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# ***Mycobacterium tuberculosis* Transcriptome In Vivo Studies – A Key to Understand the Pathogen Adaptation Mechanism**

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## **1. Introduction**

The infectious diseases caused by intracellular bacterial pathogens, such as *M. tuberculosis*, are among the most important problems in public health worldwide. The development of an infectious process depends on intricate interactions between the host defence systems and the specific systems regulating mycobacterial gene expression. Changes in expression in response to host defence are a necessary condition for the *M. tuberculosis* survival and functioning. Tracking these changes makes possible to analyze the biochemical cascades that are triggered in response to host defence mechanisms and to find the targets for designing new therapeutics and monitoring bacterial infections; in addition, these results are useful for both theoretical (for example, dynamics of the pathogen transcriptome changes during long-term persistence in the host) and applied (for example, the study of the bacterial response to various therapeutic interventions) research.

The completion of the *M. tuberculosis* genome sequence in 1998 (Cole et al., 1998) marked the beginning of the so called post genome era, the main characteristic of which are large scale studies of genome functional activity. The information on the bacterial genome organization allowed to construct macro- and microarrays containing fragments of a majority of ORFs, which enabled analysis of the pathogen transcription profile variations under different conditions. It's no wonder that the first study of the *M. tuberculosis* transcriptome using microarray technology was carried out in the first year after the publication of the genome sequence (Wilson et al., 1999). In as little as 5 years, there have been published many reports with the results of using microarrays for *in vitro* mycobacterial transcriptome analysis (for review, see (Butcher, 2004; Kendall et al., 2004)).

However, the *in vivo* analysis of mycobacterial gene expression during infection process, which is of special scientific interest, is rather complicated, and this can explain a relatively small number of such works. Experiments with analysis of a pathogen transcriptome *in vivo* are determined by the choice of: (1) an experimental model of infection; (2) a method of pathogen RNA isolation; (3) a method of analysis of RNA or cDNA enriched in bacterial transcripts. The brief features of these steps are given in the review.

## 2. RNA isolation and bacterial RNA/cDNA enrichment

The widely used scheme of an intracellular bacterial transcriptome analysis is illustrated by Fig. 1.

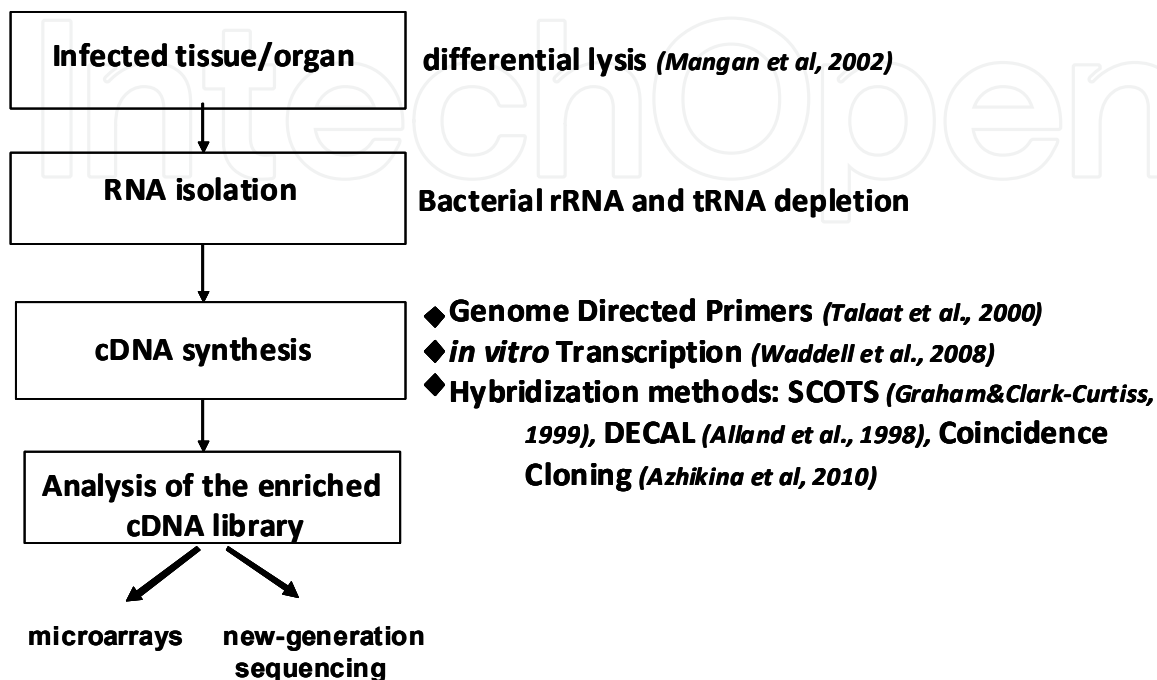


Fig. 1. Stages of intracellular transcriptome analysis (left) and main approaches used to enrich the cDNA library by bacterial transcripts (right).

The methods of RNA isolation and enrichment are described in details in our recent review (Skvortsov & Azhikina, 2010). Here we would like to emphasize that until the present time, practically all ways of preparing cDNA libraries have lead to the further microarray hybridization analysis. Hybridization-based microarray techniques are widely used in transcriptome studies, but development of novel high-throughput DNA sequencing methods (reviewed in (Shendure & Ji, 2008)) obviously demonstrated such microarrays limitations as high background levels owing to cross-hybridization, problems of rare transcript detection, and expression quantification (Shendure, 2008).

