The Prodomain of *Bordetella* FhaB, a Prototypical Two-Partner Secretion Pathway Protein, Remains Intracellular yet Affects the Conformation of the Extracellularly-Located Mature C-terminal Domain

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SUPPLEMENTARY INFORMATION

Supplemental Figure Legends

Supplemental Figure 1

Model of the FhaB/FhaC Two-Partner Secretion (TPS) system. (A) FhaB translocation across the *Bordetella* outer membrane. The FhaB preproprotein (labeled) is exported into the periplasm by the general secretory system (not shown). It is not known if the entire polypeptide is translocated into the periplasm before translocation through FhaC occurs. In this figure, the peptidoglycan layer and cytoplasmic membrane are not shown for simplicity. Also note that this figure was drawn to scale, as best as possible. Residue numbers of the FhaB proprotein are indicated. The FhaB TPS domain (orange) interacts with FhaC (purple) to initiate outer membrane translocation of FhaB as a hairpin. Upon extracellular exposure, the C-terminal portion of the TPS domain folds into a β-helix, nucleating folding of the β-helical shaft (blue) in an N- to C-terminal direction. At this point, FHA biogenesis could proceed by two routes, depending on the localization of the prodomain. (B) Possibility that the prodomain remains intracellular. After the MCD (light green) has reached the cell surface, SphB1 (yellow) and an unidentified protease (dark

green) cleave the intracellular prodomain (red) from FhaB and the prodomain is rapidly degraded. Mature FHA (labeled) can be either surface-associated or released. The mechanism for release is unknown. (C) Possibility that the prodomain reaches the extracellular space. In this scenario, prodomain cleavage and degradation would both occur extracellularly.

Supplemental Figure 2

The FhaB prodomain comprises distinct subdomains. At top is a schematic of *B. bronchiseptica* FhaB showing domain locations. At bottom, WebLogo 3.1 display of homology shared amongst TpsA proteins to the ~125 aa *B. bronchiseptica* FhaB prodomain N-terminus (PNT).

Supplemental Figure 3

Insertion of an HA epitope tag before the FhaB proline-rich region (PRR) does not affect FHA maturation or function. (A) Schematic of FhaB proteins used in experiment. Domain layout is the same as that of Figure 1A. The PRR in front of which the HA tag was introduced is labeled for the HAPRR strain. (B) Anti-MCD and anti-HA immunoblot of whole-cell lysates and concentrated supernatants. Introducing an HA tag at the C-terminus of FhaB causes instability of full-length FhaB. Moving the epitope upstream of the PRR does not affect the integrity or maturation of full-length FhaB. FHA release into the supernatants is unaffected by either location of the HA tag.

Supplemental Figure 4

Introduction of an HA tag to the FhaB prodomain suggests an intracellularly-localized prodomain. (A) Schematic of FhaB proteins used in experiment. Domain layout is the same as that of Figure 1A. (B) At the top, anti-MCD and anti-HA immuno-dot-blot of HAPRR and RBX11 strains. Normalized amounts of whole cells and boiled lysates were applied. Below, illustrations of immuno-stained samples. Layout of these illustrations is as described in Figure 5B.

Supplemental Figure 5

A small amount of prodomain is released from *B. bronchiseptica* with a deletion of the MCT. (A) Schematic of FhaB proteins used in experiment. Domain layout is the same as that of Figure 3A. (B) Anti-MCD and anti-HA immunoblot of concentrated supernatants. On the left is a merge showing release of prodomain from HA-ΔCS, HA-ΔMCT, and HA-ΔPNT strain. On the right is an image of the same immunoblot, with the 700 nm (red) channel isolated and enhanced to facilitate the visualization of prodomain bands released into the supernatant of the ΔMCT strain.

Strain list

Strain name	Description	Reference
RBX11	RB50 containing a deletion of codons 4-3203 of *fhaS*	(Julio & Cotter, 2005)
RBX11Δ*sphB1*	RB50 containing a deletion of codons 4-3203 of *fhaS* and a deletion of codons 5-1035 of *sphB1*	This work
RBX20	RB50 containing a deletion of codons 4-3705 of *fhaB* and a deletion of codons 4-3203 of *fhaS*	(Julio & Cotter, 2005)
CTHA	RBX11 containing nine codons encoding	This work

