

Supplemental Material

Characterization of mycobacterial PoID2 and PoID1 as RNA/DNA polymerases homologous to the POL domain of bacterial DNA ligase D

Hui Zhu, Hitesh Bhattarai, Han Yan, Stewart Shuman, and Michael S. Glickman

Molecular Biology and Immunology Programs, Sloan-Kettering Institute, New York, NY 10065

Supplemental Tables S1 and S2

Supplemental Figures S1, S2, and S3

Table S1

Prevalence of PoID1 and PoID2 in mycobacterial proteomes

Species	PoID1 (aa)	PoID2 (aa)
<i>Mycobacterium abscessus</i>	342	409
<i>Mycobacterium avium</i>	342	426
<i>Mycobacterium colombiense</i>	343	422
<i>Mycobacterium gilvum</i>	349	412
<i>Mycobacterium intracellulare</i>	343	422
<i>Mycobacterium kansasii</i>	345	416
<i>Mycobacterium marinum</i>	346	420
<i>Mycobacterium parascrofulaceum</i>	343	416
<i>Mycobacterium phlei</i>	349	411
<i>Mycobacterium rhodesiae</i>	349	411
<i>Mycobacterium smegmatis</i>	350	426
<i>Mycobacterium thermoresistibile</i>	344	410
<i>Mycobacterium tuberculosis</i>	346	397
<i>Mycobacterium tusciae</i>	349	428
<i>Mycobacterium ulcerans</i>	346	423
<i>Mycobacterium vanbaalenii</i>	349	412
<i>Mycobacterium xenopi</i>	358	413

Table S2

Mycobacterium smegmatis strains used in this study

Strain	Genotype	Source
mc ² 155 "wild-type"	<i>ept1</i>	Ref. 30
MGM 801	mc ² 155 <i>ligD</i> -(D136A-D138A)	Ref. 21
MGM 843	Δ <i>polD1</i>	This study
MGM 2027	mc ² 155 Δ <i>polD2</i> :: <i>loxP</i> - <i>hyg</i> - <i>loxP</i>	This study
MGM 2029	mc ² 155 Δ <i>polD2</i> :: <i>loxP</i>	This study
MGM 845	MGM801 Δ <i>polD1</i>	This study
MGM 2028	MGM801 Δ <i>polD2</i> :: <i>loxP</i> - <i>hyg</i> - <i>loxP</i>	This study
MGM 2030	MGM801 Δ <i>polD2</i> :: <i>loxP</i>	This study
MGM 846	MGM 2030 Δ <i>polD1</i>	This study

