1
Metabolic Adaptation of Human Pathogenic *Yersiniae*

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Abstract

Colonization, subsequent penetration of epithelial layers as well as persistence and proliferation in subepithelial tissues of the host by bacterial pathogens demand the expression of special sets of virulence factors. In addition, the bacteria need to adapt their metabolism to survive and replicate within the specific host niches. Activated metabolic functions and physiological adaptation processes during their life cycle and the different stages of the infection reflect the complex and dynamic nutritional resources of their environments, interbacterial competition for energy sources and onslaught of bactericidal host responses. The enteric pathogenic *Yersinia* species *Y. pseudotuberculosis* and *Y. enterocolitica* and the causative agent of plague, *Y. pestis*, have adapted to grow in many different environmental reservoirs (e.g., soil, plants, insects) and in warm-blooded animals (e.g., rodents, pigs, humans) with a preference for lymphatic tissues. In the present book chapter, we discuss metabolic adaptations of human pathogenic *yersiniae* to successfully exploit available nutrients and metabolic functions during infection and illustrate the tight link between carbon metabolism and *Yersinia* virulence. Furthermore, current knowledge about the complex regulatory networks used to coordinate and fine-tune the control of metabolic and virulence functions are presented. Deciphering the mechanisms of the function and control of bacterial metabolism within host tissues will not only increase our understanding of host–pathogen interactions, it will also facilitate the identification of potential novel drug targets for future prevention and therapeutic strategies.

Introduction

Infections of human pathogenic *yersiniae* involves a large number of specific pathogenicity factors that mediate efficient resistance against the host defense systems and enable the bacteria to colonize, invade, and multiply successfully within host tissues. The structure, function, and expression of many of these classical

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virulence factors have been characterized, and their role in pathogenicity has been studied using different animal models. However, to become a successful pathogen, *yersiniae* must also adapt their metabolic functions to the nutrient/ion composition and the physical conditions (e.g., temperature, pH, oxygen tension) of their surrounding and coordinate their metabolism with their life cycle. These unspecific strategies were long neglected, but recent use of global omic-based profiling techniques, phenotypic microarrays, and the *in vivo* analysis of metabolic mutants allowed a deeper insight into nutrient sensing, sequestration, and utilization strategies that optimize the metabolism and biological fitness of *Yersinia* during infection.

Yersinia Life Cycles and Pathogenesis

Of the 17 species of the genus *Yersinia* only *Y. pseudotuberculosis*, *Y. enterocolitica,* and *Y. pestis* are known to cause diseases in mammals [1, 2]. The two enteric pathogens *Y. pseudotuberculosis* and *Y. enterocolitica* are the causative agents of yersiniosis, a gastrointestinal disease with a variety of symptoms such as enteritis, colitis, diarrhea, and mesenteric lymphadenitis, which becomes rarely systemic. Both enteropathogenic species are well adapted to survive long term in external habitats (e.g., ground water, soil, plants, and insects) and are able to persist and replicate in various wild and domestic animals [3, 4]. A recent study analyzing a large number of genomes revealed that they are heterotrophic pathogens that are able to utilize a large variety of C-/N-/energy sources [5]. In contrast, *Y. pestis*, the causal agent of plague, which has evolved as a separate clone from *Y. pseudotuberculosis*, shows a reduced metabolic flexibility based on functional gene loss. This may reflect its unique life cycle: (i) replication within the gastrointestinal tract (proventriculus) of infected fleas and (ii) proliferation in the lymphatic system, blood, or tissues of mammals, in particular rodents [6].

All *yersiniae* are zoonotic pathogens armored with diverse cell envelope–associated virulence structures that either promote host–pathogen interactions or contribute to *Yersinia* pathogenicity by suppression of the host immune response. In case of the enteric *Yersinia* species, initial attachment and invasion of the intestinal layer is mediated by the primary invasion factor invasin (InvA), but other adhesive surface-exposed proteins, for example, homologous Inv-type adhesins (InvB/Ifp, InvC), Ail, the autotransporter adhesin YadA and the PsaA (pH6 antigen)/Myf fimbriae appear to support the dissemination process at later stages of the infection [7, 8]. In *Y. pestis* mainly adhesins Ail and PsaA contribute to host–pathogen interactions, whereas other adhesin/invasin genes, for example, *invA* and *yadA* became unfunctional [9, 10]. Moreover, all pathogenic *yersiniae* evolved mechanisms that mediate resistance against the innate immune response. Several adhesins protect the bacteria against complement killing (e.g., Ail and YadA) or prevent phagocytosis (e.g., PsaA) [7]. Furthermore, they possess a 70-kDa virulence plasmid (pYV/pCD1) that encodes the Ysc (Yersinia secretion)-Yop type III secretion system (T3SS). This needle-like delivery machine (injectisome) enables the bacteria to inject different Yops (*Yersinia* outer proteins) effector toxins from the bacterial cytoplasm

