General stress proteins: Novel function and signals for induction of stationary phase genes in *E. coli*

Örjan Persson

AKADEMISK AVHANDLING

För filosofie doktorsexamenen i mikrobiologi (examinator Thomas Nyström), som enligt fakultetssyrelsens beslut kommer att offentligt försvaras tisdagen den 25 maj 2010, kl. 10.00 i föreläsningssal Arvid Carlsson, Medicinaregatan 3, Göteborg

ISBN 978-91-628-8104-7

e-publication: http://hdl.handle.net/2077/22213

To Gullborg

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ABSTRACT

Survival during conditions when nutrients become scarce requires adaptation and expression of genes for maintenance in order for the cell to survive. Among the numerous proteins involved in adaptation and regulation under these conditions, the stationary phase sigma factor, σ^S, and the Universal stress proteins contribute to survival and bestow the cell with general stress protective functions during growth arrest. In this work we found new mechanisms for the cell to prepare and sense the intracellular environment and respond accordingly.

The *usp* genes and the *rpoS* gene (encoding σ^S) were found to be positively regulated by metabolic intermediates of the glycolysis in the central metabolic pathway. Specifically, mutations and conditions resulting in fructose-6-phosphate accumulation elicit superinduction of these genes upon carbon starvation, whereas genetic manipulations reducing the pool size of fructose-6-phosphate have the opposite effect. Under carbon starvation, transcription of the *usp* and the *rpoS* genes require and are modulated by the alarmone ppGpp. The observed positive transcriptional regulation by fructose-6-phosphate is not via alterations of the levels of ppGpp. None of the known regulators examined were found to be required for the superinduction. We suggest a novel regulatory mechanism involving the phosphorylated intermediates as a signal molecule for monitoring and subsequent regulation of stress defense genes. Based on mutational studies we also suggest that this signaling mechanism secures accumulation of required survival proteins preceding the complete depletion of the external carbon source.

Entry into stationary phase promotes a dramatic stabilization on the sigma factor σ^S. Mistranslated and oxidized proteins were shown to contribute to elevated levels of σ^S and transcription of its regulon. Furthermore, ribosomal alleles with enhanced translational accuracy attenuate induction of the RpoS regulon and prevent stabilization of σ^S. Destabilization of σ^S is governed by the ClpXP protease, for which aberrant proteins also are substrates. Mechanistically, generation of mistranslated proteins by starvation, or other means, competes for the common enzyme for degradation, and thereby sequesters the pool in favor of σ^S stabilization.

A growing body of evidence shows that there is an intimate connection between proteins required for genome stability and stationary phase survival. We show that the integral membrane protein UspB, a member of the RpoS regulon, is required for proper DNA repair as mutants lacking *uspB* are sensitive to several DNA damaging conditions. Genetic and biochemical studies demonstrate that UspB acts in the RuvABC recombination repair pathway and removing *uspB* creates a phenocopy of the DNA resolvase mutant, *ruvC,* which includes a reduced efficiency in resolving Holliday junctions. Further, we show that the *uspB* mutant phenotype can be suppressed by ectopic overproduction of RuvC and that both *ruvC* and *uspB* mutants can be suppressed by inactivating *recD*. The fact that RuvABC-dependent repair requires UspB for proper activity suggests that the σ^S-regulon works together with DNA repair pathways under stress conditions to defend the cell against genotoxic stress.

List of papers

This thesis is based on the following papers, which are referred to by their Roman numerals:

I. Metabolic control of the Escherichia coli universal stress protein response through fructose-6-phosphate. **Persson Ö**, Valadi A, Nyström T, Farewell A. Mol Microbiol. 2007 65(4):968-78.

II. The levels of fructose-6-phosphate regulate σ^S-dependent transcription upon entry to stationary phase in *E. coli*. **Persson, Ö**, Gummesson, B., Hallberg, E., Lilja, E., and Farewell, A. Manuscript

III. Decline in ribosomal fidelity contributes to the accumulation and stabilization of the master stress response regulator σ^S upon carbon starvation. Fredriksson Å, Ballesteros M, Peterson CN, **Persson Ö**, Silhavy TJ, Nyström T. Genes Dev. 2007 21(7):862-74.

IV. UspB, a member of the sigma-S regulon, is required for RuvC-dependent resolution of Holliday junctions. **Persson, Ö**, Nyström, T and Farewell, A. Under revision DNA Repair.

Other papers not included in this thesis the author published:
Increased RNA polymerase availability directs resources towards growth at the expense of maintenance. Gummesson B, Magnusson LU, Lovmar M, Kvint K, **Persson O**, Ballesteros M, Farewell A, Nyström T. EMBO J. 2009 Jul 2;28(15):2209-2219.

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1. Introduction

1.1. *E. coli* as a model organism

In 1885, the German pediatrician Theodor Escherich, isolated and cultivated a rod-shaped gram-negative bacterium; the "Bacterium coli" (later renamed *Escherichia coli*) (Lederberg 2004). Its natural habitat is the large intestine or colon of mammals and birds that it has a remarkable capacity to colonize. In number, this microorganism does not dominate in the abdomen, but is estimated to constitute only 1% of the microbial flora. However, it has been found in every species examined and in almost every single individual of those (Neidhardt 1996).

Escherichia coli has emerged as a predominant model organism in labs that study bacteria. *Escherichia coli* K-12, the dominant parental strain background in many laboratories studying *E.coli* and considered wild type, was isolated at Stanford University, CA. In contrast to its many derivatives, K-12 has not been treated with gamma or UV radiation or other mutagenic agents. Despite this, during cultivation and selection, K-12 has lost O and K antigen and is virtually unable to colonize a human gut (Smith 1975).

In its natural habitat, *E. coli* is constantly challenged with altered nutrient availability, partial anaerobiosis, and changes in pH and osmotic stress, while the temperature is fairly constant, at around 37°C. In addition, the bacteria must have capacity to sustain different environmental assaults like H_2O_2, UV light and exposure to antibiotics. In order to proliferate and survive during those conditions, cells have to adapt to the constantly changing environment and throughout its evolution, *E. coli* has perfected its adaptive strategies to these changes. Natural selection has thus equipped bacteria with systems for synchronously altering many pathways, either by up- or down-regulating gene expression in response to new environments. One mechanism of coordinate regulation is the structure of an operon, where many genes are simultaneously transcribed due to a specific stimulus. Coordinate regulation is also accomplished in *E. coli* by a variety of mechanisms including small messenger molecules such as the alarmones ppGpp and cAMP, but also by alternative sigma factors and regulons such as the SOS response that regulate sets of genes and their products. Among the proteins essential for sustaining high viability during conditions when the nutrients are scarce or lacking, the RpoS sigma factor (σ^S) and the Universal stress proteins are expressed and play a role either directly or indirectly in the process of survival in a diversity of inhospitable conditions. This thesis focuses on the regulation and function of these important proteins.

2. Aim and findings at a glance

The aim of this thesis was to find and characterize factors involved in regulation of the universal stress proteins genes *uspA*, *uspB* and the stationary phase sigma factor RpoS as well as characterize the function of UspB.

We found a new mechanism involved in the regulation of the *uspA* gene and other *usp´*s in response to carbon stress. Accumulation of fructose-6-phosphate in the central metabolic pathway, bestows a signal to the *usp* genes and positive regulation occurs (**paper I**). About one generation before cells are depleted of carbon, *uspA* is normally induced. In mutants with elevated levels of fructose-6-p the induction is stronger and superinduction occurs. This regulation is not due to alteration of the known regulators of *uspA*. How this signal is transmitted is unknown, but it implies that the cell is capable to sense and prepare accordingly before food sources become exhausted.

In a similar manner, the well characterized general stress defense gene *rpoS*, encoding the transcription factor σ^S, was also found to be regulated by metabolic accumulation of fructose-6-p (**paper II**). Like the *usp´*s the regulation occurs at the level of transcription and the protein levels correspond to the increased transcription of *rpoS*. Throughout growth an increased level of σ^S can be observed. This results in superinduction of the RpoS-dependent genes upon carbon starvation in cells with increased fructose-6-p.

On the regulation of σ^S we also found that during entry to carbon starvation, mistranslated or oxidized proteins contribute to the elevation and stability of the sigma-S (σ^S) protein (**paper III**). Aberrant proteins and sigma-S are both substrates for ClpP protease. Mechanistically, via a titration of ClpXP, decreased translational fidelity of the ribosome stabilizes the RpoS in stationary phase and elevated transcription of its regulon is observed.

Finally, the stationary phase inducible protein UspB is shown to be involved in DNA repair (**paper IV**). A mutation in *uspB* reduces the survival significantly following DNA damage. We showed by genetic and biochemical methods that a mutation in *uspB* affects the resolution of Holliday junctions following DNA damage. A possible link to the resolvase RuvC was found and a *uspB* deletion phenocopied a *ruvC* mutant under all tested conducted. The results suggest that UspB is capable of modulating the effect of the resolvase RuvC during repair of damage.

3. Growth, Metabolism and Carbon source selection

E. coli, like many other bacteria, divides by binary fission. Provided the growth conditions are unrestricted (nutrients in excess and no accumulation of toxic byproducts) *E. coli* can divide and give rise to a functional new cell in as little as 20 minutes. During these conditions the cell is basically a protein factory with the majority of resources going to ribosomal protein synthesis. To supply the demand for anabolic metabolites and energy requirements the bacterium harbors an extensive and highly dynamic metabolic network for metabolizing compounds found in the environment and converting them into different essential molecules like precursor metabolites, reducing power (NADPH) and energy transfer molecules (NADH and ATP). Different pathways are involved depending on the carbon source utilized. *E.coli* can utilize an impressively broad range of different carbon sources, from high energy carbohydrates, polyols, to two-carbon compounds or fatty acids as sole carbon and energy source. In general, by relatively simple conversions of the molecules, like phosphorylation, isomerisation and aldol cleavage etc. these different carbon sources can be fed into the central metabolic pathways of glycolysis, the pentose pathway or the tricarboxylic acid (TCA) cycle (Neidhardt 1996).

The preferred carbon source for *E.coli* cells is the monosaccharide glucose and when cells are fed with glucose, no other carbon source is simultaneously utilized (Postma and Lengeler 1985; Postma et al. 1993). The presence of glucose inhibits the expression of enzymes needed for uptake and metabolism of other carbon sources; these phenomenona are known as inducer exclusion and carbon catabolite repression. Glucose exerts this repression of utilization of other carbon sources till minute levels (μM) remain in the growth media (Ferenci 1996). The uptake of glucose via the phosphoenolpyruvate:carbonhydrate phosphotransferase (PTS) system together with cAMP-CRP mediate this response (Meadow et al. 1990; Neidhardt 1996; Bruckner and Titgemeyer 2002). Uptake of glucose is an active process via the PTS system, where glucose becomes phosphorylated by transfer of a phosphate from Enzyme IIA^{Glc} ($EIIA^{Glc}$) and enters the glycolytic pathway as glucose-6-phosphate. This results in the $EIIA^{Glc}$ protein becoming dephosphorylated and it then inhibits uptake of alternative carbohydrates and fails to activate adenylate cyclase (AC) leading to low cAMP levels (Ferenci 1996; Hogema et al. 1998) (Fig 1).

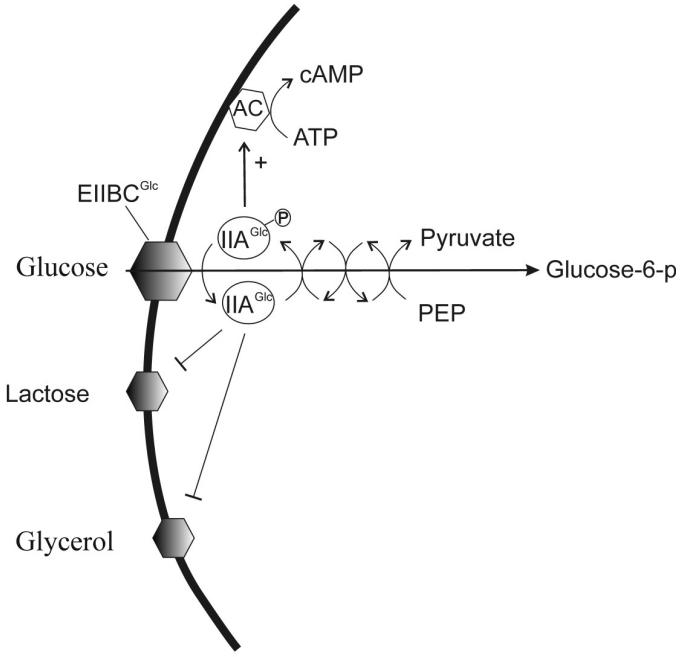

Figure 1. Mechanism of PTS-mediated catabolite repression and regulation. Glucose is rapidly phosphorylated by the PTS enzyme $EIIBC^{Glc}$ and fed into glycolysis. In this process, the protein IIA^{Glc} becomes dephosphorylated. In its dephophorylated state it binds enzymes involved in the uptake of non-PTS carbon sources (like lactose and glycerol) and thereby inhibits the utilization and uptake of these carbon sources. The dephosphorylated state of IIA^{Glc} also inhibits the synthesis of cAMP by adenylate cyclase (AC). For clarity not all steps are included. Adapted from (Hogema et al. 1998b).

In the absence of glucose, $EIIA^{Glc}$ remains phosphorylated and activates adenylate cyclase, the product of the *cya* gene (Fig 1). Active adenylate cyclase synthesizes the alarmone cAMP. cAMP with its cognate CRP (also known as CAP) protein binds operator sequences with a specific cAMP-CRP recognition sequence and thereby affects transcription of about 100 promoters in a positive or negative way (Fic et al. 2009). For positive regulation, cAMP-CRP binds the DNA and promotes transcription either by direct recruitment of RNAP to the promoter region or by facilitating RNAP-promoter complex formation (reviewed in (Busby and Ebright 1999)). In both cases the transcription is facilitated by protein-protein interaction between RNAP and CAP. In some cases, additional activators besides CAP are needed for full induction, for example the *araBAD* promoter also needs AraC and arabinose to become activated (Lobell and Schleif 1991) or relief of inducer exclusion is needed for induction of the permease *lacY* of the lactose uptake system (Hogema et al. 1998a). cAMP-CRP can also exert negative effect by directly occupying core promoter regions, like P_{gal}, (Spassky et al. 1984) or indirectly by stabilization of a repressor (Kristensen et al. 1997). By these mechanisms cAMP-CAP affects transcription of many operons involved in uptake and

metabolism of non-PTS carbon sources as well as gene products involved in processes like biofilm formation, flagellum formation, chemotaxis and nitrogen utilization (Saier 1996). Generally, the cAMP-CRP modulon increases the cell's ability to scavenge the environment for alternative carbon sources.

Whereas glucose is most efficient at catabolite repression, glycerol is considered to be a carbon source that leads to low catabolite repression (Hogema et al. 1998b; Bettenbrock et al. 2007). Uptake of glycerol is mediated via the proteins of the *glp* regulon consisting of several operons with a complex genetic structure (Weissenborn et al. 1992; Yang and Larson 1998). In contrast to other carbohydrates, glycerol is taken up by facilitated diffusion across the cytoplasmic membrane and is phosphorylated by the GlpK kinase to glycerol-3-phosphate and further dehydrogenised to the glycolytic intermediate DHAP of the central metabolic pathway (Voegele et al. 1993; Lin 1996). The kinase is allosterically regulated not only by $EIIA^{Glc}$ but also fructose1,6-bisphosphate, thus glycerol uptake is tightly regulated as long as the cell is able to take up glucose (Lin 1996). During growth on glycerol as carbon source almost all of $EIIA^{Glc}$ is in the phosphorylated state (Hogema et al. 1998), thus a complete stimulatory effect of cAMP synthesis occurs during this condition.

4. Stationary phase

In its natural habitat low nutrients availability is the prevalent situation, setting the stage for evolution under those conditions (Llorens et al.). Furthermore, in batch cultures some of the essential nutrients will eventually become exhausted. Upon depletion, the growth rate slows down and the cell reaches a phase where no net increase in cell number occurs and a balanced state between division and death is reached, called stationary phase. However, as pointed out by Groat (1986), stationary phase is only defined as a condition in which the cessation of growth occurs due to nutrient deprivation and the physiological response of the cells is different depending on the type of starvation, for example, starvation for carbon, nitrogen, phosphate or other compounds. Entry to, and maintenance in, stationary phase is not a passive process and this can clearly be exemplified by blocking protein synthesis of the cell when entering stationary phase. Adding chloramphenicol to wild type carbon starved cells reduced survival of the cultures. The earlier the protein synthesis was blocked on starved cells, the more pronounced was its effect on survival (Reeve et al. 1984; Nystrom et al. 1990). Thus, during entry to starvation the cell induces specific proteins in order to

continue divide and reproduce (e.g., to utilize an alternative nutrient source that may be present), or, if this fails, the cells induce a general stationary phase response by redirecting resources into maintenance metabolism and stress response gene expression for protection. Further, cells adapt physiologically and morphologically to this state by a number of different changes. Initiation of DNA replication is stopped, but ongoing replication and the cell cycle is completed (Kolter et al. 1993). At the same time the cell size decreases (most likely as a consequence a final division), the cells become almost coccoid and the membrane composition becomes less fluid (Kolter et al. 1993). Furthermore, aberrant and oxidized proteins accumulate as a consequence of decreased translational fidelity. Misincorporation of erroneous amino acids, translational frameshifting and stop-codon readthrough all contribute to the increased pool of mistranslated proteins (O'Farrell 1978; Barak et al. 1996; Wenthzel et al. 1998; Ballesteros et al. 2001). However, unnecessary protein synthesis is turned off quickly; in particular ribosome protein synthesis is inhibited. Cells start to scavenge for nutrients in the media as well as degrading surplus endogenous material like proteins, RNA and lipids. Degradation of these components is thought to ensure building blocks for essential stationary phase proteins. For example, at the onset of starvation, the RNase activity increases two- to eightfold, leading to extensive degradation of ribosomal RNA (rRNA) and within the first 4 hours of starvation 20-30% of the total RNA is degraded (Matin et al. 1989). In contrast to rRNA, the half-life of mRNA increases more than two-fold, regardless of whether the transcript is repressed or induced in stationary phase (Albertson and Nystrom 1994).

As a result of these changes in gene expression and physiology, stationary phase cells are more resistant towards a number of different environmental assaults, for example cold/heat shock, oxidative stress, antibiotics, osmotic stress, ethanol and UV ((Matin et al. 1989) and references therein). During stationary phase, Dps protein binds to the nucleoid and arranges the DNA into a tightly packed and condensed structure that is resistant to a broad range of different assaults, like oxidative, thermal, acid and base stresses; UV and gamma irradiation; and iron and copper toxicity (Frenkiel-Krispin et al. 2004; Kim et al. 2004; Nair and Finkel 2004). In combination with chromosome compaction undertaken by Dps, the function of Dps as a metal chelator and ferroxidase further protects the DNA. Increased HN-S concentration may also contribute to DNA condensation (Ueguchi et al. 1993).

Thus, although there are specific responses to specific starvation conditions, the general stationary phase response plays a vital role in the cells survival in stationary phase. Among

these general responses, the stationary phase sigma factor, RpoS, is the main regulator for transcription of genes involved in maintenance and survival (Hengge-Aronis 2002). However, not only RpoS regulated genes are up-regulated and crucial for survival (e.g., the universal stress protein genes). Many but not all genes that are specifically induced in stationary phase are also regulated by cAMP-CRP (Saier 1996), suggesting its role for adaptation to stationary phase. Together these responses contribute to cell survival in stationary phase.

5. Adapting and optimization to different environments

5.1. Sensing carbon metabolites

A fundamental process of the cell in order to survive and propagate in a competing environment is to efficiently adapt and respond to changes in the surroundings. One way cells can do this it to sense its intracellular and extracellular metabolic environment. For example, to avoid wasteful metabolic overflow cells constantly regulate metabolism within the central pathways and can utilize intermediates as signals. In most cases the regulation governs feedback regulation or uptake of other compounds, for example, the intracellular [PEP]/[pyruvate] ratio alters the phosphorylation state of IIA^{Glc} (Hogema et al. 1998b) and AMP as well as PEP allosterically regulates PfkA (Fraenkel 1996). Other metabolic regulators in *E. coli* are cAMP, CsrA and ArcAB, which modulates carbon metabolism during catabolic shifts of carbon sources and during anaerobic growth (Iuchi and Weiner 1996; Saier 1996). An intricate regulation of the mRNA of transcribed genes involved in acetate, glycolysis and glycogen metabolism occurs via the CsrA sRNA and the anti-CsrAs, CsrB and CsrC. However, the predominant regulatory effect of the CsrA system seems to be under conditions for acetate metabolism, even though mutations strongly affect the metabolic flow (Sabnis et al. 1995; Yang et al. 1996; Romeo 1998; Weilbacher et al. 2003).

In *B.subtilis*, carbon availability is suggested to be sensed via the levels of UDP-glucose which serves as an intracellular signal and transmits the information to the division apparatus (Weart et al. 2007; Wang and Levin 2009). In *E.coli*, no such regulation has been described, however, UDP-glucose was suggested to alter transcription from σ^S dependent genes during growth. The strains with reduced levels of, or deficient in synthesizing UDP-glucose exhibited an increased level of σ^S in growing cells (Bohringer et al. 1995).

Recently it has been discovered that the uptake of glucose itself is regulated by a feedback mechanism acting on the major PTS glucose transporter, $EIICB^{Glc}$ (PtsG). Under conditions where glucose transport exceeds the capacity of the cell to further metabolize the phosphorylated sugar, glucose transport is inhibited. This regulation is post-transcriptional and involves specific degradation of the *ptsG* mRNA, thereby reducing the *de novo* protein synthesis of $EIICB^{Glc}$ and thus the uptake of glucose. Intracellular accumulation of glucose-6-phosphate (glucose-6-p) or fructose-6-phosphate (fructose-6-p), the first metabolic intermediates after the uptake of glucose, most likely triggers this response (Kimata et al. 2001; Morita et al. 2003). However, it was also shown that replenishing the pool of metabolites downstream of the block could prevent the degradation of *ptsG* and restore its stability (Kimata et al. 2001), indicating that glucose-6-p or fructose-6-p might not be the sole signaling molecules. Further work has shown that a transcription factor, SgrR, senses the accumulation or imbalance of phosphorylated sugars and induces the expression of the small regulatory RNA, SgrS. SgrS mediates the cellular response by base-pairing with the *ptsG* mRNA and presenting it for degradation by the RNA degradosome (Vanderpool and Gottesman 2004). It was shown that the degradation in turn was dependent on RNase E and enolase, but not PNPase or RhlB of the degradosome (Morita et al. 2004).