	the HA epitope (YPYDVPDYA) following codon 3703 in *fhaB*	
HAPRR	RBX11 containing nine codons encoding the HA epitope (YPYDVPDYA) following codon 3375 in *fhaB*	This work
HAPRRΔ*sphB1*	RBX11 containing nine codons encoding the HA epitope (YPYDVPDYA) following codon 3375 in *fhaB* and a deletion of codons 5-1035 of *sphB1*	This work
BPSMAQ	Bp strain containing His7 following Q72 and AcTEV following Y1871 in FhaB	This work
BPSMAQT-N	Bp strain with His7 at Q72, AcTEV at Y1871, truncated at aa 2330, complemented to aa 2410	This work
JS20	RBX11 with a stop codon after bp 5943	
JS26	JS20::pJB51 substituting A1983C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS55	JS20::pJB127 substituting A1983C, containing codons 1815-2588 followed immediately by a stop codon	This work
JS27	JS20::pJB52 substituting V2032C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS56	JS20::pJB128 substituting V2032C, containing codons 1815-2588 followed immediately by a stop codon	This work
JS28	JS20::pJB53 substituting V2081C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS57	JS20::pJB129 substituting V2081C, containing codons 1815-2588 followed immediately by a stop codon	This work
JS48	JS20::pJB87 substituting G2132C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS58	JS20::pJB130 substituting G2132C, containing codons 1815-2588 followed immediately by a stop codon	This work
JS52	JS20::pJB93 substituting A2182C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS59	JS20::pJB131 substituting A2182C, containing codons 1815-2588 followed immediately by a stop codon	This work
JS49	JS20::pJB88 substituting A2233C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS60	JS20::pJB132 substituting A2233C, containing codons 1815-2588 followed	This work

	immediately by a stop codon	
JS51	JS20::pJB89 substituting L2284C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS61	JS20::pJB133 substituting L2284C, containing codons 1815-2588 followed immediately by a stop codon	This work
JS50	JS20::pJB90 substituting I2333C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS62	JS20::pJB134 substituting I2333C, containing codons 1815-2588 followed immediately by a stop codon	This work
JS53	JS20::pJB94 substituting A2382C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS63	JS20::pJB135 substituting A2382C, containing codons 1815-2588 followed immediately by a stop codon	This work
JS54	JS20::pJB95 substituting A2432C, containing codons 1815-3710 followed immediately by a stop codon	This work
JS64	JS20::pJB136 substituting A2432C, containing codons 1815-2588 followed immediately by a stop codon	This work
HA-ΔCS	RBX11-HAPRR containing a deletion of codons 2217-2647	This work
HA-ΔCSΔ*sphB1*	RBX11-HAPRR Δ*sphB1*containing a deletion of codons 2217-2647	This work
HA-ΔMCT	RBX11-HAPRR containing deletion of codons 2217-2471	This work
HA-ΔMCTΔ*sphB1*	RBX11-HAPRR Δ*sphB1* containing deletion of codons 2217-2471	This work
HA-ΔPNT	RBX11-HAPRR containing deletion of codons 2472-2647	This work
HA-ΔPNTΔ*sphB1*	RBX11-HAPRR Δ*sphB1* containing deletion of codons 2472-2647	This work
DH5α	*E. coli* molecular cloning strain	BRL; Gaithersburg, MD
SM10λpir	Conjugation strain	(Miller & Mekalanos, 1988)
RHO3	Conjugation strain	(López *et al*, 2009)

Plasmid list

Plasmid name	Description	Reference

pEG7	Suicide plasmid used to construct *B. pertussis* and *B. bronchiseptica* strains containing co-integrates	(Akerley *et al*, 1995)
pEG7S	*sacB*-containing allelic exchange plasmid used to construct *B. bronchiseptica* strains	(Martínez de Tejada *et al*, 1996)
pEG7S-Δ*sphB1*	pEG7S derivative used to introduce the Δ*sphB1* deletion mutation into *B. bronchiseptica* strains	(Mazar & Cotter, 2006)
pEG7-CTHA	pEG7 derivative containing nucleotides corresponding to codons 3429-3710 followed immediately by a stop codon and then 500 bp corresponding to the region immediately 3' of *fhaB* with nucleotides encoding the HA epitope following codon 3703	This work
pSS4245	pBR322-based allelic exchange plasmid for use in *Bordetella* species. Contains I-SceI cleavage site and encodes restriction endonuclease, I-SceI, under the control of the pertussis toxin (ptx) promoter	(Inatsuka *et al*, 2010)
pSS4245-HAPRR	pSS4245 derivative containing nucleotides corresponding to codons 3208-3567 with nucleotides encoding the HA epitope following codon 3375	This work
pSORTP1-Bp MCI/Y1871/AcTEV	Bp mating vector for Y1871AcTEV mutagenesis in FhaB	This work
pSORTP1-SS Template/Q72His7	Bp mating vector for Q72His7 mutagenesis in FhaB	This work
pJB48	pEG7 derivative containing nucleotides corresponding to codons 1814-3710 of *fhaB* followed immediately by a stop codon	This work
pJB101	pEG7 derivative containing nucleotides corresponding to codons 1814-2588 of *fhaB* followed immediately by a stop codon	This work
pJB28	pCR2.1 derivative containing nucleotides 5441-7410 of *fhaB*. Used for QuickChange substitutions. QuickChange derivatives were subcloned into pJB48 and pJB101.	This work
pJB51	pJB48 in which codon 1983 has been changed to encode Cys instead of Ala	This work
pJB127	pJB101 in which codon 1983 has been changed to encode Cys instead of Ala	This work
pJB52	pJB48 in which codon 2032 has been changed to encode Cys instead of Val	This work

