Kinetics of RapA inhibition by PhrA pentapeptide – curve fits -

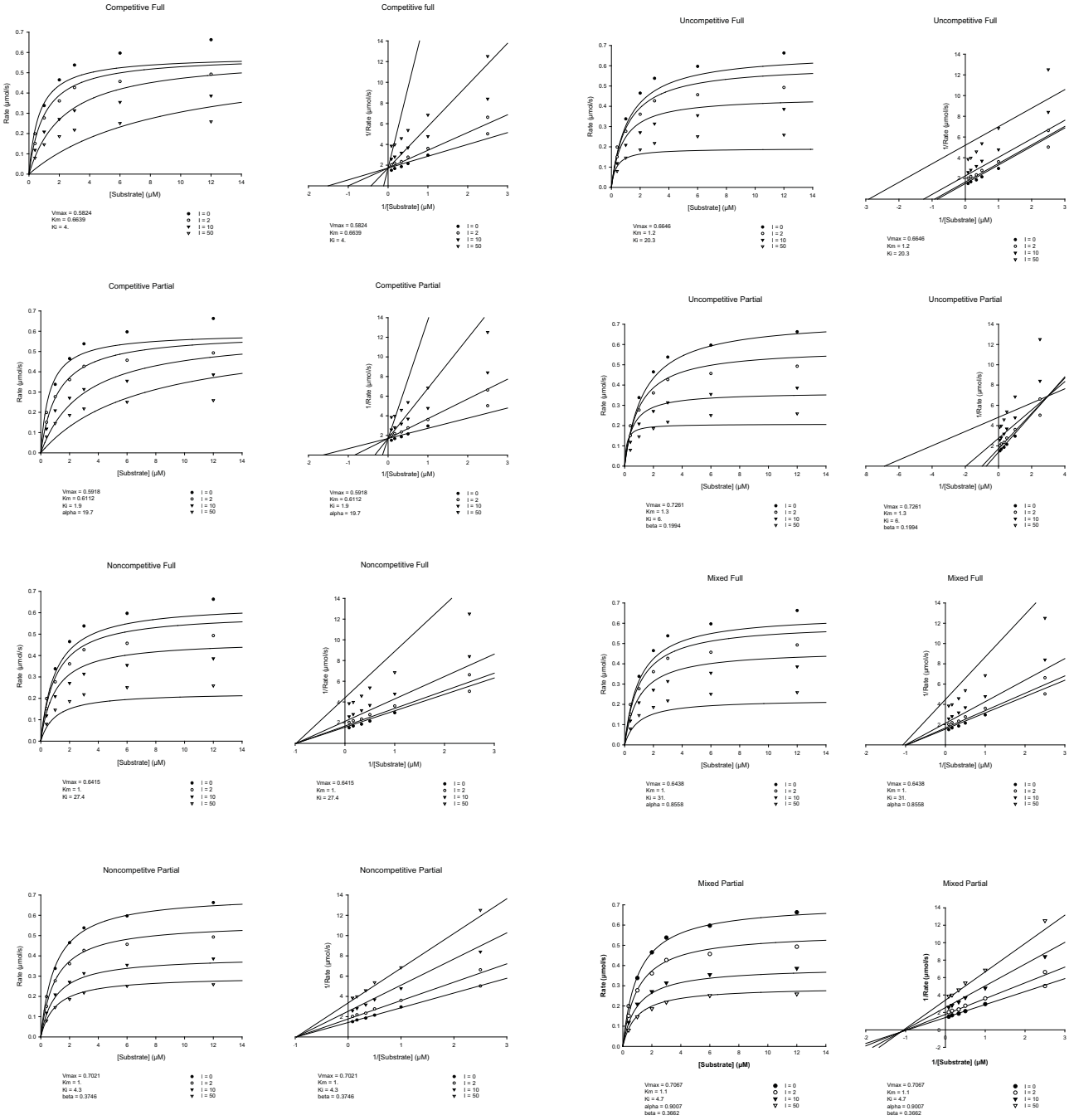

Fig. S1

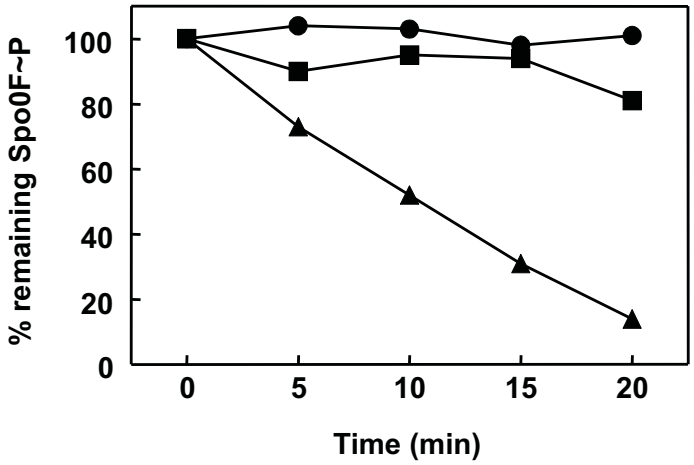

Fig. S2

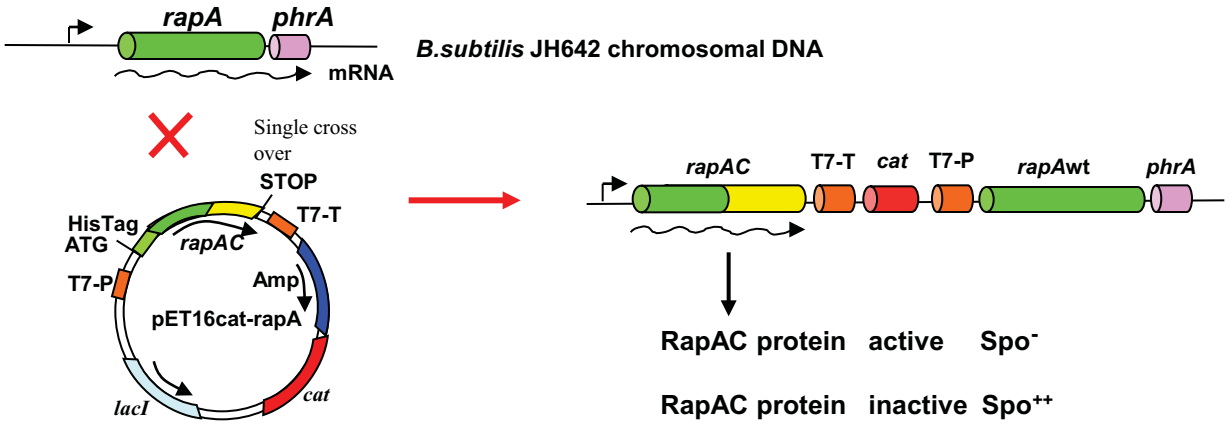
rapA
phrA
mRNA
B.subtilis JH642 chromosomal DNA
Single cross over
STOP
HisTag
ATG
rapAC
T7-T
T7-P
Amp
pET16cat-rapA
lacI
cat
rapAC
T7-T
cat
T7-P
rapAwt
phrA
RapAC protein active Spo-
RapAC protein inactive Spo++

