Suppl. materials

Proteins with CHAD Domains (Conserved Histidine α-Helical Domain) Are Attached to Polyphosphate (polyP) Granules in vivo and Constitute a Novel Family of PolyP-Associated Proteins (Phosins)

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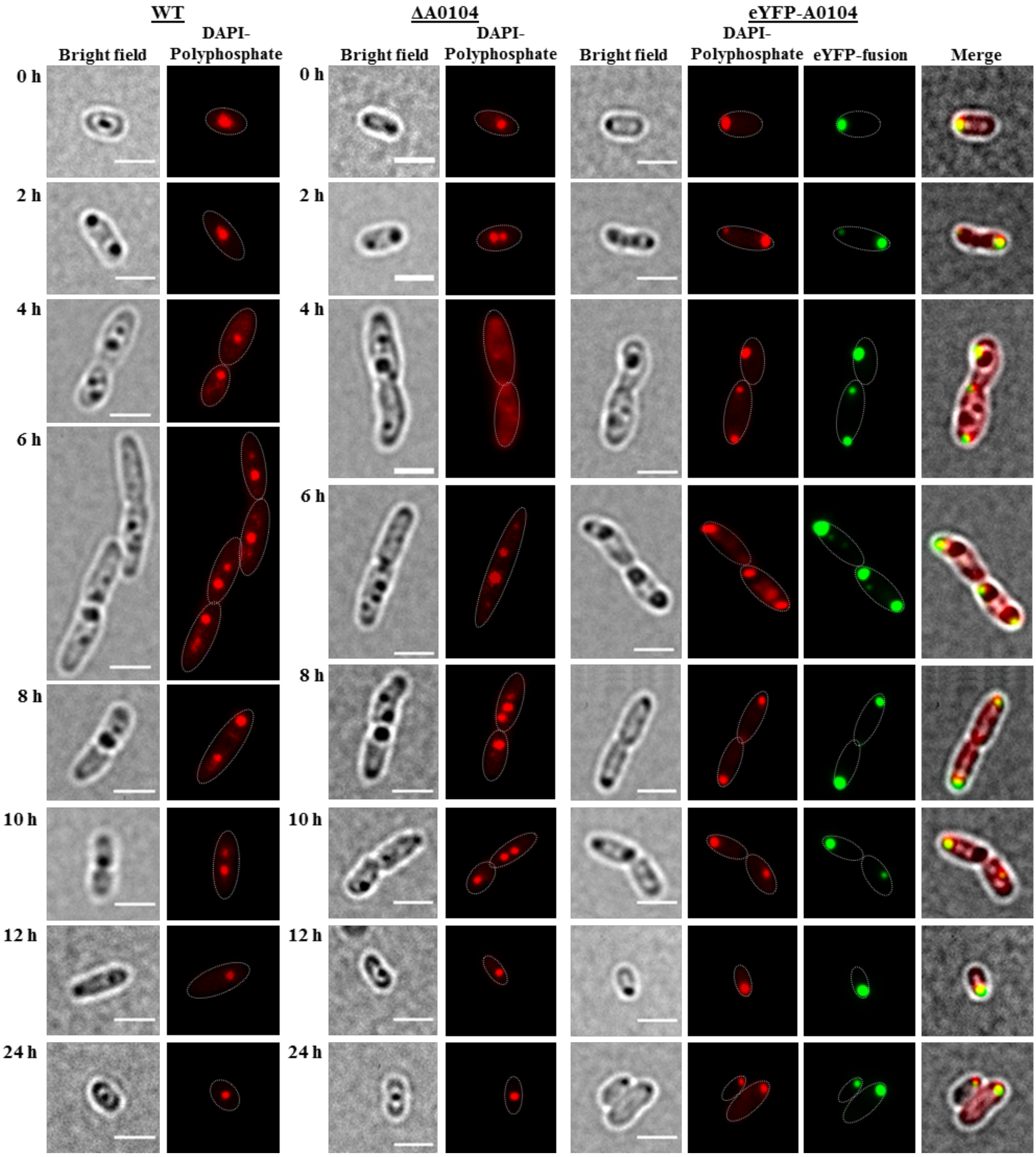
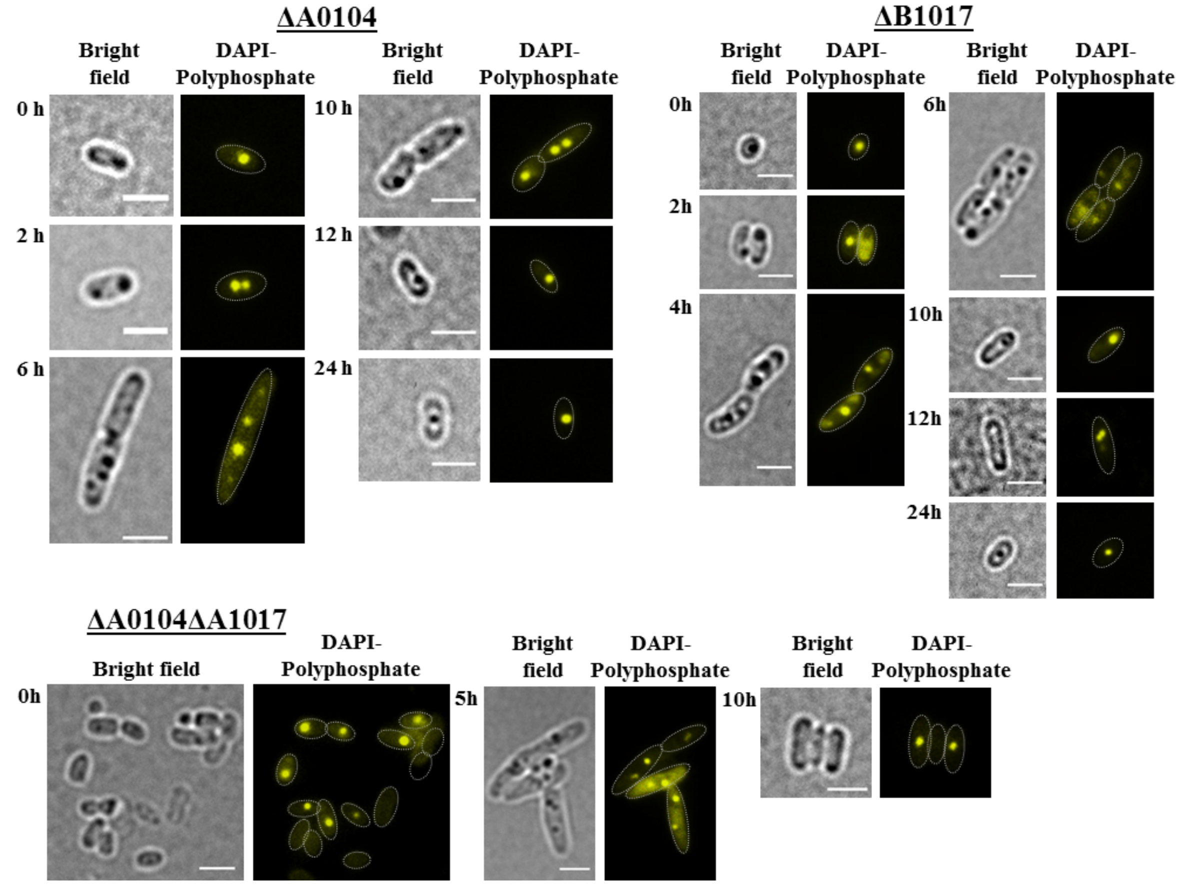
Suppl. materials Table S1: Proteins identified in polyP granule fractions isolated from *R. eutropha*