In this thesis, we show an additional function of fructose-6-p, and possibly also glucose-6-p, besides regulating the specific glucose uptake pathway. These intermediates can also function as signal molecules for positive regulation of genes involved in the general stress response (**paper I** and **II**). This highlights the importance of continuously sensing and responding to fluctuations of the upper part of the glycolysis.

5.2. Sensing starvation

There are at least three major regulatory networks responsible for regulation of the genes involved in stress protection during growth arrest: the stringent response, the heat shock regulon and the sigma-S regulon. Two of these are controlled by alternative sigma factors. The heat shock regulon is regulated by σ^{32} (*rpoH*) and the sigma-S regulon by the σ^{S} (*rpoS*), the master regulator of general stress response. Sigma factors bind RNA polymerase and direct the RNAP to a particular class of promoters (Gruber and Gross 2003) . ppGpp, the modulator of stringent response, is an epistatic factor also binding RNAP and thereby altering its activity at promoters.

5.3. The stringent response and the alarmone ppGpp

Deprivation of an amino acid, carbon source, fatty acids, phosphate or iron in a bacterial cell all results in a swift change in the major cellular overall metabolism (Xiao et al. 1991; Seyfzadeh et al. 1993; Cashel 1996; Murray and Bremer 1996; Vinella et al. 2005; Bougdour and Gottesman 2007). This pleiotropic response, initially identified in 1961 (Stent and Brenner), is known as the stringent response. Guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), collectively known as (p)ppGpp, is responsible for mediating the response including the trademark of the stringent response, the abrupt transcriptional cessation of the translational machinery components (rRNA and tRNA) (Cashel 1996). In general, ppGpp down-regulates proliferation and growth promoting processes and up-regulates genes involved in maintenance (see Fig 2). In addition, ppGpp regulates a plethora of different physiological processes as starvation survival, replication, secondary metabolism, virulence and biofilm formation (Magnusson et al. 2005; Potrykus and Cashel 2008; Wu and Xie 2009). Among the positively regulated genes are the *usp*-genes, as well as *rpoS* and many RpoS-dependent genes, like *uspB* and *bolA* (Gentry et al. 1993; Kvint et al. 2000a; Gustavsson et al. 2002).

5.3.1. Regulation of stringent response

In *E.coli*, synthesis of ppGpp is mediated by two pathways, dependent on the proteins RelA and SpoT, SpoT is also essential for degrading ppGpp (Cashel 1996) (Fig 2). During amino acid starvation, binding of uncharged tRNA in the ribosomal A site stimulates ppGpp synthase by RelA (Haseltine and Block 1973; Wendrich et al. 2002). The ribosome associated RelA protein catalyzes the phosphorylgroup transfer of phosphates from the ATP donor to the GTP or GDP (Cashel 1969; Sy and Lipmann 1973), Uncharged tRNA has also been implicated in inhibiting SpoT dependent hydrolysis of ppGpp (Richter 1980) as exhaustion of the amino acid pool reduces the SpoT hydrolase activity (Murray and Bremer 1996) and thereby increasing the stringent response.

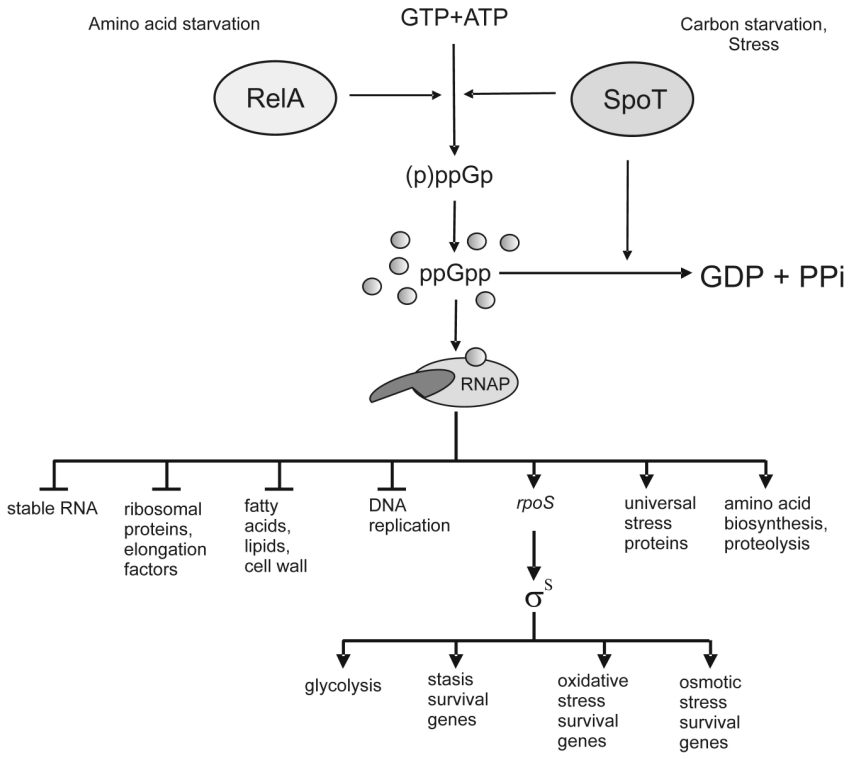

Figure 2. Summary of the synthesis of ppGpp and the effect of the alarmone on the global transcription. Adapted from (Magnusson et al. 2005).

During steady state growth the low levels of ppGpp is thought to be dictated by the SpoT hydrolyzing/synthase activity (Cashel 1969) and in the majority of the other cases when ppGpp is synthesized, excluding amino acid starvation, the accumulation is dependent on the SpoT protein ((Potrykus and Cashel 2008; Srivatsan and Wang 2008) and references therein). In many cases the regulatory mechanism is not known, i.e. how the signal is transmitted to SpoT to decrease its hydrolyzing capacity or increase its synthase activity. However, SpoT has repeatedly been co-purified with acyl carrier protein (ACP) (Gully et al. 2003; Butland et al. 2005; Gully and Bouveret 2006), indicating an interaction with ACP. ACP, a central co-factor involved in donating acyl groups for fatty acid metabolism, has been shown to bind specifically to the catalytic domain of SpoT and thus proposed to alter its enzymatic activity in favor of synthase activity due to fatty acid starvation (Battesti and Bouveret 2006). Initial results suggest that ACP interacts with SpoT during all conditions tested, both during growth and starvation conditions (Battesti and Bouveret 2006). Thus, the SpoT bound ACP might

work as a sensor of fatty acid metabolism and depending on the derivatives bound to ACP correspondingly alter the hydrolyzing/synthase capacity of SpoT by changing its structure (Battesti and Bouveret 2006). The authors also suggest a possible link to SpoT dependent accumulation of ppGpp during carbon starvation; carbon source availability could potentially also be sensed via ACP, as the pool size of the precursor molecule for fatty acid synthesis, acetyl-CoA, is drastically reduced when cells enter carbon starvation (Takamura and Nomura 1988). Further studies to pinpoint this interaction, possibly also during other stresses, have yet to be conducted. Besides ACP, ObgE (CgtA), a multi functional enzyme involved in chromosome segregation and ribosome assembly, has been suggested to regulate SpoT hydrolysis activity during growth (Jiang et al. 2007; Raskin et al. 2007). However, due to conflicting results the role of ObgE remains unclear for the moment (Persky et al. 2009).

5.3.2. The effect of the stringent response regulator, ppGpp, on RNAP

In contrast to many regulators of transcription, which bind DNA within or close to the promoter thereby affecting transcription, ppGpp directly binds to the RNAP (Chatterji et al. 1998; Artsimovitch et al. 2004). One theory of how ppGpp exerts its function is that it destabilizes the open complex between σ^{70}-programmed RNAP and the promoter, causing RNAP to fall off of promoters during the initiation process. Studies have shown that *rrn*-promoters form intrinsically unstable open complexes and thus are specifically sensitive to ppGpp (Barker et al. 2001). This theory also advocates that as a consequence of the reduced stability of the *rrn* promoters the availability of free RNAP, thought to be limiting in the cell, increases and that allows transcription from promoters are relatively poor in recruiting RNAP. These promoters would then have increased transcription and be seen as positively regulated by ppGpp (i.e., stress inducible promoters). However, the opposite has also been postulated; that the available pool of RNAP is diminished during stringent response, possibly because ppGpp increases pausing during transcription elongation (Wagner 2002; Jores and Wagner 2003). In addition, the pool of free σ^{70} programmed RNAP is thought to decrease during a stringent response due to the binding of alternative sigma factors. ppGpp has been shown to increase the ability of alternative sigma factors to compete for binding to RNAP core (Farewell et al. 1998a; Jishage et al. 2002). This model also proposes that because promoters have different capacities to load RNAP and transcribe from a promoter before they are saturated, alterations in the level of available RNAP would influence transcription rates.

rrn promoters have been shown to have a high clearance rate and estimates suggest that these promoters not are saturated under most growth conditions *in vivo*. In this model it is also proposed that since ribosomal promoters are difficult to saturate they would be especially sensitive to alterations in RNAP concentration. Experiments have shown that decreasing the levels of σ^{70} programmed RNAP mimics a stringent response (Magnusson et al. 2003), and increasing the levels gives the opposite response (Gummesson et al. 2009). These results support but do not prove this second model of passive regulation via alterations in the levels of RNAP during a stringent response.

The central regulatory role of ppGpp can easily been seen on proteomic 2-D gels or following individual transcriptional fusions know to be part of the ppGpp regulon. A $ppGpp^0$ strain entering stationary phase totally fails to down-regulate growth promoting gene transcription (Magnusson et al. 2003) and positively regulated genes like *uspA* or *uspB* are not induced when cells when enter stationary phase (Kvint et al. 2000a; Kvint et al. 2000b).

5.3.3. Other effects of the stringent response regulator, ppGpp

In addition to its role in regulating *rrn* and stress inducible promoters, ppGpp also modulates DNA replication and cell division by decreasing transcription from the stringently controlled *dnaA* promoter (Chiaramello and Zyskind 1990). The overall rate of replication is thought to be determined by the initiator protein DnaA concentration (Lobner-Olesen et al. 1989) and there is an inverse correlation between initiation of a new round of replication and the concentration of ppGpp (Schreiber et al. 1995; Ferullo and Lovett 2008). In addition to this role, *in vitro* data suggest that ppGpp inhibits DnaG primase activity in both *E.coli* and *B.subtilis* (Wang et al. 2007; Maciag et al. 2010). Moreover, ObgE seems to work in concert with ppGpp to control aspects of cell division and stringent response. One of the roles of the ObgE GTPase involves control of replication in a G-Protein like fashion (Foti et al. 2005). The finding that ObgE has a high affinity to (p)ppGpp and that an *obgE* mutant has an altered pppGpp/ppGpp ratio (Persky et al. 2009), might imply that ObgE works as an effector of the stringent response.

That ppGpp interacts with other molecules besides RNAP is also precedented by *in vitro* studies where the GTPase translational factors IF2 and EF-Tu are affected by ppGpp either by inactivating their function or increasing their accuracy (Pingoud and Block 1981; Dix and

Thompson 1986; Milon et al. 2006). This finding that ppGpp affects translation accuracy, also results in a $ppGpp^0$ strain producing more oxidized (carbonylated) proteins (Gummesson et al. 2009), a measurement of translational error. Thus, the combined *in vivo* and *in vitro* results suggest an increased accuracy of translation mediated by ppGpp during stringent conditions. However, this effect could also be indirect since ppGpp facilitates alternative sigma factors, like σ^S and σ^{32}, to compete better with σ^{70} and redirect the transcription from growth promoting genes to genes involved in maintenance function (Farewell et al. 1998a; Jishage et al. 2002) and many of the genes being regulated by these σ-factors are known to abrogate the cause of oxidized proteins (see below). Thus, the increased ribosomal accuracy together with effect of stringently regulated RNAP has on transcription might be a way for the cell to reduces synthesis of aberrant proteins.

5.4. Heat shock proteins, oxidative stress and translation fidelity

The heat shock response is important during both adverse conditions, like sudden temperature shifts and exposure to organic chemicals, as well as under non-stress conditions (Gross 1996; Hartl 1996). In response to protein misfolding in the cytoplasm, the heat shock sigma factor σ^{32} becomes stabilized and directs transcription of proteins of its regulon (Straus et al. 1987; Bukau 1993; Guisbert et al. 2004). The majority of these proteins are involved in either protein folding (chaperones) or protein degradation (proteases). These processes become increasingly important under conditions where damaged protein is produced. However, proteins always have the potential to become damaged even under favorable environmental conditions. Under aerobic conditions, the cell inevitably produces reactive oxygen species (ROS), like hydrogen peroxide, superoxide anions and radicals, as a bi-product of normal metabolic electron transport (Fridovich 1978). Oxidative damage by ROS of proteins can produce carbonylated proteins via direct metal-catalyzed oxidation (MCO) on amino acid side chains of specific amino acids, leading to potential loss of function and denaturation of proteins (Stadtman and Levine 2000; Maisonneuve et al. 2009). Once formed, a neighboring carbonylatable site is more prone to carbonylation (Maisonneuve et al. 2009), exacerbating the process of protein function degeneration. Being an irreversible process, the carbonylation process is a threat to the cell. One way the cell can combat protein carbonylation is by the means of synthesizing antioxidants, like catalase and superoxide dismutase, thereby neutralizing ROS (Dukan and Nystrom 1999; Stadtman and Levine 2000;

paper III). Since misfolded proteins constitute a signal for the heat shock response, the synthesized chaperones and proteases constitute a second way the cell can combat oxidative modification of proteins. In order to prevent detrimental accumulation and possible aggregation of denatured proteins a delicate balance between refolding and/or degrading of the damaged proteins occurs. By their capacity to refold proteins, chaperones are essential for maintaining the function of proteins (Hartl 1996). However, proteins can also be degraded by proteases if the load of protein damage is high (Gottesman 1996).

Both chaperones and proteases are required for normal adaptation when cells enter stationary phase, as both *dnaK* (a chaperone) and *clpP* (a protease) mutants display reduced survival (Spence et al. 1990; Weichart et al. 2003). It has been suggested that proteolytic degradation of damaged proteins may also be increasingly important during the entry into and during stationary phase, because of the lack of dilution of components by protein synthesis and cell division (Weichart et al. 2003). As a consequence of carbon starvation and amino acid down-shift an imminent decline in the pool size of charged tRNAs occurs. This reduced availability leads to reduced translational fidelity due to misincorporation of erroneous amino acids, translational frameshifting and stop-codon readthrough (O'Farrell 1978; Barak et al. 1996; Wenthzel et al. 1998; Ballesteros et al. 2001) as well as an induction of heat shock proteins (Matin 1991; Ballesteros et al. 2001). However, despite an up-regulation of oxidative defense proteins, those systems are not sufficient combat the levels of oxidation of damaged proteins which increase during entry to stationary phase (Fredriksson et al. 2005; Diaz-Acosta et al. 2006).

Among the many proteins under the regulatory control of σ^{32}, Clp and Lon are the major proteases responsible for degradation of cytoplasmic proteins (Maurizi 1992). Lon is the prime protease degrading misfolded and aberrant proteins and in its absence strong aggregation of proteins occurs (Tomoyasu et al. 2001). The ClpP protease assisted by the chaperones ClpA or ClpX degrades a variety of proteins, for example, proteins with abnormal N-terminal amino acids (according to the N-end rule) and incompletely synthesized proteins with SsrA-tag (Tobias et al. 1991; Gottesman et al. 1998). SsrA-tagging is a specific modification of proteins adding a short polypeptide tag to the C-terminal end of a protein, hence marking them for degradation (Tu et al. 1995; Keiler et al. 1996; Gottesman et al. 1998). ClpXP is also responsible for controlling the stability of RssB-bound RpoS, the stationary phase sigma factor (See section 5.5.1.3 and **paper III**).

5.5. RpoS regulon and general stress defense

In contrast to many specific stress responses which are induced by a specific stress with the capacity to repair or eliminate the immediate stress caused to the cell, the σ^S (RpoS) dependent stress resistance has a general protective role in the cell. For example, cells deleted for *rpoS* display reduced survival during carbon and nitrogen starvation as well as osmotic, oxidative and heat stresses (Lange and Hengge-Aronis 1991; McCann et al. 1991), indicating that RpoS governs the regulation of a large set of different stress responses.

Cessation of growth due to starvation of amino acids, phosphate, nitrogen or carbon, increases levels of the sigma factor σ^S (Hengge-Aronis 2002). Other factors like high cell density, temperature, pH shifts or high osmolarity, which do not necessarily cause growth arrest, also increase levels of σ^S (Hengge-Aronis 2002). Thus, a plethora of different stresses trigger up-regulation of σ^S (Fig 3). σ^S programmed RNAP directs transcription of genes and operons involved in protection from e.g., oxidative stress (Sak et al. 1989; Eisenstark et al. 1996), acidic stress (De Biase et al. 1999), osmotic stress (Hengge-Aronis et al. 1991; Kandror et al. 2002), DNA damage ((Merrikh et al. 2009a; Merrikh et al. 2009b) and **paper IV**) and plays a role in quorum sensing and virulence (Suh et al. 1999; Schuster et al. 2004) Not surprisingly, transcriptional genome-wide profiling has now demonstrated a large fraction (up to 10%) of the bacterial genome to be regulated by σ^S programmed RNAP, either by a direct or indirect mechanism (Patten et al. 2004; Weber et al. 2005). Further, a large fraction of the σ^S -controlled genes were found to be involved in energy metabolism (Weber et al. 2005). A more detailed study found that many metabolic genes were regulated both during growth and during entry of stationary phase (Rahman et al. 2006; Flores et al. 2008), indicating a role of σ^S not exclusively under sub-optimal growth conditions, where it is clearly up regulated. For example, the TCA cycle and parts of the pentose phosphate pathway were shown to be up-regulated in a *rpoS* mutant during growth and the strain excreted high amounts of acetate, which later could not be utilized (Rahman et al. 2006). In addition, it has been shown that an *rpoS* mutant displays an impaired ability to efficiently grow and metabolize carbon during carbon source limiting conditions (Lacour and Landini 2004).

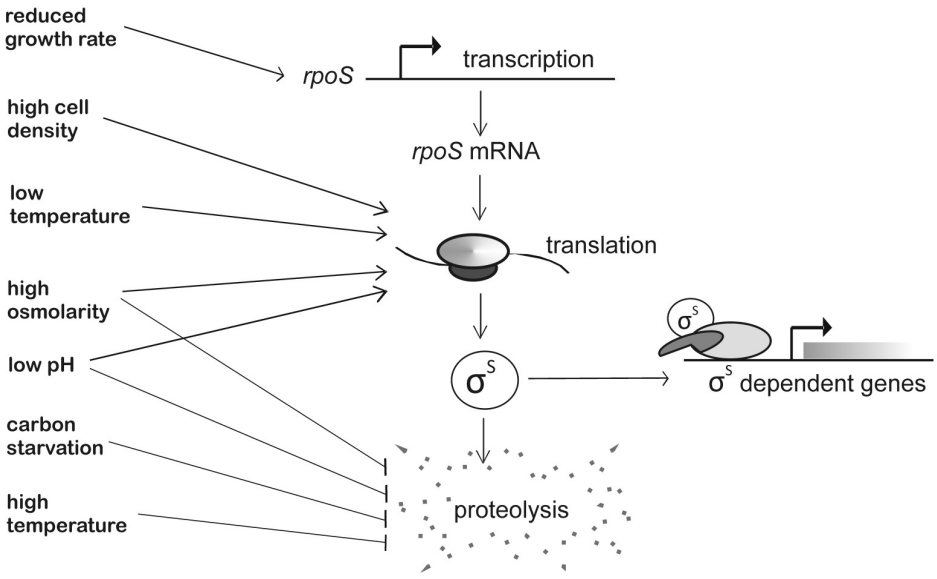

Figure 3. Summary of the regulation of σ^S. Multiple endogenous and exogenous stress conditions influence the cellular σ^S level. Elevated levels of σ^S can be obtained by increased transcription and/or translation or by inhibition of proteolysis. Adapted from (Hengge-Aronis 2002).

5.5.1. RpoS regulation

Regulation of the stationary phase sigma factor σ^S is complex and is executed at all levels: transcription, *rpoS* mRNA translation and σ^S protein stability, controlled both by *cis*-regulatory regions and *trans*-acting regulatory factors. Furthermore, it appears that different environmental signals affect regulation differently (Fig 3). Despite the extensive regulation control at all levels, altered regulation of stability of the protein is the major step affecting the total level of σ^S (Zgurskaya et al. 1997).

5.5.1.1. Transcriptional regulation

During entry into stationary phase, a 2 to 3-fold increase of *rpoS* transcript can be observed, transcribed from P*rpoS* (Lange et al. 1995; Zgurskaya et al. 1997). This promoter is located within the *nlpD* gene and is growth phase regulated (McCann et al. 1993; Takayanagi et al. 1994; Lange et al. 1995). The resulting transcript has an unusually long untranslated 5´region of 567nt, which is involved in hairpin formation and posttranscriptional regulation by occluding and inhibiting access to the ribosomal binding site ((Hengge-Aronis 2002; Majdalani et al. 2002) and references therein).

Negative control of *rpoS* transcription, exerted via the phosphorylation protein *crr* (IIA^{Glc}) suggests a possible link between *rpoS* and carbon metabolism (Ueguchi et al. 2001). This mechanism is most likely not direct; instead the signal goes through cAMP-CRP regulation of *rpoS*. Two putative cAMP-CRP sites are located in the *rpoS* promoter region. It has been reported that strains unable to make cAMP (Δ*cya*) are affected in transcription from P*rpoS*, but with inconclusive results since both positive and negative regulation of *rpoS* was reported (Lange and Hengge-Aronis 1991; McCann et al. 1993). However, in support of the negative regulation, addition of external cAMP to a *cya* mutant repressed expression of *rpoS* (Lange and Hengge-Aronis 1991b). Similarly, overproduction of CdpA leads to an increase of σ^S levels in wild type cells (though not to Δ*cya* or Δ*crp* levels) (Barth et al. 2009). CpdA is a phosphodiesterase which hydrolyses cAMP (Imamura et al. 1996). Addition of cAMP significantly improved the growth rate compared to the severely affected *cya* mutant (Lange and Hengge-Aronis 1991b). This poor growth rate in *cya* mutants complicates interpretation of these results since it is known that RpoS levels is increased in slow growing cells (Zgurskaya et al. 1997; Teich et al. 1999) perhaps via regulation by ppGpp.

