ATP-dependent motor activity of the transcription termination factor Rho from *Mycobacterium tuberculosis*

François D'Heygère, Annie Schwartz, Franck Coste, Bertrand Castaing, and Marc Boudvillain

(Supplementary information)

Address correspondence to M.B. (marc.boudvillain@cnrs-orleans.fr)

SUPPLEMENTARY METHODS

Circular Dichroism. CD spectra were recorded at 20 °C on a Jasco J-810 spectropolarimeter from 260 to 195 nm using a 1-mm quartz cell, a scan rate of 50 nm/min, a response time of 8 ms, a bandwidth of 1 nm, a resolution of 1 nm, and a protein concentration of 0.1 mg/ml in a buffer containing 10 mM sodium phosphate, pH 7.5 and 100 mM NaCl. Each spectrum represents the average of five scans, with the buffer subtracted.

Diffusion light scattering. Hydrodynamic radius measurements were made at 20 °C with a Zetasizer Nano S instrument (Malvern Instruments Ltd, UK) and a protein concentration of 1mg/ml in GF buffer.

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Plasmid	Method	Source DNA	Forward primer	Reverse primer
pET28b-	Quickchange	pET28b-MtbRho		
MtbRho/T501A	а			3-GALLOIGILALLAGLOGALLLOGILILGAL
pET28b-	Quickchange	pET28b-MtbRho		
MtbRho/T501K	а		5-00100A0ACC0001CCAAA0010ACAC001CATTIC	5-GAAAAIGACCGIGICACCIIIGGACCCGGICICGACC
pET28b-	Quickchange	pET28b-MtbRho	5' GTGCTCGTCGACGCGCCTGAGGAG	5'-CTCCTCAGGCCGCGCGCGCGACGAGGAGCAC
MtbRho/E386A	а			
pET28b-	Quickchange	pET28b-MtbRho	5' GETTEGATECTEGTEGTEGTEGTEG	5'0640400400660046647006600
MtbRho/S461A	а			
pET28b-	1 [1-75] ^b	pET28b-MtbRho	5'-GGGAATTGTGAGCGGATAAC	5'-CCGGCTGGACGACGTCGTCGGGTACCTGTCGCCTGATCTCC
Δ MtbRho	2 [220-602] ^b	pET28b-MtbRho	5'-GGAGATCAGGCGACAGGTACCCGACGACGTCGTCCAGCCGG	5'-GGCCCCAAGGGGTTATGCTAGTTATTGCTCAGCGGTGGC
pET28b-	1 [1-308]] ^b	pET28b-MtbRho	5'-GGGAATTGTGAGCGGATAAC	5'-GGGTTAAGTTCTCAAAGAGGATCCGCTTCTTGGCGTCTTCGACCG
[Mtb:Ec]Rho	2 [131-419] ^b	pET28b-EcRho	5'-CGGTCGAAGACGCCAAGAAGCGGATCCTCTTTGAGAACTTAACCC	5'-GGCCCCAAGGGGTTATGCTAGTTATTGCTCAGCGGTGGC
pET28b-	1 [1-130]] ^b	pET28b-EcRho	5'-GGGAATTGTGAGCGGATAAC	5'-CGTCAGTTTGCCGAACTCGGGTTTGTTGCGGGCGTTTTCAGG
[Ec:Mtb]Rho	2 [309-602] ^b	pET28b-MtbRho	5'-CCTGAAAACGCCCGCAACAAACCCGAGTTCGGCAAACTGACG	5'-GGCCCCAAGGGGTTATGCTAGTTATTGCTCAGCGGTGGC

Supplementary Table 1: primers used for plasmid constructs

^a Site-directed mutagenesis using the Quickchange method (Stratagene) and the indicated mutagenic primers.

^b Overhang extension PCR and standard cloning. Full insert DNA fragments were obtained by fusing the N-terminal fragment 1 to the C-terminal fragment 2 (corresponding Rho amino acids indicated in brackets) by overhang extension PCR. The insert DNA fragments were cloned into the pET28b vector plasmid after digestion of both vector and inserts with the Xbal and Xhol restriction enzymes.

