PROSPECTS
Journal of Cellular Biochemistry 112:2655–2662 (2011)

Journal of Cellular Biochemistry

Regulatory and Pathogenesis Roles of *Mycobacterium* Lrp/AsnC Family Transcriptional Factors

Wanyan Deng,[1] Honghai Wang,[2] and Jianping Xie[1,2]*

[1] *Institute of Modern Biopharmaceuticals, State Key Laboratory Breeding Base of Eco-Environment and Bio-Resource of the Three GorgesArea, School of Life Sciences, Southwest University, Chongqing 400715, China*

[2] *State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Science, Fudan University, Shanghai 200433, China*

ABSTRACT

Lrp/AsnC (leucine-responsive regulatory protein/asparagine synthase C products) family transcriptional regulators, widespread among bacteria and archaea, is also known as feast/famine regulatory protein (FFRPs). They regulate multiple cellular metabolisms globally (Lrp) or specifically (AsnC), such as amino acid metabolism, pili synthesis, DNA transactions during DNA repair and recombination, and also might be implicated in persistence. To better understanding of the pathogenesis of *M. tuberculosis*, based on our lab's work on this transcriptional factor family, these progresses are summarized, with special focus on that of *Mycobacterium* via comparative genomics. J. Cell. Biochem. 112: 2655–2662, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: LRP/ASNC; MYCOBACTERIUM TUBERCULOSIS; REGULATOR; PATHOGENESIS

Lrp/AsnC family global (Lrp)/specific (AsnC) transcriptional regulators are ubiquitous among bacteria and archaea. Lrp was firstly identified as a locus (livR) that affected the transport of branched-chain amino acids [Anderson et al., 1976]. Leucine affects both ilvIH and oppABCDF operons via a regulatory protein, hereafter we refer to it as Lrp (leucine-responsive regulatory protein). Lrp is involved in amino acid metabolism and peptide transport [Platko et al., 1990]. AsnC, the first Lrp-related protein described in *E. coli*, is a dedicated transcriptional activator of asnA involved in asparagine biosynthesis [Kolling and Lother, 1985]. Generally, Lrp-related proteins are also known as the Lrp/AsnC family proteins, epitome of the two extreme examples: global (Lrp) and specific (AsnC) [Friedberg et al., 2001].

Lrp-like protein might be limited to bacteria and archaea, since they are widespread among archaea and bacteria, but no confirmed homologs in the available eukaryal genomes. One possible explanation is they are lost rather early in the eukaryal lineage [Brinkman et al., 2003]. Members of the Lrp/AsnC family are small DNA-binding proteins. They can form diverse multimers among different organisms, such as dimers, tetramers, octamers, and hexadecamers [Leonard et al., 2001; Koike, 2004; Thaw, 2006; Reddy et al., 2007; de los Rios and Perona, 2007]. The equilibrium of multimeric state is usually affected by effectors binding [Yokoyama et al., 2007]. The member of this family has a typical N-terminal and a C-terminal domain. *E. coli* Lrp controls the expression of genes involved in amino acids metabolism, pilli biosynthesis, pyridine nucleotide transhydrogenation, mRNA translation [Calvo and Matthews, 1994] and exogenous amino acid can directly activate Lrp [Schwaiger et al., 2010]. Higher concentration of exogenous amino acid can profoundly change the metabolism of bacteria. *E. coli* can readily adapts themselves to a more heterotrophic mode with the regulation of Lrp: trigger the nutrients absorption, accelerated replication, and concomitant virulence alteration.

Mycobacterium tuberculosis, one of the most successful intracellular pathogen, can subvert host immune attack and persist in monocytes and macrophages [Malik et al., 2000]. The asymptomatic latent infections of persistent *M. tuberculosis* serve as a huge reservoir of future infections and present a bottleneck for TB eradication [Wayne and Sohaskey, 2001]. Unveil the nature of the in vivo latency is crucial to new measures against TB. Lrp/AsnC

Grant sponsor: National Key Infectious Disease Project; Grant numbers: 2008ZX10003-006, 2008ZX10003-001; Grant sponsor: Excellent PhD Thesis Fellowship of Southwest University; Grant numbers: kb2009010, ky2009009; Grant sponsor: Fundamental Research Funds for the Central Universities; Grant number: XDJK2009A003; Grant sponsor: Natural Science Foundation Project of CQ CSTC; Grant number: 2010BB5002; Grant sponsor: National Natural Science Foundation of China; Grant number: 81071316.

*Correspondence to: Jianping Xie, Institute of Modern Biopharmaceuticals, School of Life Sciences, Southwest University, Chongqing 400715, China. E-mail: georgex@swu.edu.cn

Received 25 April 2011; Accepted 13 May 2011 • DOI 10.1002/jcb.23193 • © 2011 Wiley-Liss, Inc.
Published online 23 May 2011 in Wiley Online Library (wileyonlinelibrary.com).

regulators have been established as key coordinator of nutrition availability. Therefore, it is worthwhile to investigate whether they play the same role in *Mycobacterium*. Our comparative genomic analysis demonstrated that LrpA is conserved in *Mycobacterium*, except for targets. *Mycobacterium leprae* (Fig. 1). The reduced genome of *M. leprae* lacks most of the Lrp-like regulators. The understanding of the physiological roles, target genes, and regulation of Lrp in *Mycobacterium* will provide insights into the pathogenesis of *M. tuberculosis* and other pathogenic *Mycobacterium* and clues for new drug targets.