We proposed a new method for the evaluation of sequences of bacterial pathogens specifically transcribed in infected tissues (Azhikina et al., 2010). It is based upon the coincidence cloning approach that allows isolation of representative bacterial cDNA pools from infected organs. Co-denaturation and co-renaturation of the excess of bacterial genomic DNA with the cDNA transcribed from total RNA of the infected tissue enabled selective isolation of the bacterial cDNA fraction from the sample, and a single round of coincidence cloning resulted in >1000-fold enrichment of bacterial transcripts. The enriched cDNA library is suitable for high-throughput sequencing analysis, which is less biased and more reliable than other methods including microarrays.

### 3. Experimental models of infection

Experimental models of tuberculosis used for whole transcriptome studies *in vivo* are very diverse. The most frequently used models and examples of their applications are described below.

#### 3.1 Host phagocytes (mouse and human)

The first work with a whole genome description of *M. tuberculosis* gene expression is that of Schnappinger et al. (Schnappinger et al., 2003). Activated by INF- $\gamma$  and non-activated mouse macrophages were used in this work as a model system. Rachman et al. characterized the *M. tuberculosis* genes with enhanced expression in activated and inactivated mouse macrophages both relative to each other and to mycobacteria *in vitro* (Rachman et al., 2006). Rohde et al. studied changes in the *M. tuberculosis* transcriptome at the initial stages of infection of mouse macrophages and demonstrated a dynamic enhancement in the expression level of some genes during the first 24 hours post infection (Rohde et al., 2007).

Cappelli et al. were the first to characterize the *M. tuberculosis* whole genome gene expression *in vivo* in human macrophages (Cappelli et al., 2006). Fontan et al. analyzed transcriptomes of *M. tuberculosis* from macrophages of the THP-1 cell line in 4 and 24 hours post infection (Fontan et al., 2008). In the work of Tailleux et al., the authors performed the first comparative analysis of gene expression in *M. tuberculosis* from infected macrophages and dendrite cells (Tailleux et al., 2008). An *in vivo* transcriptome comparison of two differently virulent *M. tuberculosis* strains (H37Rv and H37Ra) was first done by Li et al. (Li et al., 2010).

By the present time, the most extensive study of mycobacterial gene expression *in vivo* is that by Homolka et al. (Homolka et al., 2010). The authors performed a comparative analysis of expression profiles for 3 clinical isolates of *M. africanum*, 12 clinical isolates of *M. tuberculosis*, and two reference laboratory strains (*M. tuberculosis* H37Rv and CDC1551) in activated and non-activated mouse macrophages. This work resulted in the isolation of gene groups whose expression changes irrespective of mycobacterial strain and/or the activation status of the host macrophage.

#### 3.2 Models of *M. tuberculosis* infection in laboratory animals

In 2004, Talaat et al. first performed an analysis of whole genome *M. tuberculosis* gene expression under natural conditions in a living organism (mouse) (Talaat et al., 2004). They studied changes in the pathogen transcriptome composition at different time intervals post infection (7, 14, 21 or 28 days) and for different host genotypes (immunocompetent Balb/c mice and immunodeficient SCID mice). In 2007, Talaat et al. published a paper devoted to the analysis of *M. tuberculosis* gene expression in the lungs of Balb/c mice at later stages of the infection process (Talaat et al., 2007). In 2010, researchers of our group used a new approach to the enrichment of bacterial cDNA for analysis of *M. tuberculosis* gene expression in lung tissues of infected mice (Azhikina et al., 2010). The data obtained by us on quantitative and qualitative composition of the bacterial transcriptome were in good agreement with similar data of the Talaat's group (Talaat et al., 2007).

### 3.3 Examination of the *M. tuberculosis* transcriptome in human tissues

The report of Rachman et al. published in 2006 is thus far the only work in which *M. tuberculosis* gene expression was studied directly in the human lungs (surgical samples) (Rachman et al., 2006). In this work, the pathogen's whole genome gene expression profiles from granuloma, pericavitary lung tissue and morphologically normal lung tissue were obtained

## 4. Intracellular *M. tuberculosis* transcriptome

The data obtained by different authors in *in vivo* systems allow to single out a number of the main transcriptome features characteristic of mycobacteria persisting in macrophages. Those are primarily changes in expression of the genes involved in pathogen adaptation, as well as of the genes encoding different factors of immune response. In macrophages, mycobacteria are localized within phagosomes which makes a barrier for the immune system components, but, at the same time, complicates the access of the pathogen to nutrients and microelements. Changes in *M. tuberculosis* gene expression are aimed primarily at forming of the environment able to *in vivo* maintain the functional activity of mycobacteria.

### 4.1 Lipid metabolism

Lipid metabolism is a key process for *M. tuberculosis* which is directly or indirectly confirmed by the presence in the genome of a considerable number of lipid metabolism genes, indispensability of some genes as follows from data of transposon mutagenesis (Sasseti et al., 2003), decrease or lack of virulence observed in *M. tuberculosis* strains mutant for the genes of this functional category (Neyrolles & Guilhot, 2011), and by other factors.

One of the most characteristic changes in the expression of the genes involved in lipid metabolism is activation of the expression of the genes from clusters *fadA*, *fadB*, *fadD*, *fadE* and *echA* (Azhikina et al., 2010; Munoz-Elias & McKinney, 2006; Schnappinger et al., 2003; Tailleux et al., 2008). These genes encode enzymes involved in  $\beta$ -oxidation of fatty acids and the catabolism of cholesterol. The final products of their activity are acetyl-CoA and propionyl-CoA, the participants of the methylcitrate and tricarboxylic acids cycles.

