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Mutations in the NXF-1:NXT-1 mRNA export complex affect gene-expression driven by the *hsp-16.41* promoter

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Abstract

The NXF-1:NXT-1 heterodimer is essential for the nuclear export of mRNA. Here we describe three new alleles of *nxf-1* and one allele of *nxt-1* isolated from a forward genetic screen. These mutations cause no apparent phenotype under normal growth conditions, but partially suppress the lethality caused by heat-shock induced expression of the PEEL-1 toxin from *Phsp-16.41::peel-1*. There is also decreased expression of *Phsp-16.41::eGFP* in an *nxf-1* mutant. We propose that NXF-1:NXT-1 influences the expression of heat-shock activated genes due to a role in the recruitment of the *hsp-16.41* promoter to the nuclear pore complex during heat-shock.

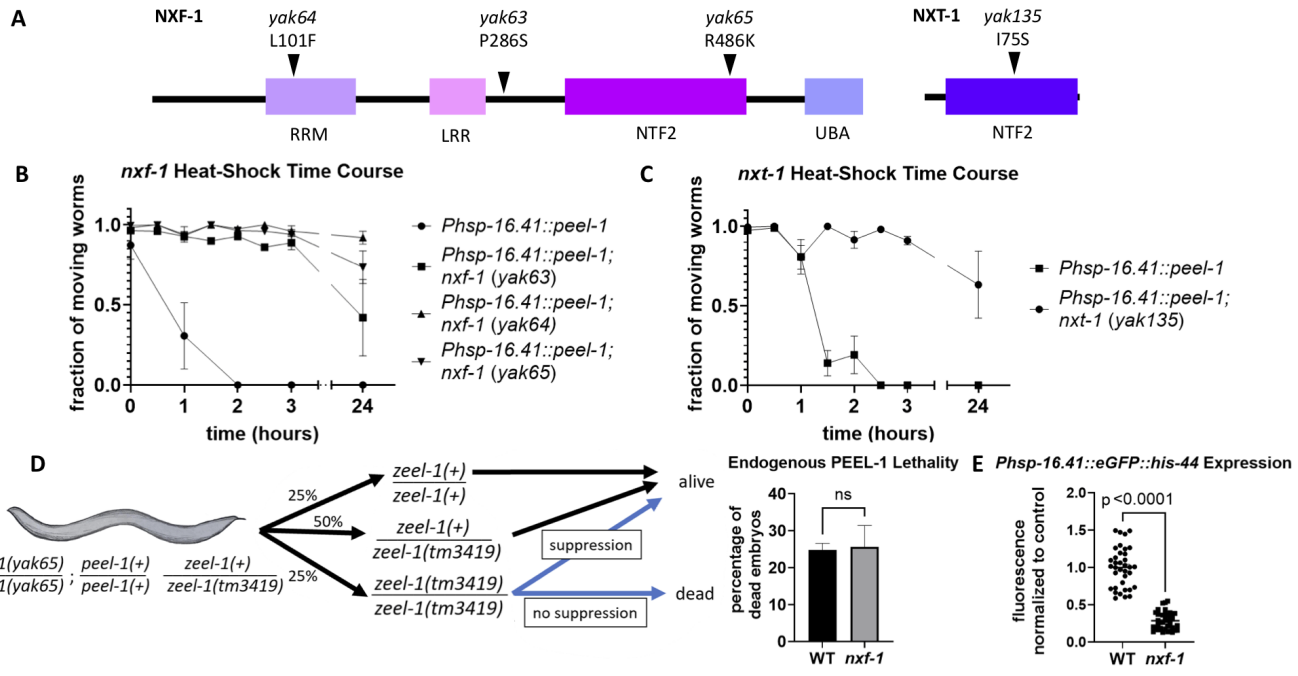

Figure 1. Missense mutations in *nxf-1* and *nxt-1* partially suppress *Phsp-16.41::peel-1* and *Phsp-16.41::eGFP::his-44* but not endogenous *peel-1*:

(A) Schematic of NXF-1 and NXT-1 indicating functional domains (see text for details) and the mutations that were identified in our screen. (B-C) Quantification of *Phsp-16.41::peel-1* lethality as the fraction of moving (living) worms at different times after a 2 hr heat shock at 34°C; n=3-5, each replicate represents 50 animals; error bars represent standard error of the mean. (D) Schematic of expected outcomes and quantification of dead progeny from animals heterozygous for the *zeel-1(tm3419)* deletion and homozygous for *nxf-1(yak65)* with endogenously expressed *peel-1*. WT control shows quantification of dead progeny from animals heterozygous for *zeel-1(tm3419)*. n=7-9, each n represents 12-127 animals; ns – not significant (p>0.05); error bars represent standard deviation. (E) Relative fluorescence of *Phsp-16.41::GFP* in an *nxf-1(yak65)* mutant normalized to control; n=3 replicates, each replicate group represents 8-17 animals; bars represent means. Statistics: unpaired two-tailed t tests.

Description

PEEL-1 is a toxin found in the sperm of *C. elegans* which is lethal to embryos lacking the antidote ZEEL-1, and PEEL-1 is also toxic to adult worms when expressed ectopically (Seidel et al., 2008, 2011). The mechanism of this lethality is not yet known. To better understand how PEEL-1 kills, we performed a genetic screen for suppressors of the lethality caused by *peel-1* expression driven by the heat-shock promoter *hsp-16.41*. Among the mutants found in this screen were four recessive partial suppressors. Three mutants (*yak63*, *yak64*, and *yak65*) were mapped to chromosome V, and one to chromosome I (*yak135*). By whole-genome sequencing we found that *yak63*, *yak64*, and *yak65* are missense mutations in *nxf-1* and that *yak135* is a missense mutation in *nxt-1* (Figure 1A).

NXF-1 and NXT-1 are nuclear export receptors which form a heterodimer essential for the nuclear export of mRNA (Katahira, 2015). NXF-1 contains four domains characterized in human, Drosophila, and yeast orthologs, whose functions are likely conserved in *C. elegans*: an RNA recognition motif (RRM) and a leucine rich repeat (LRR) region which interact with RNA and export adaptors (Katahira, 2015; Katahira et al., 2009; Viphakone et al., 2012); a nuclear transport factor 2 (NTF2)-like domain which interacts with the NTF2-like domain of NXT-1 to heterodimerize (Suyama et al., 2000); and a ubiquitin-associated (UBA)-like fold which acts with the NTF2-like domain to interact with the phenylalanine-glycine repeats found in nucleoporins (Fribourg et al., 2001) (Figure 1A). These domains work in concert to form interactions between NXF-1:NXT-1, mRNA, nucleoporins, and components of the TRanscription-EXport complexes TREX and TREX-2 (Fribourg et al., 2001; Katahira et al., 2009; Viphakone et al., 2012; Wickramasinghe et al., 2010). These interactions link mRNA transcription and processing to nuclear export.

Three of the four alleles from our screen map to known functional domains within the NXF-1:NXT-1 complex. *nxf-1(yak64)* causes an L101F substitution within the RRM of NXF-1, *nxf-1(yak65)* causes an R486K substitution within the NTF2-like domain of NXF-1, and *nxt-1(yak135)* causes an I75S substitution in NXT-1's only functional domain, the NTF2-like domain (Figure 1A). *nxf-1(yak63)* maps outside a known functional domain and causes a P286S substitution in the region between the LRR and NTF2-like domains of NXF-1.

We quantified the partial-suppression of the *nxf-1* and *nxt-1* mutants by performing a heat-shock time-course experiment. Expression of *Phsp-16.41::peel-1* was induced by heat shock, and death was measured by response to touch. All wild-type control animals died within the first three hours, while more than 80% of *nxf-1* and *nxt-1* mutant animals remained alive (Figure 1B,C). At 24 hours after heat shock, survival ranged from 42-92%. Notably, *nxf-1(yak63)* conferred the least resistance to *Phsp-16.41::peel-1*, and this allele is the only one of the four found outside of any known functional domain.