pJB128	pJB101 in which codon 2032 has been changed to encode Cys instead of Val	This work
pJB53	pJB48 in which codon 2081 has been changed to encode Cys instead of Val	This work
pJB129	pJB101 in which codon 2081 has been changed to encode Cys instead of Val	This work
pJB87	pJB48 in which codon 2132 has been changed to encode Cys instead of Gly	This work
pJB130	pJB101 in which codon 2132 has been changed to encode Cys instead of Gly	This work
pJB93	pJB48 in which codon 2182 has been changed to encode Cys instead of Ala	This work
pJB131	pJB101 in which codon 2182 has been changed to encode Cys instead of Ala	This work
pJB88	pJB48 in which codon 2233 has been changed to encode Cys instead of Ala	This work
pJB132	pJB101 in which codon 2233 has been changed to encode Cys instead of Ala	This work
pJB89	pJB48 in which codon 2284 has been changed to encode Cys instead of Leu	This work
pJB133	pJB101 in which codon 2284 has been changed to encode Cys instead of Leu	This work
pJB90	pJB48 in which codon 2333 has been changed to encode Cys instead of Ile	This work
pJB134	pJB101 in which codon 2333 has been changed to encode Cys instead of Ile	This work
pJB94	pJB48 in which codon 2382 has been changed to encode Cys instead of Ala	This work
pJB135	pJB101 in which codon 2382 has been changed to encode Cys instead of Ala	This work
pJB95	pJB48 in which codon 2432 has been changed to encode Cys instead of Ala	This work
pJB136	pJB101 in which codon 2432 has been changed to encode Cys instead of Ala	This work
pSS4245-ΔCS	pSS4245 derivative containing nucleotides corresponding to codons 2048-2216 and 2648-2808 of *fhaB*	This work
pSS4245-ΔMCT	pSS4245 derivative containing nucleotides corresponding to codons 2048-2216 and 2472-3640 of *fhaB*	This work
pSS4245-ΔPNT	pSS4245 derivative containing nucleotides corresponding to codons 2295-2471 and 2648-2808 of *fhaB*	This work

Strain construction

RBX11Δ*sphB1* was constructed by performing allelic exchange on RBX11 using plasmid pEG7SΔ*sphB1* as described (Martínez de Tejada *et al*, 1996). The strain was confirmed to be constructed as intended by PCR and nucleotide sequence analysis.

HAPRR and HAPRRΔ*sphB1* were constructed by performing allelic exchange on RBX11 and RBX11Δ*sphB1*, respectively, using plasmid pSS4245-HAPRR as described (Inatsuka *et al*, 2010). The strains were confirmed to be constructed as intended by PCR and nucleotide sequence analysis.

HA-ΔCS and HA-ΔCSΔ*sphB1* were constructed by performing allelic exchange on HAPRR and HAPRRΔ*sphB1*, respectively, using plasmid pSS4245-ΔCS. The strains were confirmed to be constructed as intended by PCR and nucleotide sequence analysis.

HA-ΔMCT and HA-ΔMCTΔ*sphB1* were constructed by performing allelic exchange on HAPRR and HAPRRΔ*sphB1*, respectively, using plasmid pSS4245-ΔMCT. The strains were confirmed to be constructed as intended by PCR and nucleotide sequence analysis.

HA-ΔPNT and HA-ΔPNTΔ*sphB1* were constructed by performing allelic exchange on HAPRR and HAPRRΔ*sphB1*, respectively, using plasmid pSS4245-ΔPNT. The strains were confirmed to be constructed as intended by PCR and nucleotide sequence analysis.