The alarmone ppGpp acts as a positive signal in *rpoS* transcription (Gentry et al. 1993; Lange et al. 1995) as well as transcription of σ^S dependent genes (Kvint et al. 2000a). Later it was suggested that ppGpp does not stimulate transcription, instead the rate of translation is positively affected by accumulation of ppGpp (Brown et al. 2002). Induction of RpoS was also shown to be affected by DksA, a protein thought to enhance the effect of ppGpp (Brown et al. 2002).

5.5.1.2. Translational regulation and sRNA's

Small regulatory RNAs can modify the activity of proteins, affect the stability and regulate transcription from mRNAs. At least three sRNAs, DsrA, RprA and OxyS, regulate RpoS translation. Together with the RNA chaperone Hfq, these RNAs base pair with *rpoS* mRNA thereby influencing the secondary structure, translation and stability. Deleting Hfq (HF-I) severely reduces σ^S levels (Muffler et al. 1996). Under oxidative stress, the small RNA OxyS represses RpoS translation. OxyS binds Hfq and might thereby alter its activity (Zhang et al. 1998). DsrA and RprA both basepair with a 5´upstream antisense element of *rpoS* RNA, thereby facilitating ribosome binding and positively regulating translation (Sledjeski et al.

2001; Majdalani et al. 2002). Furthermore, the histone like protein, H-NS, is a nucleoid-associated protein capable of binding *rpoS* mRNA and negatively regulating translation efficiency and σ^S stability (Barth et al. 1995; Yamashino et al. 1995; Brescia et al. 2004).

5.5.1.3. Protein stability

During exponential growth, when nutrients are abundant, σ^S levels are kept low. This is mainly due to rapid turnover (with a half-life of about 2 min) by the action of RssB and the protease ClpXP (Schweder et al. 1996). Entering into stationary phase or upon some stress treatments, the σ^S stability increases ~10 fold (e.g. (Lange and Hengge-Aronis 1994)). ClpXP is incapable of directly recognizing and degrading RpoS. It is assisted the adaptor protein RssB (also called SprE), which binds and delivers RpoS to ClpXP (Muffler et al. 1996a; Pratt and Silhavy 1996; Klauck et al. 2001; Zhou et al. 2001). During the process of degrading σ^S, RssB is released from the protease and recycled (Fig 4). RssB belongs to the two-component response regulators and for efficient signal transduction becomes phosphorylated at Asp58 (Bouche et al. 1998). The donor of the phosphate group and its regulation is unknown which makes RssB an orphan response regulator without any specific kinase protein. However, like many other response regulators, RssB is phosphorylated by acetyl phosphate *in vitro* (Bouche et al. 1998). Deletion of *ackA-pta* (rendering the cells acetyl phosphate free) increases the half-life of RpoS *in vivo* significantly (Bouche et al. 1998), indicating that RssB phosphorylation can be achieved by this mechanism. ArcAB, a two component system, has been shown to be capable of regulating RpoS, both by modulating the degradation of RpoS as a phosphodonor to $RssB^{D58}$ (via ArcB) and to bind and repress transcription at *rpoS*p1 (ArcA) (Mika and Hengge 2005). By this mechanism the authors suggested the ArcAB complex regulates the levels of RpoS in response to the energy balance and redox state of the quinones in the cell.

Substitutions of the conserved phosphorylation site, Asp58 of RssB, partially stabilizes the RpoS protein during exponential growth (Becker et al. 2000), demonstrating the role of Asp58 phosphorylation for the proteolytical regulation of RpoS. However, proteolytic regulation is still carried out upon, for example, carbon and phosphate starvation, in a Asp58 mutant (Peterson et al. 2004). This suggests alternative pathways of regulating the stability of RpoS. *In vivo*, σ^S is stabilized after phosphate starvation in a IraP dependent manner, independent on RssB phosphorylation (Bougdour et al. 2006). IraP, a small anti-adaptor

protein, inhibits proteolytic degradation of σ^S by binding RssB protein during nitrogen and phosphate starvation (Bougdour et al. 2006). Similar inhibition of degradation has also been identified during magnesium starvation (IraM) and oxidative or DNA damage stress (IraD) (Bougdour et al. 2008; Merrikh et al. 2009). No such anti-adaptor protein has yet been found for carbon starved cells.

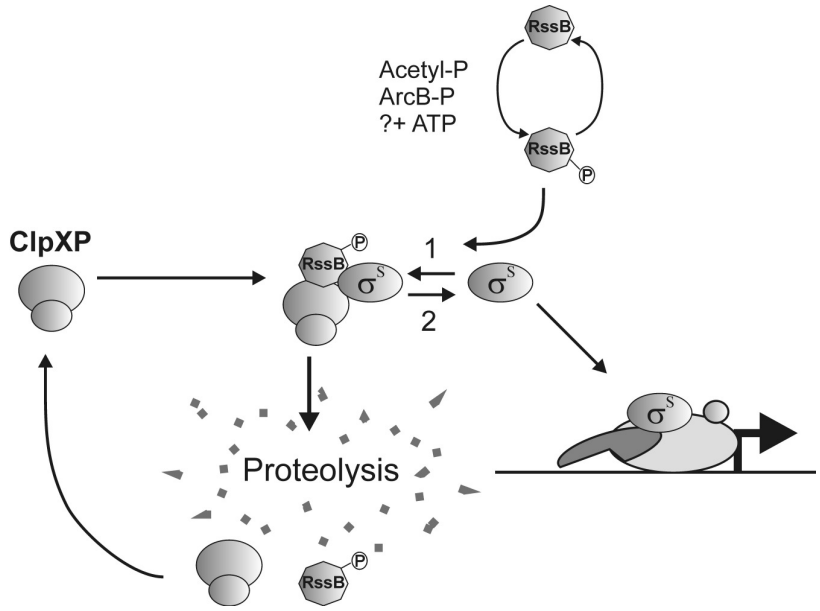

Figure 4. Post-translational regulation of σ^S. (1) During exponential phase σ^S is unfolded and degraded by ClpXP-RssB. The phosphorylated adaptor protein RssB binds to, and delivers σ^S to ClpXP for degradation. Both ClpXP and RssB are then recycled. Under starvation conditions (2) σ^S becomes stabilized, degradation is prevented and transcriptional activation of is regulon occurs.

Finally, ppGpp might also play a role in stabilizing and preventing degradation of RpoS, since σ^S competes more successfully with the house-keeping sigma, σ^{70}, for RNA polymerase when ppGpp is available (Jishage et al. 2002). The σ^S recognition site for RssB, $RpoS^{K173}$, plays a role in promoter recognition at the extended -10 region (Becker et al. 1999; Becker and Hengge-Aronis 2001). Thus, binding of σ^S to RNAP can occlude RssB dependent proteolysis. In addition, at least during phosphate starvation, transcriptional activation of IraD is ppGpp dependent (Bougdour and Gottesman 2007) and IraD induction upon starvation in LB medium is ppGpp-dependent (Merrikh 2009) indicating that ppGpp plays a role in this pathway as well.

6. Model genes and proteins

When studying global gene responses which involve regulation of many genes and proteins two complimentary approaches are often used. One is to use methods such as DNA microarrays to visualize global responses and the second is to use model genes/proteins as representatives of a class of gene/protein. This second approach allows one to study the regulation of the model gene or protein in great detail and then later assess how general this regulation is to other genes that respond to the same stimulus.

6.1. UspA and the universal stress protein family

UspA of *E. coli* is known as the paradigm protein of a superfamily that includes an ancient and conserved group of proteins not only found in the genomes of bacteria, but also in archaea, fungi, protozoa, and plants (Aravind et al. 2002; Kvint et al. 2003). The UspA domain (Pfam accession number PF00582) has been found in more than 1,000 different proteins. *E. coli* has six *usp*-family genes (*uspA, C, D, E, F and G*) and all are induced in response to stasis and stress conditions. They are all regulated by the house-keeping sigma factor, σ^{70}, and require ppGpp for induction (Gustavsson et al. 2002). Structurally they can be divided into four classes, which in most cases correspond to their function (Nachin et al. 2008). The Usp proteins of *E. coli* can form dimeric proteins and it is now evident that the Usp proteins within a class form both homodimers and heterodimers (Nachin et al. 2008). This may explain the heterogeneous functions of the individual Usp proteins (for UspA, see below). Apart from being required for stasis survival and starvation-induced stress resistance, including resistance to DNA damaging agents and oxidants (Nystrom and Neidhardt 1993; Albertson and Nystrom 1994; Kvint et al. 2003; Nachin et al. 2005), some pathogenic bacteria, e.g. *Pseudomonas aeroginosa* and *Mycobacterium tuberculosis*, require Usps to combat anaerobic energy deficiency (O'Toole and Williams 2003; Boes et al. 2006) during the persistent infections cystic fibrosis and tuberculosis. In line with the notion of Usps being important for bacterial virulence, some of the *E. coli* Usps are involved in iron homeostasis, motility and adhesion, essential features of bacterial pathogenesis (Nachin et al. 2005).

Recently, UspA-family proteins has also been shown to be required for biofilm formation and virulence in pathogenic bacteria (Chen et al. 2006; Liu et al. 2007)

6.1.1. Regulation and function of UspA

When cells enter stationary phase a drastic increase of the UspA protein occurs, easily detected on 2-D PAGE (Nystrom and Neidhardt 1992). The same pattern occurs during a large number of stress conditions, including heat shock, heavy metal exposure, oxidative and osmotic shock and starvation for carbon, DNA damage, nitrogen and phosphate (Nystrom and Neidhardt 1992; Diez et al. 2000; Gustavsson et al. 2002) and these are all the result of transcriptional activation of *uspA* (Nystrom and Neidhardt 1992). Thus, numerous conditions elicit up-regulation of *uspA*, suggesting it plays a role during these diverse conditions. Despite extensive investigations, however, no clear single function of the protein has been found. Instead a mutant of *uspA* exhibits multiple phenotypes implying a broad range of different functions including decreased survival during prolonged starvation for carbon (Nystrom and Neidhardt 1994), exposure to DNA damaging conditions (Diez et al. 2000; Gustavsson et al. 2002) and superoxide-generating compounds like H_2O_2 and PMS (Nystrom and Neidhardt 1994; Diez et al. 2000; Nachin et al. 2005) as well as an inability to efficiently respond to growth perturbation and altered utilization of carbon sources (see below).

Overproduction of UspA affects the cell physiology in several ways. Cells expressing physiological stationary phase levels of UspA, from P_{tac}-*uspA* exhibit decreased growth rate when grown in glucose minimal medium and a delay in outgrowth of stationary phase cultures (Nystrom and Neidhardt 1996). As a consequence of this overproduction, a global change in protein synthesis was observed and some proteins were shown to display altered an isoelectric point on 2-D gels suggesting a change in protein modification. UspA itself is a autophosphorylating serine and threonine phosphoprotein (Freestone et al. 1997), but it is not known whether UspA has a kinase activity and is directly involved in post-translational modification of proteins. Like overexpression of *uspA*, a mutant of *uspA* displays alterations in the pattern of protein synthesis on 2D gels and a delayed regulatory response in the synthesis of proteins during transition into stationary phase is observed when cells enter stationary phase (Nystrom and Neidhardt 1994). Thus proper levels of UspA are crucial for the cell to respond to changes in the environment. However, no further studies have been

conducted on the possible regulatory functions of UspA, even though it would have been interesting.

Mutants of *uspA* show a diauxic type of growth when grown on glucose or gluconate as carbon source (Nystrom and Neidhardt 1993). These mutants were shown to dissimilate glucose to a higher extent during growth and due to overflow metabolism excrete abnormal amounts of acetate into the media. After relief of catabolite repression (depletion of glucose), cells were able to utilize acetate as a carbon source (Nystrom and Neidhardt 1993). It should be noted, however, that this phenotype is most pronounced only in a specific strain background (JM105). Nevertheless, the result suggests that *uspA* might somehow play a role in the coupling of glucose and acetate co-metabolism. In line with this result, some of the proteins which were up-regulated after overproduction of UspA were identified and found to be involved in amino acid metabolism and the TCA cycle (Nystrom and Neidhardt 1996). In *Pseudomonas aeruginosa*, two Usp-like (37% homology to *uspA*) proteins, PA3309 and PA4352, were characterized and found to be essential for survival under anaerobic energy metabolism conditions. Mutants of PA3309 and PA4352 displayed reduced survival anearobically in the presence of pyruvate, or due to lack of an electron acceptor during shifts to anaerobic environments (Boes et al. 2006; Schreiber et al. 2006). Perhaps one function of the Usp proteins is in modulating the flow in central metabolic pathways. However, studies on a *uspA* mutant could not detect any alterations in metabolic fluxes during growth on glucose (Nanchen et al. 2008), excluding *uspA* as a general regulator under normal growth.

6.1.2. Regulation of *uspA* expression

Transcriptional expression of the *uspA* gene is positively regulated under conditions leading to starvation and is dependent on the housekeeping sigma factor, σ^{70} (Nystrom and Neidhardt 1992). During entry to stationary phase, P_{uspA} is positively regulated by the alarmone ppGpp and a $ppGpp^0$ strain (*ΔrelA, ΔspoT*) fails to induce *uspA* (Kvint et al. 2000b). Downstream of the *uspA* promoter region two operator binding sites for FadR, a regulator of the fatty acid metabolism, were identified and confirmed with footprinting analysis to be functional (Farewell et al. 1996). Conditions where FadR is inactivated significantly derepresses transcription from *uspA* in exponential phase, thus FadR exerts negative control on P_{uspA} transcription (Farewell et al. 1996). Later it was shown, however, that under conditions eliciting stringent response, the alarmone ppGpp is capable of allowing RNAP to override the

repressive effect of FadR (Kvint et al. 2000b). Thus, FadR regulation most likely only plays a role in setting *uspA* transcription levels in growing cells and when cells are using fatty acids as a carbon source. *uspA* has also been shown to be regulated under some conditions in response to DNA damage and/or cell division defects (Diez et al. 1997). A mutation in *ftsK,* encoding a DNA translocase, exhibits superinduction from P_{uspA} and this superinduction is dependent on RecA (Diez et al. 2000). However, the positive regulation of *uspA* by RecA is atypical in the sense that regulation is LexA independent and only occurs, at least under conditions tested, in conjunction with the *ftsK* mutant (Diez et al. 2000). Finally, stability of the *uspA* messenger RNA is affected by CspC and CspE, two cold shock proteins constitutively expressed at normal temperatures. Overexpression of either of these proteins stabilizes and deletions decrease the transcript (Phadtare and Inouye 2001; Phadtare et al. 2006).

Further characterization of the regulation of P_{uspA} indicates that *uspA* is also positively regulated by metabolic intermediates. Accumulation of fructose-6-p (and possibly also glucose-6-p) intermediates at the start of glycolysis, increase the induction from P_{uspA} upon entry to stationary phase due to carbon starvation (**paper I**). This was shown most dramatically in mutants that accumulate these intermediates but is also shown to play a role in stationary phase induction in wild type cells.

6.2. UspB

6.2.1. Function and regulation

Located next to *uspA* on the *E. coli* chromosome, with promoter start sites separated by 135 bp, the *uspB* gene is transcribed divergently relative to the *uspA* gene. Transcription of the *uspB* gene is induced by a large variety of stresses conditions (see below) and consequently it was named universal stress protein B (Farewell et al. 1998b). However, based on sequence homology, *uspB* is not part of the growing *uspA*-superfamily (Kvint et al. 2003). UspB protein is only found in close relatives to *E.coli*. A BLAST-P search for orthologs of the cytoplasmic domain show that UspB exists in Enterobacteriaceae, but also in *Vibrio* (Gammaproteobacteria). Interestingly most of those bacteria also have a *uspA* family member next to *uspB* and like *E.coli* divergently transcribed, suggesting that the two genes were chromosomally linked in an ancestor of these species.

Transcription from the *uspB* promoter is regulated by the stationary phase sigma factor (σ^S) and ppGpp (Farewell et al. 1998; Kvint et al. 2000a). ppGpp is required for induction of both σ^S (Gentry et al. 1993; Lange et al. 1995; Hirsch and Elliott 2002) and transcription from σ^S -dependent promoters, like *uspB* (Kvint et al. 2000a; Jishage et al. 2002). Consequently, during exponential growth in rich media transcription of *uspB* is very low and is strongly induced when the cells enter stationary phase (Farewell et al. 1998b). The same induction pattern is seen when cells are starved for glucose, nitrogen, phosphate or exposed to osmotic or oxidative stress. However, none of these conditions require functional UspB protein for cell survival (Farewell et al. 1998b).

The *uspB* gene product is a small protein (111 aa) with a protein mass of about 14kDa. The amino acid structure suggests that the protein harbors two membrane spanning sequences, located in the very end of C and N termini of the protein. The membrane spanning domains have now been confirmed and the final protein is anchored in the cytoplasmic membrane with a 65 amino acid loop facing the cytoplasm (Daley et al. 2005). Functional characterization of the protein has so far not been so successful but genetic studies have shed light on the function of UspB. One phenotype is that UspB is needed for resistance to ethanol during stationary phase (Farewell et al. 1998b). Further, during repetitive freeze thaw cycles a beneficial effect of reduced *uspB* expression for culture survival was found (Sleight et al. 2008). However, why mutation in *uspB* is favored during these conditions remain unsolved. Both ethanol and hypothermic stress lead to alterations in membrane composition toward increased membrane fluidity (Sinensky 1974; Ingram and Vreeland 1980; Hazel 1995). Ethanol, like thermal shock, also causes protein denaturation and induces the heat shock response (Bukau 1993; Gross 1996; Feder and Hofmann 1999). Thus one possibility is that *uspB* mutants not are able to properly alter their membrane composition when entering stationary phase. However, no alteration in membrane composition could be detected in an *uspB* mutant in either stationary phase or after repetitive freeze thaw cycles (Sleight et al. 2008). Similarly, no difference in survival could be observed following heat shock (Farewell et al. 1998b). At the moment its unknown how the UspB works to increase the survival following ethanol and freeze/thawing. Recent data indicates a novel functional role of UspB. A *uspB* mutant displays sensitivity towards many agents known to affect the integrity of the DNA and data suggests that the protein is taking part of the repair mechanism of such DNA lesions (**paper IV**).

7. DNA damage

The maintenance of DNA integrity is vital for the viability and to avoid a high frequency of alterations in the genome of the organism. The genome is constantly inflicted with DNA damage from endogenous sources like metabolic byproducts, misincorporation of incorrect bases during replication, and spontaneous deamination, depurination or depyrimidination, as well as various environmental factors (Friedberg 2006). Cells have evolved a battery of defense mechanisms in order to prevent maintenance of lesions leading alterations in the blueprint. Examples of this are reversal of base damage, excision repair, translesion repair, and strand break repair.

In many cases the DNA damage interferes with the ongoing replication of the DNA and a cell has to overcome this situation. Thus the processes of DNA replication and DNA repair must cooperate and work in synergy in order for the cell minimize genomic rearrangements and avoid lethal situations. Moreover, following DNA damage, fast growing organisms with multiple simultaneous rounds of replication like *E.coli* can take advantage of the multiple chromosomes as a blueprint in order to repair the DNA by non-mutagenic mechanisms.

7.1. Replication, not that smooth

E. coli, like most other prokaryotic organisms, has one circular chromosome and in order for the cell to divide and produce two daughter cells the genome must be duplicated. By initiating a new round of replication before cells have divided, thus producing multiple simultaneous rounds of replication , cells are able to divide faster than it takes for one round of replication (Helmstetter 1996). Replication is highly controlled event and is regulated at the level of initiation (Katayama and Sekimizu 1999). Initiation of replication occurs at a fixed, specific site, *oriC*. DnaA and other specific initiator proteins, bind the *oriC* region and melt a AT- rich sequence, form an open complex and prime the DNA for assembly of the components of the DNA polymerase machinery (Bramhill and Kornberg 1988). Once bound, the replisomes initiate the replication. In *E.coli*, replication is a bidirectional process, thus two replisomes are actively synthesizing the new DNA in opposite directions from *oriC*. Eventually those replisomes will meet at the terminus region (*ter*) (Messer et al. 2001).

The classical view of replication is that replication occurs in a semi-discontinuous way due to the antiparallel structure of DNA. Catalyzed by DNA polymerase, *de novo* DNA is

synthesized in a 5'- 3' direction; the leading strand is synthesized as a continuous chain and the lagging strand discontinuously in shorter (1-2kb) Okazaki fragments. Over the last decade, it has been established that the interruption of replication forks is a frequent event, and even under conditions when cells are not exposed to genotoxic compounds replication can be stalled or interrupted (Sandler and Marians 2000). *In vivo* studies where replication restart proteins, for example, PriA and PriC, have been deleted, provide evidence that replication restart is a frequent and essential mechanism for proper replication of the DNA (Sandler 2000; Gregg et al. 2002; Rangarajan et al. 2002), as those mutants display a severe decrease in replication accuracy or synthetic lethality in combination with mutant alleles of replication proteins. Furthermore, those *pri* mutants rapidly acquired suppressor mutations in the replisome assembly machinery (Sandler et al. 1996; Sandler et al. 1999), demonstrating the fundamental process of replication reloading for survival and that replication is not a continuous process from *oriC* till the *ter* site. Furthermore, various environmental perturbations can introduce DNA lesions and further inhibit DNA replication. Whether these hindrances lead to replisome stalling or collapse has been controversial and has led to many models, including polymerase switching, replication restart, template switching, overriding of the lesion and replisome reactivation (for ex (Gabbai and Marians 2010; Kowalczykowski 2000; Courcelle and Hanawalt 2003; Wang 2005; Lovett 2007; Michel et al. 2007)).

Further support of the importance of DNA repair during replication comes from findings with mutants that forms dimeric chromosomes (Steiner and Kuempel 1998). Homologous recombination during replication of the DNA, if causing an uneven number of cross-overs, results in chromosomal dimerization, which can prevent proper segregation of the genome to the daughter cells and is thought be a lethal event. To circumvent this potential demise, XerC and XerD function as site specific resolvases and specifically resolve the dimerization near the terminus of replication at a site called *dif* before cells can start divide and form the septum. Mutants with defects in *dif* or *xer* display an 15% lethality and this number is interpreted to directly correspond to dimeric genomes (Steiner and Kuempel 1998). These experiments provide strong evidence that homologous recombination is a common event during replication.

