

Supplementary Figures

Parasitology

Analyses of the essential C82 subunit uncovered some differences in RNA polymerase III transcription between *Trypanosoma brucei* and *Leishmania major*

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Fig. S1. C82 sequence comparisons among eukaryotes. (A) Percent identities among amino acid sequences of C82. (B) Sequence alignment of the N-terminal region of C82. The trypanosomatid-specific loop is shown in bold letters. The sequences analyzed were: *T. brucei* (Tb, Tb927.2.2990), *L. major* (Lm, LmjF.27.2600), *Schizosaccharomyces pombe* (Sp, SPAPB1E7.03), *Saccharomyces cerevisiae* (Sc, NP_015516), *Homo sapiens* (Hs, NP_006459.3), *Mus musculus* (Mm, NP_083201), *Rattus norvegicus* (Rn, XP_008759532), *Arabidopsis thaliana* (At, NP_566914), *Caenorhabditis elegans* (Ce, NP_491807), *Plasmodium falciparum* (Pf, ETW47020.1), *Toxoplasma gondii* (Tg, XP_002366133), and *Naegleria fowleri* (Nf, KAF0973179).

Fig. S2. Sequence comparison of C82 in trypanosomatids. (A) C82 protein sequence alignment. (B) Percent identities among amino acid sequences of C82. The species analyzed were: *Leishmania braziliensis* (Lbr, LbrM.27.2720), *Leishmania tarentolae* (Lta, LtaP27.2650), *Leishmania mexicana* (Lmx, LmxM.27.2600), *Leishmania major* (Lmj, LmjF.27.2600), *Leishmania donovani* (Ld, LdCL_270033100), *Crithidia fasciculata* (Cfa, CFAC1_210027500), *Leptomonas seymouri* (Lse, Lsey_0405_0050), *Trypanosoma cruzi* (Tcr, TcCLB.509127.40), *Trypanosoma rangeli* (Tra, TRSC58_03156), *Trypanosoma brucei* (Tbr, Tb927.2.2990), and *Trypanosoma vivax* (Tvi, TvY486_0200650).

Fig. S3. Two isoforms of C82 are present in the *Leishmania* species. (A) Sequence comparison between C82 (LmjF.27.2600) and C82-short (LmjF. 02.0680) in *L. major*. The peptide specific to C82-short is indicated with the black bar at the boundaries of the eWH3 and eWH4 domains. (B) Sequence alignment of C82-short. (C) Percent identities among amino acid sequences of C82-short. The species analyzed were: *L. major* (Lmj, LmjF.02.0680), *L. aethiopica* (Lae, LAEL147_000034900), *L. arabica* (Lar, LARLEM1108_020011500), *L. braziliensis* (Lbr, LbrM.02.0660), *L. donovani* (Ldo, LdBPK_020650.1), *L. gerbilli* (Lgr, LGELEM452_020011500), *L. infantum* (Lin, LINF_020012100), *L. mexicana* (Lmx, LmxM.02.0680), *L. tropica* (LtrLTRL590_020012900), and *L. turanica* (Ltu, LTULEM423_020011100).

Fig. S1

A

	Tb 563 aa	Lm 604 aa	Sp 591 aa	Sc 654 aa	Hs 534 aa	Mm 533 aa	Rn 533 aa	At 523 aa	Ce 544 aa	Pf 915 aa	Tg 823 aa	Nf 611 aa
Tb	100	32.78	13.97	12.25	13.64	14.25	14.53	16.32	12.43	8.80	18.45	16.83
Lm		100	13.62	13.32	16.05	16.09	16.09	15.34	16.62	11.22	21.04	17.59
Sp			100	25.78	22.57	22.57	22.36	17.73	20.08	15.26	19.85	22.20
Sc				100	19.25	18.45	18.87	16.05	16.19	15.74	15.50	18.54
Hs					100	94.00	94.00	21.14	26.04	15.25	18.34	24.53
Mm						100	97.37	20.55	26.29	15.29	18.16	24.17
Rn							100	20.76	26.29	15.29	18.38	24.79
At								100	19.44	18.28	17.56	24.15
Ce									100	16.30	14.44	21.41
Pf										100	18.03	17.41
Tg											100	19.53
Nf												100