#	**Identified protein**	**Gene no**	**MW**	**Δ*phaC1***	**Δ*phaX*Δ*phaC1***
1	**hypothetical protein H16_A0104 (PtpA)**	A0104	35 kDa	x	x
2	ABC transporter ATPase	A0290	30 kDa	x	x
3	hypothetical protein	A0407	12 kDa	x	x
4	hypothetical protein	A1108	25 kDa	x	x
5	**hypothetical protein (PPK2c)**	A1212 (PPK2c)	42 kDa	x	x
6	**hypothetical protein (PPK2d)**	A1271 (PPK2d)	34 kDa	x	
7	dimethylaniline monooxygenase	A2109	49 kDa	x	
8	alkaline phosphatase	A2182	48 kDa		x
9	**polyphosphate kinase (PPK1a)**	A2437 (PPK1a)	78 kDa	x	x
10	phosphate transporter permease subunit PstC	A2443	34 kDa		x
11	transcriptional regulator	B0011	27 kDa		x
12	EPS I polysaccharide export protein, putative tyrosine-protein kinase	B0012	87 kDa	x	x
13	exopolysaccharide export protein	B0024	43 kDa	x	x
14	glycosyl transferase	B0025	45 kDa		x
15	aminotransferase	B0031	41 kDa	x	x
16	hypothetical protein	B0037	38 kDa	x	x
17	hypothetical protein	B0055	44 kDa	x	x
18	putative double-glycine peptidase	B0057	26 kDa	x	x
19	LysR family transcriptional regulator	B0484	33 kDa		x
20	LysR family transcriptional regulator	B0535	33 kDa		x
21	phenol hydroxylase P1 protein	B0540	37 kDa	x	x
22	phenol hydroxylase P3 protein	B0542	59 kDa	x	
23	catechol 2,3-dioxygenase	B0546	35 kDa	x	x
24	4-hydroxy-2-ketovalerate aldolase	B0552	38 kDa	x	
25	response regulator	B0621	25 kDa		x
26	long-chain-fatty-acid-CoA ligase	B0714	58 kDa		x
27	L-aspartate dehydrogenase	B0736	28 kDa		x
28	outer membrane protein (porin)	B1077	39 kDa	x	x
29	patatin-like phospholipase	B1090	31 kDa	x	x
30	transcriptional regulator	B1131	26 kDa	x	
31	transcriptional regulator	B1193	37 kDa	x	
32	AsnC family transcriptional regulator	B1366	17 kDa		x
33	Short chain CoA dehydrogenase	B1696	26 kDa		x
34	transcriptional regulator	B1787	37 kDa	x	
35	bb3-type cytochrome oxidase, subunit I	B2061	65 kDa	x	x
36	AraC family transcriptional regulator	B2258	37 kDa	x	x
37	AraC family transcriptional regulator	B2287	34 kDa	x	x
38	ATP-dependent DNA ligase	B2352	98 kDa	x	
39	hypothetical protein	B2377	19 kDa		x
40	phosphatase	B2398	70 kDa		x
41	LysR family transcriptional regulator	B2512	33 kDa	x	x

