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Structural insights into a new homodimeric self-activated GTPase family

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The human XAB1/MBD*in* GTPase and its close homologues form one of the ten phylogenetically distinct families of the SIMIBI (after signal recognition particle, MinD and BioD) class of phosphate-binding loop NTPases. The genomic context and the partners identified for the archaeal and eukaryotic homologues indicate that they are involved in genome maintenance—DNA repair or replication. The crystal structure of PAB0955 from *Pyrococcus abyssi* shows that, unlike other SIMIBI class G proteins, these highly conserved GTPases are homodimeric, regardless of the presence of nucleotides. The nucleotide-binding site of PAB0955 is rather rigid and its conformation is closest to that of the activated SRP G domain. One insertion to the G domain bears a strictly conserved GPN motif, which is part of the catalytic site of the other monomer and stabilizes the phosphate ion formed. Owing to this unique functional feature, we propose to call this family as GPN-loop GTPase.

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INTRODUCTION

Owing to the crucial cellular roles of GTPases, there has been tremendous efforts over the past two decades to understand their structures and function (Bourne *et al*, 1991; Vetter & Wittinghofer, 2001). Among the superfamily of phosphate-binding loop (P-loop) GTPases, 28 families have been phylogenetically defined (Leipe *et al*, 2002). The best known families include molecular switches such as the Ras-like small G proteins, heterotrimeric G proteins, elongation factor G domains (TRAFAC class), as well as signal recognition particle (SRP), its receptor (SR) and hydrogenase accessory protein B (HypB) that adopt a distinct topology (SIMIBI (after signal recognition particle, MinD and BioD) class).

A human GTPase, XPA binding protein 1/MBD2 interacting protein (XAB1/MBD*in*), has been shown to interact with Xeroderma pigmentosum protein A (XPA), a protein involved in nucleotide excision repair (Nitta *et al*, 2000), and with MBD2, a component of the methyl-CpG-binding protein 1 (MeCP1) large protein complex that represses transcription of densely methylated genes (Lembo *et al*, 2003). XAB1/MBD*in* has been shown to counteract the inhibitory effect of MBD2 at methylated promoters. Furthermore, deletion of the gene coding for its closest homologue in *Saccharomyces cerevisiae* (*Yjr072c*) is lethal (Giaever *et al*, 2002), indicating that these eukaryotic GTPases are involved in a crucial cellular mechanism. Archaeal homologues have also been identified. The amino-acid sequences of PAB0955 from *Pyrococcus abyssi* and human XAB1/MBD*in* share 27% identity (see supplementary Fig S1 online). The *pab0955* gene is located between genes encoding the replicative helicase minichromosome maintenance protein (MCM) and the cell-division protein MinD. In several distinct