B

Trypanosomatid-specific loop

Tb	MS-RRAEAL-----GALNPQQQQI I HDEHPRMIGVQNELLETSVSEQLSPLAGFLVRTLNRCGP-LTLLKALAEAVRQE Q EPFGNPLRSSKG G GGASVGGSVN--EAVKQ	99
Lm	MP-FFCVGD-----AATCPSEVCEELAAKPTTAHHQLIISTVT H QLGPLAGAI C RTLIQSGP-MSLKDISDAVHRDEEVRAAAAANRASATGEKTAVPPTHQ	98
Sp	-----MSQYAVELCEILVEEFFGDCCSAVASALLRHGR-LTIPMLQKR-----	42
Sc	M-DELLGEALS A ENQTGESTVESEKLVTPEDVMTISSLEQRTLNPDLFLYKELVKAHLGERAASVIGMLVALGR-LSVRELVEK-----	82
Hs	-----MTQAEIKLCSLLLQEHFGEIVEKIGVHLIRTGS-QPLRVIAHD-----	42
Mm	-----MTQABIKLCSLLLQEHFGEIVEKIGVHLIRTGS-QPLRVIAHD-----	42
Rn	-----MTQABIKLCSLLLQEHFGEIVEKIGVHLIRTGS-QPLRVIAHD-----	42
At	MSMSEFGIVYAIHIIITVQFGSVVKVCECLLRKG P -LSSRDISRLAESD-----	49
Ce	MGGKYETQMCVIIIEDIFGKIVAKV M E T ILKESQLSTLFYKLK--GQI-----	48
Pf	MKNELDYLYKIICDIFGSCCS D IVEIILYGNKLSIYELINL--SSY-----	46
Tg	MFRYEVOQFACLLLED A FGPVVSTCARLLLHG S -LTILELRD L FLAVPSASGVFPPLSRC S RNAAAFA--SGCGRPSTHA-----	77
Nf	MN--KRTL A LEI I TDQFGDVIGKVGGVLMQFGP-QTMAEL-----IQA-----	40

Tb	VSSPIPL---PVTAPS D IVTDAIKEIMTRLLLHRRVQH-----	135
Lm	VGSSFHVNLGDIASVN D VIHEVAVKELVTRLVHVR L HA-----	137
Sp	-----TSLPGPKIRQALVSLM Q H M VLYTV V ENVR-EVT-----	76
Sc	-----IDGMDVDSVK T TLVSLTQLRCV K Y L QETAI-----	115
Hs	-----TGTSLDQVKKALCVL V QHN L VSYQV-HKRGV V VEYAQCS-----	80
Mm	-----TKASLDQVKKALCVL V HH N L V YHV-HKRGV V VEYAQCS-----	80
Rn	-----TKASLDQVKKALCVL V HH N L V YHV-HKRGV V VEYAQCS-----	80
At	-----NHNKVDI L Y L IQHNCVQAFS-----IEPP-----	75
Ce	-----SNTLIRKS V STL N GFVTF-----HLDTN-----	73
Pf	-----EFNVVRNV V LLCLLIHNIVDV N IYSKSIECNHLSPE-----	82
Tg	-----GQDVYPLVRNALLVLLQHN L L V TPIDGQ G APSLRAAPQ-----	116
Nf	-----TSMQFPHIRNALLVLIQHN M C I YDKNIYLQ-KLE-----	74

B

Fig. S3

A

		Trypanosomatid-specific loop	
LmjF.27.2600	MPFFCVGDAATCPSEVCEELAAKPIAHQLIISTVTHQPLAGAICRTLISQSGPMSLKDISDAVHRDEEVRAVAAAANRASATGEKTTAVPPTHQVG	100	
LmjF.02.0680	-----	0	
	eWH1		
LmjF.27.2600	SSFHVNLGDIASVNDVIHEVAKELVTRLVVHRLIHADPATHLYEIRYGSAILLRVLFPLLLHCA	199	
LmjF.02.0680	ROQYGE-AAKCIVLLVYOLGVVPPRAAVQVALSRTMGAIIMVLRERVRASEEYIRRLLECTAHSACTEAVKNRLMEVARA-----	44	
	eWH2		
LmjF.27.2600	PSITRDAIEYAVVRMVEDGLVPVLNLPTT-APGAATAANAARLADLRFSHGSN-ADL-----WNATIPYSVGLEAALHYLFNDAIEQAVAERYADGRL	292	
LmjF.02.0680	--HCRETTRMRITWDALRQVTEGTDIAASEELHCLIN--PRRPTGRSPASPFFPCSTRMHARRRRSPDACTSSLSKCHQKVERMDTQAVAERYADGRL	140	
	Insertion loop		
LmjF.27.2600	ALTEVRALRRRDPNSGYTEAVASFQELAELPMTAGVRRDRSGELRMGTDNVPSSSVEAVQRCLQRLCOPIVFYSCDAAAPMTSAAGISPFTTMPSTR	392	
LmjF.02.0680	GLTEVRALRRRDPASGYTE-----VRRDRSGEPRMGTDNVPSSSVEAVQRCLQRLCOPIVFYRCDAAPMSAAGISPFTTMPSTR	224	
	eWH3		
LmjF.27.2600	ALLVKPHSQSNFYAIDHVTAVHLLQEAVCERVVYSRYGVLGVRIMKLLLQHHFLEERTLAEQSIATYVKREVLHQMFKDGFLLQQEVPRTSALVERSAK	492	
LmjF.02.0680	ALLVKPHSQSNFYAIDHVTAVHLLQEAVCERVVHSRYGVLGVRIMKLLLQHHFLEERTLAEQSIATYVKREVLHQMFKDGFLLQQEVPRTSALVERSAK	324	
	eWH4		
LmjF.27.2600	ASVYLWGGLWSATLLPVVRERLAKTLTIWAKLREAQQQASAVAKHAAGGRPTPAAGSSINSSAAHEEIEAQWKYGMSRNIIQQALQAQRAVTGLQSCVM	592	
LmjF.02.0680	ASVYLWGGLWSATLLPVVRERLAKTLTIWAKLREAQQQASAVAKHAAGGRPTPAAGSSINSSAAHEEIEAQWKYGMSRNIIQQALQAQRAVTGLQSCVM	424	
	Cleft loop		
LmjF.27.2600	SLMRLLLIVDFF-----	604	
LmjF.02.0680	SLMRLLLIVDFFFKGVRRTVERQTSVGVVGVASLSPYRCFGCHARQGDGAVRPARRPSPLFSPSCAPARVVRGA	501	
	C. coil		
LmjF.27.2600	-----		
LmjF.02.0680	-----		