Fig.S3

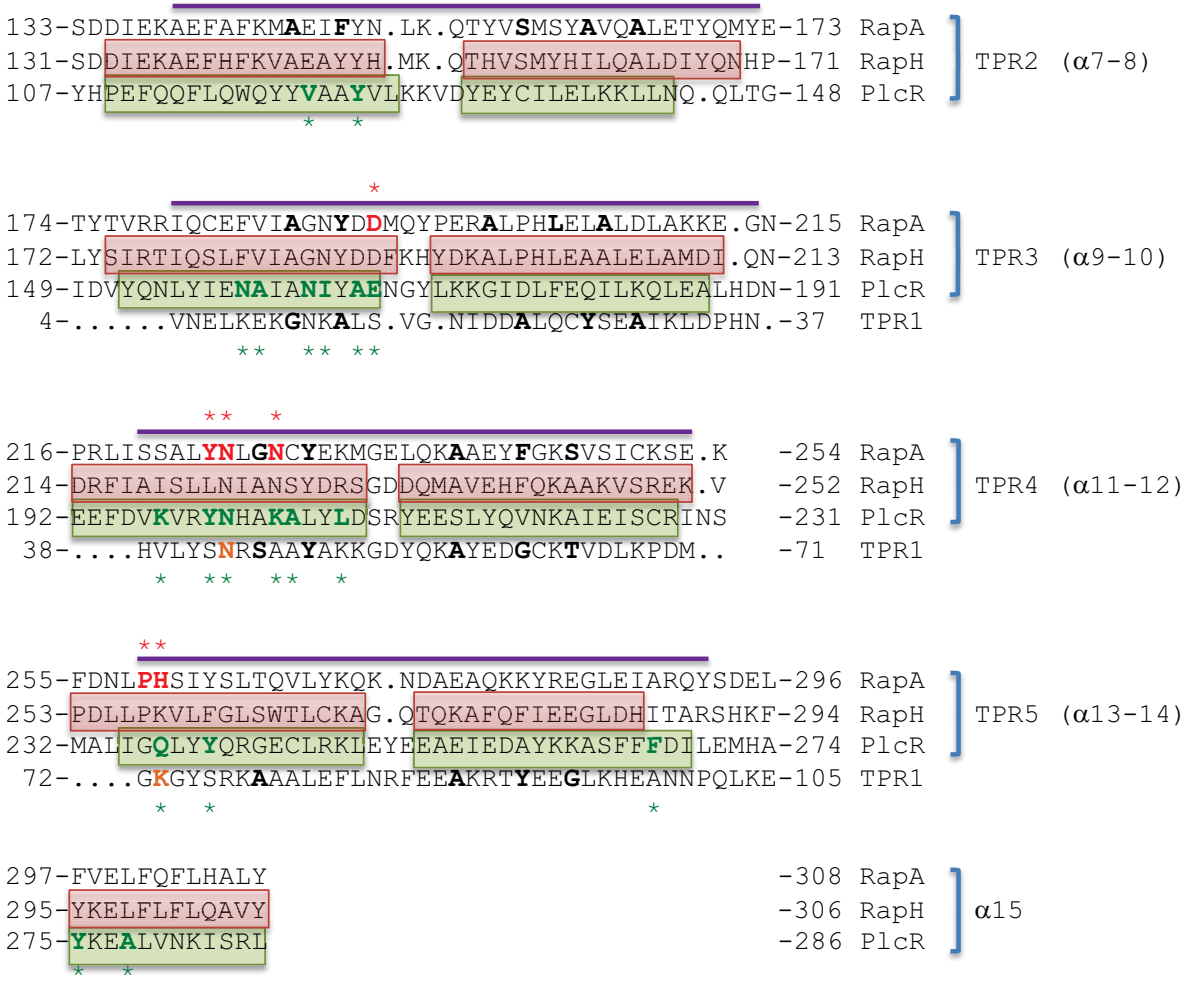
133-SDDIEKAEFAFKMAEIFYN.LK.QTYVSMSYAVQALETYQMYE-173 RapA
131-SDDIEKAEFHFKVAEAYYH.MK.QTHVSMYHILQALDIYQNHP-171 RapH
107-YHPEFQQFLQWQYYVAAYVLKKVDYEYCILELKKLLNQ.QLTG-148 PlcR
TPR2 (α7-8)
174-TYTVRRIQCEFVIAGNYDDMQYPERALPHLELALDLAKKE.GN-215 RapA
172-LYSIRTIQSLFVIAGNYDDFKHYDKALPHLEAALELAMDI.QN-213 RapH
149-IDVYQNLYIENAIANIYAENGYLKKGIDLFEQILKQLEALHDN-191 PlcR
4-......VNELKEKGNKALS.VG.NIDDALQCYSEAIKLDPHN.-37 TPR1
TPR3 (α9-10)
216-PRLISSALYNLGNCYEKMGELQKAAEYFGKSVSICKSE.K -254 RapA
214-DRFIAISLLNIANSYDRSGDDQMAVEHFQKAAKVSREK.V -252 RapH
192-EEFDVKVRYNHAKALYLDSRYEESLYQVNKAIEISCRINS -231 PlcR
38-....HVLYSNRSAAYAKKGDYQKAYEDGCKTVDLKPDM.. -71 TPR1
TPR4 (α11-12)
255-FDNLPHSIYSLTQVLYKQK.NDAEAQKKYREGLEIARQYSDEL-296 RapA
253-PDLLPKVLFGLSWTLCKAG.QTQKAFQFIEEGLDHITARSHKF-294 RapH
232-MALIGQLYYQRGECLRKLEYEEAEIEDAYKKASFFFDILEMHA-274 PlcR
72-....GKGYSRKAAALEFLNRFEEAKRTYEEGLKHEANNPQLKE-105 TPR1
TPR5 (α13-14)
297-FVELFQFLHALY -308 RapA
295-YKELFLFLQAVY -306 RapH
275-YKEALVNKISRL -286 PlcR
α15

