



PhD Program between the Freie Universität Berlin (FUB) and the China Scholarship Council (CSC)

Open PhD position at FUB for CSC scholarship candidates 2017

Please note: the PhD position is only offered to Chinese PhD candidates for application in the framework of the FUB-CSC PhD Program.

<u>Department/Institute:</u>	Department of Biology, Chemistry, Pharmacy / Institute for Chemistry and Biochemistry
<u>Subject area:</u>	Structural biochemistry, molecular biology, host-virus interactions
<u>Name of Supervisor:</u>	Prof. Markus C. WAHL, PHD (Mr.)
<u>Number of open PhD positions:</u>	1
<u>Type of the PhD Study:</u>	Full-time (4 years)
<u>Project title:</u>	Host RNA-binding/processing factors in viral replication and innate immunity

PhD Project description:

Summary. Replication of viruses relies on host gene expression and gene regulation machineries, which in turn utilize many RNA-binding and RNA-processing factors. At the same time, host RNA-binding/processing factors are part of the cellular innate immune system that forms the first line of defense against pathogens based on the recognition of pathogen-specific structures. The aims of this project are (1) to identify new host RNA-binding/processing factors involved in the propagation of selected viruses (measles, rubella and coronaviruses) that initiate infections via the respiratory tract, (2) to delineate molecular interfaces and mechanisms of capture of the host factors by the pathogens, (3) to uncover new RNA-binding/processing factors involved in innate immune responses and (4) to clarify the molecular mechanisms by which they act. Identifying novel host-pathogen interactions may pave the way for new strategies towards therapeutic interventions.

Background. Measles virus is a single-stranded, negative-sense RNA virus that infects humans and, despite global vaccination campaigns, remains a serious health threat with an estimated 120,000 fatal cases annually worldwide. Rubella virus is a single-stranded, positive-sense RNA virus, and rubella infections during the first trimester of pregnancy lead to birth of 100,000 children per year with congenital rubella syndrome, of which the underlying molecular processes are poorly understood. Viral mutations might render present vaccinations ineffective and therapeutic measures for unprotected cases are presently lacking. Coronaviruses are single-stranded, positive-sense RNA viruses that infect a wide range of mammals and birds and can lead to respiratory syndromes like SARS and MERS in humans, for which there are presently no therapies. Alternative strategies for antiviral drug development are therefore urgently needed. Here, we aim at identifying host RNA-binding/processing factors utilized by the viruses or involved in the initial responses to fend off infections, to develop new strategies for therapeutic intervention.

To make economic use of their compact genomes, viruses have evolved elaborate mechanisms, including overlapping genes and promoters, coding on sense and antisense strands, translational frameshifting, stop-codon read-through, editing, alternative splicing¹ or, in the case of coronaviruses, discontinuous transcription that involves targeted RNA polymerase template switching². To implement these mechanisms, viruses hijack and reprogram gene expression and gene regulatory machineries of the host. These host machineries rely on a large number of RNA-binding/processing factors. Not surprisingly, therefore, genome-wide RNAi screens and functional genomics approaches have identified, among others, host RNA-binding/processing factors that are involved in infection and replication of influenza A and HIV viruses³⁻⁵. E.g., the Smu1-RED splicing factor complex interacts with influenza A virus polymerase and knock-down of these proteins led to decreased levels of spliced NS2 mRNA⁶. Furthermore, the HIV-1 Tat protein interacts with the spliceosomal biogenesis factor SART3 to support HIV-1 gene expression and viral replication⁷. However, no comprehensive screen for such host cell factors has so far been performed for measles, rubella or coronaviruses.

