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Molecular Chaperones
A short resume of common folding helpers

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Without the help of all these people I would not have achieved such an accomplishment.

Thank you all for your continuous support!

Declaration of Authenticity

I hereby declare that the thesis submitted is my own unaided work, that I have not used other than the declared sources/ resources, that I have explicitly marked all material which has been quoted either literally or by content from the used sources, and that all direct or indirect sources used are acknowledged as references.

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Introduction

Christopher M. Dobson once wrote in an article that “Folding and unfolding are crucial ways of regulating biological activity and targeting proteins to different cellular locations. Aggregation of misfolded proteins that escape the cellular quality-control mechanisms is a common feature of a wide range of highly debilitating and increasingly prevalent diseases.”[1]

Only correctly folded proteins possess a long-term stability and are able to interact with their correspondent partners in a proper way.[1] The origin of a wide variety of pathological conditions can be linked to proteins which did not remain correctly folded, aggregated or did not achieve the biological self-assembly in the first place.[1]

“Although all the information necessary for a protein to attain its native structure is contained in its amino acid sequence, efficient protein folding *in vivo* requires the participation of various factors, including molecular chaperones, folding catalysts and proteases.”[2]

This Bachelor thesis is addressed to **molecular chaperones** and their future scope of application for medical treatment. Today there are numerous opportunities for the field of application, like the design of new antibacterial molecules to fight multiresistant bacteria, to prevent aggregation diseases, including Alzheimer’s disease and Parkinson’s disease and even to understand the relationship of protein quality control to complex biological processes such as ageing or to form the basis of anticancer vaccines.[2–5] Molecular chaperones are of fundamental interest to biotechnology and fall into two general categories: foldases and holdases.[5] Foldases catalyze chemical reactions and holdases isolate aggregation-prone polypeptides. [5]

The next pages are dedicated to representatives of **periplasmic folding helpers** and **stress-induced periplasmic chaperones** (“[...] that specifically function under stress conditions [...]”[2]) and should give a basic understanding to a reader with a common knowledge in protein chemistry, biochemistry or biotechnology.

General regulations

The periplasmic compartment

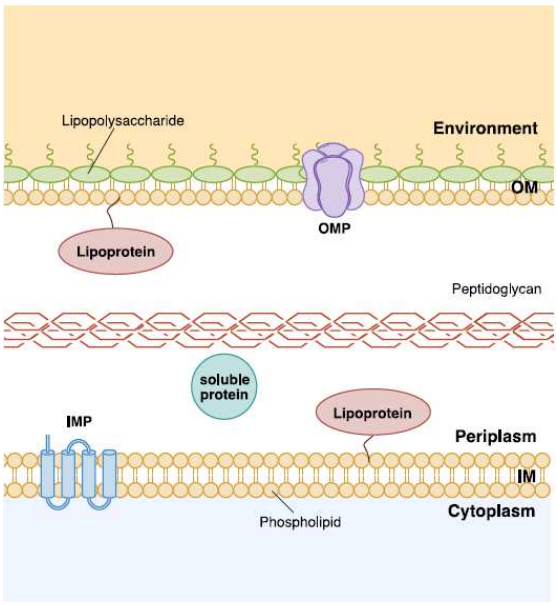

Figure 1: General structure of the *E. coli* envelope (p. 1518)[2]

"Gram-negative bacteria have two cellular compartments: the cytoplasm and the periplasm, an extracytoplasmic compartment located between the inner cytoplasmic membrane (IM) and the outer membrane (OM) of the cell."[6] The periplasmic compartment is an aqueous compartment with an oxidizing environment which does not contain ATP and it is the preferred location within *E. coli* where disulfide bonds can spontaneously form.[5,6] Nevertheless, this unique characteristics the conditions of the compartment can still adapt to those of the external environment and therefore functions as link between the inner compartment of the cell and the external environment.[5,6] Most proteins destined for secretion or transport to the outer membrane are synthesized in the cytosol and translocated in an unfolded form across the cytoplasmic membrane into the periplasm[5,7]. These technique can be used to manufacture recombinant therapeutic proteins such as antibodies and virulence factors, before they are inserted into the outer membrane.[5,7]

Due to the ability of the periplasm to adapt to external conditions (high temperatures, extremes in pH or the presence of toxic molecules) the cell is able to sense stress and response to the alterations of their environment.[6] This is achieved by monitoring the build-up of protein-folding intermediates and degradation or refolding of the misfolded proteins by chaperones.[6] Without the assistance of molecular chaperones, the proper folding of some periplasmic proteins would be almost impossible and therefore lethal for the cell.[2]

Envelope stress response systems

The *E. coli* cell envelope is a protective barrier, spread from the inner cell membrane to the outer membrane and is able to respond to environmental assaults by a complex stress-sensing system.[8–11] There are many signal transduction systems, but the three most important for periplasmic stress response are the σ^E, Cpx and Bae system.[9,10]

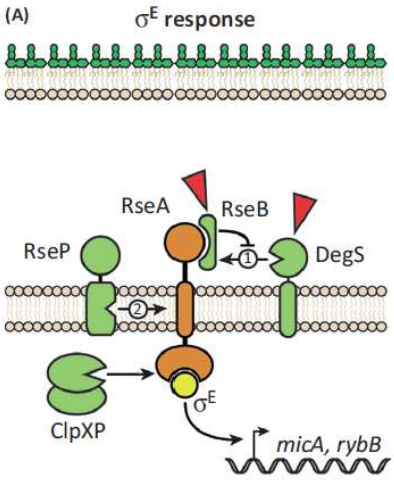

Figure 2: sigma stress response (p. 234)[9]

The σ-factor is an important stress response system and in charge of the up regulation of several target genes by the recruitment of RNA polymerase, in order to enhance the production of periplasmic folding helpers.[9,12] σ^E (or σ^{24}) is part of the heat shock response system in periplasmic compartments leading to (elevated) transcription of several genes.[12] The first gene identified to be affected by σ^E was *degP* (or *htrA*), with a promoter almost similar to that of the cytoplasmic σ-factor (σ^{32}), which is also induced in response to heat.[12,13] The σ^E system is closely linked to the Cpx two-component system and the two pathways seem to be antagonistic and display high signal linkages.[9] This could be because both systems try to maintain homeostasis of the outer and inner membrane and therefore protect the energy-generating functions of the cell.[9]

The conjugative plasmid expression (Cpx) system specifically responds when the peptidoglycan integrity at the inner membrane is challenged and also functions as a surveillance system of misfolded proteins in the envelope.[11,14] The Cpx two-component system consist of the sensor histidine kinase CpxA and the cytoplasmic response regulator CpxR.[9,11,15,16] Misfolded and/or mislocalized proteins are responsible for the activation of the Cpx pathway and lead to the phosphorylisation of CpxA, which acts as a histidine autokinase.[15] The phosphorylated CpxA initiates the transcription of protein folding and degrading factors (e.g. DegP, DsbA) by transferring the phosphate to the CpxR response regulator.[14,15] Beside CpxA and CpxR there is also CpxP, a small periplasmic inhibitor protein capable to interact with the sensing domain of CpxA.[15] CpxP is not required for signal transduction but is expressed at high transcription rates of *cpxRA* genes or at the absence of envelope stress to set the Cpx-pathway in an off state either at the presence of many folding helpers or to save energy when everything is under control.[15] This could be an indication for a negative feedback-loop or a repressor system.

