4

Guénard S, Muller C, Monlezun L, Benas P, Broutin I, Jeannot K & Plésiat P (2014) Multiple mutations lead to MexXY-OprM-dependent aminoglycoside resistance in clinical strains of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **58:** 221–8

Gutierrez A, Laureti L, Crussard S, Abida H, Rodríguez-Rojas A, Blázquez J, Baharoglu Z, Mazel D, Darfeuille F, Vogel J & Matic I (2013) β-Lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity. *Nat. Commun.* **4:** 1610

Hanawalt PC (2002) Subpathways of nucleotide excision repair and their regulation. *Oncogene* **21:** 8949–8956

Hancock RE, Decad GM & Nikaido H (1979) Identification of the protein producing transmembrane diffusion pores in the outer membrane of Pseudomonas aeruginosa PA01. *Biochim. Biophys. Acta* **554:** 323–31 Available at: http://www.ncbi.nlm.nih.gov/pubmed/114220 [Accessed June 18, 2015]

Hancock REW & Brinkman FSL (2002) Function of pseudomonas porins in uptake and efflux. *Annu. Rev. Microbiol.* **56:** 17–38

Heller RC & Marians KJ (2006) Replication fork reactivation downstream of a blocked nascent leading strand. *Nature* **439:** 557–62

Heurlier K, Dénervaud V, Haenni M, Guy L, Krishnapillai V & Haas D (2005) Quorum-sensing-negative (*lasR*) mutants of *Pseudomonas aeruginosa* avoid cell lysis and death. *J. Bacteriol.* **187:** 4875–83

Higuchi K, Katayama T, Iwai S, Hidaka M, Horiuchi T & Maki H (2003) Fate of DNA replication fork encountering a single DNA lesion during *oriC* plasmid DNA replication *in vitro*. *Genes Cells* **8:** 437–49

Hirai K, Suzue S, Irikura T, Iyobe S & Mitsuhashi S (1987) Mutations producing resistance to norfloxacin in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **31:** 582–6

Holloway BW (1955) Genetic recombination in *Pseudomonas aeruginosa*. *J. Gen. Microbiol.* **13:** 572–81

Hosaka M, Gotoh N & Nishino T (1995) Purification of a 54-kilodalton protein (OprJ) produced in NfxB mutants of *Pseudomonas aeruginosa* and production of a monoclonal antibody specific to OprJ. *Antimicrob. Agents Chemother.* **39:** 1731–5

Howard-Flanders P, Boyce RP & Theriot L (1966) Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. *Genetics* **53:** 1119–1136

Huang H & Hancock RE (1993) Genetic definition of the substrate selectivity of outer membrane porin protein OprD of *Pseudomonas aeruginosa*. *J. Bacteriol.* **175:** 7793–800

Ikenaga M, Ishii Y, Tada M, Kakunaga T & Takebe H (1975) Excision-repair of 4-nitroquinolin-1-oxide damage responsible for killing, mutation, and cancer. *Basic Life Sci.* **5B:** 763–771

Imai M, Tago Y, Ihara M, Kawata M & Yamamoto K (2007) Role of the 5' – > 3' exonuclease and Klenow fragment of *Escherichia coli* DNA polymerase I in base mismatch repair. *Mol. Genet. Genomics* **278:** 211–20

Imlay JA (2008) Cellular defenses against superoxide and hydrogen peroxide. *Annu. Rev. Biochem.* **77:** 755–76

Imlay JA (2015) Diagnosing oxidative stress in bacteria: not as easy as you might think. *Curr. Opin. Microbiol.* **24C:** 124–131

Imlay JA & Linn S (1988) DNA damage and oxygen radical toxicity. *Science* **240:** 1302–9

Indiani C, Langston LD, Yurieva O, Goodman MF & O'Donnell M (2009) Translesion DNA polymerases remodel the replisome and alter the speed of the replicative helicase. *Proc. Natl. Acad. Sci. U. S. A.* **106:** 6031–8

Indiani C, McInerney P, Georgescu R, Goodman MF & O'Donnell M (2005) A sliding-clamp toolbelt binds high- and low-fidelity DNA polymerases simultaneously. *Mol. Cell* **19:** 805–15

Indiani C & O'Donnell M (2013) A proposal: Source of single strand DNA that elicits the SOS response. *Front. Biosci.* **18:** 312–323

Ippoliti PJ, Delateur NA, Jones KM & Beuning PJ (2012) Multiple strategies for translesion synthesis in bacteria. *Cells* **1:** 799–831

Jacobs M a, Alwood A, Thaipisuttikul I, Spencer D, Haugen E, Ernst S, Will O, Kaul R, Raymond C, Levy R, Chun-Rong L, Guenthner D, Bovee D, Olson M V & Manoil C (2003) Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U. S. A.* **100:** 14339–44

Jacoby GA (2009) AmpC beta-lactamases. *Clin. Microbiol. Rev.* **22:** 161–82

Jakimowicz D, Majka J, Messer W, Speck C, Fernandez M, Martin MC, Sanchez J, Schauwecker F, Keller U, Schrempf H & Zakrzewska-Czerwińska J (1998) Structural elements of the *Streptomyces oriC* region and their interactions with the DnaA protein. *Microbiology* **144 (Pt 5:** 1281–90

Jalal S, Ciofu O, Hoiby N, Gotoh N & Wretlind B (2000) Molecular mechanisms of fluoroquinolone resistance in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Antimicrob. Agents Chemother.* **44:** 710–2

Jankovic M, Kostic T & Savic DJ (1990) DNA sequence analysis of spontaneous histidine mutations in a *polA1* strain of *Escherichia coli* K12 suggests a specific role of the GTGG sequence. *Mol. Gen. Genet.* **223:** 481–6

Jaouen T, Dé E, Chevalier S & Orange N (2004) Pore size dependence on growth temperature is a common characteristic of the major outer membrane protein OprF in psychrotrophic and mesophilic *Pseudomonas* species. *Appl. Environ. Microbiol.* **70:** 6665–9

Jarosz DF, Godoy VG, Delaney JC, Essigmann JM & Walker GC (2006) A single amino acid governs enhanced activity of DinB DNA polymerases on damaged templates. *Nature* **439:** 225–8

Jeiranian HA, Schalow BJ, Courcelle CT & Courcelle J (2013) Fate of the replisome following arrest by UV-induced DNA damage in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **110:** 11421–6

Joyce C (2004) DNA polymerase I, Bacterial. In *Encyclopedia of Biological Chemistry* pp 720–725.

Joyce CM & Grindley ND (1984) Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. *J. Bacteriol.* **158:** 636–43

Juan C, Maciá MD, Gutiérrez O, Vidal C, Pérez JL & Oliver A (2005) Molecular mechanisms of beta-lactam resistance mediated by AmpC hyperproduction in *Pseudomonas aeruginosa* clinical strains. *Antimicrob. Agents Chemother.* **49:** 4733–8

Juurik T, Ilves H, Teras R, Ilmjärv T, Tavita K, Ukkivi K, Teppo A, Mikkel K & Kivisaar M (2012) Mutation frequency and spectrum of mutations vary at different chromosomal positions of *Pseudomonas putida*. *PLoS One* **7:** e48511

El Karoui M, Biaudet V, Schbath S & Gruss A Characteristics of Chi distribution on different bacterial genomes. *Res. Microbiol.* 150: 579–87

Kato T & Shinoura Y (1977) Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. *Mol. Gen. Genet.* **156:** 121–31

Keren I, Wu Y, Inocencio J, Mulcahy LR & Lewis K (2013) Killing by bactericidal antibiotics does not depend on reactive oxygen species. *Science* **339:** 1213–6

Kim J, Yoshimura SH, Hizume K, Ohniwa RL, Ishihama A & Takeyasu K (2004) Fundamental structural units of the *Escherichia coli* nucleoid revealed by atomic force microscopy. *Nucleic Acids Res.* **32:** 1982–92

Kim S, Dallmann HG, McHenry CS & Marians KJ (1996) Coupling of a replicative polymerase and helicase: a tau-DnaB interaction mediates rapid replication fork movement. *Cell* **84:** 643–50