Table 1 | Crystallographic statistics

	Native apo	GDP	GTP-γS	GTP^{EDTA}	PiGDP	GDP^{GTP293}	MgGDP
Data collection							
Wavelength (Å)	0.933	1.542	0.979	0.934	0.979	0.979	0.934
Space group	$P3_221$	$P2_1$	$P3_221$	$P3_221$	$P2_12_12$	$P3_221$	$P2_1$
Unit-cell parameters	$a=b=60.3$ Å, $c=117.1$ Å	$a=59.1$ Å, $b=84.9$ Å, $c=60.2$ Å, $\beta=95^\circ$	$a=b=60.2$ Å, $c=115.9$ Å	$a=b=60.71$ Å, $c=116.8$ Å	$a=58.82$ Å, $b=84$ Å, $c=53.16$ Å	$a=b=60.8$ Å, c $=117.2$ Å	$a=59.47$ Å, $b=85.21$ Å, $c=60.57$ Å, $\beta=94.61^\circ$
Resolution (Å)	50–2.15 (2.23–2.15)	50–2.30 (2.38–2.30)	50–2.08 (2.15–2.08)	20–2.40 (2.46–2.40)	100–2.80 (2.90–2.80)	100–2.40 (2.45–2.40)	50–1.75 (1.80–1.75)
No. of observations	140,472 (10,187)	88,157 (8,488)	83,727 (5,701)	117,734 (8,425)	50,000 (4,710)	93,234 (5,405)	282,790 (18,469)
No. of unique reflections	13,998 (1,345)	26,182 (2,560)	12,892 (1,245)	10,088 (696)	6,729 (618)	9,976 (574)	59,865 (5,075)
$\langle I\rangle/\langle\sigma(I)\rangle$	8.2 (2.2)	5.2 (2.1)	6.6 (2.3)	20.7 (7)	15.19 (4.6)	19.55 (5.76)	12.47 (5.91)
Completeness (%)	100 (100)	99.9 (100)	87.6 (94)	98.5 (98.2)	97.8 (93.8)	99 (98.8)	98.6 (93.5)
R_{merge} (%)	5.7 (34.8)	6.8 (36.1)	6.7 (28.4)	7.5 (40)	9.3 (45.5)	6.7 (40.4)	9.8 (37.2)
Refinement							
Resolution (Å)	15–2.15 (2.23–2.15)	15–2.30 (2.38–2.30)	15–2.08 (2.15–2.08)	15–2.40 (2.46–2.40)	15–2.80 (2.90–2.80)	15–2.40 (2.45–2.40)	15–1.75 (1.80–1.75)
No. of reflections	12,996	23,201	11,234	8,981	6,017	8,930	53,677
Free set	685	2,607	1,224	994	663	981	6,086
Asymmetric unit content	Monomer	Dimer	Monomer	Monomer	Monomer	Monomer	Dimer
R_{work}	0.232	0.186	0.208	0.236	0.225	0.211	0.195
R_{free}	0.299	0.265	0.295	0.325	0.346	0.324	0.247
R_{cryst}	0.235	0.190	0.209	0.242	0.232	0.218	0.196
Average B-factor (Å^2) r.m.s.d.	53.6	34.9	45.4	48.9	46.8	50.1	33.8
Bonds (Å)	0.010	0.011	0.009	0.009	0.008	0.009	0.008
Angles (deg)	1.246	1.373	1.297	1.192	1.175	1.185	1.215
PDB entry code	1YR6	1YRA	1YR7	1YR8	1YR9	2OXR	1YRB

Values in parentheses are for the highest resolution shell.
PDB, Protein Data Bank

archaea, DNA replication-related genes are located in the close neighbourhood of the *pab0955* homologous gene (see supplementary Fig S2 online). Such observations strengthen the idea that these GTPases are indeed involved in crucial mechanisms at the DNA level, possibly related to DNA replication/repair and conserved from archaea to human.

To gain insight into this new GTPase family, we solved the crystal structure of PAB0955, both free and in complex with different nucleotides. This provides us with different snapshots along the hydrolysis pathway and shows that PAB0955 is the archetype for a new family of homodimeric GTPases, called the GPN-loop GTPase family.

RESULTS

The PAB0955-nucleotide complex structures

The crystal structures of the nucleotide-free form of PAB0955, as well as six complexes with different nucleotides, were determined (Table 1). Two complexes co-crystallized with GDP contained either GDP alone (PAB0955-GDP) or GDP and a Mg^{2+} ion (PAB0955-MgGDP). One complex was obtained with GTP-γS and three others were co-crystallized with GTP. The latter ones contain either GDP and one phosphate ion in the vicinity of the nucleotide (crystals grown at 277 K, PAB0955-PiGDP) or GDP and one Mg^{2+} ion (crystals grown at 293 K, PAB0955-GDP^{GTP293}). The presence of GDP in these crystals suggests catalytic activity of the protein in

