

Supplemental Materials

Molecular Biology of the Cell

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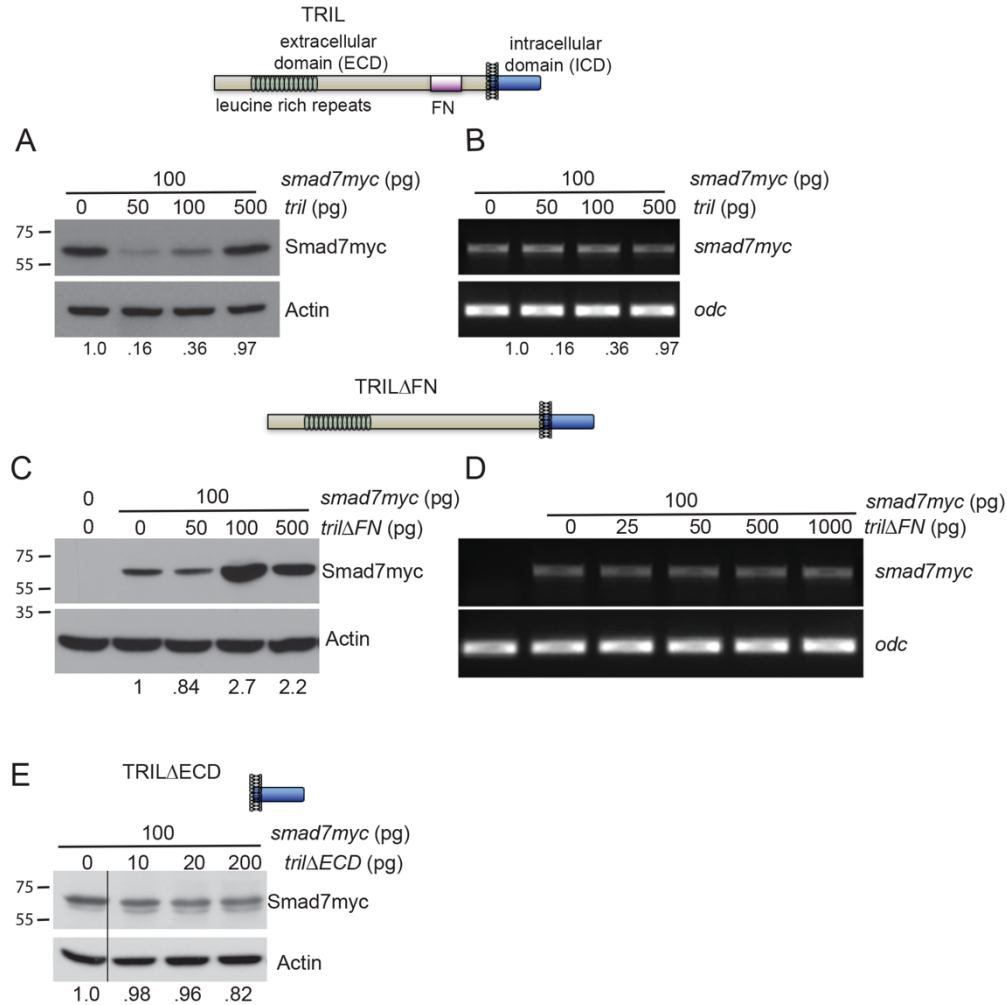


Figure S1. Ectopic Tril and Tril Δ FN induce changes in steady state levels of Smad7myc protein but not RNA. (A-E) *smad7myc* RNA was injected into two-cell embryos alone or together with RNA encoding Tril (A-B) Tril Δ FN (C-D) or Tril Δ ECD (E). (A, C, E) Immunoblots of lysates from stage 11 embryos (15 per group) were probed with anti-Myc antibodies, and then reprobbed for β -Actin. The relative level of Smad7myc, normalized to actin and reported relative to that in embryos injected with Smad7myc alone is indicated below each lane. In panel E, all lanes are from the same immunoblot, aligned following removal of an intervening lane (following the second lane, marked by a black bar). (B, D) The steady state level of *smad7myc* RNA was analyzed by semi-quantitative RT-PCR at stage 11.

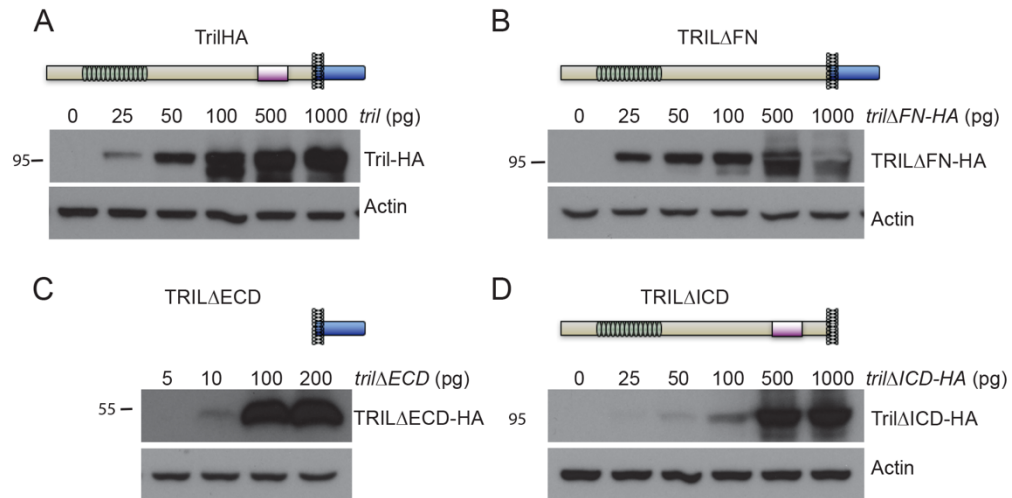


Figure S2. Dose dependent increase in Tril protein in embryos injected with increasing doses of *tril* RNA. Two-cell *Xenopus* embryos were injected with increasing doses of RNA encoding HA-epitope tagged wild type or deletion mutant forms of Tril, as indicated at the top of each gel. Immunoblots of lysates from stage 11 embryos were probed with anti-HA antibodies, and then reprobed for β -Actin. Immunoblots shown in panels A-C are from sibling embryos that were injected and analyzed in parallel and blots were exposed for an identical amount of time. The immunoblot in panel D is from embryos injected and analyzed on a different date.