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M. IUteus IJDERPEEVT DMQRSVDGEV IASTFDEPAD DHTTLAELAI ERAKKLVEMG KDVVILLDSN TRLGRAYNLA APASGRILSG GVDSALYPP KRFFGAARNU E. coli ILDERPEEVT DIERVAGEV VASTFDEPAS RHVQVAEMVI ERAKRLVEMK KDVIILLDSI TRLARAYNLV VPASGKVLG GVDSALHRP KRFFGAARNU B. subtilis IJDERPEEVT DIERVAGEV VSSTFDEVPE NHKVAELVL ERAKRLVEKK KDVIILDSI TRLARAYNLV I <u>PPSGRTLSG GID</u> PAAFHRP KRFFGAARNU Malker B Q-loop 601 TAT Catalytic Glu Arg Valve 651 M. tuberculosis EEGGSLTILA TAMVETGSRM DEVIFEEFKG TGNAELKLDR KLAERRVFPA VDVNPSGTRK DELLLSPDEF AIVHKLRRVL SGLDSHQAID LLMSQLRKTK E. coli EEGGSLTILA TALVETGSRM DEVIFEEFKG TGNMELRLSR HLAERRIFPA VDVNASGTRR EEALLSQEEV KIMWKLRRVL SGLEQQQAID LLTNKKDTA E. coli EEGGSLTILA TALVETGSRM DEVIFEEFKG TGNMELHLSR KIAERRVFPA IDYNRSGTRK EELLTQEEL QKMWILRKII HPMGEIDAME FLINKLAMTK B. subtilis EEGGSLTILA TALVDTGSRM DDVIYEEFKG TGNMELHLSR KIAERRVFPA IDYNRSGTRK EELLTQEEL QKMWILRKII HPMGEIDAME FLINKLAMTK R-loop Arg finger 701 M. tuberculosis NNYEFLVQVS KTTFGSMDSD E. coli NNYEFLVQVS KTTGSMDSD E. coli TNDDFFEMMK RS	М. М. В.	tuberculosis luteus coli subtilis	401 ^{5Y} QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYYGLLHVE	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL	41 ETSTERL ETDPKLI ERGNGSTEDL ETKPNFL 51	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV	SPPKAGKTTI SPPKAGKTMI APPKAGKTML APPKAGKTML Walker A	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA	NNPECHLMVV NNPEVHLMMV NHPDCVLMVL NQPEAELIVL
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B. subtilis LVDERPEEVT DIERSVAGDV VSSTFDEVPE NHIKVAELVL ERAMRLVEHK KD <u>VIIIMDSI</u> TRLARAYNLV I <u>PPSGRTLSG GIB</u> PAAFHRP KRFFGAARNI T catalytic Glu Arg Valve Walker B Q-loop 601 TNA EEGGSLTIIA TANVETGSTG DTVIFEEFKG M. luteus ENGGSLTIIA TALVETGSTM DEVIFEEFKG EEGGSLTIIA TALVETGSTM DEVIFEEFKG EEGGSLTIIA TALVETGSTM DEVIFEEFKG EEGGSLTIIA TALVETGSTM DEVIFEEFKG S coli EEGGSLTIIA TALIDTGSTM DEVIFEEFKG B. subtilis EEGGSLTIIA TALVDTGSTM DDVIYEEFKG TGNMELRLSR HLAERRIFPA VDVNASGTRR EEALLSQEEV KIMWKLRRVL SGLEQQQALD LLTNKIKDTA E. coli EEGGSLTIIA TALVDTGSTM DDVIYEEFKG TGNMELHLSR KIAEKRVFPA IDYNRSGTRK EELLTQEEL QKMWILRKII HPMGEIDAME FLINKLAMTK B. subtilis EEGGSLTIIA TALVDTGSTM DDVIYEEFKG TGNMELHLDR SLAERRIFPA IDIRRSGTRK EELLVPKEHL DRLWSIRKTM SDSPDFAE KFMRKMKKTK R-loop Arg finger 701 M. tuberculosis NNYEFLVQVS KTTPGSMDSD E. coli TNDDFFEMMK RS	М. Е. В. М.	tuberculosis luteus coli subtilis tuberculosis luteus	401 ^{5Y} OKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT	NTD SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPPS	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTSVAELAI	4: ETSTERL ERGNGSTEDL ETKPNFL 5: ERAKRLVEQG ERAKRLVEQG	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 Bcm KDVVVLIDSI RDVVVLIDSI	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV Bcm TRLGRAYNNA TRLGRAYNNA	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A TTTT SPASGRILSG APASGRILSG	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA	NNPECHLMVV NNPEVHLMMV NHPDCVLMVL NQPEAELIVL KRFLGAARNI KKFFGAARNI
Kalker B Q-loop 601 T 601 T <t< th=""><th>М. Е. В. М. Е.</th><th>tuberculosis luteus coli subtilis tuberculosis luteus coli</th><th>401 ^{5Y} OKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LIDERPEEVT</th><th>NTD SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV EMQRLVKGEV</th><th>CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT</th><th>PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTSVAELAI RHVQVAEMVI</th><th>4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEMG</th><th>51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 BCm KDVVVLDSI RDVVVLDSI RDVVVLDSI KDVVLLDSI</th><th>IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV Bcm TRLGRAYNNA TRLGRAYNNA TRLARAYNTV</th><th>SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG VPASGKVLTG</th><th>LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP</th><th>NNPECHLMVV NNPEVHLMMV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI</th></t<>	М. Е. В. М. Е.	tuberculosis luteus coli subtilis tuberculosis luteus coli	401 ^{5Y} OKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LIDERPEEVT	NTD SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV EMQRLVKGEV	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTSVAELAI RHVQVAEMVI	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEMG	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 BCm KDVVVLDSI RDVVVLDSI RDVVVLDSI KDVVLLDSI	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV Bcm TRLGRAYNNA TRLGRAYNNA TRLARAYNTV	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG VPASGKVLTG	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP	NNPECHLMVV NNPEVHLMMV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI