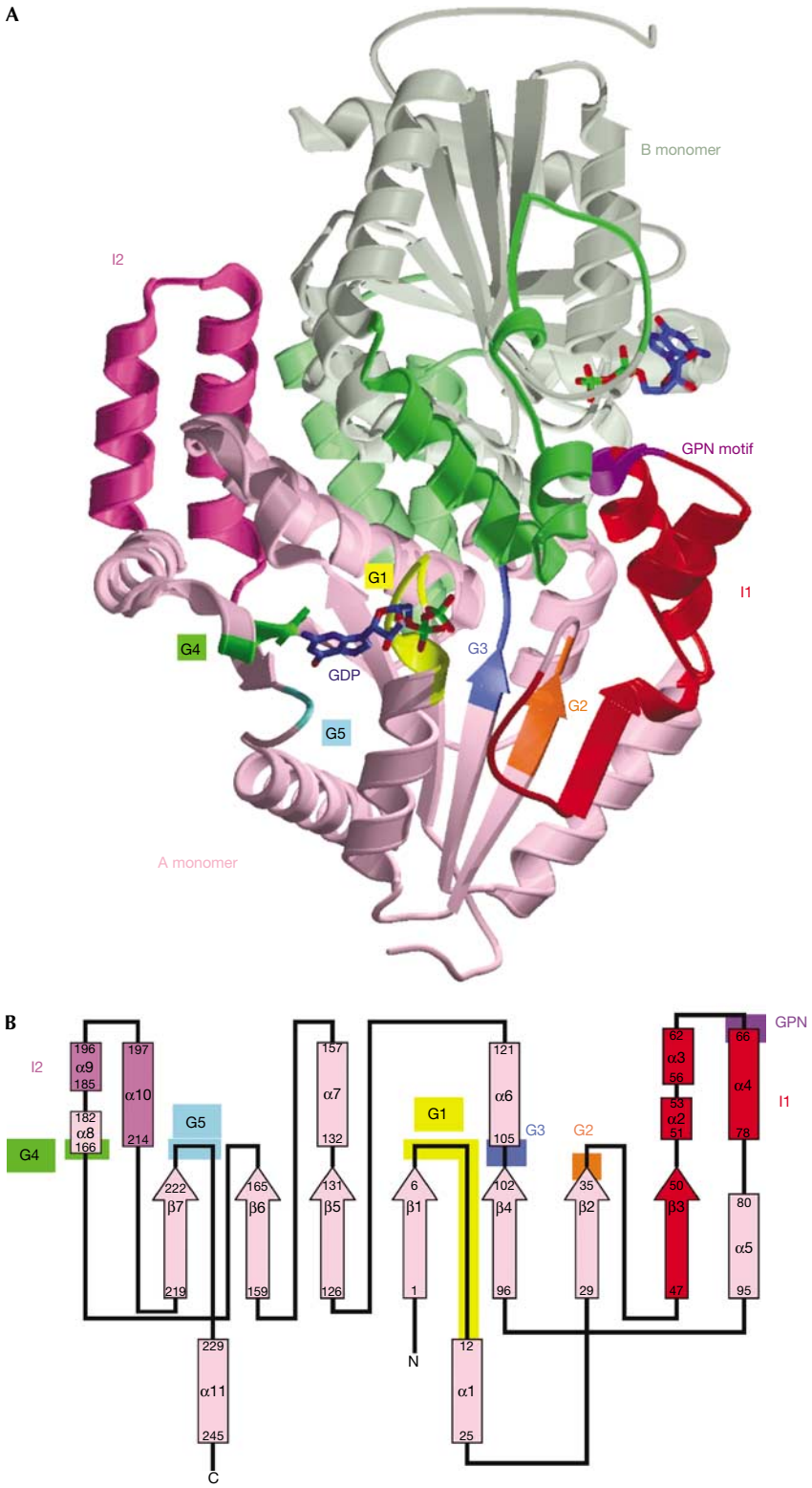

Fig 1 | Overall structure and topology of PAB0955 GPN-loop GTPase. (**A**) View of the PAB0955-GDP dimer. Monomer's A and B are shown in pink and light green, respectively. GDP molecules are shown as sticks coloured according to atom type (light blue for carbon, blue for nitrogen, red for oxygen, green for phosphorus). The G1, G2, G3, G4 and G5 motifs (A monomer) are shown in yellow, orange, blue, green and cyan, respectively. The two insertions I1 and I2 are depicted in fully saturated and partially saturated colours respectively. (**B**) Topology of PAB0955. G1–G5 boxes are shown with the same colour scheme.

