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Dual tagging of GFP and Degron on endogenous COSA-1 in *Caenorhabditis elegans* as a crossover investigation tool

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Abstract

COSA-1 is essential for accurate meiosis in *C. elegans*. Two null mutants (*cosa-1(me13)* and *cosa-1(tm3298)*) have been notably studied. These null mutants exhibit severe meiotic defects, hindering the observation of the subtle or dynamic nature of COSA-1 function. To overcome these limitations, we developed a *C. elegans* strain with inducible COSA-1 degradation using the Auxin-Inducible Degron (AID) system. This strain exhibits normal fertility and COSA-1::GFP foci. Auxin treatment successfully depletes COSA-1, resulting in a 96% decrease in progeny viability and 12 univalent chromosomes in diakinesis oocytes. This strain serves as a valuable tool for studying the dynamics of COSA-1.

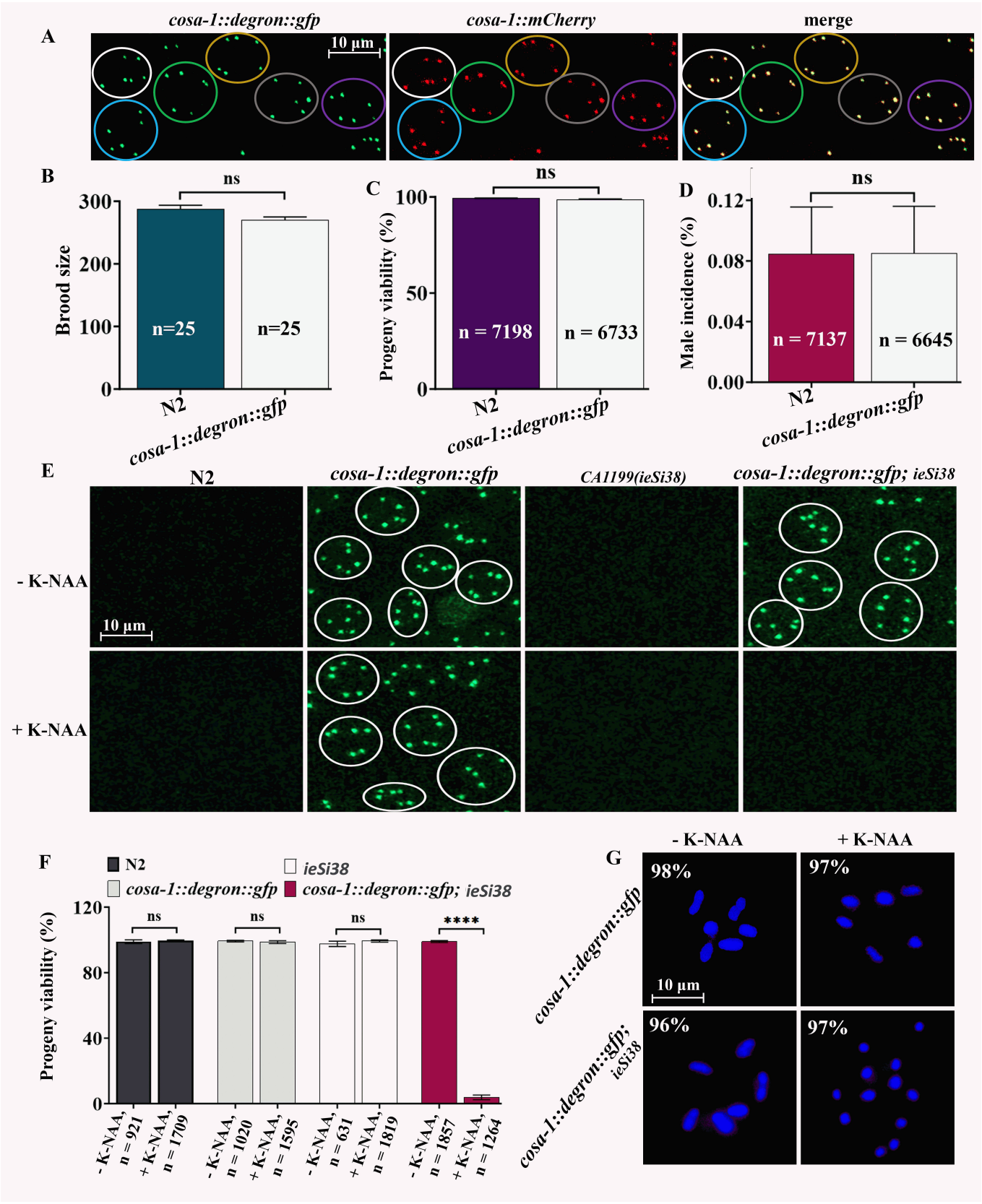

Figure 1. Cytological and phenotypic characterization of the *cosa-1::degron::gfp* strain:

(A) Fluorescence images depict the colocalization of COSA-1::Degron::GFP and COSA-1::mCherry foci in the late pachytene zones of the germline in live worm. Rings of the same color indicate the same nucleus. Images were captured using a Zeiss