into the cytosol of host cells, in particular professional phagocytes [11]. *Yersinia* pathogenicity relies on the following crucial functions of translocated Yop effector proteins: (i) antiphagocytic activity by manipulation and destruction of the actin cytoskeleton; (ii) suppression of cytokine production by macrophages, dendritic cells, and neutrophils; and (iii) induction of host cell death [11].

Carbon Metabolism and Links to *Yersinia* Pathogenesis

External reservoirs, vector and animal environments colonized by *Yersinia* have likely driven the evolution of metabolic pathways to maximize present nutritional opportunities. Variations in certain metabolic functions might thus be a consequence of the adaptation to a specific host or host niche. A selective advantage can be gained either by acquisition of new metabolic functions, for example, by horizontal gene transfer, or by loss of function mutations that change the metabolic abilities of the pathogen. Furthermore, changes in the control mechanisms implicated in metabolic adaptation and regulatory strategies linking metabolic and virulence traits could manipulate the pathogen's response to varying nutrient availabilities in the environment.

Food Sources, Nutrient Sequestration, and Utilization

Animal tissues contain a large variety of different energy sources (e.g., sugars, amino acids, lipids, proteins) and can be regarded as a rich source of food for bacteria. In particular the digestive tract of mammals is nutrient rich and contains a large diversity of different nutritional substrates, which can be metabolized by enteric *yersiniae*. However, the pathogens have to compete successfully with the perfectly adapted resident microbiota. About 10^{14} bacteria form a complex microbial ecosystem of more than 400 species, in which strictly anaerobic bacteria degrade complex polysaccharides into simple carbohydrates, which are readily absorbed by the mammalian small intestine or used by other (facultative anaerobic) commensals such as *Escherichia coli* [12]. Furthermore, the host can rapidly change the availability of nutrients in host tissues based on the induction of inflammation and hypoxic conditions triggered by the immune response [13], and it can restrict access to essential ions such as magnesium, manganese, zinc, and iron [14, 15]. As a consequence, *Yersinia* needs to sense, retrieve, and metabolize nutrients more efficiently, or alternatively it must grow on available substrates, which are not used by other members of the competing microbiota. An important characteristic of many bacterial pathogens, including *Yersinia*, is their ability to sense and initiate use of readily digestible carbon sources by sophisticated global regulatory systems: (i) **carbon catabolite repression** (CCR) triggered in response of the availability of simple sugars, for example, glucose [16, 17] and (ii) the **carbon storage regulator/regulator of secondary metabolites** system (Csr/Rsm) [18, 19] (see also below: Coordinated control of carbon metabolism and virulence).

Metabolic Pathways of *Yersinia* Crucial for Virulence

All pathogenic *Yersinia* species possess a highly flexible and robust metabolic system with many redundant or alternative catabolic and biosynthetic pathways, which allow them to respond very rapidly and efficiently to changing nutrient concentrations. Simple sugars can be utilized via glycolysis (Embden–Meyerhof pathway), the pentose phosphate pathway and the Entner–Doudoroff pathway. They can further be catabolized by aerobic or anaerobic respiration via a complete tricarboxylic acid (TCA) cycle and a functional glyoxylate bypass, or via fermentation [20–22]. Many enzymes and metabolic pathways are conserved among the different *Yersinia* species, but several characteristic differences were also observed. Due to the loss of multiple metabolic genes, for example, the glucose 6-phosphate dehydrogenase gene *zwf Y. pestis* is unable to use glucose via the pentose phosphate pathway [20]. It further lacks the methionine salvage and the urease pathway, aspartase to mediate catabolism of glutamate to aspartate and is unable to synthesize several amino acids, including glycine, threonine, L-valine and L-isoleucine, L-phenylalanine, and L-methionine [23, 24], which makes the pathogen more dependent on mechanisms accessing host nutrients. An important specific feature of *Y. enterocolitica* is its ability to metabolize 1,2-propanediol and ethanolamine by cobalamin-dependent enzymes under anaerobiosis using tetrathionate as terminal electron acceptor [5]. Tetrathionate production is strongly induced upon inflammation [25], indicating that these metabolic properties are advantageous for *Y. enterocolitica* to outcompete the microbiota of the intestine. In contrast, *Y. pseudotuberculosis* and *Y. pestis* are able to metabolize itaconate by converting it into pyruvate and acetyl-CoA. Itaconate contributes to the antimicrobial activity of macrophages as it inhibits isocitrate lyase, a key enzyme of the glyoxylate cycle. Thus, itaconate degradation could allow *Yersinia* to persist in macrophages [26].