Kiser TH, Obritsch MD, Jung R, MacLaren R & Fish DN (2010) Efflux pump contribution to multidrug resistance in clinical isolates of *Pseudomonas aeruginosa*. *Pharmacotherapy* **30:** 632–8

Klockgether J, Munder A, Neugebauer J, Davenport CF, Stanke F, Larbig KD, Heeb S, Schöck U, Pohl TM, Wiehlmann L & Tümmler B (2010) Genome diversity of *Pseudomonas aeruginosa* PAO1 laboratory strains. *J. Bacteriol.* **192:** 1113–21

Kohanski MA, DePristo MA & Collins JJ (2010a) Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. *Mol. Cell* **37:** 311–20

Kohanski MA, Dwyer DJ & Collins JJ (2010b) How antibiotics kill bacteria: from targets to networks. *Nat. Rev. Microbiol.* **8:** 423–35

Köhler T, van Delden C, Curty LK, Hamzehpour MM & Pechere JC (2001) Over-expression of the MexEF-OprN multidrug efflux system affects cell-to-cell signaling in *Pseudomonas aeruginosa*. *J. Bacteriol.* **183:** 5213–22

Köhler T, Epp SF, Curty LK & Pechère JC (1999) Characterization of MexT, the regulator of the MexE-MexF-OprN multidrug efflux system of *Pseudomonas aeruginosa*. *J. Bacteriol.* **181:** 6300–5

Köhler T, Michéa-Hamzehpour M, Henze U, Gotoh N, Curty LK & Pechère JC (1997) Characterization of MexE-MexF-OprN, a positively regulated multidrug efflux system of *Pseudomonas aeruginosa*. *Mol. Microbiol.* **23:** 345–54

Konrad EB (1977) Method for the isolation of *Escherichia coli* mutants with enhanced recombination between chromosomal duplications. *J. Bacteriol.* **130:** 167–72

Konrad EB & Lehman IR (1974) A conditional lethal mutant of *Escherichia coli* K12 defective in the 5' leads to 3' exonuclease associated with DNA polymerase I. *Proc. Natl. Acad. Sci. U. S. A.* **71:** 2048–51

Koorits L, Tegova R, Tark M, Tarassova K, Tover A & Kivisaar M (2007) Study of involvement of ImuB and DnaE2 in stationary-phase mutagenesis in *Pseudomonas putida*. *DNA Repair (Amst).* **6:** 863–8

Kornberg A & Baker TA (2005) DNA Replication University Science Books

Kowalczykowski SC, Dixon DA, Eggleston AK, Lauder SD & Rehrauer WM (1994) Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.* **58:** 401–65

Kresge N, Simoni RD & Hill RL (2005) Arthur Kornberg's Discovery of DNA Polymerase I. *J. Biol. Chem.* **280:** e46

Krwawicz J, Arczewska KD, Speina E, Maciejewska A & Grzesiuk E (2007) Bacterial DNA repair genes and their eukaryotic homologues: 1. Mutations in genes involved in base excision repair (BER) and DNA-end processors and their implication in mutagenesis and human disease. *Acta Biochim. Pol.* **54:** 413–434

Kuban W, Banach-Orlowska M, Bialoskorska M, Lipowska A, Schaaper RM, Jonczyk P & Fijalkowska IJ (2005) Mutator phenotype resulting from DNA polymerase IV overproduction in *Escherichia coli*: preferential mutagenesis on the lagging strand. *J. Bacteriol.* **187:** 6862–6

Kurth I & O'Donnell M (2013) New insights into replisome fluidity during chromosome replication. *Trends Biochem. Sci.* **38:** 195–203

Lambert M-L, Suetens C, Savey A, Palomar M, Hiesmayr M, Morales I, Agodi A, Frank U, Mertens K, Schumacher M & Wolkewitz M (2011) Clinical outcomes of health-care-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. *Lancet. Infect. Dis.* **11:** 30–8

Lee J-Y & Ko KS (2012) OprD mutations and inactivation, expression of efflux pumps and AmpC, and metallo-β-lactamases in carbapenem-resistant *Pseudomonas aeruginosa* isolates from South Korea. *Int. J. Antimicrob. Agents* **40:** 168–72

Lee YS, Han JS, Jeon Y & Hwang DS (2001) The arc two-component signal transduction system inhibits in vitro *Escherichia coli* chromosomal initiation. *J. Biol. Chem.* **276:** 9917–23

Lehman IR, Bessman MJ, Simms ES & Kornberg A (1958) Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *Escherichia coli*. *J. Biol. Chem.* **233:** 163–70

Leonard AC & Mechali M (2013) DNA Replication Origins. *Cold Spring Harb. Perspect. Biol.* **5:** a010116–a010116

Leu FP, Georgescu R & O'Donnell M (2003) Mechanism of the *E. coli* tau processivity switch during lagging-strand synthesis. *Mol. Cell* **11:** 315–27

Lewis RA, Bignell CR, Zeng W, Jones AC & Thomas CM (2002) Chromosome loss from par mutants of *Pseudomonas putida* depends on growth medium and phase of growth. *Microbiology* **148:** 537–548

Li XZ, Nikaido H & Poole K (1995) Role of *mexA-mexB-oprM* in antibiotic efflux in P*seudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **39:** 1948–53

Lin CG, Kovalsky O & Grossman L (1997) DNA damage-dependent recruitment of nucleotide excision repair and transcription proteins to *Escherichia coli* inner membranes. *Nucleic Acids Res.* **25:** 3151–8

Lindahl T & Barnes DE (2000) Repair of Endogenous DNA Damage. *Cold Spring Harb. Symp. Quant. Biol.* **65:** 127–134

Lister PD, Wolter DJ & Hanson ND (2009) Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin. Microbiol. Rev.* **22:** 582–610

Liu Y & Imlay JA (2013) Cell death from antibiotics without the involvement of reactive oxygen species. *Science* **339:** 1210–3

Llanes C, Hocquet D, Vogne C, Benali-Baitich D, Neuwirth C & Plésiat P (2004) Clinical strains of *Pseudomonas aeruginosa* overproducing MexAB-OprM and MexXY efflux pumps simultaneously. *Antimicrob. Agents Chemother.* **48:** 1797–802

Lodge JM, Minchin SD, Piddock LJ & Busby SJ (1990) Cloning, sequencing and analysis of the structural gene and regulatory region of the *Pseudomonas aeruginosa* chromosomal ampC beta-lactamase. *Biochem. J.* **272:** 627–31

López de Saro FJ & O'Donnell M (2001) Interaction of the beta sliding clamp with MutS, ligase, and DNA polymerase I. *Proc. Natl. Acad. Sci. U. S. A.* **98:** 8376–80

López E & Blázquez J (2009) Effect of subinhibitory concentrations of antibiotics on intrachromosomal homologous recombination in *Escherichia coli. Antimicrob. Agents Chemother.* **53:** 3411–5

López E, Elez M, Matic I & Blázquez J (2007) Antibiotic-mediated recombination: ciprofloxacin stimulates SOS-independent recombination of divergent sequences in *Escherichia coli. Mol. Microbiol.* **64:** 83–93

Lu TK & Collins JJ (2009) Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. *Proc. Natl. Acad. Sci.* **106:** 4629–4634

De Lucia P & Cairns J (1969) Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. *Nature* **224:** 1164–6

Lusetti SL & Cox MM (2002) The bacterial RecA protein and the recombinational DNA repair of stalled replication forks. *Annu. Rev. Biochem.* **71:** 71–100

Lyamichev V, Brow MA & Dahlberg JE (1993) Structure-specific endonucleolytic cleavage of nucleic acids by eubacterial DNA polymerases. *Science* **260:** 778–83

Lynch MJ, Drusano GL & Mobley HL (1987) Emergence of resistance to imipenem in *Pseudomonas aeruginosa. Antimicrob. Agents Chemother.* **31:** 1892–6