601 RNA M. tuberculosis M. luteus EEGGSLTIIA TALVETGSTG EEGGSLTIIA TALVETGSTG EEGGSLTI	М. Е. В. М. Е.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 T QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LIDERPEEVT LVDERPEEVT	NTD SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV	<pre>CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE</pre>	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTSVAELAI RHVQVAEMVI NHIKVAELVL	41 ETSTERL ETDPKLI ETKPNFL 51 ERAKRLVEQG ERAKRLVEMG ERAKRLVEMG ERAKRLVEHK	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 EDVVVLIDSI RDVVVLIDSI KDVVLLDSI KDVIILIDSI	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLGRAYNNA TRLARAYNLV	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG VPASGKVLTG I <u>PPSGRTLSG</u>	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI KRFFGAARNI
601 M. tuberculosis M. luteus EEGGSLTIIA TAMVETGSTG DTVIFEEFKG E. coli EEGGSLTIIA TALUETGSTM DEVIFEEFKG B. subtilis EEGGSLTIIA TALUDTGSTM DEVIFEEFKG R-loop M. tuberculosis NNYEFLVQVS KTTPGSMDSD E. coli B. subtilis TNDDFFEMMK RS	M. E. B. M. E.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 ⁵ Y QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LIDERPEEVT LIDERPEEVT Catalytic Glu Arg Valve	NTD SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPPS VASTFDEPAS VSSTFDEVPE	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL	41 ETSTERL ETDPKLI ERGNGSTEDL ETKPNFL 51 ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 BC KDVVVLIDSI RDVVVLDSM KDVILLDSI KDVILLDSI KDVILLDSI Walker B	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLGRAYNLA TRLARAYNLV	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG VPASGKVLTG I <u>PPSGRTLSG</u> Q-loop	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI
M. tuberculosis M. luteus EEGGSLTIIA TAMVETGSTG DTVIFEEFKG M. luteus EEGGSLTIIA TALVETGSRM DEVIFEEFKG E. coli EEGGSLTIIA TALUDTGSRM DEVIFEEFKG B. subtilis EEGGSLTIIA TALUDTGSRM DEVIYEEFKG R-loop M. tuberculosis M. luteus E. coli TONDEFEMIK RS E. coli B. subtilis TONDEFEDIIN OFFEMIK RS	M. E. B. M. E. B.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 ^{5Y} QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LIDERPEEVT LIDERPEEVT Catalytic Glu Arg Valve	NTD - SINGGSVEDA EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV	KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL	43 ETSTERL ETDPKLI ERGNGSTEDL ETKPNFL 53 ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 BOVVVLIDSI RDVVVLIDSI KDVIILLDSI KDVIILLDSI Walker B	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNLV	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A TTTT SPASGRILSG APASGRILSG VPASGKVLTG IPPSGRTLSG Q-loop	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP	NNPECHLMVV NNPEVHLMMV NQPEAELIVL KRFLGAARNI KRFFGAARNI KRFFGAARNI
M. luteus ENGGSLTILA TALVETGSRM DEVIFEEFKG E. coli EEGGSLTIIA TALIDTGSKM DEVIYEEFKG B. subtilis EEGGSLTIIA TALUDTGSRM DUVIYEEFKG R-loop DUVIYEEFKG M. tuberculosis NNYEFLVQVS KTTPGSMDSD E. coli TNDDFFEMIK RS B. subtilis TNDDFFEDILN OEWKOANLSS APP	M. E. B. M. E. B.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 ^{5'Y} QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT Catalytic Glu Arg Valve 601	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV	KRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPPS VASTFDEPAS VSSTFDEVPE	PLYPNQRLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 BOVVVLIDSI RDVVVLIDSI KDVIILDSI KDVIILDSI Walker B 51	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLGRAYNLA TRLARAYNLV	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG VPASGKVLTG I <u>PPSGRTLSG</u> Q-loop	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI
