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Mechanism of Rab1b deactivation by the *Legionella pneumophila* GAP LepBEmerich Mihai Gazdag^{1*}, Alexandra Streller^{1*}, Ina Haneburger², Hubert Hilbi², Ingrid R. Vetter¹, Roger S. Goody¹⁺ & Aymelt Itzen^{1,3++}¹Max-Planck-Institute of Molecular Physiology, Department of Physical Biochemistry, Dortmund, ²Max von Pettenkofer-Institute, Ludwig-Maximilians-University, Munich, and ³Center for Integrated Protein Science Munich, Chemistry Department, Technische Universität München, Garching, Germany

Legionella pneumophila is an intracellularly surviving pathogen that releases about 270 different proteins into the host cell during infection. A set of secreted proteins takes control of the vesicular trafficking regulator Rab1. *Legionella* LepB inactivates Rab1 by acting as a GTPase-activating protein (GAP). We present the crystal structure of the Rab1b:LepB complex together with a thorough biochemical analysis and show that the GAP domain of LepB consists of an unusual fold. LepB acts by an atypical RabGAP mechanism that is reminiscent of classical GAPs and therefore sets the protein apart from mammalian TBC-like GAPs. Surprisingly, LepB can function as a GAP for Rab3, Rab8, Rab13 and Rab35, too, suggesting that it has a broader cellular role than previously thought.

Keywords: vesicular trafficking; Legionnaires' disease
GTPase; pathogen vacuole

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INTRODUCTION

Bacterial pathogens have evolved intriguing mechanisms to interfere with various signalling pathways of eukaryotic host cells. *L. pneumophila*, the causative agent of Legionnaires' disease, resides in phagocytic cells by establishing a replicative organelle, called the *Legionella*-containing vacuole (LCV) [1–3]. For the generation of the LCV, *Legionella* releases ~270 bacterial proteins via a type IV secretion system into the cytosol of the host to modify vesicle trafficking and signalling pathways such as

apoptosis [4]. As many as six type IV secreted *Legionella* proteins target the small GTPase Rab1, a key regulator of the secretory vesicular trafficking machinery: DrrA (also known as SidM) activates Rab1 [5,6] and posttranslationally modifies the GTPase by adenylation [7], while AnkX phosphocholates Rab1 [8], and LidA binds Rab1 with exceptionally high affinity [9]. The covalent modifications of Rab1 are reversible, and *L. pneumophila* produces the effectors SidD and Lem3 that deadenylylate and dephosphocholate Rab1 [10–13], respectively. In addition, LepB is an antagonist of DrrA and deactivates Rab1 [14].

Rab proteins are the largest subfamily of small GTPases and control vesicular trafficking events by switching between inactive and active forms that are bound to GDP or GTP, respectively (for a review on Rab proteins see Novic & Hutagalung [15]). They are localized to intracellular membranes by means of carboxy-terminally attached geranylgeranyl moieties. Rab GDP/GTP exchange factors (GEFs) activate Rabs by catalyzing the displacement of the tightly bound GDP followed by subsequent loading with GTP. After fulfilling their function in vesicular trafficking, Rabs are switched off by GTPase-activating enzymes (GAPs) that stimulate the intrinsic GTP hydrolysis activity. Tight temporal and spatial control over Rab deactivation is essential for Rab function. As there are more than 60 different Rabs in humans, RabGAPs have evolved with specificity towards Rab and Rab subfamily proteins. The known RabGAPs are related in sequence and molecular structure, and belong to the TBC family (Tre-2/Bub2/Cdc16) in humans. The protein TBC1D20, for example, is the specific RabGAP for Rab1 [16]. Interestingly, *Legionella* has evolved the RabGAP LepB [14] that is entirely unrelated in amino-acid (aa) sequence to the TBC family, suggesting the possibility of different structures and enzymatic mechanisms for the stimulation of GTP hydrolysis. LepB is a 1,294 aa protein that is secreted continuously during infection, accumulates on LCVs for more than 12 h and acts as a specific GAP for Rab1 [14].

