Junior Research Group VI
Function and regulation of interferon-induced molecular complexes

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Interferon-induced proteins are involved in cell autonomous resistance against viruses, bacteria and protozoa. The aim of this project is to understand the regulation of these proteins by post-translational modifications. These covalent modifications by either lipids or proteins from the ubiquitin family regulate the assembly of dynamin related Mx and guanylate-binding proteins (GBPs) as well as PML nuclear bodies (PML-NBs) which are in the focus of our research.

Introduction
Upon infection animals and plants activate an array of non-specific defence mechanisms on the cell based as well as the systemic level. This innate immune system does not confer long-lasting immunity against the pathogen but detains the dissemination of the pathogen which can reside in different compartments of the infected host cell (Fig. 1). In vertebrates, interferons and other cytokines regulate the activation of the innate immune system as well as the cross-talk with the adaptive immune response. In mammals there are over 300 interferon-stimulated genes (ISGs). Much less is known, however, about the molecular mechanisms by which these anti-viral and anti-microbial systems detect and combat the pathogens and how these in return try to evade them.

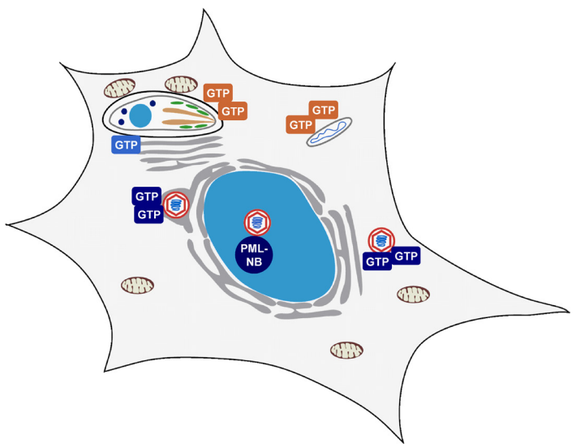

Fig. 1: Intracelullar pathogens and interferon-induced resistance factors. Many eukaryotic, bacterial and viral pathogens reside and replicate in specific compartments within the host cell. Among the several hundred resistance factors which are upregulated in response to interferons are also the dynamin-related Mx and guanylate-binding proteins (dark and light blue), the IRG GTPases (red) and the PML protein in the nucleus.

PML nuclear bodies
PML-NBs are a sub-nuclear compartment defined by the presence of the PML protein which was initially identified as part of a fusion protein with the retinoic acid receptor (RAR) α in cells from patients suffering from acute promyelocytic leukaemia (APL). They control the transcription and replication of chromosomal and viral DNA in the nucleus by regulated sequestration and release of proteins. The modification of the PML protein by the small ubiquitin-like modifier (SUMO) protein is essential for the assembly of PML-NBs. Several viruses such as herpes simplex virus or treatment of cells with arsenic trioxide lead to the degradation of PML and the dispersal of PML-NBs. Thus, it was thought that SUMOylation stabilises the protein by preventing its targeting for the proteasome. PML and other PML-NB proteins also contain SUMO interaction motifs (SIMs).

SUMO-dependent ubiquitin ligases
In collaboration with the group of Jürgen Dohmen at the Institute for Genetics we found that chain forming SUMO-2 conjugates are prone to degradation by the proteasome but the mono-SUMO-1 conjugates are not (Uzunova *et al.* 2007). We identified a class of proteins named "ubiquitin ligases for SUMO conjugates" or ULS proteins. These proteins are classified by the presence of a RING domain with ubiquitin E3-ligase activity and one or more SIMs (Miteva *et al.* 2010). We found that the human RING finger protein RNF4, which localises to PML-NBs, is a ULS protein (Weisshaar *et al.* 2008,). It specifically ubiquitylates SUMO-2/3 modified PML *in vitro* and this process leads to PML degradation in response to arsenic treatment *in vivo* (Fig. 2).
This process is the molecular basis for the degradation of the PML-RARα fusion protein in treatment of APL patients. Furthermore, we could show that arsenic leads to an increase of total SUMO-2/3 conjugate levels to a similar extent as ethanol or hydrogen peroxide. However, when cells were exposed to osmotic stress PML degradation was not activated. Instead, PML-NBs became larger and more abundant and showed an enhanced accumulation of SUMO-1. These data suggest a differential response of so far unidentified signalling processes to external stimuli. As a result PML-NBs can switch their function between protein storage and protein degradation depending on the SUMOylation state of the resident proteins and by the recruitment and activation of ULS proteins.