E. coli EEGGSLTIIA TALIDTGSKM DEVIYEEFKG B. subtilis EEGGSLTIIA TALIDTGSKM DEVIYEEFKG R-loop Arg finger 701 M. tuberculosis NNYEFLVQVS KTTFGSMDSD E. coli TNDDFFEMMK RS	М. Е. В. М. Е. В.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis tuberculosis	401 ^{5Y} QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LIDERPEEVT TT catalytic Glu Arg Valve 601 EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK 61 KIAERRVFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 EDVVVLIDSI RDVVVLIDSI KDVIILDSI KDVIILDSI Walker B 51 VDVNPSGT R K	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLGRAYNNA TRLARAYNTV TRLARAYNLV DELLLSPDEF	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG UPASGKVLTG IPPSGRTLSG Q-loop AIVHKLRRVL	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI KRFFGAARNI
B. subtilis EEGGSLTILA TALVDTGSRM DDVIYEEFKG TGNMELHLDR SLAERRIFPA IDIRRSGTRK EELLVPKEHL DRLWSIRKTM SDSPDFAE KFMRKMKKKK R-loop Arg finger 701 M. tuberculosis NNYEFLVQVS KTTPGSMDSD M. luteus SNAEFLMLVS KTTLGSKGDD E. coli TNDDFFEMMK RS B. subtilis TNODFFEMMK RS	M. E. B. M. E. B.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus	401 ^{5Y} OKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LIDERPEEVT Catalytic Glu Arg Valve 601 EEGGSLTIIA ENGGSLTILA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV RNA TAMVETGSTG TALVETGSRM	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE DTVIFEEFKG DEVIFEEFKG	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNMELRLSR	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK 6: KIAERRVFPA HLAERRIFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 RDVVVLDSI RDVVVLDSI RDVVILDSI KDVIILDSI Walker B 51 VDVNPSGTRK VDVNASGTRR	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNLV TRLARAYNLV DELLLSPDEF EEALLSQEEV	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG UPASGKVLTG UPSGRTLSG Q-loop AIVHKLRRVL	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQQALD	NNPECHLMVV NNPEVHLMMV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA
R-loop Arg finger 701 M. tuberculosis NNYEFLVQVS KTTPGSMDSD M. luteus SNAEFLMLVS KTTLGSKGDD E. coli TNDDFFEMMK RS B. subtilis TNDDFFEMMK RS	M. E. B. M. E. B.	tuberculosis coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus coli	401 T QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT G01 EEGGSLTIIA EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV RNA TAMVETGSTG TALVETGSRM TALIDTGSKM	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE DTVIFEEFKG DEVIFEEFKG DEVIFEEFKG	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNAELKLDR TGNMELRLSR	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK 6: KIAERRVFPA HLAERRIFPA KIAEKRVFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 BCM KDVVLLDSI RDVVLLDSI KDVILLDSI KDVILLDSI Walker B 51 VDVNPSGTRK VDVNASGTRR IDYNRSGTRK	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNLV DELLLSPDEF EEALLSQEEV EELLTTQEEL	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG VPASGKVLTG I <u>PPSGRTLSG</u> Q-loop AIVHKLRRVL KIMWKLRRVL	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQQALD HPMGEIDAME	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFLGAARNI KRFFGAARNI KRFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA FLINKLAMTK
finger 701 M. tuberculosis NNYEFLVQVS KTTPGSMDSD M. luteus SNAEFLMLVS KTTLGSKGDD E. coli TNDDFFEMMK RS B. subtilis TNODFFEDILN OFWKOANLSS APP	M. E. B. M. E. B. M. E. B.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 T QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT Catalytic Glu Arg Valve 601 EEGGSLTIIA EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV RNA TAMVETGSRM TALVETGSRM TALVDTGSRM	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE DTVIFEEFKG DEVIFEEFKG DEVIYEEFKG DDVIYEEFKG	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNAELKLDR TGNMELRLSR TGNMELHLSR	4: ETSTERL ETDPKLI ERGNGSTEDL ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK KIAERRVFPA KIAERRVFPA SLAERRIFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 ECM KDVVVLLDSI KDVVVLDSI KDVIILDSI Walker B 51 VDVNPSGTRK UDVNRSGTRK IDIRRSGTRK	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNLV DELLLSPDEF EEALLSQEEV EELLTTQEEL EELLVPKEHL	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A RNA TTIT SPASGRILSG APASGRILSG UPASGKVLTG I <u>PPSGRTLSG</u> Q-loop AIVHKLRRVL KIMWKLRRVL QKMWILRKII DRLWSIRKTM	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQQALD HPMGEIDAME SDSPDFAE	NNPECHLMVV NNPEVHLMMV NQPEAELIVL KRFIGAARNI KRFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA FLINKLAMTK KFMRKMKKTK