Hu MEAARALRLLLVVCGCLALPPLAEPVCPERCDCQHPOHLLCTNRGLRVVPKTS^{SLPS}PHDVL^{TYS}LG^{GNF}
Xl MAKAAFIDLL-VLLAC-TLAWVVEPKCLEPCDCQHLOHILCSNRGLLSVPKSSQILSASGTKTYS^{LG}GNF

Hu ITNITAFDFHRLGQLRRDLQYNQIRSLHPKTFEKL^{SRLEELY}LG^{NNLL}QALAPGTLAPLRKLRILYANG
Xl ISNISVLDVVFHFPQLQRDLQYNQIRSIHLKAFEKLP^{ELEELY}LG^{NNLL}TTLAPGALAPLRKLRKVLNVNG

Hu NEISRLSRGSFEGLES^{LVKLR}LDGNALGALPDAVFAPLGNLLYLHLESNRIRFLGKNAFAQ^{LGKLR}FNL
Xl NRLHNISRASF^{SNLAALIKLR}LDGNDIQNLQGS^{PFSALS}NLLYLHLENNKITNISKNVFTGLGKLRLLSL

Hu SANELQPSLRHAATFAPLRSLSSLILSANNLQHLGPRIFQHL^{PRLGLLSLRGNQL}THLAPEAFWGLEALR
Xl SGNP-QSFLRQP-TFLPLRSLSTLTMAGNQLQQLG^{PSMFNGLQRLSRLI}ILSSNQISAIQTKTFLGLDLLQ

Hu ELRLEGNRLSQLPTALLEPLHSLEALDLSGNELSALHPATFGHL^{GRLRELSLRNNALS}ALSGDIFAASPA
Xl ELHLDGNKLVQLPEGVLVPLHNLEVLNLSRNAISHLHP^{EMFKGLMRLKVL}DLQHNMLRYLSGQTFAGNPV

Hu LYRLDLGNGWTCDCRRLRGLKRW-MGDWHSQGRLLTVFVQCRHP^{ALRGKYLDYLD}DDQ-QLQNGSCADPS
Xl LYRLQLDGNRWNCDC^{HLDLKHWILGTL}HPRSRMLTVFVQ^{CEPQKVAGKYLDY}LEDAYLLGVGGCQVST

Hu PSASLTADR-----RRQPLPTAAGEEMTPPAGLAEELPPQP^{QLQQQGR}FLAGVA-----
Xl TPAGQEQIKNSTLRDKHIGIHQPGKGD^{RDLKNG-ADILRAQPKTEEKSLHL}PTLPSEVSPALETLALRQQ

Hu -----WDGAA-RELVGNRSALRLSRRGPG-----LQQ^{SPS}-----VAAAAGPA
Xl ALVTKWPSSTNRDSTAKNRGLETSR^{KGKGSVKNAAEHSRKLHLLSQ}PVHPTQSKVKQMSVLI^{PASS}NLP

Hu PQSLDLHK-KPQRGRPTRADPALAEP^{TPT-----ASPGSAPSPAGDPWQRATKHRL}-----GTE
Xl PHSESLHSEKPSQLDPPSVV^{PYTDDLK SAYATLQHNDTTDKPIHHDKTLHQSPSDTLLPNYNSFQQAEGD}

Hu HQERAAQS-DGGAGLPPLVSDPCDFNK^{FILCNLTVEAVGADSASVRWAVREHRS}PRPLGGARFRL^{LDFRE}
Xl PMHPAPETLHQVAPF^{PSLLSDPCEFNKLYLVNLSVESVGSSTARVRW---}QTI^{SVHTQGPVLF}RVLYERF

Hu GQQPKFHRFVYLP^{ESSDSATLREL}RGDTPYLVCVEGVLGGRVCPVAPRDHCAGLVTLP^{EAGSRGGVDYQL}
Xl GQTGRFQRFVYPRGRVESL^{TLOELTGKTPYLVCVESII}GGRACPVAPRDHCIGIVTLP^{SEDDRPLLNYQV}

Hu ITLALLTVNALIVLLALAAWASRWLRRKLRARRKGGAPVHVRHMYSTRRPLRSMGTGVSADFSGFQSHRP
Xl LALSLLAVNALLLLLGLVAWGSRLAHRK-WGRRR--PPVHVRQMYSTRRPFYRSVGTGVSTDFSGFQSHRP

Hu RTTVCALSEADLIEFP-CDRF^{MDSAGGGAGGSLRREDRLLQRFAD}
Xl RTTVCALGEADLIEFP^{CDRFRE-----GGNIHRED-LLQRFAD}

Figure S3. Sequence alignment of Human and *Xenopus* Tril. Human (*Hu*) and *Xenopus laevis* (*Xl*) share 45% amino acid identity overlap with the greatest homology (77% identify) in the intracellular domain. Sequence encoding the predicted transmembrane domain is highlighted in red, potential di-leucine like motifs are underlined.