Martins-Pinheiro M, Galhardo RS, Lage C, Lima-Bessa KM, Aires KA & Menck CFM (2004) Different patterns of evolution for duplicated DNA repair genes in bacteria of the *Xanthomonadales* group. *BMC Evol. Biol.* **4:** 29

Maseda H, Saito K, Nakajima A & Nakae T (2000) Variation of the mexT gene, a regulator of the MexEF-oprN efflux pump expression in wild-type strains of *Pseudomonas aeruginosa. FEMS Microbiol. Lett.* **192:** 107–12

Maseda H, Sawada I, Saito K, Uchiyama H, Nakae T & Nomura N (2004) Enhancement of the *mexAB-oprM* efflux pump expression by a quorum-sensing autoinducer and its cancellation by a regulator, MexT, of the *mexEF-oprN* efflux pump operon in *Pseudomonas aeruginosa. Antimicrob. Agents Chemother.* **48:** 1320–8

Masuda N, Gotoh N, Ohya S & Nishino T (1996) Quantitative correlation between susceptibility and OprJ production in NfxB mutants of *Pseudomonas aeruginosa. Antimicrob. Agents Chemother.* **40:** 909–13

Masuda N, Sakagawa E & Ohya S (1995) Outer membrane proteins responsible for multiple drug resistance in *Pseudomonas aeruginosa. Antimicrob. Agents Chemother.* **39:** 645–9

Matsui E, Kawasaki S, Ishida H, Ishikawa K, Kosugi Y, Kikuchi H, Kawarabayashi Y & Matsui I (1999) Thermostable flap endonuclease from the archaeon, *Pyrococcus horikoshii*, cleaves the replication fork-like structure endo/exonucleolytically. *J. Biol. Chem.* **274:** 18297–309

Matsuo Y, Eda S, Gotoh N, Yoshihara E & Nakae T (2004) MexZ-mediated regulation of *mexXY* multidrug efflux pump expression in *Pseudomonas aeruginosa* by binding on the *mexZ-mexX* intergenic DNA. *FEMS Microbiol. Lett.* **238:** 23–8

Maul RW, Sanders LH, Lim JB, Benitez R & Sutton MD (2007) Role of *Escherichia coli* DNA polymerase I in conferring viability upon the *dnaN*159 mutant strain. *J. Bacteriol.* **189:** 4688–95

McGlynn P & Guy CP (2008) Replication forks blocked by protein-DNA complexes have limited stability in vitro. *J. Mol. Biol.* **381:** 249–55

McGlynn P & Lloyd RG (2002) Genome stability and the processing of damaged replication forks by RecG. *Trends Genet.* **18:** 413–9

McInerney P, Johnson A, Katz F & O'Donnell M (2007) Characterization of a triple DNA polymerase replisome. *Mol. Cell* **27:** 527–38

McInerney P & O'Donnell M (2004) Functional uncoupling of twin polymerases: mechanism of polymerase dissociation from a lagging-strand block. *J. Biol. Chem.* **279:** 21543–51

McInerney P & O'Donnell M (2007) Replisome fate upon encountering a leading strand block and clearance from DNA by recombination proteins. *J. Biol. Chem.* **282:** 25903–25916

Mellon I & Hanawalt PC (1989) Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* **342:** 95–8

Michel B (2005) After 30 years of study, the bacterial SOS response still surprises us. *PLoS Biol.* **3:** e255

Michel B, Ehrlich SD & Uzest M (1997) DNA double-strand breaks caused by replication arrest. *EMBO J.* **16:** 430–8

Mine T, Morita Y, Kataoka A, Mizushima T & Tsuchiya T (1999) Expression in *Escherichia coli* of a new multidrug efflux pump, MexXY, from *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **43:** 415–7

Montón Silva A, Lapenta F, Stefan A, Dal Piaz F, Ceccarelli A, Perrone A & Hochkoeppler A (2015) Simultaneous ternary extension of DNA catalyzed by a trimeric replicase assembled in vivo. *Biochem. Biophys. Res. Commun.* **462:** 14–20

Moolenaar GF, Moorman C & Goosen N (2000) Role of the *Escherichia coli* nucleotide excision repair proteins in DNA replication. *J. Bacteriol.* **182:** 5706–14

Morimatsu K & Kowalczykowski SC (2003) RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: a universal step of re-combinational repair. *Mol. Cell* **11:** 1337–47

Morimatsu K, Wu Y & Kowalczykowski SC (2012) RecFOR proteins target RecA protein to a DNA gap with either DNA or RNA at the 5' terminus: implication for repair of stalled replication forks. *J. Biol. Chem.* **287:** 35621–30

Morimyo M (1982) Anaerobic incubation enhances the colony formation of a *polA recB* strain of *Escherichia coli* K-12. *J. Bacteriol.* **152:** 208–14

Morimyo M & Shimazu Y (1976) Evidence that the gene *uvrB* is indispensable for a polymerase I deficient strain of *Escherichia coli* K-12. *Mol. Gen. Genet.* **147:** 243–50

Morita Y, Cao L, Gould VC, Avison MB & Poole K (2006a) *nalD* encodes a second repressor of the *mexAB-oprM* multidrug efflux operon of *Pseudomonas aeruginosa*. *J. Bacteriol.* **188:** 8649–54

Morita Y, Komori Y, Mima T, Kuroda T, Mizushima T & Tsuchiya T (2001) Construction of a series of mutants lacking all of the four major mex operons for multidrug efflux pumps or possessing each one of the operons from *Pseudomonas aeruginosa* PAO1: MexCD-OprJ is an inducible pump. *FEMS Microbiol. Lett.* **202:** 139–43

Morita Y, Sobel ML & Poole K (2006b) Antibiotic inducibility of the MexXY multi-drug efflux system of *Pseudomonas aeruginosa*: involvement of the antibiotic-inducible PA5471 gene product. *J. Bacteriol.* **188:** 1847–55

Morita Y, Tomida J & Kawamura Y (2012) MexXY multidrug efflux system of *Pseudomonas aeruginosa*. *Front. Microbiol.* **3:** 408

Moriya S, Imai Y, Hassan AK & Ogasawara N (1999) Regulation of initiation of *Bacillus subtilis* chromosome replication. *Plasmid* **41:** 17–29

Nagata Y, Mashimo K, Kawata M & Yamamoto K (2002) The roles of Klenow processing and flap processing activities of DNA polymerase I in chromosome instability in *Escherichia coli* K12 strains. *Genetics* **160:** 13–23

Napolitano R, Janel-Bintz R, Wagner J & Fuchs RP (2000) All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. *EMBO J.* **19:** 6259–65

Nelson KE, Weinel C, Paulsen IT, Dodson RJ, Hilbert H, Martins dos Santos VAP, Fouts DE, Gill SR, Pop M, Holmes M, Brinkac L, Beanan M, DeBoy RT, Daugherty S, Kolonay J, Madupu R, Nelson W, White O, Peterson J, Khouri H, et al (2002) Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. *Environ. Microbiol.* **4:** 799–808

Nestorovich EM, Sugawara E, Nikaido H & Bezrukov SM (2006) *Pseudomonas aeruginosa* porin OprF: properties of the channel. *J. Biol. Chem.* **281:** 16230–7

Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* **67:** 593–656

Nikaido H (2009) Multidrug resistance in bacteria. *Annu. Rev. Biochem.* **78:** 119–46

Nikaido H, Nikaido K & Harayama S (1991) Identification and characterization of porins in *Pseudomonas aeruginosa*. *J. Biol. Chem.* **266:** 770–9

Nohmi T (2006) Environmental stress and lesion-bypass DNA polymerases. *Annu. Rev. Microbiol.* **60:** 231–253

Nohmi T, Battista JR, Dodson LA & Walker GC (1988) RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation. *Proc. Natl. Acad. Sci. U. S. A.* **85:** 1816–20

Nordmann P & Guibert M (1998) Extended-spectrum beta-lactamases in *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* **42:** 128–31

Nowosielska A, Calmann M a, Zdraveski Z, Essigmann JM & Marinus MG (2004) Spontaneous and cisplatin-induced recombination in *Escherichia coli*. *DNA Repair (Amst).* **3:** 719–728