BPSMAQ was constructed by performing allelic exchange on BPSM using plasmids pSORTP1-Bp MCI/Y1871/AcTEV and pSORTP1-SS Template/Q72His7. The strains were confirmed to be constructed as intended by PCR and nucleotide sequence analysis

BPSMAQT-N was constructed by introducing pEG7-FI1a/C&R-N into BPSMAQ by conjugation and selecting co-integrates on BG-Sm-Gm agar as described (Akerley *et al*, 1995) Integration of plasmids at the correct site in the chromosome was confirmed by PCR.

CTHA was constructed by introducing pEG7-CTHA into RBX11 by conjugation and selecting co-integrates on BG-Sm-Gm agar. Integration of the plasmids at the correct site in the chromosome was confirmed by PCR.

JS26, JS55, JS27, JS56, JS28, JS57, JS48, JS58, JS52, JS59, JS49, JS60, JS51, JS61, JS50, JS62, JS53, JS63, JS54, and JS64 were constructed by introducing pJB51, pJB127, pJB52, pJB128, pJB53, pJB129, pJB87, pJB130, pJB93, pJB131, pJB88, pJB132, pJB89, pJB133, pJB90, pJB134, pJB94, pJB135, pJB95, and pJB136, respectively, into JS20 by conjugation and selecting co-integrates on BG-Sm-Gm agar. Integration of the plasmids at the correct site in the chromosome was confirmed by PCR.

Plasmid construction

pJB28 is a pCR2.1 derivative that contains a PCR-amplified 2.0 kb fragment of RBX11 *fhaB* (corresponding to codons 1814 through 2470).

The following plasmids are pEG7 derivatives used to construct co-integrate strains. Sequences cloned into this plasmid between the gentamicin resistance gene and ampicilin resistance gene are described.

pJB48 contains PCR-amplified 2.0 kb, and 3.8 kb fragments of RBX11 *fhaB* (corresponding to codons 1814 through 2470 and codons 2471-3710 followed by a STOP codon) from RBX11 ligated together.

pJB101 contains PCR-amplified 2.0 kb, and 350 bp fragments of RBX11 *fhaB* (corresponding to codons 1814 through 2470 and codons 2471-2588 followed by a STOP codon) from RBX11 ligated together.

pJB51, pJB52, pJB53, pJB87, pJB93, pJB88, pJB89, pJB90, pJB94 and pJB95 contain PCR-amplified 2.0 kb, and 3.8 kb fragments of RBX11 *fhaB* (corresponding to codons 1814 through 2470 and codons 2471-3710 followed by a STOP codon) from RBX11 ligated together with a QuickChange mutagenesis of the residues specified in plasmid list.

pJB127, pJB128, pJB129, pJB130, pJB131, pJB132, pJB133, pJB134, pJB135 and pJB136 contain PCR-amplified 2.0 kb, and 350 bp fragments of RBX11 *fhaB* (corresponding to codons 1814 through 2470 and codons 2471-2588 followed by a stop codon) from RBX11 ligated together with a QuickChange mutagenesis of the residues specified in plasmid list.

pEG7-CTHA contains PCR-amplified 0.6 kb (corresponding to codons 3429 through 3703) and 0.5 kb fragments of RBX11 *fhaB* (corresponding to codon 3703 extending to

457 bp 3' of STOP codon) ligated together with nucleotides encoding an HA epitope following codon 3703.

The following plasmids are pSS4245 derivatives used for allelic exchange in *B. bronchiseptica*. Sequences cloned into this plasmid between the I-SceI cleavage site and the tetracycline resistance gene are described.

pSS4245-HAPRR contains PCR-amplified 0.5 kb (corresponding to codons 3208 through 3375) and 0.6 kb fragments of RBX11 *fhaB* (corresponding to codons 3375 through 3567) ligated together with nucleotides encoding an HA epitope following codon 3375.

pSS4245-ΔCS contains PCR-amplified 0.5 kb (corresponding to codons 2048 through 2216) and 0.5 kb fragments of RBX11 *fhaB* (corresponding to codons 2648 through 2808) ligated together.

pSS4245-ΔMCT contains PCR-amplified 0.5 kb (corresponding to codons 2048 through 2216) and 0.5 kb fragments of RBX11 *fhaB* (corresponding to codons 2472 through 2640) ligated together.

pSS4245-ΔPNT contains PCR-amplified 0.5 kb (corresponding to codons 2295 through 2471) and 0.5 kb fragments of RBX11 *fhaB* (corresponding to codons 2648 through 2808) ligated together.