To investigate the catalytic mechanism of LepB, we determined the atomic structure of its GAP domain in complex with Rab1b, revealing an unusual RabGAP mechanism. On the basis of the

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complex structure and aa sequence alignments of various Rab proteins, we have further investigated the specificity of LepB. LepB also stimulates GTP hydrolysis on Rab3, Rab8, Rab13 and Rab35. Therefore, the RabGAP activity of LepB might have consequences for the function of more Rab proteins than previously appreciated.

RESULTS

The RabGAP domain of LepB

LepB is predicted to contain an extended coiled-coil region between aa 619–1,231 (Fig 1A). We were able to locate RabGAP activity in the domain amino terminal to the coiled-coil comprising aa 2–618. Using limited proteolysis, we could further narrow down the domain to aa 299–618 (LepB₂₉₉₋₆₁₈) (supplementary Fig S1a online). The further generation and analysis of truncated LepB fragments yielded a minimal GAP domain spanning residues 317–618 (LepB₃₁₇₋₆₁₈). To investigate the catalytic mechanism of LepB, we aimed at determining the crystal structure of its GAP domain. As the production of well-diffracting protein crystals failed, we introduced surface entropy reduction (SER) mutations into LepB₃₁₇₋₆₁₈ (see Methods for details) to obtain crystals of higher quality. The SER mutants (K457A E458A K460A) did not affect the catalytic activity of LepB₃₁₇₋₆₁₈ (supplementary Fig S1b online). We were able to determine the structure of the RabGAP domain of LepB₃₁₇₋₆₁₈ by crystallizing the protein in its native and selenomethionine-labelled form for phase determination. The crystals contain three molecules LepB per asymmetric unit that are very similar to each other with r.m.s. values of 0.849–2.133 for 886 C α residues. LepB₃₁₇₋₆₁₈ has a fold that has not been observed previously and consists of 11 α -helices that assemble in an L-shaped form in which the long arm is constituted by four N-terminal helices and the short arm by seven α -helices (Fig 1B). As the structure of LepB₃₁₇₋₆₁₈ is unrelated to TBC domains, it suggests the possibility of a GTP hydrolysis mechanism different from this family of RabGAPs (Fig 1C). The catalytic activity of LepB₃₁₇₋₆₁₈ on Rab1b is comparable to other RabGAPs (see below) and differed slightly between Rab1b loaded with GTP and Rab1b loaded with a fluorescent analogue of GTP (mant-GTP) (supplementary Fig S1a online) [12]. As Rab1b:mantGTP is a worse substrate than Rab1b:GTP, a steric hindrance of the mant-group on Rab1b–LepB complex formation seems likely. This observation also highlights a difference to TBC-family RabGAPs, which accept Rab:mantGTP complexes just as well as physiological substrates.

The catalytic mechanism of LepB

For further elucidation of LepB function, we determined the crystal structure of Rab1b (aa 3–174, Rab1b₃₋₁₇₄) in complex with the SER mutant of LepB₃₁₇₋₆₁₈, GDP and beryllium fluoride to 2.7 Å. Rab1b₃₋₁₇₄ binds in the inner corner of the L-shaped LepB₃₁₇₋₆₁₈, with the Rab1b switch I region running parallel to the short arm and the switch II helix parallel to the long arm of LepB₃₁₇₋₆₁₈ (Fig 2A). The binding interface with Rab1b₃₋₁₇₄ is mainly formed by α -helices α_{3L} and α_{5L} and the α_{3L} – α_{4L} loop of LepB (subscript L denotes LepB), and the mode of binding to Rab1b is clearly different from TBC-domain-containing GAPs such as seen in the Rab33b:Gyp1 complex (supplementary Fig S2a online). Interestingly, the α_{3L} – α_{4L} loop arginine R444_L protrudes into the nucleotide-binding pocket of Rab1b₃₋₁₇₄, making contacts to the GDP α - and β -phosphates (Fig 2B). Such arginine fingers have