THE STRUCTURE OF Lrp/AsnC PROTEIN

Crystal structures for nine proteins of the Lrp/AsnC family are currently available. The hyperthermophilic archaeon *Pyrococcus furiosus* LrpA is the first member of the Lrp/AsnC family and the first transcriptional regulator from a hyperthermophile, whose crystal structure has been determined [Leonard et al., 2001]. Resolution of another seven members followed: *Pyrococcus OT3* FL11 [Koike, 2004], *B. subtilis* LrpC [Thaw, 2006], *E. coli* AsnC (Fig. 2A) [Thaw, 2006], *E. coli* Lrp [de los Rios and Perona, 2007], *Neisseria meningitides* NMB0573 [Ren et al., 2007], *M. tuberculosis* LrpA [Reddy et al., 2007], *Sulfolobus tokodaii* strain 7 ST1022 [Nakano et al., 2007], and *P. horikoshii* FL11 [Yamada et al., 2009]. Lrp/AsnC monomer contains two domains: an N-terminal DNA-binding motif with a common helix-turn-helix fold, and a C-terminal ligand binding domain with a typical αβ-sandwich fold, which forms a large proportion of the dimmer interface as a novel ligand-binding site. The latter regulatory domain involved in amino acid metabolism and designated as RAM (*r*egulation of *a*mino acid *m*etabolism) [Ettema et al., 2002]. These two domains are joined by a hinge with one β-stand [Leonard et al., 2001] or two flexible linker [Reddy et al., 2007] with variable amino acid sequence in length [de los Rios and Perona, 2007]. This linker of all known crystal is well conserved, indicating a conformational rigidity to some extent [Peeters and Charlier, 2010]. The C-terminal ligand binding domain adopts a βαββαβ fold in which four-stranded anti-parallel β-sheet flanked by two α-helices.

Most Lrp/AsnC-like possess a highly symmetrical octamer with subtle difference, which is ranked by a closed internal ring of four dimmers. *PyrococcusOT3* FL11 is an exception, which organized as disk or helical cylinder with six dimers each turn [Koike, 2004]. Several members have been cocrystallized with amino acids or DNA (Table I), but their transcriptional regulation mechanism remain unknown. Although AsnC was cocrystallized with asparagine [Thaw, 2006], its association state [Thaw, 2006] and autoregulation of asnC [Kolling and Lother, 1985] irrelevant to asparagine, implying that ligand is not indispensable to octamer formation and function. Interestingly, *E. coli* Lrp crystallized with DNA possess an open conformation with linear array of four dimmers [de los Rios and Perona, 2007], this may provide a structural basis for Lrp/AsnC protein binding to its target DNA sequences. Moreover, FL11 from *P. horikoshii* cocrystallized with arginine and lysine with slight difference in conformation (Table I), closed quaternary association like *Bacillus subtilis* LrpC (Fig. 2A) will form a DNA-binding motif, which is parallel to the domains in the open octamer [Yamada et al., 2009]. In addition, *M. tuberculosis* feast/famine regulatory protein LrpA (Rv3291c) Gly102Thr mutant adopts an unusual "open" quaternary conformation but the Glu104Ala mutant retains the closed octamer observed in the native protein [Shrivastava et al., 2009]. These might implicate that the transition into open octamer from closed may be a crucial step for DNA–protein interaction and underlying the molecular mechanism of Lrp/AsnC transcriptional regulatory proteins.

THE EXPRESSION REGULATION OF Lrp/AsnC GENES

Interaction with the pre-initiation complex of transcription is a conventional way to modulate gene expression. Most Lrp/AsnC family transcription factor positively or negatively autoregulate themselves in a global (Lrp) or specific (AsnC) manner, and often affected by small molecules. Microarray data suggested that Lrp functions largely in the regulation of stationary phase-induced genes, such as those responsive to starvation, high concentrations of organic acids, and osmotic stress [Tani et al., 2002]. Additionally 11 transcription factors were identified under the control of Lrp and most of them participate in amino acid metabolism and small molecule transport [Cho et al., 2008]. Therefore, as a global transcriptional factors, Lrp ranks high up in the hierarchy of transcriptional regulatory network [Martinez-Antonio and Collado-Vides, 2003]. These suggest that Lrp plays an indispensable role in various metabolic events. Unlike most Lrp/AsnC family member, *B. subtilis* LrpC is positively autoregulated and mainly expressed in stationary growth phase [Beloin et al., 2000] impervious to leucine [Lopez-Torrejon et al., 2006].

Since epigenetic states of DNA can be modulated by environmental factors, change the reversible DNA methylation patterns of both prokaryotes and eukaryotes provides a way to modulate gene expression [Jaenisch and Bird, 2003]. DNA adenine methyltransferase (Dam) and leucine responsive regulatory protein (Lrp) are two key regulators controlling the expression of pap operon by a reversible OFF/ON phase switch mechanism [Blyn et al., 1989]. Lrp moves between proximal and distal sites to control pap pilin transcription and is regulated by PapI and Dam. Dam perfectly binds to and methylates proximal GATC site. Low-level Lrp leads to a phase switch resulting in pili expression [Peterson and Reich, 2008]. Previous study has shown that CpxR-P competes with Lrp for binding to both proximal and distal pap DNA binding sites, inhibiting pap transcription in vitro and pili expression in vivo [Hernday et al., 2004]. These may shell light on the mechanisms of epigenetic regulation for the expression of genes which are controlled by environmental factors.