701 M. tuberculosis NNYEFLVQVS KTTPGSMDSD M. luteus SNAEFLMLVS KTTLGSKGDD E. coli TNDDFFEMMK RS B. subtilis TNDDFFEDIIN OFWKDANLSS APP	M. E. B. M. E. B. M. E. B.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 ^{5'Y} QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT Catalytic Glu Arg Valve 601 EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV RNA TALVETGSTM TALVETGSSM TALVDTGSSM R-loop	KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE DTVIFEEFKG DEVIFEEFKG DEVIFEEFKG DEVIYEEFKG	PLYPNQRLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNMELRLSR TGNMELHLDR	4: ETSTERL ETKPNFL ETKPNFL 5: ERAKRLVEQG ERAKRLVEQG ERAKRLVEHK ERAMRLVEHK KIAERRVFPA KIAERRVFPA SLAERRIFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 EDVVVLIDSI RDVVVLIDSI RDVVLLDSI Walker B 51 VDVNPSGTRK VDVNASGTRR IDYNRSGTRK IDIRRSGTRK Arq	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNTV TRLARAYNTV DELLLSPDEF EEALLSQEEV EELLTTQEEL EELLVPKEHL	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG UPASGKVLTG UPASGKVLTG Q-loop AIVHKLRRVL KIMWKLRRVL QKMWILRKII DRLWSIRKTM	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA T GVDSTALYPP GVDSTALYPP GVDSTALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQALD HPMGEIDAME SDSPDFAE	NNPECHLMVV NNPEVHLMMV NQPEAELIVL KRFFGAARNI KRFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA FLINKLAMTK KFMRKMKKTK
M. tuberculosis NNYEFLVQVS KTTPGSMDSD M. luteus SNAEFLMLVS KTTLGSKGDD E. coli TNDDFFEMMK RS B. subtilie TNDDFFEDIIN OFWKOANLSS APP	M. E. B. M. E. B. M. E.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 ^{5YY} QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT Catalytic Glu Arg Valve 601 EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVKGEV DMQRSVKGEV DMQRSVKGEV DMQRSVKGEV EMQRLVKGEV DIERSVAGDV TAMVETGSTG TALVETGSTM TALVETGSTM TALVETGSTM TALVETGSTM	KRRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE DTVIFEEFKG DEVIFEEFKG DEVIFEEFKG DDVIYEEFKG	PLYPNQRLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNAELKLDR TGNMELRLSR TGNMELHLDR	4: ETSTERL ETPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK KIAERRVFPA HLAERRIFPA KIAEKRVFPA SLAERRIFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 RDVVVLIDSI RDVVVLIDSI KDVIILDSI Walker B 51 VDVNPSGTRK VDVNASGTRR IDYNRSGTRK IDIRRSGTRK Arg finge	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNTV TRLARAYNTV DELLLSPDEF EEALLSQEEV EELLTTQEEL EELLVPKEHL	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A SPASGRILSG APASGRILSG UPASGKVLTG I <u>PPSGRTLSG</u> Q-loop AIVHKLRRVL KIMWKLRRVL KIMWKLRRVL DRLWSIRKTM	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA T GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQQAID HPMGEIDAME SDSPDFAE	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFIGAARNI KRFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA FLINKLAMTK KFMRKMKKTK
M. luteus SNAEFLMLVS KTTLGSKGDD E. coli TNDDFFEMMK RS B. subtilie TNDFFEDIIN OFWKOANLSS APP	М. В. М. В. М. В. М. В.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 ^{5Y} QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LIDERPEEVT TC catalytic Glu Arg Valve 601 EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV TAMVETGSTG TALVETGSTM TALVETGSTM TALVETGSTM TALVETGSTM TALVETGSTM	KRRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE DTVIFEEFKG DEVIFEEFKG DEVIFEEFKG DDVIYEEFKG	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNAELKLDR TGNMELHLSR TGNMELHLDR	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEMK ERAMRLVEHK KIAERRVFPA HLAERRIFPA KIAERRVFPA SLAERRIFPA	51 TRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 EDVVVLLDSI RDVVVLLDSI RDVVILLDSI KDVIILLDSI Walker B 51 VDVNPSGTRK VDVNSGTRK IDJNRSGTRK IDJRRSGTRK Arg finge	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLGRAYNNA TRLARAYNTV TRLARAYNTV DELLLSPDEF EEALLSQEEV EEELLTTQEEL EELLVPKEHL	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A FTTTT SPASGRILSG APASGRILSG UPASGKVLTG IPPSGRTLSG Q-loop AIVHKLRRVL KIMWKLRRVL QKMWILRKII DRLWSIRKTM	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQQALD HPMGEIDAME SDSPDFAE	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFIGAARNI KKFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA FLINKLAMTK KFMRKMKKTK