Enhanced expression of the isocitrate lyase gene *icl1* is also related to the methylcitrate cycle. Isocitrate lyase is a key enzyme of the glyoxylate cycle that is activated when the main carbon source are fatty acids. During this process, activated acetate (acetyl-CoA) is being stepwise converted into malate through the stage of glyoxylic acid formation. Malate can be converted into pyruvate by the enzymatic activity of the *pckA* gene product, whose enhanced expression was also observed *in vivo* in mycobacteria (Marrero et al., 2010; Schnappinger et al., 2003; Tailleux et al., 2008). Apart from the maintenance of the glyoxylate bypass, the activity of isocitrate lyase is also needed to utilize cytotoxic propionyl-CoA accumulated during the life cycle of mycobacteria (Savvi et al., 2008). This utilization is possible due to the ability of isocitrate lyase to function as 2-methylisocitrate lyase that facilitates the conversion of propionyl-CoA into succinate. Propionyl-CoA can be also metabolized by a conversion into methylmalonyl-CoA and then into succinate, or included into certain components of the cell wall, such as phthiocerol dimycocerosate (PDIM) or sulfolipid-1 (SL-1) (Russell et al., 2010).



Among other genes of lipid metabolism expressed predominantly *in vivo*, there are *desA* genes encoding desaturases of fatty acids (Homolka et al., 2010; Li et al., 2010; Rachman et al., 2006; Schnappinger et al., 2003). The *papA* and *pks* genes, whose protein products are needed for synthesis of polyketides as components of the *M. tuberculosis* cell wall (Bhatt et al., 2007; Hatzios et al., 2009; Sirakova et al., 2001), are practically always transcribed in experiments. However, the level of their transcription *in vivo* varies, possibly reflecting variations in the lipid metabolism depending upon specific conditions (Azhikina et al., 2010; Homolka et al., 2010; Rohde et al., 2007; Tailleux et al., 2008). Interestingly, transcription of these genes is decreased in the avirulent *M. bovis* BCG and *M. tuberculosis* H37Ra strains as compared with the virulent *M. tuberculosis* H37Rv strain (Li et al., 2010; Rohde et al., 2007).

#### 4.2 Energy metabolism: Cell respiration

According to data obtained in studies of *in vivo M. tuberculosis* gene expression, energy metabolism of mycobacteria is undergoing a significant transformation during infection process. A characteristic of this transformation is a gradual decrease in the level of the type I NADH dehydrogenase (*nuoA-N*) gene expression and increase in expression of the nitrate reductase gene cluster *narGHJI* and of the *narK2* gene, the product of which is a nitrate transporter protein (Azhikina et al., 2010; Schnappinger et al., 2003; Tailleux et al., 2008). Such a metabolic shift most probably suggests that ETC is being reoriented to the using of nitrate electrons as a finite acceptor. Also, in most cases, aa3 type cytochrome c oxidase (*ctaBECD*) and cytochrome c reductase (*qcrCAB*) gene expression is downregulated (Garton et al., 2008; Schnappinger et al., 2003; Shi et al., 2005).

#### 4.3 Protein biosynthesis and cell growth

Decreased expression of ribosomal protein genes (*rpl*, *rpm*, *rps*) indicates a reduced need for the synthesis of new proteins. Usually this phenomenon occurs in conditions non optimal for the pathogen (dendrite cells, activated macrophages) and correlates with decreased expression level of the ATP-synthase (*atpA-H*) gene and slowdown of *M. tuberculosis* replication (Homolka et al., 2010; Tailleux et al., 2008).

#### 4.4 Defense mechanisms, DNA replication

The compartment (early phagolysosome) of *M. tuberculosis* residing at persisting in macrophages represents a rather non-aggressive environment with practically no hydrolytic activity and pH 6.2-6.4. Nevertheless, the mycobacteria are under the influence of many stress factors like active forms of oxygen and nitrogen or the apoptotic death of the host cell. The effect of stress factors induces an upregulation of genes of the DNA repair and recombination (*dinF/G*) systems (Rachman et al., 2006; Schnappinger et al., 2003; Talaat et al., 2004), as well as chaperon genes (*groES*, *groEL1/2*, *dnaJ/K*, *hspX*) (Fontan et al., 2008; Garton et al., 2008; Homolka et al., 2010; Karakousis et al., 2004; Rohde et al., 2007; Tailleux et al., 2008). Certain data indicate that this effect is not a specific reaction on intracellular conditions, but part of adaptive response to stress (Boshoff et al., 2004; Waddell et al., 2004).

#### 4.5 Cell wall, membrane, and transport

The cell wall and inner plasma membrane are components of the complex cell envelope of mycobacteria. The inner plasma membrane contains a lot of transport systems. Some genes of these systems were observed to be upregulated, among them the *irtA/B* genes encoding carboxymycobactin transporters (Li et al., 2010; Schnappinger et al., 2003; Tailleux et al., 2008), the *sugI*, *Rv2040c* and *Rv2041c* genes of carbohydrate transporters (Azhikina et al., 2010; Schnappinger et al., 2003; Tailleux et al., 2008), and the *narK2* gene of the nitrate transporter protein (Azhikina et al., 2010; Garton et al., 2008; Tailleux et al., 2008; Talaat et al., 2007).

Enhanced expression of the genes of siderophore-synthesis enzymes, mycobactin and carboxymycobactin (*mbtA-J*, *mbtL-N*), could be explained by limited access to iron (Azhikina et al., 2010; Schnappinger et al., 2003; Tailleux et al., 2008).

#### 4.6 Factors of *Mycobacterium tuberculosis* virulence

The *M. tuberculosis* virulence depends upon various metabolic processes that provide for successful infection process. The pathogen survival in host cells directly depends on avoiding host defence mechanisms and getting nutrient substances from host tissues for the existence and reproduction. A considerable damage of host tissues and organs at later stages of infection is also needed as it facilitates dissemination of the infection. We will consider the factors that directly affect the host organism in order to suppress host defence mechanisms. One of such immune response modulators is the ESAT-6 protein (Sorensen et al., 1995) encoded by the *esxA* gene and secreted by the type VII secretion system (T7SS) ESX-1 (Abdallah et al., 2007; Simeone et al., 2009). ESAT-6 is one of the immune modulation key factors suggested to be involved in lysis of phagolysosome membranes and macrophage outer membranes thus facilitating spread of mycobacteria from one host cell to another (de Jonge et al., 2007; Kinshikar et al., 2010). The *in vivo* expression of the *esx* cluster genes is under constant control and can be both decreased and increased depending on the conditions (Fontan et al., 2008; Rohde et al., 2007; Schnappinger et al., 2003; Tailleux et al., 2008).