Due to the diverse DNA binding patterns and locations, multiple regulation strategies are speculated. However, only two mechanisms have been elucidated at the molecular level [Dahlke and Thomm, 2002; Ouhammouch, 2003]. *Pyrococcus* LrpA targets approximately promoter region to form TBP-TFB-LrpA ternary, restraining the binding of RNA polymerase (RNAP) to TBP-TFB, thereby blocking the transcription of pre-initiation complex [Dahlke and Thomm,

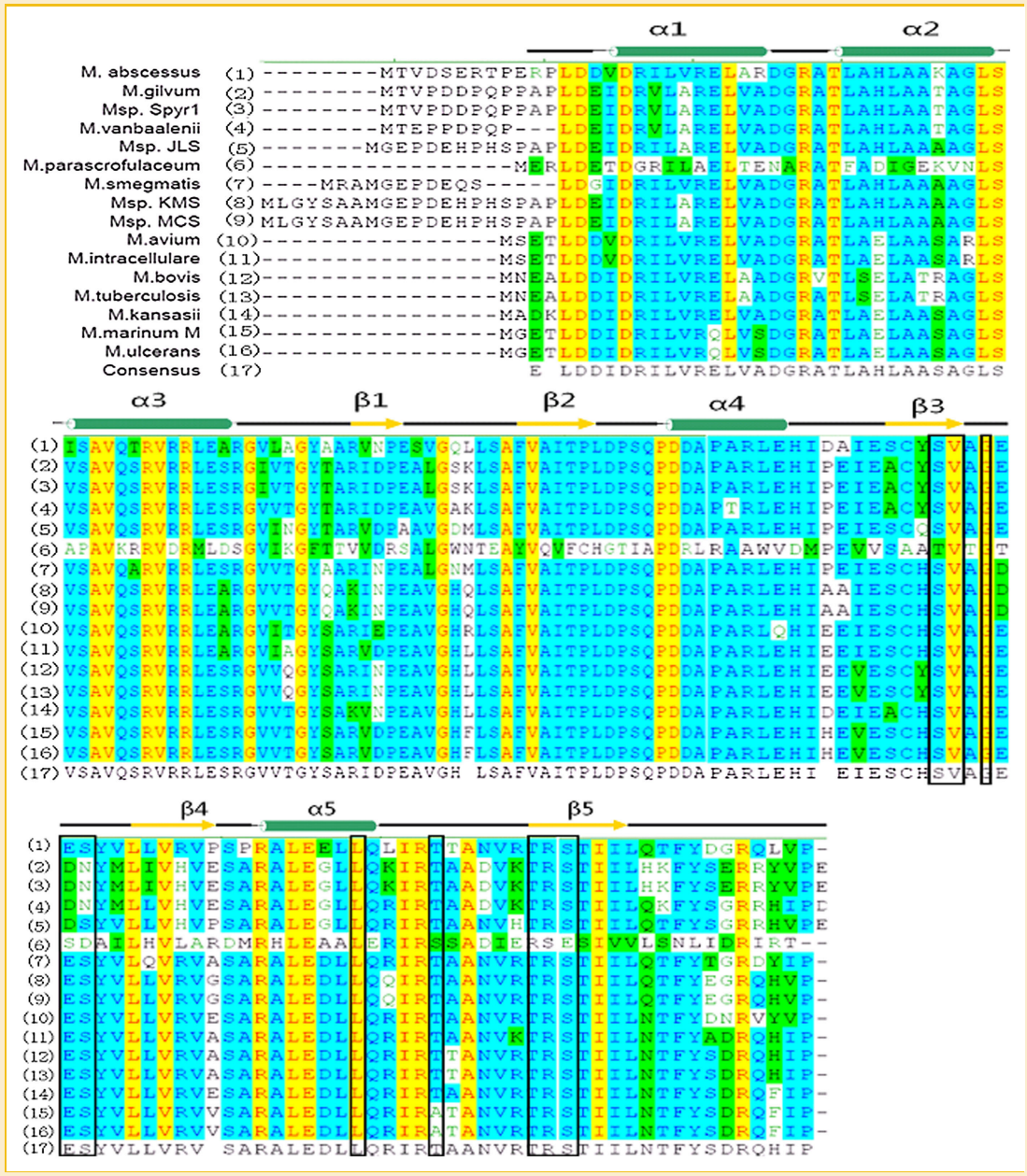

Fig. 1. Structure-based multiple alignment of amino acid sequences of *Mycobacterium* LrpA proteins. Elements of secondary structure in MTB LrpA are shown as labeled cylinders (α-helices) and arrows (β-stands). Residues found to be important in the formation of the ligand-binding site are indicated by closed boxes. The identities of aligned proteins are as follows: *M. abscessus* (*Mycobacterium abscessus* ATCC1997); *M. gilvum* (*Mycobacterium gilvum* PYR-GCK); Msp. Spyr1 (*Mycobacterium* sp. Spyr1); *M. vanbaalenii* (*Mycobacterium vanbaalenii* PYR-1); Msp. JLS (*Mycobacterium* sp. JLS); *M. parascrofulaceum* (*Mycobacterium parascrofulaceum* ATCC BAA-614); *M. smegmatis* (*Mycobacterium smegmatis* str. MC2 155); Msp. KMS (*Mycobacterium* sp. KMS); Msp. MCS (*Mycobacterium* sp. MCS); *M. avium* (*Mycobacterium avium* 104); *M. tuberculosis* (*Mycobacterium tuberculosis* H37Rv); *M. kansasii* (*Mycobacterium kansasii* ATCC 12478); *M. marinum* M (*Mycobacterium marinum* M); *M. ulcerans* (*Mycobacterium ulcerans* Agy99). The sequences were obtained from GenBank database (http://www.ncbi.nlm.nih.gov/). The alignment was generated by the program Vector NTI.