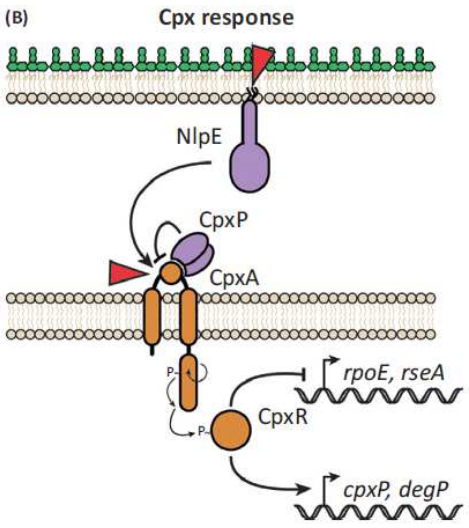

Figure 3: Cpx stress response (p. 234)[9]

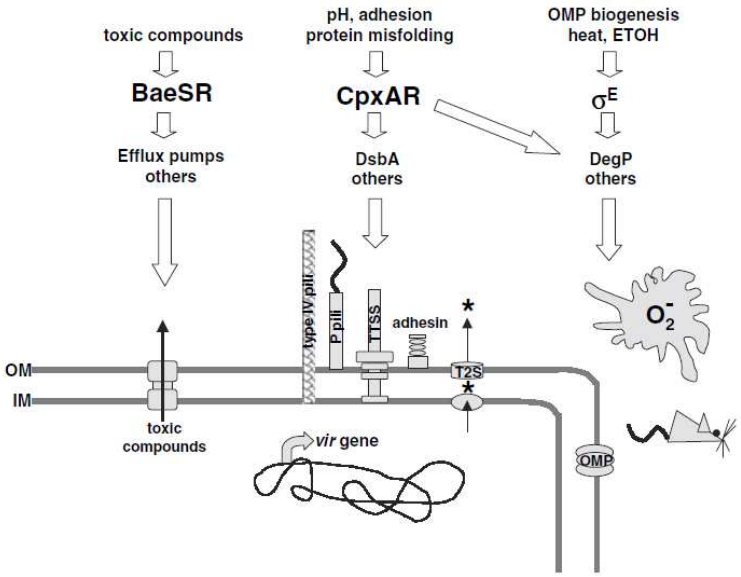

Figure 4: Envelope stress responses (p. 1120)[10]

Beside the Cpx-pathway there is a second two-component system, the bacterial adaptive envelope stress response system (Bae system).[10] Like the Cpx system, it also consists of a membrane localized histidine kinase (BaeS) and a response regulator (BaeR).[10] The Bae system is responsible for the activation of multi-drug efflux pumps, through its regulation of specific genes in the presence of antimicrobial compounds, such as β-lactam antibiotics.[10] "In addition, the Bae response protects *E. coli* from other envelope perturbants, including indole and misfolded proteins, through unidentified mechanisms."[10]

Overview of common folding helpers

Periplasmic folding helpers

SurA

SurA (**sur**vival factor **A**[7]), a periplasmic protein, originally isolated as a protein essential for survival in stationary phase, was later described both as a chaperone that is involved in the maturation and assembly of LamB and as a peptidyl-prolyl *cis-trans* isomerise (PPIase).[2,6,17] "LamB is a trimeric outer membrane porin for maltodextrins [(maltoporin[18])] as well as the bacteriophage λ receptor in *Escherichia coli*."[6] SurA is important in maintaining this outer membrane integrity by converting the unstable trimers of LamB (as well as OmpC and OmpF) to stable trimers.[17,19] Beside SurA, there are three more known periplasmic *cis-trans* prolyl isomerases, FkpA, PpiA and PpiD.[20] These isomerases specifically facilitate the conversion of apparent unfolded monomers to folded monomers of outer membrane proteins by increasing the rate of transition of proline residues between the *cis* and *trans* states.[17,20] Periplasmic isomerases are not essential for growth under laboratory conditions and a disruption of the *surA* gene alone is not lethal.[17,20] Simultaneous null mutations of PPIases (*surA* and *ppiD* genes) could be lethal because the periplasmic proteins have significant roles

in survival in environmental and pathogenic niches.[17,20] The lethality by the combined null mutation could be caused by the high sequence similarity to the catalytic domain of parvulin and the fact, that PpiD and SurA interact with similar model peptides, and therefore must have partially overlapping substrate specificities.[19] If one PPIase is silenced the other isomerase could still proceed, but a simultaneous null mutation is lethal, indicating that *surA* is responsible for an essential activity that is encoded by redundant genes.[17]

Skp

Skp (**S**eventeen **K**ilodalton **P**rotein, in literature also referred to OmpH and HlpA[21]) primarily known for its role in OMP biogenesis, by selectively binding to unfolded OMPs, was originally described as a histone-like protein that binds DNA.[2,5,21] From pH 3 to pH 11 Skp form a stable trimer and also interacts stably with a number of OMPs (e.g. LamB, OmpA, OmpF, OmpG and Omp85[2,5,7,22]) by encapsulating them partially and protecting them from the aqueous environment until delivery to the β-barrel assembly machinery (BAM-complex; "The Bam complex is likely responsible for the assembly of β-barrel proteins into the OMs of virtually all Gram-negative bacteria"[23]).[5,7,24] Skp also assists in folding soluble proteins in the bacterial periplasm and increases the active yields of many recombinant proteins by reducing aggregation but does not alter the observed folding rates of Skp-sensitive proteins.[5]

The functional form of Skp is a homo-trimeric quaternary structure composed of a central β-barrel-like core domain from which three α-helical formations diverge.[2,5,21,22] In literature the structure is often described as "jellyfish-like" with α-helical tentacles.[2,5,21] Independent from the visual appearance, the sequence of Skp is quite unique compared to any other known chaperon and does not display sequence similarity.[21]