Nowosielska A, Smith SA, Engelward BP & Marinus MG (2006) Homologous re-combination prevents methylation-induced toxicity in *Escherichia coli*. *Nucleic Acids Res.* **34:** 2258–68

O'Donnell M (2006) Replisome architecture and dynamics in *Escherichia coli*. *J. Biol. Chem.* **281:** 10653–6

Ochs MM, Lu CD, Hancock RE & Abdelal AT (1999a) Amino acid-mediated induction of the basic amino acid-specific outer membrane porin OprD from *Pseudomonas aeruginosa*. *J. Bacteriol.* **181:** 5426–32

Ochs MM, McCusker MP, Bains M & Hancock RE (1999b) Negative regulation of the *Pseudomonas aeruginosa* outer membrane porin OprD selective for imipenem and basic amino acids. *Antimicrob. Agents Chemother.* **43:** 1085–90

Okazaki R, Arisawa M & Sugino A (1971) Slow joining of newly replicated DNA chains in DNA polymerase I-deficient *Escherichia coli* mutants. *Proc. Natl. Acad. Sci. U. S. A.* **68:** 2954–7

Ollivierre JN, Fang J & Beuning PJ (2010) The Roles of UmuD in Regulating Muta-genesis. *J. Nucleic Acids* **2010:**

Pagès J-M, James CE & Winterhalter M (2008) The porin and the permeating anti-biotic: a selective diffusion barrier in Gram-negative bacteria. *Nat. Rev. Microbiol.* **6:** 893–903

Pagès V & Fuchs RP (2003) Uncoupling of leading- and lagging-strand DNA replication during lesion bypass in vivo. *Science* **300:** 1300–3

Pagès V, Mazón G, Naiman K, Philippin G & Fuchs RP (2012) Monitoring bypass of single replication-blocking lesions by damage avoidance in the *Escherichia coli* chromosome. *Nucleic Acids Res.* **40:** 9036–43

Paris Ü, Mikkel K, Tavita K, Saumaa S, Teras R & Kivisaar M (2015) NHEJ enzymes LigD and Ku participate in stationary-phase mutagenesis in *Pseudomonas putida*. *DNA Repair (Amst).* **31:** 11–8

Pasca MR, Dalla Valle C, De Jesus Lopes Ribeiro AL, Buroni S, Papaleo MC, Bazzini S, Udine C, Incandela ML, Daffara S, Fani R, Riccardi G & Marone P (2012) Evaluation of fluoroquinolone resistance mechanisms in *Pseudomonas aeruginosa* multidrug resistance clinical isolates. *Microb. Drug Resist.* **18:** 23–32

Patel PH, Suzuki M, Adman E, Shinkai A & Loeb LA (2001) Prokaryotic DNA polymerase I: evolution, structure, and 'base flipping' mechanism for nucleotide selection. *J. Mol. Biol.* **308:** 823–37

Perron K, Caille O, Rossier C, Van Delden C, Dumas J-L & Köhler T (2004) CzcR-CzcS, a two-component system involved in heavy metal and carbapenem resistance in *Pseudomonas aeruginosa*. *J. Biol. Chem.* **279:** 8761–8

Petrova V, Chen SH, Molzberger ET, Tomko E, Chitteni-Pattu S, Jia H, Ordabayev Y, Lohman TM & Cox MM (2015) Active displacement of RecA filaments by UvrD translocase activity. *Nucleic Acids Res.* **43:** 4133–49

Pitcher RS, Brissett NC & Doherty AJ (2007) Nonhomologous end-joining in bacteria: a microbial perspective. *Annu. Rev. Microbiol.* **61:** 259–82

Poole K (2004) Efflux-mediated multiresistance in Gram-negative bacteria. *Clin. Microbiol. Infect.* **10:** 12–26

Poole K (2011) *Pseudomonas aeruginosa*: Resistance to the Max. *Front. Microbiol.* **2:** 65

Poole K, Gotoh N, Tsujimoto H, Zhao Q, Wada A, Yamasaki T, Neshat S, Yamagishi J, Li XZ & Nishino T (1996a) Overexpression of the *mexC-mexD-oprJ* efflux operon in *nfxB*-type multidrug-resistant strains of *Pseudomonas aeruginosa*. *Mol. Microbiol.* **21:** 713–24

Poole K, Krebes K, McNally C & Neshat S (1993) Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. *J. Bacteriol.* **175:** 7363–72

Poole K, Tetro K, Zhao Q, Neshat S, Heinrichs DE & Bianco N (1996b) Expression of the multidrug resistance operon *mexA-mexB-oprM* in *Pseudomonas aeruginosa*: *mexR* encodes a regulator of operon expression. *Antimicrob. Agents Chemother.* **40:** 2021–8

Possoz C, Filipe SR, Grainge I & Sherratt DJ (2006) Tracking of controlled *Escherichia coli* replication fork stalling and restart at repressor-bound DNA in vivo. *EMBO J.* **25:** 2596–604

Postow L, Ullsperger C, Keller RW, Bustamante C, Vologodskii A V & Cozzarelli NR (2001) Positive torsional strain causes the formation of a four-way junction at replication forks. *J. Biol. Chem.* **276:** 2790–6

Pumbwe L, Everett MJ, Hancock RE & Piddock LJ (1996) Role of *gyrA* mutation and loss of OprF in the multiple antibiotic resistance phenotype of *Pseudomonas aeruginosa* G49. *FEMS Microbiol. Lett.* **143:** 25–8

Purssell A, Fruci M, Mikalauskas A, Gilmour C & Poole K (2015) EsrC, an envelope stress-regulated repressor of the *mexCD-oprJ* multidrug efflux operon in *Pseudomonas aeruginosa*. *Environ. Microbiol.* **17:** 186–98

Purssell A & Poole K (2013) Functional characterization of the NfxB repressor of the *mexCD-oprJ* multidrug efflux operon of *Pseudomonas aeruginosa*. *Microbiology* **159:** 2058–73

Qiu Z & Goodman MF (1997) The *Escherichia coli polB* locus is identical to *dinA*, the structural gene for DNA polymerase II. Characterization of Pol II purified from a *polB* mutant. *J. Biol. Chem.* **272:** 8611–7

Ramos JL, Duque E, Godoy P & Segura A (1998) Efflux pumps involved in toluene tolerance in *Pseudomonas putida* DOT-T1E. *J. Bacteriol.* **180:** 3323–9

Rangarajan S, Woodgate R & Goodman MF (1999) A phenotype for enigmatic DNA polymerase II: a pivotal role for pol II in replication restart in UV-irradiated *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **96:** 9224–9

Rangarajan S, Woodgate R & Goodman MF (2002) Replication restart in UV-irradiated *Escherichia coli* involving pols II, III, V, PriA, RecA and RecFOR proteins. *Mol. Microbiol.* **43:** 617–28

Reyes-Lamothe R, Sherratt DJ & Leake MC (2010) Stoichiometry and architecture of active DNA replication machinery in *Escherichia coli*. *Science* **328:** 498–501

Rivera E, Vila L & Barbé J (1996) The *uvrB* gene of *Pseudomonas aeruginosa* is not DNA damage inducible. *J. Bacteriol.* **178:** 5550–5554

Rivera E, Vila L & Barbé J (1997) Expression of the *Pseudomonas aeruginosa uvrA* gene is constitutive. *Mutat. Res.* **377:** 149–155

Robins P, Pappin DJ, Wood RD & Lindahl T (1994) Structural and functional homology between mammalian DNase IV and the 5'-nuclease domain of *Escherichia coli* DNA polymerase I. *J. Biol. Chem.* **269:** 28535–8

Robu ME, Inman RB & Cox MM (2001) RecA protein promotes the regression of stalled replication forks in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **98:** 8211–8

Rodríguez-Martínez J-M, Poirel L & Nordmann P (2009a) Extended-spectrum cephalo-sporinases in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **53:** 1766–71