2002]. However, *Sulfolobus solfataricus* Lrs14 binds to multiple sequences of its own promoter and inhibits the transcription of pre-initiation complex by via precluding RNAP binding or attenuate its ability to initiate transcription through the overlap TATA box. This is reminiscent of a negatively autoregulated repressor [Napoli et al., 1999]. These alternative repression mechanisms are shared by other Lrp/AsnC regulators as well [Yokoyama et al., 2007]. *M. jannaschii* Ptr2 is the first bacterial-type regulator with archaea origin that can activate its cognate eukaryote-like transcription machinery in vitro, which is a potent transcriptional activator via recruitment of TATA-binding protein to its promoter [Ouhammouch, 2003].

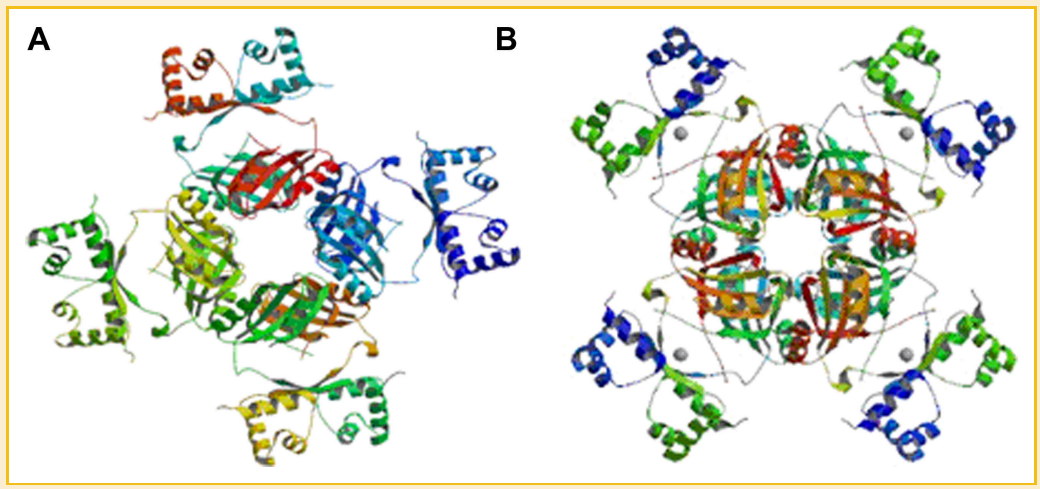

Fig. 2. The structure of LrpC and AsnC [Thaw, 2006]. A: The structure of *Bacillus subtilis* LrpC (PDB 2CFX). B: AsnC cocrystallized with aspartate (PDB 2CG4). These structural information sourced from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do).

THE PHYSIOLOGICAL FUNCTIONS OF Lrp/AsnC

Despite LrpAsnC homologs are abundant in bacteria and archaea genomes, the physiological role and the identity of the target genes of most remain elusive. The well-known *E. coli* Lrp is a global regulator involved in diverse regulatory patterns which are directly affected by leucine [Platko et al., 1990]. AsnC was the first reported member of this family whose activity is triggered by asparagine and with unclear roles in aspartate metabolism [Kolling and Lother, 1985]. It is very difficult to precisely predict the roles of Lrp/AsnC homologs due to their multiple functions in regulation and beyond that (Table II), such as transportation, energy, central metabolism, DNA repair and recombination, bacterial persistence and virulence.

Lrp/AsnC PROTEINS REGULATE "FEAST/FAMINE" AMINO ACID METABOLISM

The Lrp/AsnC transcription factor family links bacterial metabolism to environmental cues, especially amino acid resulting availability and functions during the transition between feast and famine. The regulation by FL11, a member of Lrp/AsnC family in hyperthermophilic archaeon *Pyrococcus OT3*, is a case of point. Lysine regulates the FL11 association state (octamer or dimer), thereby activating (feast) or repressing (famine) the transcription of its target genes [Yokoyama et al., 2007]. This "feast and famine" regulation affected by exogenous leucine and alanine is also observed in *E. coli* [Berthiaume et al., 2004]. Lrp can also be triggered by amino acids in addition to leucine, such as Met, Ile, His, and Thr [Hart and Blumenthal, 2011]. *M. tuberculosis* LrpA (Rv3291c) is a putative Lrp/AsnC transcriptional regulatory protein with expression level inversely correlated with bacterial growth rate, the epitome of feast/famine regulatory mode [Slayden et al., 2006]. LrpA oligomer state transition between hexadecameric and octameric/lower-order oligomers is regulated by Phe [Shrivastava et al., 2009].

Lrp/AsnC PROTEINS REGULATE CENTRAL METABOLISM

In addition to genes necessary for amino acid metabolism, Lrp/AsnC gene family also contains genes relate to other biological process, such as central metabolism [Schut et al., 2001; Ouhammouch, 2003; Yokoyama et al., 2007; Peeters et al., 2009; Schwaiger et al., 2010]. ATP synthesis regulated by *Pyrococcus OT3* FL11 depends on the lysine concentration [Yokoyama et al., 2007]. Lrp from *Halobacterium salinarum* R1 repress gldA1 (glycerol dehydrogenase) and activate korAB (oxoglutarate ferredoxin oxidoreductase α-subunit/β-subunit) to regulate central metabolism [Schwaiger et al., 2010]. Ptr2 weakly binds to ferredoxin (fdxA) and rubredoxin 2 (rb2) with oxide reductase activity [Ouhammouch, 2003; Ouhammouch et al., 2005]. Gene disruption mutant analysis has shown that Ss-LrpB (from *S. solfataricus*) activates a pyruvate ferredoxin oxidoreduc-

