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On the use of *Legionella*/*Rickettsia* chimeras to investigate the structure and regulation of *Rickettsia* effector RalF

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ABSTRACT

A convenient strategy to interrogate the biology of regulatory proteins is to replace individual domains by an equivalent domain from a related protein of the same species or from an ortholog of another species. It is generally assumed that the overall properties of the native protein are retained in the chimera, and that functional differences reflect only the specific determinants contained in the swapped domains. Here we used this strategy to circumvent the difficulty in obtaining crystals of *Rickettsia prowazekii* RalF, a bacterial protein that functions as a guanine nucleotide exchange factor for eukaryotic Arf GTPases. A RalF homolog is encoded by *Legionella pneumophila*, in which a C-terminal capping domain auto-inhibits the catalytic Sec7 domain and localizes the protein to the *Legionella*-containing vacuole. The crystal structures of domain-swapped chimeras were determined and used to construct a model of *Legionella* RalF with a RMSD of less than 1 Å with the crystal structure, which validated the use of this approach to build a model of *Rickettsia* RalF. In the *Rickettsia* RalF model, sequence differences in the capping domain that target it to specific membranes are accommodated by a shift of the entire domain with respect to the Sec7 domain. However, local sequence changes also give rise to an artifactual salt bridge in one of the chimeras, which likely explains why this chimera is recalcitrant to activation. These findings highlight the structural plasticity whereby chimeras can be engineered, but also underline that unpredictable differences can modify their biochemical responses.

1. Introduction

Rickettsia prowazekii (Rp), the intracellular bacteria responsible for epidemic typhus, invades endothelial cells and replicates in the host cytosol by mechanisms that remain largely unknown (reviewed in (Sahni et al., 2013)). Notably, although the Rp genome encodes most components of a type IV secretion system, how translocated effector proteins subvert or exploit eukaryotic cellular processes is still poorly understood. One of these effectors is RalF, which modulates the actin cytoskeleton when expressed in cells (Alix et al., 2012). RpRalF comprises a Sec7 domain homologous to eukaryotic guanine nucleotide exchange factors (GEF) for Arf proteins, a family of small GTPases that controls most aspects of cellular traffic in eukaryotes (reviewed in (Cherfils, 2014; Donaldson and Jackson, 2011)) (Fig. 1A). RpRalF was discovered based on its sequence homology with an effector secreted by *Legionella pneumophila* (LpRalF) (Nagai et al., 2002). LpRalF functions as a bacterial-encoded ArfGEF that activates host Arf GTPases at the surface of the *Legionella*-containing vacuole (LCV) where this pathogen hides and replicates (Nagai et al., 2002). The Sec7 domain of LpRalF is followed by a capping domain unrelated to any eukaryotic domain, which blocks access to the active site in solution (Amor et al., 2005) (Fig. 1A). Binding of this domain to the membrane of the LCV locates LpRalF to this organelle and releases auto-inhibition (Alix et al., 2012; Folly-Klan et al., 2013). Like LpRalF, RpRalF activates Arf GTPases by its Sec7 domain and has a capping domain that is auto-inhibitory in solution and converts RpRalF into an active ArfGEF on membranes (Alix et al., 2012; Folly-Klan et al., 2013). This underlines that although LpRalF and RpRalF have similar biochemical functions, they have different effector functions in the host cell (Alix et al., 2012).

In vitro, LpRalF activates Arf GTPases both on anionic membranes that resemble those of the phagosome, and on membranes with packing defects that resemble those of vesicles from the endoplasmic reticulum which are incorporated in the