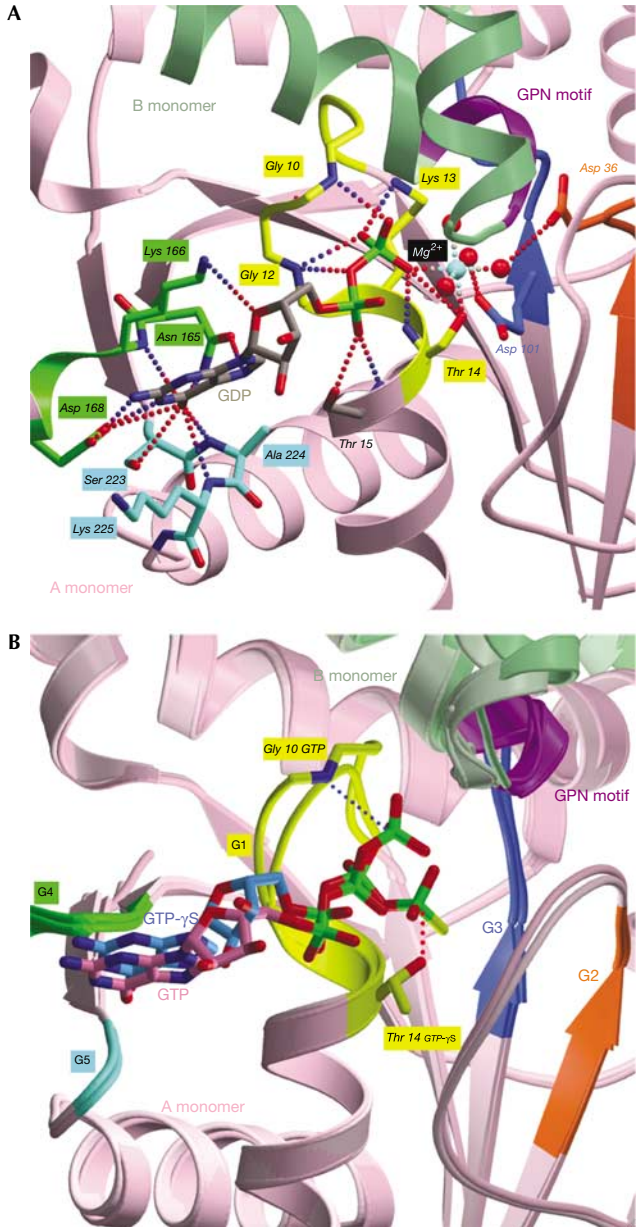

Fig 2 | The PAB0955 nucleotide-binding site. (**A**) Detailed view of the nucleotide-binding site of PAB0955-MgGDP. G1–G5 boxes are shown with the same colour scheme used in Fig 1. Hydrogen bonds are represented as dotted lines. The Mg^{2+} ion is shown in cyan and water molecules are shown in red. (**B**) Close-up view of the superposition of PAB0955–GTP^{EDTA} (GTP with pink carbon atoms) and PAB0955–GTPγS structures (GTP-γS with blue carbon atoms). Superposition has been carried out on the 154 residues forming the Rossmann fold core of the protein.

either the crystallization solution or the crystals. The last complex, obtained with crystals grown in the presence of both EDTA and GTP, contains GTP (PAB0955–GTP^{EDTA}).

Overall PAB0955 structure description

PAB0955 is a 248-residue α/β monodomain protein, which adopts the canonical 'Rossmann fold' shared by all the P-loop-containing NTP hydrolases. The ^{7}GTAGSGKT^{14} motif, located close to the amino terminus of the polypeptide chain, constitutes the P-loop that binds the nucleotide phosphate groups. The core of the protein consists of a central six-stranded parallel β-sheet surrounded by six α-helices. Two insertions are grafted to this core (residues 41–80 and 183–217) and form two protrusions to the globular shape of the monomer (Fig 1). The first insertion (I1) is reminiscent of the insertion box domain identified for SRP G domains (Freymann *et al*, 1997). The second insertion (I2) has no equivalent in other GTPases and has the least conserved amino-acid sequence among the PAB0955 homologues. The protein is dimeric both in solution (Gras *et al*, 2005) and in all the crystal forms. The homodimer is either crystallographic, as observed in the $P3_221$ and the $P2_12_12$ crystal forms, or non-crystallographic, as observed in the $P2_1$ crystal form. The accessible surface buried at the interface of the dimer is 4420 Å^2 and the two insertions contribute 39% to this interface; 64% of the interface is contributed by hydrophobic residues. The two nucleotide-binding sites contained in the PAB0955 dimer are close to the interface of the dimer but are not connected with each other. The structure of PAB0955 is well conserved in either its apo form or in complex with different nucleotides. The calculated root mean square difference (r.m.s.d.) for the 154 residues making up the core of one monomer ranges from 0.16 to 0.58 Å. Loops involved in the nucleotide-binding site (the P-loop, Lys 40 next to the G2 motif and the ^{64}YGPNGA^{69} loop) show moderate structural differences with maximum Cα displacement of 1.08, 1.98 and 1.16 Å, respectively. The I2 region is the most flexible with an r.m.s.d. of approximately 2.8 Å for the three conformations observed in PAB0955-MgGDP (two independent molecules) and PAB0955-PiGDP structures. The structures derived from crystals belonging to the $P3_221$ space group have sparse electron density for this region. An extensive search of the Protein Data Bank led to the identification of only 15 GTPases having a fully parallel β-sheet. These include tubulin, FtsZ and three members of the SIMIBI class: HypB, SRP and SR G domains. A search using DALI confirmed that the fifty-four homologue (Ffh) fragment of SRP of *Thermus aquaticus* (Freymann *et al*, 1997) is the GTPase that is structurally most similar to PAB0955 (Ffh: r.m.s.d. = 1.94 Å for 136 Cα; HypB: r.m.s.d. = 1.98 Å for 126 Cα) and emphasizes that PAB0955 belongs to the SIMIBI class. However, the mode of dimerization observed for PAB0955 differs markedly from that observed for the SRP/SR complex (Egea *et al*, 2004) or HypB (Gasper *et al*, 2006). When the Ffh SRP G domain and HypB A monomer are superposed on PAB0955 A monomer, the orientation of FtsY SR G domain and HypB B monomer, relative to PAB0955 B monomer, differ by a rotation of 66° and 173°, respectively. The relative position of the two nucleotide-binding sites also differs, as exemplified by the distance between the two β-phosphate atoms: 22.0 Å for PAB0955, 17.5 Å for HypB and 9.1 Å for Ffh/FtsY.