TABLE I. The Slight Difference of Several Typical LrpAsnC Crystal Structures

Classification	Subunit	Oligomer	Cocrystallization	Refs.
P. furiosus LrpA	Dimer	Highly symmetrical closed octamers	–	Leonard et al. [2001]
PyrococcusOT3 FL11	Dimer	May disk-shaped octamers	DNA	Koike [2004]; Yokoyama et al. [2007]
B. subtilis LrpC	Dimer	Highly symmetrical closed octamers similar to nucleosome	–	Thaw [2006]
E. coli AsnC	Dimer	Highly symmetrical closed octamers	Aspartate	Thaw [2006]
E. coli Lrp	Dimer	Open, linear array of four dimers	DNA	de los Rios and Perona [2007]
M. tuberculosis LrpA	Dimer	Highly symmetrical closed octamers	–	Reddy et al. [2007]
P. horikoshii FL11	Dimer	Octamers with open (associate with arginine) or closed (associate with lysine) conformation	Lysine and arginine	Yamada et al. [2009]

TABLE II. The Roles of Lrp/AsnC Regulators

Organism	Name	Biological process	Refs.
M. jannaschii	Ptr2	Electron transport	Ouhammouch [2003]; Ouhammouch et al. [2005]
S. solfataricus	Ss-LrpB	Central metabolism and transport	Peeters et al. [2009]
B. subtilis	LrpC	DNA transactions during repair and recombination	Lopez-Torrejon et al. [2006]
M. tuberculosis	LrpA	Bacteria persistence	Parti et al. [2008]; Reddy et al. [2007]
Pyrococcus OT3	FL11	Amino acid metabolism, central metabolism and ATP biosynthesis	Yokoyama et al. [2007]

tase operon *porDAB* and two permease genes: Sso2127 and Sso2126 [Peeters et al., 2009]. Other Lrp/AsnC-homologues regulators, belonging to different archaeal lineages, also regulate genes encoding subunits of the class of 2-oxoacid: ferredoxin oxidoreductases, ubiquitous among archaea and involved in catalyzing different central metabolic reactions [Schut et al., 2001].

THE ROLES OF Lrp/AsnC PROTEINS IN DNA REPAIR AND RECOMBINATION

Previous studies have shown that *B. subtilis* LrpC is a sequence-independent DNA-binding protein which targets curved DNA and promotes DNA bending and forms intermolecular bridges between different DNAs including linear and supercoiled DNA molecules therefore, LrpC can also be considered as a chromatin-associated protein which modulates DNA transactions during recombination [Tapias et al., 2000]. Moreover, DNA binding experiments have demonstrated that LrpC forms looped and/or sandwich structures with circular ssDNA and supercoiled dsDNA, binding high affinity to different recombination final product NAD and AMP intermediates such as 4-way and Y-junction. The former is a key intermediate in nearly all homologous recombination processes. In addition, lrpC deletion mutant is more sensitive to DNA damaging agents such as methyl methanesulfonate (MMS) or 4-nitroquinoline-1-oxide (4NQO) than wild strain [Lopez-Torrejon et al., 2006]. Together, LrpC may be crucial in DNA transactions during DNA repair and recombination.

Lrp/AsnC PROTEINS PLAY A POTENTIAL ROLE IN BACTERIAL PERSISTENCE AND VIRULENCE

Bacterial adaptation to nutrient limitation and increased population densities is central to survival and virulence. *M. tuberculosis* Rv3291c (LrpA), a well-characterized Lrp/AsnC family transcriptional regulators responsive to nutrient limitation, is significantly up-regulated (16-fold) during starvation [Betts et al., 2002]. lat (Rv3290c), lysine amino transferase encoding gene—located upstream of Rv3291c (LrpA), is up-regulated 42-fold after 96 h of starvation [Betts et al., 2002]. Mtb LrpA directly binds to *lat* promoter, which has a putative motif homologous to other genes differentially regulated under starvation, demonstrating that the *lat* operon (Fig. 3) is a direct target of LrpA [Reddy et al., 2007]. These implicate that LrpA might be involved *M. tuberculosis* transition to persistence.

M. fortuitum mutant deficient in virulence and persistence in a murine infection model could be complemented by *M. tuberculosis* Rv3291c. This suggests that LrpA is crucial to the long-term persistence of *M. tuberculosis* in vivo and may be a novel therapeutic target against persistent *M. tuberculosis* [Parti et al., 2008]. Lrp can also act as a sensor to relay butyrate stimulation to the PchA-Ler regulatory network that governs the LEE genes involving in the regulation of enterohemorrhagic *Escherichia coli* (EHEC) virulence genes [Nakanishi et al., 2009]. Taken together, these may suggest that members of Lrp/AsnC family have important roles in bacteria virulence and persistence.

Lrp/AsnC PROTEIN AS POTENTIAL ANTI-TB DRUG TARGETS

The absence of Lrp/AsnC protein in eukaryotic genomes may derive from their increasing sophisticated transcriptional machinery. The distribution of Lrp/AsnC transcriptional regulators is uneven among different microbe. Some genomes have over a dozen of Lrp-like genes, whereas others have only a few or none. The number of Lrp/

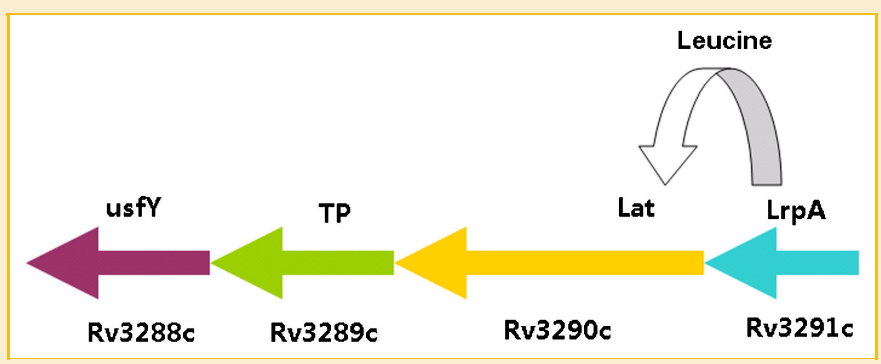

Fig. 3. The components of lat operon. The definition of the genes is as follows: Rv3288c, hypothetical protein with unknown function; Rv3289c, a possible transmembrane protein (TP); Rv3290c, lysine aminotransferase; Rv3291c (LrpA), Lrp/AsnC family transcriptional regulator (http://genolist.pasteur.fr/TubercuList/).