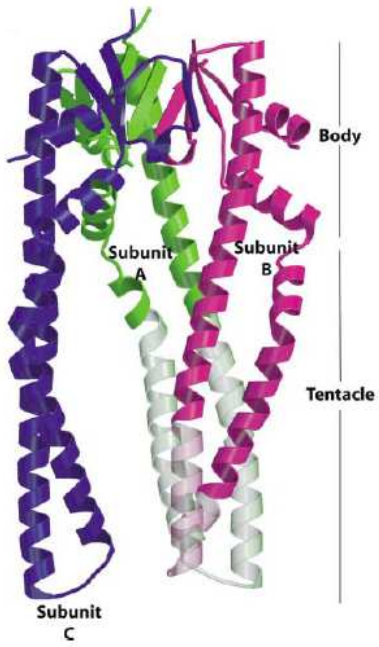

Figure 5: Side view of Skp trimer (p. 369)[21]

DegP

DegP (for "**deg**radation", in literature also referred to protease Do[25]), a homologue of the heat shock protein HtrA (high temperature requirement protease A), is a key periplasmic protease and also functions as a chaperone at lower temperature.[2,26] The protease degrades proteins that

remain unfolded in the periplasm or prevents aggregation of proteins under stress conditions and thereby protecting the cell from their detrimental effects.[2,21,27] Below 28°C DegP behaves mainly as chaperon, but above this temperature the function as a protease predominates and it degrades unfolded proteins.[2,26] The synthesis of DegP is linked to the Cpx (conjugation plasmid expression) systems and the "stress" sigma factor, σ^E, which is secreted when the cell undergoes extracellular stress that result in protein misfolding.[2,25] As mentioned before, "Cpx is a stress response system that controls cell envelope damages via proteases and folding helpers activation."[2]

The compact structure of DegP is achieved by a disulfide bridge between residues Cys57 and Cys69.[26] DegP occurs either as a trimer or as a hexamer and can exist in active and inactive forms.[26] The trimeric DegP is the minimal functional unit and capable of performing protease and chaperonic activities, the hexameric DegP is just formed in the absence of substrate.[26,27] In the presence of substrate several trimers can oligomerize into 12-mer and 24-mer globular cages consisting of either four or eight identical trimeric units.[27] To gain access to the catalytic site, the DegP substrates must be partially unfolded with the presence of paired hydrophobic amino acids (Ile, Val, Met, Leu, Ala).[2,25]

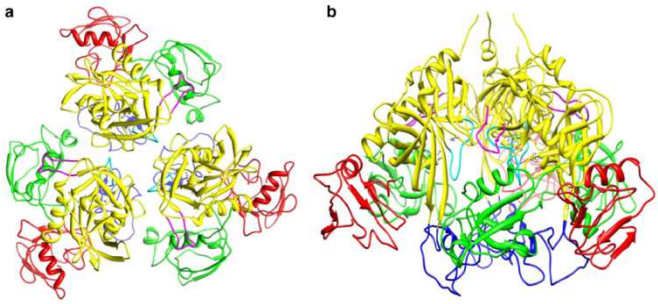

Figure 6: DegP-trimer in top (a) and side (b) view (p. 331)[26]

FkpA

FkpA is a member of the **FK**506-Binding-**P**roteins (FKBP family) of the peptidyl-prolyl *cis-trans* isomerises (PPIases) and is involved in gene expression, signal transduction and protein secretion.[2,28,29] Its PPIase activity was found to be among the highest of any such enzyme but the chaperonic effect is presumed to be independent of this activity.[30,31] FkpA prevents the aggregation of the early folding intermediates and can reactivate inactive proteins by its folding-assisting functions.[31] Gene deletion studies by X. Ge *et al.* revealed that FkpA increases in binding rate and affinity as chaperone for OMPs at the temperature of 44°C and is therefore functionally redundant with SurA for cell growth and OMP biogenesis under heat shock conditions.[32] The study also showed, that FkpA act as a multicopy suppressor for a lethal phenotype at heat shock conditions of a Δ*surA* Δ*skp* double-deletion strain of *E. coli* but did not influence the lethality at normal growth temperatures (30°C and 37°C) and result in

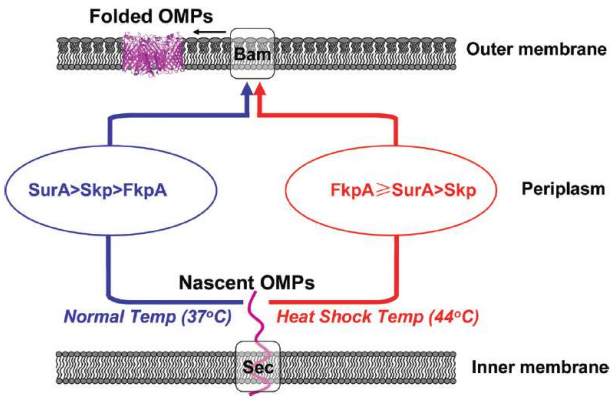

Figure 7: Scjematic illustration for the temperatue dependentcy of FkpA (p. 678)[32]

defect OMPs.[32] The unfolded precursor form, the unfolded mature form and the folded form of the OmpC as well as the OmpF bound to FkpA and therefore could reflect the folding process of the proteins assisted by FkpA.[32] The data presented by X. Ge *et al*. could lead to the provisional conclusion that the chaperon activity of FkpA vary from a low activity at the normal temperature up to a 20-fold higher activity at the heat shock temperature and is therefore even more importantly as SurA or Skp for OMP biogenesis at high temperatures.[32]

LolA

LolA, a lipoprotein-specific periplasmic carrier protein, assists the transport of lipoproteins across the periplasm to the outer membrane by forming a water-soluble lipoprotein-LolA complex in the periplasm.[2,33–35]

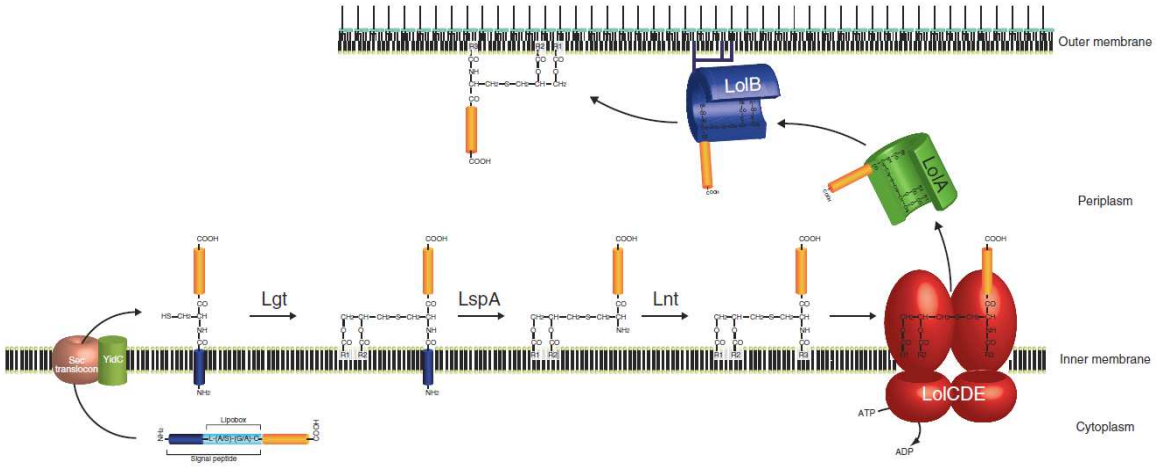

Figure 8: Biosynthesis and outer membrane localization of lipoproteins (p. 1045)[33]

