No.	K22-3082	
研究課題名	Analysis of the mechanisms of co-infection with Mycobacterium and HIV in human macrophage linage cells	
研究代表者	Mi Kaixia (Institute of Microbiology, Chinese Academy of Sciences・教授)	
研究組織	受入教員	川口 寧 (東京大学医科学研究所・教授)
	分担者	Kaixia Mi (Institute of Microbiology, Chinese Academy of Sciences・Principal Investigator)
	分担者	Xintong Zhou (Institute of Microbiology, Chinese Academy of Sciences・Assistant Professor)
	分担者	Yasushi Kawaguchi (Division of Molecular Virology・Professor)
	分担者	Jin Gohda (Research Center for Asian Infectious Diseases・Project Associate Professor)

(Form 2-2)

Principal Investigator

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Select type of report▼

Report

Our research focuses on the interaction between *Mycobacterium tuberculosis* (Mtb) and HIV-1. We previously discovered that infection with the BCG strain of Mtb upregulates transcription of HIV-1 latent provirus in HIV-1 latently infected model cells derived from human monocyte cells (THP-1 cells). This finding indicates that latent HIV-1 can be reactivated by Mtb infection.

In this reporting period, we expanded our investigation into the signaling transduction pathways involved in HIV-1 reactivation following Mtb infection and conducted an in-depth analysis of the mycobacterial LprG gene's functions in bacterial-host interactions.

Key Findings

HIV-1 Reactivation by Mtb Infection

We established that TLRs recognize Mtb-derived pathogen structures and induce innate immune responses during Mtb infection. MyD88, an adaptor protein essential for signal transduction through almost all TLRs, was investigated by establishing MyD88-deficient HIV-1 latent model THP-1 cells using a CRISPR/Cas9 system. Our results demonstrate that Mtb infection induces reactivation of latent HIV-1 through both TLR-dependent and independent mechanisms, highlighting the crucial role of TLRs in Mtb-HIV co-infection.

LprG Gene Function Analysis

I. Effect of the LprG Gene on Bacterial Growth

By knocking out the LprG gene in *Mycobacterium bovis* BCG, we found that this gene has no significant effect on the growth of BCG. This finding aligns with previous research results and further confirms that the *LprG* gene is non-essential for BCG growth processes.

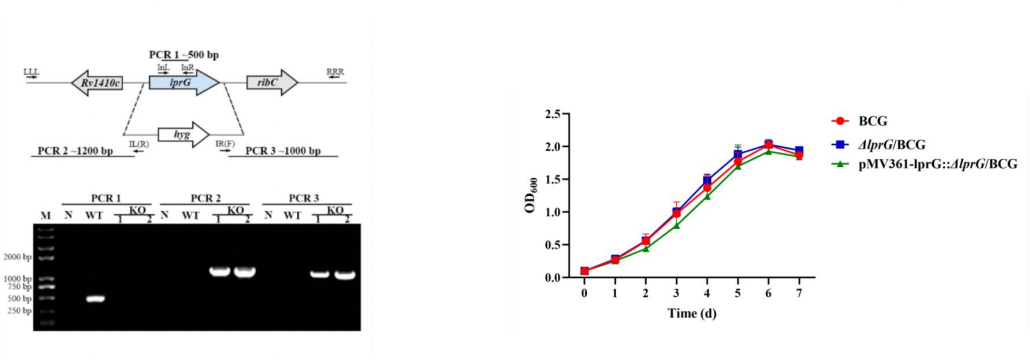

Fig 1. Effect of LprG on mycobacterial growth.

II. Role of LprG in bacterial entry and intracellular survival

We discovered that mycobacterial LprG, a secrted protein, significantly affect both mycobacterial entry into host cells and survival within host cell.

