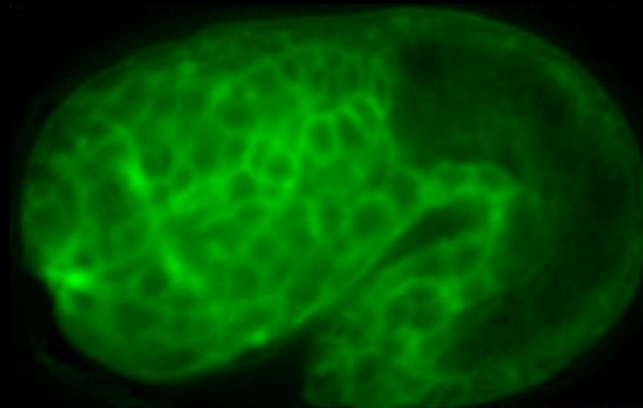


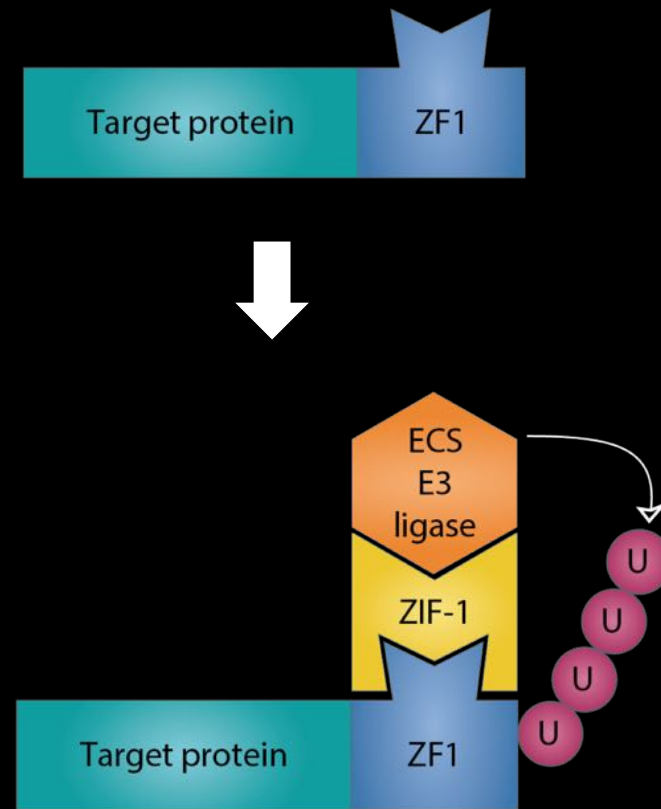
Conditional degradation of endogenous proteins in *C. elegans* using the ZF1/ZIF-1 system

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NYU School of Medicine



Questions addressed today:

- What is ZF1-mediated protein degradation?
- How do we use ZF1-mediated conditional degradation to produce loss-of-function phenotypes?
- What are some considerations when using ZF1-mediated degradation?



Nance and Frøkjær-Jensen (*in press*)

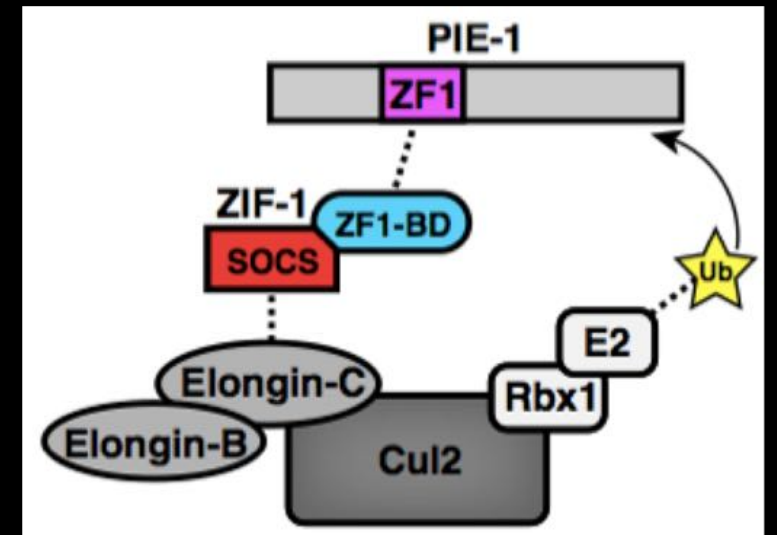
What is ZF1-mediated protein degradation?