7.2. DNA damage caused by UV irradiation

UV light is probably the single most extensively used model system for understand the biological consequences of DNA damage and due to its evolutionary and environmental significance it is highly relevant (Friedberg 2006). UV light can be divided into three subcategories based on wavelength: UV-A (320 to 400nm), UV-B (295 to 320 nm) and UV-C (100 to 295 nm). Most of the research on DNA damage (including **paper IV**) is done with germicidal lamps with a peak of emission at 254nm (UV-C). At this wavelength, DNA is the main cellular chromophore, with an absorption peak at 260 nm (Friedberg 2006). Proteins do not efficiently absorb energy at this wavelength, resulting in a more specific and direct effect on the DNA. The predominant photoproducts of the DNA cause by emission of UV-C is a covalent bond formation of a four-member (C5,C6 saturation) ring structure between two adjacent pyrimidines residues, called cyclobutane pyrimidine dimers (CPDs) (Friedberg 2006). CPDs are primarily formed between two thymine residues (T<>T) but can also be formed between cytosine residues (C<>C) or thymine cytosine (T<>C). Studies on plasmid DNA reveal a ratio of 68:29:3, with T<>T the most abundant and C<>C least (Mitchell et al. 1992). Another photoproduct formed after UV-C illumination is pyrimidine-(6-4)-pyrimidone photoproducts [(6-4)PPs)], which is a linkage between C6 and C4 of two adjacent pyrimidines (Friedberg 2006). Studies of the frequency of formation between CPDs and (6-4)PPs shows that the ratio is about 3:1 in cells exposed for UV-C (Mitchell and Nairn 1989), with variation depending on the specific DNA sequence.

UV-A and UV-B also cause CPDs in bacteria, though the effect is less specific than for UV-C (Tyrrell 1973). UV-A causes oxidative DNA damage, indirectly via reactive oxygen species (ROS) or other photosensitizer molecules, most notably production of the 8-oxo-7,8-dihydroguanine (8-oxo-gua), a highly mutagenic adduct (Friedberg 2006). Those lesions are repaired by the mechanism of recognition and base excision repair by *mut* genes in combination with exonucleases (Bai and Lu 2007).

Following UV irradiation a transient arrest in DNA synthesis occurs and is resumed after some minutes (Khidhir et al. 1985; Courcelle et al. 2005). Pyrimidine dimers formed after UV irradiation can cause both gaps and double strand breaks in the DNA if not excised and repaired. However, the dimers produced after UV are not the direct cause of mutations in the DNA. Instead, ongoing replication proceeding past the lesions produces gaps. If the primary lesion is formed on the lagging strand and not is excised before the replication machinery

encounters the lesion, daughter strand gaps are formed. Left unrepaired, these daughter strand gaps can later be converted to double strand breaks, if a new replication fork encounters the daughter strand gap (Fig 5). Repair of two closely located CPDs by UvrABC (NER enzymes, see below) might also cause the DNA to dissociate (Bonura and Smith 1975a; Bonura and Smith 1975b) and thus, higher doses of UV irradiation can cause double strand breaks.

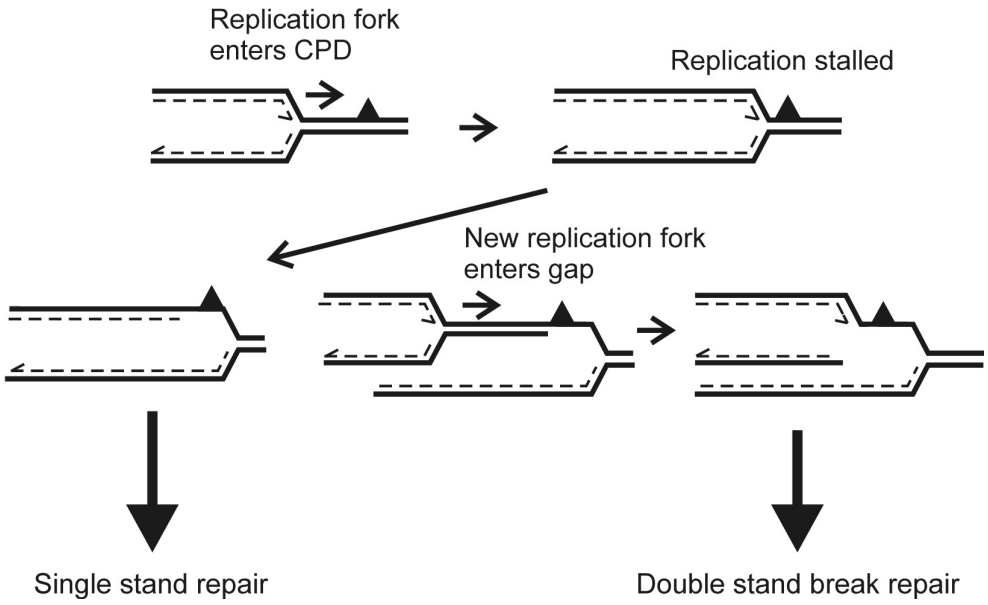

Figure 5. Mechanisms and formation of single strand junctions and double strand breaks following DNA lesions, such as CPDs (triangles). For detailed repair and processing of the DNA damage, see Fig.6. Adapted from (Ishioka et al. 1998)

Not only do the formed CPDs by UV irradiation affects the replication and repair machinery. Rapidly growing cells have a high rate of transcription and thus RNAP is transcribing the same DNA as is being replicated. RNA polymerases can potentially inhibit the repair of CPDs by blocking and physically hindering the repair proteins from access. If the RNAP is not dislocated it can further the potency the initial effect of the DNA damage. Studies of *relA, spoT* and *dksA* mutant show a severely reduced survival in different repair mutants (McGlynn and Lloyd 2000; Trautinger et al. 2005). This observed effect is most likely a consequence of a lack of destabilization effect on RNAP in these mutants, as stringent

suppressor mutations in RNAP or a hydrolyzing deficient SpoT allele could partially restore the survival (McGlynn and Lloyd 2000; Trautinger and Lloyd 2002; Trautinger et al. 2005).

7.3. UV produced lesions are recognized and excised by the NER system

In *E.coli* nucleotide excision repair is mediated by the UvrABC protein complex (Van Houten et al. 2005). UvrABC has the capacity to recognize a lesion or structural distortion in DNA like CPDs, (6-4)PPs and crosslinking with mitomycin C (Hanawalt and Haynes 1965; Hanawalt 1993). Via a multistep process these proteins recognize the adduct, make contact and make a dual incision in the DNA. Later the incised oligonucleotide is removed, DNA polymerase I fills in the gap and ligase seals the newly synthesized DNA with the parental strand (Van Houten et al. 2005; Truglio et al. 2006).

7.4. SOS response; RecA and its function

Following production of UV induced lesions, the cell repairs the DNA damage using the nucleotide excision repair system. However if the imposed load of lesions exceeds the capacity of NER, the ongoing replication fork will inevitable cause gaps or breaks in the DNA. RecA protein monomers bind and form a filament on single stranded DNA (ssDNA) and catalyze strand exchange with homologous duplex DNA (Cox 2007b; Cox 2007a). As the nucleoprotein filament is formed, the multi-functional RecA also acts as a co-protease facilitating auto-cleavage of the LexA repressor (Sassanfar and Roberts 1990; Little 1991) as well as UmuD (Pham et al. 2002). LexA protein is inactivated and UmuD is activated by this cleavage. This results in transcriptional activation of the approximately 40 SOS regulon genes (Courcelle et al. 2001) as well as the transcriptional and post-translational activation of the SOS mutagenesis translesion polymerase, DNA PolV (*umuC* of SOS regulon and UmuD') (Smith and Walker 1998; Friedberg 2006). RecA also plays a structural role by maintaining the replication fork at lesions. Filamentation of RecA has been shown to facilitate non-mutagenic repair and allows the resumption of replication (Chow and Courcelle 2007). RecA can also facilitate the bypass of DNA lesions by PolV (Pham et al. 2002; Fujii et al. 2006; Schlacher et al. 2006). Accumulation of PolV is slow (Sommer et al. 1998; Opperman et al. 1999), providing time for non-mutagenic repair of the lesions.

However, if this fails the PolV can rescue a potentially lethal event by using translesion synthesis to bypass the lesion and allowing replication to continue (Courcelle et al. 2005).

7.5. Processing of DNA breaks (RecBCD and RecFOR)

A battery of different repair mechanisms to combat the inevitable occurrence of DNA damage has been developed in the cell. Most repair processes involve the DNA binding protein RecA for recombination of the processed DNA. Cells with an inactivated RecA protein display a hypersensitive phenotype following DNA damage with severe degradation of the genome, illustrating its central role in DNA repair. Even though RecA can bind and has high affinity towards ssDNA, in many cases the DNA must first be processed before the RecA protein forms filaments and D-loops with daughter strands, thereby promoting homologous recombination. In *E.coli*, the two major mechanisms for recombinational DNA repair and processing of DNA are the RecBCD and RecFOR pathways. RecBCD is involved in double strand break repair and RecFOR in the repair of single strand breaks and gaps. However, RecFOR also has the capacity to repair DSBs in RecBC mutant carrying suppressor mutations in *sbcB* and *sbcC* (or *sbcD*) genes (Kushner et al. 1971; Lloyd and Buckman 1985) as well as under *in vitro* conditions (Handa et al. 2009). Thus, under some conditions they have overlapping functions.

Biochemically, RecR interacts with both RecF and RecO and the resulting complex(es) facilitates presynaptic formation by directing RecA nucleation onto gapped SSB-coated ssDNA (Umezu et al. 1993; Umezu and Kolodner 1994). RecF(R) recognizes the 5´ end of an ssDNA-dsDNA junction and RecRO assists the loading of RecA molecules onto ssDNA, allowing 5´-3´ assembly and replacement the SSB proteins (Morimatsu and Kowalczykowski 2003; Sakai and Cox 2009). However, *in vitro*, RecO together with RecR seem to be sufficient for mediating RecA filamentation and displacement of SSB-coated ssDNA is under those conditions even inhibited by RecF (Umezu et al. 1993; Umezu and Kolodner 1994; Sakai and Cox 2009). Moreover, overexpression of RecOR suppresses the reduced survival of a *recF* mutant after UV stress (Sandler and Clark 1994), indicating that RecF is not always essential.

However, studies conducted *in vivo* following a sub-lethal dose of UV irradiation show that the replication fork fails to recover in single mutants of any of the *recFOR* genes (Rangarajan

et al. 2002; Chow and Courcelle 2004). Thus, under natural conditions all three proteins are required for presynaptic formation as well as for protecting the DNA from degradation. In contrast to processing of synthetically produced DNA molecules under *in vitro* experiments, in *in vivo* conditions the RecFOR recombination pathway needs additional enzymes to process the DNA into a presynaptic intermediate. Both the helicase RecQ and the exonucleases RecJ are needed for processing the nascent DNA at blocked replication forks, before loading of RecFOR (Courcelle and Hanawalt 1999). In wild type cells both RecJ and RecQ are essential for non-mutagenic repair and by processing the nascent DNA are thought to serve to lengthen the substrate that can be recognized by RecFOR and RecA (Courcelle et al. 2006). In cells lacking any of the RecFOR proteins, the nascent lagging DNA strand is extensively degraded by the RecQ and RecJ proteins (Chow and Courcelle 2004; Chow and Courcelle 2007).

Genetic studies of the RecF pathway indicate that the gene products of *recF*, *recO* and *recR* belong to the same epistasic group. Single and double mutants display the same reduced survival after UV irradiation (Lloyd et al. 1988; Mahdi and Lloyd 1989) and single mutants are neither defective in conjugation nor transduction (Kuzminov 1999). RecFOR is required for proper SOS induction as a mutation in any of the genes causes a delayed response after DNA damage, followed by SOS superinduction (Whitby and Lloyd 1995). During unstressed conditions, these mutant strains display a chronic elevation of the SOS response (Whitby and Lloyd 1995) and a *recF* mutant shows an increased mutation rate as well (Southworth and Bridges 1984; Volkert 1989).

The RecBCD enzyme (Exonuclease V) is a heterotrimeric helicase/nuclease capable of processing blunt or near blunt dsDNA ends into a substrate for homologous recombination in DNA repair and during transduction as well as conjugational events (Willetts and Mount 1969; Lloyd et al. 1987). Once bound to the DNA substrate, the ATP-dependent RecBCD is capable of unwinding and degrading the DNA at a remarkable speed (up to 2.000 bp/s), making it the fastest helicase known. *In vitro* studies have shown that both RecB and RecD possess helicase activity albeit with different polarity and translocation speed. The faster RecD subunit translocates the DNA in the 5´-3´direction. The RecB protein has a 3´-5´ DNA helicase activity and a C-terminal nuclease motif which possesses all endonuclease activity of the holoenzyme (Yu et al. 1998a; Yu et al. 1998b; Dillingham et al. 2003; Taylor and Smith 2003). When bound to DNA, the RecBCD complex subunits RecB and RecD unwind and separate the duplex and the distally located nuclease motif degrades both of the DNA

strands. The endonuclease preferentially degrades the 3´-terminated end, but occasionally also degrades the 5´ end. This seemingly contradictory event, to degrade both strands, is attenuated when the complex encounters the recombinational hot spot sequence 5´-GCTGGTGG-3´, called the Chi site (χ) (Stahl and Stahl 1977; Dixon and Kowalczykowski 1995). An occupied 3´ Chi site prevents degradation of this end and favors 5´degradation. Further, Chi modified RecBCD now facilitates the loading of RecA on the 3´ ssDNA, which polymerizes in the opposite direction of the growing 3´end further facilitates the degradation of the 5´ end (Dixon and Kowalczykowski 1991; Dixon and Kowalczykowski 1995). The RecA loaded ssDNA searches for homologous DNA and by forming D-loops promotes recombinational repair.

Inactivating RecBCD function by mutations in *recB* or *recC*, causes cells to become extremely sensitive to DNA damaging agents like UV light and gamma irradiation. These mutants display poor viability and are highly reduced in homologous recombination, thus the processing and recombinational event by this enzyme is crucial for efficient recovery of the cell (Emmerson 1968; Willetts and Mount 1969; Capaldo et al. 1974). In contrast to *recB* or *recC* mutants, inactivation of RecD does not create hypersensitivity to DNA damaging agents (Lloyd et al. 1988; Lovett et al. 1988). Cells lacking the RecD subunit of the enzyme are capable of processing DSBs, albeit with reduced kinetic activity (Korangy and Julin 1994). Further, it has been shown that the RecBC(D^-) enzyme is devoid of nuclease activity, does not recognize Chi-sites and is unable to regulate RecA loading on ssDNA (Masterson et al. 1992; Churchill et al. 1999; Amundsen et al. 2000). Under some circumstances (or in some assays) a *recD* mutant displays an increased hypermutability (Biek and Cohen 1986; Thaler et al. 1989), possibly indicating the functional role the protein possess in the complex besides the helicase activity.

Considering the fundamental functions of RecBCD, the extremely low amount of RecBCD (approximately 10 molecules/cell) in the cell is somewhat surprising (Eichler and Lehman 1977; Taylor and Smith 1980). Moreover, expression of *recBCD* is not induced upon stress and overexpression studies of RecBCD even show that too much RecBCD causes an increase in genomic degradation and impaired DNA repair and homologous recombination (Dermic et al. 2005). Thus, maintaining low levels of RecBCD seems essential to the cell.

7.6. Branch migration and resolution by RuvABC and RecG

Once DNA pairing and strand exchange with the sister chromosome is accomplished with the assistance of RecA, processing of the unstable D-loop is critical. Branch migration can either dissolve the structure and stabilize it. By moving the D-loop in opposite direction of the growing RecA strand invasion, branch migration dissolves the structure. Alternatively, by extending the region and forming so called Holliday junctions the structure is stabilized. The directionality and how this is controlled is largely unknown, but in *E.coli,* RecG and RuvAB helicases have been postulated to displace RecA, catalyze the branch migration and form Holliday junctions (Sharples et al. 1999; van Gool et al. 1999; McGlynn and Lloyd 2002). Depending on the DNA lesion and DNA repair mechanism involved, the DNA structure must then either be resolved by an endonuclease enzyme or extended by forming a Holliday junction and ligated (Fig 6). The biochemical formation of the RuvAB complex and the resolution of the crystal structure have greatly facilitated the understanding of RuvAB branch migration. In short, a tetrameric form of RuvA binds specifically to Holliday junctions and makes contact with the phosphate backbone in the cross-over section, thereby serving as an identifier of Holliday junctions (Iwasaki et al. 1992; Parsons and West 1993). RuvA also targets RuvB to the junction (Parsons and West 1993). RuvB, harboring the helicase activity, forms two hexameric rings on either side of RuvA and acts as a motor of branch migration. Two divergently oriented RuvB rings translocate the DNA through RuvA by an ATP dependent pump mechanism (Stasiak et al. 1994; Yamada et al. 2004). Assisted by the RuvAB proteins, the endonuclease RuvC scans the DNA during branch migration and creates dual incisions on two strands of the same polarity (Bennett and West 1995a; Bennett and West 1995b). Endonucleatic cutting occurs most efficiently at the sequence 5´(A/T)T**T**(**G/**C) (Shah et al. 1994). Depending on how it cuts the Holliday junction, RuvC resolution results in either a patched or spliced product (Shah et al. 1994).

Mutants of any of the three genes of *ruvABC* display sensitivity and filamentous growth following exposure of DNA damaging agents, like UV-, gamma radiation and mitomycin C (Lloyd et al. 1984; Shurvinton et al. 1984; Ishioka et al. 1998). In general, double mutations of *ruv* in combination with mutant in *recF*, *recJ*, *recB* or *recC* do not display an additive effect following UV irradiation, whereas an addictive effect occurs in an *uvrA, ruv* mutant (Lloyd et al. 1984; Ryder et al. 1994). Like many other DNA repair mutant, mutations in *ruv* are only modestly affected in conjugational crosses. However, when combined with mutants involved in processing of ssDNA or dsDNA a strong synergistic effect is observed (Lloyd et

al. 1984; Lloyd 1991; Zahradka et al. 2002). Thus, RuvABC play an important role in processing of Holliday junctions following recombinational repair and homologous recombination. As mentioned above, genetic studies following UV irradiation show that a similar phenotype is caused by a *ruvC* mutation as a mutation in either *ruvA* or *ruvB*, indicating a prerequisite for all three proteins in proper Holliday junction resolving activity (Lloyd 1991). However, this does not mean that RuvAB and RuvC always work in concert and several studies have found disparate results following inactivation of either RuvABC or RuvC (for example (Lloyd 1991; Seigneur et al. 1998; Lovett 2006) and **paper IV**). In most of those cases, branch migration by RuvAB can fulfill the requirements for repair of a lesion.

RecG is a dsDNA translocase capable of promoting branch migration of DNA structures. Unlike the case with RuvAB, however, no associated resolvase for Holliday junctions has been identified and RecG has no intrinsic ability to cleave junctions (Lloyd and Sharples 1993; Sharples et al. 1994). Thus, the function of RecG in DNA repair is still under debate. Like in *ΔruvAB* mutants, survival assays after DNA damage conducted on a *ΔrecG* mutant show a modest deficiency in survival and recombination (Lloyd 1991).

Double *recG ruvAB* mutant is severely affected compared to the individual mutants and displays a synergistic effect with a hypersensitive phenotype, indicating an overlapping function of the proteins and that branch migration is essential for efficient repair following DNA damage (Lloyd 1991). Moreover, RecG can, like RuvAB, promote branch migration of four way structures and D-loops *in vitro* (Lloyd and Sharples 1993b; Lloyd and Sharples 1993a). In addition, RecG has affinity for and is capable of migrating transcriptional RNA/DNA hybrids, R-loops. However, it has been proposed that RecG, in contrast to RuvAB, frequently aborts the strand exchange formed by RecA (Whitby et al. 1993). It was recently shown that RecG inhibits PriA-dependent initiation of replication restart (iSDR) at D- and R-loops after UV stress (Rudolph et al. 2009). As a result, a *recG* mutant accumulates branched DNA structures derived from decontrolled re-initiation of replication (Rudolph et al. 2009a; Rudolph et al. 2009b), and has multiple replication forks originating from non *oriC* sites. This confirms previous findings of iSDR in *recG thyA*$^{-}$ strains and the dependence on PriA in this condition(Asai and Kogoma 1994; Masai et al. 1994).