MtuPolD1	MAAAAEELDVDGIAVRLTSPDRMYFPKLG--SHGTKRRLVEYYFAVAGGPMLTALRDRPT	58
MsmPolD1	MASAAATELDVDGVKVRFTNPDKVYFPKLG--KNGTKGKLVVEYYLSVASGPMLALLRDRPV	59
MtuPolD2	MAAP-VSLDVHGRQVIVTHPGRVVFPANHNRKGYTKFDLVRYYLAVAEG-AMRGVAGRPM	61
MsmPolD2	MMAGALTLELDGLTVSVTNPKVIFPEYDGRAAVTKLDDLVNYYRAVADG-ALRGVFGSRPM	72
PaeLigD	ASAGASRAATAGVRISHPQRLIDPSIQ----ASKLELAEFHARYAD-LLLRDLRERPV	589
MtuPolD1	HLQRFDPDGVDGGEQIYQKRIPRHRPDYLQTCRVTFPSPGRMADALKVTHPAAIVWAAQMGTI	118
MsmPolD1	HLQRFDPDGIEGEEIYQKRVPQKHPDYLETVCVTFPSPGRTADALKITHPSSIIWAAQMGTV	119
MtuPolD2	ILKRFVKGISAEAVFQKRAPANRPDWVDVAELHYASGRSAAEAVIHDAAGLAWVINLGCV	121
MsmPolD2	ILKRFVKGIATEAIFQKRAPEKRPDFIEVAELKYRSGTSAKEAVLRNTAGLAWAVNLGCV	132
PaeLigD	SLVRGPDGIGGELFFQKHAARLKI PGIVQLDPALDPGHP-PLLQIRSAEALVGAVQMGSI	648
MtuPolD1	TLHPWQVRCPDTEHPDELRI DLPQPGTGfVEARTVAVDVLRVLDLGLVGYPKTSGGR	178
MsmPolD1	TLHPWQVRCPDTEHPDELRV DLPQPGTGfKEARTVACDVLKPLLDELGLVGYPKTSGGR	179
MtuPolD2	DLNPHPVLAGDLHPDELRV DLPMPGVAWQRVVEVAL-VVREVLEDYGLTAWPKTSGSR	180
MsmPolD2	DLNPHPVRADDLHPDELRV DLPMPGVEWQQILDVAM-VAREVLSDHGLTAWPKTSGSR	191
PaeLigD	EFHTWNASLANLERPDRFVL DLPDPALPWKRMLE-ATQLSLTLLDELGLRAFLKTSGGK	707
MtuPolD1	GIHVFLRIATDWDfVEVRRAGIALAREVERRAPDAVTTSWWKEERGARIFIDFNQNARDR	238
MsmPolD1	GVHVFLRIKQWDFIEVRRAGIALAREVERRAPDAVTTSWWKEERGERLFI DYNQNARDR	239
MtuPolD2	GFHVYARIAPCWSFPQVRLAAQTVAREVERRLPDAATSRWWKEEREG-VFVDFNQNAKDR	239
MsmPolD2	GFHIYARISRDWPYAKVRLAAQTVAREVERRAPDLATAHWWKEEREG-VFVDFNQNAKDR	250
PaeLigD	GMHLLVPLERRHGWDEVKDFAQAISQHLARLMPERFSAVSGPRNRVKGKIFVDYLRNSRGA	767
MtuPolD1	TMASAYSVRPTPIATVSMPLTWEELAGADP-DDYTMTTVPELVKI--RDPWPAGMDDVAQ	295
MsmPolD1	TFASAYSVRKTPPIATVSMPLSWDELNRNADP-DDYTMTNTVPDLLAG--RDPWADIDSVQQ	296
MtuPolD2	TVASAYSVRATPDARVSTPLHWEEVPGCDP-AVFTMATVPSRLAD--IGDPWAGMDDAVG	296
MsmPolD2	TVASAYSVRATPDARVSTPLRWDEVPTCRP-EEFTIATVPDRFAE--IGDPWEGMDTAVG	307
PaeLigD	STVAAYSVRAREGLPVSVPVFFREELDSLQGANQWNLRSPLPQRLDELAGDDPWADYAGTRQ	828

Figure S1. **Mycobacterial PoID1 and PoID2 are homologous to the LigD POL domain.** The amino acid sequences of MtuPolD1, MsmPolD1, MtuPolD2 and MsmPolD2 are aligned to the homologous segment of the POL domain of *Pseudomonas aeruginosa* (Pae) LigD. Gaps in the alignment are denoted by -. Positions of side chain identity/similarity in all five proteins are denoted by •. The active site aspartate that was mutated to alanine in MsmPolD1 and MsmPolD2 is indicated by | above the alignment. Counterpart of amino acids in bacterial LigD that contact manganese or ATP are highlighted in yellow; amino acids that contact DNA are highlighted in cyan.