ZF1 (Zinc Finger 1) - degron

- Identified for its role in targeting germline protein PIE-1 for degradation in somatic cells of early embryo (Reese et al. 2000)

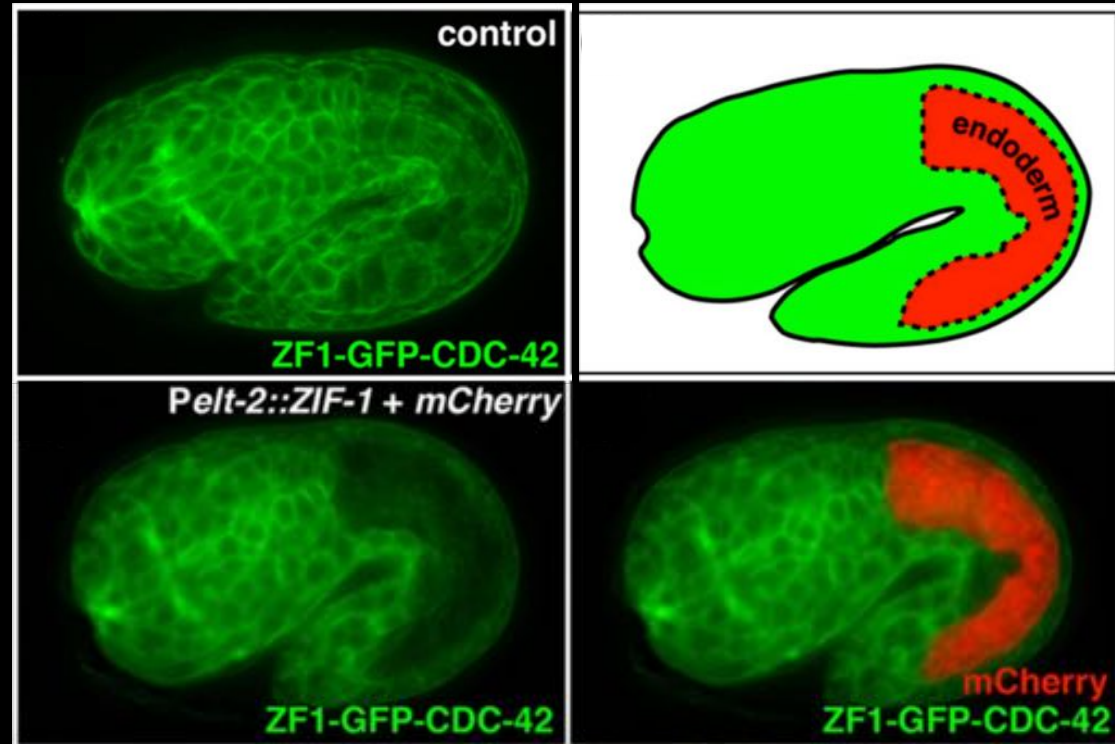
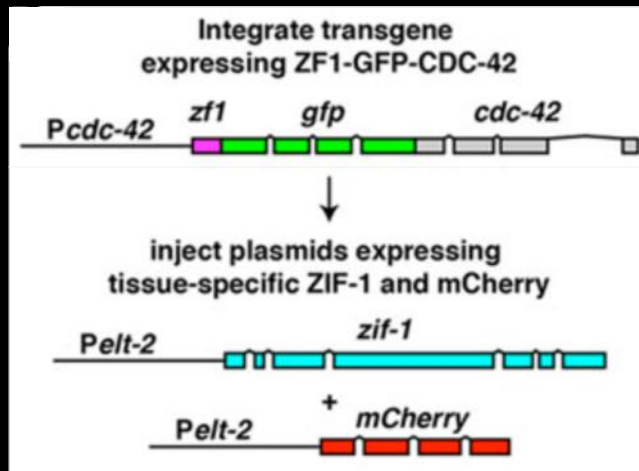
ZIF-1 – adaptor protein

- Maternally expressed SOCS-box adaptor protein that binds ZF1 domains
- Recruits ZF1-containing protein to an ECS (Elongin-C, Cul2, SOCS-box family) E3 ubiquitin ligase complex for degradation