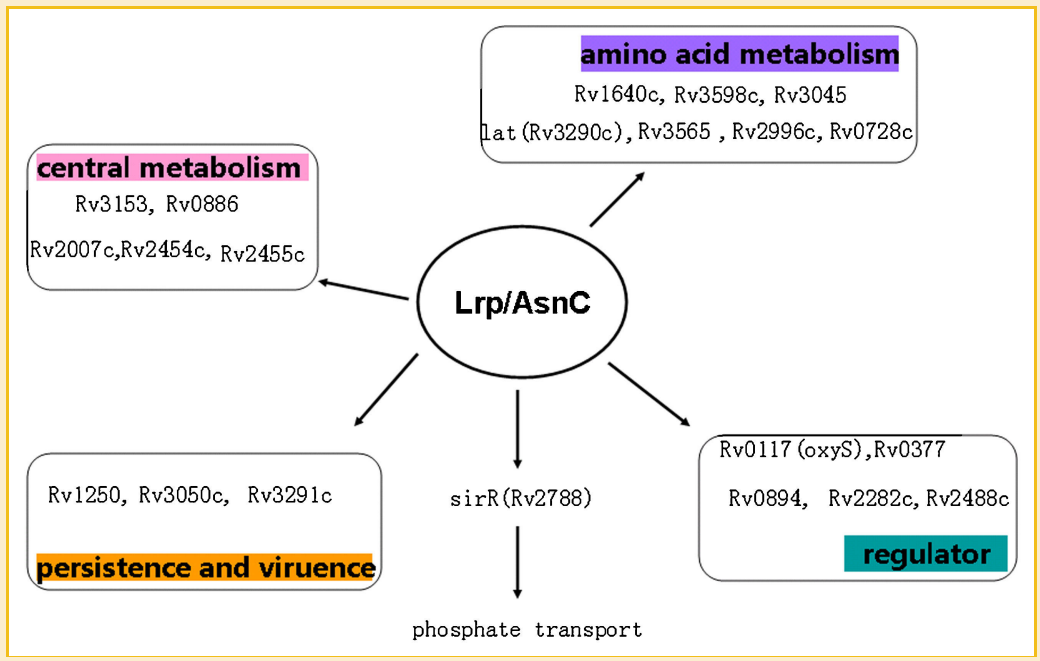

Fig. 4. The predicated targets of Lrp/AsnC in *M. tuberculosis*. Rv3153, NADH dehydrogenase subunit I; Rv0886, NADPH:adrenodoxin oxidoreductase FprB; Rv2007c, ferredoxin FDXA; Rv2455c, oxidoreductase alpha subunit; Rv1250; Rv3050c; Rv3291c; Rv2788, transcriptional repressor SIRR; lat (Rv3290c), Rv3565, aspartate aminotransferase; Rv1640c, lysyl-tRNA synthetase; Rv3598c, lysyl-tRNA synthetase; Rv3045 (adhC); Rv0117, oxidative stress response regulatory protein oxyS; Rv0377/ Rv2282c, LysR family transcriptional regulator; Rv2488c/Rv0894, LuxR family transcriptional regulator.

AsnC in particular genome might be correlated with specific lifestyle. Those with few or even without Lrp/AsnC might totally depend on their host for amino acids and other key metabolites. The absence of Lrp homologues in the six lactic acid bacterial genomes, whose survival require exogenous amino acids [Brinkman et al., 2003], partly supports our speculation. New TB drugs capable of shortening the current 6 months are highly desirable and hindered by our poor understanding of the metabolism of the persistent bacteria. Five ORFs in *M. tuberculosis* genome were annotated as "Lrp-like" or "AsnC-like" transcription regulators: Rv2779c, Rv0212c (nadR), Rv2324, Rv3050c, and Rv3291c (LrpA). In consideration of the widespread of these ORFs among most *Mycobacterium* may suggest that their essential role in persistence and their function somewhat like the global metabolic regulator of *E. coli* Lrp.

Gene and protein expression profiling analysis have shown that Rv2779c was up-regulated by starvation, this suggests a role in nutrient response [Betts et al., 2002]. Higher activity of L-alanine dehydrogenase (Ald) in Rv2779c recombinant *Mycobacteria smegmatis* [Feng et al., 2002] implicates that Rv2779c might be a regulator of alanine metabolism. Rv3050c is regulated by *sig*E and its expression is up-regulated twofold in H37Rv than sigE mutant during macrophage infection [Fontan et al., 2008]. *M. tuberculosis* Rv3291c (LrpA) was reported to have a potential role in persistence [Parti et al., 2008]. LrpA was up-regulated 2.9-fold in anaerobic conditions [Muttucumaru et al., 2004]. Interestingly, LrpA preferentially binds to aromatic amino acids [Shrivastava and Ramachandran, 2007], which are of great significance to the pathogen survival [Parish and Stoker, 2002]. Aromatic amino acid metabolism crucial enzymes, such as those from the Shikimate pathway, have been recognized as important promising targets for antibiotics [Ducati et al., 2007]. Since Lrp/AsnC in *M. tuberculosis* might play a role in the bacterial virulence and latence infection with multiple functions (Fig. 4), we conclude it deserves to be considered as a novel anti-TB drug target. These suggest that LrpA and other members of this family might be novel drug targets against tuberculosis and deserve further in-depth exploration.