Nutritional Virulence: Nutritional Adaptation Important for Pathogenesis

Various "omic" approaches and transcriptional profiling studies with pathogenic *yersinia*e grown *in vitro* under different virulence-relevant conditions revealed numerous metabolic pathways and adaptive metabolic responses, which could contribute to pathogenesis. Important initial studies addressed temporal changes during a temperature shift from 26 to 37 °C, mimicking transmission of *Y. pestis* from the flea to mammals. They revealed that not only virulence genes but also numerous metabolic functions are under thermal control [27, 28]. Genes encoding for enzymes involved in nitrogen assimilation were strongly downregulated, whereas those required for efficient catabolism of amino acids were induced in *Y. pestis* grown *in vitro* at 37 °C. Some of these enzymes are responsible for the majority of released metabolic ammonia via reactions that directly or indirectly promote deamination during formation of α-keto acids entering the TCA cycle.

A thermal upshift caused a downregulation of glycolysis, whereby terminal oxidation of the available energy sources (carbohydrates, amino acids, and lipids) in the nutrient-rich medium was favored. This first *in vitro* study indicated that, in nature, *Y. pestis* prefers fermentative pathways in the flea vector, while oxidative catabolism is favored during rapid proliferation in the lymphatic systems of the mammalian host [27]. Moreover, differential expression of catabolic enzymes suggests that different sugars (e.g., maltose, gluconate, ribose) are utilized after temperature transition, and this metabolic switch appears to be crucial to trigger virulence. Two equivalent transcriptomic studies were directed to identify metabolic functions of *Y. pestis* required during septicaemic plague in humans and of *Y. pseudotuberculosis* during systemic infections. *In vitro* growth in media containing human plasma showed that in particular genes related to purine/pyrimidine metabolism were upregulated in plague bacilli and supported a previous report demonstrating that purine metabolism is crucial for *Y. pestis* pathogenicity [29, 30]. In *Y. pseudotuberculosis,* genes supporting the consumption of the plasma glucose (e.g., the glucose-specific phosphotransferase system (PTS)) were strongly upregulated [31]. This indicated that high growth rate aerobic cultivations on glucose induce an "overflow metabolism" channeling the carbon flow toward byproduct formation and secretion to balance accumulation of reducing equivalents (NADH) through the TCA cycle. In fact, our recent fluxome approach revealed that *Y. pseudotuberculosis* does not accumulate and excrete acetate like *E. coli* when grown on glucose; it spills large amount of pyruvate (46% of the glucose uptake). Preliminary results indicate that excretion of pyruvate by *Y. pseudotuberculosis* is achieved by a sustained glycolytic flux that is accompanied by a bottleneck in the TCA and a downregulation of acetate formation (Bücker *et al.*, [32]).

Over the past years also *in vivo* gene expression profiling was performed to gain a better insight into host–pathogen interactions and the metabolic activities that support persistence and replication of *Y. pestis* in the flea [33] and the mammalian host [34–36]. Numerous metabolic genes involved in the catabolism of amino acids, in particular the L-glutamate group (e.g., glutamine, histidine, arginine, proline) were found to be upregulated in *Y. pestis* located in the proventriculus of infected fleas [33] (Figure 1.1). This was interpreted as a special adaptation to the flea gut, which contains protein and lipid rich meals with relatively low amount of carbohydrates. Utilization of the L-glutamate group amino acids involves enzymes of the TCA cycle, which are upregulated in the flea vector [33]. In contrast, catabolism of carbohydrates seems less important as most sugar uptake systems are repressed or only slightly expressed. Only chitobiose, a PTS sugar present in the flea's proventriculus spines, is efficiently imported and metabolized (Figure 1.1).