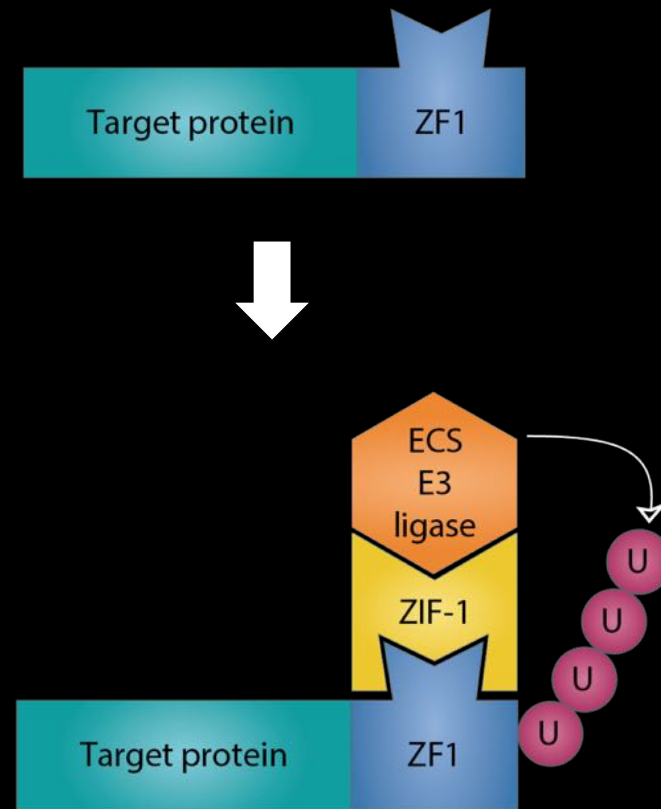
Armenti et al. 2014

Transgenic expression of *zif-1* can induce degradation of ZF-1 tagged proteins later in development



Questions addressed today:

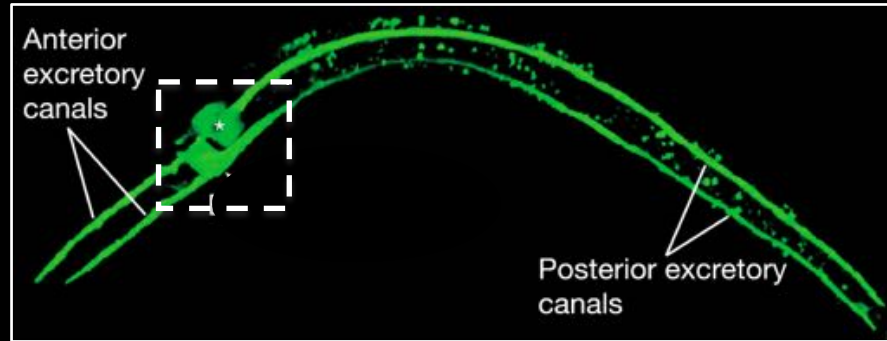
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Nance and Frøkjær-Jensen (*in press*)