One more important system of immune response modulation is the expression of the PE/PPE family proteins that possess immunogenic properties (Bottai & Brosch, 2009; Sampson, 2011; Voskuil et al., 2004). The level of their expression is also dependent upon specific conditions (Azhikina et al., 2010; Fontan et al., 2008; Schnappinger et al., 2003; Tailleux et al., 2008; Voskuil et al., 2004).

#### 4.7 Transcription regulation

The adaptation to changing conditions of the environment is mainly underlain by the activity of signal and regulatory systems of the bacterium, and therefore by changing expression of transcription regulatory systems' genes. Among genes of the regulatory systems, 13 genes of sigma factors (Manganelli et al., 2004; Rodrigue et al., 2006) are of special interest. Their differential expression was repeatedly observed in *in vivo* experiments. For instance, *sigH* was upregulated in artificial granulomas in mice and in human macrophages (Karakousis et al., 2004), whereas *sigB* and *sigE* were also upregulated

in mouse phagosomes (Rohde et al., 2007), as well as in artificial mouse granulomas and in the mouse lung, respectively (Karakousis et al., 2004; Talaat et al., 2004).

Other transcription regulatory genes whose upregulation was observed in *in vivo* experiments are *whiB3* (Fontan et al., 2008; Rohde et al., 2007), *ethR*, *ideR*, *kstR* and *relA* (Fontan et al., 2008; Schnappinger et al., 2003; Tailleux et al., 2008). In addition, *M. tuberculosis* has 12 two-component regulatory systems (Tucker et al., 2007). Two of them, *phoPR* (Gonzalo-Asensio et al., 2008) and *dosRS* (*devRS*) (Park et al., 2003), were studied more thoroughly than others. It was shown that functional activity of the *phoPR* system supports the *M. tuberculosis* virulence by regulating the metabolism of complex lipids and the work of the ESX secretion systems (Gonzalo-Asensio et al., 2008). The positive transcription regulator *phoP* was observed to induce genes under its control in a low-acid (pH 6.4) environment of mouse macrophage phagosomes (Rohde et al., 2007). Not less important is the two component regulatory system *dosRS* responsible for expression regulation of about 50 genes (Park et al., 2003). Enhanced expression of these genes was repeatedly observed in the course of mycobacterial infection of macrophages (Fontan et al., 2008; Rohde et al., 2007; Schnappinger et al., 2003; Tailleux et al., 2008). Moreover, *dosRS* regulon genes were expressed practically in all other conditions: in *M. tuberculosis* from artificial mouse granulomas, samples of mouse lung tissues and surgical samples of human lung, as well as in samples of sputum and in some experiments *in vitro* (Garton et al., 2008; Homolka et al., 2010; Karakousis et al., 2004; Li et al., 2010; Talaat et al., 2007; Timm et al., 2003). The functional role of this regulon is still not quite clear, but its activity was suggested to be important for *M. tuberculosis* adaptation to variations in the redox status during the infection process (Bacon et al., 2004; Bacon & Marsh, 2007; Rustad et al., 2009).

## **5. Profiling of *Mycobacterium tuberculosis* gene expression during infection in genetically different mouse models**

We have carried out a comparative study of *M. tuberculosis* transcriptomes in order to reveal the features of expression profiles that correlate with progressing disease, and also to understand the difference between efficient and defective defence mechanisms at the level of bacterial gene expression. To this end, at different stages of the infection process, we performed a comparative quantitative and qualitative analysis of the sequences transcribed during infection of mice sensitive (inefficient immune response) and resistant (efficient response) to these bacteria (Skvortsov et al., 2010).

We have compared transcriptomes of *M. tuberculosis* H37Rv in infected mice of two lineages, I/StSnEgYCit (I/St) and C57BL/6JCit (B6). These lineages have been earlier described in detail (Kondratieva et al., 2010), and the B6 lineage was shown to be more resistant to the infection by *M. tuberculosis* than I/St. In particular, the infection process in B6 mice was less aggressive, and the infected mice had a longer survival.

Female mice of both lineages were aerogenically infected with *M. tuberculosis* bacteria. In 4 and 6 weeks post infection, the infected mice were killed, and total lung RNA isolated. Samples of total RNA from lung tissues of I/St and B6 mice were used to synthesize cDNA enriched in fragments of bacterial cDNA using coincidence cloning procedure (Azhikina et al., 2010). As a result, three cDNA libraries were obtained, which represented transcriptomes of *M. tuberculosis* from lung tissues of I/St mice on week 6 post infection



(CC6(SUS)) and from lung tissues of B6 mice on weeks 4 and 6 post infection (CC4(RES) and CC6(RES), respectively). The libraries were analyzed using the 454 pyrosequencing technology. A general scheme of the experiment is shown in Fig. 2, and general characteristics of the libraries analyzed are presented in Table 1. In total, sequences of 190031 cDNA fragments were determined: 73410 from CC4(RES), 75655 from CC4(SUS), and 40966 from CC6(RES).