The polyP granule fraction and other cell fractions (membrane, membrane-associated, soluble fraction) of *R. eutropha ΔphaC1* and of *R. eutropha ΔphaX+ΔphaC1* were prepared and proteome-analyzed as described recently (1). The table shows only those proteins of the polyP fraction that were absent in all other cell fractions. The “x” indicates in which of the two strains the respective protein was identified. Proteins for which a colocalization with polyP granules was confirmed in vivo (via fusion with eYFP) are shown in bold letters. *R. eutropha* Δ*phaC1* was used for analysis because the presence of PHB granules in a *phaC1*-positive background complicates the isolation of polyP granules. The *R. eutropha ΔphaX+ΔphaC1* strain forms much more polyP granules than *phaX*-harboring strains (1) and was included in the analysis to increase the sensitivity of polyP protein detection. Most of the proteins shown in this table have been identified previously (1), except for the A0104 protein.



Suppl. Fig. S2: Time course of polyP formation and localization of eYFP-A0104 in *R. eutropha*. From left to right: *R. eutropha* wild type, *R. eutropha* ΔA0104, and *R. eutropha* harboring pBBR1MCS2-P*phaC*-*eyfp*-A0104. Cells were grown in NB medium at 30°C. Samples were taken at time points as indicated, stained with DAPI and immediately imaged. For better visibility in merged figures, the DAPI-polyP specific signals (normally yellowish) are provided in red color. Note, dark globular structures visible in bright field can represent either PHB granules or polyP granules. The shape of the cells was highlighted in most fluorescent images by a white dotted line. Scale bars correspond to 2 µm.



Suppl. Fig. S3: Time course of polyP formation in ΔA0104, ΔB1017 and ΔA0104+ΔB1017 cells of *R. eutropha*. Cells were grown in NB medium at 30°C. Samples were taken at time points as indicated, stained with DAPI and immediately imaged. Note, all cells formed polyP granules. Numbers and localizations of formed polyP granules corresponded to wild type cells. Scale bar corresponds to 2 µm.

REFERENCE

1. **Tumlirsch T**, **Sznajder A**, **Jendrossek D**. 2015. Formation of polyphosphate by polyphosphate kinases and its relationship to poly(3-hydroxybutyrate) accumulation in *Ralstonia eutropha* strain H16. Appl Environ Microbiol **81**:8277–8293.