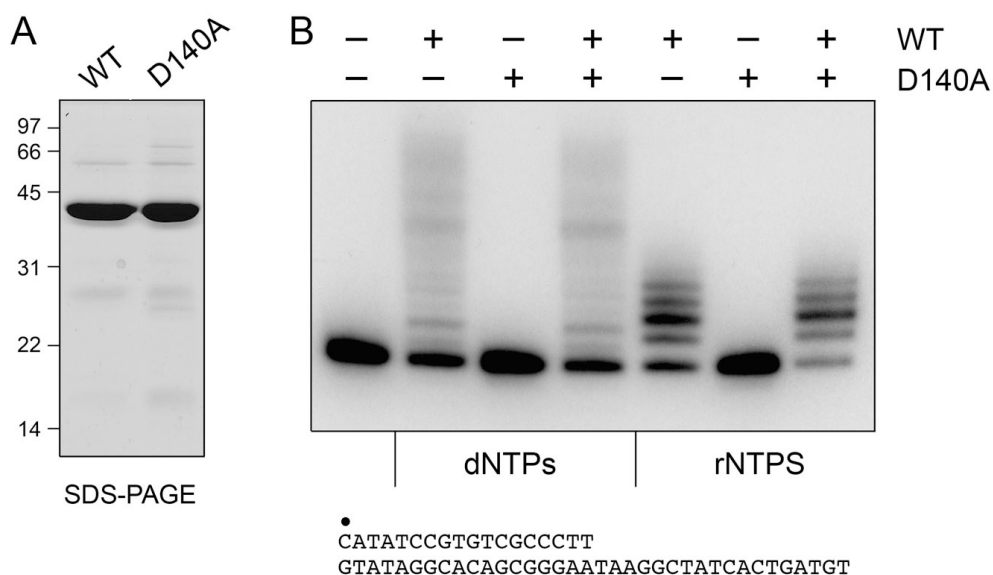


Figure S2. **Polymerase activity of recombinant MsmPolD1.** (A) Aliquots (5 μ g) of the phosphocellulose preparations of wild-type MsmPolD1 and mutant D140A were analyzed by SDS-PAGE. The Coomassie Blue-stained gel is shown. The positions and sizes (kDa) of marker polypeptides are indicated on the *left*. (B) Polymerase reaction mixtures (20 μ l) containing 50 mM Tris-HCl (pH 7.5), 1.25 mM CoCl_2 , 1 pmol of ^{32}P -labeled primer-template DNA substrate, 100 μM each of dATP, dGTP, dCTP and dTTP (dNTPs) or 100 μM each of ATP, GTP, CTP and UTP (rNTPs), and 400 ng of MsmPolD1 (WT or D140A, where indicated by +) were incubated for 10 min at 37°C. The products were resolved by PAGE and visualized by autoradiography.

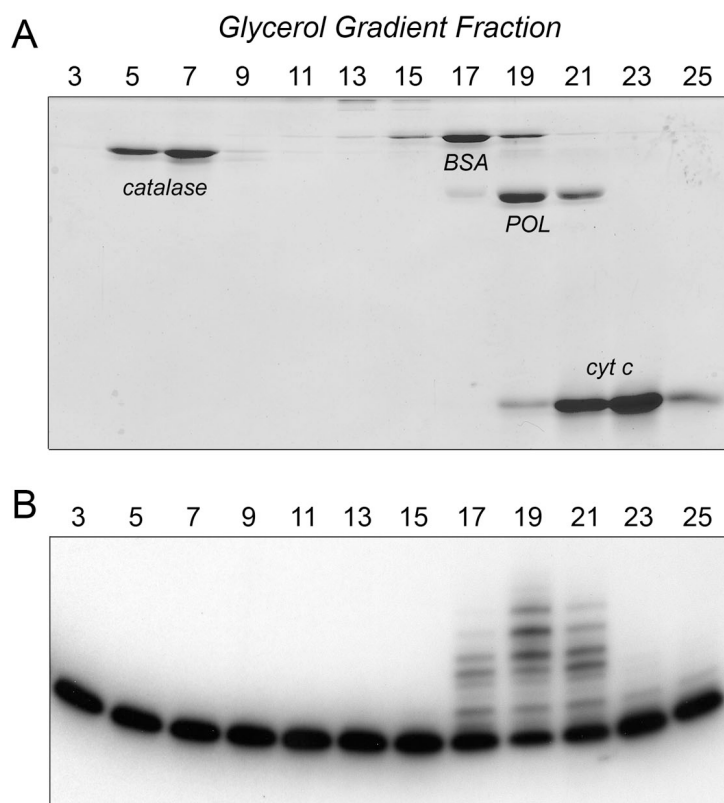


Figure S3. **MsmPold1 sediments as a monomer.** Glycerol gradient sedimentation of a mixture of MsmPold1, catalase, BSA and cytochrome c was performed as described under Experimental Procedures. (A) Aliquots (15 μ l) of the odd-numbered gradient fractions were analyzed by SDS-PAGE. The Coomassie Blue-stained gel is shown. (B) Polymerase reaction mixtures (20 μ l) containing 50 mM Tris-HCl (7.5), 1.25 mM CoCl_2 , 1 pmol of ^{32}P -labeled primer-template DNA substrate, 100 μ M each of dATP, dGTP, dCTP and dTTP, and 2 μ l of the odd-numbered gradient fractions were incubated for 10 min at 37°C. The reaction products were resolved by PAGE and visualized by autoradiography.