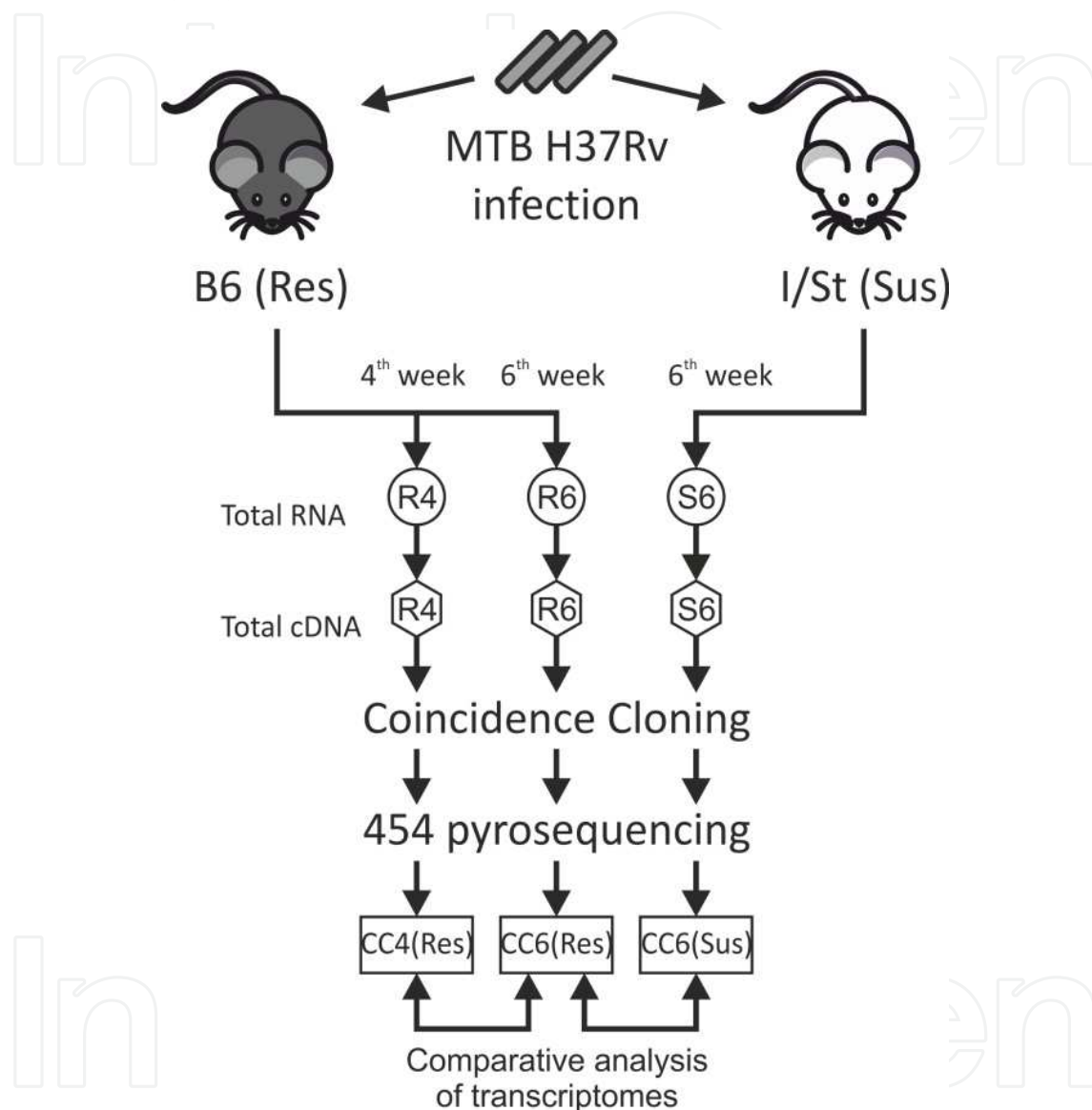


Fig. 2. Scheme of the transcriptomes comparison experiment. Res - genetically resistant mice, Sus - genetically susceptible mice, CC - library enriched with bacterial cDNA

Using a standalone BLASTn algorithm, the nucleotide sequences obtained were mapped to the genome sequence of *M. tuberculosis* H37Rv (GenBank version: AL123456.2). The mapping revealed that the CC4(RES), CC6(SUS) and CC6(RES) samples contained 14990 (20.42%), 43618 (57.65%) and 34234 (83.57%) *M. tuberculosis* sequences, respectively.

The results obtained demonstrate that the technology used allowed us to considerably enrich the cDNA samples with bacterial sequences.

| Library  | CC4(RES) | CC6(SUS) | CC6(RES) |
|--|----------|----------|----------|
| Total sequences read   | 73410    | 75655    | 40966    |
| <i>M. tuberculosis</i> -specific (unique) sequences                            | 14990    | 43618    | 34234    |
| <i>M. tuberculosis</i> -specific (unique) sequences, % of total sequences read | 20,4     | 57,7     | 83,6     |
| The number of <i>M. tuberculosis</i> genes expressed (at least one reading)    | 1012     | 1353     | 1940     |
| Genes expressed, % of the total number of genes in the library                 | 25,2     | 33,7     | 48,3     |

Table 1. Results of sequencing and mapping of the CC4(RES), CC6(RES) and CC6(SUS) libraries

Of 4012 *M. tuberculosis* genes and 7 pseudogenes, in the CC4(RES) sample 1012 (25.2%), in CC4(SUS) - 1353 (33.7%), and in CC6(RES) - 1940 (48.3%) genes were expressed. 1428 (35,5%) genes were not expressed in either of the samples, whereas 469 (11.7%) genes were expressed in each sample.

The distribution of the expressed genes between functional categories is shown in Fig. 3. Mobile elements (insertion sequences and phages) were excluded from the analysis.