Rodríguez-Martínez J-M, Poirel L & Nordmann P (2009b) Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **53:** 4783–8

Ross C, Pybus C, Pedraza-Reyes M, Sung H-M, Yasbin RE & Robleto E (2006) Novel role of Mfd: effects on stationary-phase mutagenesis in *Bacillus subtilis*. *J. Bacteriol.* **188:** 7512–20

Rudolph CJ, Upton AL & Lloyd RG (2007) Replication fork stalling and cell cycle arrest in UV-irradiated *Escherichia coli*. *Genes Dev.* **21:** 668–81

Rupp WD, Wilde CE, Reno DL & P H-F (1971) Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. *J. Mol. Biol.* **61:** 25–44

Ryan VT, Grimwade JE, Camara JE, Crooke E & Leonard AC (2004) *Escherichia coli* prereplication complex assembly is regulated by dynamic interplay among Fis, IHF and DnaA. *Mol. Microbiol.* **51:** 1347–59

Ryan VT, Grimwade JE, Nievera CJ & Leonard AC (2002) IHF and HU stimulate assembly of pre-replication complexes at *Escherichia coli oriC* by two different mechanisms. *Mol. Microbiol.* **46:** 113–24

Rybenkov V V (2014) Maintenance of chromosome structure in *Pseudomonas aeruginosa*. *FEMS Microbiol. Lett.* **356:** 154–65

Sadikot RT, Blackwell TS, Christman JW & Prince AS (2005) Pathogen-host inter-actions in *Pseudomonas aeruginosa* pneumonia. *Am. J. Respir. Crit. Care Med.* **171:** 1209–23

SaiSree L, Reddy M & Gowrishankar J (2000) *lon* incompatibility associated with mutations causing SOS induction: null *uvrD* alleles induce an SOS response in *Escherichia coli*. *J. Bacteriol.* **182:** 3151–3157

Saito K, Yoneyama H & Nakae T (1999) *nalB*-type mutations causing the over-expression of the MexAB-OprM efflux pump are located in the mexR gene of the *Pseudomonas aeruginosa* chromosome. *FEMS Microbiol. Lett.* **179:** 67–72

Sanders LH, Rockel A, Lu H, Wozniak DJ & Sutton MD (2006) Role of *Pseudomonas aeruginosa dinB*-encoded DNA polymerase IV in mutagenesis. *J. Bacteriol.* **188:** 8573–85

Sassanfar M & Roberts JW (1990) Nature of the SOS-inducing signal in *Escherichia coli*. The involvement of DNA replication. *J. Mol. Biol.* **212:** 79–96

Saumaa S, Tarassova K, Tark M, Tover A, Tegova R & Kivisaar M (2006) Involvement of DNA mismatch repair in stationary-phase mutagenesis during prolonged starvation of *Pseudomonas putida*. *DNA Repair (Amst).* **5:** 505–14

Savery NJ (2007) The molecular mechanism of transcription-coupled DNA repair. *Trends Microbiol.* **15:** 326–333

Savir Y & Tlusty T (2010) RecA-mediated homology search as a nearly optimal signal detection system. *Mol. Cell* **40:** 388–96

Sawada I, Maseda H, Nakae T, Uchiyama H & Nomura N (2004) A quorum-sensing autoinducer enhances the *mexAB-oprM* efflux-pump expression without the MexR-mediated regulation in *Pseudomonas aeruginosa*. *Microbiol. Immunol.* **48:** 435–9

Schröder W, Goerke C & Wolz C (2013) Opposing effects of aminocoumarins and fluoroquinolones on the SOS response and adaptability in *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **68:** 529–38

Selby CP, Witkin EM & Sancar A (1991) *Escherichia coli mfd* mutant deficient in 'mutation frequency decline' lacks strand-specific repair: *in vitro* complementation with purified coupling factor. *Proc. Natl. Acad. Sci. U. S. A.* **88:** 11574–8

Sharma V, Sakai Y, Smythe KA & Yokobayashi Y (2013) Knockdown of *recA* gene expression by artificial small RNAs in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **430:** 256–9

Shen C-H, Chiang Y-C, Hsu C-H & Yang M-K (2007) Identification and charac-terization of two *uvrA* genes of *Xanthomonas axonopodi*s pathovar *citri*. *Mol. Genet. Genomics* **277:** 149–60

Shiba T, Ishiguro K, Takemoto N, Koibuchi H & Sugimoto K (1995) Purification and characterization of the *Pseudomonas aeruginosa* NfxB protein, the negative regulator of the nfxB gene. *J. Bacteriol.* **177:** 5872–7

Shimizu M, Gruz P, Kamiya H, Kim S-R, Pisani FM, Masutani C, Kanke Y, Harashima H, Hanaoka F & Nohmi T (2003) Erroneous incorporation of oxidized DNA precursors by Y-family DNA polymerases. *EMBO Rep.* **4:** 269–73

Skarstad K & Boye E (1993) Degradation of individual chromosomes in *recA* mutants of *Escherichia coli*. *J. Bacteriol.* **175:** 5505–5509

Skurnik D, Roux D, Cattoir V, Danilchanka O, Lu X, Yoder-Himes DR, Han K, Guillard T, Jiang D, Gaultier C, Guerin F, Aschard H, Leclercq R, Mekalanos JJ, Lory S & Pier GB (2013) Enhanced *in vivo* fitness of carbapenem-resistant *oprD* mutants of *Pseudomonas aeruginosa* revealed through high-throughput sequencing. *Proc. Natl. Acad. Sci. U. S. A.* **110:** 20747–52

Smith GR (2012) Chi Sites and Their Consequences. In *Bacterial Genomes: Physical Structure and Analysis* pp 50–66.

Sobel ML, Hocquet D, Cao L, Plesiat P & Poole K (2005a) Mutations in PA3574 (*nalD*) lead to increased MexAB-OprM expression and multidrug resistance in laboratory and clinical isolates of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **49:** 1782–6

Sobel ML, Neshat S & Poole K (2005b) Mutations in PA2491 (*mexS*) promote MexT-dependent *mexEF-oprN* expression and multidrug resistance in a clinical strain of *Pseudomonas aeruginosa*. *J. Bacteriol.* **187:** 1246–53

Sommer S, Boudsocq F, Devoret R & Bailone A (1998) Specific RecA amino acid changes affect RecA-UmuD'C interaction. *Mol. Microbiol.* **28:** 281–91

Srikumar R, Li XZ & Poole K (1997) Inner membrane efflux components are responsible for beta-lactam specificity of multidrug efflux pumps in *Pseudomonas aeruginosa*. *J. Bacteriol.* **179:** 7875–81

Srikumar R, Paul CJ & Poole K (2000) Influence of mutations in the *mexR* repressor gene on expression of the MexA-MexB-oprM multidrug efflux system of *Pseudomonas aeruginosa*. *J. Bacteriol.* **182:** 1410–4

Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrock-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, et al (2000) Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406:** 959–64

Strateva T & Yordanov D (2009) *Pseudomonas aeruginosa* – a phenomenon of bacterial resistance. *J. Med. Microbiol.* **58:** 1133–48

Sugawara E, Nagano K & Nikaido H (2012) Alternative folding pathways of the major porin OprF of *Pseudomonas aeruginosa*. *FEBS J.* **279:** 910–8

Sugawara E, Nestorovich EM, Bezrukov SM & Nikaido H (2006) *Pseudomonas aeruginosa* porin OprF exists in two different conformations. *J. Biol. Chem.* **281:** 16220–9

Sutton MD (2010) Coordinating DNA polymerase traffic during high and low fidelity synthesis. *Biochim. Biophys. Acta* **1804:** 1167–79

Tago Y, Imai M, Ihara M, Atofuji H, Nagata Y & Yamamoto K (2005) *Escherichia coli* mutator (Delta)*polA* is defective in base mismatch correction: the nature of in vivo DNA replication errors. *J. Mol. Biol.* **351:** 299–308