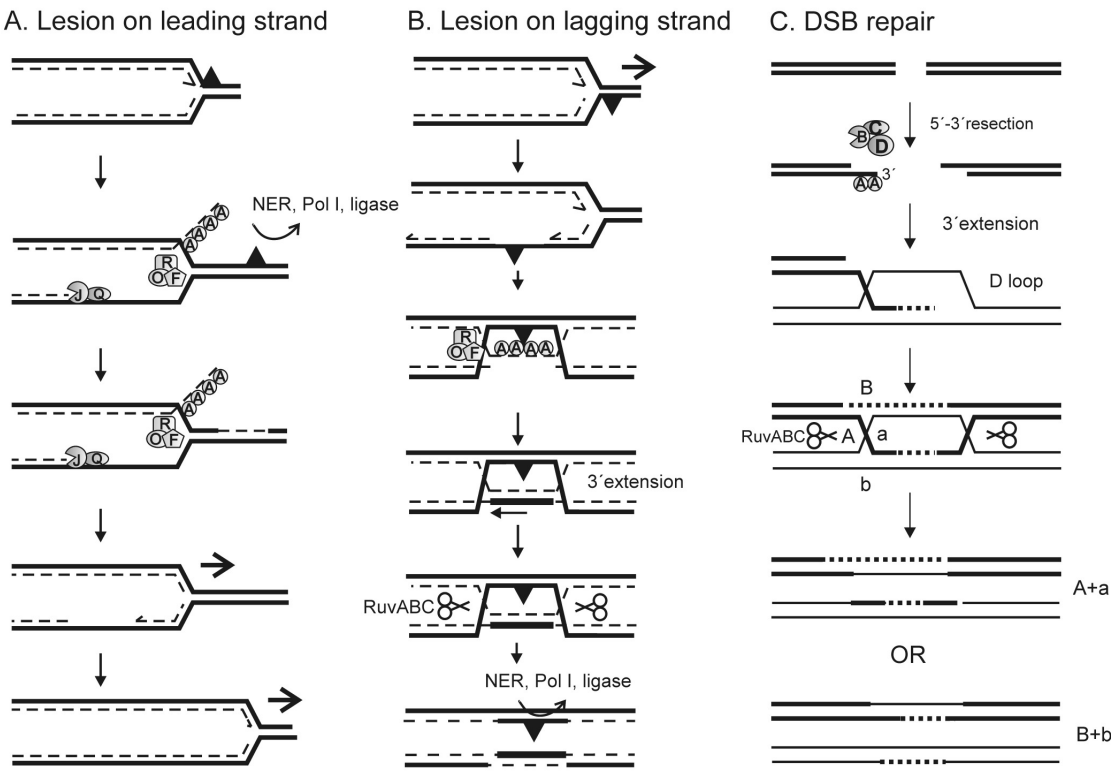

Figure 6. Models for processing of DNA lesions and repair. (A) Following CPD formation on a leading strand, as depicted here, the replication fork is arrested. RecJQ partially degrades the lagging strand and nucleotide excision repair proteins can then repair the DNA. RecFOR assists and facilitates filamentation of RecA on 3´ leading strand till the lesion is repaired and the replisome can be assembled again. (B) Following CPD formation on the lagging strand, the non-arresting lesion leaves a gap. Promoted by RecFOR, RecA-ssDNA formation and strand invasion occurs. Following 3´extension and ligation of DNA, the cross-over can be resolved by RuvABC and the lesions can be repaired by the NER system. (C) Following a double strand break, RecBCD processes the ends and forms a 3´overhang loaded with RecA. The ssDNA-RecA forms a D-loop with a homologous region of a sister chromosome. Following DNA extension and ligation, the cross-over can be resolved by RuvABC. Depending of the location of the incision the outcome is either a patched (A+a) or spliced (B+b). Models adapted from (Meddows et al. 2004; Donaldson et al. 2006).

recG mutants displays a severe filamentous phenotype after UV stress, which can partly be suppressed in a helicase deficient PriA300 allele (Rudolph et al. 2009). Thus, the predominant helicase activity of RecG is thought to inhibit PriA-mediated illegitimate replication initiation (Rudolph et al. 2009a; Rudolph et al. 2009b; Zhang et al. 2010), even though it is capable of branch migration of Holliday junctions. In addition to their role in the repair of DNA lesions via Holliday junction formation, both RuvAB and RecG have been suggested to be capable of replication fork regression of blocked forks (McGlynn et al. 2001; Michel et al. 2007). By inverting and forming a Holliday junction of the nascent DNA caused

by a stalled replication fork, followed by processing by RuvABC or a RecBCD, the cell can restore the fork via a PriA-dependent mechanism (Michel et al. 2007; Atkinson and McGlynn 2009). However, these mechanisms have been proposed to take place in various replication mutants with increased replication fork collapse (Seigneur et al. 1998; Flores et al. 2001) and might not occur under more common conditions, like UV irradiation of wild type cells (Donaldson et al. 2004; Chow and Courcelle 2007).

8. Results and discussion

8.1. Paper I: Accumulation of the glycolytic intermediate fructose-6-p serves as a positive regulatory signal to *uspA* transcription.

One of the goals of this work is to understand the regulation of stationary phase inducible genes. To this end, we screened a transposon mutant library for mutants that altered expression from our reporter construct P*uspA-lacZ* and found one mutant that showed higher expression from *PuspA*. This mutant has a mutation in the gene encoding for the glycolytic enzyme phosphoglucose isomerase (Pgi) and was shown to up-regulate the transcription of *uspA* when grown on glucose. Pgi catalyzes the first enzymatic step in the glycolysis and drive the reversible reaction glucose-6-p to fructose-6p, and is the only known enzyme doing this reaction (Fig 7A). Cells inactivated in this enzymatic step accumulate high levels of glucose-6-p when grown on glucose (Morita et al. 2003) and display a severe reduction in growth rate compared to wild type cells (Hanson and Rose 1980; Kabir and Shimizu 2003). *pgi* mutants grown on glucose are unable to feed glucose-6-phosphate into the glycolytic pathway and must use the pentose phosphate pathway as the primary route for glucose catabolism. However, the pentose phosphate pathway is incapable in converting the intermediates fast enough to avoid accumulation of glucose-6-p and sole use of this pathway also results in NADPH imbalance, both thought to affect growth rate of the cell (Canonaco et al. 2001; Hua et al. 2003; Kabir and Shimizu 2003). In order to study the regulation of *uspA* in a *pgi* mutant we first needed to find a carbon source which minimized the growth rate effect since *uspA* is growth rate regulated (Nystrom and Neidhardt 1992; Tao et al. 1999). Defined rich media with glycerol as carbon source was shown to fulfill our demands as a *pgi* mutant displayed similar growth rate as wild type. A *pgi* mutant superinduces expression from P*uspA* in this medium as it does in glucose medium, i.e., log phase expression is similar to a wild type cell but the *pgi* mutant induces expression approximately two-fold more than

wild type upon entry to stationary phase (Fig 7B). We also observed that both normal induction and superinduction of *uspA* occurred early in transition phase before the depletion of carbon.

To understand how a *pgi* mutant modulates *uspA* transcription, we first examined known regulators of *uspA*. Transcription of *uspA* is positively correlated with the levels of ppGpp and strongly dependent on it (Kvint et al. 2000b). Carbon starvation stimulates synthesis of ppGpp making this alarmone a strong candidate for the observed superinduction. However, elevated transcription of *uspA* was still observed in a *pgi* mutant even in a ppGpp^{O} background indicating that *pgi* regulation occurs independently of the stringent response. Direct measurements of ppGpp verified this observation, and even showed that the levels are lower in a *pgi* mutant compared to wild type. Thus, we concluded that the superinduction of *uspA* does not depend on ppGpp in a *pgi* mutant. Likewise, we verified that superinduction was independent of RecA/FtsK and FadR, both known to regulate *uspA* transcription (Farewell et al. 1996; Diez et al. 2000). Thus, we concluded that the metabolic block in the *pgi* mutant stimulates induction of *uspA* via a previously unknown mechanism.

A *pgi* mutant would be expected to have numerous alterations in the levels of glycolytic intermediates and next we did a series of experiments aimed at testing whether we could correlate *uspA* superinduction to any specific metabolite. A potential candidate for a signaling molecule, UDP-glucose, was previously suggested to alter transcription from σ^S dependent genes; strains with reduced levels, or deficient in synthesizing, of UPD-glucose exhibits an increased level of σ^S in growing cells (Bohringer et al. 1995). Inactivation of enzymes in the pathway for UDP-glucose synthesis, by single mutations in *pgm,* or *galU*, which convert glucose-6-p to glucose-1-p and glucose-1-p to UDP-glucose respectively (see Fig 7A) showed that the signal is unrelated to levels of UDP-glucose. By the same logic, adding small amount of glucose (0.0004%) to a *pgi* mutant (increasing production of UDP-glucose) did not alter the superinduction of P*uspA*, whereas this was previously shown to repress σ^S dependent gene expression (Bohringer et al. 1995).

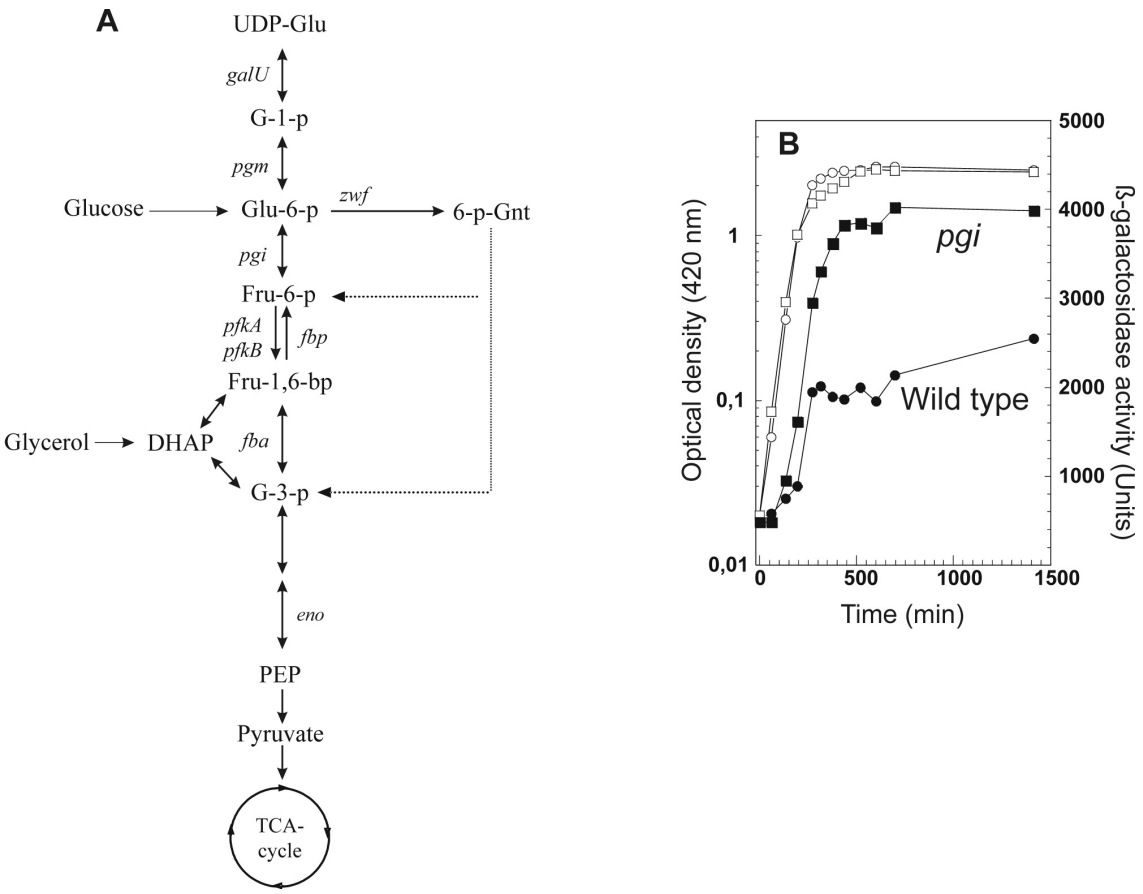

Figure 7. (A). Schematic representation of the central metabolic pathways in *E.coli*. Relevant steps in the pathway are shown and the genes encoding relevant enzymes are indicated. Abbreviations used: 6-p-Gnt, 6-phosphoglucono-δ–lactone; UDP-Glu, UDP-glucose; Glu-1-p, glucose-1-phosphate; Glu-6-p, glucose-6-phosphate; Fru-6-p, fructose-6-phosphate; Fru-1,6-phosphate; DHAP, dihydroxyacetone phosphate; G-3-p, Glycerol-3-phosphate; PEP, phosphoenolpyruvate; TCA, tricarboxylic acid. (B). Expression of *uspA* in wild type and a *pgi* mutant. Cell density (open symbols) and P*uspA-lacZ* (filled symbols) of indicated strains.

We hypothesized that accumulation of either fructose-6-p or glucose-6-p of the central glycolytic pathway functions as a positive signal for *uspA* expression. Double mutants of *pgm*, *zwf* as well as a complete block in Pfk activity were shown to increase *uspA* expression when grown on carbon sources expected to accumulate levels of glucose-6-p and fructose-6-p. Because superinduction occurred in a *pgi* mutant grown on glycerol medium, we can conclude that fructose-6-p is sufficient for superinduction, though we cannot rule out that glucose-6-p can also cause superinduction. Direct measurement of fructose-6-p showed that the timing of the superinduction of *uspA* corresponds to the accumulation of the intermediate in a strain lacking Pgi activity. To further demonstrate that the intracellular accumulations of

fructose-6-p in a *pgi* mutant stimulates transcription of *uspA*, we decided to ectopically lower the intracellular levels of this intermediate. By overproducing the main enzyme converting fructose-6-p into fructose-1,6-p, PfkA, in a *pgi* mutant the expression of *uspA* was suppressed to the levels of wild type cells.

Finally, we also show that the effect of fructose-6-p is not specific to only *uspA* nor to mutants in glycolysis. Besides *uspA*, three other σ^{70}-dependent stationary phase inducible *usp´*s exhibited superinduction in a *pgi* mutant. However, it should be noted that not every *usp* gene responded this way: *uspE* did not. Further, indications suggest that in wild type cells the intermediate plays a role in the normal induction of P*uspA* upon entry into stationary phase, as overproduction of PfkA abolished normal induction in stationary phase. These results indicate that fructose-6-p plays a signaling role in wild type cells upon entry to stationary phase and that this mechanism may be widespread.

8.2. Paper II: The levels of fructose-6-phosphate regulate σ^S-dependent transcription upon entry to stationary phase in *E. coli*

We wished to expand our understanding of the regulation of stationary phase inducible genes by fructose-6-P and chose to examine RpoS regulated genes since the regulation of this regulon is well studied. We found, that similar to the σ^{70} dependent *usp*-genes, the σ^S dependent genes *uspB* and *katE* were also shown to be superinduced in a *pgi* mutant. The transcriptional up-regulation of those genes was, like *uspA*, shown to occur during entry of stationary phase in a *pgi* mutant when grown of glycerol as a carbon source. Thus, we hypothesized that those genes are positively regulated by accumulation of fructose-6-p. Indeed, this was found to be correct. In all conditions tested when we expect accumulation of fructose-6-p (and glucose-6-p in some cases) the *uspB* gene was shown to be superinduced. Double mutations in *zwf, pgm*, but neither of these single mutants displayed superinduction of *uspB*. So did the cells mutated in *pfkAB* when grown on glucose as carbon source. Finally, titration of fructose-6-p, by overproduction of PfkA in a *pgi* background, reduced the expression of *uspB*. Overexpression of PfkA in a wild type also could reduce *uspB* expression in a wild type strain as well. These results strongly indicate that accumulation of this intermediate do affect the transcription of σ^S-dependent genes, much like the positive regulation of the σ^{70} regulated genes in **paper I**.

Since our model gene *uspB* is regulated by the alternative sigma factor σ^S (Farewell et al. 1998b), we decided to investigate if the transcriptional regulation of *uspB* was due to altered levels of the σ^S protein. By using immuno-detection of the RpoS protein, we found that the level of σ^S was elevated throughout growth in a *pgi* mutant. Intrigued by this result we measured the levels of *rpoS* by different *rpoS* construct fused to *lacZ*. These fusions are reported to reflect the different regulatory steps of *rpoS*. We found that the *pgi* mutant displayed elevated expression of all the *rpoS-lacZ* fusions in transition and stationary phase. Since the transcriptional fusion of P*rpoS-lacZ* was superinduced in a *pgi* mutant, accumulation of fructose-6-p most likely stimulates transcription from P*rpoS*. This result was confirmed by Northern blot where we found that the *rpoS* mRNA is elevated in a *pgi* mutant. A more then two fold superinduction of *rpoS* is seen when cells enter transition phase. Thus transcriptional activation of *rpoS* is positively regulated by accumulation of fructose-6-p.

8.2.1. Speculations Paper I and II

In **paper I** and **II**, we show that the general stress protein genes *uspA* and *rpoS* respond to the levels of fructose-6-p. Increased intracellular levels positively regulate the transcription of those genes, whereas diminished levels reduce the transcription. However, it is also possible that glucose-6-p acts in a similar fashion; this could not be determined in our experiments. It has been reported that both glucose-6-p and fructose-6-p, play a central role in the regulation of the uptake system of glucose. The *ptsG* mRNA is specifically degraded when the capacity of the cell to take up glucose exceeds the metabolic flow of central metabolic pathway (Kimata et al. 2001; Morita et al. 2003), i.e., when accumulation of glucose-6-p or fructose-6-p intermediates occurs. Contrary to these results, we observe an increased transcriptional level of our genes studied, and unlike *ptsG*, our model genes are affected when cells are grown on glyconeogenetic carbon sources (e.g., glycerol). Further, no degradation of *uspA* or *rpoS* mRNA could be seen on Northern blots. Thus, this makes it unlikely that *uspA* and *rpoS* are part of this same regulatory system. However, we cannot absolutely exclude a model where a repressor of *uspA* and *rpoS* is under regulation of this system.

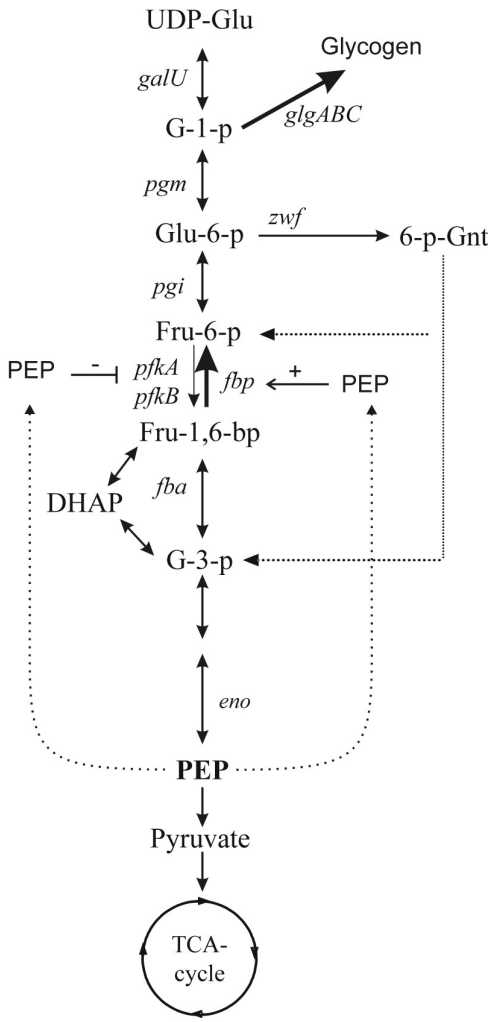

Figure 8. Glyconeogenetic redirection of the central metabolic pathway under entry to transition phase in *E.coli* wild type cell. For details see text. For abbreviations used, see Fig 7.

UspA and the RpoS regulon are both involved in promoting high viability under conditions when cells remain in stationary phase. Using intermediates as signal molecules to sense and respond to changes in the environment could be a way for the cell to coordinate and regulate its metabolic capacity. Thus, it makes sense for the cell to respond and up-regulate the expression of stress proteins using metabolic signals when nutrient become scarce. But how could fructose-6-p be a signal mechanism for starvation? A highly tentative explanation could be that this molecule accumulates normally (in a wild type cell) under condition when cells enter stationary phase at least due to carbon starvation. It is known that glycogen accumulates in stationary phase (Govons et al. 1969; Govons et al. 1973; Preiss et al. 1975). Studies have shown that the activity of Fbp increases transiently under transition phase and

simultaneously PfkA activity deceases (Fraenkel and Horecker 1965; Sabnis et al. 1995), thus a likely redirection of the central metabolism into glycogen production occurs (Fig 8). This combined altered activity of Fbp and PfkA might then lead to a transient accumulation of fructose-6-p. In support of this argument, we observed that overproduction of PfkA both in *pgi* and wild type reduces the expression of *uspA* and *rpoS*. In addition, it is known that Fbp is under positive, and PfkA under negative, allosteric regulation by PEP (Blangy et al. 1968; Hines et al. 2007). In addition, interestingly, transcriptional profiling has demonstrated that during growth on glucose in a *pgi* mutant, enolase (*eno*), the glycolytic enzyme converting 2-phophoglycerate to PEP, is one of the most induced genes (Kabir and Shimizu 2003). This might lead to an increase in PEP and also drive the accumulation of fructose-6-p under those conditions (Fig 8). Under conditions when cells are growing on glyconeogenetic carbon sources, like glycerol, the level of PEP is high, and possibly inhibits PfkA whereas Fbp is stimulated (Blangy et al. 1968; Hines et al. 2007). Thus, fructose-6-p might be a highly regulatable sensor for the cells metabolic status and thus a good choice as a regulatory signal when cells experience a change in growth conditions such as are experienced upon entry to stationary phase.

It should be noted that accumulation of the glycolytic intermediates, fructose-6-p and glucose-6-p, is for unknown reasons toxic to the cell. However, we chose growth conditions where we did not observe any growth retardation in the *pgi* mutant to avoid secondary effects. We interpreted this lack of growth rate defect as the cells not accumulating high enough levels of fructose-6-p to become toxic. Reducing sugars have the capacity to form Maillard reactions and by nonenzymatic glycosylation of proteins produce advanced glycosylation endproducts (AGE) and *in vivo* and *in vitro* studies have shown that reducing sugars have an effect on mutation rates and results in insertions and deletions in the genome (Lee and Cerami 1987; Lee and Cerami 1991; Levi and Werman 2001). Among the phosphorylated sugars, fructose-6-p was shown to be most reactive (Levi and Werman 2001; Levi and Werman 2003). Thus it is possible that observed elevated levels of *uspA* and RpoS in the *pgi* mutant is a consequence of either oxidative protein or DNA damage, both conditions know to cause transcriptional activation of *uspA* and stabilization of RpoS ((Nystrom and Neidhardt 1992; Diez et al. 2000; Merrikh et al. 2009a) and **paper III**), caused by the reducing sugars. However, preliminary experiments could not detect any alteration in carbonylation levels (a measure of oxidative damage) in crude extracts of total protein of a *pgi* mutant following growth on glycerol (unpublished).

8.3. Paper III: Decline in ribosomal fidelity contributes to the accumulation and stabilization of the master stress response regulator σ^S upon carbon starvation

Cessation of growth has in numerous studies been shown to elicit induction of the σ^S regulon (reviewed in (Navarro Llorens et al. ; Matin 1991). The main regulatory component of this regulon, the RpoS sigma factor, σ^S, redirects the transcription of genes during those conditions. RpoS itself is under a complex regulatory control and is regulated at all possible levels. A key step in this regulation is on the level of stability of σ^S and involves the control of degradation of RpoS by RssB recognition and ClpXP proteolysis. Despite extensive characterization of the regulation of this process, the signal mechanism during carbon starvation accumulation of σ^S in not fully known. Recently, it has been shown that the anti-adaptor proteins IraP, IraM and IraD can inhibit proteolytic degradation by occupying the RssB protein during various stresses (Bougdour et al. 2006; Bougdour et al. 2008; Merrikh et al. 2009b). However, no such specific anti-adaptor has been identified during RpoS stabilization during carbon starvation.