We then asked whether these partial suppressors could also suppress endogenously expressed *peel-1*. *nxf-1(yak65)* was chosen due to its relatively strong suppression of *Phsp-16.41::peel-1* lethality. *nxf-1(yak65)* animals heterozygous for a deletion of the PEEL-1 antidote *zeel-1(tm3419)* were generated and allowed to self-reproduce. For controls, we generated *zeel-1(tm3419)*/+ animals and selfed them. Mendelian independent assortment predicts that 25% of the progeny would be *zeel-1(tm3419)* homozygotes and would succumb to endogenous PEEL-1 toxicity unless suppressed by *nxf-1(yak65)*. Animals with at least one copy of the antidote *zeel-1(+)* would remain unaffected by PEEL-1 (Seidel et al., 2008). We found that about 25% of the progeny of *zeel-1(tm3419)*/+; *nxf-1(yak65)* animals succumbed to endogenous *peel-1*, matching controls (Figure 1D). Thus, although *nxf-1(yak65)* suppresses *peel-1* lethality when the toxin is expressed ectopically under the heat-shock promoter *hsp-16.41*, *nxf-1(yak65)* does not suppress endogenously expressed *peel-1*. We also generated *nxf-1(yak65)* mutants carrying *Pexp-3::peel-1* which expresses PEEL-1 in the egg-laying muscles (Seidel et al., 2011). Eight out of nine control *Pexp-3::peel-1* animals in a wild-type background had an observable egg-laying defect as expected for egg-laying muscle death caused by PEEL-1. Similarly, seven of nine *nxf-1(yak65); Pexp-3::peel-1* animals also had egg-laying defects, indicating that *nxf-1(yak65)* does not suppress PEEL-1 when expressed in egg-laying muscles. These results suggest that the suppression of *Phsp-16.41::peel-1* by mutations in *nxf-1* and *nxt-1* is due to an effect on the heat-shock-induced expression of *peel-1* rather than suppression of PEEL-1 toxicity per se.

To confirm that *nxf-1(yak65)* affects the expression of heat-shock induced genes, we generated *nxf-1(yak65)* animals expressing GFP under the *hsp-16.41* promoter. The average fluorescence of these worms upon heat-shock was reduced by 72% compared to worms with wild-type *nxf-1* (Figure 1E), further supporting the idea that *nxf-1* affects expression from the *hsp-16.41* promoter.

Because NXF-1:NXT-1 appears to specifically affect expression of genes driven by the *hsp-16.41* promoter, the NXF-1:NXT-1 complex appears to be acting at the level of the DNA in contrast to its more widely described role in exporting mRNA. A plausible mechanism is that NXF-1:NXT-1 may be involved in recruiting the *hsp-16.41* promoter to nuclear pores for efficient transcription and export of mRNA driven by this promoter. In *C. elegans*, localization of the bidirectional heat-shock promoter *hsp-16.2/41* at the nuclear pore complex was shown to occur during heat-shock (Rohner et al., 2013). This localization depended on a component of the TREX-2 complex, which was proposed to act as a tether between the *hsp-16.2/41* promoter and the nuclear pore complex (Rohner et al., 2013). Interestingly, the human TREX-2 component GANP directly interacts

with NXF1 (Wickramasinghe et al., 2010), suggesting that TREX-2 and NXF-1:NXT-1 may together recruit the *hsp-16.2/41* promoter. However, we note that two of our *nxf-1* alleles (*yak64* and *yak63*) are located in N-terminal regions of this protein, while a C-terminal fragment of human NXF1 carrying just its NTF2 and UBA domains was sufficient for binding to GANP (Wickramasinghe et al., 2010).

The *nxf-1* and *nxt-1* mutants we isolated are overtly wild-type. By contrast, loss-of-function mutations or RNAi knockdown of *nxf-1* and *nxt-1* cause severe defects in mRNA export and lethality (Tan et al., 2000; Zheleva et al., 2019), suggesting that our mutations may specifically perturb recruitment of the *hsp-16.41* promoter without affecting the general mRNA export functions of NXF-1:NXT-1.

Methods

Strain maintenance: C. elegans worms were maintained at 20°C or room temperature (~23°C) on NGM agar plates seeded with lawns of OP50 bacteria (Brenner, 1974).

Suppressor screen: Strains XZ1174 and XZ1372, which both contain two single-copy insertions of *Phsp-16.41::peel-1*, were mutagenized with ENU or EMS as described (Brenner, 1974; De Stasio & Dorman, 2001). F2 animals were heat-shocked at 34° for two hours, and survivors were isolated. *yak63* was isolated from EMS mutagenesis, while *yak64*, *yak65*, and *yak135* were isolated from ENU mutagenesis. All of these mutations are recessive.

