Dormant Mycobacterium tuberculosis: a quiescent form with an eye open

Tuberculosis (TB) is the leading cause of mortality by a single pathogen in humans. *Mycobacterium tuberculosis* (Mtb), the etiologic agent of TB, causes millions of new cases each year. TB is an ancient disease and Mtb is highly adapted to the human body. When Mtb infects a new host, in the vast majority of cases it establishes a latent infection. It is estimated that approximately one third of human population is infected by Mtb, constituting a huge reservoir of the disease. During the latent infection, Mtb resides within granulomatous lesions in a non-replicative condition called dormancy. This form is highly resistant to harsh environments including low oxygen tension, nutrient starvation, high nitric oxide level, anti-TB drugs. A common *in vitro* model to study Mtb dormant physiology and gene expression is the Wayne model, which consists in incubating Mtb cultures in tubes with tightly closed caps (see insert in Fig. 1), since low oxygen concentration is a main dormancy inducer.

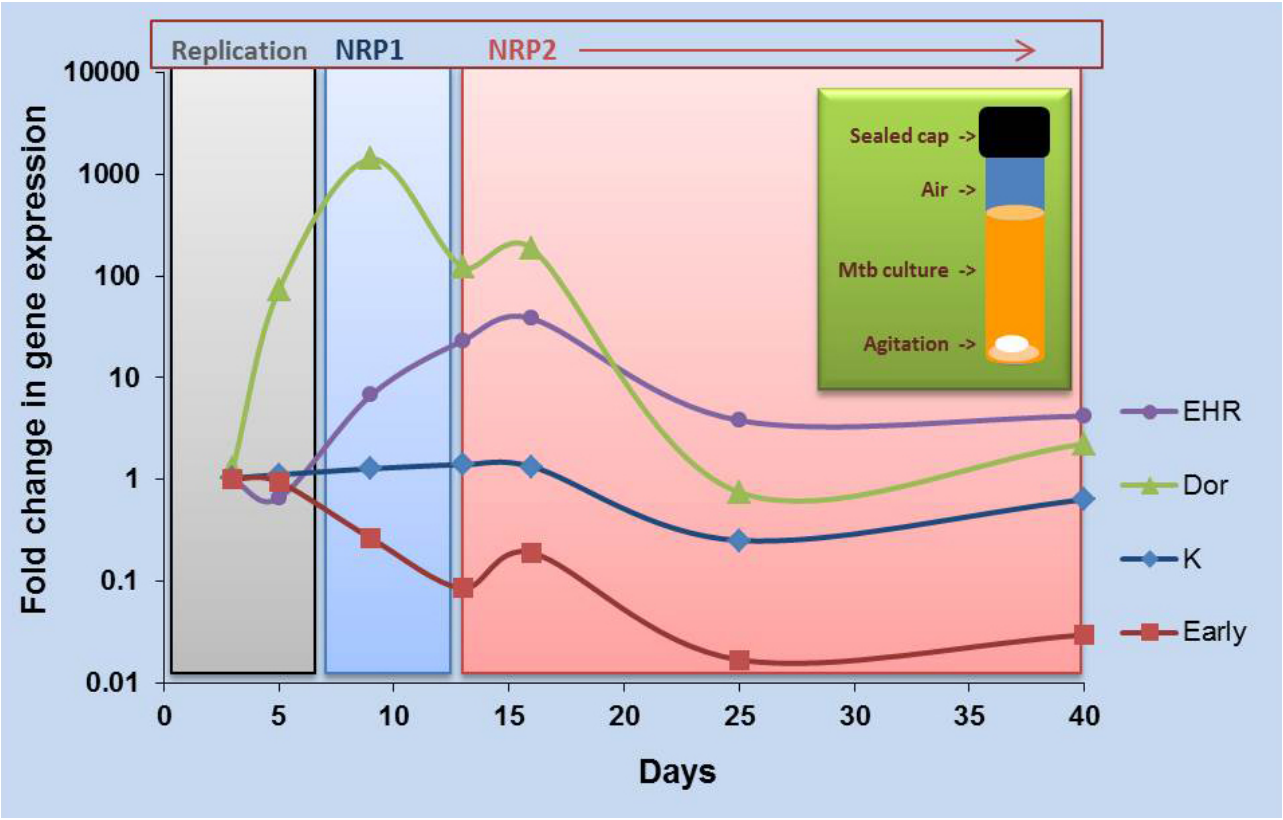

Fig. 1. The 4 patterns of gene expression in Mtb cultured in the Wayne model (insert).