LSM800 confocal microscope with a 63x oil immersion objective (scale bar = 10 µm). (B) Analysis of brood size for N2 and *cosa-1::degron::gfp* strains. (C) Analysis of progeny viability. (D) Analysis of male frequency. Statistical comparisons for data sets in panels A, B, and C were conducted using an unpaired Student's T-test (using Kolmogorov-Smirnov test), with $^{ns}P > 0.05$ in all cases compared to N2. The bar graphs indicate the mean ± SEM. (E) Fluorescence images of K-NAA-induced degradation of COSA-1 are shown in live worm. (F) Progeny viability analysis of worm strains following treatment with 4 mM K-NAA. The *cosa-1::degron::gfp*; *ieSi38* strain exhibited a 96% reduction in viability following K-NAA treatment (****$P < 0.0001$ compared to all other groups). The bar graphs indicate the mean ± SEM ($^{ns}P > 0.05$; ****P < 0.0001, 2-way ANOVA with Dunnett's multiple comparisons test). The experiment was conducted three times. (G) Images of diakinesis nuclei show chromosome morphology in the strains, with or without K-NAA treatment (n = 100 per treatment). Images were captured using a DM6B fluorescence microscope (Leica, Germany) with a 100x oil immersion objective, and GFP filter (scale bar = 10 µm).

Description

Crossover (CO) recombination between homologous chromosomes plays a crucial mechanical role in guiding their segregation during meiosis I division (Martinez-Perez and Colaiácovo, 2009). In the worm *C. elegans*, the protein COSA-1 plays a crucial role in promoting meiotic crossovers at designated CO sites (Yokoo et al., 2012). These sites undergo robust homeostasis and interference, ensuring the formation of only one CO per chromosome pair (Hillers and Villeneuve, 2003). Two *cosa-1* mutants have been notably studied: *cosa-1(me13)* and *cosa-1(tm3298)* (Yokoo et al., 2012). The *cosa-1(me13)* mutant is characterized by a specific G-to-A transition, leading to a premature stop at codon 148. This mutation results in severe meiotic defects, with a striking 97% rate of inviable embryos in self-progeny. Additionally, *cosa-1(me13)* exhibits a significantly elevated frequency of XO male offspring (38%) compared to the negligible 0.2% observed in wild-type worms. Wild-type diakinesis oocytes typically display six DAPI-stained bodies, corresponding to the six pairs of homologous chromosomes held together by chiasmata. In stark contrast, *cosa-1(me13)* oocytes exhibit an average of 11 resolvable DAPI-stained bodies, indicating the absence of chiasmata. The deletion allele *cosa-1(tm3298)* is characterized by a 335 bp deletion and an additional G-to-A transition, and exhibits a comparable phenotype. In *cosa-1(tm3298)*, all of exon 3 is deleted, and the transition alters the splice acceptor site adjacent to exon 4. The likely spliced mRNA (in which exon 2 is spliced to exon 5) would contain a frameshift after codon 24, resulting in the termination of translation after 30 additional codons. While the study of *cosa-1(me13)* and *cosa-1(tm3298)* null mutants has provided valuable insights, significant limitations persist. For instance, the null mutations completely render the COSA-1 protein non-functional, leading to severe defects, and preventing the observation of the subtle or dynamic nature of COSA-1 function. Additionally, null mutations are irreversible and effective throughout the organism's lifecycle, making it impossible to dissect the specific contribution of COSA-1 at different stages of development or its response to specific stimuli. To overcome these limitations and gain a more comprehensive understanding of COSA-1 function, a more flexible and dynamic approach, such as the Auxin-Inducible Degron (AID) system, is needed.