E. coli TNDDFFEMMK RS	м. Е. В. М. Е. В.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 ^{5Y} OKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN 601 EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV DIERSVAGDV RNA TAMVETGSTG TALVETGSRM TALIDTGSKM TALIDTGSKM TALIDTGSKM	CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE Ben DTVIFEEFKG DEVIFEEFKG DEVIYEEFKG	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNMELRLSR TGNMELHLDR	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK KIAERRVFPA HLAERRIFPA KIAEKRVFPA SLAERRIFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 RDVVVLIDSI RDVVVLIDSI KDVVILIDSI KDVIILIDSI Walker B 51 VDVNPSGTRK UDVNSGTRK IDIRRSGTRK Arg finge	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNTV TRLARAYNTV DELLLSPDEF EEALLSQEEV EELLTTQEEL EELLVPKEHL	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A TTIT SPASGRILSG APASGRILSG UPASGKVLTG I <u>PPSGRTLSG</u> Q-loop AIVHKLRRVL KIMWKLRRVL QKMWILRKII DRLWSIRKTM	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQQALD HPMGEIDAME SDSPDFAE	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA FLINKLAMTK KFMRKMKKTK
B subtilisNORENTIN OEWKOANISS APP	М. Е. В. М. Е. В. М. В.	tuberculosis coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 T OKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN -RYFALLKVN 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT Catalytic Glu Arg Valve 601 EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV IERSVAGDV RNA TAMVETGSTG TALVETGSRM TALIDTGSKM TALIDTGSKM R-loop	<pre>CTD KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE DTVIFEEFKG DEVIFEEFKG DEVIYEEFKG</pre>	PLYPNQRLRL PLYPQERLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNMELRLSR TGNMELHLDR	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEHK ERAMRLVEHK KIAERRVFPA HLAERRIFPA KIAEKRVFPA SLAERRIFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 BCM KDVVLLDSI RDVVVLDSI RDVVLLDSI KDVIILDSI Walker B 51 VDVNPSGTRK UDVNSGTRK IDIRRSGTRK Arg finge	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNLV TRLARAYNLV DELLLSPDEF EEALLSQEEV EELLTTQEEL EELLVPKEHL	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A FRNA FASGRILSG APASGRILSG VPASGKVLTG I <u>PPSGRTLSG</u> Q-loop AIVHKLRRVL KIMWKLRRVL QKMWILRKII DRLWSIRKTM	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQQALD HPMGEIDAME SDSPDFAE	NNPECHLMVV NNPEVHLMVV NQPEAELIVL KRFLGAARNI KKFFGAARNI KRFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA FLINKLAMTK
	M. E. B. M. E. B. M. E. B.	tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis tuberculosis luteus coli subtilis	401 ^{5'Y} QKFNPLVRLD QKFNALVKIS -RYFALLKVN -RYYGLLHVE 501 Bcm LVDERPEEVT LVDERPEEVT LVDERPEEVT LVDERPEEVT Catalytic Glu Arg Valve 601 EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA EEGGSLTIIA	NTD - SINGGSVEDA SVNGQPAVEH EVNFDKPENA AVNGDDPESA DMQRSVKGEV DMQRSVDGEV EMQRLVKGEV DIERSVAGDV RNA TAMVETGSTG TALVETGSTM TALIDTGSKM TALIDTGSKM R-Loop KTTPGSMDSD KTTLGSKGDD BS	KKRPEFGKLT PQRVEFGKLV RNKILFENLT KERVHFPALT IASTFDRPPS IASTFDRPAD VASTFDEPAS VSSTFDEVPE DTVIFEEFKG DEVIFEEFKG DEVIYEEFKG	PLYPNQRLRL PLHANSRLRM PLYPDRQMVL DHTSVAELAI DHTTLAELAI RHVQVAEMVI NHIKVAELVL TGNAELKLDR TGNMELRLSR TGNMELHLDR	4: ETSTERL ETDPKLI ETKPNFL 5: ERAKRLVEQG ERAKRLVEMG ERAKRLVEMK ERAMRLVEHK KIAERRVFPA HLAERRIFPA KIAEKRVFPA SLAERRIFPA	51 TTRVIDLIMP GPRVIDLVSP TARVLDLASP STRIMDMMAP 51 BCM KDVVLLDSI RDVVLLDSI KDVILLDSI KDVILLDSI Walker B 51 VDVNPSGTRK VDVNASGTRK IDIRRSGTRK Arg finge	IGKGQRALIV IGKGQRGLIV IGRGQRGLIV VGFGQRGLIV TRLGRAYNNA TRLGRAYNNA TRLARAYNLV DELLLSPDEF EEALLSQEEV EELLTTQEEL EELLVPKEHL	SPPKAGKTTI SPPKAGKTMI APPKAGKTML Walker A FIII SPASGRILSG APASGRILSG UPASGKVLTG UPASGKVLTG Q-loop AIVHKLRRVL KIMWKLRRVL QKMWILRKII DRLWSIRKTM	LQDIANAITR LQSIANAIKT LQNIAQSIAY LKEIANSITA GVDSTALYPP GVDSTALYPP GVDSSALYPP GVDANALHRP GIDPAAFHRP SGLDSHQAID SGLEQQQALD HPMGEIDAME SDSPDFAE	NNPECHLMVV NNPEVHLMMV NQPEAELIVL KRFFGAARNI KRFFGAARNI KRFFGAARNI LLMSQLRKTK LLTNKIKDTA FLINKLAMTK KFMRKMKKTK

