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Endogenous expression and localization of HIS-72::mTurquoise2 in *C. elegans*

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Abstract

To generate a non-red/green fluorescent fusion histone protein in *C. elegans*, we have generated a C-terminal mTurquoise2-tagged HIS-72 at the endogenous locus using CRISPR. We found that HIS-72::mTurquoise2 localizes in a similar pattern to the previously published HIS-72::GFP strain.

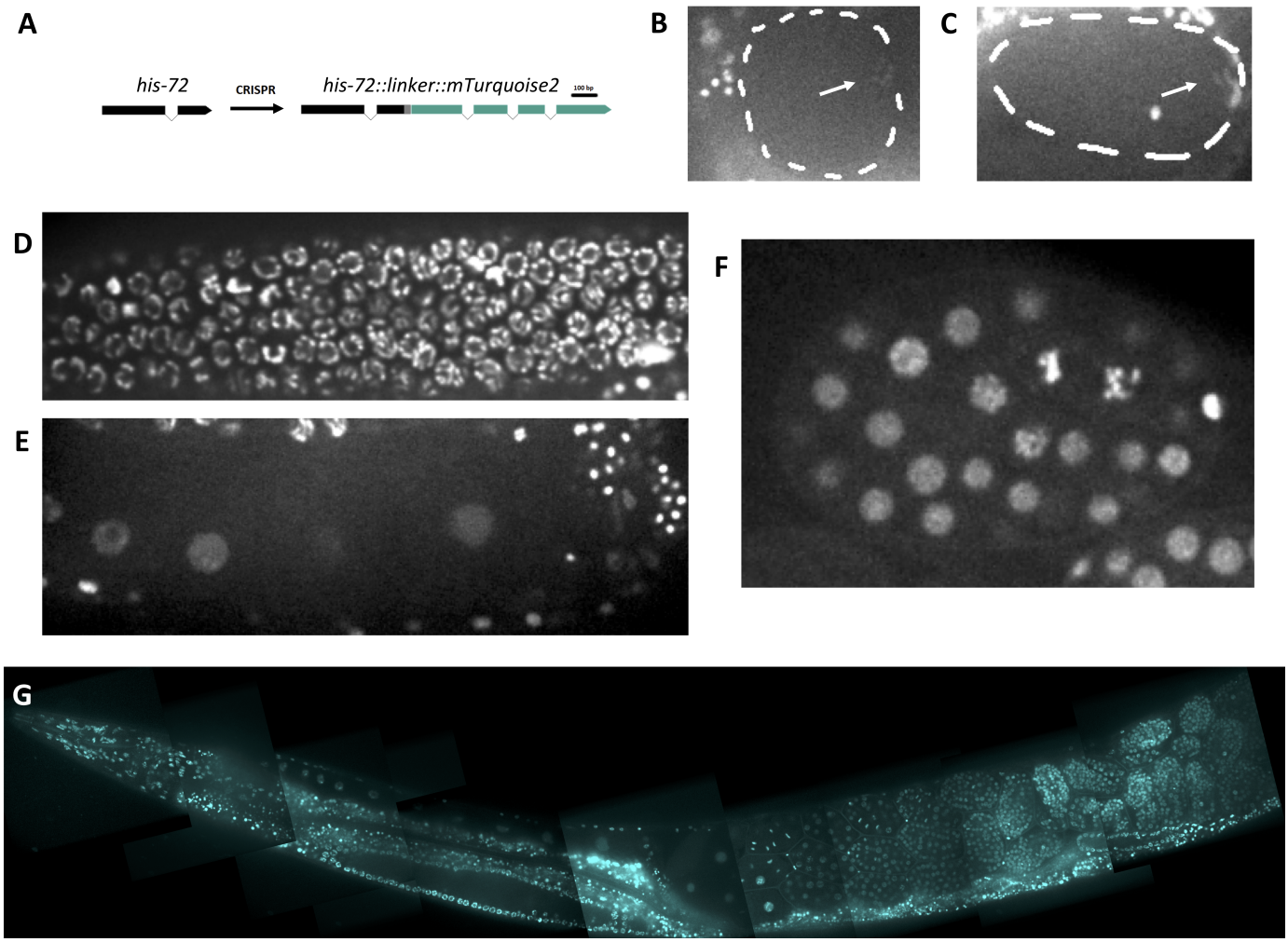

Figure 1. Endogenous HIS-72::linker::mTurquoise2: (A) Schematic of *his-72* edit (B-F) Images of HIS-72::linker::mTurquoise2 localization: in a prometaphase I embryo (B, arrow indicates meiotic chromosomes), in an anaphase I embryo (C, arrow indicates meiotic chromosomes), at the distal gonad arm (D), in the proximal gonad arm (E), and in a 50-100 cell embryo (F). (G) HIS:-72::linker::mTurquoise2 in an adult worm.

Description

In fixed samples, chromatin is most often visualized by using a fluorescent DNA-binding dye like DAPI, which emits blue light (Kapuscinski 1995). In live fluorescence microscopy, however, a fluorescently tagged histone is often used instead. Histones form nucleosomes to package chromatin, and are involved in the organization of the genome (Chen *et al.* 2021). Most frequently, histones are fused to red or green fluorescent proteins, though the use of a blue or cyan fluorescent protein instead would allow for three (or more) channel imaging (Kanda *et al.* 1998, Das *et al.* 2003, Ooi *et al.* 2006).