Transcriptional profiling of *Y. pestis* located in the bubo in a rat model as well as in the lung of a murine pneumonic infection model was used to characterize the metabolic adaptation of *Y. pestis* to its mammalian host [34–36]. Notable is the strong induction of genes involved in iron acquisition (e.g., hemin uptake operon) and amino acid biosynthesis (e.g., histidine, glutamate, and aspartate), and downregulation of the TCA cycle and the ATP-proton motive force during pneumonic plague development [35, 36] (Figure 1.1). In parallel, genes encoding the

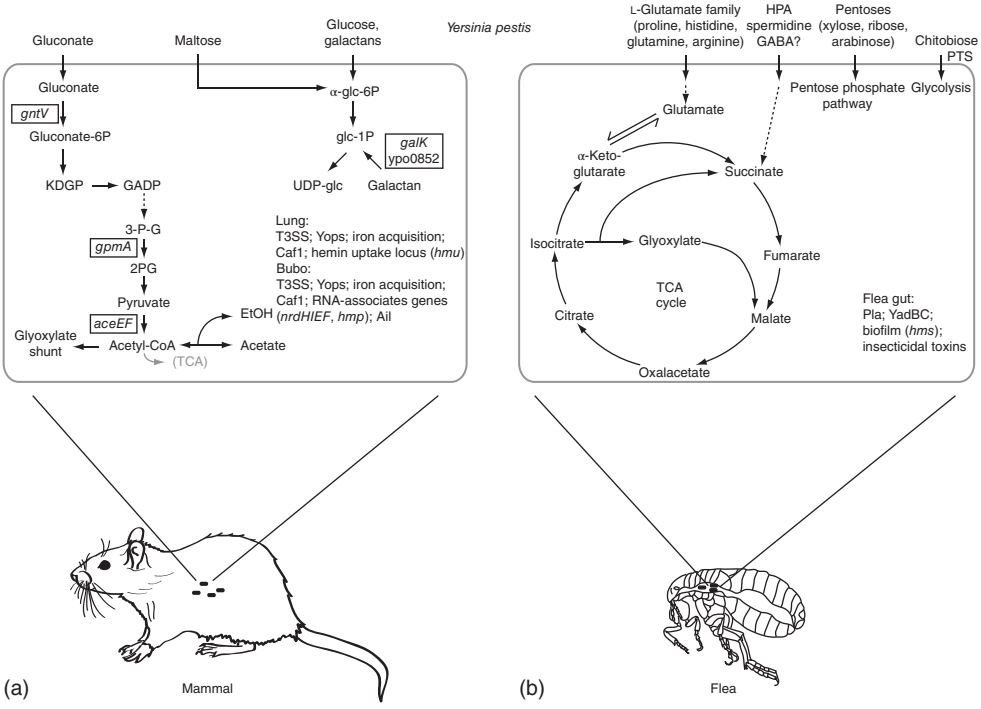

Figure 1.1 Metabolic pathways and virulence factors of *Y. pestis,* which are significantly induced in the mammalian host and the flea gut. Specific metabolic pathways and pathogenicity traits upregulated *in vivo* are presented, which are considered to be crucial for the colonization of the lung or bubo of the mammalian host (a) and the flea gut (b). Abbreviations: BarA/UvrY (nutrient-responsive two-component system); Csr (carbon storage regulator); Crp (cAMP receptor protein); GADP (glyceraldehyde-3P); Hfq: RNA chaperone; KDGP (2-dehydro-3-deoxy-gluconate-6P); M-cell (microfold cell); 3-P-G (3-phosphoglycerate); 2PG (2-phosphoglycerate); PhoP/PhoQ (ion-responsive two-component system), PsaA (pH6 antigen); Yops (*Yersinia* outer proteins); T3SS (type III secretion system); and TCA (tricarboxylic acid cycle).