Fig 1 | Structure of the GAP domain of LepB. (A) Domain architecture of LepB with a detailed depiction of the Rab1-GAP domain. The position of catalytically relevant amino acids R444 (arginine finger) and E449 (stabilizes the catalytic glutamine of Rab1b) is indicated. (B) Cartoon representation of the crystal structure of LepB₃₁₇₋₆₁₈ (K457A, E458A and K460A) coloured from blue (N terminus) to red (C terminus) (N, C: N and C terminus, respectively). (C) Cartoon representation of the crystal structure of the Rab1-GAP domain of Gyp1 (N, C: N and C terminus, respectively). The GAP-domain Gyp1 (a TBC-domain GAP of Rab1/Ypt1 [17], PDB ID 2G77) shows no structural similarity to LepB (B), suggesting the possibility of a different catalytic GAP mechanism. GAP, GTPase-activating protein.

been observed to be relevant for the catalytic activity of TBC-family RabGAPs [17], and in keeping with this, the mutant LepB₃₁₇₋₆₁₈ R444A is indeed severely impaired for Rab1b:GTP hydrolysis (Fig 2C). However, TBC proteins also provide a glutamine residue in *trans* that positions a water molecule for attack of the GTP- γ -phosphate (supplementary Fig S2b online). In the case of LepB₃₁₇₋₆₁₈ no glutamine is provided, but the conserved glutamine 67 of Rab1b (Q67_R, subscript R denotes

Fig 2 | LepB operates by a different catalytic mechanism compared with classical RabGAPs. (A) Cartoon representation of the crystal structure of the complex between Rab1b₃₋₁₇₄, LepB₃₁₇₋₆₁₈ (SER-mutant), GDP and BeF₃ (grey, orange: N- and C-terminal subdomains of the LepB GAP domain; green: Rab1b₃₋₁₇₄ with switch I (purple), switch II (yellow) and P-loop (blue)). (B) Interactions between GDP, BeF₃, Mg²⁺, Rab1b₃₋₁₇₄ and LepB₃₁₇₋₆₁₈ in the nucleotide-binding pocket. LepB provides an arginine finger (R444_L) that is involved in GTP hydrolysis. The structure suggests that Rab glutamine Q67_R is also involved in catalysis, which is mechanistically different from TBC-domain-containing proteins. (C) GTP-hydrolysis kinetics of 0.5 μM Rab1b_{wt} catalyzed by 2.5 μM LepB₂₉₉₋₆₁₈ and 2.5 μM LepB₂₉₉₋₆₁₈ R444A monitored by the change of Rab1b tryptophan fluorescence, demonstrating the significance of the arginine finger R444_L of LepB for GAP activity. (D) The *cis*-glutamine of Rab1b is important for GTP hydrolysis by LepB, but not TBC1D20. The figures compares the catalytic activities (k_{cat}/K_M) of LepB and TBC1D20 in dependence of wild-type and the Q67A mutant of Rab1. The Q67R substitution affects catalysis by LepB (14,400 × reduction) 293 times more severely than for TBC1D20 (49 × reduction). GAP, GTPase-activating protein.

14,400 under identical conditions (k_{cat}/K_M (Rab1b_{wt}, LepB₂₉₉₋₆₁₈) = $3.6 \cdot 10^6 \text{ M}^{-1} \text{ s}^{-1}$; k_{cat}/K_M (Rab1b Q67A, LepB₂₉₉₋₆₁₈) = $2.5 \cdot 10^2 \text{ M}^{-1} \text{ s}^{-1}$). Thus, LepB operates by a catalytic mechanism reminiscent of classical but not TBC-domain GAPs.

The specificity of LepB

The binding of Rab1b₃₋₁₇₄ to LepB₃₁₇₋₆₁₈ involves relatively few aa residues of Rab1b, most of which are conserved among Rab1b, Rab3a, Rab8a, Rab10, Rab13 and Rab35 (Fig 3A). We therefore speculated that LepB might also serve as a GAP for at least some of these proteins. To this end, we tested Rab3a, Rab8a and Rab13, and found that these Rabs are also very good substrates of LepB₃₁₇₋₆₁₈ albeit with ~10 times lower activity than Rab1b (Fig 3B): k_{cat}/K_M (Rab3a) = k_{cat}/K_M (Rab8a) = $0.36 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, k_{cat}/K_M (Rab13) = $0.54 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$. Even if these GTPases are worse substrates for LepB than Rab1b (k_{cat}/K_M (Rab1b) = $3.6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$), LepB₂₉₉₋₆₁₈ has a similar activity on them as TBC1D20₁₋₃₆₂. Also, Rab35 is a substrate for LepB₃₁₇₋₆₁₈ but with ~100 times less activity compared with Rab1b (k_{cat}/K_M (Rab35) = $0.028 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$). These observations suggest that other Rab proteins might be physiological targets of LepB during infection of human cells by *Legionella*. Interestingly, Rab8a is also found on the LCV during infection [20] and hence might be an actual interaction partner of LepB in addition to Rab1b.