The lipoproteins are transported there by the **l**ipoprotein **o**uter membrane **l**ocalization (Lol) pathway, which is also responsible for the naming of the transport system.[34,36] LolA plays an important role in the sorting of lipoproteins and their localisation at the outer membrane.[35] In *E. coli*, LolA and four additional proteins (LolB-LolE) are involved in the transport of lipoproteins to the periplasmic surface of the membrane, but only LolA is a periplasmic chaperone, whereas the other proteins are driven by ATP hydrolysis.[2,36,37] LolCDE function as

an ABC-transporter and release OMPs from the inner membrane into the periplasm to LolA, causing a formation of a complex with a 1:1 stoichiometry.[34,37] The lipoprotein-LolA complex interacts in the next step with the OM receptor LolB, thereby transfers the lipoprotein to LolB and gets in a final step localized to the outer membrane.[35,37] The LolA/B fold is assumed to be flexible which would allow the protein to bind one to three acyl chains in a hydrophobic cavity and the remaining chains internally and therefore fit perfectly to the hydrophobic cavity of this complex.[34]

LolA was the first identified component of the Lol-pathway and verified as a key factor in periplasmic lipoprotein transport by observing the lethality of a *lolA* knockout strain due to the accumulations of outer membrane lipoproteins in the inner membrane.[34] The amino acid residue at the second position directs the localisation of the protein.[35] An Asp residue is specific for an attachment to the inner membrane, whereas the other 19 remaining residues direct lipoproteins to the outer membrane.[35] And an Arg residue at position 43 plays a major role at the transfer process of lipoproteins to the outer membrane receptor LolB.[35]

DsbA and DsbB

The Dsb system (for "**dis**ulfide **b**ond") possesses both disulfide oxidation and isomerisation pathways and represents a further key step in the protein-folding pathways by enabling the formation of a disulfide bond between two cysteine residues to stabilize a protein structure.[38,39]

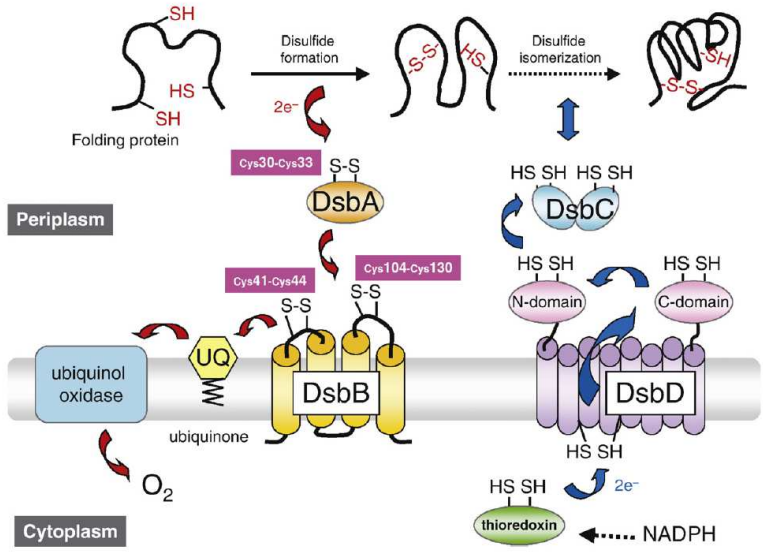

Figure 9: disulfide bond acquisition (p. 521)[42]

The oxidative pathway in *E. coli* is formed by the two proteins DsbA and DsbB and is involved in the maturation process of at least 300 different proteins secreted to the periplasm.[38,40] DsbA has the second highest redox potential among proteins with a thioredoxin (TRX) domain and functions as strong thiol oxidant by supporting the formation of disulfide bonds into newly synthesized proteins.[38–41] "The thioredoxin-fold of DsbA consists of a fivestranded β-sheet and three α-helices."[39] The catalytic CXXC motive

(Cys30-Pro31-His32-Cys33) is hyper-reactive and located at the N-terminus of the first α-helix of the TRX domain.[38,41,42] It represents a characteristic catalytic motive, found in most periplasmic Dsb proteins and is surrounded by a groove with an uncharged surface.[39]

The main disulfide donor in the periplasmic space, DsbA, has a broad substrate specificity and binds primarily reduced peptides via hydrophobic interactions to form disulfide bonds through disulfide exchange.[38,39,42] The formation of the disulfide bonds occur within seconds after the synthesis of the peptide and simultaneously lead to the reduction of DsbA.[39] The reduced form of DsbA is much more inflexible as the oxidized form, but only has to be recognized by the membrane protein DsbB for reoxidation.[39,43] Beside a similar core structure and a CXXC motive, DsbB also has a second pair of cysteins to oxidize the reduced DsbA and therefore regenerate it.[43,44] To regenerate a disulfide bond in DsbA, DsbB first oxidizes the CXXC motif of DsbA, than transport the electrons from the first pair of Cys residues (Cys104 and Cys130) to the second (Cys41 and Cys44) and finally transfer the electrons, by using ubiquinone as cofactor, to oxygen.[39–41]

DsbC and DsbD

The Dsb oxidative system is not specific and can introduce non-native disulfide bonds in substrates that possess more than two cysteins.[38,39] "For example, a protein with four pairs of cysteins has less than a 1% chance of attaining the correct four disulfides by random oxidation."[40] These incorrect disulfide bonds have to be corrected by the proofreading mechanism of the two Dsb isomerases, DsbC and DsbG, to prevent protein misfolding and aggregation.[38,39,44] The isomerases share 24% sequence identity as well as 49% sequence similarity and both have a CXXC active site motive.[40,44] Together, they form a V-shape dimer with a hydrophobic cavity which is kept in its active reduced form by the membrane protein DsbD to facilitate a nucleophilic attack at non-native disulfide by Cys98 of DsbC.[38,39,41,44] Each monomer of the V-shape dimer contains four conserved cystein residues, but only Cys98 and Cys101 of DsbC are arranged in a CXXC catalytic motive in the C-terminal domain.[39] The C-terminal domain also has a TRX-fold whereas six-stranded anti-parallel β-sheets of the N-terminal domain form the dimerization domain of the dimer.[39] The dimerisation domain is involved in the chaperone activity of DsbC and prevent the oxidation of the V-shape dimer by DsbB.[39]

The integral membrane protein DsbD can bind to the V-cavity of DsbC and has three different domains: an N-terminal (α-domain), a transmembrane (β-domain) and a C-terminal (γ-domain).[39,41] The Cys100 of the α-domain of DsbD (DsbDα) is able to interact with the