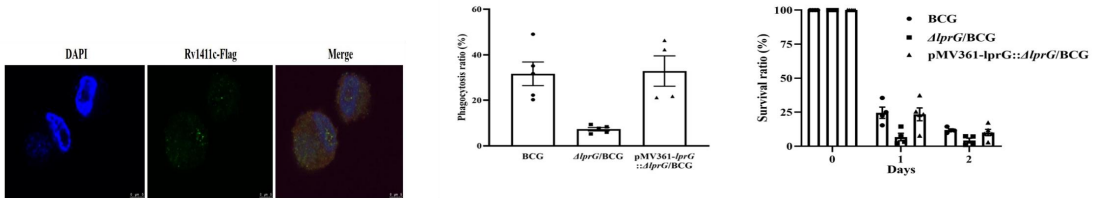

Fig 2. LprG's effect on mycobacterial entry and survival

III. LprG-CypA interaction and its functional significance

Our research revealed that LprG protein directly interacts with host Cyclophilin A (CypA) protein. This interaction substantially impacts mycobacterial survival and reproduction within macrophages. Through extensive experimentation, we confirmed the critical role of LprG-CypA interaction in bacterial adaptation to the host environment.

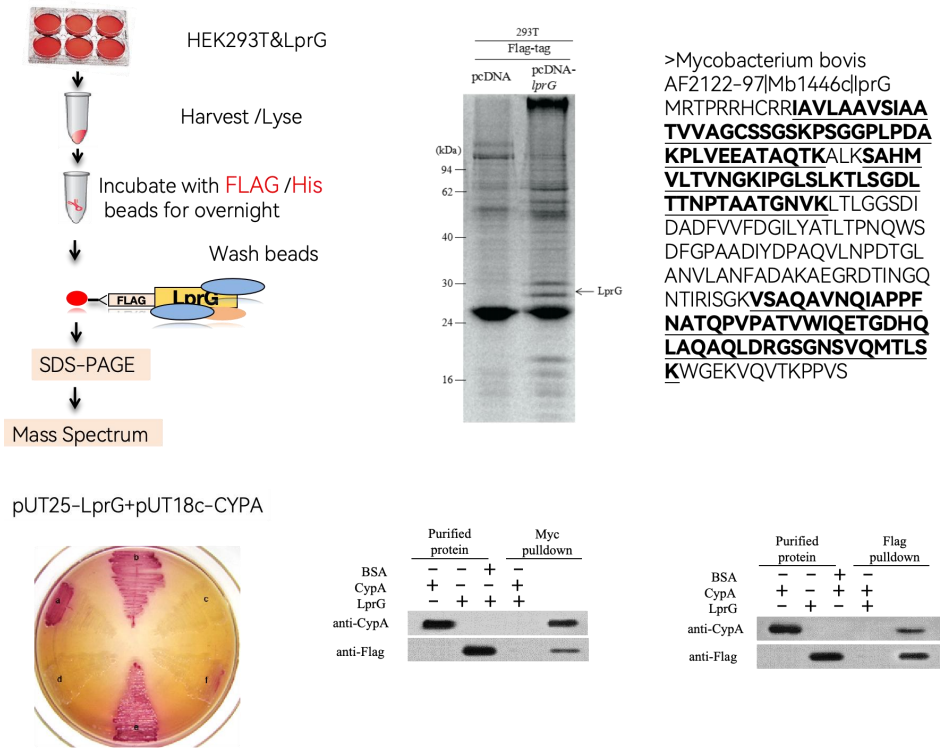

Fig 3. The interaction of LprG with CypA

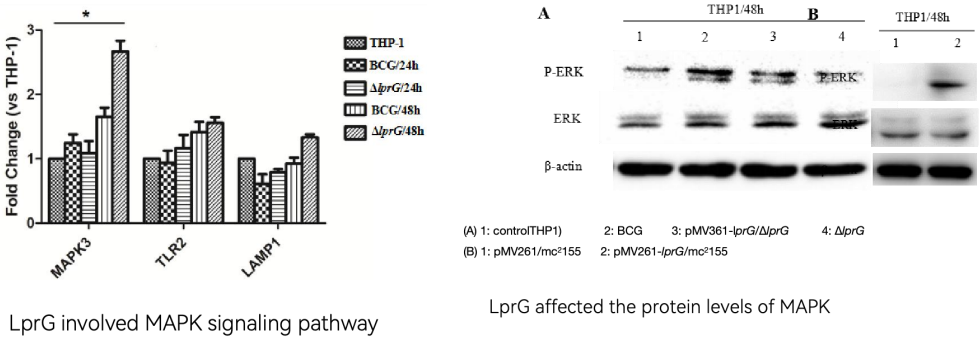

Fig 4. LprG-CypA interaction affect mycobacterial survival within macrophages

IV. Molecular Basis of LprG-CypA interaction

1. Bioinformatics analysis

Our prediction results show that the LprG protein contains intrinsically disordered regions (IDRs), which may promote phosphorylation modifications. In addition, LprG shows a tendency to aggregate, potentially related to its function. In contrast, CypA is an ordered protein.