Using ZF-mediated degradation to study essential proteins in larval canal cell

C. elegans excretory canal

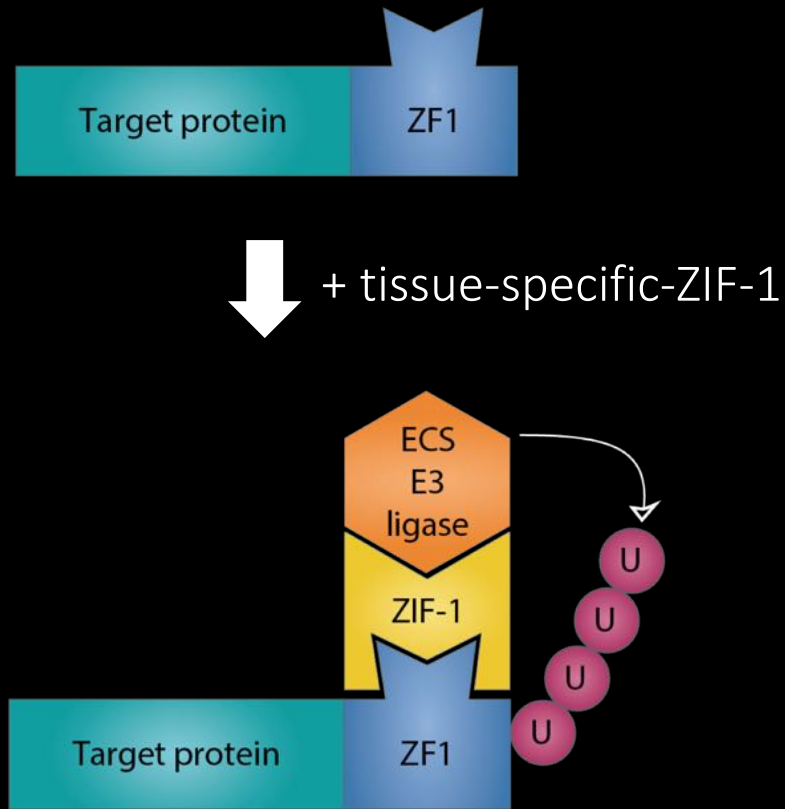


WormAtlas

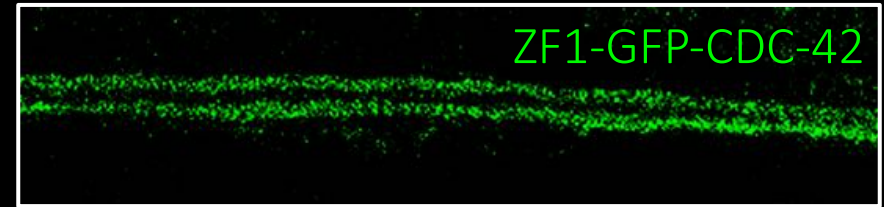
Question: what is the role of PAR polarity proteins and exocyst trafficking complex in the excretory canal?

Problem: *par* and exocyst null mutants arrest during embryogenesis

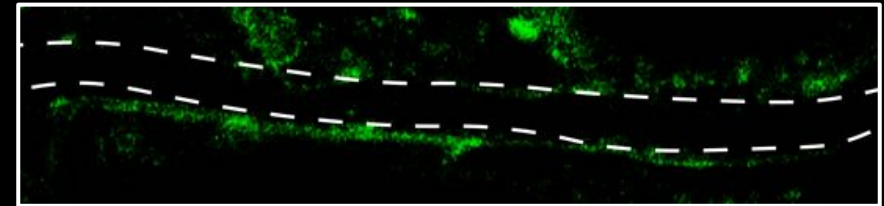
ZIF-1 expression in excretory cell causes robust degradation of ZF1-tagged protein



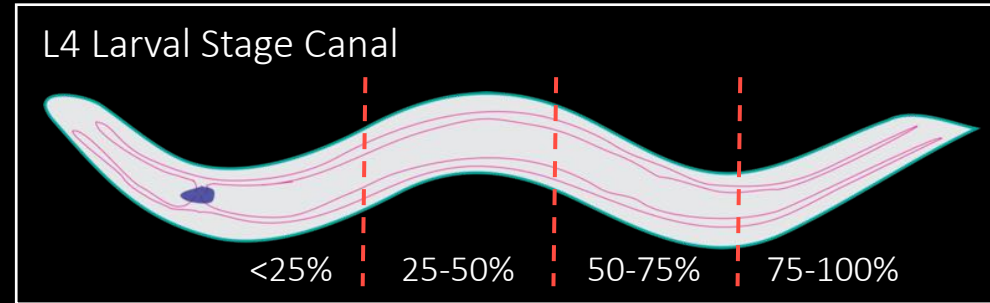
control



exc-ZIF-1



Canal cell depletion of essential trafficking proteins causes measurable phenotypes

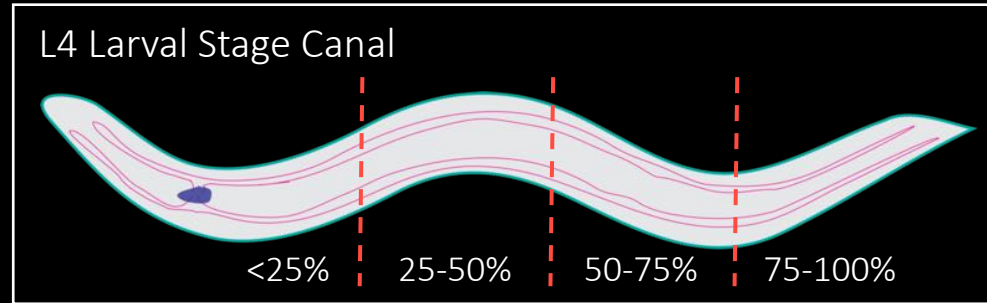


canal length (% relative to body length)

	<25%	25-50%	50-75%	75-100%
control	0	0	2	98
SEC-5 ^{canal (-)}	68	32	0	0
RAL-1 ^{canal (-)}	14	64	4	18