B

	eWH2	Insertion loop	
Lmj	MGAIIMVLRERVRASEEYIRRLEGAHTSACLEAVLNRLMEVARAHCRETRMRTWDALRQVWEGETDIAASEELHCGLINPERRTRGRSPASPPPCS-TRM		99
Lae	-----		0
Lar	-----MTRMRTWDALRQVWEGMEDIAASEELHCGLINPERRTRGRSSASPPRSTCGM		52
Lbr	-----	-MNR-	3
Ldo	-----		0
Lgr	-----		0
Lin	-----		0
Lmx	-----		0
Ltr	MGPLIMVLREGVRVPVEYIRGLEGAHSACLEAVLNRLMEVARARCRETKPMRTWDALRRVWEGMEDIAASEELHCGLINPERRTRGRSSASPPRSTCGM		100
Ltu	MDPIIMVLRERVRVSEEEYIRRLEGAHSACLEAVLNRLMEVARARCRETRMRTWDALRQVWEGMEDIAASEELHCGLINPQRRTGRSSASPPRSTCGM		100
	eWH3		
Lmj	HARRRRRSDTACTSSLSKCHQKVERMDTPQAAERYADGRL-----GLTFVRALRRRIAP-ASGYTEAVRDRSGEPRMGTDNVFSSSVEAV		185
Lae	MHRRRPRPS---AVQTDFG-----PLTLVRALRRCIAP-SSGYTEAVRDRSGEELRMGTDNVFSSSVEAV		60
Lar	HARRRRRADTACTSSLSKCHQNVERMDTPQAAERYADGRL-----ALTLVRALRRCIAP-DSGYTEAVRDRSGEPRTGTDNVFSSSAEAV		138
Lbr	-----SADQHVAAKYPDKRRVRTSPHPSQGREIKTHMRRRLRGREGGDTGQVADGECGGEPRVGTDSVSSGAEAV		77
Ldo	-----	-MGTDNVFSSSVEAV	14
Lgr	-----		0
Lin	-----		14
Lmx	-----		14
Ltr	HARRRRRAGTACTSSLSKCHRKVERMDAPQAAERYADGRL-----ALTLVRALRRRVAP-NSGYTEAVRDRSGEELRMGTDNVFSSVVAV		186
Ltu	HARRRRRADTACTSSLSKCHQKVERMDTPQAAERYADGRL-----ALTLVRALRRRVAP-DSGYTEAVRDRSGEPRMGTDNVFSSSVEAV		186
	eWH4		
Lmj	QRCLQRLCQPIV-FYRCDAAAPMSSAACAGISPSPFTTMSSTRALLVKPHQSNSFYAIDHVTAVHLLQEAVCERVVHSRYGVLGVRIMKLLLQHHFLEERTLA		284
Lae	QRCLQRLCQPIV-FYSCDAAAPMTSAAGISPFTTMSSTRALLVKPHQSNSFYAIDHVTAVHLLQEAVCERVVYSRYGVLGVRIMKLLLQHHFLEERTLA		159
Lar	QRCLQRLCQSVI-FYSCDAAAPMTSAAGISPFTTMSSTRALLVKPHQSNSFYAIDHVTAVHLLQEAVCERVVYSRYGVLGVRIMKLLLQHHFLEERTLA		237
Lbr	RRCLERLCQPVALFAGCNAAAPMTAAGISPFTTMSSTRALLVKPHQSNSFYAIDHVTAVHLLQEAVCERVVYSRYGVLGVRIMKLLLQHHFLEERTLA		177
Ldo	QRCLQRLCQPIV-FYSCDTAAPMTSAAGISPFTTMSSTRALLVKPHQSNSFYAIDHVTAVHLLQEAVCERVVYSRYGVLGVRIMKLLLQHHFLEERTLA		113
Lgr	-----	-MKLLLQHHFLEERTLA	16
Lin	QRCLQRLCQPIV-FYSCDAAAPMTSAAGISPFTTMSSTRALLVKPHQSNSFYAIDHVTAVHLLQEAVCERVVYSRYGVLGVRIMKLLLQHHFLEERTLA		113