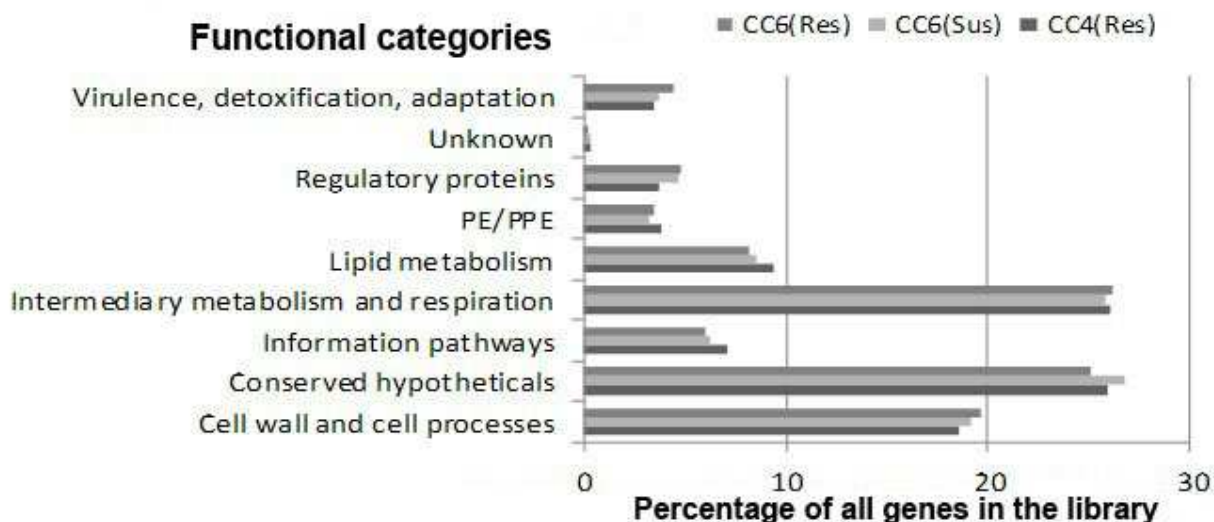


Fig. 3. Assignment to biochemical categories of the *M. tuberculosis* H37Rv genes expressed in samples of lung tissue of B6 mice on weeks 4 and 6 post infection and of I/St mice on week 6 post infection (CC4(RES), CC6(RES) and CC6(SUS) libraries, respectively)

### 5.1 Genes whose expression is enhanced during infection

The comparison of *M. tuberculosis* transcriptomes during infection in a genetically stable mouse lineage (CC6(RES) vs CC4(RES)) and in genetically different mice (CC6(RES) vs CC6(SUS)), described above, was aimed at the search of genes whose expression is enhanced in the course of infection, specifically in B6 mice on week 6 as compared with

other time points. The comparison of CC6(RES) vs CC4(RES) allowed to reveal 226 genes upregulated in infected tissues of B6 mice. A similar comparison of CC6(RES) vs CC6(SUS) revealed 253 genes upregulated in the CC6(RES) sample (Fig. 4). We concentrated our search on *M. tuberculosis* genes:

1. with expression upregulated only in one of genetically different hosts;
2. with expression upregulated irrespective of genetic features of the host organism. These genes represent a kind of basic set of genes responsible for a universal compensatory reaction of *M. tuberculosis* to unfavourable conditions of the environment. They are termed CUG –Commonly Upregulated Genes.

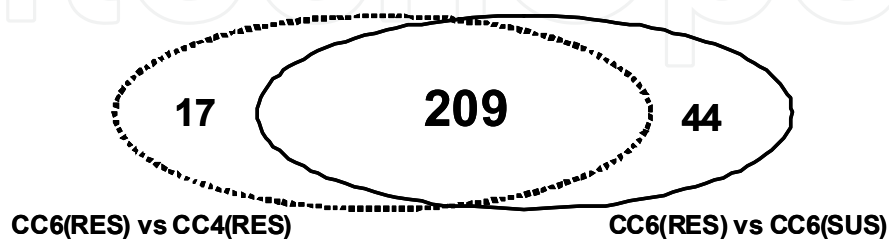


Fig. 4. Venn diagram illustrating the number of upregulated mycobacterial genes in comparisons CC6(RES) vs CC4(RES) (dotted) and CC6(RES) vs CC6(SUS) (solid)

### 5.1.1 Genes upregulated only in one of genetically different hosts

We found 17 genes with enhanced expression in CC6(RES) vs CC4(RES) and 44 genes in CC6(RES) vs CC6(SUS). Such a statistics probably reflects the fact that in the first case bacterial genes are expressed in one and the same microenvironment, whereas in the second case microenvironments are different resulting in a greater number of genes upregulated.

Genes, the expression of which is enhanced in the CC6(RES) sample only relative to CC4(RES), mostly belong to categories of cell wall and cell processes, intermediary metabolism and respiration, and lipid metabolism. The protein products of 12 out of 17 genes were detected in a fraction of cell membrane or cell wall where they mainly fulfil transport and defence functions. For example, the *embA* gene codes for indolyacetyltransferase EmbA involved in the synthesis of arabinan, and mutations in this gene cause resistance to ethambutol. Also, the *Rv3273* gene encodes carbonate dehydratase that participates in sulfate transport (TubercuList, <http://tuberculist.epfl.ch>). In the analysis performed, we failed to detect metabolic pathways specifically activated at later infection times as compared to early stages.

Comparing the bacterial transcriptomes within different hosts we found greater variety of biochemical pathways. An increase in energy exchange is reflected in enhanced expression of the genes of three NADH-dehydrogenase subunits (*nuoH*, *nuoI*, *nuoL*), as well as in increased activity of the tricarboxylic acid cycle and in upregulation of the *Rv1916* gene. The *Rv1916* gene is the second part of the *aceA* (*icl2*) gene which in *M. tuberculosis* H37Rv is divided into two modules, *Rv1915* and *Rv1916* (*aceAa* и *aceAb*), each of which can be expressed independently. Among other important differences, one can highlight enhanced expression of genes, the products of which are responsible for lipid and amino acid metabolism and catabolism (*lipV*, *lipF*, *Rv2531c*), and genes of DNA repair enzymes (*recO*, *recB*).