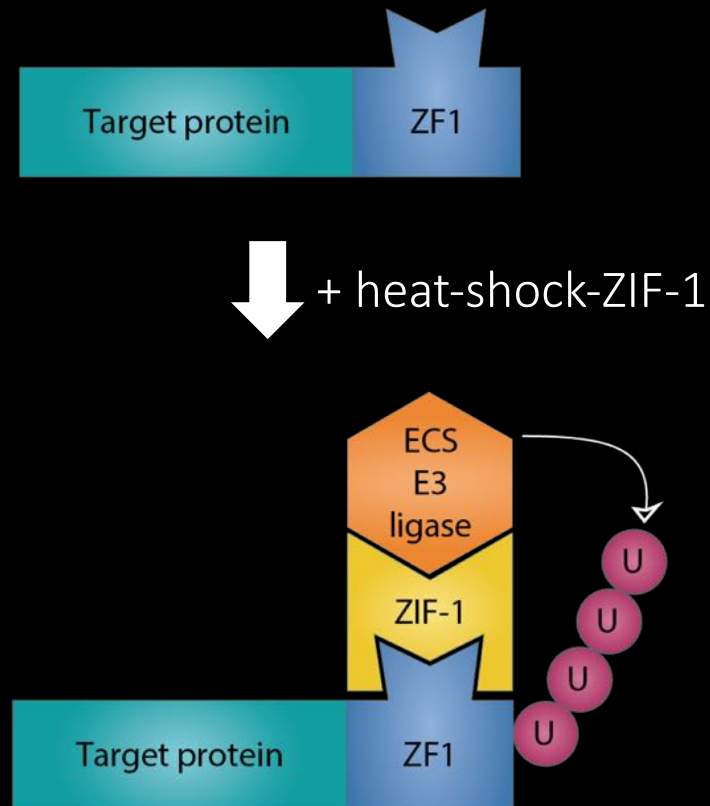
100%
0%

Phenotype of SEC-5-ZF1 is not enhanced by one copy of *sec-5* null allele

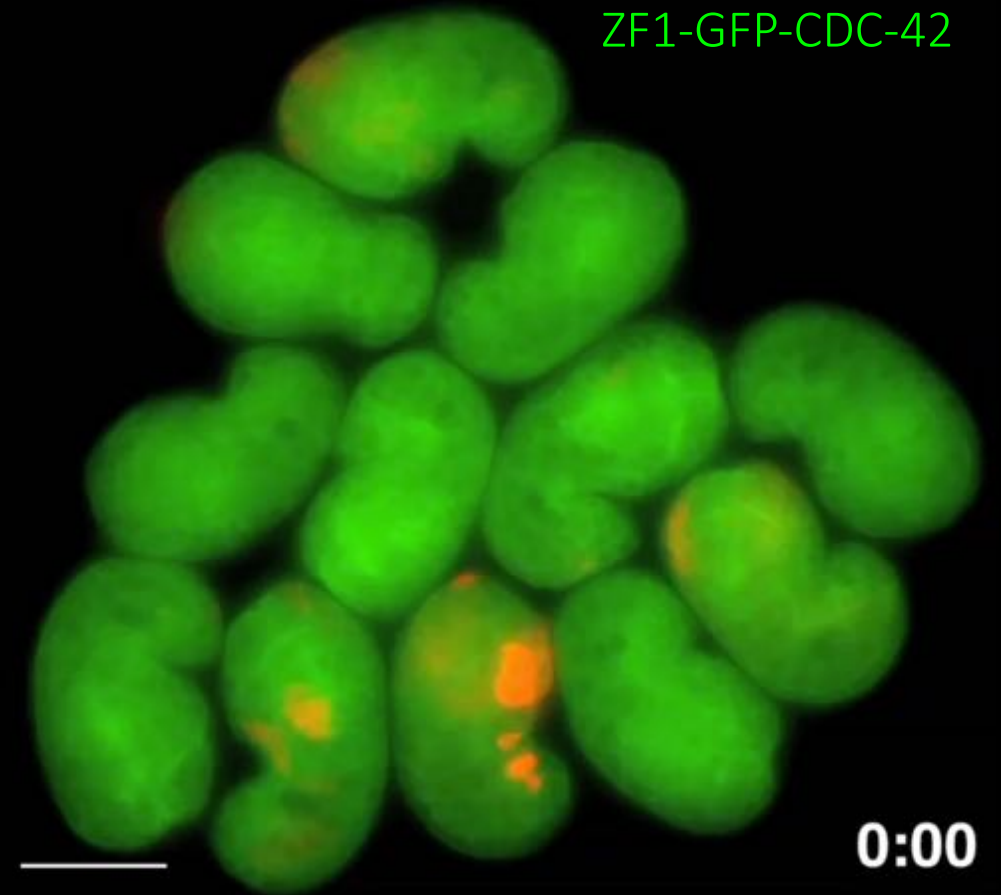


	<10%	25-50%	50-75%	75-100%	
<i>sec-5-zf1-yfp; exc::ZIF-1</i>	68	32	0	0	(n = 80)
<i>sec-5(tm1443)/sec-5-zf1-yfp; exc::ZIF-1/+</i>	61	29	10	0	(n = 49)
<i>sec-5-zf1-yfp; exc::ZIF-1/+</i>	62	38	0	0	(n = 21)