Lmx	---QPLCQTVV-FYSCDAAAPMTSAAGISPFTTMSSTRALLVKPHQSNSFYAIDHVTAVHLLQEAVCERVVYSRYGVLGVCIMKLLLQHHFLEERTLA		109
Ltr	QRCLQRLCQPIV-FV-----		200
Ltu	QRCLQRLCQPIV-FYSCDPAAPMTSAAGISPFTTMSSTRALLVKPHQSNSFYAIDHVTAVHLLQEAVCERVVYSRYGVLGVRIMKLLLQHHFLGERTLA		285
	Cleft loop	C. coil	
Lmj	EQSIATYVKVREVLHQMFKDGFLLQQEVPRTSALVERSAKASVYLWGLSWSATLLPVVRERLAKTLTIAWAKLREAQQQASAVAKHAAGRPTPAAGSSI		384
Lae	EQSIATYVKVREVLHQMFKDGFLLQQEVPRTSALVERSAKASVYLWGLSWSATLLPVVRERLAKLTIAWAKLREAQQQASAVAKPAAGVRPTPAAGSSI		259
Lar	EQSIATYVEVREVLHQMFKDGFLLQQEVPRTSALVERSAKASVYLWGLSWSATLLPVVRERLAKLTIAWAKLREAQQQASAVAKPAAGVRPTPAAGSSI		337
Lbr	EQSVATYVKAREVLHQMFKDGFLQQEVPRTSALVERSAKASVYLWGLSWSATLLPAVVRERLAKLTIAWAKLREAQQQASAVAKPAACVRSSPAASGGI		277
Ldo	EQSIATYVKVREVLHQMFKDGFLLQQEVPRTSALVERSAKASVYLWGLSWSATLLPAVVRERLAKLTIAWAKLREAQQQH-----		192
Lgr	EQSIATYVKVREVLHQMFKDGFLLQQEVPRTSALVERSAKASVYLWGLSWSATLLPVVRERLAKALTIAWAKLREAQQQASAVAKPAAGVRPTPAAGSSI		116
Lin	EQSIATYVKVREVLHQMFKDGFLLQQEVPRTSALVERSAKASVYLWGLSWSATLLPVVRERLAKLTIAWAKLREAQQH-----		192
Lmx	EQSVATYVKVREVLHQMFKDGFLLQQEVPRTSALVERPAKASVYLWGGG-----		158
Ltr	-----		200
Ltu	EQSIATYVKAREVLHQMFKDGFLLQQEVPRTSALVERSAKASVYLWGLSWSATLLPVVRERLAKLTIAWAKLREAQLQASAVAKPAAGVRPTPAAGSSI		385
	C. coil		
Lmj	NSSAAHAAEEIEAQWKYGMSRNIIQQALQAQRAVTGLQSCVMSLMRLLLIVDFF	FFKGVRRTVERQTSVGVVGVASLSLSPYRCFGCHARQGDGAVRPARRP	484
Lae	NSSAAHAAEEIEAQWKYGMSRNIIQQALQAQRAVTGLQSCVMSLMRLLLIVDFF		311
Lar	NSSAAHAAEEIEAQWKYGMSRNIIQQALQAQRAVTGLQSCVMSLMRLLLIVDFF		389
Lbr	KSSAAHAAEEVEAQWKSGMSRSIQQALQTQRTITGLQSCVMSLMRLLLIVDFF		329
Ldo	-----		192
Lgr	NSSAAHAAEEIEAQWKYGISRNIIQQALQAQRAVTGLQSCVMSLMRLLLIVDFF		168
Lin	-----		192
Lmx	-----		158
Ltr	-----		200
Ltu	NSSAAHAAEEIEAQWKYGMSRNIPQALQAQRAVTGLQSCVMSLMRLLLIVVFF		437
Lmj	SPLFSPSCAPARVVRGA	501	
Lae	-----	311	
Lar	-----	389	
Lbr	-----	329	
Ldo	-----	192	
Lgr	-----	168	
Lin	-----	192	
Lmx	-----	158	
Ltr	-----	200	
Ltu	-----	437	

C