REFERENCES

Anderson JJ, Quay SC, Oxender DL. 1976. Mapping of two loci affecting the regulation of branched-chain amino acid transport in *Escherichia coli* K-12. J Bacteriol 126:80–90.

Beloin C, Exley R, Mahe AL, Zouine M, Cubasch S, Le Hegerat F. 2000. Characterization of LrpC DNA-binding properties and regulation of *Bacillus subtilis* lrpC gene expression. Am Soc Microbiol 182:4414–4424.

Berthiaume F, Crost C, Labrie V, Martin C, Newman EB, Harel J. 2004. Influence of L-leucine and L-alanine on Lrp regulation of foo, coding for F1651, a Pap homologue. J Bacteriol 186:8537–8541.

Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K. 2002. Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. Mol Microbiol 43:717–731.

Blyn LB, Braaten BA, White-Ziegler CA, Rolfson DH, Low DA. 1989. Phase-variation of pyelonephritis-associated pili in *Escherichia coli*: Evidence for transcriptional regulation. EMBO J 8:613–620.

Brinkman AB, Ettema TJ, de Vos WM, de Vos Oost J. 2003. The Lrp family of transcriptional regulators. MicroReview 48:287–294.

Calvo JM, Matthews RG. 1994. The leucine-responsive regulatory protein, a global regulator of metabolism in *Escherichia coli*. Am Soc Microbiol 58:466–490.

Cho BK, Barrett CL, Knight EM, Park YS, Palsson BO. 2008. Genome-scale reconstruction of the Lrp regulatory network in *Escherichia coli*. Proc Natl Acad Sci USA 105:19462–19467.

Dahlke I, Thomm M. 2002. A *Pyrococcus* homolog of the leucine-responsive regulatory protein, LrpA, inhibits transcription by abrogating RNA polymerase recruitment. Nucleic Acids Res 30:701–710.

de los Rios S, Perona JJ. 2007. Structure of the *Escherichia coli* leucine-responsive regulatory protein Lrp reveals a novel octameric assembly. J Mol Biol 366:1589–1602.

Ducati RG, Basso LA, Santos DS. 2007. Mycobacterial shikimate pathway enzymes as targets for drug design. Curr Drug Targets 8:423–435.

Ettema TJ, Brinkman AB, Tani TH, Rafferty JB, Van Der Oost J. 2002. A novel ligand-binding domain involved in regulation of amino acid metabolism in prokaryotes. J Biol Chem 277:37464–37468.

Feng Z, Caceres NE, Sarath G, Barletta RG. 2002. *Mycobacterium smegmatis* L-alanine dehydrogenase (Ald) is required for proficient utilization of alanine as a sole nitrogen source and sustained anaerobic growth. J Bacteriol 184: 5001–5010.

Fontan PA, Aris V, Alvarez ME, Ghanny S, Cheng J, Soteropoulos P, Trevani A, Pine R, Smith I. 2008. *Mycobacterium tuberculosis* sigma factor E regulon modulates the host inflammatory response. J Infect Dis 198:877–885.

Friedberg D, Midkiff M, Calvo JM. 2001. Global versus local regulatory roles for Lrp-related proteins: Haemophilus influenzae as a case study. J Bacteriol 183:4004–4011.

Hart BR, Blumenthal RM. 2011. Unexpected coregulator range for the global regulator Lrp of *Escherichia coli* and *Proteus mirabilis*. J Bacteriol 193:1054–1064.

Hernday AD, Braaten BA, Broitman-Maduro G, Engelberts P, Low DA. 2004. Regulation of the pap epigenetic switch by CpxAR: Phosphorylated CpxR inhibits transition to the phase ON state by competition with Lrp. Mol Cell 16:537–547.

Jaenisch R, Bird A. 2003. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. Nat Genet 33(Suppl): 245–254.

Koike H. 2004. The archaeal feast/famine regulatory protein: Potential roles of its assembly forms for regulating transcription. Proc Natl Acad Sci 101:2840–2845.

Kolling R, Lother H. 1985. AsnC: An autogenously regulated activator of asparagine synthetase A transcription in *Escherichia coli*. J Bacteriol 164:310–315.

Leonard PM, Smits SH, Sedelnikova SE, Brinkman AB, de Vos WM, van der Oost J, Rice DW, Rafferty JB. 2001. Crystal structure of the Lrp-like transcriptional regulator from the archaeon *Pyrococcus furiosus*. EMBO J 20: 990–997.

Lopez-Torrejon G, Martinez-Jimenez MI, Ayora S. 2006. Role of LrpC from *Bacillus subtilis* in DNA transactions during DNA repair and recombination. Nucleic Acids Res 34:120–129.

Malik ZA, Denning GM, Kusner DJ. 2000. Inhibition of Ca(2+) signaling by *Mycobacterium tuberculosis* is associated with reduced phagosome-lysosome fusion and increased survival within human macrophages. J Exp Med 191: 287–302.

Martinez-Antonio A, Collado-Vides J. 2003. Identifying global regulators in transcriptional regulatory networks in bacteria. Curr Opin Microbiol 6:482–489.

Muttucumaru DG, Roberts G, Hinds J, Stabler RA, Parish T. 2004. Gene expression profile of *Mycobacterium tuberculosis* in a non-replicating state. Tuberculosis (Edinb) 84:239–246.