Supplementary figure 1: Alignment of representative Rho sequences (excerpt from Ref. 13). Color coding of major motifs is the same as in Figure 1. Key conserved residues are highlighted in grey. The small PBS insert (aa 281-286) and T501 residue (in R-loop) in _{Mtb}Rho are highlighted in light red. Numbers above sequences correspond to positions in the alignment (not in individual sequences).



Supplementary figure 2: Single-point mutant E386A and WT _{Mtb}Rho have comparable structural, oligomerization, and ATP binding behaviors. **(A)** Chromatograms of the E386A and WT _{Mtb}Rho proteins analyzed by gel filtration on a Sephacryl S-300 HR column. **(B)** Diffusion light scattering and **(C)** circular dichroism spectra of the E386A and WT _{Mtb}Rho proteins. **(D)** Retention of [γ^{32} P]ATP:Rho complexes on a nitrocellulose (NC) membrane. Rho proteins (10 or 200 nM) were incubated with 0.1 nM [γ^{32} P]ATP in helicase buffer supplemented with 1 mM MgCl₂ for 10 min at 30°C before being filtered through a NC membrane using a slot-blot apparatus. Note that the amounts of ATP retained with proteins on the NC membrane are significantly higher for _{Ec}Rho and _[Mtb:Ec]Rho. This suggests that the _{Ec}Rho motor binds ATP much tighter than the _{Mtb}Rho motor and is consistent with the ~10-fold lower K_{m,ATP} measured for ATP hydrolysis by _{Ec}Rho than by _{Mtb}Rho