By using different alleles affected in the translational accuracy of the ribosome, we found that the stability of σ^S was affected by the accuracy of ribosomal activity during entry to and during carbon starvation. It was previously shown that an *rpsL141* ribosomal mutant, which possesses increased translational accuracy synthesizes less oxidative protein leading to the idea that either mistranslated proteins are more susceptible to oxidation, or that oxidation tags mistranslated proteins for future degradation (Dukan et al. 2000). In this work, we show that an *rpsL141* mutant also influences the levels of σ^S and σ^S -regulated genes in a negative way. The opposite result was found for a ribosomal allele with reduced proof-reading activity, *rpsD12*, i.e. it showed a positive effect on the levels of RpoS compared to wild type. The *rpsD12* allele also displayed elevated σ^S levels in exponential phase. Thus, these results indicate that the production of aberrant proteins affects the levels of RpoS.

In order to elucidate on what level the regulation of RpoS is affected, we utilized a transcriptional, transcriptional/translational and full control fusion of *rpoS* fused to the reporter *lacZ*. We found that a strong effect of the *rpsL141* allele on expression of the full length protein fusion construct of *rpoS*, but not on the other constructs. A six-fold reduction in expression of the full-length *rpoS* fusion was observed in *rpsL141* compared to wild type. This construct harbors a functional RssB recognition site for post-transcriptional regulation. We concluded that the regulation of RpoS must be on the post-translational level of σ^S. This

was confirmed by measuring the stability of RpoS. The *rpsL141* allele conferred an elevated increase in RpoS degradation following carbon starvation.

Since the protease ClpXP is involved in the controlled degradation of RssB-bound RpoS as well as the degradation of aberrant and ssrA-tagged proteins, we speculated that during carbon starvation the ClpXP protease might be occupied in the degradation of aberrant proteins. RpoS can thereby escape degradation by a titration mechanism, provided that some of the components in this regulatory circuit are limiting. Experiments with chemically induced aberrant protein synthesis supported this idea, since addition of canavanine (an amino acid analog producing misfolded proteins) had a positive effect on RpoS stability. Modulation of the levels of ClpX and ClpP also supported this theory. Inactivation of ClpX, the chaperone facilitating aberrant protein degradation by ClpP, positively influenced the stability of RpoS, both in wild type and *rpsL141*. Overproduction of the protease subunit ClpP of ClpXP destabilized RpoS and was dependent on functional RssB for the degradation of RpoS. In a similar manner, ectopic overproduction of ClpXP substrate, ssrA-tagged proteins, was shown to stabilize RpoS in an otherwise wild type background. Taken together, these results indicate that synthesis of aberrant proteins during carbon starvation may be a way for the cell to modulate the stability of the RpoS protein and contributes to the understanding of how RpoS is regulated under those conditions.

8.3.1. Speculations paper III

A fundamental property of the suggested titration model is that it is strongly dependent on the assumption that heat shock proteins have a limited capacity to degrade their substrates during the brief transition into stationary phase. Previous results have shown that the ClpX and ClpP proteins are not induced in response to starvation for carbon when entering stationary phase (Schweder et al. 1996; Mandel and Silhavy 2005). It has also been suggested that RssB is a limiting factor in regulating the degradation of RpoS and a small increase in transcription of *rpoS* could itself stabilize the RpoS protein (Pruteanu and Hengge-Aronis 2002; Hengge 2009). Thus, there are reasons to believe that any of those components could be limiting for the cell. In addition, several studies on translation fidelity have observed a reduced accuracy of protein synthesis when cells enter stationary phase (O'Farrell 1978; Barak et al. 1996; Wenthzel et al. 1998; Ballesteros et al. 2001). Based on these observations, the total load of aberrant proteins might then saturate the capacity of the protease machinery

and thereby indirectly stabilize RpoS (Fig 9). Overproduction of the protease ClpP supports this notion, as an increased degradation capacity of RpoS was observed. In line with this, occupying the ClpXP with ssrA-tagged GFP also stabilizes the RpoS protein.

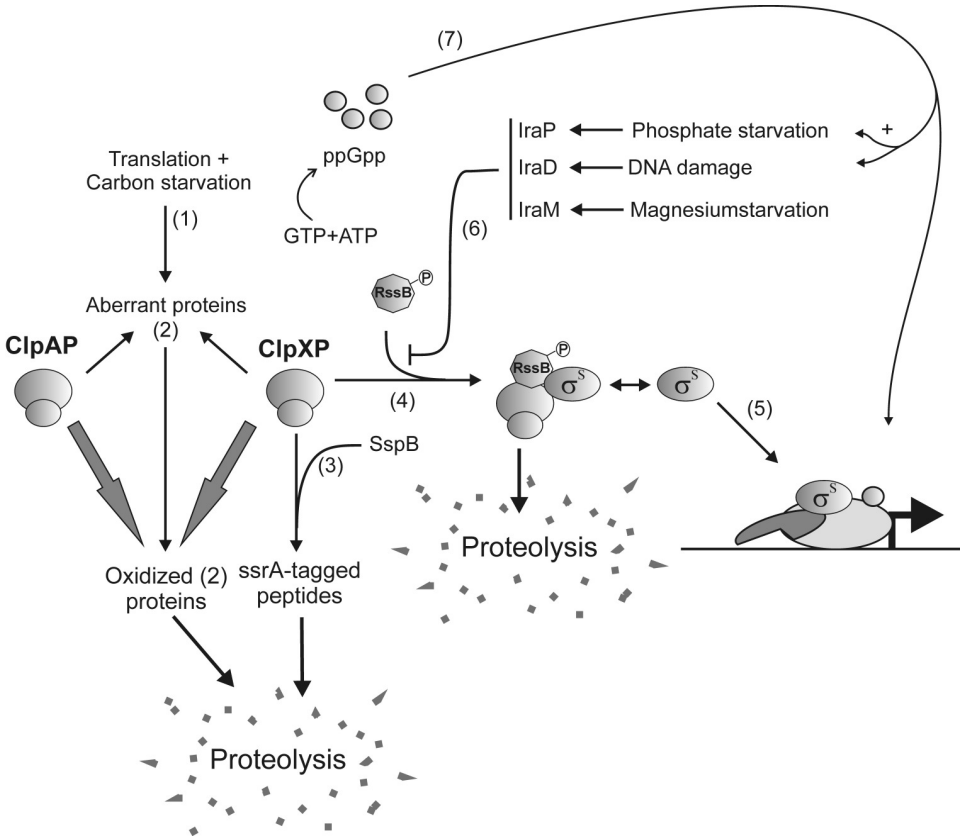

Figure 9. Schematic model of σ^S stabilization upon starvation. (1) Upon starvation an increase in translational error occurs, resulting in misfolded and mistranslated proteins. Aberrant proteins are prone to be oxidatively modified. (2) These misfolded proteins constitute substrates for, and sequester the ClpXP and ClpAP proteases. (3) In addition, ssrA-tagged peptides also compete for ClpXP as a substrate. (4) As a consequence, ClpXP is titrated and less ClpXP is available of proteolytical degradation of RssB-bound σ^S, indirectly causing the levels of σ^S to increase. (5) σ^S binds RNAP and directs it to transcribe its regulon. (6) During phosphate, magnesium and other starvation conditions, σ^S is stabilized by a mechanism where Ira proteins sequester the RssB protein and thereby prevent degradation of σ^S. (7) Accumulation of ppGpp, as an outcome of starvation, besides stimulating transcription of IraP and IraD, also increases competition of σ^S to RNAP and could help to stabilize σ^S.

Thus, by this model we propose that sudden carbon starvation can serve as a passive mechanism for modulating σ^S-stability, not by affecting the RssB factor, but by acting at the level of the ClpXP protease (Fig 9). In support of this model, inactivation of the DnaK heat-shock chaperone results in diminished levels of σ^S, as *dnaK* cells have an increased level of

aberrant proteins which occupy the ClpXP protease (Rockabrand et al. 1998). Following the stabilization of σ^S under these conditions, genes of its regulon like *katE* and *dps* are up-regulated and are involved in resistance towards oxidative stress (Sak et al. 1989; Altuvia et al. 1994). By up-regulating these genes before cells enter stationary phase, the organism can prevent further oxidative attack in the cell. This might prevent cumulative oxidative protein damage. Thereby, superfluous activities from the ATP-dependent ClpXP protease and chaperones are prevented.

8.4. Paper IV: Characterization of a *uspB* mutant indicates that the phenotype is linked to DNA damage

As previously mentioned, UspB is known to be involved in two processes. Firstly, it plays a role in resistance to ethanol since a *uspB* mutant in stationary phase dies upon exposure to ethanol. Secondly, UspB has a negative effect on survival after repeated freeze thawing. Both these stresses affect the cytoplasmic membrane where UspB is anchored. Thus, we decided to test if UspB was important for membrane structure and function. We compared survival of a *uspB* mutant to an otherwise isogenic wild type strain during exposure to compounds known to affect the membrane, but found no differences (unpublished). However, when we exposed a *uspB* mutant to the DNA damaging agent mitomycin C a reduced survival was found.

Further characterization following exposure of genotoxic agents showed that a *uspB* mutant was sensitive to UV irradiation, ciprofloxacin and bleomycin. This phenotype indicates a functional role of the protein in DNA repair or resistance towards such compounds. In order to test if the phenotype observed following UV irradiation is specific to CPD formation in DNA in the *uspB* mutant, we analyzed the cells for enzymatic photoreactivation and levels of CPDs. A *uspB* mutant incubated in light showed an almost complete suppression of the sensitive phenotype following UV irradiation. Thus, the enzyme DNA photolyase, which utilizes visible light for reversion of the dimers (Friedberg 2006), is sufficient to reverse the effect of UV on a *uspB* mutant and its' sensitivity is specifically caused by CPDs and not spurious effects like oxidative stress. A similar level of CPDs, measured with the TDH-2 antibodies, were found to be formed in *uspB* and wild type cells directly after UV treatment. Following post-UV incubation both *uspB* and wild type repaired lesions with the same kinetics indicating that NER is not affected by a *uspB* mutation.

The genotoxic compounds used for characterization of a *uspB* mutant all have the potential effect of causing a single or double strand break as well obstruction of ongoing replication. Bleomycin causes a direct break of DNA and the other agents cause breaks in an indirect manner. We thus focused our attention on recombinational repair pathways and started by comparing survival following UV irradiation of mutants of known repair pathways with *uspB* and double mutants thereof. Inactivation of the RecF pathway genes *recF* and *recR* in combination with a *uspB* mutation showed small additive effect with regards to survival. A stronger additive effect was observed when the DNA processing proteins of the RecF pathway, *recJ* an *recQ* was deleted, suggesting that UspB does not take part of this pathway. However, mutants in the double strand break pathway, either *recB* or *recC,* in combination with a *uspB* mutation showed no additive effect on sensitivity following UV irradiation. The same results were found for mutants involved in Holliday junction resolvation, the *ruvABC* genes, i.e. no additive phenotype in combination with a *uspB* mutant. These results encouraged us to focus on these pathways. We found that a *uspB* mutant always showed the same phenotype as a *ruvC* mutant following exposure to different genotoxic chemicals. Moreover, a *uspB* mutant displayed a chronic two fold elevation of SOS response and reduced efficiency in Hfr conjugation. Similar results have been reported for *ruvC* (Lloyd 1991; Asai and Kogoma 1994). Thus, several experiments indicate that UspB might work in the same pathway as RuvC. Besides genetic evidence of that UspB is involved in the repair of DNA lesion, we wished to demonstrate it in a more direct way by physical methods. Results from the Courcelle laboratory has demonstrated that a *ruvC* mutant exhibits problems in resolving Holliday junction intermediates following replication of UV irradiated cells (Donaldson et al. 2006). By using the same two-dimensional agarose technique, we found that a *uspB* mutant, much like *ruvC*, exhibits problems in resolving the intermediates. Cells deleted for *uspB* were shown to display an increased level of unresolved intermediates following UV irradiation and a delay in repair of those.

Finally, we showed that expression of an ectopic copy of *ruvC* could suppress the UV sensitivity of a *uspB* mutant. The opposite was not true; *uspB* overexpression could not complement a *ruvC* mutant. These results suggest that UspB functions upstream of RuvC. Possibly UspB could either alter the transcription of RuvC or affect its function. By fusion studies of *ruvC* and Q-PCR, we showed that UspB does not affect the transcriptional or translational levels of *ruvC*. Thus, UspB might affect the function of RuvC at the protein level. We also checked whether *uspB* itself is regulated by DNA damage and found that it is

not. During the course of studies we also found that a *recD* mutant suppressed the sensitive phenotype of both a *uspB* and *ruvC* mutation. Further characterization demonstrated that it was only those mutants which were suppressed by Δ*recD*; a double mutant of either *ruvB* or *ruvABC* or a *recG* mutant in combination with *recD* was not suppressed.

8.5. Speculations paper IV

Our results in **paper IV** suggest that the UspB protein is required to maintain the integrity of the DNA in both unstressed conditions as well as under conditions following DNA damage. Several assays indicate that UspB has a functional role in DNA repair mechanisms when the cellular pathways for recombinational crossover and the subsequent resolution of Holliday junctions are active. The biochemical and genetic data further suggests that UspB is involved in resolution of Holliday junctions. Still, it is hard to imagine how a membrane protein could be directly involved in DNA repair mechanisms, especially since no single study has demonstrated that the mechanism of DNA repair involves the cytoplasmic membrane. The most plausible function for UspB is that it alters the activity of RuvC. However, several *in vitro* assays demonstrates that the resolvase RuvC and its two associated proteins RuvA and RuvB are sufficient for Holliday junction resolution (Eggleston et al. 1997; van Gool et al. 1998; Zerbib et al. 1998), making it hard to envisage a direct function of UspB for the processes of branch migration and resolvation. Unlike *ruvAB*, the *ruvC* gene is not part of the inducible SOS response regulon and is expressed at low levels ((Shinagawa et al. 1988; Sharples and Lloyd 1991; West 1997) **paper IV**). Estimates are that there are about 50 dimers of RuvC per cell (West 1997), thus a small functional alteration of the protein could influence its activity. One mechanism could be that UspB sequesters an unknown allosteric repressor for RuvC and thereby alters its activity *in vivo*. Overexpression data of RuvC in a *uspB* mutant background support this theory, since high concentrations of RuvC protein suppresses the *uspB* phenotype. Alternatively, UspB could interact directly, or indirectly via another protein, with RuvC and thereby facilitate its function.

uspB is part of the σ^S regulon and thus becomes induced when entering stationary phase. However, the levels of UspB produced under exponential phase are crucial for proper survival and repair of DNA damage under these conditions. The induction pattern, though, indicates that under non-proliferating conditions, e.g. stationary phase, increased function of UspB is desirable. Under these non-proliferative conditions about 20% of the genome is

broken down and resynthesized within a day (Tang et al. 1979) demonstrating that the stationary phase not is a complete passive, dormant state on the DNA level. Several genes involved in DNA repair and metabolism are under control of RpoS (Villarroya et al. 1998; Saint-Ruf et al. 2004), and it has been shown that SOS response is up-regulated (Taddei et al. 1995) further indicating that DNA metabolism is active during stationary phase. However, during stationary phase when the nutrients are scarce, repair of lethal lesions on DNA like single stand and double strand breaks are fundamental for survival. UspB could under these conditions, by its function in facilitating resolution by RuvC, thereby mitigate the stress. During these conditions perfect restoration of the genome might not be crucial, exemplified by the induction of the *dinB* gene, encoding the error prone DNA polymerase IV, during stationary phase (Layton and Foster 2003). Translesion synthesis by Pol IV is capable of bypassing lesions, exhibits reduced fidelity and thus causes increased mutagenesis in stationary phase, at leased measured as F´*lac* reversions (Layton and Foster 2003; Galhardo et al. 2009). During this mutagenic repair the processing of DNA is dependent on RecBCD as well and RuvABC (Foster et al. 1996; Harris et al. 1996; He et al. 2006). UspB could secure and increase the efficiency of recombinational repair of such mechanisms, especially since the reversion is dependent on functional RuvABC.

9. Concluding remarks

To properly adapt to alterations in the environment is a fundamental process for the cell in order to maintain viability and survive. In its natural habitat bacteria are seldom provided with optimal nutritional conditions and non-differentiating bacteria like *E.coli* can respond to nutrient deprivation by entering stationary phase. How cells sense and respond to the impending nutrient deprivation in order to adapt to the new requirements is complex and involves multiple signaling systems. Besides the effect of stringent control, by ppGpp, a major effect on the molecular level is modulated by increasing levels of σ^S, the stationary phase sigma factor. Both σ^S and ppGpp contribute to the redirection and expression of genes involved in, for example, maintenance metabolism and general stress resistance, motility and altered morphology (Lange and Hengge-Aronis 1991b; Lange and Hengge-Aronis 1991a; Weber et al. 2005; Magnusson et al. 2007). However, the sensing-signal mechanism for adapting to and the expression of genes during entry into stationary phase is less well characterized. Sensing and responding to the intracellular metabolic intermediates, which

could reflect the extracellular environment contributes to the overall regulatory effect for stationary phase genes, like the Universal stress protein genes, *usp´*s, and the σ^S regulon (**paper I** and **II**). This mechanism appears to operate before the cells completely run out of carbon and further work is need to determine how the cells sense this dwindling level of carbon. In a similar sensing-signaling mechanism, the cell monitors the status of the erroneous protein synthesis (due to e.g. nutrient deprivation) and is capable of transmitting the information to σ^S (**paper III**). Together these two systems allow the cell to express stationary phase genes and increase survival.

A novel function for UspB was discovered (**paper IV**). Observations indicate that the UspB protein is involved in the late process of DNA damage repair during resolution of DNA strand exchange. This DNA damage sensitive phenotype was observable in both stationary phase conditions, where the UspB protein is highly expressed, and in exponential phase, where UspB levels are low, indicating that the function of UspB is critical for proper DNA repair under all growth conditions. The elevated levels of UspB and other stationary phase inducible genes involved in DNA damage repair like DNA polymerase IV (*dinB*) (Layton and Foster 2003), β subunit of DNA polymerase III (*dnaN*) (Villarroya et al. 1998) and Exo III, abasic endonuclease and 3' exonucleases (*xth*) (Sak et al. 1989) indicates that DNA damage may be a common problem in stationary phase. Further studies will need to explore and characterize the functional role of UspB under stationary phase conditions and determine how UspB exerts its effect in the cell in more detail.

10. Acknowledgments

First of all I would like to express my gratitude towards my supervisor Anne, and to Thomas, for an inspiring atmosphere and letting me know about the field of bacterial genetics. Your vast knowledge about *E.coli* and molecular microbiology is truly impressive and stimulating.

I'm grateful for the opportunity to have had you, Anne, for my supervisor. From the very first start, when attending your lectures, I was impressed the way you can explain difficult things in an understandable way. A rare skill, but you possess it. Your patience in managing such a stubborn creature as me is impressive. Your warm personality and capability to fix problems, major or minor of mine, is also extraordinary.

Thomas, for bearing with me under all difficult times and never giving up faith. You kept me running for the extra time I required to finish this thesis. Thank you so much, without your generous help it would not have been possible.

I also want to thank present and past members of the group. Together you all make the lab such great place to be. It's a true joy spending time at this lab. My former office mate Beidong, for your subtle humor and caring personality. And for taking command over the drinking events, (it was you, right?). Laurence, my (almost exclusive) companion regarding Facebook-matters. And for making me aware that growth curves is not the only way to study bacterial genetics!! Bertil, for letting me defeat you and end up as the Bowling Champion of the lab, at least once. Malin H, you need big improvements regarding the 9 ball-rules! Lisa M, for (totally unaware?) introducing me to the world of MTB. This, by letting me haul a container-sized trunk in the middle of the overcrowded streets of Manhattan. Micke, your enthusiasm for everything and easy-going character is truly inspiring. Åsa, simply for you being you (It's positive!). Kristian, for your ability to make an ordinary conversation… more! Antonio, I miss you and your great reluctant humor over here. Ana, for being such a warm and fun person. I'll miss the lunch conversations. Xin-Xin, for your excellent cooking and nice warm personality. Veronica, your remarkable energy concerning everything. Junsheng, your humble approach to everything. Manu, for making the lab a more interesting place. David, the impressing elbow grease task in reporting about all bad movies seen during the weekends. Alfredo, for ground breaking studies of how to replica plate!! Martin, the high capacity man with a big portion of humor. Elin K-J, for being a positive and happy person and the small chats in the corridor. Lisa L, for a friendly personality and nice pastries.

I also want to thank the numerous 10-90 point students, who have struggled and made the initial studies on different projects.

Göteborgs Kex, for ruining my economy on cookies, but providing me with energy for writing a thesis and “transfetter” enough for a life time.

My family, the Perssons; Mamma and Pappa for giving me the basic interest in biology from the very beginning, like “Mulle-skolan”. Dad, I hope to see you more from now on. And my brother Frank for the pioneering and pawing work for me in life!

LVG, for putting me on course again. Thank you all.

My second family, the Forsmarks, for the open and welcoming atmosphere.

Agneta for finding and sorting out the mysterious world of economy and invoice-matters. Invaluable!

Administrational staff, especially Ann, Helena; you are always so helpful and professional. And rarely did I leave your office without a smile.

Lars and Bruno for ctlr-alt-del related matter.

My **Annabelle**, for enriching my life much, much, much more. This, despite your cunning habit of frequently opening (and almost finishing) the yummy box of cereals (which I’ve been looking forward to for weeks) before finishing the plain, boring ones (which I respectfully consume)! Your otherwise altruistic approach to humankind and to life, it rules.

11. References

Albertson, N.H. and Nystrom, T. 1994. Effects of starvation for exogenous carbon on functional mRNA stability and rate of peptide chain elongation in Escherichia coli. *FEMS Microbiol Lett* **117**(2): 181-187.

Altuvia, S., Almiron, M., Huisman, G., Kolter, R., and Storz, G. 1994. The dps promoter is activated by OxyR during growth and by IHF and sigma S in stationary phase. *Mol Microbiol* **13**(2): 265-272.

Asai, T. and Kogoma, T. 1994. Roles of ruvA, ruvC and recG gene functions in normal and DNA damage-inducible replication of the Escherichia coli chromosome. *Genetics* **137**(4): 895-902.

Bai, H. and Lu, A.L. 2007. Physical and functional interactions between Escherichia coli MutY glycosylase and mismatch repair protein MutS. *J Bacteriol* **189**(3): 902-910.