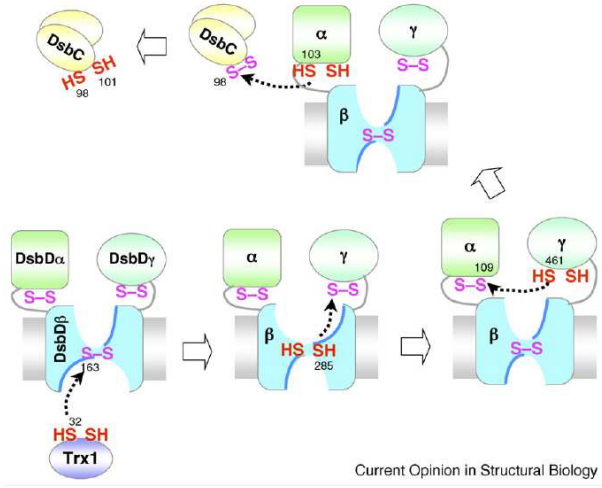

Figure 10: Dsb domains (p. 455)[41]

Cyc98 and can form a disulfide bond.[41] This bond is necessary to transfer electrons from DsbD to the periplasmic protein disulfide isomerases.[39] In return DsbD receives electrons from the cytoplasmic TRX which is kept reduced by a NADPH dependent TRX reductase.[39] This cascade is necessary to generate a kinetic barrier and to prevent the oxidation of DsbD by DsbA. [39]

Stress-induced periplasmic chaperones

HdeA and HdeB

HdeA stands for "**H**NS-**d**ependent **e**xpression **A**" and is a periplasmic chaperone that seem to specifically function under stress conditions at low pH.[2,45–49] The expression of the gene *hdeA* is induced under acidic conditions by a pH below 3 and repressed by the histone-like nucleoid structuring protein (HNS protein), which is also responsible for the naming.[45,46,49] The activation of the chaperone is triggered by the dissociation of the dimeric HdeA into its monomers between pH 3 and 1,5 and thereby it exposes its hydrophobic surfaces to bind denatured substrate.[46,49–52] The dissociation into monomers is fully reversible by increasing the pH- value over 3.[46,51] Under physiological conditions, the chaperone is inactive and unable to bind to other proteins.[46,51] Beside HdeA there is a second highly expressed and related protein, HdeB, which also belongs to the *hdeAB* acid stress operon.[51,52] HdeA and HdeB have a similar size (9,7 kDa and 9 kDa after signal sequence cleavage) and share a high structure similarity, despite the low sequence identity of 13%, but operate at different pH optima (pH 2 and pH 3).[47,49,51,52] HdeA and HdeB cause a over 100- to 1000-fold improvement in acid survival and protect some periplasmic proteins, like the chaperone SurA, alcohol dehydrogenase or glyceraldehydes-3-phosphate dehydrogenase, from acid-induced aggregation.[47,52]

Spy

The small periplasmic protein, **s**pheroplast **p**rotein **y** (Spy), is an ATP independent chaperone with a mostly unknown physiological function.[53–56] Even at the presence of sub-

stoichiometric concentrations Spy is, *in vitro*, capable to inhibit both aggregation and promote folding, but, *in vivo*, the *spy*-deletion mutant (*Δspy*) did not show any signs of a defect, neither under normal growth conditions nor under stress conditions.[54,57] Structure analysis show that Spy shares 25,5% structure identity with the negative regulator of the conjugation plasmid expression system (CpxP), suggesting that Spy could play a major role in the envelope stress response (ESR) systems, and therefore be classified as stress-induced periplasmic chaperone.[54] When the cell undergoes a spheroplast formation (an almost completely removed cell wall), the two ESR systems, Cpx and Bae (bacterial adaptive response two-component system), regulate the overexpression of Spy.[54,58] Conversely, the spheroplast protein y cannot be detected in normal cells.[54] Beside the induction during the spheroplasting, the *spy* expression could be activated by an exposure to alkaline pH, unfolding agents like ethanol, a copper shock for a few minutes or in the presence of zinc.[53–55]

Even though the function of Spy is not yet understood, the crystal structure is well-known.[54] The protein occurs as a stable dimer with N- and C-terminal LTXXQ (Leu-Thr-X-X-Gln) motifs, stabilizing the overall fold.[53,54,56] The antiparallel dimer is formed by four α-helices, arranged in a long kinked hairpin-like structure and incorporate three conserved regions that contain identical residues.[54] Based on the structure identity with CpxP and the similar conserved sequence motifs, it is likely that Spy and CpxP even share the same fold.[54]

Medical treatment

The origin of protein expression

The use of the periplasmic compartment in order to produce biological active proteins was developed around 1980 with the synthesis and secretion of human proinsulin.[59] The production of human proinsulin was a major breakthrough in science and demonstrated that it was possible to use and engineer a bacterial host cell to fulfil special requirements to make them ready for industrial use. During the production of a highly expressed recombinant protein there are numerous issues, like the formation of insoluble inclusion bodies.[60] Since the early 1990 scientists try to increase the active yield of protein and one of the most promising ways to do this, is to apply stress to the cell or engineer the cell to independently produce folding helpers.[60]

scFvs (single-chain variable fragments)

Nowadays there is a strong trend to manufacture genetically engineered antibodies, including Fab fragments (**F**ragment **a**ntigen **b**inding), Fv fragments (**v**ariable **f**ragments) and scFvs (**s**ingle-**c**hain **v**ariable **f**ragment**s**).[61–66] Those fragments are part of the paratope, the upper tips of the antibodies.[63] On the other hand the region of the paratope is designed to connect to a specific epitope, a region displayed on the corresponding antigen.[63] Accordingly this mechanism is part of the immune system and build a variable and highly adaptable defence system, that is capable to detect, identify and neutralize infected cells and foreign objects.[63] All of the previous mentions antibody fragments can also serve as an anti-carcinogen alternative to full-length monoclonal antibodies and possess several unique properties.[61–63] One of the most significant abilities is, that these "cropped" antibodies retain their original antigen-binding activity (compared to the full-length monoclonal antibody) and therefore are extremely valuable for therapeutic applications or medical diagnostic.[62] They also got a potentially better tumor penetration, a rapid blood clearance and a reduced immunogenicity over whole antibodies.[61,62]

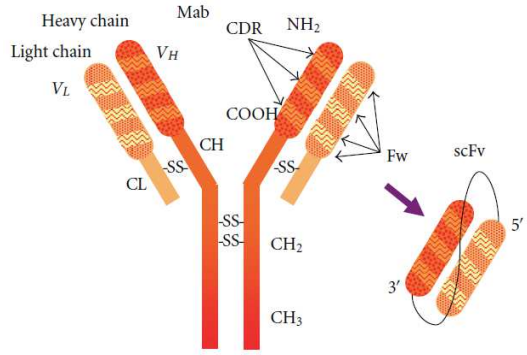

Figure 11: Antibody model (p. 3)[63]