As a first line of defense against viral infections, pattern recognition receptors (PRRs) of the innate immune system recognize pathogen-associated molecular patterns and activate signaling cascades that induce protective cellular responses. Apart from the well-characterized membrane-bound PRRs (e.g. toll-like and C-type lectin receptors) or cytoplasmic PRRs (e.g. NOD-like and RIG-I-like receptors), several RNA-binding/processing factors of the host gene expression/regulation machineries have also been identified as components of their innate immune systems. E.g., recent findings revealed that host RNA degradation and surveillance mechanisms, such as nonsense-mediated decay (NMD), which are cornerstones of post-transcriptional gene regulation in eukaryotes, are also involved in detecting and combating infecting viruses^{8,9}. Other RNA quality control pathways, such as non-stop decay or no-go decay, and the associated

degradative machineries also have the potential for contributing to the cellular defense against virus invasion^{9,10}. Moreover, several transcription and splicing factors have been implicated in innate immunity. E.g., the spliceosomal RNA helicase, *Brr2*, has been shown to cooperate with TANK kinase 1 (TBK1) in recognizing viral RNA and promoting an antiviral innate immune response¹¹, while the related transcriptional ASCC3 helicase was found as a negative regulator of the host defense system¹². We have previously studied the structures of some of these proteins¹³⁻¹⁵.

Research strategy. To identify host RNA-binding/processing proteins hijacked by measles, rubella or coronaviruses, as well as to detect host RNA-binding/processing proteins involved in combating these viruses, we propose to perform targeted RNAi screens, using a siRNA library against around 200 human RNA-binding/processing proteins. The siRNA-transfected cells will be infected with measles, rubella or coronaviruses, and assayed for virus propagation using established RT-PCR assays and reporter viruses enabling read-out by live cell fluorescence microscopy. Proteins whose knock-down leads to a defect or enhancement in virus propagation will be scored as potential host cell interaction or innate immunity factors, respectively. Candidate proteins will be further investigated using diverse biochemical and genetics tools to determine molecular mechanisms that drive the virus life cycle in the host cell or that restrict virus propagation. We also plan to elucidate the interaction interfaces of the involved host and viral factors at atomic resolution. We will then target these interfaces by site-directed mutagenesis to interfere with interactions, assess the consequences of such manipulation for virus infectivity and propagation and test whether other viruses target the same host proteins. Moreover, after identifying interfering mutations, the corresponding mutated virus variants will be generated by reverse genetics and the phenotypes of these viruses will be studied. Eventually, we plan to develop chemical compounds that interfere with host-virus interactions or that support innate immune responses.

References

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- 4 Karlas, A. et al. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature* 463, 818-U132, (2010).
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- 7 Liu, Y., Li, J. L., Kim, B. O., Pace, B. S. & He, J. J. HIV-1 tat protein-mediated transactivation of the HIV-1 long terminal repeat promoter is potentiated by a novel nuclear tat-interacting protein of 110 kDa, Tip110. *J Biol Chem* 277, 23854-23863, (2002).
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- 14 Absmeier, E. et al. The large N-terminal region of the *Brr2* RNA helicase guides productive spliceosome activation. *Genes Dev* 29, 2576-2587, (2015).
- 15 Mozaffari-Jovin, S. et al. Inhibition of RNA helicase *Brr2* by the C-terminal tail of the spliceosomal protein *Prp8*. *Science* 341, 80-84, (2013).

Language requirements:

The doctoral thesis can be written in English or German. English is the working language in our lab. Proficiency in German is not required (neither required in the lab nor for the thesis). English requirements: IELTS 6.5 or TOEFL 95

Academic requirements:

We welcome applications from students holding a Bachelor or Master's degree in Biochemistry or a related area of the Life Sciences. While students typically enter a PhD program with a Master's degree, in principle students can join after their Bachelor's degree. Students need to have a strong theoretical background in Biochemistry and Molecular Biology. Students need to have basic biochemical and molecular biological laboratory skills. For example, they should have practical experience with molecular cloning, recombinant protein production and protein purification. Additional background in Structural Biology or Bioinformatics is a plus.

Information of the professor or research group leader:

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Please note:

In a first step the complete application should be submitted to the Beijing Office for evaluation by January 4th, 2017. Please don't contact the professor before. He/She will get in contact with you after having received the complete application in January.