Tam VH, Schilling AN, LaRocco MT, Gentry LO, Lolans K, Quinn JP & Garey KW (2007) Prevalence of AmpC over-expression in bloodstream isolates of *Pseudomonas aeruginosa*. *Clin. Microbiol. Infect.* **13:** 413–8

Tanaka M, Narumi I, Funayama T, Kikuchi M, Watanabe H, Matsunaga T, Nikaido O & Yamamoto K (2005) Characterization of pathways dependent on the *uvsE, uvrA1,* or *uvrA2* gene product for UV resistance in *Deinococcus radiodurans*. *J. Bacteriol.* **187:** 3693–7

Tang M, Shen X, Frank EG, O'Donnell M, Woodgate R & Goodman MF (1999) UmuD'(2)C is an error-prone DNA polymerase, *Escherichia coli* pol V. *Proc. Natl. Acad. Sci. U. S. A.* **96:** 8919–24

Tark M, Tover A, Koorits L, Tegova R & Kivisaar M (2008) Dual role of NER in muta-genesis in *Pseudomonas putida*. *DNA Repair (Amst).* **7:** 20–30

Tark M, Tover A, Tarassova K, Tegova R, Kivi G, Hõrak R & Kivisaar M (2005) A DNA polymerase V homologue encoded by TOL plasmid pWW0 confers evolutionary

fitness on *Pseudomonas putida* under conditions of environmental stress. *J. Bacteriol.* **187:** 5203–13

Tegova R, Tover A, Tarassova K, Tark M & Kivisaar M (2004) Involvement of error-prone DNA polymerase IV in stationary-phase mutagenesis in *Pseudomonas putida*. *J. Bacteriol.* **186:** 2735–44

Terán W, Felipe A, Segura A, Rojas A, Ramos J-L & Gallegos M-T (2003) Antibiotic-dependent induction of *Pseudomonas putida* DOT-T1E TtgABC efflux pump is mediated by the drug binding repressor TtgR. *Antimicrob. Agents Chemother.* **47:** 3067–72

Terzi HA, Kulah C & Ciftci IH (2014) The effects of active efflux pumps on antibiotic resistance in *Pseudomonas aeruginosa*. *World J. Microbiol. Biotechnol.* **30:** 2681–7

Tian Z-X, Mac Aogáin M, O'Connor HF, Fargier E, Mooij MJ, Adams C, Wang Y-P & O'Gara F (2009a) MexT modulates virulence determinants in *Pseudomonas aeruginosa* independent of the MexEF-OprN efflux pump. *Microb. Pathog.* **47:** 237–41

Tian Z-X, Fargier E, Mac Aogáin M, Adams C, Wang Y-P & O'Gara F (2009b) Trans-criptome profiling defines a novel regulon modulated by the LysR-type trans-criptional regulator MexT in *Pseudomonas aeruginosa*. *Nucleic Acids Res.* **37:** 7546–59

Timinskas K, Balvočiūtė M, Timinskas A & Venclovas Č (2014) Comprehensive analysis of DNA polymerase III α subunits and their homologs in bacterial genomes. *Nucleic Acids Res.* **42:** 1393–413

Timmins J, Gordon E, Caria S, Leonard G, Acajjaoui S, Kuo M-S, Monchois V & McSweeney S (2009) Structural and mutational analyses of *Deinococcus radiodurans* UvrA2 provide insight into DNA binding and damage recognition by UvrAs. *Structure* **17:** 547–558

Trias J & Nikaido H (1990) Protein D2 channel of the *Pseudomonas aeruginosa* outer membrane has a binding site for basic amino acids and peptides. *J. Biol. Chem.* **265:** 15680–4

Truglio JJ, Croteau DL, Van Houten B & Kisker C (2006) Prokaryotic nucleotide excision repair: the UvrABC system. *Chem. Rev.* **106:** 233–52

Uchida K, Furukohri A, Shinozaki Y, Mori T, Ogawara D, Kanaya S, Nohmi T, Maki H & Akiyama M (2008) Overproduction of *Escherichia coli* DNA polymerase DinB (Pol IV) inhibits replication fork progression and is lethal. *Mol. Microbiol.* **70:** 608–22

Uwate M, Ichise Y, Shirai A, Omasa T, Nakae T & Maseda H (2013) Two routes of MexS-MexT-mediated regulation of MexEF-OprN and MexAB-OprM efflux pump expression in *Pseudomonas aeruginosa*. *Microbiol. Immunol.* **57:** 263–72

Vaisman A, McDonald JP, Huston D, Kuban W, Liu L, Van Houten B & Woodgate R (2013) Removal of misincorporated ribonucleotides from prokaryotic genomes: an unexpected role for nucleotide excision repair. *PLoS Genet.* **9:** e1003878

Veaute X, Delmas S, Selva M, Jeusset J, Le Cam E, Matic I, Fabre F & Petit M-A (2005) UvrD helicase, unlike Rep helicase, dismantles RecA nucleoprotein filaments in *Escherichia coli*. *EMBO J.* **24:** 180–189

Visse R, de Ruijter M, Ubbink M, Brandsma JA & van de Putte P (1993) The first zinc-binding domain of UvrA is not essential for UvrABC-mediated DNA excision repair. *Mutat. Res.* **294:** 263–74

Vivona JB & Kelman Z (2003) The diverse spectrum of sliding clamp interacting proteins. *FEBS Lett.* **546:** 167–172

Wang J, Zhou J, Qu T, Shen P, Wei Z, Yu Y & Li L (2010) Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Chinese hospitals. *Int. J. Antimicrob. Agents* **35:** 486–91

Warner DF, Ndwandwe DE, Abrahams GL, Kana BD, Machowski EE, Venclovas C & Mizrahi V (2010) Essential roles for *imuA'*- and *imuB*-encoded accessory factors in DnaE2-dependent mutagenesis in *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U. S. A.* **107:** 13093–8

Waters LS, Minesinger BK, Wiltrout ME, D'Souza S, Woodruff R V & Walker GC (2009) Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiol. Mol. Biol. Rev.* **73:** 134–54

Westfall LW, Carty NL, Layland N, Kuan P, Colmer-Hamood JA & Hamood AN (2006) *mvaT* mutation modifies the expression of the *Pseudomonas aeruginosa* multidrug efflux operon *mexEF-oprN. FEMS Microbiol. Lett.* **255:** 247–54

Wolański M, Donczew R, Zawilak-Pawlik A & Zakrzewska-Czerwińska J (2014) *oriC*-encoded instructions for the initiation of bacterial chromosome replication. *Front. Microbiol.* **5:** 735

Wolff E, Kim M, Hu K, Yang H & Miller JH (2004) Polymerases leave fingerprints: analysis of the mutational spectrum in *Escherichia coli rpoB* to assess the role of polymerase IV in spontaneous mutation. *J. Bacteriol.* **186:** 2900–5

Woodgate R & Ennis DG (1991) Levels of chromosomally encoded Umu proteins and requirements for in vivo UmuD cleavage. *Mol. Gen. Genet.* **229:** 10–6

Woodgate R & Sedgwick SG (1992) Mutagenesis induced by bacterial UmuDC proteins and their plasmid homologues. *Mol. Microbiol.* **6:** 2213–8

Wrzesiński M, Nowosielska A, Nieminuszczy J & Grzesiuk E (2005) Effect of SOS-induced Pol II, Pol IV, and Pol V DNA polymerases on UV-induced mutagenesis and MFD repair in *Escherichia coli* cells. *Acta Biochim. Pol.* **52:** 139–47

Xavier DE, Picão RC, Girardello R, Fehlberg LCC & Gales AC (2010) Efflux pumps expression and its association with porin down-regulation and beta-lactamase production among *Pseudomonas aeruginosa* causing bloodstream infections in Brazil. *BMC Microbiol.* **10:** 217

Xu Y, Grindley ND & Joyce CM (2000) Coordination between the polymerase and 5'-nuclease components of DNA polymerase I of *Escherichia coli*. *J. Biol. Chem.* **275:** 20949–55

Yeeles JTP & Marians KJ (2011) The *Escherichia coli* replisome is inherently DNA damage tolerant. *Science* **334:** 235–8