The following plasmids are pSORTP1 derivatives used for allelic exchange in *B. pertussis*. Sequences cloned into this plasmid between the gentamicin resistance gene and ampicilin resistance gene are described.

pSORTP1-Bp MCI/Y1871/AcTEV contains PCR-amplified 600 bp fragments flanking the codon for Y1871 of RBX11 *fhaB* with nucleotides encoding an AcTEV cleavage site inserted following Y1871.

pSORTP1-SS Template/Q72His7 contains PCR-amplified 600 bp fragments flanking the codon for Q72 of RBX11 *fhaB* with nucleotides encoding a His7 epitope inserted following Q72.

References

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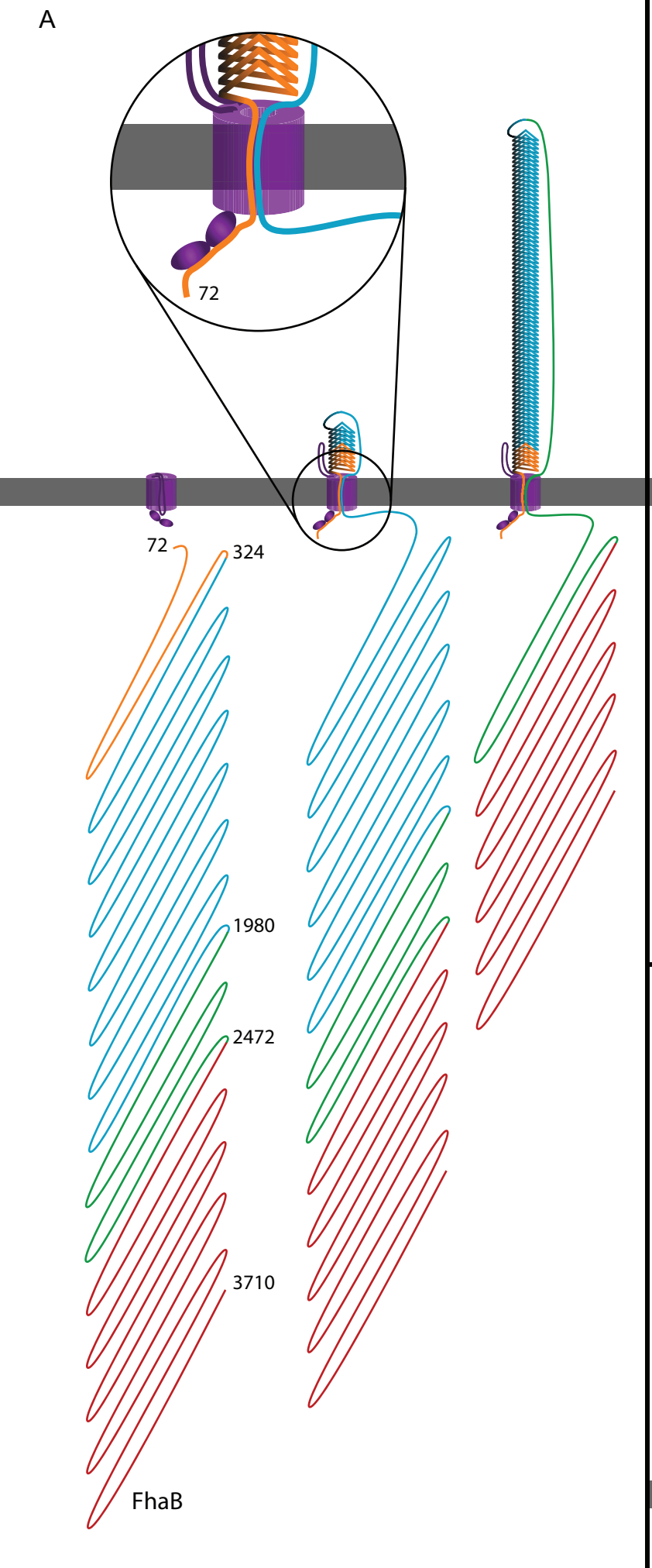
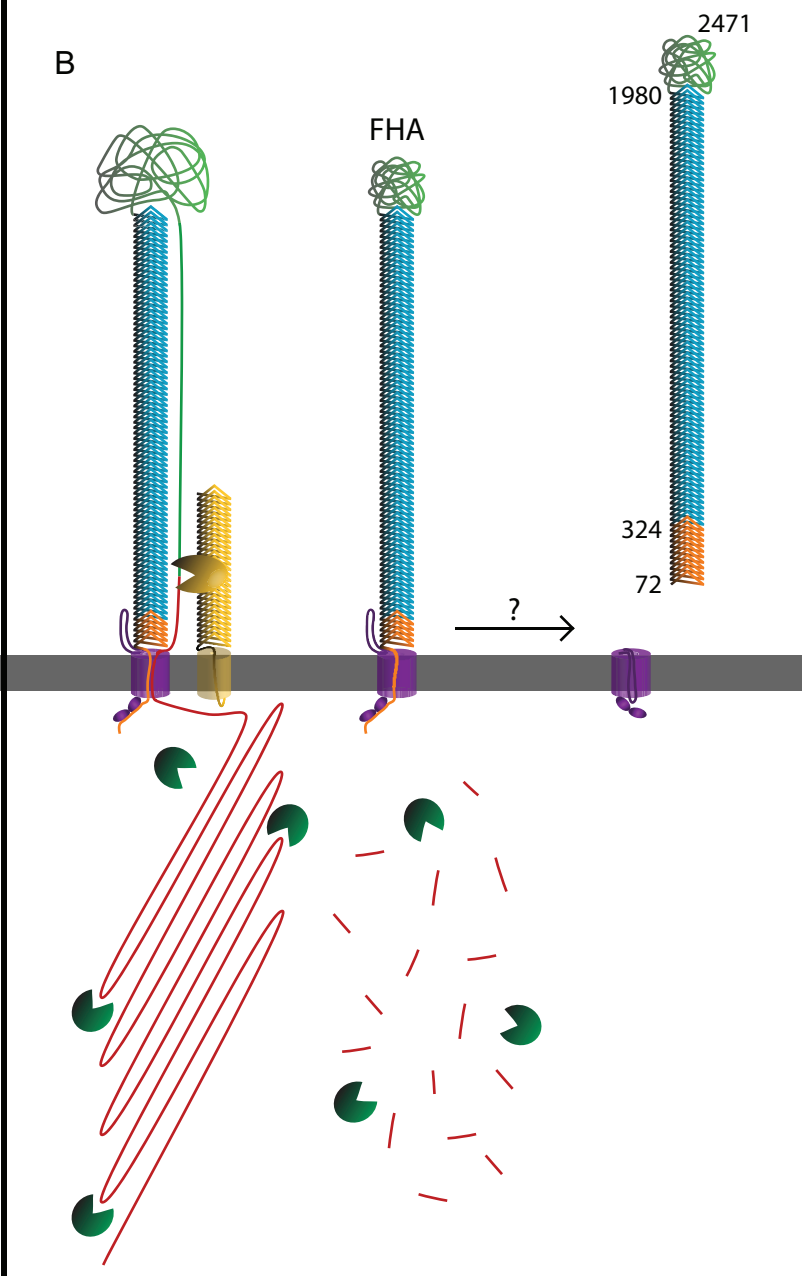
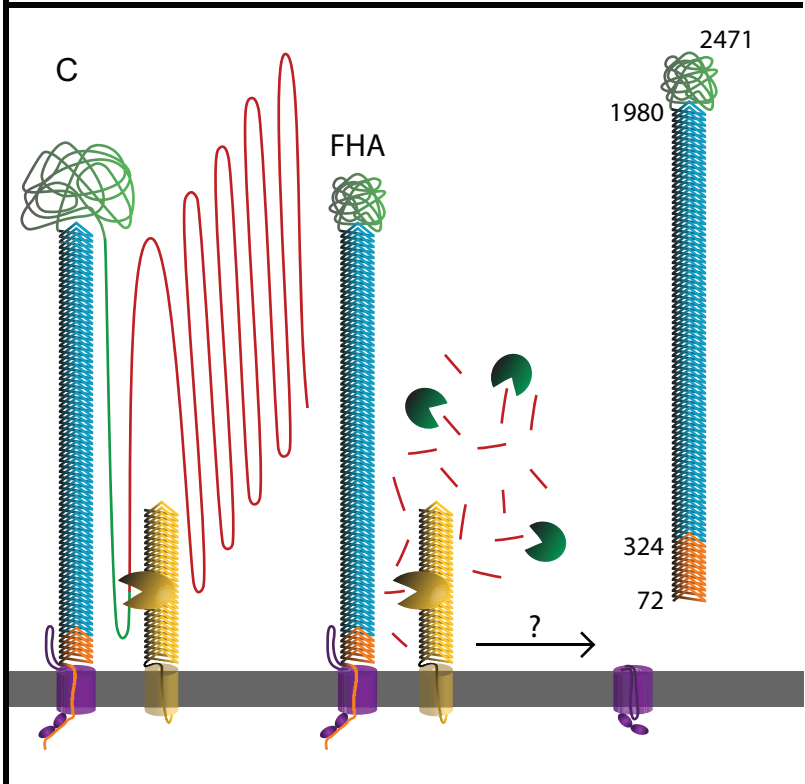
SUPPLEMENTAL FIGURE 1