A special focus in today's research lies on single-chain variable fragments.[61–66] "An antibody in scFv (single chain fragment variable) format consist of variable regions of heavy (V_H) and light (V_L) chains, which are joined together by a flexible peptide linker [...]."[63] There are many advantages and disadvantages for the utilization of scFvs at medical treatment and especially for the design of the expression system in different host cells (bacterial, mammalian cell, yeast, plant and insect cells).[63] scFvs can also be economically expressed within a standard laboratory equipment, possess well-established fermentation methods and provide yields up to 10-30% of total cellular protein.[61,63] The expression of the scFv antibody using a bacterial host cell can take place directly in the cytoplasm or into the periplasmic space of *E. coli*.[63] A cytoplasmic expression excludes the need of a signal peptide to guide the peptide to its destination and enables a very high expression rate of the polypeptide.[63] Due to the reducing environment of the bacterial cytoplasm, the formation of insoluble inclusion bodies cannot be prohibited and a rearrangement of the disulfide bonds in order to correctly fold the polypeptide is necessary.[63] "To overcome this problem, a signal peptide is used to direct secretion of the scFv antibody into the periplasmic space [...]."[63] Various folding helpers, such as disulfide

isomerases (can alternatively link the two peptides) are able to assist the proper folding in the periplasmic space and therefore periplasmic expression is a very common technique in scFv expression.[63]

Co-expression of chaperones

A secretion of scFv antibodies into the periplasmic space often goes hand in hand with a co-expression of molecular chaperones.[64–66] A single-chain variable fragment against human IGF-1R (type 1 insulin-like growth factor receptor) needs the co-expression of a least one Dsb-chaperone in order to correctly fold into the periplasmic space of *E. coli*.[64] The co-expression of DsbC alone can already recover up to 50% of the misfolded polypeptides.[64] A multiple co-expression of DsbA and DsbC in combination with host specific chaperones and foldases obtained the best results and showed a maximum solubility up to 80% of the scFvs.[64] The recovered antigen-fragments presented full antigen-binding activity and therefore confirmed the positive influence of Dsb members to the solubility and activity of scFvs.[64]
Beside members of the Dsb family, some other chaperones (like SkpA) improved antigen binding activity compared to when at least one of the chaperones is missing.[64,65] The co-expression of SkpA enables "to obtain higher yields of soluble antibody fragments from cultures without the need for supplementation [such as sucrose, betaine, or sorbitol] of the culture medium during expression."[65] The co-expression of SkpA is often linked to an IPTG-inducible LacZ promoter to start the expression of the chaperones and the scFvs at an appropriate cell density in order to maximise the active yields.[65] Toxic accumulations of poorly folded protein residues can be reduced when Skp chaperones were co-expressed during the production phase of scFvs.[65,66]

Closing words

Beside their assisting abilities in protein folding, molecular chaperones are able to do a lot more. There are still many parts to reveal and uncover but the field of application growth with every day. The future scope of science suggests a major involvement in developing effective methods to inhibit cancer cell growth, induce adjusted cell death and to fight or even cure debilitating diseases.[64]

Finally I can only say: "*I am thrilled to know what the future holds*."

List of abbreviations

ABC	ATP-binding cassette
Ala	alanine (amino acid)
Arg	arginine (amino acid)
Asp	aspartic acid (amino acid)
ATP	adenosine triphosphate
Bae system	bacterial adaptive envelope stress response system
BeaR	response regulator of the Bea system
BeaS	membrane localized histidine kinase of the Bea system
BAM	β-barrel assembly machinery
CDR	Complementarity Determining Region
Cpx system	conjugation/ conjugative plasmid expression system
CpxA	sensor histidine kinase A of the Cpx system
CpxP	small periplasmic inhibitor protein of the Cpx system
CpxR	cytoplasmic response regulator of the Cpx system
cpxRA	gene with the information for the CpxA and CpxR protein
CXXC motive	amino acid structure motive (Cys-X-X-Cys)
Cys	cysteine (amino acid)
Dsb	for “disulfide bond” (DsbA, DsbB, ...)
DegP	for “degradation”, in literature also referred to protease Do
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
ESR	envelope stress response
Fab	Fragment antigen binding
FK506	“(Tacrolimus) is commonly used as an immunesuppressant to prevent the rejection of organ transplants.”[67]
FkpA	FK506-binding-protein
Fv	variable fragments
Gln	glutamine (amino acid)
HdeA	HNS-dependent expression A
HlpA	histone like protein A, also reffered to Skp
HNS	histone-like nucleoid structuring
HtrA	high temperature requirement protease A
IGF-1R	type 1 insulin-like growth factor receptor

Ile	isoleucine (amino acid)
IM	inner membrane/ cytoplasmic membrane
IMP	integral membrane protein
IPTG	isopropyl β-D-1-thiogalactopyranoside
kDa	kilo Dalton, unified atomic mass unit
LacZ	lac operon Z, "the gene product of *lacZ* is β-galactosidase"[68]
LamB	trimeric outer membrane porin for maltodextrins
Leu	leucine (amino acid)
Lgt	phosphatidylglycerol:prolipoprotein diacylglyceryl transferase
Lnt	apolipoprotein *N*-acyltransferase
Lol pathway	lipoprotein outer membrane localization pathway
Lol	lipoprotein-specific periplasmic carrier protein of the Lol-pathway (LolA, LolB, ...)
LspA	lipoproteinspecific signal peptidase A
LTXXQ motive	amino acid structure motive (Leu-Thr-X-X-Gln)
Met	methionine (amino acid)
NADPH	nicotinamide adenine dinucleotide phosphate
OM	outer membrane
OMP	outer membrane protein (OmpC, OmpF, ...)
OmpH	outer membrane protein H, also reffered to Skp
pH	pondus hydrogenii, potential of hydrogen
PPIase	peptidyl-prolyl *cis-trans* isomerise (PpiA, PpiD, ...)
RNA	ribonucleic acid
scFv	single-chain variable fragment
Skp	seventeen kilodalton protein
Spy	spheroplast protein y
SurA	survival factor A
T2S	type II secretion
Thr	threonine (amino acid)
TRX	thioredoxin
TTSS	type III secretion systeme
Val	valine (amino acid)
V_H	variable region of the heavy chain of an antibody
V_L	variable region of the light chain of an antibody