Ballesteros, M., Fredriksson, A., Henriksson, J., and Nystrom, T. 2001. Bacterial senescence: protein oxidation in non-proliferating cells is dictated by the accuracy of the ribosomes. *EMBO J* **20**(18): 5280-5289.

Barak, Z., Gallant, J., Lindsley, D., Kwieciszewki, B., and Heidel, D. 1996. Enhanced ribosome frameshifting in stationary phase cells. *J Mol Biol* **263**(2): 140-148.

Barth, E., Gora, K.V., Gebendorfer, K.M., Settele, F., Jakob, U., and Winter, J. 2009. Interplay of cellular cAMP levels, {sigma}S activity and oxidative stress resistance in Escherichia coli. *Microbiology* **155**(Pt 5): 1680-1689.

Battesti, A. and Bouveret, E. 2006. Acyl carrier protein/SpoT interaction, the switch linking SpoT-dependent stress response to fatty acid metabolism. *Mol Microbiol* **62**(4): 1048-1063.

Becker, G., Klauck, E., and Hengge-Aronis, R. 2000. The response regulator RssB, a recognition factor for sigmaS proteolysis in Escherichia coli, can act like an anti-sigmaS factor. *Mol Microbiol* **35**(3): 657-666.

Bettenbrock, K., Sauter, T., Jahreis, K., Kremling, A., Lengeler, J.W., and Gilles, E.D. 2007. Correlation between growth rates, EIIACrr phosphorylation, and intracellular cyclic AMP levels in Escherichia coli K-12. *J Bacteriol* **189**(19): 6891-6900.

Biek, D.P. and Cohen, S.N. 1986. Identification and characterization of recD, a gene affecting plasmid maintenance and recombination in Escherichia coli. *J Bacteriol* **167**(2): 594-603.

Blangy, D., Buc, H., and Monod, J. 1968. Kinetics of the allosteric interactions of phosphofructokinase from Escherichia coli. *J Mol Biol* **31**(1): 13-35.

Bohringer, J., Fischer, D., Mosler, G., and Hengge-Aronis, R. 1995. UDP-glucose is a potential intracellular signal molecule in the control of expression of sigma S and sigma S-dependent genes in Escherichia coli. *J Bacteriol* **177**(2): 413-422.

Bouche, S., Klauck, E., Fischer, D., Lucassen, M., Jung, K., and Hengge-Aronis, R. 1998. Regulation of RssB-dependent proteolysis in Escherichia coli: a role for acetyl phosphate in a response regulator-controlled process. *Mol Microbiol* **27**(4): 787-795.

Bougdour, A., Cunning, C., Baptiste, P.J., Elliott, T., and Gottesman, S. 2008. Multiple pathways for regulation of sigmaS (RpoS) stability in Escherichia coli via the action of multiple anti-adaptors. *Mol Microbiol* **68**(2): 298-313.

Bougdour, A. and Gottesman, S. 2007. ppGpp regulation of RpoS degradation via anti-adaptor protein IraP. *Proc Natl Acad Sci U S A* **104**(31): 12896-12901.

Bougdour, A., Wickner, S., and Gottesman, S. 2006. Modulating RssB activity: IraP, a novel regulator of sigma(S) stability in Escherichia coli. *Genes Dev* **20**(7): 884-897.

Bramhill, D. and Kornberg, A. 1988. A model for initiation at origins of DNA replication. *Cell* **54**(7): 915-918.

Brown, L., Gentry, D., Elliott, T., and Cashel, M. 2002. DksA affects ppGpp induction of RpoS at a translational level. *J Bacteriol* **184**(16): 4455-4465.

Busby, S. and Ebright, R.H. 1999. Transcription activation by catabolite activator protein (CAP). *J Mol Biol* **293**(2): 199-213.

Canonaco, F., Hess, T.A., Heri, S., Wang, T., Szyperski, T., and Sauer, U. 2001. Metabolic flux response to phosphoglucose isomerase knock-out in Escherichia coli and impact of overexpression of the soluble transhydrogenase UdhA. *FEMS Microbiology Letters* **204**(2): 247-252.

Cashel, M. 1969. The control of ribonucleic acid synthesis in Escherichia coli. IV. Relevance of unusual phosphorylated compounds from amino acid-starved stringent strains. *J Biol Chem* **244**(12): 3133-3141.

Cashel, M.G., D.R. Hernandes, V.J. Vinella D. 1996. The stringent response. in *Escherichia and Salmonella: Cellular and molecular biology* (ed. F.C. Neidhardt), pp. 1458-1496. ASM Press.

Chiaramello, A.E. and Zyskind, J.W. 1990. Coupling of DNA replication to growth rate in Escherichia coli: a possible role for guanosine tetraphosphate. *J Bacteriol* **172**(4): 2013-2019.

Chow, K.H. and Courcelle, J. 2007. RecBCD and RecJ/RecQ initiate DNA degradation on distinct substrates in UV-irradiated Escherichia coli. *Radiat Res* **168**(4): 499-506.

Courcelle, C.T., Belle, J.J., and Courcelle, J. 2005. Nucleotide excision repair or polymerase V-mediated lesion bypass can act to restore UV-arrested replication forks in Escherichia coli. *J Bacteriol* **187**(20): 6953-6961.

Courcelle, C.T., Chow, K.H., Casey, A., and 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**(24): 9154-9159.

Courcelle, J. and Hanawalt, P.C. 1999. RecQ and RecJ process blocked replication forks prior to the resumption of replication in UV-irradiated Escherichia coli. *Mol Gen Genet* **262**(3): 543-551.

Courcelle, J., Khodursky, A., Peter, B., Brown, P.O., and Hanawalt, P.C. 2001. Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient Escherichia coli. *Genetics* **158**(1): 41-64.

Dermic, D., Halupecki, E., Zahradka, D., and Petranovic, M. 2005. RecBCD enzyme overproduction impairs DNA repair and homologous recombination in Escherichia coli. *Res Microbiol* **156**(3): 304-311.

Diez, A., Gustavsson, N., and Nystrom, T. 2000. The universal stress protein A of Escherichia coli is required for resistance to DNA damaging agents and is regulated by a RecA/FtsK-dependent regulatory pathway. *Mol Microbiol* **36**(6): 1494-1503.

Diez, A.A., Farewell, A., Nannmark, U., and Nystrom, T. 1997. A mutation in the ftsK gene of Escherichia coli affects cell-cell separation, stationary-phase survival, stress adaptation, and expression of the gene encoding the stress protein UspA. *J Bacteriol* **179**(18): 5878-5883.

Dixon, D.A. and Kowalczykowski, S.C. 1991. Homologous pairing in vitro stimulated by the recombination hotspot, Chi. *Cell* **66**(2): 361-371.

-. 1995. Role of the Escherichia coli recombination hotspot, chi, in RecABCD-dependent homologous pairing. *J Biol Chem* **270**(27): 16360-16370.

Donaldson, J.R., Courcelle, C.T., and Courcelle, J. 2006. RuvABC is required to resolve holliday junctions that accumulate following replication on damaged templates in Escherichia coli. *J Biol Chem* **281**(39): 28811-28821.

Dukan, S., Farewell, A., Ballesteros, M., Taddei, F., Radman, M., and Nystrom, T. 2000. Protein oxidation in response to increased transcriptional or translational errors. *Proc Natl Acad Sci U S A* **97**(11): 5746-5749.

Eggleston, A.K., Mitchell, A.H., and West, S.C. 1997. In vitro reconstitution of the late steps of genetic recombination in E. coli. *Cell* **89**(4): 607-617.

Farewell, A., Diez, A.A., DiRusso, C.C., and Nystrom, T. 1996. Role of the Escherichia coli FadR regulator in stasis survival and growth phase-dependent expression of the uspA, fad, and fab genes. *J Bacteriol* **178**(22): 6443-6450.

Farewell, A., Kvint, K., and Nystrom, T. 1998a. Negative regulation by RpoS: a case of sigma factor competition. *Mol Microbiol* **29**(4): 1039-1051.

-. 1998b. uspB, a new sigmaS-regulated gene in Escherichia coli which is required for stationary-phase resistance to ethanol. *J Bacteriol* **180**(23): 6140-6147.

Ferenci, T. 1996. Adaptation to life at micromolar nutrient levels: the regulation of Escherichia coli glucose transport by endoinduction and cAMP. *FEMS Microbiol Rev* **18**(4): 301-317.

Fic, E., Bonarek, P., Gorecki, A., Kedracka-Krok, S., Mikolajczak, J., Polit, A., Tworzydlo, M., Dziedzicka-Wasylewska, M., and Wasylewski, Z. 2009. cAMP receptor protein from escherichia coli as a model of signal transduction in proteins--a review. *J Mol Microbiol Biotechnol* **17**(1): 1-11.

Flores, M.J., Bierne, H., Ehrlich, S.D., and Michel, B. 2001. Impairment of lagging strand synthesis triggers the formation of a RuvABC substrate at replication forks. *EMBO J* **20**(3): 619-629.

Foster, P.L., Trimarchi, J.M., and Maurer, R.A. 1996. Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in Escherichia coli. *Genetics* **142**(1): 25-37.

Fraenkel, D.G. 1996. Glycolysis. in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (ed. F.C. Neidhardt, Ingraham, J.L., Low, K.b., Magansanik, B., Schaechter, M., and Umberger, H.E.), pp. 189-198. American Society for Microbiology Press., Washington,D.C.

Fraenkel, D.G. and Horecker, B.L. 1965. Fructose-1,6-Diphosphatase and Acid Hexose Phosphatase of Escherichia coli. *J Bacteriol* **90**(4): 837-842.

Freestone, P., Nystrom, T., Trinei, M., and Norris, V. 1997. The universal stress protein, UspA, of Escherichia coli is phosphorylated in response to stasis. *J Mol Biol* **274**(3): 318-324.

Fridovich, I. 1978. The biology of oxygen radicals. *Science* **201**(4359): 875-880.

Friedberg, E. 2006. *DNA repair and mutagenesis*. ASM press.

Galhardo, R.S., Do, R., Yamada, M., Friedberg, E.C., Hastings, P.J., Nohmi, T., and Rosenberg, S.M. 2009. DinB upregulation is the sole role of the SOS response in stress-induced mutagenesis in Escherichia coli. *Genetics* **182**(1): 55-68.

Gentry, D.R., Hernandez, V.J., Nguyen, L.H., Jensen, D.B., and Cashel, M. 1993. Synthesis of the stationary-phase sigma factor sigma s is positively regulated by ppGpp. *J Bacteriol* **175**(24): 7982-7989.

Gottesman, S. 1996. Proteases and their targets in Escherichia coli. *Annu Rev Genet* **30**: 465-506.

Gottesman, S., Roche, E., Zhou, Y., and Sauer, R.T. 1998. The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system. *Genes Dev* **12**(9): 1338-1347.

Govons, S., Gentner, N., Greenberg, E., and Preiss, J. 1973. Biosynthesis of bacterial glycogen. XI. Kinetic characterization of an altered adenosine diphosphate-glucose synthase from a "glycogen-excess" mutant of Escherichia coli B. *J Biol Chem* **248**(5): 1731-1740.

Govons, S., Vinopal, R., Ingraham, J., and Preiss, J. 1969. Isolation of mutants of Escherichia coli B altered in their ability to synthesize glycogen. *J Bacteriol* **97**(2): 970-972.

Groat, R.G., Schultz, J.E., Zychlinsky, E., Bockman, A., and Matin, A. 1986. Starvation proteins in Escherichia coli: kinetics of synthesis and role in starvation survival. *J Bacteriol* **168**(2): 486-493.

Gruber, T.M. and Gross, C.A. 2003. Multiple sigma subunits and the partitioning of bacterial transcription space. *Annu Rev Microbiol* **57**: 441-466.

Gummesson, B., Magnusson, L.U., Lovmar, M., Kvint, K., Persson, O., Ballesteros, M., Farewell, A., and Nystrom, T. 2009. Increased RNA polymerase availability directs resources towards growth at the expense of maintenance. *EMBO J*.

Gustavsson, N., Diez, A., and Nystrom, T. 2002. The universal stress protein paralogues of Escherichia coli are co-ordinately regulated and co-operate in the defence against DNA damage. *Mol Microbiol* **43**(1): 107-117.

Hanawalt, P.C. 1993. "Close-fitting sleeves"--recognition of structural defects in duplex DNA. *Mutat Res* **289**(1): 7-15.

Hanawalt, P.C. and Haynes, R.H. 1965. Repair Replication of DNA in Bacteria: Irrelevance of Chemical Nature of Base Defect. *Biochem Biophys Res Commun* **19**: 462-467.

Handa, N., Morimatsu, K., Lovett, S.T., and Kowalczykowski, S.C. 2009. Reconstitution of initial steps of dsDNA break repair by the RecF pathway of E. coli. *Genes Dev* **23**(10): 1234-1245.

Hanson, R.L. and Rose, C. 1980. Effects of an insertion mutation in a locus affecting pyridine nucleotide transhydrogenase (pnt::Tn5) on the growth of Escherichia coli. *J Bacteriol* **141**(1): 401-404.

Harris, R.S., Ross, K.J., and Rosenberg, S.M. 1996. Opposing roles of the holliday junction processing systems of Escherichia coli in recombination-dependent adaptive mutation. *Genetics* **142**(3): 681-691.

Hartl, F.U. 1996. Molecular chaperones in cellular protein folding. *Nature* **381**(6583): 571-579.

He, A.S., Rohatgi, P.R., Hersh, M.N., and Rosenberg, S.M. 2006. Roles of E. coli double-strand-break-repair proteins in stress-induced mutation. *DNA Repair (Amst)* **5**(2): 258-273.

Helmstetter, C. 1996. *Timing of synthetic activities in the cell cycle.* . ASM press.

Hengge-Aronis, R. 2002. Signal transduction and regulatory mechanisms involved in control of the sigma(S) (RpoS) subunit of RNA polymerase. *Microbiol Mol Biol Rev* **66**(3): 373-395, table of contents.

Hengge, R. 2009. Proteolysis of sigmaS (RpoS) and the general stress response in Escherichia coli. *Res Microbiol* **160**(9): 667-676.

Hines, J.K., Fromm, H.J., and Honzatko, R.B. 2007. Structures of activated fructose-1,6-bisphosphatase from Escherichia coli. Coordinate regulation of bacterial metabolism and the conservation of the R-state. *J Biol Chem* **282**(16): 11696-11704.

Hogema, B.M., Arents, J.C., Bader, R., Eijkemans, K., Inada, T., Aiba, H., and Postma, P.W. 1998a. Inducer exclusion by glucose 6-phosphate in Escherichia coli. *Mol Microbiol* **28**(4): 755-765.

Hogema, B.M., Arents, J.C., Bader, R., Eijkemans, K., Yoshida, H., Takahashi, H., Aiba, H., and Postma, P.W. 1998b. Inducer exclusion in Escherichia coli by non-PTS substrates: the role of the PEP to pyruvate ratio in determining the phosphorylation state of enzyme IIAGlc. *Mol Microbiol* **30**(3): 487-498.

Hua, Q., Yang, C., Baba, T., Mori, H., and Shimizu, K. 2003. Responses of the central metabolism in Escherichia coli to phosphoglucose isomerase and glucose-6-phosphate dehydrogenase knockouts. *J Bacteriol* **185**(24): 7053-7067.

Imamura, R., Yamanaka, K., Ogura, T., Hiraga, S., Fujita, N., Ishihama, A., and Niki, H. 1996. Identification of the cpdA gene encoding cyclic 3',5'-adenosine monophosphate phosphodiesterase in Escherichia coli. *J Biol Chem* **271**(41): 25423-25429.

Ishioka, K., Fukuoh, A., Iwasaki, H., Nakata, A., and Shinagawa, H. 1998. Abortive recombination in Escherichia coli ruv mutants blocks chromosome partitioning. *Genes Cells* **3**(4): 209-220.

Iuchi, S. and Weiner, L. 1996. Cellular and molecular physiology of Escherichia coli in the adaptation to aerobic environments. *J Biochem* **120**(6): 1055-1063.

Jiang, M., Sullivan, S.M., Wout, P.K., and Maddock, J.R. 2007. G-protein control of the ribosome-associated stress response protein SpoT. *J Bacteriol* **189**(17): 6140-6147.

Jishage, M., Kvint, K., Shingler, V., and Nystrom, T. 2002. Regulation of sigma factor competition by the alarmone ppGpp. *Genes Dev* **16**(10): 1260-1270.

Jores, L. and Wagner, R. 2003. Essential steps in the ppGpp-dependent regulation of bacterial ribosomal RNA promoters can be explained by substrate competition. *J Biol Chem* **278**(19): 16834-16843.

Kabir, M.M. and Shimizu, K. 2003. Gene expression patterns for metabolic pathway in pgi knockout Escherichia coli with and without phb genes based on RT-PCR. *J Biotechnol* **105**(1-2): 11-31.

Keiler, K.C., Waller, P.R., and Sauer, R.T. 1996. Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. *Science* **271**(5251): 990-993.

Kimata, K., Tanaka, Y., Inada, T., and Aiba, H. 2001. Expression of the glucose transporter gene, ptsG, is regulated at the mRNA degradation step in response to glycolytic flux in Escherichia coli. *EMBO J* **20**(13): 3587-3595.

Kolter, R., Siegele, D.A., and Tormo, A. 1993. The stationary phase of the bacterial life cycle. *Annu Rev Microbiol* **47**: 855-874.

Korangy, F. and Julin, D.A. 1994. Efficiency of ATP hydrolysis and DNA unwinding by the RecBC enzyme from Escherichia coli. *Biochemistry* **33**(32): 9552-9560.

Kristensen, H.-H., Valentin-Hansen, P., and Sogaard-Andersen, L. 1997. Design of CytR regulated, cAMP-CRP dependent class II promoters in Escherichia coli: RNA polymerase-promoter interactions modulate the efficiency of CytR repression. *Journal of Molecular Biology* **266**(5): 866-876.

Kuzminov, A. 1999. Recombinational repair of DNA damage in Escherichia coli and bacteriophage lambda. *Microbiol Mol Biol Rev* **63**(4): 751-813, table of contents.

Kvint, K., Farewell, A., and Nystrom, T. 2000a. RpoS-dependent promoters require guanosine tetraphosphate for induction even in the presence of high levels of sigma(s). *J Biol Chem* **275**(20): 14795-14798.

Kvint, K., Hosbond, C., Farewell, A., Nybroe, O., and Nystrom, T. 2000b. Emergency derepression: stringency allows RNA polymerase to override negative control by an active repressor. *Mol Microbiol* **35**(2): 435-443.

Kvint, K., Nachin, L., Diez, A., and Nystrom, T. 2003. The bacterial universal stress protein: function and regulation. *Curr Opin Microbiol* **6**(2): 140-145.

Lacour, S. and Landini, P. 2004. SigmaS-dependent gene expression at the onset of stationary phase in Escherichia coli: function of sigmaS-dependent genes and identification of their promoter sequences. *J Bacteriol* **186**(21): 7186-7195.

Lange, R., Fischer, D., and Hengge-Aronis, R. 1995. Identification of transcriptional start sites and the role of ppGpp in the expression of rpoS, the structural gene for the sigma S subunit of RNA polymerase in Escherichia coli. *J Bacteriol* **177**(16): 4676-4680.

Lange, R. and Hengge-Aronis, R. 1991a. Growth phase-regulated expression of bolA and morphology of stationary-phase Escherichia coli cells are controlled by the novel sigma factor sigma S. *J Bacteriol* **173**(14): 4474-4481.

-. 1991b. Identification of a central regulator of stationary-phase gene expression in Escherichia coli. *Mol Microbiol* **5**(1): 49-59.

Layton, J.C. and Foster, P.L. 2003. Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in Escherichia coli. *Mol Microbiol* **50**(2): 549-561.

Lederberg, J. 2004. E.coli K-12. *Microbiology today* **31**.

Lin , E.C.C. 1996. Dissimilatory Pathways for Sugars, Polyols, and Carboxylates. in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (ed. F.C. Neidhardt, Ingraham, J.L., Low, K.b., Magansanik, B., Schaechter, M., and Umberger, H.E.), pp. 307-326. American Society for Microbiology Press., Washington,D.C.

Llorens, J.M.N., Tormo, A., and Martínez-García, E. Stationary phase in gram-negative bacteria. *FEMS Microbiology Reviews* **9999**(9999).

Lloyd, R.G. 1991. Conjugational recombination in resolvase-deficient ruvC mutants of Escherichia coli K-12 depends on recG. *J Bacteriol* **173**(17): 5414-5418.

Lloyd, R.G., Benson, F.E., and Shurvinton, C.E. 1984. Effect of ruv mutations on recombination and DNA repair in Escherichia coli K12. *Mol Gen Genet* **194**(1-2): 303-309.

Lloyd, R.G., Buckman, C., and Benson, F.E. 1987. Genetic analysis of conjugational recombination in Escherichia coli K12 strains deficient in RecBCD enzyme. *J Gen Microbiol* **133**(9): 2531-2538.

Lloyd, R.G., Porton, M.C., and Buckman, C. 1988. Effect of recF, recJ, recN, recO and ruv mutations on ultraviolet survival and genetic recombination in a recD strain of Escherichia coli K12. *Mol Gen Genet* **212**(2): 317-324.

Lobell, R.B. and Schleif, R.F. 1991. AraC-DNA looping: orientation and distance-dependent loop breaking by the cyclic AMP receptor protein. *J Mol Biol* **218**(1): 45-54.

Lobner-Olesen, A., Skarstad, K., Hansen, F.G., von Meyenburg, K., and Boye, E. 1989. The DnaA protein determines the initiation mass of Escherichia coli K-12. *Cell* **57**(5): 881-889.

Lovett, S.T. 2006. Replication arrest-stimulated recombination: Dependence on the RecA paralog, RadA/Sms and translesion polymerase, DinB. *DNA Repair (Amst)* **5**(12): 1421-1427.

Lovett, S.T., Luisi-DeLuca, C., and Kolodner, R.D. 1988. The genetic dependence of recombination in recD mutants of Escherichia coli. *Genetics* **120**(1): 37-45.

Magnusson, L.U., Farewell, A., and Nystrom, T. 2005. ppGpp: a global regulator in Escherichia coli. *Trends Microbiol* **13**(5): 236-242.

Magnusson, L.U., Gummesson, B., Joksimovic, P., Farewell, A., and Nystrom, T. 2007. Identical, independent, and opposing roles of ppGpp and DksA in Escherichia coli. *J Bacteriol* **189**(14): 5193-5202.