Fig. S4

Legend to Supplementary Figures

Fig. S1: Curve fit analysis of inhibition equations fit to the data obtained with RapA and the PhrA pentapeptide. The SigmaPlot software was used to determine the type of competition that best fit the data obtained experimentally and plotted as Michaelis-Menten and Lineweaver-Burk kinetics.

Fig. S2: RapCA1 does not dephosphorylate Spo0F~P. The dephosphorylation assay was carried out as described in Materials and Methods and samples were taken at the indicated time points. Spo0F~P (2.5 μM) was incubated alone (-●-) with RapAwt (-▲-) or with RapCA1 (-■-) (0.5 μM). Percentage of remaining Spo0F~P was calculated from the quantitation (pixel values) of each time point using the ImageQuant Software.

Fig. S3: Schematic representation of the mechanism of a single cross over integration using plasmid pET16cat carrying the *rapAC* genes. Integration of these plasmids results in a duplication of the *rapA* gene and interruption of the *rapA phrA* transcript at the 3' end of the *rapA* sequence (on the left side of the vector), and *rap* and *phr* genes lacking an active promoter (on the right side of the vector). If PhrA is not produced and if the Rap protein is active against Spo0F~P, the cells will be sporulation deficient (spo^-). On the contrary, if the Rap protein is inactive against Spo0F~P, a hypersporulation phenotype (spo^{++}) will be observed similar to the phenotype of the *rapA* deletion mutant (4).

Fig. S4: Amino acid sequence alignment of the TPR domains involved in peptide binding in RapA, RapH, PlcR and the TPR1 module of the Hop protein that specifically recognizes the C-terminal heptapeptide of Hsp70 (2,3,5). The purple lines delineate the predicted 34 amino acids of the TPR domains of RapA based on the position of the consensus residues. TPR consensus amino acids are in bold in RapA and TPR1 and they occupy position 8, 20 and 27 (small side chain residues: A, G or S), position 11 (bulky, charged, small hydrophobic residues: Y, F, H, K, A, L) and position 24 (bulky, hydrophobic residues: Y, F, L or I). Notably, the TPR sequences in PlcR are degenerated and therefore conservation of the consensus is not always observed (1). The extent of the α helices as defined by the crystal structures of RapH and PlcR are indicated by the red and green boxes, respectively. Residues highlighted in red and the asterisks within the RapA sequence indicate the amino acids that affect PhrA activity when mutated. Residues highlighted in green and the asterisks within the PlcR sequence indicate residues relevant for PlcR-PapR interaction based on the structure of the complex. Residues in orange within the TPR1 sequence indicate positions critical for peptide binding that correspond to similar residues in RapA. The TPR number and a helix number for the Rap and PlcR proteins are shown on the right of the brakets.