We sought to generate an endogenously tagged histone line with a blue or cyan fluorescent protein. mTurquoise2 is a relatively recently engineered cyan fluorescent protein with a high quantum yield (Goedhart *et al*. 2012). Thus, we constructed a HIS-72::mTurquoise2 fluorescent fusion protein using a design similar to that of another group who also tagged HIS-72 (Figure 1A, Dickinson *et al.* 2013; see Design section). We report similar localization of HIS-72::mTurquoise2 to previously generated HIS-72 fluorescent fusion strains in most nuclei of the animal (Figure 1G). HIS-72::mTurquoise2 shows chromatin labeling in the germline and all stages of the cell cycle in the early embryo (Figure 1B-F). We observed that HIS-72::mTurquoise2 signal is significantly reduced on chromatin during the meiosis I and II divisions as compared with other stages (Figure 1B,C), which is not observed in other exogenously expressed fluorescently tagged histone lines (Bai and Bembenek 2017, Bembenek *et al.* 2007). This may reflect the role of different histone variants in meiotic chromosome function.

In sum, we have constructed a HIS-72::mTurquoise2 fluorescent fusion strain for use to the *C. elegans* community.

Methods

Request a detailed protocol

CRISPR/Cas9 Gene editing

We followed the CRISPR/Cas9 protocol generated by the Seydoux lab for C-terminal mTurquoise2 tagging of the *C. elegans* his-72 gene (Paix *et al.* 2015). The repair template was amplified from the pDD377 plasmid, a gift from Bob Goldstein (Addgene plasmid #91823). Tagging of his-72 was done in the wild type N2 background. The transgenic strain was crossed to the N2 strain for 5 generations to remove potential off target mutations. All guide RNAs and oligos were obtained commercially.

The primer sequences for amplifying the repair template are listed below:

his-72::mTurquoise2_Forward (*DS_030*):

TGCAACTCGCCAGACGCATCAGAGGAGAACGTGCTGGAGCATCGGGAGCCTCAGGAGCATCGATGGTAAGTAAGGGCG

his-72::mTurquoise2_Reverse (*DS_031*):

ATTAAAAGTGCTTCGAGAATTGGTGATGGAGCTTACTTGTAGAGCTCGTCCATTCC

The flexible linker sequence: GGAGCATCGGGAGCCTCAGGAGCATCG (AA seq: GASGASGAS)

The target sequence (minus PAM) used in the crRNA (*Termed DS_g004; see Dickinson et al., 2013*): GAGCTTAAGCACGTTCTCCG

NOTE: This design only tags HIS-72 isoform a (Y49E10.6a, see WormBase).

Microscopy

Gravid adult worms were mounted on agar pads according to standard methods. Imaging was performed on a spinning disk confocal system consisting of a Nikon Eclipse inverted microscope with a 60×1.40 NA objective, a CSU-22 spinning disk system, and a Photometrics EM-CCD camera from Visitech International. Images were acquired by Metamorph (Molecular Devices) and analyzed by ImageJ/Fiji Bio-Formats plugins (National Institutes of Health) (Schindelin *et al.* 2012). The cyan/blue channel included a 450/50 emission filter with maximum blocking at 405 nm, and maximum intensity from 425-475 nm.

Reagents

JAB191: *his-72*(*erb77*[his-72::linker::mTurquoise2])

This strain will be made available to the CGC.

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References

Bai X, Bembenek JN. 2017. Protease dead separase inhibits chromosome segregation and RAB-11 vesicle trafficking. Cell Cycle 16: 1902-1917. PMID: 28820333.

Bembenek JN, Richie CT, Squirrell JM, Campbell JM, Eliceiri KW, Poteryaev D, Spang A, Golden A, White JG. 2007. Cortical granule exocytosis in *C. elegans* is regulated by cell cycle components including separase. Development 134: 3837-48. PMID: 17913784.

Chen P, Li W, Li G. 2021. Structures and Functions of Chromatin Fibers. Annu Rev Biophys 50: 95-116. PMID: 33957053.

Das T, Payer B, Cayouette M, Harris WA. 2003. In vivo time-lapse imaging of cell divisions during neurogenesis in the developing zebrafish retina. Neuron 37: 597-609. PMID: 12597858.

Dickinson DJ, Ward JD, Reiner DJ, Goldstein B. 2013. Engineering the *Caenorhabditis elegans* genome using Cas9-triggered homologous recombination. Nat Methods 10: 1028-34. PMID: 23995389.

Goedhart J, von Stetten D, Noirclerc-Savoye M, Lelimousin M, Joosen L, Hink MA, van Weeren L, Gadella TW Jr, Royant A. 2012. Structure-guided evolution of cyan fluorescent proteins towards a quantum yield of 93%. Nat Commun 3: 751. PMID: 22434194.

Kanda T, Sullivan KF, Wahl GM. 1998. Histone-GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells. Curr Biol 8: 377-85. PMID: 9545195.

Kapuscinski J. 1995. DAPI: a DNA-specific fluorescent probe. Biotech Histochem 70: 220-33. PMID: 8580206.

Ooi SL, Priess JR, Henikoff S. 2006. Histone H3.3 variant dynamics in the germline of *Caenorhabditis elegans*. PLoS Genet 2: e97. PMID: 16846252.

Paix A, Folkmann A, Rasoloson D, Seydoux G. 2015. High Efficiency, Homology-Directed Genome Editing in *Caenorhabditis elegans* Using CRISPR-Cas9 Ribonucleoprotein Complexes. Genetics 201: 47-54. PMID: 26187122.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9: 676-82. PMID: 22743772.

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