Nakanishi N, Tashiro K, Kuhara S, Hayashi T, Sugimoto N, Tobe T. 2009. Regulation of virulence by butyrate sensing in enterohaemorrhagic *Escherichia coli*. Microbiology 155:521–530.

Nakano N, Kumarevel T, Matsunaga E, Shinkai A, Kuramitsu S, Yokoyama S. 2007. Purification, crystallization and preliminary X-ray crystallographic analysis of ST1022, a putative member of the Lrp/AsnC family of transcriptional regulators isolated from *Sulfolobus tokodaii* strain 7. Acta Crystallogr Sect F Struct Biol Cryst Commun 63:964–966.

Napoli A, van der Oost J, Sensen CW, Charlebois RL, Rossi M, Ciaramella M. 1999. An Lrp-like protein of the hyperthermophilic archaeon *Sulfolobus solfataricus* which binds to its own promoter. J Bacteriol 181:1474–1480.

Ouhammouch M. 2003. Activation of archaeal transcription by recruitment of the TATA-binding protein. Proc Natl Acad Sci 100:5097–5102.

Ouhammouch M, Langham GE, Hausner W, Simpson AJ, El-Sayed NM, Geiduschek EP. 2005. Promoter architecture and response to a positive regulator of archaeal transcription. Mol Microbiol 56:625–637.

Parish T, Stoker NG. 2002. The common aromatic amino acid biosynthesis pathway is essential in *Mycobacterium tuberculosis*. Microbiology 148:3069–3077.

Parti R, Shrivastava R, Srivastava S, Subramanian A, Roy R, Srivastava B, Srivastava R. 2008. A transposon insertion mutant of *Mycobacterium fortuitum* attenuated in virulence and persistence in a murine infection model that is complemented by Rv3291c of *Mycobacterium tuberculosis*. Microb Pathog 45:370–376.

Peeters E, Charlier D. 2010. The Lrp family of transcription regulators in archaea. Archaea 2010:750457.

Peeters E, Albers S-V, Vassart A, Driessen AJM, Charlier D. 2009. Ss-LrpB, a transcriptional regulator from *Sulfolobus solfataricus*, regulates a gene cluster with a pyruvate ferredoxin oxidoreductase-encoding operon and permease genes. Mol Microbiol 71:972–988.

Peterson SN, Reich NO. 2008. Competitive Lrp and Dam assembly at the pap regulatory region: Implications for mechanisms of epigenetic regulation. J Mol Biol 383:92–105.

Platko JV, Willins DA, Calvo JM. 1990. The ilvIH operon of *Escherichia coli* is positively regulated. J Bacteriol 172:4563–4570.

Reddy MCM, Gokulan K, Jacobs WR, Ioerger TR, Sacchettini JC. 2007. Crystal structure of *Mycobacterium tuberculosis* LrpA, a leucine-responsive global regulator associated with starvation response. Protein Sci 17:159–170.

Ren J, Sainsbury S, Combs SE, Capper RG, Jordan PW, Berrow NS, Stammers DK, Saunders NJ, Owens RJ. 2007. The structure and transcriptional analysis of a global regulator from *Neisseria meningitidis*. J Biol Chem 282:14655–14664.

Schut GJ, Menon AL, Adams MW. 2001. 2-keto acid oxidoreductases from *Pyrococcus furiosus* and *Thermococcus litoralis*. Methods Enzymol 331:144–158.

Schwaiger R, Schwarz C, Furtwangler K, Tarasov V, Wende A, Oesterhelt D. 2010. Transcriptional control by two leucine-responsive regulatory proteins in *Halobacterium salinarum* R1. BMC Mol Biol 11:40.

Shrivastava T, Ramachandran R. 2007. Mechanistic insights from the crystal structures of a feast/famine regulatory protein from *Mycobacterium tuberculosis* H37Rv. Nucleic Acids Res 35:7324–7335.

Shrivastava T, Dey A, Ramachandran R. 2009. Ligand-induced structural transitions, mutational analysis, and 'open' quaternary structure of the *M. tuberculosis* feast/famine regulatory protein (Rv3291c). J Mol Biol 392:1007–1019.

Slayden RA, Knudson DL, Belisle JT. 2006. Identification of cell cycle regulators in *Mycobacterium tuberculosis* by inhibition of septum formation and global transcriptional analysis. Microbiology 152:1789–1797.

Tani TH, Khodursky A, Blumenthal RM, Brown PO, Matthews RG. 2002. Adaptation to famine: A family of stationary-phase genes revealed by microarray analysis. Proc Natl Acad Sci USA 99:13471–13476.

Tapias A, Lopez G, Ayora S. 2000. *Bacillus subtilis* LrpC is a sequence-independent DNA-binding and DNA-bending protein which bridges DNA. Nucleic Acids Res 28:552–559.

Thaw P. 2006. Structural insight into gene transcriptional regulation and effector binding by the Lrp/AsnC family. Nucleic Acids Res 34:1439–1449.

Wayne LG, Sohaskey CD. 2001. Nonreplicating persistence of *Mycobacterium tuberculosis*. Annu Rev Microbiol 55:139–163.

Yamada M, Ishijima SA, Suzuki M. 2009. Interactions between the archaeal transcription repressor FL11 and its coregulators lysine and arginine. Proteins 74:520–525.

Yokoyama K, Ishijima SA, Koike H, Kurihara C, Shimowasa A, Kabasawa M, Kawashima T, Suzuki M. 2007. Feast/famine regulation by transcription factor FL11 for the survival of the hyperthermophilic archaeon *Pyrococcus* OT3. Structure 15:1542–1554.