The AID system leverages the auxin-triggered SCF-TIR1 E3 ligase complex to recognize and ubiquitinate AID-tagged proteins, leading to their conditional and rapid proteasomal degradation in a controlled and inducible manner (Nishimura et al., 2020). The AID system offers rapid and specific reversible protein depletion, tight control of protein levels (the rate of degradation is directly proportional to the concentration of auxin), and compatibility with a wide range of cell types and organisms. In this study, we constructed the *cosa-1::degron::gfp C. elegans* strain using the CRISPR-Cas9 system (Friedland et al., 2013; Zhang et al., 2015). This strain displayed six green fluorescence protein (GFP) foci in the late pachytene nuclei of the germline, corresponding to COSA-1 (Fig. 1A). We had previously reported an endogenously tagged mCherry-tagged COSA-1 in *C. elegans*, where we confirmed the colocalization of mCherry and GFP in the *gfp::cosa-1; cosa-1::mCherry* strain (Ezechukwu et al., 2022). To further confirm that the foci in the *cosa-1::degron::gfp* strain are indeed COSA-1, we crossed the strain with the *cosa-1::mCherry* strain to obtain the codominant heterozygous strain (*cosa-1::mCherry/cosa-1::degron::gfp*), as they are both endogenous tags. Our results showed colocalization of GFP and mCherry proteins at the CO-designated foci (Fig. 1A). To verify that the transgenesis did not cause defects in the *cosa-1::degron::gfp* strains, we analyzed the fertility phenotype of the worms. The transgenesis did not affect brood size (Fig. 1B), progeny viability (Fig. 1C) or male frequency (Fig. 1D) when compared to the wild-type ($P > 0.05$, in all cases). To verify if the AID system actually works in the *cosa-1::degron::gfp; ieSi38* strain, we exposed L4 worms to 4 mM auxin (1-naphthaleneacetic acid potassium salt (K-NAA)), and analyzed the COSA-1 distribution as well as the progeny viability following 16 hours of auxin treatment. Our results showed that all COSA-1 foci disappeared in the *cosa-1::degron::gfp; ieSi38* strain (Fig. 1E). The progeny viability of the auxin-treated *cosa-1::degron::gfp; ieSi38* strain decreased by 96% ($P < 0.0001$) (Fig. 1F), and most (~97%) of the diakinesis nuclei exhibited 12 univalent chromosomes (Fig. 1G). In addition to the AID system, this strain offers the advantage of a GFP tag at the endogenous location for COSA-1 microscopy, as the prominently used *gfp::cosa-1* strain AV630 (*meIs8 [pie-1p::GFP::cosa-1 + unc-119(+)]* II) was generated by the microparticle bombardment method.

Methods

The CRISPR/Cas9 method was employed to construct the *cosa-1::degron::gfp* strain. Initially, the *cosa-1* sgRNA recognition site TGTCAGAGATGGTAGTTACG was selected near the termination codon of the *cosa-1* gene. The template sequence of pU6-cosa-1 C-sgRNA was then constructed by fusion PCR following the method described by Ward (2014). Similarly, the *cosa-1::degron::gfp* repair template was generated by fusion PCR: ~1000 bp upstream DNA sequence before the stop codon of the cosa-1 gene (PCR from N2 genomic DNA with *cosa-1::degron::gfp* UF/*cosa-1::degron::gfp* UR primers) was added before the start codon of the degron::gfp coding sequence (PCR from PYH-0114 plasmid with Degron::GFP F/Degron::GFP R primers), and ~1000 bp downstream DNA sequence after the stop codon of the *cosa-1* gene (PCR from N2 genomic DNA with *cosa-1::degron::gfp* DF/*cosa-1::degron::gfp* DR primers) was added after the stop codon of the degron::gfp coding sequence. Subsequently, a mixture of pDD162 (Peft-3::Cas9, 50 ng/µL), pCFJ90 (Pmyo-2::mCherry, 2.5 ng/µL), and pCFJ104 (Pmyo-3::mCherry, 5 ng/µL) plasmids (Dickinson and Goldstein, 2016), along with pU6-cosa-1 C-sgRNA (50 ng/µL) and *cosa-1::degron::gfp* repair template (50 ng/µL), was microinjected into N2 young adult worms. The F1 progeny expressing pCFJ90 and pCFJ104 plasmids were selected under the Olympus SZX2-ILLB fluorescence microscope and individually plated (1 worm per plate) to lay eggs for 2 days. Subsequently, worms were selected for lysis and PCR screening using the primers listed in Regents. The PCR products of *cosa-1::degron::gfp* transgenic worms were sequenced for confirmation, and the transgenic worm was backcrossed to wild-type four times before use. The distribution of COSA-1::GFP was confirmed using confocal microscope. The fertility phenotype (brood-size, progeny viability and male frequency) of the strain was analyzed using established protocols (Ezechukwu et al., 2022). To confirm the effectiveness of the AID system in the strain, synchronized L1 larval stage animals were initially cultured on standard nematode growth media (NGM) plates seeded with *E. coli* OP50. At the L4 stage, the worms were transferred to OP50-seeded 4 mM K-NAA NGM agar plates and cultured for 16 hours. Following this, the depletion of COSA-1 was confirmed using confocal microscope, and the progeny viability of the strain was analyzed.

Reagents

Strain	**Genotype**	**Available from**	
N2	Bristol *Caenorhabditis elegans*	CGC	
XSW955	*cosa-1(wsh7 [cosa-1::mCherry]) III*	Wang Lab	
XSW974	*wsh30(cosa-1::degron::gfp) III*	Wang Lab	
CA1199	*ieSi38 [sun-1p::TIR1::mRuby::sun-1 3'UTR + Cbr-unc-119(+)] IV*	CGC	
XSW1010	*wsh30[cosa-1::degron::gfp]; ieSi38[sun-1p::TIR1::mRuby::sun-1 3'UTR + Cbr-unc-119(+)] IV*	Wang Lab	

Primers	**Sequence (5'-3')**	**Description**	
degron+gfp VR		CTTCGGGCATGGCACTCT	For *cosa-1::degron::gfp* transformants screening
cosa-1+mch -F4	CTGCGCGAAAAAGGTAACTGC		
cosa-1+mch -R4	ACGTGACAGGAAATTGCGAA		

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