References

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Table S1: Oligonucleotide primers used in this study

Name	Sequence[a]
OM EcoRI SWAP	5'-AGTGCGAATTCGTCTTTCGGGGTCAACC-3'
OM3' Kpn	5'-AAACAGGTACCTTAGATTTCAATTTCATACAAACCTTCAC-3'
OMexp3'	5'-GGCTGGATCCAAACAAATAACAAACAACTTAGATTTC-3'
OMexp5'	5'-GGTGAACATATGAAGAGTGGGGTAATTCCTTCTTCAGCG-3'
OL5' Bam	5'-GGGGGAGGATCCGAGGATGAAGCAGACGATTCCGTC-3'
OL3' Bam	5'-AAACCTGGGATCCATTTAGATTTCATATAAACAATCTCC-3'
OM5'Kpn	5'-CCCAGGGTACCGTAGAAATCAAGAGTGAAGAAG-3'
RapC BsmA	5'-ACCCCCGTCTCGAAATGGCACTGAATGATTC-3'
RapA BsmA	5'-AATCCGTCTCGATTTCGTTATTGCAGGTAATTATG-3'
RapA 3'Pst	5'-CAACACTGCAGACATCCATTTAGATTTCATATAAAC-3'
RapA5'Pst	5'-GAGAACTGCAGGTATCTTCAGCCCTTCAATG-3'
RapASOE-70	5'-GGCAGAAATAAATTCCCGCTGCTTAAATTCATACATTCCTCG-3'
RapCSOE-70	5'-CGAGGAATCTATGAATTTAAGCAGCGGGAATTTATTTCTGCC-3'
RapASOE-122	5'-GATAATCCAGCATAAGCTGGTGCCTGAACTCCATTAAAG-3'
RapCSOE-122	5'-CTTTAATGGAGTTCAGGCACCAGCTTATGCTGGATTATC-3'
RapASOE+105	5'-GATCGTCTGTTTCTTTAGCGATTTCCAATCCTTCACGAT-3'
RapCSOE+105	5'-ATCGTGAAGGATTGGAAATCGCTAAAGAAACAGACGATG-3'
RapAC5 3'	5'-AGGGCTAAATCCTCAATATAAGGATACAG-3'
RapAC5 5'	5'-CTGTATCCTTATATTTGAGGATTTAGCCCT-3'
M193A	5'-AGGTAATTATGATGATGCGCAGTATCCAGCAAGAGCATTGC-3'
M193Acompl	5'-GCAATGCTCTTTCTGGATACTGCGCATCATCATAATTACCT-3'
Q194A	5'-TAATTATGATGATATGGCGTATCCAGAAAGAGCATTGCC-3'
Q194Acompl	5'-GGCAATGCTCTTTCTGGATACGCCATATCATCATAATTA-3'
Y224A	5'-ATCAGTTCTGCCCTAGCGAATCTCGGAAACTGCTATGAG-3'
Y224Acompl	5'-CTCATAGCAGTTTCCGAGATTCGCTAGGGCAGGACTGAT-3'
N225A	5'-AGTTCTGCCCTATATGCGCTCGGAAACTGCTATGAGAAAATGG-3'
N225Acompl	5'-CCATTTTCTCATAGCAGTTTCCGAGCGCATATAGGGCAGAACT-3'
N228A	5'-TATATAATCTCGGAGCGTGCTATGAGAAAATGGGTGAACTGC-3'
N228Acompl	5'-GCAGTTCACCCATTTTCTCATAGCACGCTCCGAGATTATATA-3'
K232AF	5'-AAACTGCTATGAGGCGATGGGTGAACTGCAAAAGG-3'
K232AR	5'-CCTTTTGCAGTTCACCCATCGCCTCATAGCAGTTT-3'
E253AF	5'-TTCTATTTGCAAGTCGGCGAAGTTCGATAATCTTCCGC-3'
E253AR	5'-GCGGAAGATTATCGAACTTCGCCGACTTGCAAATAGAA-3'
H260AF	5'-AAGTTCGATAAYCTTCCGGCGTCTATCTACTCTTTAACACAAG-3'
H260AR	5'-CTTGTGTTAAAGAGTAGATAGACGCCGGAAGATTATCGAACTT-3'

a) Restriction enzyme sites are underlined.