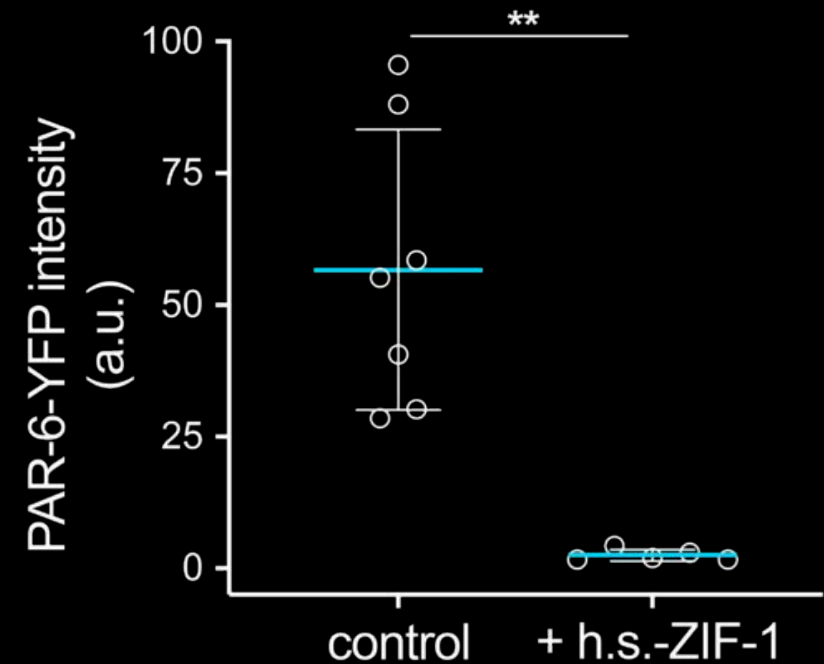
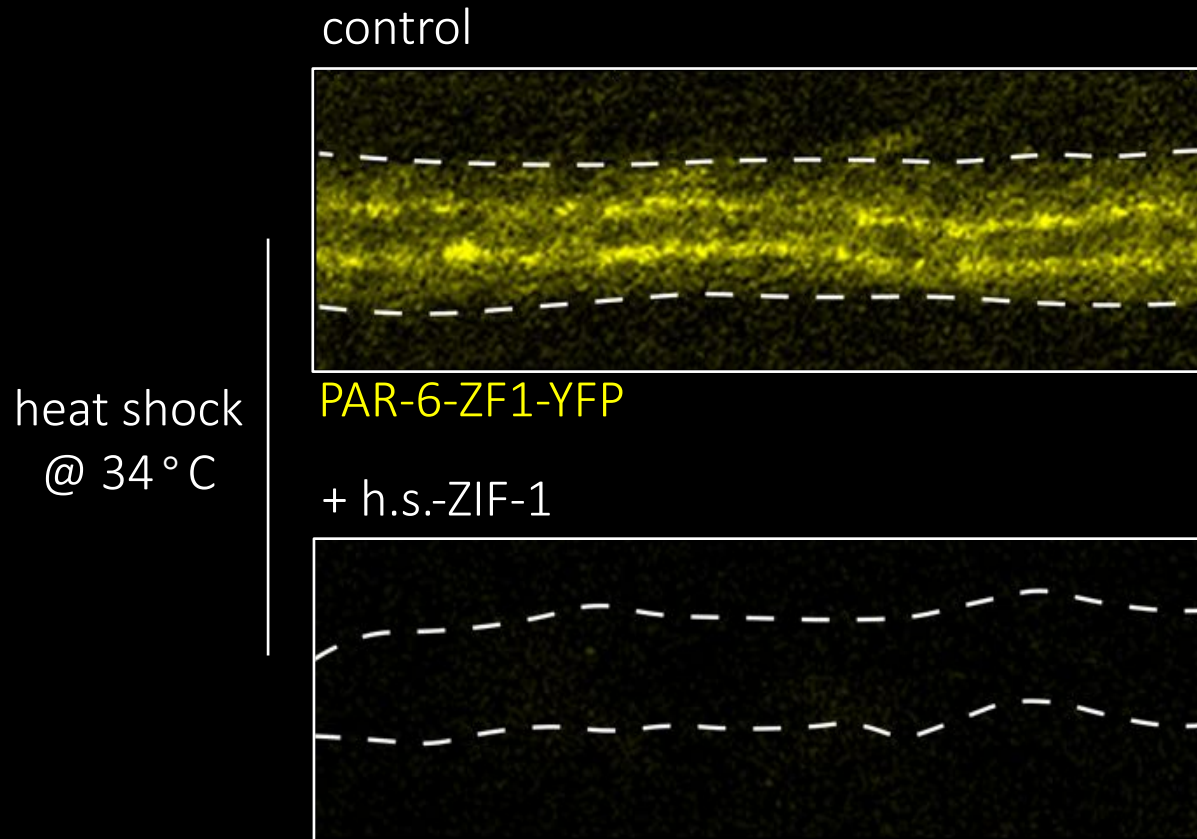
Phsp::ZIF-1 enables inducible protein degradation after heat-shock