Yim G, McClure J, Surette MG & Davies JE (2011) Modulation of *Salmonella* gene expression by subinhibitory concentrations of quinolones. *J. Antibiot. (Tokyo).* **64:** 73–8

Yoshimura F & Nikaido H (1982) Permeability of *Pseudomonas aeruginosa* outer membrane to hydrophilic solutes. *J. Bacteriol.* **152:** 636–42

Ysern P, Clerch B, Castaño M, Gibert I, Barbé J & Llagostera M (1990) Induction of SOS genes in Escherichia coli and mutagenesis in *Salmonella typhimurium* by fluoroquinolones. *Mutagenesis* **5:** 63–6

Zhang S & Sundin GW (2004) Mutagenic DNA repair potential in *Pseudomonas* spp., and characterization of the *rulAB*Pc operon from the highly mutable strain *Pseudomonas cichorii* 302959. *Can. J. Microbiol.* **50:** 29–39

Ziha-Zarifi I, Llanes C, Köhler T, Pechere JC & Plesiat P (1999) *In vivo* emergence of multidrug-resistant mutants of *Pseudomonas aeruginosa* overexpressing the active efflux system MexA-MexB-OprM. *Antimicrob. Agents Chemother.* **43:** 287–91

SUMMARY IN ESTONIAN

DNA kahjustuste reparatsioon ja genoomi terviklikkuse tagamine pseudomonaadides

Efektiivne ja täpne DNA replikatsioon ja õigeaegne DNA kahjustuste eemaldamine on erakordse tähtsusega genoomi terviklikkuse säilitamisel. Kõrgelt koordineeritud DNA reparatsiooni mehhanismid võimaldavad rakkudel ellu jääda ka ulatuslike rakuväliselt indutseeritud DNA kahjustuste korral, kuid on ilmselgelt vajalikud ka spontaanselt tekkivate DNA kahjustustega võitlemiseks. Nukleotiidi väljalõike reparatsioon NER on üks põhilisi DNA reparatsioonisüsteeme, mis tunneb ära ja parandab laia spektrit DNA kahjustusi, mis on reeglina põhjustatud eksogeensete tegurite poolt. Vähemalt kuue valgu koostöö tulemusena eemaldatakse DNA kaksikahelast 12–13 nukleotiidi pikkune üksikahelaline kahjustust sisaldav lõik (osalevad valgud UvrA, UvrB, UvrC ja UvrD) ning selle asemele sünteesitakse uus DNA ahel, mis ühendatakse vana ahelaga kokku (osalevad DNA polümeraas Pol I ja DNA ligaas). Pol I on multifunksionaalne ensüüm, mille põhifunktiooniks DNA kahjustuste puudumisel on Okazaki fragmentidest RNA praimerite eemaldamine ja nende asemele uute DNA lõikude süntees. DNA kahjustuste korral on Pol I osalus oluline ka DNA reparatsioonilisel sünteesil aluse väljalõike reparatsiooni BER ja NER radades. Seega, nii NER kui ka Pol I on potentsiaalselt olulised geneetilise informatsiooni säilitamiseks rakkude paljunemisel.

Genoomi terviklikkust ja rakkude heaolu võib hinnata nii rakkude morfoloogia, eluvõime, mutatsioonide tekkesageduse kui ka ja rekombinatsiooniliste sündmuste sageduse põhjal. Kuna homoloogiline rekombinatsioon (HR) on oluline DNA kahjustuste tõttu seiskununud replikatsiooni taasalustamisel ning DNA kahjustustega kaasnevate üksik- ja kaheahelaliste DNA katkete parandamisel, viitab HR sageduse suurenemine kindla valgu puudumisel selle valgu rollile genoomi tervislikkuse säilitamisel. Kuna puudus eksperimentaalne testsüsteem HR sündmuste detekteerimiseks pseudomonaadides ja teistes bakterites, mis ei ole võimelised kasutama laktoosi süsinikuallikana, sai selle töö üheks eesmärgiks konstrueerida vastav testsüsteem ning selgitada erinevate NER-i raja valkude ja Pol I osalust genoomi stabiilsuses. Kuna *P. putida* genoom kodeerib lisaks Pol I-le ja replikatiivsele DNA polümeraasile Pol III-le kolme spetsialiseeritud DNA polümeraasi, Pol II, Pol IV ja DnaE2, mis võiksid Pol I puudumist osaliselt komplementeerida, selgitasime nende DNA polümeraaside osalust DNA sünteesil ja mutatsioonide tekitamisel Pol I funktsioonide puudumisel. Lisaks, kuigi DNA kahjustuste tolereerimiseks ja reparatsiooniks eksisteerivad erinevad mehhanismid, on kahjustuste hulka võimalik vähendada ka sel viisil, et hoida DNA-d kahjustatavate ühendite taset rakkudes võimalikult madalal nende ühendite rakkudest aktiivse väljaviimisega. Näiteks *P. aeruginosa* on tuntud oma võime poolest muutuda resistentseks paljudele antimikroobsetele ühenditele, mis on tihti saavutatav tänu mutatsioonidele väljavoolu (ingl. k. *efflux*) pumpade

ekspressiooni reguleerivates geenides, mis võimaldab antibiootikumide ja teiste rakule kahjulike ühendite väljapumpamist rakkudest. Uurides *P. aeruginosa* MPAO1 põhjal konstrueeritud NER-defektse tüve tundlikkust DNA-d kahjustatavate ühendite suhtes täheldasime nende kemikaalide oodatust tunduvalt väiksemat mõju rakkude eluvõimele. Sellest tulenevalt tekkis vajadus võrrelda *P. aeruginosa* metsiktüve PAO1 erinevate laboratoorsete alaliinide kasvu rakkudele kahjulike ühendite juuresolekul ning selgitada kasvuerinevuste põhjusi.

Saadud tulemused võib kokku võtta järgmiselt:

1. Konstrueerisime testsüsteemi, mis võimaldab uurida HR toimumist erinevate kromosoomi piirkondade vahel nii *P. putida* kasvavates kui ka statsionaarse faasi rakkudes. Antud testsüsteem võimaldab uurida faktoreid, mis mõjutavad HR sagedust (s.h. näiteks alleelide vaheline kaugus, DNA lokaalse struktuuri omadused, transposooni insertsiooniga katkestatud geeni funktsioon). Näitasime, et kromosoomisiseselt toimuvad HR sündmused põhiliselt kasvavates rakkudes ning on pärsitud statsionaarse faasi rakkudes, mis on erinev kromosoomi ja plasmiidi vahelisest HR-i dünaamikast. Nimelt suurenes HR sagedus kromosoomi ja plasmiidi vahel bakterite nälgimisel. Edasi kasutasime konstrueeritud testsüsteemi selleks, et selgitada erinevate DNA metabolismi valkude osalust HR pärssimisel ja genoomi terviklikkuse säilitamisel.

2. NER-i raja valgud UvrA, UvrB ja UvrC on vajalikud genoomi terviklikkuse tagamisel nii kasvavates kui ka statsionaarse faasi *P. putida* rakkudes. Nende valkude puudumine on rakkudele kahjulik ning avaldub rakkude kahanenud eluvõimelisuses, rakkude filamenteerumises ning suurenenud HR sageduses. Sellist efekti NER valkude puudumisel ei ole teistes organismides varem kirjeldatud. Samas toimub NER-defektsete tüvede kiire adapteerumine, mille tulemusena taastub bakteritel normaalne fenotüüp ning väheneb HR sagedus. Huvitav on see, et kui algsetel mitteadapteerunud tüvedel on HR suurenenud ka bakterite nälgimise perioodil, siis adapteerunud tüvedel on see statsionaarse faasi rakkudes pärsitud. Kuigi ka Pol I puudumine on rakkude kahjulik sarnaselt NER valkude puudumisele, on HR Pol I-defektsuse korral nälgivates rakkudes endiselt pärsitud, mis rõhutab veel kord just UvrA ja UvrB valkude olulisusele statsionaarses faasis genoomi terviklikkuse säilitamisel. Lisaks, kuna bakterite adapteerumine ei suurendanud DNA kahjustuste taluvust, viitab see NER-valkude osalemisele protsessides, mis on käesolevas töös uuritud DNA kahjustuste eemaldamisest erinev.