Nucleotide-binding mode

The overall nucleotide-binding mode is well conserved in the six PAB0955 complexes. Fig 2A shows the five consensus motifs in PAB0955—G1–5—involved in GTP binding of GTPases (Bourne *et al*, 1991). The G2 motif is Asp 36 at the carboxy-terminal end of the β2-strand and is highly conserved in SIMIBI family proteins: Asp 135 in *T. aquaticus* Ffh, Asp 69 in HypB. It stabilizes the

Mg^{2+} ion through a water-mediated hydrogen bond. The G3 motif (^{101}DTPGQ^{105} in PAB0955) is structurally rather well conserved in all PAB0955 structures, as it participates in the interface of the dimer. The presence of a proline in this motif probably enhances the rigidity of this loop, whereas in Ras-like small G proteins, this G3 motif (switch II) is flexible (Vetter & Wittinghofer, 2001). The conformation of the G3 motif observed in PAB0955 also differs from that of SRP Ffh and HypB. The G4 (^{165}NKVD^{168}) and G5 (^{223}SAK^{225}) motifs specifically stabilize the guanine and the ribose groups, as shown in Fig 2A. PAB0955 G4 and G5 motifs are very similar to those of HypB (^{167}NKID^{170} and ^{199}SLK^{201}), both in sequence and in structure. In the SRP Ffh G domain, the G5 motif differs significantly in both aspects. Surprisingly, the position of γ-monothiophosphate in PAB0955–GTPγS differs by 3.26 Å from that of γ-phosphate in PAB0955–GTP^{EDTA} (Fig 2B) and is close to that of the coordinated Mg^{2+} ion observed in PAB0955–MgGDP structure, thus preventing Mg^{2+} binding. Consequently, the conformation of the GTP-γS bound to PAB0955 is unlikely to be the one adopted by GTP before hydrolysis; this is in contrast to other GTPases to which it binds with a conformation very similar to that of GTP and other analogues (Ihara *et al*, 1998).

GTPase activity of PAB0955

Specific nucleotide hydrolysis in the presence of protein was detected *in vitro* by determining the GDP:GTP (or ADP:ATP) ratio. The protein originates from a hyperthermophilic organism; therefore, the kinetics were measured at 80 °C. A weak intrinsic GTPase activity (0.012 μmol of GTP hydrolysed in GDP per min and per mg of protein) was detected, whereas no ATPase activity could be observed. This activity is consistent with that observed for the PAB0955 human orthologue XAB1/MBD*in* (Nitta *et al*, 2000). As already observed for some GTPases, such as Era (Sood *et al*, 1994), PAB0955 is able to phosphorylate itself *in vitro* at 80 °C only in the presence of GTP and Mg^{2+} ions (see supplementary information online).

Search for protein partners related to DNA metabolism

The partners identified for human XAB1/MBD*in* have no homologues in *P. abyssi*; therefore, a search for PAB0955 protein partners was undertaken. Pull-down assays with cellular extracts of *P. abyssi* and screening by surface plasmonic resonance led to the identification of three interacting partners: DNA topoisomerase VI (subunit B), DNA primase DnaG and RF-C (small subunit). An equilibrium dissociation constant of approximately 0.5 nM was obtained only with the former protein (see supplementary information online).