Supplementary figure 3: The MtbRho enzyme is a directional helicase requiring a 5'-ssRNA tail upstream from its duplex target. Unwinding experiments were performed in the presence of ATP under standard conditions (see methods) with substrate D which contains the same 14bp duplex region than substrate C but located upstream rather than downstream from the ssRNA tail (see diagram, inset). Although the duplex in substrate D is slightly less stable than in substrate C, MtbRho does not accelerate duplex unwinding with this substrate (left graph). The same is true for $_{Ec}$ Rho which even displays a slight 'annealing' activity with substrate D as evidenced by a bell-shaped unwinding curve. By contrast, the non-directional DEAD-box protein Ded1 indiscriminately increases unwinding of the duplexes in substrates C and D. Experiments with Ded1 were performed as described in Ref. 44.



Supplementary figure 4: Single-round transcriptions with bead-affixed transcription complexes containing biotinylated λ tR1 DNA templates. The ³²P-labeled complexes, halted at +24 (by privation of CTP from the initiation mixture), were immobilized and purified on streptavidin-coated magnetic beads. They were then incubated with the Rho variants indicated above gel lanes before being 'chased' with 75 µM rNTPs. The supernatant (*S*) and bead (*B*) fractions were then separated on a Magrack (GE-healthcare) before analysis by denaturing PAGE. The presence of the Rho-dependent transcripts in supernatants confirms that they stem from termination rather than transcriptional pausing or arrest. (A) Comparison of the RNA release efficiencies of the various Rho proteins. (B) Effect of the presence of BCM (0 or 750 µM) in reactions performed with the representative WT MtbRho or the [Mtb:Ec]Rho chimera.



Supplementary figure 5: Normalized electrophoregrams of transcription termination reactions performed under standard 'chase' conditions (as in Figure 6B).



Supplementary figure 6: Upstream shift of the termination window obtained with the [Mtb:Ec]Rho chimera as a function of BCM concentration. Graphs show normalized fractions of products released at representative termination positions as a function of BCM concentration (data averaged from 3 independent experiments).



Supplementary figure 7: Characterization of the single-point mutant S461A of $_{Mtb}$ Rho by (A) gel filtration on a Sephacryl S-300 HR column, (B) diffusion light scattering, (C) circular dichroism, and (D) steady-state ATP hydrolysis measurements. The Q-loop mutation greatly alters the capacity of $_{Mtb}$ Rho to unwind duplex C (E) or to trigger transcription termination (F). Data for WT $_{Mtb}$ Rho are shown for comparison.