Phsp::ZIF-1 + *mCherry* (extrachromosomal array)



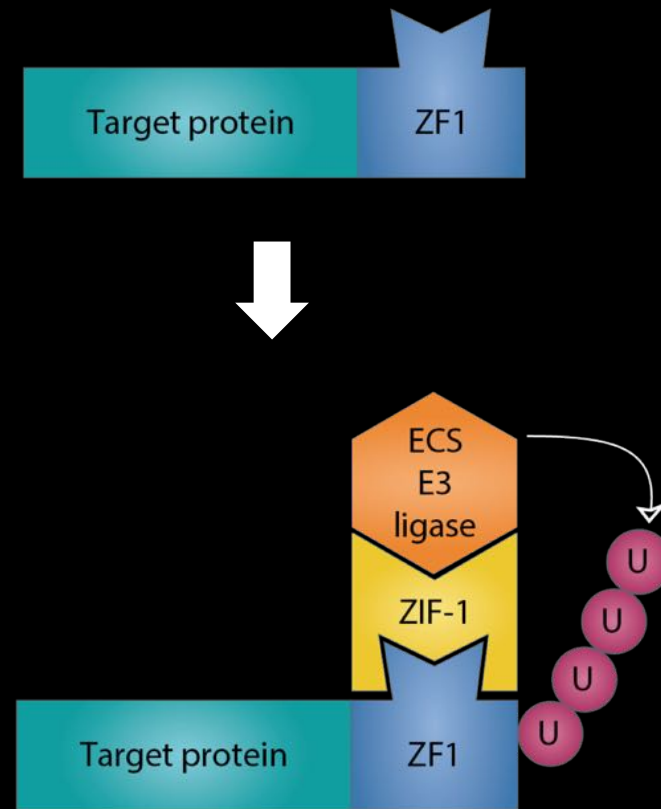
Heat shock expression of *zif-1* enables protein depletion during larval stages



- Heat shock inducible protein degradation enables us to examine epistasis between essential proteins later in development

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Considerations when using ZF1-mediated degradation

ZF1-tagged allele

- the degron tag may interfere with function of protein
- use this strain as control to compare to degraded animals
- monitor degradation with fluorescent protein or antibody