DISCUSSION

The new GPN-loop GTPase family

At least 64 sequences homologous to PAB0955 were identified by PSI-BLAST analysis. Sequence alignment of PAB0955 homologues suggests that their topology should be similar to that of PAB0955, with a core adopting a Rossmann fold and two insertions made of 40–43 and 35–52 residues, respectively. The stable dimeric form observed for PAB0955 is likely to be conserved in these homologues. Among the 45 residues contributing to the interface of the dimer, 20% are strictly conserved and 27% are homologous (supplementary Fig S1 online).

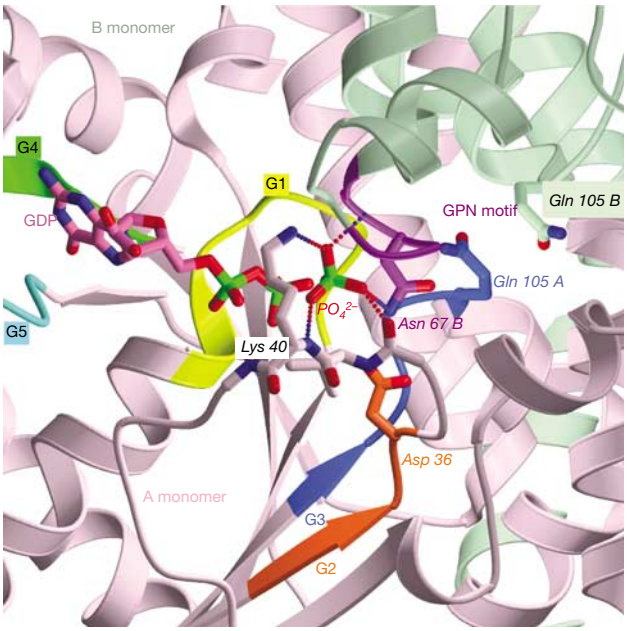

Fig 3 | View of the PAB0955 residues stabilizing the phosphate ion in the PAB0955-PiGDP structure. Hydrogen bonds are represented as dotted lines. G1 to G5 boxes are shown with the colour scheme used in Fig 1; the GPN motif is shown in purple.

Remarkably, a strictly conserved GPN motif in the I1 region is located at the dimer interface, close to the nucleotide-binding site of the other monomer (Fig 2A). In the PAB0955–GTP^{EDTA} structure, the $N^{\delta 2}$ atom of Asn 67 (molecule B) is 5.9 Å away from the closest GTP γ-phosphate oxygen atom bound to monomer A. In the PAB0955-PiGDP structure, it forms a hydrogen bond with the phosphate ion (molecule A, Fig 3). This Asn 67 seems to have the same role as that attributed to Gln 61 of the G3 motif in Ras (Prive *et al*, 1992), which is thought to stabilize the phosphate intermediate. The G3 motif of PAB0955 also contains a Gln 105, but it is approximately 7 Å away from both the γ-phosphate group in PAB0955–GTP^{EDTA} and the phosphate ion in PAB0955-PiGDP. The presence of a proline in the G3 motif and its involvement in the dimer interface prevents PAB0955 Gln 105 from getting closer to the nucleotide and fulfilling the role of Gln61 in Ras. However, as the amine group of Gln 105 (molecule A) is hydrogen bonded to the amine group of Asn 67 (molecule B), it might indirectly have a limited role in the catalysis. Remarkably, HypB Asn 124, the equivalent of Ras Gln 61, points away from the nucleotide and clearly cannot have the same role. HypB His 154 (molecule B), hypothesized to activate the water molecule that can act as a nucleophile (Gasper *et al*, 2006), is strikingly close to Asn 67 (molecule B) in PAB0955. The strict conservation of the GPN motif throughout the whole family further supports an essential catalytic role for Asn 67.

Another consensus sequence, (V/I/L)N(L/M)D(T/P) (^{33}VNLDT^{37} in PAB0955), constitutes the G2 motif of GPN-loop GTPases. Val 33 and Leu 35 form a hydrophobic cluster with Ile 48 and Ile 100, whereas Asn 34 blocks the main chain conformation of Asp 36 through two hydrogen bonds between the Asn 34 amine group and the NH and C = O of Asp 36. This stable motif forces