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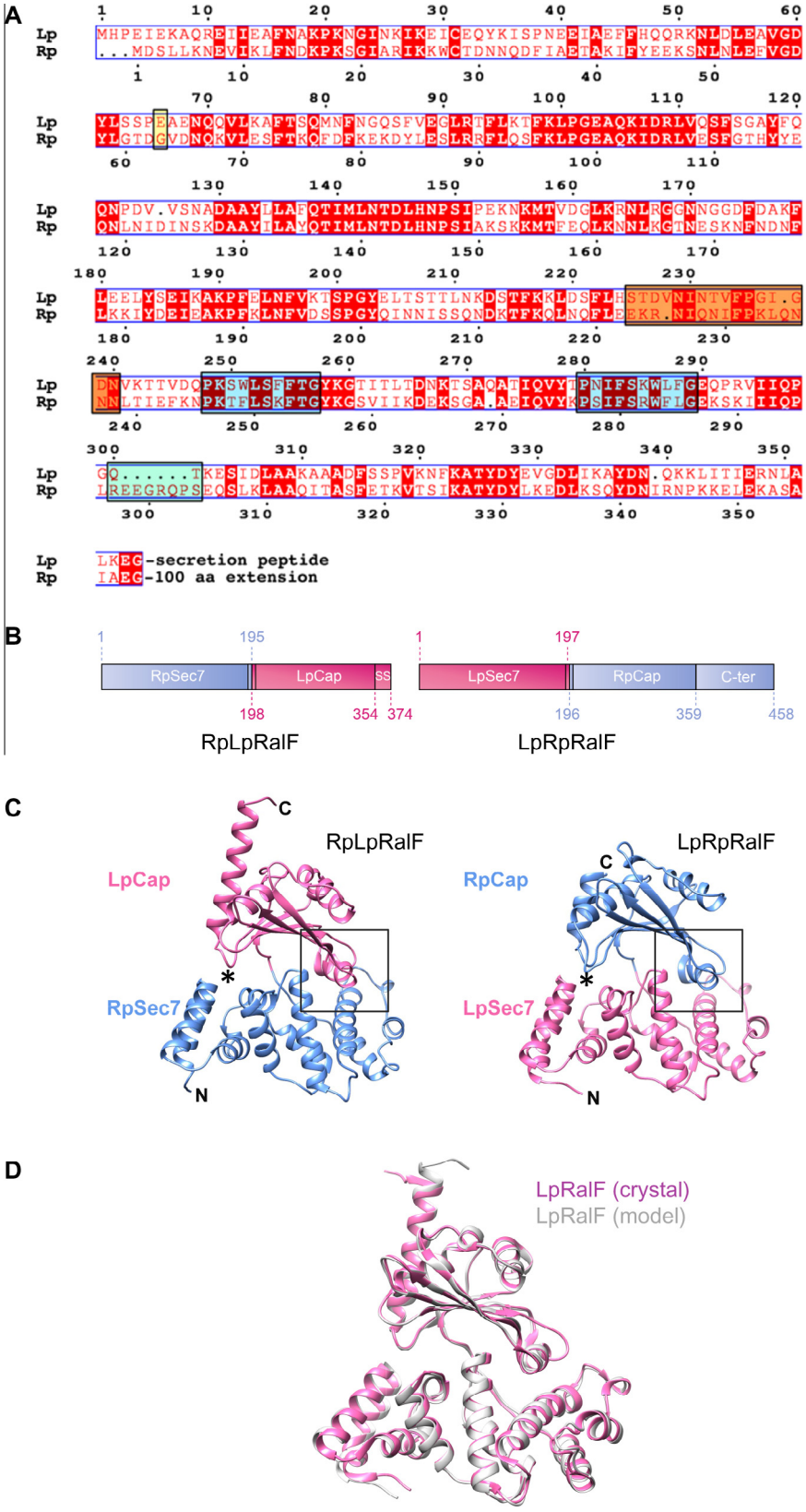

Fig.1. Structure of the LpRpRalF and RpLpRalF chimeras. (A) Sequence alignment of *Legionella pneumophila* (Lp) and *Rickettsia prowazekii* (Rp) RalF proteins. The Dot/Icm secretion motif of LpRalF, which is not visible in the electron density of RpLpRalF, and the 100-amino acid extension of RpRalF, which is cleaved in the crystal of LpRpRalF, are not shown. The auto-inhibitory helices of the aromatic cluster are boxed in blue, the insertion in $RpRalF^{cap}$ in green and the regions in the capping domains that have different conformation in RpRalF and LpRalF in orange. The residue boxed in yellow corresponds to a sequence difference that may lead to increased auto-inhibition in the LpRpRalF chimera (see text and Fig. 3B). (B) Design of the chimeras used in this study. (C) Crystal structures of the LpRpRalF and RpLpRalF chimeras. Both chimeras have an auto-inhibited conformation similar to that of LpRalF. Domains from *Legionella* RalF are in magenta, domains from *Rickettsia* RalF in blue. The auto-inhibitory helices are boxed. The KATY motif is indicated with an asterisk. (D) Superposition of the crystal structure of LpRalF (in magenta, PDB entry 1XSZ (Amor et al., 2005)) and the model of LpRalF constructed with the chimeras (in gray).