Rab1-modifications interfere with LepB activity

It has been observed previously that posttranslational modifications of Rab1b by the *Legionella* enzymes DrrA (adenylation on Y77_R) and AnkX (phosphocholination on S76_R) impair binding to LepB and therefore inhibit GAP-mediated GTP hydrolysis [7,12]. These observations can now be explained by the structure of the Rab1b₃₋₁₇₄:GDP:BeF₃⁻:LepB₃₁₇₋₆₁₈ complex. Adenylation of Rab1b by DrrA leads to the attachment of an AMP moiety to Y77_R that would sterically clash with α-helices α_{6L} and α_{7L} of LepB₃₁₇₋₆₁₈, explaining the severe influence of Rab1b adenylation on the interaction with LepB (Fig 4). Although S76_R has only a minor role in the generation of the binding

Rab1b) is used as a surrogate that is held in place by the conserved residue E449 of LepB (Fig 2B). This arrangement suggests a catalytic mechanism similar to classical GAPs of small GTPases (that is, non-TBC-GAPs), in which the internal conserved glutamine of the GTPase is needed for GTP hydrolysis [18,19]. Accordingly, when using the hydrolysis-impaired, constitutively active Rab1b₃₋₁₇₄ Q67A mutant, TBC1D20₁₋₃₆₂ is still able to inactivate Rab1b by stimulating GTP hydrolysis, whereas LepB₂₉₉₋₆₁₈ under the same catalytic conditions is not (Fig 2D). The Rab1b Q67A mutation reduces the catalytic activity of TBC1D20₁₋₃₆₂ by a factor of ca. 46 in comparison to Rab1b_{wt} (k_{cat}/K_M (Rab1b_{wt}, TBC1D20₁₋₃₆₂) = $7.3 \cdot 10^4 \text{ M}^{-1} \text{ s}^{-1}$; k_{cat}/K_M (Rab1b Q67A, TBC1D20₁₋₃₆₂) = $1.5 \cdot 10^3 \text{ M}^{-1} \text{ s}^{-1}$). However, the catalytic activity of LepB₂₉₉₋₆₁₈ is reduced by a factor of

Fig 3 | LepB recognizes several Rab proteins. (A) Sequence alignment of LepB substrates (Rab1b, Rab3, Rab8, Rab10 and Rab13) versus non-LepB substrates (Rab5, Rab7 and Rab9). Amino acids involved in LepB binding are indicated by black arrows, yellow colours are conserved amino acids for LepB binding. Red colours indicate amino acids that distinguish LepB binders from non-LepB binders. (Top row: amino-acid numbering according to the Rab1 sequence.) (B) Catalytic activities (k_{cat}/K_M) of indicated Rab proteins for LepB₂₉₉₋₆₁₈ measured by the change in tryptophan fluorescence of Rab proteins (assay conditions: $c(\text{LepB}_{299-618}) = 0.5 \mu M$, $c(\text{Rab}) = 5 \mu M$).

Fig 4 | The Rab1b:LepB complex structures explains the consequences of Rab1 posttranslational modifications. A hypothetical model between Rab1b (cartoon) adenylylated (AMP) at Y77_R [7] in complex with the LepB₃₁₇₋₆₁₈ (surface representation) is shown. The hydroxyl group (red sphere) of S76_R can be phosphocholinated by *Legionella* AnkX [8]. Inset: detailed depiction of residues surrounding S76_R and Y77_R. The potential presence of an AMP moiety would result in severe clashes with the side chain of the residues I483_L, Q486_L and T497_L from $\alpha 5_L$ and $\alpha 6_L$ helices. This explains the inhibitory potential of adenylation for LepB-stimulated GTP hydrolysis [7,12]. The phosphocholinated S76_R side chain could in principle be accommodated without clashes. However, the observed LepB inhibition by Rab1 phosphocholination could also be owing to electrostatic effects.

interface between Rab1b₃₋₁₇₄ and LepB₃₁₇₋₆₁₈, the effect of phosphocholination at this residue is still significant (Fig 4) [12].