Y. pestis specific antiphagocytic F1 protein capsule (Caf1), as well as the T3SS/Yop apparatus important for resistance against the innate immune response are highly expressed. A similar strong induction of the Caf1 capsule and the T3SS/Yop machinery was also observed in the rat bubo [34]. Furthermore, *Y. pestis* induces a protective response to reactive nitrogen species (RNS), which are released by polymorphonuclear neutrophils (PMNs) in the buboes [34]. This is reflected by an upregulation of the ribonucleotide reductase genes (*nrdHIEF* operon) and *hmp*, which encodes a flavohemoglobin that detoxifies RNS. To further investigate the importance of genes upregulated during bubonic plague, a mutant library was constructed and tested in a rodent model of bubonic plague [34, 37]. Virulence testing revealed that *Y. pestis* depends mainly on the catabolism of carbohydrates (i.e., glucose, galactans, and gluconate) [37] (Figure 1.1). Since the terminal part

(*gpmA*, *aceEF*), but not the upper part (*pgi*, *pfkA*) of the glycolysis pathway was essential for competition with the wildtype *in vivo*, it was suspected that gluconate is metabolized to glyceraldehyde-3-phosphate, pyruvate, acetyl-CoA, and acetate, whereby the galactans and glucose are most likely channeled toward UDP-glucose synthesis [37] (Figure 1.1). Additional results, demonstrating unimportance of certain TCA cycle genes (e.g., *gltA*, *acnA,* and *fumC*) and constitutive expression of the glyoxylate shunt suggest that *Y. pestis* shifts to anaerobic respiration or fermentation during colonization of rodents [34].

Coordinated Control of Carbon Metabolism and Virulence

Rapid changes in environments encountered by *yersiniae* in their external habitats, during the vector-associated lifestyle and within the intestine/lymphatic tissues in mammals request a fast bacterial response to adjust metabolic and virulence traits. To overcome this challenge, it is no wonder that *Yersinia* and other bacteria use the availability of ions and nutrients as well as certain metabolic cues to coordinately control their metabolism and virulence function. For example, virulence factors can be activated via the stringent response through (p)ppGpp under nutrient-limiting conditions, such as amino acid and fatty acid starvation [38]. Furthermore, the synthesis and activity of certain transcriptional regulators and RNA elements (e.g., Fur, Zur, riboswitches) can be controlled by metal ions or small metabolites to modulate expression of metabolic or virulence functions. Many virulence genes are also under CCR control and are regulated by the global transcription factors cyclic adenosine monophophate (cAMP) receptor protein (Crp) and CsrA. They coordinate the uptake and utilization of alternative carbon sources and enable the bacteria to adjust their pathogenic properties in accordance to the availability of readily utilizable sugars [16, 18].

Importance of Ions

All pathogenic *Yersinia* species are characterized by a strong induction of numerous iron uptake and sequestration systems during the infection of mammals, indicating the importance for *Yersinia* to acquire iron [31, 34–36, 39]. The ferric uptake regulator Fur represses most of the iron uptake systems in the presence of iron and controls genes of various noniron metabolic and physiological functions including biofilm formation in *Y. pestis* [40–42]. Although Fur was also shown to control expression of the T3SS in related pathogens [43, 44], Fur-mediated regulation of T3S in *Yersinia* has not been described. However, most recently, a new regulator, IscR, was found to control expression of LcrF, the major regulator of the T3SS-associated genes in *Y. pseudotuberculosis*. It has been suggested that IscR senses iron, O_2, and/or reactive oxygen species concentrations in order to optimize T3S synthesis [45].

Sensing of magnesium ions is another important feature of *Yersinia* to adapt virulence and metabolic gene expression. The pleiotropic two-component system (TCS) PhoP/PhoQ is composed of the membrane-bound sensor kinase PhoQ that