Magnusson, L.U., Nystrom, T., and Farewell, A. 2003. Underproduction of sigma 70 mimics a stringent response. A proteome approach. *J Biol Chem* **278**(2): 968-973.

Maisonneuve, E., Ducret, A., Khoueiry, P., Lignon, S., Longhi, S., Talla, E., and Dukan, S. 2009. Rules governing selective protein carbonylation. *PLoS One* **4**(10): e7269.

Mandel, M.J. and Silhavy, T.J. 2005. Starvation for different nutrients in Escherichia coli results in differential modulation of RpoS levels and stability. *J Bacteriol* **187**(2): 434-442.

Matin, A. 1991. The molecular basis of carbon-starvation-induced general resistance in Escherichia coli. *Mol Microbiol* **5**(1): 3-10.

Matin, A., Auger, E.A., Blum, P.H., and Schultz, J.E. 1989. Genetic basis of starvation survival in nondifferentiating bacteria. *Annu Rev Microbiol* **43**: 293-316.

Maurizi, M.R. 1992. Proteases and protein degradation in Escherichia coli. *Experientia* **48**(2): 178-201.

McGlynn, P. and Lloyd, R.G. 2000. Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. *Cell* **101**(1): 35-45.

-. 2002. Genome stability and the processing of damaged replication forks by RecG. *Trends Genet* **18**(8): 413-419.

Meddows, T.R., Savory, A.P., and Lloyd, R.G. 2004. RecG helicase promotes DNA double-strand break repair. *Mol Microbiol* **52**(1): 119-132.

Merrikh, H., Ferrazzoli, A.E., Bougdour, A., Olivier-Mason, A., and Lovett, S.T. 2009a. A DNA damage response in Escherichia coli involving the alternative sigma factor, RpoS. *Proc Natl Acad Sci U S A* **106**(2): 611-616.

Merrikh, H., Ferrazzoli, A.E., and Lovett, S.T. 2009b. Growth phase and (p)ppGpp control of IraD, a regulator of RpoS stability, in Escherichia coli. *J Bacteriol*.

Mitchell, D.L., Jen, J., and Cleaver, J.E. 1992. Sequence specificity of cyclobutane pyrimidine dimers in DNA treated with solar (ultraviolet B) radiation. *Nucleic Acids Res* **20**(2): 225-229.

Mitchell, D.L. and Nairn, R.S. 1989. THE BIOLOGY OF THE (6-4) PHOTOPRODUCT. *Photochemistry and Photobiology* **49**(6): 805-819.

Morita, T., El-Kazzaz, W., Tanaka, Y., Inada, T., and Aiba, H. 2003. Accumulation of glucose 6-phosphate or fructose 6-phosphate is responsible for destabilization of glucose transporter mRNA in Escherichia coli. *J Biol Chem* **278**(18): 15608-15614.

Murray, K.D. and Bremer, H. 1996. Control of spoT-dependent ppGpp synthesis and degradation in Escherichia coli. *J Mol Biol* **259**(1): 41-57.

Nachin, L., Brive, L., Persson, K.C., Svensson, P., and Nystrom, T. 2008. Heterodimer formation within universal stress protein classes revealed by an in silico and experimental approach. *J Mol Biol* **380**(2): 340-350.

Nachin, L., Nannmark, U., and Nystrom, T. 2005. Differential roles of the universal stress proteins of Escherichia coli in oxidative stress resistance, adhesion, and motility. *J Bacteriol* **187**(18): 6265-6272.

Nanchen, A., Schicker, A., Revelles, O., and Sauer, U. 2008. Cyclic AMP-dependent catabolite repression is the dominant control mechanism of metabolic fluxes under glucose limitation in Escherichia coli. *J Bacteriol* **190**(7): 2323-2330.

Navarro Llorens, J.M., Tormo, A., and Martinez-Garcia, E. Stationary phase in gram-negative bacteria. *FEMS Microbiol Rev*.

Neidhardt, F.C. 1996. *Escherichia coli and Salmonella; cellular and molecular biology*. ASM, Washington DC.

Nystrom, T., Flardh, K., and Kjelleberg, S. 1990. Responses to multiple-nutrient starvation in marine Vibrio sp. strain CCUG 15956. *J Bacteriol* **172**(12): 7085-7097.

Nystrom, T. and Neidhardt, F.C. 1992. Cloning, mapping and nucleotide sequencing of a gene encoding a universal stress protein in Escherichia coli. *Mol Microbiol* **6**(21): 3187-3198.

-. 1993. Isolation and properties of a mutant of Escherichia coli with an insertional inactivation of the uspA gene, which encodes a universal stress protein. *J Bacteriol* **175**(13): 3949-3956.

-. 1994. Expression and role of the universal stress protein, UspA, of Escherichia coli during growth arrest. *Mol Microbiol* **11**(3): 537-544.

-. 1996. Effects of overproducing the universal stress protein, UspA, in Escherichia coli K-12. *J Bacteriol* **178**(3): 927-930.

O'Farrell, P.H. 1978. The suppression of defective translation by ppGpp and its role in the stringent response. *Cell* **14**(3): 545-557.

Parsons, C.A. and West, S.C. 1993. Formation of a RuvAB-Holliday junction complex in vitro. *J Mol Biol* **232**(2): 397-405.

Persky, N.S., Ferullo, D.J., Cooper, D.L., Moore, H.R., and Lovett, S.T. 2009. The ObgE/CgtA GTPase influences the stringent response to amino acid starvation in Escherichia coli. *Mol Microbiol* **73**(2): 253-266.

Peterson, C.N., Ruiz, N., and Silhavy, T.J. 2004. RpoS proteolysis is regulated by a mechanism that does not require the SprE (RssB) response regulator phosphorylation site. *J Bacteriol* **186**(21): 7403-7410.

Pham, P., Seitz, E.M., Saveliev, S., Shen, X., Woodgate, R., Cox, M.M., and Goodman, M.F. 2002. Two distinct modes of RecA action are required for DNA polymerase V-catalyzed translesion synthesis. *Proc Natl Acad Sci U S A* **99**(17): 11061-11066.

Postma, P.W. and Lengeler, J.W. 1985. Phosphoenolpyruvate:carbohydrate phosphotransferase system of bacteria. *Microbiol Rev* **49**(3): 232-269.

Postma, P.W., Lengeler, J.W., and Jacobson, G.R. 1993. Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol Rev* **57**(3): 543-594.

Potrykus, K. and Cashel, M. 2008. (p)ppGpp: still magical? *Annu Rev Microbiol* **62**: 35-51.

Preiss, J., Greenberg, E., and Sabraw, A. 1975. Biosynthesis of bacterial glycogen. Kinetic studies of a glucose-1-phosphate adenylyltransferase (EC 2.7.7.27) from a glycogen-deficient mutant of Escherichia coli B. *J Biol Chem* **250**(19): 7631-7638.

Pruteanu, M. and Hengge-Aronis, R. 2002. The cellular level of the recognition factor RssB is rate-limiting for sigmaS proteolysis: implications for RssB regulation and signal transduction in sigmaS turnover in Escherichia coli. *Mol Microbiol* **45**(6): 1701-1713.

Rahman, M., Hasan, M.R., Oba, T., and Shimizu, K. 2006. Effect of rpoS gene knockout on the metabolism of Escherichia coli during exponential growth phase and early stationary phase based on gene expressions, enzyme activities and intracellular metabolite concentrations. *Biotechnol Bioeng* **94**(3): 585-595.

Raskin, D.M., Judson, N., and Mekalanos, J.J. 2007. Regulation of the stringent response is the essential function of the conserved bacterial G protein CgtA in Vibrio cholerae. *Proc Natl Acad Sci U S A* **104**(11): 4636-4641.

Reeve, C.A., Amy, P.S., and Matin, A. 1984. Role of protein synthesis in the survival of carbon-starved Escherichia coli K-12. *J Bacteriol* **160**(3): 1041-1046.

Richter, D. 1980. Uncharged tRNA inhibits guanosine 3',5'-bis (diphosphate) 3'-pyrophosphohydrolase [ppGppase], the spoT gene product, from Escherichia coli. *Mol Gen Genet* **178**(2): 325-327.

Rockabrand, D., Livers, K., Austin, T., Kaiser, R., Jensen, D., Burgess, R., and Blum, P. 1998. Roles of DnaK and RpoS in Starvation-Induced Thermotolerance of Escherichia coli. *J Bacteriol* **180**(4): 846-854.

Romeo, T. 1998. Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule CsrB. *Mol Microbiol* **29**(6): 1321-1330.

Rudolph, C.J., Upton, A.L., Harris, L., and Lloyd, R.G. 2009. Pathological replication in cells lacking RecG DNA translocase. *Mol Microbiol* **73**(3): 352-366.

Ryder, L., Whitby, M.C., and Lloyd, R.G. 1994. Mutation of recF, recJ, recO, recQ, or recR improves Hfr recombination in resolvase-deficient ruv recG strains of Escherichia coli. *J Bacteriol* **176**(6): 1570-1577.

Sabnis, N.A., Yang, H., and Romeo, T. 1995. Pleiotropic regulation of central carbohydrate metabolism in Escherichia coli via the gene csrA. *J Biol Chem* **270**(49): 29096-29104.

Saier, M.H.R., T.M. Reizer, J. 1996. Regulation of Carbon Utilization. in *Escherichia and Salmonella: Cellular and molecular biology* (ed. F.C. Neidhardt), pp. 1325-1340. ASM Press.

Saint-Ruf, C., Taddei, F., and Matic, I. 2004. Stress and survival of aging Escherichia coli rpoS colonies. *Genetics* **168**(1): 541-546.

Sak, B.D., Eisenstark, A., and Touati, D. 1989. Exonuclease III and the catalase hydroperoxidase II in Escherichia coli are both regulated by the katF gene product. *Proc Natl Acad Sci U S A* **86**(9): 3271-3275.

Sandler, S.J. and Clark, A.J. 1994. RecOR suppression of recF mutant phenotypes in Escherichia coli K-12. *J Bacteriol* **176**(12): 3661-3672.

Sandler, S.J. and Marians, K.J. 2000. Role of PriA in Replication Fork Reactivation in Escherichia coli. *J Bacteriol* **182**(1): 9-13.

Schweder, T., Lee, K.H., Lomovskaya, O., and Matin, A. 1996. Regulation of Escherichia coli starvation sigma factor (sigma s) by ClpXP protease. *J Bacteriol* **178**(2): 470-476.

Seigneur, M., Bidnenko, V., Ehrlich, S.D., and Michel, B. 1998. RuvAB acts at arrested replication forks. *Cell* **95**(3): 419-430.

Seyfzadeh, M., Keener, J., and Nomura, M. 1993. spoT-dependent accumulation of guanosine tetraphosphate in response to fatty acid starvation in Escherichia coli. *Proc Natl Acad Sci U S A* **90**(23): 11004-11008.

Shah, R., Bennett, R.J., and West, S.C. 1994. Genetic recombination in E. coli: RuvC protein cleaves Holliday junctions at resolution hotspots in vitro. *Cell* **79**(5): 853-864.

Sharples, G.J., Ingleston, S.M., and Lloyd, R.G. 1999. Holliday junction processing in bacteria: insights from the evolutionary conservation of RuvABC, RecG, and RusA. *J Bacteriol* **181**(18): 5543-5550.

Sharples, G.J. and Lloyd, R.G. 1991. Resolution of Holliday junctions in Escherichia coli: identification of the ruvC gene product as a 19-kilodalton protein. *J Bacteriol* **173**(23): 7711-7715.

Shinagawa, H., Makino, K., Amemura, M., Kimura, S., Iwasaki, H., and Nakata, A. 1988. Structure and regulation of the Escherichia coli ruv operon involved in DNA repair and recombination. *J Bacteriol* **170**(9): 4322-4329.

Shurvinton, C.E., Lloyd, R.G., Benson, F.E., and Attfield, P.V. 1984. Genetic analysis and molecular cloning of the Escherichia coli ruv gene. *Mol Gen Genet* **194**(1-2): 322-329.

Sleight, S.C., Orlic, C., Schneider, D., and Lenski, R.E. 2008. Genetic basis of evolutionary adaptation by Escherichia coli to stressful cycles of freezing, thawing and growth. *Genetics* **180**(1): 431-443.

Smith, B.T. and Walker, G.C. 1998. Mutagenesis and more: umuDC and the Escherichia coli SOS response. *Genetics* **148**(4): 1599-1610.

Smith, H.W. 1975. Survival of orally administered E. coli K 12 in alimentary tract of man. *Nature* **255**(5508): 500-502.

Spassky, A., Busby, S., and Buc, H. 1984. On the action of the cyclic AMP-cyclic AMP receptor protein complex at the Escherichia coli lactose and galactose promoter regions. *EMBO J* **3**(1): 43-50.

Srivatsan, A. and Wang, J.D. 2008. Control of bacterial transcription, translation and replication by (p)ppGpp. *Curr Opin Microbiol* **11**(2): 100-105.

Stahl, F.W. and Stahl, M.M. 1977. Recombination pathway specificity of Chi. *Genetics* **86**(4): 715-725.

Steiner, W.W. and Kuempel, P.L. 1998. Cell division is required for resolution of dimer chromosomes at the dif locus of Escherichia coli. *Mol Microbiol* **27**(2): 257-268.

Stent, G.S. and Brenner, S. 1961. A genetic locus for the regulation of ribonucleic acid synthesis. *Proc Natl Acad Sci U S A* **47**: 2005-2014.

Sy, J. and Lipmann, F. 1973. Identification of the synthesis of guanosine tetraphosphate (MS I) as insertion of a pyrophosphoryl group into the 3'-position in guanosine 5'-diphosphate. *Proc Natl Acad Sci U S A* **70**(2): 306-309.

Taddei, F., Matic, I., and Radman, M. 1995. cAMP-dependent SOS induction and mutagenesis in resting bacterial populations. *Proc Natl Acad Sci U S A* **92**(25): 11736-11740.

Takamura, Y. and Nomura, G. 1988. Changes in the intracellular concentration of acetyl-CoA and malonyl-CoA in relation to the carbon and energy metabolism of Escherichia coli K12. *J Gen Microbiol* **134**(8): 2249-2253.

Tang, M.S., Wang, T.C., and Patrick, M.H. 1979. DNA turnover in buffer-held Escherichia coli and its effect on repair of UV damage. *Photochem Photobiol* **29**(3): 511-520.

Tao, H., Bausch, C., Richmond, C., Blattner, F.R., and Conway, T. 1999. Functional genomics: expression analysis of Escherichia coli growing on minimal and rich media. *J Bacteriol* **181**(20): 6425-6440.

Teich, A., Meyer, S., Lin, H.Y., Andersson, L., Enfors, S., and Neubauer, P. 1999. Growth rate related concentration changes of the starvation response regulators sigmaS and ppGpp in glucose-limited fed-batch and continuous cultures of Escherichia coli. *Biotechnol Prog* **15**(1): 123-129.

Thaler, D.S., Sampson, E., Siddiqi, I., Rosenberg, S.M., Thomason, L.C., Stahl, F.W., and Stahl, M.M. 1989. Recombination of bacteriophage lambda in recD mutants of Escherichia coli. *Genome* **31**(1): 53-67.

Tobias, J.W., Shrader, T.E., Rocap, G., and Varshavsky, A. 1991. The N-end rule in bacteria. *Science* **254**(5036): 1374-1377.

Tomoyasu, T., Mogk, A., Langen, H., Goloubinoff, P., and Bukau, B. 2001. Genetic dissection of the roles of chaperones and proteases in protein folding and degradation in the Escherichia coli cytosol. *Mol Microbiol* **40**(2): 397-413.

Trautinger, B.W., Jaktaji, R.P., Rusakova, E., and Lloyd, R.G. 2005. RNA polymerase modulators and DNA repair activities resolve conflicts between DNA replication and transcription. *Mol Cell* **19**(2): 247-258.

Trautinger, B.W. and Lloyd, R.G. 2002. Modulation of DNA repair by mutations flanking the DNA channel through RNA polymerase. *EMBO J* **21**(24): 6944-6953.

Tu, G.F., Reid, G.E., Zhang, J.G., Moritz, R.L., and Simpson, R.J. 1995. C-terminal extension of truncated recombinant proteins in Escherichia coli with a 10Sa RNA decapeptide. *J Biol Chem* **270**(16): 9322-9326.

Tyrrell, R.M. 1973. Induction of pyrimidine dimers in bacterial DNA by 365 nm radiation. *Photochemistry and Photobiology* **17**(1): 69-73.

Ueguchi, C., Kakeda, M., and Mizuno, T. 1993. Autoregulatory expression of the Escherichia coli hns gene encoding a nucleoid protein: H-NS functions as a repressor of its own transcription. *Mol Gen Genet* **236**(2-3): 171-178.

Ueguchi, C., Misonou, N., and Mizuno, T. 2001. Negative control of rpoS expression by phosphoenolpyruvate: carbohydrate phosphotransferase system in Escherichia coli. *J Bacteriol* **183**(2): 520-527.

Umezu, K., Chi, N.W., and Kolodner, R.D. 1993. Biochemical interaction of the Escherichia coli RecF, RecO, and RecR proteins with RecA protein and single-stranded DNA binding protein. *Proc Natl Acad Sci U S A* **90**(9): 3875-3879.

Umezu, K. and Kolodner, R.D. 1994. Protein interactions in genetic recombination in Escherichia coli. Interactions involving RecO and RecR overcome the inhibition of RecA by single-stranded DNA-binding protein. *J Biol Chem* **269**(47): 30005-30013.

Wagner, R. 2002. Regulation of ribosomal RNA synthesis in E. coli: effects of the global regulator guanosine tetraphosphate (ppGpp). *J Mol Microbiol Biotechnol* **4**(3): 331-340.

van Gool, A.J., Hajibagheri, N.M., Stasiak, A., and West, S.C. 1999. Assembly of the Escherichia coli RuvABC resolvasome directs the orientation of holliday junction resolution. *Genes Dev* **13**(14): 1861-1870.

van Gool, A.J., Shah, R., Mezard, C., and West, S.C. 1998. Functional interactions between the holliday junction resolvase and the branch migration motor of Escherichia coli. *EMBO J* **17**(6): 1838-1845.

Van Houten, B., Croteau, D.L., DellaVecchia, M.J., Wang, H., and Kisker, C. 2005. 'Close-fitting sleeves': DNA damage recognition by the UvrABC nuclease system. *Mutat Res* **577**(1-2): 92-117.

Wang, J.D. and Levin, P.A. 2009. Metabolism, cell growth and the bacterial cell cycle. *Nat Rev Microbiol* **7**(11): 822-827.

Weart, R.B., Lee, A.H., Chien, A.C., Haeusser, D.P., Hill, N.S., and Levin, P.A. 2007. A metabolic sensor governing cell size in bacteria. *Cell* **130**(2): 335-347.

Weber, H., Polen, T., Heuveling, J., Wendisch, V.F., and Hengge, R. 2005. Genome-wide analysis of the general stress response network in Escherichia coli: sigmaS-dependent genes, promoters, and sigma factor selectivity. *J Bacteriol* **187**(5): 1591-1603.

Weichart, D., Querfurth, N., Dreger, M., and Hengge-Aronis, R. 2003. Global role for ClpP-containing proteases in stationary-phase adaptation of Escherichia coli. *J Bacteriol* **185**(1): 115-125.

Weilbacher, T., Suzuki, K., Dubey, A.K., Wang, X., Gudapaty, S., Morozov, I., Baker, C.S., Georgellis, D., Babitzke, P., and Romeo, T. 2003. A novel sRNA component of the carbon storage regulatory system of Escherichia coli. *Mol Microbiol* **48**(3): 657-670.

Weissenborn, D.L., Wittekindt, N., and Larson, T.J. 1992. Structure and regulation of the glpFK operon encoding glycerol diffusion facilitator and glycerol kinase of Escherichia coli K-12. *J Biol Chem* **267**(9): 6122-6131.

Wenthzel, A.M., Stancek, M., and Isaksson, L.A. 1998. Growth phase dependent stop codon readthrough and shift of translation reading frame in Escherichia coli. *FEBS Lett* **421**(3): 237-242.

West, S.C. 1997. Processing of recombination intermediates by the RuvABC proteins. *Annu Rev Genet* **31**: 213-244.

Whitby, M.C. and Lloyd, R.G. 1995. Altered SOS induction associated with mutations in recF, recO and recR. *Mol Gen Genet* **246**(2): 174-179.

Villarroya, M., Perez-Roger, I., Macian, F., and Armengod, M.E. 1998. Stationary phase induction of dnaN and recF, two genes of Escherichia coli involved in DNA replication and repair. *Embo J* **17**(6): 1829-1837.

Willetts, N.S. and Mount, D.W. 1969. Genetic analysis of recombination-deficient mutants of Escherichia coli K-12 carrying rec mutations cotransducible with thyA. *J Bacteriol* **100**(2): 923-934.

Vinella, D., Albrecht, C., Cashel, M., and D'Ari, R. 2005. Iron limitation induces SpoT-dependent accumulation of ppGpp in Escherichia coli. *Mol Microbiol* **56**(4): 958-970.

Xiao, H., Kalman, M., Ikehara, K., Zemel, S., Glaser, G., and Cashel, M. 1991. Residual guanosine 3',5'-bispyrophosphate synthetic activity of relA null mutants can be eliminated by spoT null mutations. *J Biol Chem* **266**(9): 5980-5990.

Yang, B. and Larson, T.J. 1998. Multiple promoters are responsible for transcription of the glpEGR operon of Escherichia coli K-12. *Biochim Biophys Acta* **1396**(1): 114-126.

Yang, H., Liu, M.Y., and Romeo, T. 1996. Coordinate genetic regulation of glycogen catabolism and biosynthesis in Escherichia coli via the CsrA gene product. *J Bacteriol* **178**(4): 1012-1017.

Zahradka, D., Zahradka, K., Petranovic, M., Dermic, D., and Brcic-Kostic, K. 2002. The RuvABC resolvase is indispensable for recombinational repair in sbcB15 mutants of Escherichia coli. *J Bacteriol* **184**(15): 4141-4147.

Zerbib, D., Mezard, C., George, H., and West, S.C. 1998. Coordinated actions of RuvABC in Holliday junction processing. *J Mol Biol* **281**(4): 621-630.

Zgurskaya, H.I., Keyhan, M., and Matin, A. 1997. The sigma S level in starving Escherichia coli cells increases solely as a result of its increased stability, despite decreased synthesis. *Mol Microbiol* **24**(3): 643-651.