Gene identification: Mutations were mapped to chromosomes using marker strains EG8040 and EG8041 (Frøkjær-Jensen et al., 2014). *yak63*, *yak64*, and *yak65* were mapped to chromosome V, and *yak135* was mapped to chromosome I. A complementation test suggested that *yak64* and *yak65* belong to the same gene. *yak63*, *yak64*, and *yak65* were outcrossed five times, and *yak135* three times using strain XZ1047 to generate strains XZ63, XZ64, XZ65, and XZ135. Genomic DNA was isolated from these strains using the Hobert lab protocol found at http://hobertlab.org/wp-content/uploads/2013/02/Worm_Genomic_DNA_Prep.pdf. Whole-genome sequencing was performed by Novogene and the data were analyzed using the Galaxy web platform (version 18.09) at https://usegalaxy.org/. Mutations were confirmed by Sanger sequencing. The *nxf-1* mutations are as follows: *yak63* is a C to T mutation leading to P286S, *yak64* is a C to T mutation leading to L101F, and *yak65* is a G to A mutation leading to R486K. The *nxt-1* mutation *yak135* is a T to G mutation leading to I75S.

Heat-shock: 50 gravid adults of strains XZ63, XZ64, XZ65, and XZ135 along with controls XZ1174 or XZ1372 were placed on new plates at 34°C for two hours and then moved to 20°C or room temperature. Animals were probed gently by worm pick, and response to touch was measured either as movement or no movement. Animals were assayed immediately following heat-shock and then every thirty minutes for three hours, with a final measurement at 24 hours. This experiment was performed a minimum of three times for each strain.

Endogenous peel-1 suppression assays: To test whether *nxf-1(yak65)* suppressed endogenously expressed *peel-1*, we first generated strain XZ2281 that is homozygous for *nxf-1(yak65)* and in which the *zeel-1(tm3419)* deletion is balanced by the hT2 translocation carrying a GFP marker (*qIs48*) and a recessive lethal mutation. Similar to the control strain QX1319 *zeel-1/hT2[let GFP]*, we found that all XZ2281 surviving animals had GFP, indicating that *zeel-1/zeel-1; nxf-1(yak65)* animals are inviable and that *nxf-1(yak65)* does not suppress endogenous *peel-1*. To better quantify this result, animals of genotype *zeel-1(tm3419)/oxTi638[Peft-3::tdTomato::H2B]; nxf-1(yak65)* were constructed via a series of crosses: N2 X EG7832 X EG7766 X XZ65 X XZ2281. Nine *zeel-1(tm3419)/+ ; nxf-1(yak65)* L4 hermaphrodites were grown individually and allowed to produce progeny. The number of dead progeny over the total number of progeny was used to quantify PEEL-1-induced lethality. For control, AFS216 *zeel-1(tm3419) peel-1(cle6)*, was crossed to N2 males. AFS216 contains the same *zeel-1(tm3419)* deletion, as well as a *peel-1* early stop-codon insertion and frameshift that is irrelevant for this cross. Seven L4 hermaphrodites produced from this cross were singled to new plates and live and dead progeny were counted.

Phsp-16.41::GFP fluorescence assays: Strains XZ2418 and XZ2457 carrying a single-copy insertion of *Phsp-16.41::eGFP::H2B* were heat-shocked for 2 hours at 34°C. Animals were then placed at room temperature for 2 hours before mounting to agarose slides with 50 mM sodium azide. Entire animals were imaged using a NikonTi2-E Crest X-light V2 spinning disk confocal microscope by excitation at 488 nm and capture with a 510/20 filter. Each image was taken as a Z series with 0.8 µm steps. Images were analyzed using FIJI utilizing the Z-project sum slices tool (Schindelin et al., 2012). ROIs were drawn around each animal and measured for mean gray value. Background was subtracted from these values using the same ROIs placed outside of the animal. Control worm mean-gray values after background subtraction were averaged for each replicate and used to normalize all values for that replicate.