responds to low magnesium and phosphorylates the cytoplasmic response regulator PhoP. It further recognizes low pH environments and host-secreted cationic antimicrobial peptides (CAMPs) [46]. Transcription of the *Y. pestis phoP* gene is significantly upregulated in the lung in an intranasally challenged plague model in mice [36] and in infected fleas [33, 47] and is essential for the formation of a normal foregut-blocking flea infection [33, 47]. Although the PhoP/PhoQ system was shown to be essential for the survival and proliferation of all pathogenic *Yersinia* species in macrophages and neutrophils *in vitro* [48–50], the role of the PhoP/PhoQ system for *Yersinia* pathogenesis is less clear. *phoP* mutants of *Y. pestis* GB and the *Y. pseudotuberculosis* derivative 32 777 were strongly attenuated in virulence, whereas loss of a functional *phoP* gene did not affect the pathogenicity of *Y. pestis* CO92 and the *Y. pseudotuberculosis* strain YPIII [48, 50–52]. This strongly suggests that the different outcomes are the result of strain-specific differences that remodel regulation and/or composition of the PhoP/PhoQ regulon. This is supported by recent findings from our laboratory, demonstrating the presence of strain-specific variations in the PhoP-mediated control of the Csr system affecting expression of numerous metabolic, stress adaptation, and virulence functions in *Y. pseudotuberculosis* [53].

Importance of the Csr System

The important global posttranscriptional Csr system is composed of the RNA-binding protein CsrA and Csr-type sRNAs (CsrB and CsrC in *Y. pseudotuberculosis*). CsrA recognizes conserved (N)GGA motifs in the loop portions of RNA hairpin structures that are mostly found in close vicinity to the ribosomal binding site in the target mRNA. Binding of CsrA affects translation and/or stability of the mRNA. The Csr-RNAs contain several CsrA-binding sites and can eliminate CsrA function by sequestration of CsrA from its target mRNAs [18, 19]. The Csr system controls many genes involved in metabolism and virulence in *Yersinia* similar to many other pathogens [18, 19]. A recent transcriptomic approach revealed that about 20% of the CsrA-dependent genes of *Y. pseudotuberculosis* are involved in metabolic processes [18] (Figure 1.2). The *Y. pseudotuberculosis* Csr system is further implicated in the first steps of the infection process through regulation of the global virulence gene regulator RovA, which activates the synthesis of the primary entry factor invasin and the PsaA fimbriae (Figure 1.2) [54, 55]. Preliminary data further indicate that the Csr system is also crucial for the expression of the *Yersinia* Ysc-Yop/T3SS machinery (R. Steinmann, unpublished results).

Based on the crucial role of the Csr system, it is not surprising that the expression of the Csr components is tightly regulated in response to environmental parameters. Both Csr-RNAs are controlled by different regulatory mechanisms in response to ions and availability of C-sources. The TCS PhoP/PhoQ activates *csrC* transcription in a Mg^{2+}-dependent manner [53]. Furthermore, CsrC synthesis is repressed in the absence of iron (A.K. Heroven, unpublished results). Expression of the CsrB RNA is induced by the TCS BarA/UvrY [54]. The UvrY/BarA system is activated by metabolic end products such as formate and acetate in *E. coli* or by an imbalance of the TCA cycle in *Pseudomonas* [56, 57]. The signal(s) to which the *Yersinia*