In this culture model, Mtb undergoes an initial phase of replication, followed by a growth arrest (non-

replicating persistence stage 1, NRP-1) when hypoxic conditions are established, and finally, when oxygen tension becomes extremely low, by an NRP-2 stage. We studied the expression of a panel of Mtb genes at different stages of dormancy depicting four major transcription patterns (Fig. 1). A number of genes remained constant (K) throughout the 40 days examined, encompassing general transcription factors and metabolism, suggesting an active metabolic state despite the absence of replication. A set of genes known as the dormancy regulon (Dor) was highly upregulated early in dormancy. Among these genes, the Dor transcriptional regulatory factor regulator *devR*, the a-crystallin (*acr*) (a highly overexpressed heat shock gene with a role in protein stabilization), a triglyceride synthase responsible for intracellular lipid accumulation. A second and wider set of genes was upregulated in the NRP-2 stage, the so-called enduring hypoxic response (EHR). Finally, genes highly expressed in growing bacteria (Early), like those encoding for the common antigens Esat-6 and Ag85B, were downregulated during dormancy.

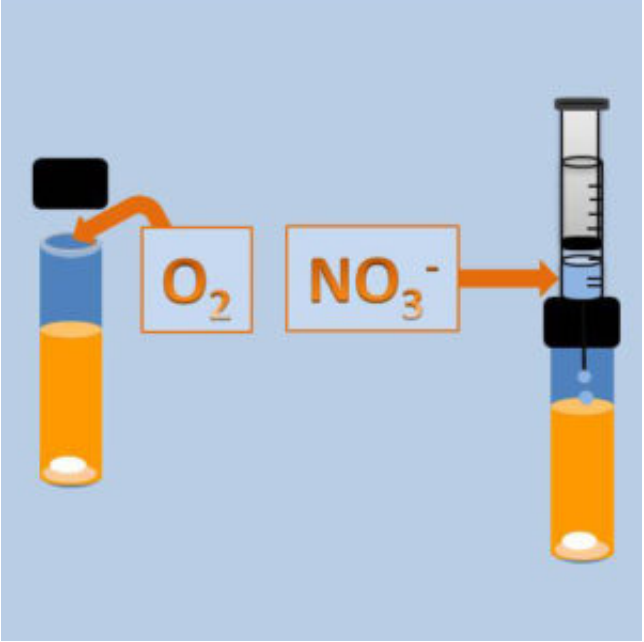

Fig. 2. Induction of respiration of dormant Mtb by O2 or NO3-

Despite dormancy is a quiescent state, we found that dormant Mtb responded very quickly to stimuli reactivating respiration. We used two methods to prove this (Fig. 2). First, after opening the tube cap to allow oxygen to diffuse in the media of late Wayne cultures, transcription was activated as soon as one hour after oxygen exposure, in particular for those genes responsible for general transcription, replication (*dnaA* and *ftsZ*), and reactivation (*rpf* genes). By consequence, replication was restored very quickly, with a very short time lapse. It is known that in the absence of oxygen, Mtb uses NO_3^- as final electron acceptor in the anaerobic respiration. So we added nitrates to late anaerobic cultures by using a syringe in order to not introduce oxygen in the cultures. Under these

conditions, nitrates strongly activated transcription of many genes, such as *esat-6*, *acr*, *icl*, *fad26*, and, in particular, *narG*, a nitrate reductase.

Overall, we showed that the switch to the dormancy phenotype consists in a huge rearrangement of genes involved in expression and metabolism, but conditions allowing increased respiration and growth mimicking those occurring for example when the host immune system has weakened, quickly activate a cell division program similar to that observed during reactivation from latent infection to active TB disease.

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