Reagents

Reagents:

Strain	Genotype	Source
N2	wild-type *Caenorhabditis elegans*	CGC
XZ63	*oxSi507[Phsp-16.41::peel-1, Cb-unc-119] II ; oxSi280[Phsp-16.41::peel-1, Cb-unc-119] IV ; nxf-1(yak63) V*	This study
XZ64	*oxSi507[Phsp-16.41::peel-1, Cb-unc-119] II ; oxSi280[Phsp-16.41::peel-1, Cb-unc-119] IV ; nxf-1(yak64) V*	This study
XZ65	*oxSi507[Phsp-16.41::peel-1, Cb-unc-119] II ; oxSi280[Phsp-16.41::peel-1, Cb-unc-119] IV ; nxf-1(yak65) V*	This study
XZ135	*nxt-1(yak135) I ; oxSi507[Phsp-16.41::peel-1, Cb-unc-119] II ; oxSi280[Phsp-16.41::peel-1, Cb-unc-119] IV ; him-5(e1490) V*	This study
XZ1047	*oxSi507[Phsp-16.41::peel-1, Cb-unc-119] II ; unc-119(ed9) III ; oxSi280[Phsp-16.41::peel-1, Cb-unc-119] IV ; him-5(e1490) V*	This study
XZ1174	*oxSi507[Phsp-16.41::peel-1, Cb-unc-119] II ; oxSi280[Phsp-16.41::peel-1, Cb-unc-119] IV*	This study
XZ1372	*yakTi4[Phsp-16.41::eGFP::H2B, NeoR] I ; oxSi507[Phsp-16.41::peel-1, Cb-unc-119] II ; oxSi280[Phsp-16.41::peel-1, Cb-unc-119] IV*	This study
XZ2281	*zeel-1(tm3419) I / hT2[bli-4(e937) let-?(q782) qIs48] I; III ; nxf-1(yak65) V*	This study
XZ2297	*nxf-1(yak65) V*	This study
XZ2418	*nxf-1(yak65) V; yakTi6[Phsp-16.41::eGFP::H2B, NeoR]*	This study
XZ2457	*yakTi6[Phsp-16.41::eGFP::H2B, NeoR]*	This study
XZ2463	*unc-119(ed9) III ; nxf-1(yak65) V; oxEx1504[Pexp-3::peel-1 Cb-unc-119, Pmyo-3::mCherry, Pmyo-2::mCherry, Prab-3::mCherry]*	This study
EG6301	*qqIr5[niDf9 zeel-1(-) peel-1(-)]; ttTi5605 II ; unc-119(ed9) III ; oxEx1504[Pexp-3::peel-1 Cb-unc-119, Pmyo-3::mCherry, Pmyo-2::mCherry, Prab-3::mCherry]*	Erik Jorgensen
EG7766	*oxTi77[Peft-3::GFP::H2B unc-18(+)] V ; unc-18(md299) X*)	Erik Jorgensen
EG7832	*oxTi638[Peft-3::tdTomato::H2B cb-unc-119(+)] I ; unc-119(ed3) III*	CGC
EG7833	*oxTi559[Peft-3::tdTomato::H2B cb-unc-119(+)] I ; unc-119(ed3) III*	CGC

EG7958	*unc-119(ed3) III; oxTi710[Peft-3::tdTomato::H2B Cb-unc-119(+)] V*	CGC
EG8040	*oxTi302[Peft-3::mCherry cb-unc-119(+)] I ; oxTi75[Peft-3::GFP::H2B unc-18(+)] II ; oxTi411[Peft-3::tdTomato::H2B cb-unc-119(+)] III unc-119(ed3) III; him-8(e1489) IV*	CGC
EG8041	*oxTi76[Peft-3::GFP::H2B unc-18(+)] IV ; oxTi405[Peft-3::tdTomato::H2B cb-unc-119(+)] V him-5(e1490) V ; oxTi421[Peft-3::mCherry cb-unc-119(+)] X*	CGC
AFS216	*zeel-1(tm3419) I peel-1(cle6) I*	Aaron Severson
QX1319	*zeel-1(tm3419) I / hT2[bli-4(e937) let-?(q782) qIs48] I; III*	Leonid Kruglyak

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