DISCUSSION

The structure of Rab1b in complex with LepB reveals a previously unknown mechanism for the deactivation of the Rab family of small GTPases. In contrast to TBC-domain-containing GAPs, LepB does not act via a glutamine provided in *trans* but uses the

cis-glutamine Q67_R to accelerate the intrinsic GTP-hydrolysis activity of Rab1b, a mechanism that is much more reminiscent of the action of many classical GAPs acting on non-Rab GTPases [18]. This finding leads to the question of why TBC-domain-containing RabGAPs have evolved with a different catalytic mechanism instead of functioning in a similar manner to other GAPs. We speculate that mammalian cells could possibly contain non-TBC-RabGAPs with a catalytic mechanism similar to classical GAPs and/or LepB.

Supporting this speculation, LepB originally was identified as a 'Legionella eukaryotic protein' that might have been acquired by *trans*-kingdom horizontal gene transfer [21].

Interestingly, not only the LepB-substrate Rab1 but also Rab8 appears on the LCV at early stages of infection [20]. Rab8 controls late secretory trafficking between the *trans* Golgi network and the plasma membrane. Moreover, Rab8 appears to be functionally relevant for LCV formation, as its depletion by RNA interference affects the LCV composition. Rab proteins are in the GTP state when bound to a membrane but GDP-bound when cytosolic, and therefore Rab8 probably requires activation and deactivation at the LCV similarly to Rab1. Our findings suggest that LepB could mediate the deactivation of Rab8 at the LCV and thereby contribute to its membrane cycling. In summary, the relaxed substrate spectrum of LepB provides a mechanistic rationale for the notion that LCVs communicate not only with the early secretory pathway and the endoplasmic reticulum, but also with the late secretory pathway [20]. In addition, as Rab1 and Rab8 are not the only biochemical substrates of LepB, it will be interesting to analyse whether Rab3, Rab10, Rab13 and Rab35 might also be involved in the biogenesis or maintenance of the LCV.

METHODS

Cloning, protein expression and purification. Rab1b-encoding DNA was codon-optimized for expression in *Escherichia coli* by omitting rare aa codons (Mr Gene, Regensburg, Germany). Genes that encoded human Rab1b_{wt}, truncated Rab1b₃₋₁₇₄, and *L. pneumophila* LepB₂₉₉₋₆₁₈, were subcloned into a modified pET19 vector that contained an N-terminal hexahistidine tag and a Tobacco Etch Virus protease cleavage sequence. Genes encoding LepB₂₋₆₁₈, LepB₃₁₇₋₆₁₈, and LepB₃₂₅₋₆₁₈ were subcloned into a pOPINF vector that contained an N-terminal hexahistidine tag and a PreScission protease cleavage sequence. To improve the crystallization properties of LepB₃₁₇₋₆₁₈, we have eliminated aa clusters with high conformational entropy by site-directed mutagenesis (SER method see refs [22,23]). On the basis of the prediction of the SERp server [22], the cluster spanning residues 457KEHK₄₆₀ was substituted for 457AAHA₄₆₀. A fusion protein of Rab1b₃₋₁₇₄ and LepB₃₁₇₋₆₁₈ K457A E458A K460A was produced using overlap extension PCR with a linker that contained a PreScission cleavage sequence. The catalytic activity of the SER mutant LepB₂₉₉₋₆₁₈ K457A E458A K460A for Rab1b was comparable to the activity of wild-type LepB₂₉₉₋₆₁₈ (supplementary Fig S1b online).