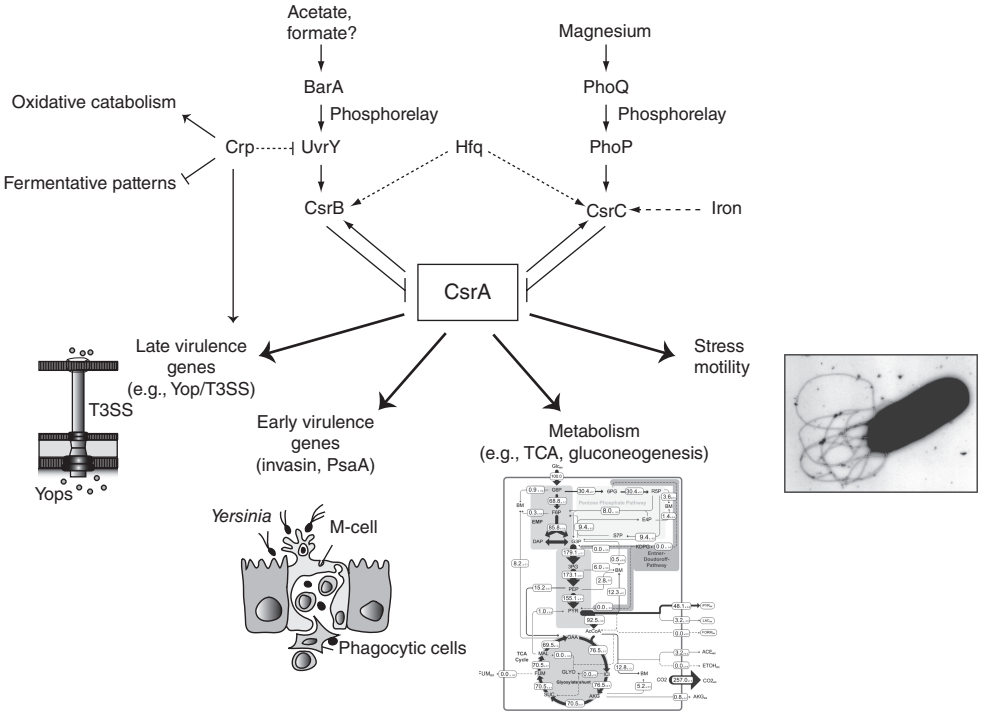

Figure 1.2 Schematic overview of the environmental sensing and signal transduction system and the regulatory cascade with implicated control factors that are known to coordinate expression of metabolic functions and virulence-associated traits of *Y. pseudotuberculosis*. All sensory and regulatory components are also encoded in the other human pathogenic *Yersinia* species, but the function of some of them has still not been experimentally verified.

BarA/UvrY system responds are still unknown, but it has been shown that the *uvrY* gene of *Y. pestis* is highly expressed in the lung, but not in the liver and spleen of infected mice. This indicates that metabolites and/or ions that are present in a certain host niche are able to induce this TCS during infection [36]. Thirdly, Crp regulates expression of *csrC* and *csrB* in an opposite manner, and promotes a tight link between carbon metabolism and regulation of virulence in *Yersinia* [58].

Importance of CCR and the cAMP-Crp Complex

The global transcriptional regulator Crp controls metabolism and pathogenicity in all three human pathogenic *Yersinia* species. The adenylate cyclase catalyzes the synthesis of cAMP in the absence of glucose or other efficiently utilized sugars. Binding of the signal metabolite cAMP activates Crp [59]. At least 6% of the genes in *Y. pestis* and *Y. pseudotuberculosis* are controlled by the cAMP–Crp complex. This includes genes required for growth on different C-sources, survival under carbon, nitrogen, and phosphate limitation as well as virulence [58, 60]. In a recent study, we

could demonstrate that Crp of *Y. pseudotuberculosis* promotes oxidative catabolism of many different C-sources, whereas it represses fermentative patterns [58]. In *Y. pestis*, Crp regulates the T3SS/Yop machinery and the plasminogen activator protease Pla. Consistently, loss of *crp* strongly affects the development of bubonic and pneumonic plague [60–63]. A *Y. enterocolitica crp* mutant strain is severely attenuated in an oral infection model. It has been suggested that the influence of Crp on the expression of the flagellar, Ysc/Yop, and Ysa T3SS might contribute to the loss of virulence [64]. Similarly, mice infected with a *Y. pseudotuberculosis crp* mutant developed no disease symptoms. Crp is required for colonization and/or persistence in the mesenteric lymph nodes (MLNs) and organs later during infection [58].

Importance of Posttranscriptional Regulation Strategies

While numerous transcription factors have been characterized with regard to metabolic and virulence control, only recently attention has been drawn to post-transcriptional control mechanisms involving sensory and regulatory RNAs. A recent study comparing the global transcriptome and proteome response of *Y. pseudotuberculosis* and *Y. pestis* grown under physiologically relevant temperatures revealed that regulation of the metabolism and the translational machinery seems to underlie a conserved posttranscriptional control. This includes proteins of the purine and pyrimidine metabolism, glycolysis/gluconeogenesis, pyruvate metabolism, the TCA cycle, and amino-acyl tRNA biosynthesis [65]. Importance of posttranscriptional control mechanisms became also evident through the analysis of the role of Hfq, an RNA chaperone that controls RNA–RNA and RNA–protein interactions as well as the stability and translation of RNAs [66]. Hfq contributes to virulence of all pathogenic *Yersinia* species, for example, it is implicated in the posttranscriptional regulation of T3SS/Yop machinery in *Y. pestis* and *Y. pseudotuberculosis* [67, 68], and modulates the early stage virulence cascade, including RovA (Figure 1.2), by the control of CsrB and CsrC levels (A.K. Heroven, unpublished results). Hfq influence also seems to occur through Crp as it is required for efficient synthesis of Crp. The underlying posttranscriptional mechanism is still not understood, but it involves the 5′ untranslated region (UTR) of the *crp* mRNA [63].