Image index

Bibliography

1. Dobson, C. M. Protein folding and misfolding. *Nature* **426,** 884–890 (2003).
2. Goemans, C., Denoncin, K. & Collet, J.-F. Folding mechanisms of periplasmic proteins. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1843,** 1517–1528 (2014).
3. Hartl, F. U., Bracher, A. & Hayer-Hartl, M. Molecular chaperones in protein folding and proteostasis. *Nature* **475,** 324–332 (2011).
4. Saibil, H. R. Chaperone machines in action. *Curr. Opin. Struct. Biol.* **18,** 35–42 (2008).
5. Entzminger, K. C., Chang, C., Myhre, R. O., McCallum, K. C. & Maynard, J. A. The Skp Chaperone Helps Fold Soluble Proteins *in Vitro* by Inhibiting Aggregation. *Biochemistry (Mosc.)* **51,** 4822–4834 (2012).
6. Rouvière, P. E. & Gross, C. A. SurA, a periplasmic protein with peptidyl-prolyl isomerase activity, participates in the assembly of outer membrane porins. *Genes Dev.* **10,** 3170–3182 (1996).
7. Qu, J., Mayer, C., Behrens, S., Holst, O. & Kleinschmidt, J. H. The Trimeric Periplasmic Chaperone Skp of Escherichia coli Forms 1:1 Complexes with Outer Membrane Proteins via Hydrophobic and Electrostatic Interactions. *J. Mol. Biol.* **374,** 91–105 (2007).
8. Cell envelope - Wikipedia. Available at: https://en.wikipedia.org/wiki/Cell_envelope. (Accessed: 19th March 2017)
9. Grabowicz, M. & Silhavy, T. J. Envelope Stress Responses: An Interconnected Safety Net. *Trends Biochem. Sci.* **42,** 232–242 (2017).

10. Raivio, T. L. MicroReview: Envelope stress responses and Gram-negative bacterial pathogenesis: Envelope stress responses and virulence. *Mol. Microbiol.* **56,** 1119–1128 (2005).
11. Delhaye, A., Collet, J.-F. & Laloux, G. Fine-tuning of the Cpx envelope stress response is required for cell wall homeostasis in Escherichia coli. *MBio* **7,** e00047–16 (2016).
12. Duguay, A. R. & Silhavy, T. J. Quality control in the bacterial periplasm. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1694,** 121–134 (2004).
13. Heusipp, G., Nelson, K. M., Schmidt, M. A. & Miller, V. L. Regulation of *htrA* expression in *Yersinia enterocolitica*. *FEMS Microbiol. Lett.* **231,** 227–235 (2004).
14. Raivio, T. L. Everything old is new again: An update on current research on the Cpx envelope stress response. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1843,** 1529–1541 (2014).
15. Buelow, D. R. & Raivio, T. L. Cpx Signal Transduction Is Influenced by a Conserved N-Terminal Domain in the Novel Inhibitor CpxP and the Periplasmic Protease DegP. *J. Bacteriol.* **187,** 6622–6630 (2005).
16. Price, N. L. & Raivio, T. L. Characterization of the Cpx Regulon in Escherichia coli Strain MC4100. *J. Bacteriol.* **191,** 1798–1815 (2009).
17. Bitto, E. & McKay, D. B. Crystallographic structure of SurA, a molecular chaperone that facilitates folding of outer membrane porins. *Structure* **10,** 1489–1498 (2002).
18. WikiGenes - Collaborative Publishing. *WikiGenes - Collaborative Publishing* Available at: http://www.wikigenes.org/. (Accessed: 15th February 2017)
19. Stymest, K. H. & Klappa, P. The periplasmic peptidyl prolyl cis-trans isomerases PpiD and SurA have partially overlapping substrate specificities: Substrate interactions of periplasmic PPIases. *FEBS J.* **275,** 3470–3479 (2008).
20. Justice, S. S. *et al.* Periplasmic Peptidyl Prolyl cis-trans Isomerases Are Not Essential for Viability, but SurA Is Required for Pilus Biogenesis in Escherichia coli. *J. Bacteriol.* **187,** 7680–7686 (2005).
21. Walton, T. A. & Sousa, M. C. Crystal structure of Skp, a prefoldin-like chaperone that protects soluble and membrane proteins from aggregation. *Mol. Cell* **15,** 367–374 (2004).
22. Jarchow, S., Lück, C., Görg, A. & Skerra, A. Identification of potential substrate proteins for the periplasmic Escherichia coli chaperone Skp. *PROTEOMICS* **8,** 4987–4994 (2008).
23. Assembly of Outer Membrane β-Barrel Proteins: the Bam Complex. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4231818/. (Accessed: 17th February 2017)

24. Structural basis of outer membrane protein insertion by the BAM complex. - PubMed - NCBI. Available at: https://www.ncbi.nlm.nih.gov/pubmed/26901871. (Accessed: 17th February 2017)
25. Jones, C. H. *et al.* Escherichia coli DegP Protease Cleaves between Paired Hydrophobic Residues in a Natural Substrate: the PapA Pilin. *J. Bacteriol.* **184,** 5762–5771 (2002).
26. Rai, N. & Ramaswamy, A. Temperature dependent dynamics of DegP-trimer: A molecular dynamics study. *Comput. Struct. Biotechnol. J.* **13,** 329–338 (2015).
27. Iwanczyk, J., Leong, V. & Ortega, J. Factors Defining the Functional Oligomeric State of Escherichia coli DegP Protease. *PLoS ONE* **6,** e18944 (2011).
28. FKBP - Lexikon der Biochemie - Spektrum der Wissenschaft. Available at: http://www.spektrum.de/lexikon/biochemie/fkbp/2204. (Accessed: 24th February 2017)
29. Kallscheuer, N., Bott, M., van Ooyen, J. & Polen, T. Single-Domain Peptidyl-Prolyl *cis* / *trans* Isomerase FkpA from Corynebacterium glutamicum Improves the Biomass Yield at Increased Growth Temperatures. *Appl. Environ. Microbiol.* **81,** 7839–7850 (2015).
30. Bothmann, H. The Periplasmic Escherichia coli Peptidylprolyl cis,trans-Isomerase FkpA. I. INCREASED FUNCTIONAL EXPRESSION OF ANTIBODY FRAGMENTS WITH AND WITHOUT cis-PROLINES. *J. Biol. Chem.* **275,** 17100–17105 (2000).
31. Ramm, K. & Plückthun, A. The periplasmic Escherichia coli peptidylprolyl cis, trans-isomerase FkpA II. Isomerase-independent chaperone activity in vitro. *J. Biol. Chem.* **275,** 17106–17113 (2000).
32. Ge, X. *et al.* Identification of FkpA as a Key Quality Control Factor for the Biogenesis of Outer Membrane Proteins under Heat Shock Conditions. *J. Bacteriol.* **196,** 672–680 (2014).
33. Narita, S. ABC Transporters Involved in the Biogenesis of the Outer Membrane in Gram-Negative Bacteria. *Biosci. Biotechnol. Biochem.* **75,** 1044–1054 (2011).
34. Zückert, W. R. Secretion of Bacterial Lipoproteins: Through the Cytoplasmic Membrane, the Periplasm and Beyond. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1843,** 1509–1516 (2014).
35. Miyamoto, A., Matsuyama, S. & Tokuda, H. Mutant of LolA, a Lipoprotein-Specific Molecular Chaperone of Escherichia coli, Defective in the Transfer of Lipoproteins to LolB. *Biochem. Biophys. Res. Commun.* **287,** 1125–1128 (2001).
36. Konovalova, A. & Silhavy, T. J. Outer membrane lipoprotein biogenesis: Lol is not the end. *Philos. Trans. R. Soc. B Biol. Sci.* **370,** 20150030 (2015).