3. HR ärahoidmiseks *P. putida* rakkudes on samuti olulised transkriptsiooniga seotud NER-i (TC-NER) põhiensüümi Mfd ja UvrD helikaasi funktsioonid, kuid nende valkude mõju HR sagedusele avaldub ainult kasvavates rakkudes.

4. Võrreldes bakteriga *E. coli* on *P. putida*'l kaks UvrA homoloogi: UvrA ja UvrA2. UvrA2 ortoloogid esinevad mitmete bakterite genoomides, kuid nende roll bakterirakkudes on siiani jäänud ebaselgeks. Uurides UvrA2 mõju HR toimumisele *P. putida* rakkudes leidsime, et UvrA2 ei ole HR ärahoidmiseks

oluline vähemalt indutseeritud kahjustuste puudumisel. UvrA2 roll ei ilmnenud ka UvrA valgu puudumisel.

5. Pol I puudumisel on bakterite kasv häiritud rikkal söötmel, kuid bakterid on eluvõimelised minimaalsöötmel, mis näitab, et bakterite aeglase kasvu tingimustes on teised süsteemid võimelised Pol I puudumist kompenseerima. Pol I defektsete rakkude kasvu rikkal söötmel saab taastada, vähendades rakkudes reaktiivsete hapniku radikaalide (ROS) hulka, mis viitab Pol I olulisusele endogeenselt tekkivate ROS-ide tolereerimisel. Nägime, et Pol I puudumisel suureneb nii HR kui ka mutatsioonide tekkesagedus. Samas, kuigi spetsialiseeritud DNA polümeraasid Pol II, Pol IV ja DnaE2 ei mõjutanud mutatsioonisagedust Pol I puudumisel, näitas Rif^r mutatsioonide spektri võrdlus, et sel juhul osalevad DNA replikatsioonis ka Pol II ja Pol IV. DnaE2 osalus DNA replikatsioonis oli detekteeritav ainult indutseeritud DNA kahjustuste olemasolul.

6. *P. aeruginosa* laboratoorsete metsiktüvede genoomis tekkinud muutused põhjustavad erinevaid fenotüüpe PAO1 alaliinidel. Neljast alaliinist (MPAO1, PAO1-L, PAO1-DSM ja PAO1-UT) esines MPAO1 alaliinil *nfxC*-tüüpi fenotüüp, millega kaasneb resistentsus klooramfenikooli ning ciprofloksatsiini suhtes, mis on seotud antibiootikumide aktiivse väljapumpamisega MexEF-OprN pumba abil. MexEF-OprN pumba aktiveerimine toimus tänu aktiivsust taastavale mutatsioonile selle operoni aktivaatorit kodeerivas *mexT* geenis. Kuna MexT aktiivsus on täiendavalt reguleeritud, sisaldab MPAO1 tundmatuks jäänud mutatsiooni, mis võimaldab *nfxC*-fenotüübi avaldumist erinevalt MPAO1 transposoon-derivaatidest, millel on MPAO1-ga identne *mexT* järjestus. Samas puudus PAO1-UT alaliinil, kus MexEF-OprN süsteem on samuti üleekspresseeritud, vastav fenotüüp, ehk resistentsus klooramfenikoolile ja ciprofloksatsiinile. Oleme näidanud, et antud tüves esineb suur deletsioon, mis hõlmab DNA kahjustusest indutseeritava *imuA-imuB-dnaE* operoni järjestused ning samuti klooramfenikooli atsetüültransferaasi kodeeriva geeni. See deletsioon võib põhjustada antud tüve tundlikust klooramfenikooli suhtes vaatamata MexEF-OprN pumba üleekspressioonile. Laboratoorsete metsiktüvede fenotüüpide varieeruvus raskendab oluliselt erinevate töörühmade poolt saadud tulemuste võrdlust.

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PUBLICATIONS

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2. List of publications
Sidorenko, J., Ukkivi, K. and Kivisaar, M., (2015). NER enzymes maintain genome integrity and suppress homologous recombination in the absence of exogenously induced DNA damage in *Pseudomonas putida*. *DNA Repair*. 25, 15–26.

Tavita, K., Mikkel, K., Tark-Dame, M., Jerabek, H., Teras, R., Sidorenko, J., Tegova, R., Tover, A., Dame, R.T. and Kivisaar, M., (2012). Homologous recombination is facilitated in starving populations of *Pseudomonas putida* by phenol stress and affected by chromosomal location of the recombination target. *Mutation Research*. 737, 1–2, 12–24

Sidorenko, J**.,** Jatsenko, T., Saumaa, S., Teras, R., Tark-Dame, M., Hõrak, R. and Kivisaar, M., (2011). Involvement of specialized DNA polymerases Pol II, Pol IV and DnaE2 in DNA replication in the absence of Pol I in *Pseudomonas putida*. *Mutation Research*. 714, 1–2, 63–77

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Vene, eesti ja inglise keel; itaalia keel kesktasemel ja saksa keel algtasemel

1. Peamised uurimisvandkonnad

Minu põhiline uurimisvaldkond on DNA reparatsioonimehhanismid ja DNA kahjustuste tolereerimine bakterirakkudes. Olen uurinud DNA reparatsiooni-süsteemide (nukleotiidi väljalõike reparatsioon (NER) ja aluse väljalõike re-paratsioon (BER) ja DNA polümeraaside osalust genoomi terviklikkuse taga-mises ning nende mõju mutatsiooniprotsessidele pseudomonaadides, mulla-bakteris *Pseudomonas putida* ning oportunistlikus inimise patogeenis *P. aeru-ginosa*. Samuti olen uurinud antibiootikumiresistentsuse kujunemise mehha-nisme bakteris *P. aeruginosa*, k. a. väljavoolu (ingl. k. *efflux*) pumpade üle-ekspressiooni.

2. Publikatsioonide loetelu

Sidorenko, J., Ukkivi, K. and Kivisaar, M., (2015). NER enzymes maintain genome integrity and suppress homologous recombination in the absence of exogenously induced DNA damage in *Pseudomonas putida*. *DNA Repair*. 25, 15–26.

Tavita, K., Mikkel, K., Tark-Dame, M., Jerabek, H., Teras, R., Sidorenko, J., Tegova, R., Tover, A., Dame, R.T. and Kivisaar, M., (2012). Homologous recombination is facilitated in starving populations of *Pseudomonas putida* by phenol stress and affected by chromosomal location of the recombination target. *Mutation Research*. 737, 1–2, 12–24

Sidorenko, J**.,** Jatsenko, T., Saumaa, S., Teras, R., Tark-Dame, M., Hõrak, R. and Kivisaar, M., (2011). Involvement of specialized DNA polymerases Pol II, Pol IV and DnaE2 in DNA replication in the absence of Pol I in *Pseudomonas putida*. *Mutation Research*. 714, 1–2, 63–77

3. Saadud uurimistoetused ja stipendiumid

FEMS-i ja Biomeditsiini ja biotehnoloogia doktorikooli stipendiumid erinevatel konverentsidel osalemiseks:

"13th International Conference on Pseudomonas", 4.–7. September 2011, Sydney, Austraalia

"Microbial Stress: from Molecules to Systems", 10.–13. Mai 2012, Belgiraat, Itaalia

"Fourth Microbial Genome Maintenance Meeting", 26.–29. Aprill 2013, Oslo, Norra

Eesti InimeseGeneetika Ühingu aastakonverentsid (2010–2014)

Baltimaade Mikrobioloogide Kongress (2013, 2014)

4. Muu teaduslik erialane ja organisatsiooniline tegevus

Alates 2010 Eesti Mikrobioloogide Ühingu liige

Alates 2011 Geneetika praktikumi (LOMR.03.023) juhendaja Tartu Ülikooli Molekulaar- ja Rakubioloogia Instituudis

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