In addition to the Csr-type sRNAs, other conserved sRNAs could influence *Yersinia* metabolism. Among them are SgrS and Spot42, that are implicated in the regulation of sugar metabolism [69, 70], and GcvB shown to control the amino acid metabolism [71]. Furthermore, *Yersinia* possesses two RyhB homologs. RyhB is a key player for adaptation to iron-limiting conditions in *E. coli* and other *Enterobacteriaceae*, in which it prevents the synthesis of nonessential iron-containing proteins and induces the production of iron-scavenging siderophores [72]. Although the RyhB RNAs are highly expressed in *Y. pestis* within infected lungs (but not in the spleen), their loss had no obvious effect on the dissemination capacity and survival of the bacteria after subcutaneous and intranasal infection. This could be explained by the fact that *Yersinia* possesses several redundant iron uptake systems [73, 74].

The importance of posttranscriptional regulation strategies in the adaptation process of *Yersinia* virulence and metabolism was further supported by observations made in a recent study investigating the regulation of T3SS in *Y. enterocolitica*. Schmid *et al*. could demonstrate that components of the secretion machinery are able to directly interfere with metabolic enzymes [75]. YscM1 (LcrG in *Y. pestis* and *Y. pseudotuberculosis*) and YscM2 are functionally equivalent regulators of the T3SS [76]. Both YscM1 and YscM2 bind to phosphoenolpyruvate carboxylase (PEPC). PEPC is involved in the padding of the oxaloacetate pool in the TCA cycle under virulence conditions. *In vitro*, binding of YscM1 was found to inhibit the function of PEPC [75]. YscM1 and YscM2 participate in the central metabolism of *Y. enterocolitica* as mutants in *yscM1* and *yscM2* displayed increased rates of (i) pyruvate formation via glycolysis or the Entner–Doudoroff pathway, (ii) oxaloacetate formation via the TCA, and (iii) amino acid biosynthesis. It has been suggested that the altered PEPC activity is required for the metabolic adaptation process of *Yersinia* during the infection. In the first phase, *Yersinia* produces massive amounts of Yops to prepare against the phagocytic attack. To do so, PEPC is active in order to refill the TCA for the amino acid synthesis ("loading phase"). After cell contact, the preproduced Yops are rapidly secreted to inhibit the phagocytic cells. In order to maintain the energy charge, anaplerosis is prevented via inhibition of PEPC ("shooting phase"). The cycle starts again when new Yops are needed [75]. *Vice versa*, the availability of amino acids can also influence T3S. Secretion of Yop proteins can be induced by the amino acids glutamate, glutamine, aspartate, and asparagine, feeding into the TCA cycle [77].

Conclusions

Due to the rapid development of antibiotic resistance and emergency of more and more multiresistant bacterial pathogens, new anti-infective strategies are urgently needed. Strategies to adjust the *in vivo* metabolism to nutrient availability in the infected tissues belong to the most fundamental features of bacterial pathogenicity. So far, antivirulence strategies have been developed that inhibit the synthesis or function of crucial virulence factors, such as T3SSs, but important metabolic functions or control systems could also be exploited for antimicrobial therapy since they are a prerequisite for virulence.

Although many aspects of the metabolism of *Yersinia* and related pathogens are already known, this approach is still in its infancy stage. One reason is that our knowledge about the metabolism of these pathogens during the different stages of the infection is still scarce and often inconsistent data have been published that hamper our general understanding. This is based on the fact that identified metabolic genes in *in vivo* high-throughput screens were often not further investigated and the advantage of gene loss was rarely studied. In addition, cultivation conditions, used strain isolates, and animal model systems, as well as the type of infection modes, varied significantly between the studies, which complicate the

identification of important metabolic pathways and regulatory systems. In conclusion, novel approaches and strategies need to be developed in the future, which allow us to follow the metabolism of the pathogen and the host over the course of an infection. Promising techniques are transcriptome profiling approaches, which use deep-sequencing technologies (RNA-Seq), use of radiolabeled C-sources for *in vivo* metabolomics, and the establishment of more "human-like" infection models (e.g., *ex vivo* systems).

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