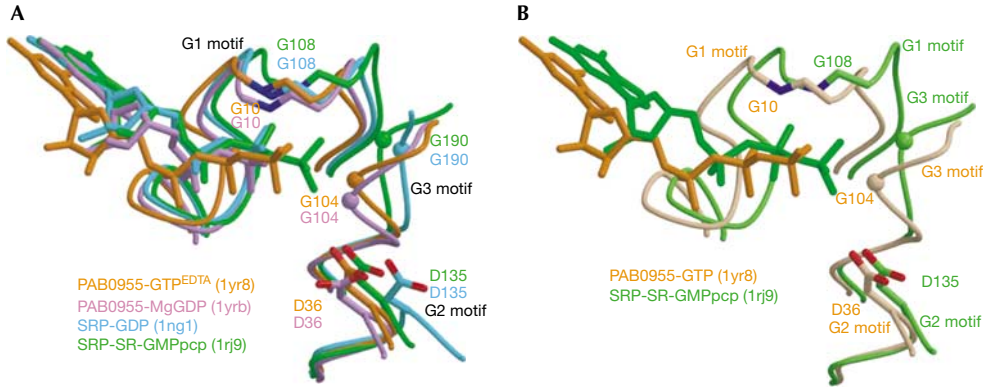

Fig 4 | Comparison of PAB0955 and SAP G domain nucleotide-binding sites. (**A**) Close-up view of the nucleotide-binding site of the superposed PAB0955–GTP^{EDTA}, PAB0955-MgGDP, Ffh–GDP (1ng1) and Ffh-GMPpcp when bound to FtsY (1rj9) structures, shown in orange, purple, blue and green, respectively. (**B**) Close-up view of the nucleotide-binding site of PAB0955–GTP^{EDTA}, shown in orange, and Ffh in the Ffh–FtsY complex, shown in green. The concerted shift of the γ-phosphate group and the G1 and G3 motifs is clearly seen.

the conserved Asp 36 side chain to point towards the Mg^{2+} ion. In summary, the presence of the $GX_2GXGK(T/S)$, (V/I/L)N(L/M)D, GPN, DXPGQ and (N/S/T)KXD motifs constitutes the signature of a GPN-loop GTPase.

An activated conformation for the nucleotide-binding site

As illustrated by the comparison of Ffh–GDP (inactivated state) and Ffh-GMPpcp in the Ffh-GMPpcp-FtsY complex (activated state), SRP G domains undergo conformational changes for their G2 and G3 motifs on activation (Egea *et al*, 2004). In PAB0955, the conformation of the G2 motif and the position of Asp36 are conserved, and are much closer to that of Asp135 in the Ffh/FtsY complex (Fig 4A). Furthermore, the relative positions of the γ-phosphate group and the G1 and G3 motifs in both PAB0955–GTP^{EDTA} and Ffh–GMPpcp structures (Fig 4B) are very similar. Therefore, structurally PAB0955 is significantly closer to the activated conformation of Ffh.

The G domain superposition of the PAB0955 dimer with those of Ras–RasGAP (Scheffzek *et al*, 1997) and SRP-SR (Egea *et al*, 2004) complexes shows that no steric clash prevents a putative partner for bringing an 'arginine finger', such as GP120 RasGAP Arg 789 or SRP Ffh Arg 138, to activate PAB0955 further. No positively charged residue is seen in the vicinity of the phosphate groups owing to PAB0955 dimerization; this is in contrast to the HypB, where Lys 153 (molecule B) hydrogen bonds the γ-phosphate (Gasper *et al*, 2006). Compared with small GTPases and SRP, the higher GTPase activity of PAB0955 might therefore originate from the fact that Asn 67 is already stabilized in its optimal conformation. The hydrogen bond between Gln 105 (molecule A) and Asn 67 (molecule B) might also enhance the ability of Asn67 to stabilize the negatively charged γ-phosphate in the transition state. The dimeric state of PAB0955 partly mimics the Ras–RasGAP interaction by stabilizing the G2 and G3 motifs and bringing the GPN motif close to the nucleotide. To our knowledge, PAB0955 is the first example of a structurally characterized GTPase dimeric in either apo or holo states. This observation suggests that the PAB0955 GTPase activity should be controlled *in vivo* by an as yet unknown mechanism.