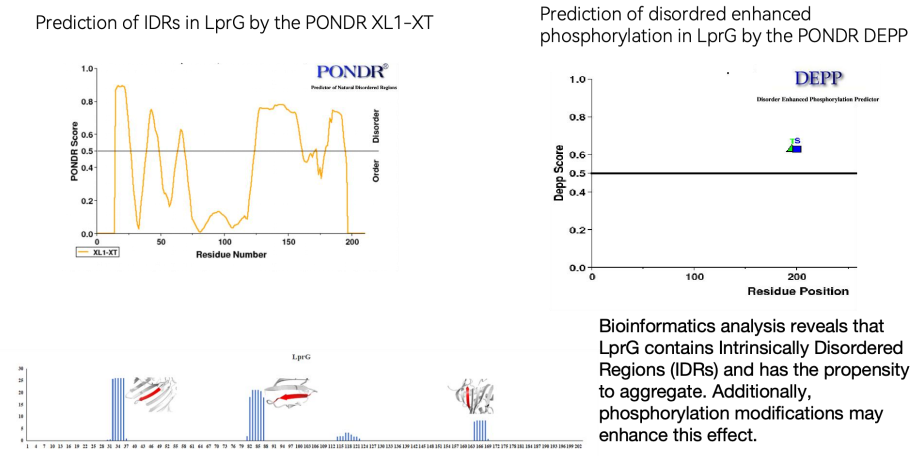

Fig 4.1.1 Bioinformatics analysis of LprG

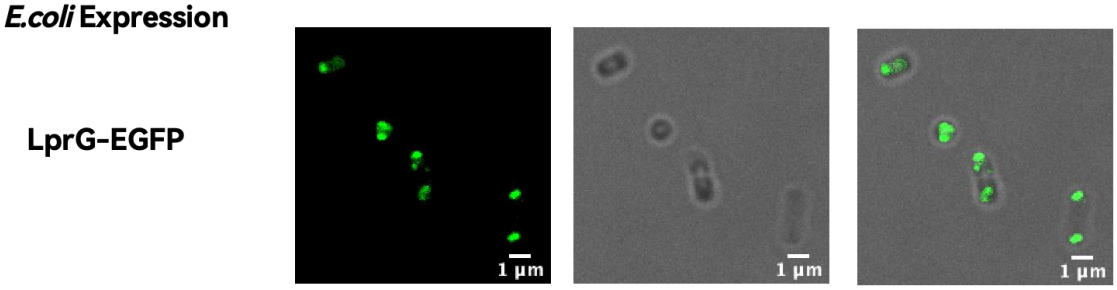

Fig 4.1.2 The propensity of LprG condensates in *E. coli.*

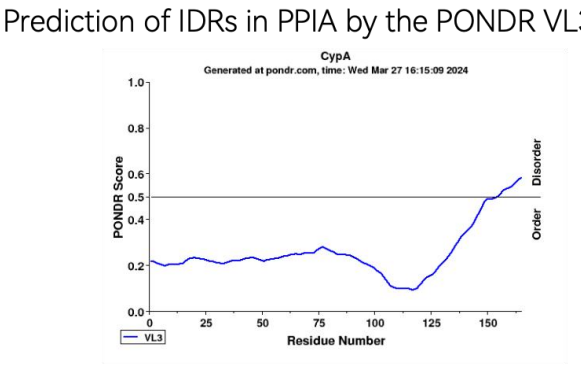

Fig 4.2.1 Bioinformatics analysis of CypA

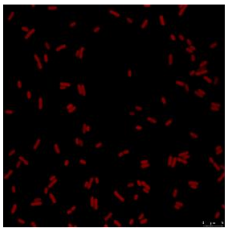

Fig 4.2.2 *E. coli* expressing CypA-mCherry

Notably, CypA aggregates and form co-condensates with LprG.