Proteins were expressed in *E. coli* BL21-Codon-Plus(DE3)-RIL at 20 °C after induction with 0.2 mM isopropyl-β-D-thiogalactopyranoside. His-tagged proteins were purified from the bacterial lysates by Ni-NTA chromatography using a 5 ml Hi-Trap Ni-NTA column (GE Healthcare) pre-equilibrated with buffer A (50 mM Hepes pH 8.0, 150 mM NaCl, 2 mM β-mercaptoethanol and additionally, 1 μM GDP and 1 mM MgCl₂ for Rab1b constructs). Elution of proteins was carried out with a linear gradient of buffer B (buffer A, containing 500 mM imidazole). Protein-containing fractions were pooled and the His-tag was cleaved off overnight at 4 °C with Tobacco Etch Virus or PreScission protease while dialysing against buffer A. The sample was passed over a 5 ml Hi-Trap Ni-NTA column pre-equilibrated with buffer A to remove the His-tag, uncleaved protein and protease. Proteins

were further purified by size-exclusion chromatography (Superdex 75, GE Healthcare) pre-equilibrated with buffer containing 20 mM Hepes pH 7.5, 150 mM NaCl, 2 mM DTE and, in addition, 1 μM GDP and 1 mM MgCl₂ for Rab1b constructs. Fractions containing pure protein were pooled, concentrated and stored at -80 °C. The low affinity between Rab1 and LepB did not allow complex purification using gel filtration; therefore, the Rab1b₃₋₁₇₄:LepB₃₁₇₋₆₁₈ K457A E458A K460A complex was purified as fusion protein to ensure an 1:1 protein ratio. Crystallization screens were performed with the fusion construct and a sample cleaved using catalytic amounts of PreScission protease. Crystals were only identified in conditions that led to cleavage of the linker. Proteins that were labelled with selenomethionine were expressed using the methionine biosynthesis inhibition method [24] and purified as described above.

Crystallization and structure determination. Initial crystallization conditions for LepB₃₁₇₋₆₁₈ K457A E458A K460A and the Rab1b₃₋₁₇₄:LepB₃₁₇₋₆₁₈ K457A E458A K460A complex were determined with the JCSG Core I-IV and the PEGs suites (Qiagen) using the sitting-drop vapour diffusion method with a reservoir volume of 75 μl and a drop volume of 0.1 μl protein and 0.1 μl reservoir solution at 20 °C. Crystal-producing conditions were further optimized using the hanging-drop vapour diffusion method with a reservoir volume of 500 μl and a drop volume of 1 μl protein plus 1 μl reservoir solution. Finally, native LepB₃₁₇₋₆₁₈ K457A E458A K460A (30 mg ml⁻¹) was crystallized in 0.1 M Bicine pH 7.5, 0.75 M LiCl and 10% (w/v) PEG 6,000 at 20 °C, selenomethionine-labelled LepB₃₁₇₋₆₁₈ K457A E458A K460A (28 mg ml⁻¹) was crystallized in 0.1 M Bicine pH 7.5, 1.25 M LiCl and 6 % (w/v) PEG 6,000 at 20 °C. Right before setting up the crystallization experiments of Rab1b₃₋₁₇₄:LepB₃₁₇₋₆₁₈ K457A E458A K460A, the protein complex was supplemented with 2 mM BeF₂ and 20 mM NaF (Beryllium fluoride and GDP mimic GTP and therefore stabilize the Rab1:LepB complex). In addition, PreScission protease was added to the complex in an 1:25 ratio, to cleave the fusion protein Rab1b₃₋₁₇₄:LepB₃₁₇₋₆₁₈ K457A E458A K460A *in situ*. Best diffracting crystals of the protein complex were grown in 0.1 M sodium cacodylate pH 6.4, 0.2 M MgCl₂ and 17% (w/v) PEG 8,000 at 20 °C. Crystals were flash-cooled in liquid nitrogen and diffraction data were collected on beamline X10SA of the Swiss Light Source (Paul Scherrer Institute, Villigen, Switzerland) and processed with XDS [25]. The structure of LepB₃₁₇₋₆₁₈ K457A E458A K460A was solved by the single anomalous diffraction method using data collected at the selenium absorption edge and SOLVE/RESOLVE [26]. The initial phases from six selenium sites (two per molecule) were extended to the higher-resolution native data set in DM of the CCP4 package [27]. The initial model was completed by hand in Coot [28] and refined in PHENIX [29] using the TLS option (without NCS). The structure of LepB₃₁₇₋₆₁₈ K457A E458A K460A and the one of Rab1b₃₋₁₇₄ were used as models for maximum likelihood molecular replacement (Phaser [30], CCP4) to obtain an initial model of the protein complex, which then was refined with Phenix.refine [29]. Ligands like GDP, Mg²⁺ and beryllium fluoride were added manually in Coot [28]. Data collection and refinement statistics are summarized in Table 1. The atomic structures of LepB and Rab1b:LepB complex were deposited in the Protein Data Bank under the accession numbers 4I1M and 4I1O, respectively.