Such a picture is quite predictable as the microenvironment in a resistant host is a hostile habitat which can explain the need in more active repair systems. Increased gene expression of lipolytic enzymes (*lipF*, *lipV*, *plcA*), enzymes of the tricarboxylic acid cycle and *aceAb* may suggest a forced usage of lipids as the source of energy and carbon.

### 5.1.2 CUGs – genes needed for *M. tuberculosis* adaptation to different host defense mechanisms

We have revealed 209 genes upregulated in both comparisons. According to the results of transposon mutagenesis, the products of 44 out of these genes are essential in *M. tuberculosis* (Sassetti et al., 2003), the list of these genes is given in Table 2. *Rv3569c*, *Rv3537*, and *Rv3563* were earlier shown to be essential for survival in mouse macrophages (TubercuList, <http://tuberculist.epfl.ch>)

A bit less than one third of the 209 genes belong to the conserved hypothetical (59 genes) and unknown (2 genes) categories. In spite of unknown functions, the genes of these categories may be considered potential therapeutic targets, since their low homology to genes of other microorganisms suggests that they are characteristic just of mycobacteria or even *M. tuberculosis*.

The function of the PE/PPE family proteins is not quite clear, but they are suggested to be needed for antigenic variability in mycobacteria (Karboul et al., 2008). Nevertheless, the *Rv0152c* and *Rv0355c* genes had a high expression level in the CC6(RES) sample, and they were also expressed in the CC4(RES) and CC4(SUS) samples. *Rv3135* encodes a protein essential in *M. tuberculosis* H37Rv that may suggest some additional functions beyond antigenic variability

One more feature of CUGs is an increased activity of amino acid metabolism pathways. It is not clear if the stimulation of this metabolism enzyme expression is due to the absence of available amino acids (and the necessity of their synthesis) or their availability (and the possibility to use them). Poor nutrient conditions of the environment are supported by a high level gene expression of various systems of acquisition and accumulation of nutrients, e.g. such as phosphate (*pstS1*) or iron (*irtA*, *mbtC*, *mbtE*, *mbtF*). A shortage of phosphate is indicated by enhanced expression of the *senX3* gene, a sensor component of the *senX3* *regX3* two-component system that activates the so called stringent response under phosphorus deficiency. The expression of lipid metabolism genes (*fadD*, *fadE*, *lipU*, *lipJ*) suggests a switch to using lipids as a major source of energy and carbon. Enhanced expression of the *narH* and *narK3* genes implies a switch to anaerobic respiration characteristic of latent infection. Finally, the *secA2* gene is also worth mentioning. This gene codes for translocase SecA2 which is a component of the *M. tuberculosis* secondary transport system Sec that provides for, in particular, secretion of superoxide dismutase SodA and catalase katG. A live vaccine based on an *M. tuberculosis* mutant for the *secA2* gene (Hinchey et al., 2011) showed high efficiency and safety in animal trials. Summarizing, it can be said that CUGs reflect characteristic features of infection in a mouse model. An exception is increased expression of the *atpF* and *atpH* genes, although, according to some reports, their expression decreases in the course of infection as energy demand of the pathogen goes down upon transition to the state of latent infection.

| Gene           | Name           | Function | Description   |
|----------------|----------------|----------|---|
| <i>Rv0015c</i> | <i>pknA</i>    | RP       | transmembrane serine/threonine-protein kinase A   |
| <i>Rv0020c</i> | <i>fhaA</i>    | IMaR     | aspartate aminotransferase  |
| <i>Rv0072</i>  | <i>Rv0072</i>  | IMaR     | diaminopimelate epimerase   |
| <i>Rv0089</i>  | <i>Rv0089</i>  | CWaCP    | Possible integral membrane efflux protein   |
| <i>Rv0145</i>  | <i>Rv0145</i>  | LM       | Possible acyl-CoA dehydrogenase Fad19   |
| <i>Rv0261c</i> | <i>narK3</i>   | CWaCP    | Probable integral membrane nitrite extrusion protein narK3 (nitrite facilitator)                              |
| <i>Rv0332</i>  | <i>Rv0332</i>  | CH       | Conserved hypothetical protein  |
| <i>Rv0465c</i> | <i>Rv0465c</i> | RP       | Probable transcriptional regulatory protein   |
| <i>Rv0509</i>  | <i>hemA</i>    | IMaR     | Probable glutamyl-trna reductase hemA   |
| <i>Rv0525</i>  | <i>Rv0525</i>  | CH       | hypothetical protein  |
| <i>Rv0526</i>  | <i>Rv0526</i>  | IMaR     | Possible thioredoxin protein  |
| <i>Rv0558</i>  | <i>menH</i>    | IMaR     | ubiquinone/menaquinone biosynthesis methyltransferase   |
| <i>Rv0645c</i> | <i>mmaA1</i>   | LM       | Methoxy mycolic acid synthase 1 mmaa1 (methyl mycolic acid synthase 1) (mmA1) (hydroxy mycolic acid synthase) |
| <i>Rv0684</i>  | <i>fusA1</i>   | IP       | elongation factor EF-2  |
| <i>Rv0712</i>  | <i>Rv0712</i>  | CH       | Conserved hypothetical protein  |
| <i>Rv0998</i>  | <i>Rv0998</i>  | CH       | Conserved hypothetical protein  |
| <i>Rv1026</i>  | <i>Rv1026</i>  | VDA      | hypothetical protein  |
| <i>Rv1106c</i> | <i>Rv1106c</i> | IMaR     | 3-beta-hydroxysteroid dehydrogenase   |
| <i>Rv1186c</i> | <i>Rv1186c</i> | CH       | hypothetical protein  |
| <i>Rv1202</i>  | <i>dapE</i>    | IMaR     | succinyl-diaminopimelate desuccinylase  |
| <i>Rv1232c</i> | <i>Rv1232c</i> | CH       | hypothetical protein  |
| <i>Rv1306</i>  | <i>atpF</i>    | IMaR     | ATP synthase subunit B  |
| <i>Rv1307</i>  | <i>atpH</i>    | IMaR     | Probable ATP synthase delta chain   |
| <i>Rv1348</i>  | <i>irtA</i>    | CWaCP    | Probable iron-regulated transporter irtA  |
| <i>Rv1514c</i> | <i>Rv1514c</i> | CH       | Conserved hypothetical protein  |
| <i>Rv1629</i>  | <i>polA</i>    | IP       | DNA polymerase I  |
| <i>Rv1663</i>  | <i>pks17</i>   | LM       | Probable polyketide synthase pks17  |
| <i>Rv1712</i>  | <i>Cmk</i>     | IMaR     | cytidylate kinase   |
| <i>Rv1828</i>  | <i>Rv1828</i>  | CH       | hypothetical protein  |
| <i>Rv2382c</i> | <i>mbtC</i>    | LM       | Polyketide synthase MTBC  |
| <i>Rv2476c</i> | <i>Gdh</i>     | IMaR     | probable NAD-dependent glutamate dehydrogenase  |
| <i>Rv2499c</i> | <i>Rv2499c</i> | IMaR     | Possible oxidase regulatory-related protein   |
| <i>Rv2727c</i> | <i>miaA</i>    | IMaR     | tRNA delta(2)-isopentenylpyrophosphate transferase  |
| <i>Rv2934</i>  | <i>ppsD</i>    | LM       | Phenolphthiocerol synthesis type-I polyketide synthase ppsD   |
| <i>Rv3001c</i> | <i>ilvC</i>    | IMaR     | ketol-acid reductoisomerase   |
| <i>Rv3135</i>  | <i>PPE50</i>   | PE/PPE   | PPE family protein  |
| <i>Rv3285</i>  | <i>accA3</i>   | LM       | Probable bifunctional protein acetyl-/propionyl-coenzyme A carboxylase (alpha chain)                          |