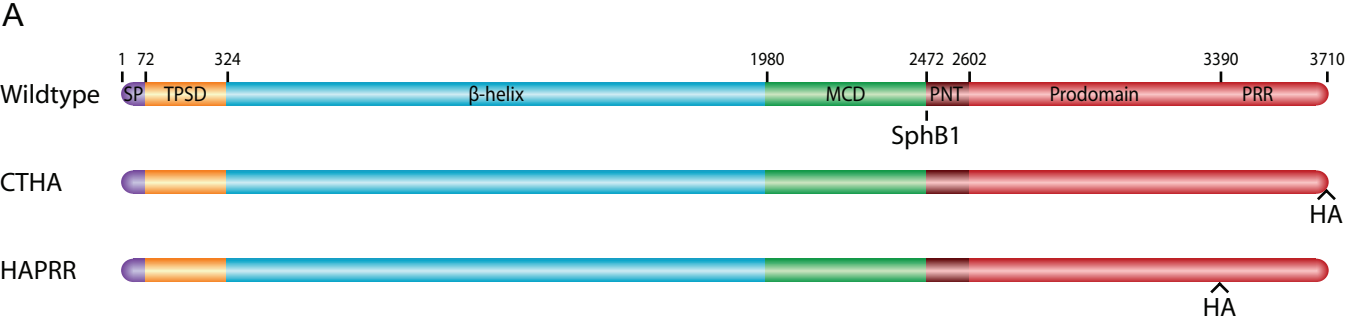


SUPPLEMENTAL FIGURE 2

WebLogo 3.1

SUPPLEMENTAL FIGURE 3

A



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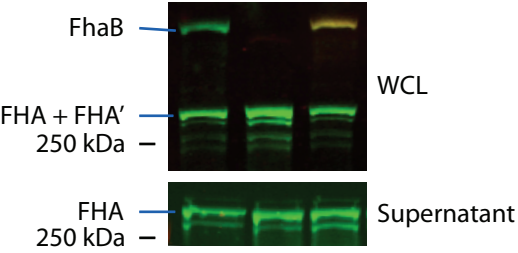
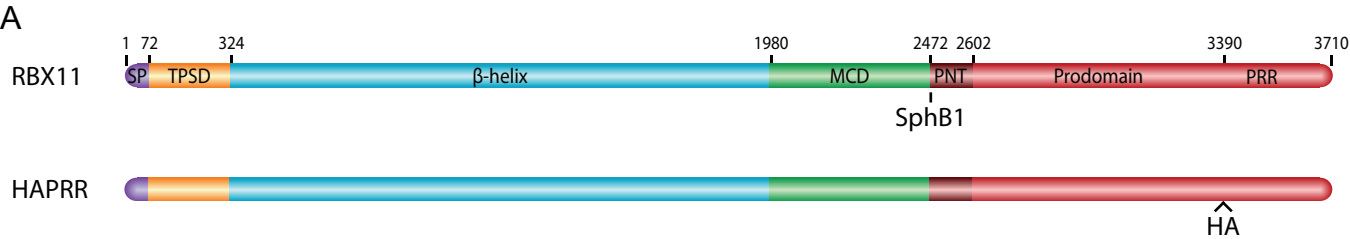
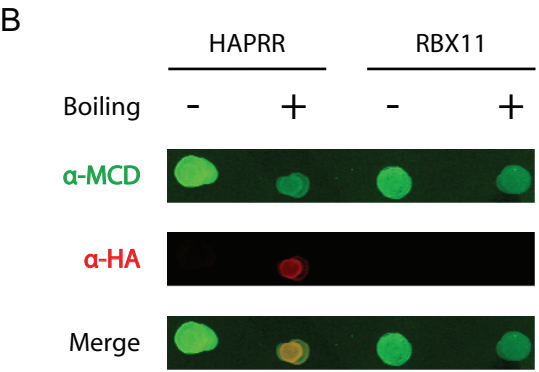
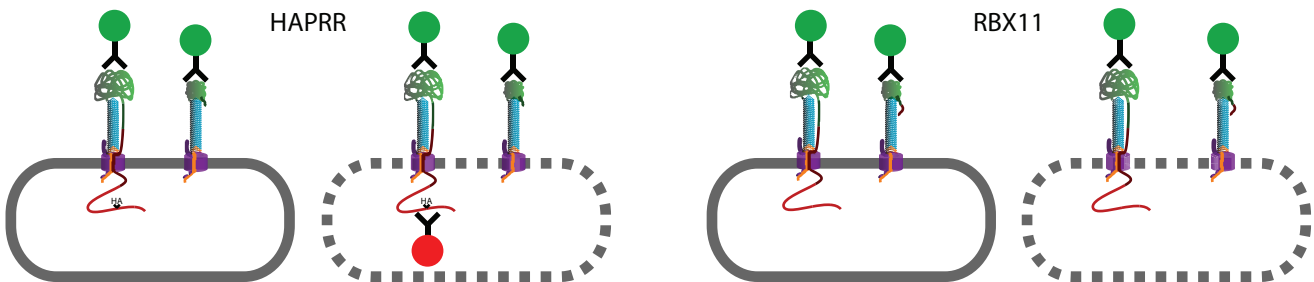