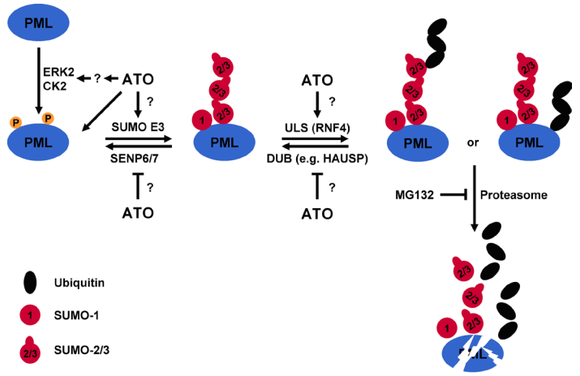

Fig. 2: Arsenic stimulates the degradation of PML by RNF4 mediated ubiquitylation. Phosphorylation of PML results in an enhanced SUMOylation of PML by SUMO2/3 chains. These chains recruit the ubiquitin ligase RNF4 leading to the ubiquitylation of the SUMO chains and of PML. Due to its reactivity arsenic (As_2O_3, ATO) could interfere with this process at several sites.

Membrane binding of human GBP1
The guanylate-binding proteins show a co-operative hydrolysis of GTP to GMP (Kunzelmann *et al.* 2006). The protein forms dimers in the GTP bound state which can assemble into tetramers. In mammalian cells GBP is subjected to post-translational modifications. In the case of human GBP1 the C-terminal CaaX motif serves as a signal for isoprenylation.
We have established *in vitro* and *in vivo* systems to produce lipid-modified hGBP1. The lipid modified hGBP1 binds to synthetic liposomes selectively in the activated state and displays a preference for PI(P)P. Thus, we are able to reproduce the *in vivo* situation, where hGBP1 is recruited to the Golgi complex after activation and interferon stimulation. We also found that the protein is efficiently recruited to newly forming phagosomes (Fig. 3) at the plasma membrane.

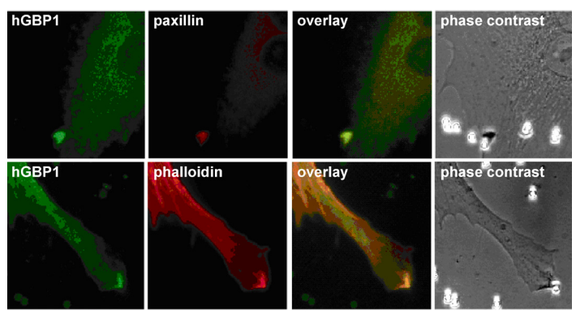

Fig. 3: Membrane binding of hGBP1. Phagosome recruitment of hGBP1 in primary human fibroblasts induced with interferon-γ.

This recruitment does not require activation by aluminium fluoride but a functional GTPase-domain and lipid modification. These data underline a functional similarity of the guanylate-binding proteins and the IRG proteins.

Perspectives
We want to resolve the biological function of interferon induced protein complexes in innate immunity on a molecular level. The assembly and localisation of these complexes is regulated by the post-translational modification of their key components. This is equally true for the cytosolic guanylate-binding proteins as well as the PML nuclear bodies. Having systems at hand to produce the proteins in the modified form, we are able to analyse the regulation of their biochemical properties such as substrate turnover, membrane binding and interactions with other proteins *in vitro* and to validate these data in cellular assays.

Selected publications
Kunzelmann, S., Praefcke, G.J.K., and Herrmann, C. (2006). Transient kinetic investigation of GTP hydrolysis catalyzed by interferon-gamma induced human guanylate binding protein 1 (hGBP1). J. Biol. Chem. 281, 28624-28635.

Uzunova, K., Göttsche, K., Miteva, M., Weißhaar, S.R., Glanemann, C., Schnellhardt, M., Niessen, M., Scheel, M., Hofmann, K., Johnson, E.S., Praefcke, G.J.K., and Dohmen, R.J. (2007). Ubiquitin-dependent proteolytic control of SUMO conjugates. J. Biol. Chem. 282, 34167-34175.

Weisshaar, S.R., Keusekotten, K., Krause, A., Horst, C., Springer, H.M., Göttsche, K., Dohmen, R.J., and Praefcke, G.J.K. (2008). Arsenic trioxide stimulates SUMO-2/3 modification leading to RNF4-dependent proteolytic targeting of PML. FEBS Letters 582, 3174-3178.

Fres, J.M., Muller, S., and Praefcke, G.J. (2010). Purification of the CaaX-modified, dynamin-related large GTPase hGBP1 by coexpression with farnesyltransferase. J Lipid Res 51, 2454-2459.

Miteva, M., Keusekotten, K., Hofmann, K., Praefcke, G.J.K., and Dohmen, R.J. (2010). SUMOylation as a signal for polyubiquitylation and proteasomal degradation. Chapter in: Conjugation and deconjugation of ubiquitin family modifiers, Landes Bioscience.