| Gene           | Name           | Function | Description   |
|----------------|----------------|----------|---|
| <i>Rv3411c</i> | <i>guaB2</i>   | IMaR     | inositol-5-monophosphate dehydrogenase  |
| <i>Rv3515c</i> | <i>fadD19</i>  | LM       | Probable fatty-acid-coA ligase fadD19 (fatty-acid-coa synthetase) (fatty-acid-coa synthase) |
| <i>Rv3593</i>  | <i>lpqF</i>    | CWaCP    | Probable conserved lipoprotein lpqF   |
| <i>Rv3627c</i> | <i>Rv3627c</i> | CH       | hypothetical protein  |
| <i>Rv3658c</i> | <i>Rv3658c</i> | CWaCP    | Probable conserved transmembrane protein  |
| <i>Rv3793</i>  | <i>embC</i>    | CWaCP    | Integral membrane indolylacetylinoitol arabinosyltransferase                                |
| <i>Rv3799c</i> | <i>accD4</i>   | LM       | Probable propionyl-CoA carboxylase beta chain 4   |

RP - regulatory proteins; IMaP - intermediate metabolism and respiration; CWaCP - cell wall and cell processes; LM - lipid metabolism; IP - information pathway; VDA - virulence, detoxification, adaptation; PE/PPE - PE/PPE protein families; CH - conserved hypotheticals

Table 2. CUGs, found as essential according (Sasseti et al., 2003)

## 6. Conclusion

Infectious diseases caused by intracellular pathogenic bacteria represent a significant challenge to health care. The course of the infection depends not only on the protective mechanisms, but also on the specific expression of bacterial genes. Altered expression as a response to the immune reaction of the host organism is critical for the survival and functioning of pathogenic bacteria. Understanding of *M. tuberculosis* transcriptional responses to different stimuli and aggressiveness of the environment gives the opportunity to describe the adaptation mechanisms necessary for bacterial successful survival and colonization of the host.

*M. tuberculosis* transcription profiling obtained in different conditions allows to define the core set of adaptive genes (we called it “commonly upregulated genes”), which characterize different phases of *M. tuberculosis* intracellular life - from primary infection through latency to reactivation. The expression of these genes can be considered as a universal reaction of mycobacteria to various stress factors of the environment. Accumulation and analysis of such data is the surest way to proceeding and developing effective approaches towards diagnostics and treatment of tuberculosis.

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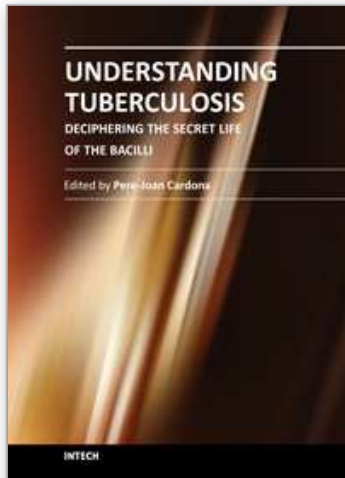
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## **Understanding Tuberculosis - Deciphering the Secret Life of the Bacilli**

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*Mycobacterium tuberculosis*, as recent investigations demonstrate, has a complex signaling expression, which allows its close interaction with the environment and one of its most renowned properties: the ability to persist for long periods of time under a non-replicative status. Although this skill is well characterized in other bacteria, the intrinsically very slow growth rate of *Mycobium tuberculosis*, together with a very thick and complex cell wall, makes this pathogen specially adapted to the stress that could be generated by the host against them. In this book, different aspects of these properties are displayed by specialists in the field.

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