Table 1 | Data collection and refinement statistics

	LepB _{317–618} K457A E458A K460A			LepB _{317–618} SeMet* K457A E458A K460A			Rab1b _{3–174} /LepB _{317–618} K457A E458A K460A		
<i>Data collection</i> [†]									
Space group	P4 ₂ 2 ₁ 2			P4 ₂ 2 ₁ 2			P3 ₁		
<i>Cell dimensions</i>									
<i>a</i> , <i>b</i> , <i>c</i> (Å)	152.7, 152.7, 129.5			152.7, 152.7, 129.6			124.4,124.4,147.4		
α, β, γ (°)	90, 90, 90			90, 90, 90			90, 90, 120		
Resolution (Å) [§]	49.8–2.8 (2.87–2.8)			49.8–3.4 (3.49–3.4)			49.16–2.7 (2.8–2.7)		
R _{mrgd} -F	7.1 (46.4)			13.6 (76.0)			8.5 (47.7)		
<i>I</i> /σ(<i>I</i>)	24.4 (3.51)			15.8 (2.6)			19.47 (3.67)		
Completeness (%)	100 (100)			100 (100)			100 (100)		
Redundancy	13.3 (13.1)			13.5 (12.9)			10.51 (10.15)		
Molecules/AU	3			3			8		
<i>Refinement</i>									
Resolution (Å)	2.8						2.7		
No. of reflections	38,034						69,986		
<i>R</i> _{work} / <i>R</i> _{free}	19.95/23.80						21.62/24.55		
<i>No. of atoms</i>									
Protein	6,489						14,006		
Ligand/ion	7						174		
Water	28						1,446		
<i>B-factors</i>									
Protein	76.57						50.40		
Ligand/ion	104.34						35.65		
Water	51.52						48.82		
<i>r.m.s.d.</i> [§]									
Bond lengths (Å)	0.003						0.014		
Bond angles (°)	0.676						1.063		
<i>PDB ID</i>	411M						411O		

Abbreviation: r.m.s.d., root-mean-square distance.

[†]Data collection statistics for single-wavelength anomalous dispersion data refer to unmerged Friedel pairs; *data sets were collected from a single crystal;[§]values in parentheses refer to the highest resolution shell; [§]r.m.s.d. values.

GAP assays. Rab1b_{wt} was exchanged preparatively against GTP or mant-GTP, respectively, as described previously [7]. LepB-stimulated GTP hydrolysis of Rab proteins was examined using reversed phase chromatography and fluorescence spectroscopy. The fluorescence measurements were carried out at 25 °C in a buffer containing 20 mM Hepes pH 7.5, 100 mM NaCl, 2 mM MgCl₂ and 2 mM DTE. The fluorescence intensity change of mant-GTP ($\lambda_{\text{ex}} = 365$ nm, $\lambda_{\text{em}} = 440$ nm or 420 nm cut-off filter) or intrinsic Rab-tryptophan ($\lambda_{\text{ex}} = 297$ nm, $\lambda_{\text{em}} = 340$ nm or 320 nm cut-off filter) was used as a signal for GTP hydrolysis using a Fluoromax-3 fluorescence spectrometer (Horiba Jobin Yvon) or a stopped-flow apparatus (SX.18MV-R, Applied Photophysics). The observed changes in fluorescence intensity were fitted to a first-order exponential function using Origin7.0 (OriginLab).

The catalytic efficiencies of enzymes have been calculated by dividing the rate constants by the enzyme assay concentration (condition: $c(\text{GAP}) < c(\text{Rab1:GTP}) \ll K_M$).

Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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