Snapshots of the hydrolysis pathway

The set of PAB0955 structures provides us with snapshots of the GTP hydrolysis pathway. The PAB0955–GTP^{EDTA} structure is a plausible view of the GTP position before hydrolysis. The absence of the Mg^{2+} ion, intentionally removed with EDTA to block hydrolysis, should not have an impact on the γ-phosphate position, as the nucleotide is sterically constrained by the G3 motif. The PAB0955-PiGDP represents the next step. The GTP present in the crystallization solution has been hydrolysed and a free phosphate ion is located 5.2 Å away from the position of the GTP γ-phosphate group observed in the PAB0955–GTP^{EDTA} structure. It is stabilized by five hydrogen bonds involving Gly 38 and Lys 40 (molecule A), as well as Asn 67 and Gly 68 (molecule B) GPN loop (Fig 3), thus confirming that this pocket at the interface of the dimer helps to stabilize the phosphate ion just after GTP hydrolysis. After hydrolysis of the GTP, a 1.1 Å positional shift of the α and β phosphate groups deeper into the nucleotide-binding site towards the G3 loop is observed. The PAB0955-GDP^{GTP293} structure might represent the next snapshot, with GTP fully hydrolysed and no phosphate ion observed. The GDP molecule is shifted back and superposes well with the GTP molecule in PAB0955-GTP^{EDTA}, possibly illustrating one step towards the release of the nucleotide, which is probably driven by the mutual electrostatic repulsion of GDP and Pi. These snapshots illustrate how the nucleotide adapts to the rather rigid PAB0955 structure along the hydrolysis pathway, pointing out a marked difference with Ffh in which the G3 motif is more flexible.

Although structurally well conserved, eukaryal and archaeal GPN-loop GTPases might be involved in different biological functions, as they seem to have different partners. It seems that along the emergence of the eukaryotic branch and its further evolution, different partners were lost and new ones recruited. PAB0955 is a good structural template for their eukaryal homologues; therefore, it will help to explain how XAB1/MBD*in* functions in the near future. Altogether, our results allowed this new dimeric GTPase family to be structurally and mechanistically characterized. The fact that this GTPase is dimeric regardless of the presence of a bound nucleotide and that rather small structural

changes are observed on nucleotide binding indicate an as yet unobserved mode of action, which is different from the dimerization-dependent switch mechanism described for the closest members of the SIMIBI family such as SRP/SR and HypB. Therefore, unveiling its precise cellular role in eukaryotes remains an exciting challenge.

METHODS

GTP hydrolysis. Recombinant PAB0955 was obtained as described previously (Gras *et al*, 2005). The GTPase activity was assessed in triplicate by incubating various amounts of purified PAB0955 (0.2–1.0 μM) with 200 μM GTP in 50 mM EPPS buffered at pH 7.0 containing 400 mM potassium glutamate and 5 mM $MgCl_2$ at 80 °C (see supplementary information online).

Crystallization. Native crystals, either nucleotide free or co-crystallized with GDP and GTP-γS, were obtained as described previously (Gras *et al*, 2005). Derivative crystals and native PAB0955, co-crystallized with GTP or GDP and $NaWO_4$, were obtained as described in the supplementary information online.

Crystallographic data collection. Before being flash-cooled in liquid nitrogen, crystals were soaked in a cryoprotecting solution containing mother liquor with PEG4000 concentration increased to 30–35% (w/v). Three crystallographic data sets were collected on two DTPA-BMA-Gd-soaked crystals at three different wavelengths on the beam-line BM-30A of the European Synchrotron Radiation Facilities (ESRF) using a MarCCD detector. Another data set was collected on a DTPA-BMA-Gd co-crystallized crystal on the beam-line ID14-eh4 of the ESRF using an ADSC Q4 CCD detector (supplementary Table S1 online). The data collection of the native PAB0955, PAB0955–GTPγS and PAB0955-GDP crystals was described previously (Gras *et al*, 2005). For the PAB0955-GDP^{GTP293} and PAB0955-PiGDP crystals, crystallographic data were collected on the beam-line BM-30A of the ESRF. For the PAB0955–GTP^{EDTA} and PAB0955-MgGDP crystals, crystallographic data were collected on the beam-line ID14-eh1 of the ESRF using an ADSC Q4 CCD detector (Table 1).

Structure determination and refinement. The nucleotide-free PAB0955 structure was solved using SIRAS/MAD techniques, with crystallographic data being collected with the native and the Gd-derivatized crystals as described in the supplementary information online. All the PAB0955-nucleotide complex structures were solved by molecular replacement with AMoRe (Navaza, 1994) by using the apo-form of PAB0955 as the initial model. The refinement protocol used for all structures included several cycles of refinement with REFMAC (CCP4, 1994) followed by manual model rebuilding with O (Jones *et al*, 1991), until no interpretable electron density could be identified in the residual map. Final refinement statistics and PDB entry codes are summarized in Table 1.

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

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