SUPPLEMENTAL FIGURE 4

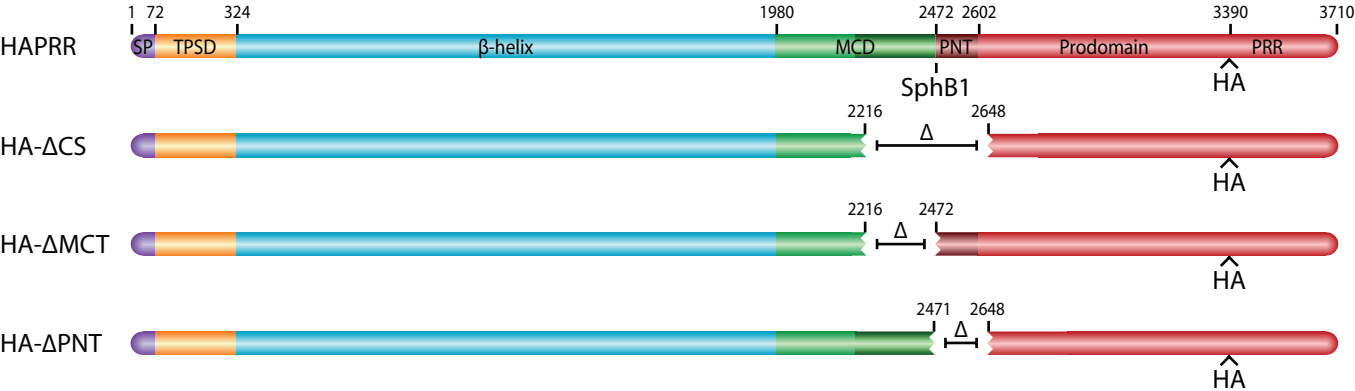






SUPPLEMENTAL FIGURE 5

A



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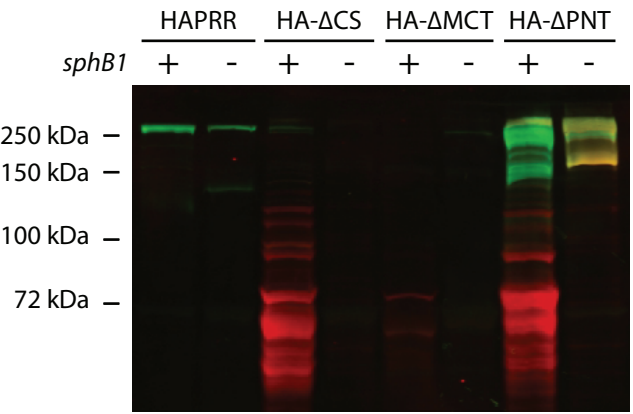
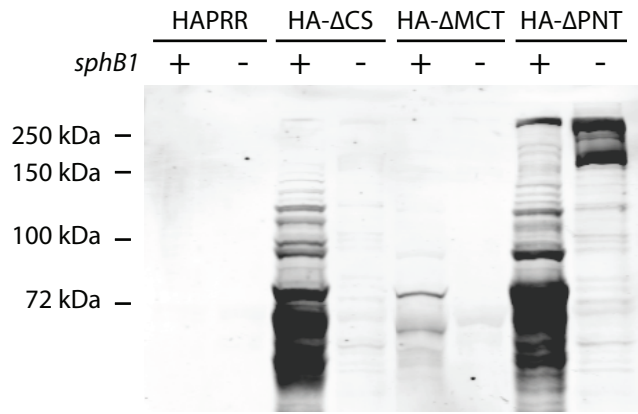


Supernatant



Supernatant