37. Tokuda, H. & Matsuyama, S. Sorting of lipoproteins to the outer membrane in E. coli. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1693,** 5–13 (2004).
38. Heras, B. *et al.* DSB proteins and bacterial pathogenicity. *Nat. Rev. Microbiol.* **7,** 215–225 (2009).
39. Messens, J. & Collet, J.-F. Pathways of disulfide bond formation in Escherichia coli. *Int. J. Biochem. Cell Biol.* **38,** 1050–1062 (2006).
40. Nakamoto, H. & Bardwell, J. C. A. Catalysis of disulfide bond formation and isomerization in the Escherichia coli periplasm. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1694,** 111–119 (2004).
41. Ito, K. & Inaba, K. The disulfide bond formation (Dsb) system. *Curr. Opin. Struct. Biol.* **18,** 450–458 (2008).
42. Inaba, K. & Ito, K. Structure and mechanisms of the DsbB–DsbA disulfide bond generation machine. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1783,** 520–529 (2008).
43. Inaba, K. *et al.* Crystal Structure of the DsbB-DsbA Complex Reveals a Mechanism of Disulfide Bond Generation. *Cell* **127,** 789–801 (2006).
44. Heras, B., Kurz, M., Shouldice, S. R. & Martin, J. L. The name's bond……disulfide bond. *Curr. Opin. Struct. Biol.* **17,** 691–698 (2007).
45. Quan, S. & Bardwell, J. C. A. Chaperone discovery. *BioEssays* **34,** 973–981 (2012).
46. Hong, W. *et al.* Periplasmic Protein HdeA Exhibits Chaperone-like Activity Exclusively within Stomach pH Range by Transforming into Disordered Conformation. *J. Biol. Chem.* **280,** 27029–27034 (2005).
47. Hong, W., Wu, Y. E., Fu, X. & Chang, Z. Chaperone-dependent mechanisms for acid resistance in enteric bacteria. *Trends Microbiol.* **20,** 328–335 (2012).
48. HdeA family - Wikipedia. Available at: https://en.wikipedia.org/wiki/HdeA_family. (Accessed: 10th March 2017)
49. Gajiwala, K. S. & Burley, S. K. HDEA, a periplasmic protein that supports acid resistance in pathogenic enteric bacteria. *J. Mol. Biol.* **295,** 605–612 (2000).
50. Bardwell, J. C. A. & Jakob, U. Conditional disorder in chaperone action. *Trends Biochem. Sci.* **37,** 517–525 (2012).
51. Wang, W. *et al.* Salt Bridges Regulate Both Dimer Formation and Monomeric Flexibility in HdeB and May Have a Role in Periplasmic Chaperone Function. *J. Mol. Biol.* **415,** 538–546 (2012).
52. Kern, R., Malki, A., Abdallah, J., Tagourti, J. & Richarme, G. Escherichia coli HdeB Is an Acid Stress Chaperone. *J. Bacteriol.* **189,** 603–610 (2007).

53. Srivastava, S. K., Lambadi, P. R., Ghosh, T., Pathania, R. & Navani, N. K. Genetic regulation of spy gene expression in Escherichia coli in the presence of protein unfolding agent ethanol. *Gene* **548,** 142–148 (2014).
54. Kwon, E., Kim, D. Y., Gross, C. A., Gross, J. D. & Kim, K. K. The crystal structure *Escherichia coli* Spy: The Crystal Structure of EcSpy. *Protein Sci.* **19,** 2252–2259 (2010).
55. Yamamoto, K., Ogasawara, H. & Ishihama, A. Involvement of multiple transcription factors for metal-induced spy gene expression in Escherichia coli. *J. Biotechnol.* **133,** 196–200 (2008).
56. Appia-Ayme, C. *et al.* ZraP is a periplasmic molecular chaperone and a repressor of the zinc-responsive two-component regulator ZraSR. *Biochem. J.* **442,** 85–93 (2012).
57. Powers, E. T. & Balch, W. Protection from the outside. *Nature* **471,** 42–43 (2011).
58. Spheroplast - Wikipedia. Available at: https://en.wikipedia.org/wiki/Spheroplast. (Accessed: 18th March 2017)
59. Chan, S. J. *et al.* Biosynthesis and periplasmic segregation of human proinsulin in Escherichia coli. *Proc. Natl. Acad. Sci.* **78,** 5401–5405 (1981).
60. Blackwell, J. R. & Horgan, R. A novel strategy for production of a highly expressed recombinant protein in an active form. *FEBS Lett.* **295,** 10–12 (1991).
61. Freyre, F. M. *et al.* Very high expression of an anti-carcinoembryonic antigen single chain Fv antibody fragment in the yeast Pichia pastoris. *J. Biotechnol.* **76,** 157–163 (2000).
62. Cheng, C.-M. *et al.* Functional Production of a Soluble and Secreted Single-Chain Antibody by a Bacterial Secretion System. *PLoS ONE* **9,** e97367 (2014).
63. Ahmad, Z. A. *et al.* scFv Antibody: Principles and Clinical Application. *Clin. Dev. Immunol.* **2012,** 1–15 (2012).
64. Sun, X.-W., Wang, X.-H. & Yao, Y.-B. Co-expression of Dsb proteins enables soluble expression of a single-chain variable fragment (scFv) against human type 1 insulin-like growth factor receptor (IGF-1R) in E. coli. *World J. Microbiol. Biotechnol.* **30,** 3221–3227 (2014).
65. Mavrangelos, C. *et al.* Increased Yield and Activity of Soluble Single-Chain Antibody Fragments by Combining High-Level Expression and the Skp Periplasmic Chaperonin. *Protein Expr. Purif.* **23,** 289–295 (2001).
66. Andrew, H. & Harris, William J. Escherichia coli Skp Chaperone Coexpression Improves Solubility and Phage Display of Single-Chain Antibody Fragments. *Protein Expr. Purif.* **15,** 336–343 (1998).

67. FK506 (Tacrolimus) - Calcineurin inhibitor - immunosuppressor FK-506. Available at: http://www.invivogen.com/fk506. (Accessed: 24th February 2017)
68. lac operon - Wikipedia. Available at: https://en.wikipedia.org/wiki/Lac_operon. (Accessed: 23rd June 2017)