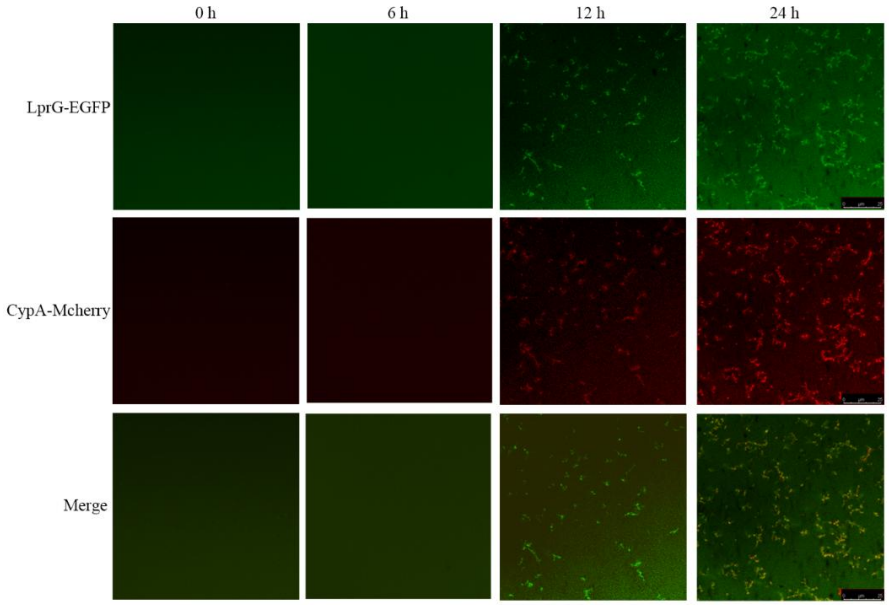

Fig 5. CypA and LprG form co-condensates

V. CypA-mediated survival mechanisms

Through proteome analysis of wild-type BCG and BCG::Δ*Rv1411c* (LprG-deficient) infected-macrophages, we discovered that LprG restrict CypA function, modulates host immune responses to BCG's infection, and affects lipid metabolism pathways.

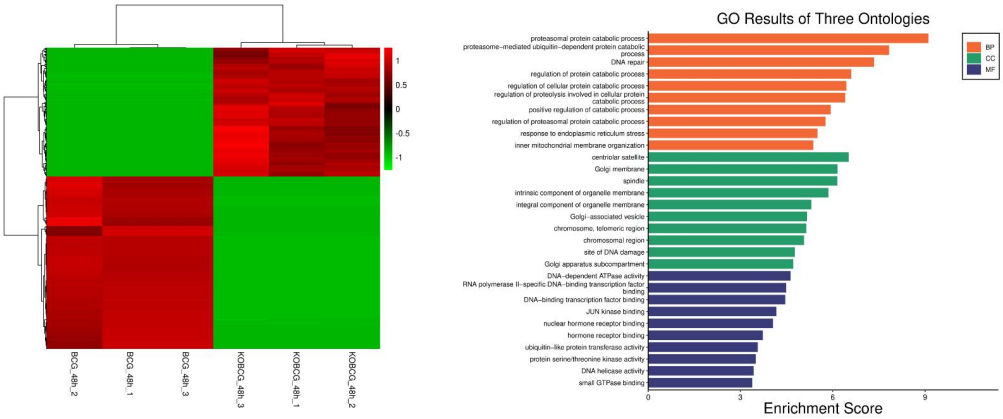

Fig 6. The proteomic analysis of BCG and BCG::Δ*Rv1411c* infected-macrophages

Conclusion

Our research provides significant insights into the interaction between mycobacteria and HIV-1 in co-infected hosts. We showed that Mtb infection induces HIV-1 reactivation through TLR-dependent and independent pathways. Furthermore, we characterized the mycobacterial LprG protein, which forms aggregates with host CypA, suppressing its biological activity. This interaction modifies the host's immune response to mycobacterial infection.

The secreted mycobacterial protein LprG forms aggregates with CypA, resulting in the suppression of CypA's biological activity. Consequently, this interaction modifies the host's immune response to mycobacterial infections.

These findings advance our understanding of Mtb-HIV co-infection mechanisms and provide potential targets for therapeutic interventions.