Development of an automated siRNA screening of host macrophages genes involved in *Mycobacterium tuberculosis* infection.

Jean-Philippe Carralot[1], Chang-Bok Lee[2], Tae Kyu Kim[1], Annette Boese[3], Boris Lenseigne[4], Auguste Genovesio[4], Peter Sommer[3], Thierry Christophe[2] and Priscille Brodin[1].
[1]Biology of Intracellular Pathogens Inserm Avenir Group, [2]Screening Technologies and Pharmacology, [3]Cell biology of Retrovirus, [4]Image Mining
Institut Pasteur Korea, 39-1 Hawolgok-dong, Seongbuk-gu, Seoul, 136-791, Korea.

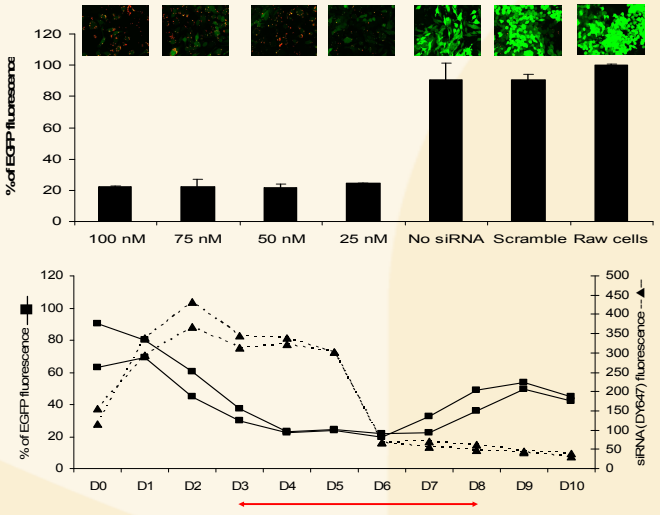
Introduction

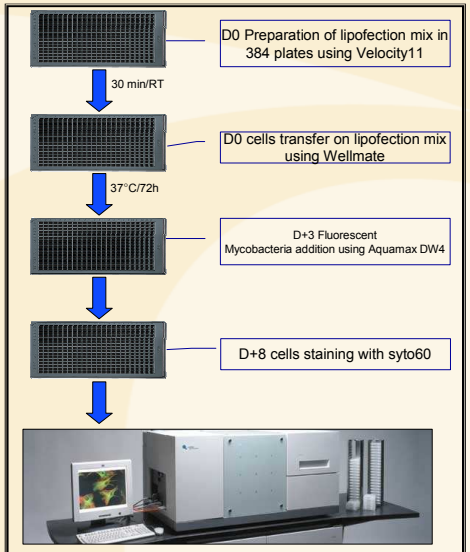
Mycobacterium tuberculosis, the etiological agent of Tuberculosis in humans, is able to invade and to actively replicate in the host macrophage a key player of the immune system specialized in infectious organisms' clearance. The successful survival of *M. tuberculosis* into macrophages is linked to its ability to manipulate the host phagosome by preventing its maturation in an acidic, hydrolytic phagolysosome.

In order to identify host genes required for *M. tuberculosis* infection and persistence, we developed a phenotypic cell-based assay in murine cells and adapted it for high throughput and high content screening purposes.

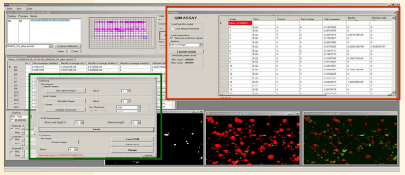
Transfection efficiency

Raw 264.7 cells expressing EGFP were transfected with DY647-labeled siRNA against EGFP. Pictures were taken at 488 and 635 nm on automated confocal microscope OPERA . Cells and siRNA fluorescences were analyzed using IM software.





Reading 4h and 5 days after infection on Opera, automated confocal microscope.

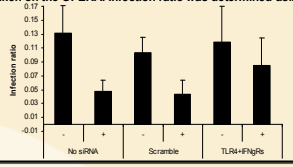


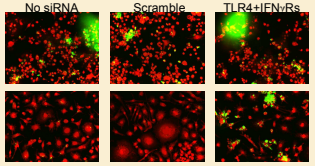
Data mining, results interpretation, hit identification

Scheme of the HTS siRNA screening

Proof of concept

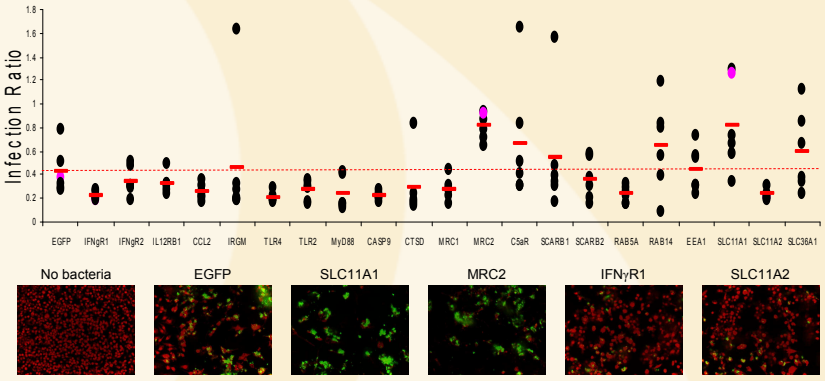
Raw 264.7 cells were transfected with 100nM of control siRNAs. Three days after transfection, cells were treated(+) or not (-) with LPS and IFNγ. The next day cells were cells were infected with *Mycobacterium tuberculosis* H37Rv expressing EGFP. Five days after infection, cells were stained with syto60 and pictures were taken on the OPERA. Infection ratio was determined using IM software.





siRNA controls

Raw 264.7 cells were transfected with 100nM of each control siRNAs. Three days after transfection, cells were infected with *M. tuberculosis* H37Rv expressing EGFP. Five days after infection, cells were stained with syto60 and pictures were taken on the OPERA. Infection ratio was determined using IM software.



Conclusions

This assay will allow the High-Through Put screening (~8000 genes/day) of genome-wide trasncripts involved in *M. tuberculosis* infection.

These screenings should provide a comprehensive picture of host's interactions with the tubercle bacillus and pave the way to further screens on host-pathogens interactions investigation.

RNAi Europe Conference, Barcelona 20-21st Sept. 2007
Poster #168