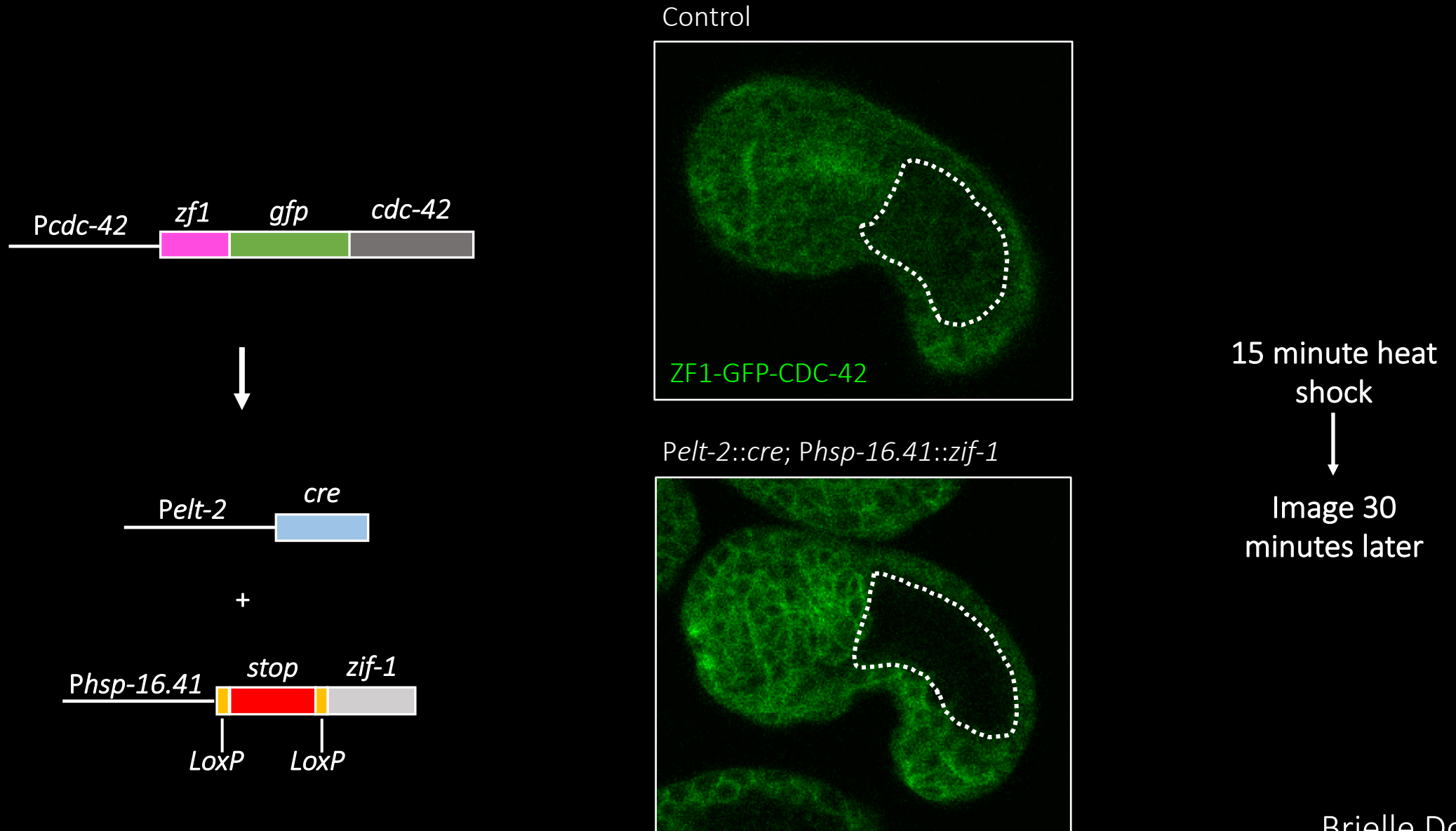
ZIF-1 adaptor

- be sure you have a strong/specific promoter
- Can use extrachromosomal arrays, but be sure to visually monitor degradation
- Integrated high copy arrays are best for robust and consistent degradation

Considerations when using ZF1-mediated degradation

- Should not use in germ line to avoid degrading PIE-1/other proteins with Zinc Finger domains
- Maternally expressed ZF1-tagged proteins are degraded by endogenous ZIF-1: complicates use of proteins required for cell viability or division.
 - Recently overcome this using *zif-1* mutants (Sallee et al. 2018)
- Degron must be in same cellular compartment as ZIF-1/E3 ligase complex
 - Enables the specific labeling of membrane-wrapped structures (Beer et al. 2019, bioRxiv)
- Not yet able to combine temporal and spatial control -- we are currently addressing this

Spatial and temporal control of ZF1-mediated degradation



ZF1-mediated degradation plasmids available on Addgene

pSA109 - drives ZIF-1 protein in *C. elegans* intestinal cells

pSA110 - drives mCherry in *C. elegans* intestinal cells

pSA107 - drives ZIF-1 protein in *C. elegans* ubiquitously

pSA108 - drives mCherry in *C. elegans* ubiquitously

pSA101 - drives ZIF-1 protein in *C. elegans* neurons

pSA102 - drives mCherry in *C. elegans* neurons

pSA120 - *C. elegans* heat-shock driven ZIF-1 and mCherry co-expressed in the same operon

pJN601 - zf1::yfp fusion with floxed unc-119(+) within an intron of yfp. For creating zf1::yfp knock-ins using unc-119 as a marker

ZF1-mediated degradation strains available at CGC

FT1450 - xnIs23 [cdc-42p::ZF1::GFP::cdc-42 + unc-119(+)]. xnEx342 [rab-3p::zif-1 + rab-3p::mCherry + rol-6(su1006)]

FT1481 - xnIs23 [cdc-42p::ZF1::GFP::cdc-42 + unc-119(+)]. xnEx350 [elt-2p::zif-1 + elt-2p::mCherry + rol-6(su1006)]

FT1547 - xnIs23 [cdc-42p::ZF1::GFP::cdc-42 + unc-119(+)]. xnEx380 [hsp-16.41p::zif-1::SL2::mCherry + rol-6(su1006)]

FT1607 - xnIs520 [cdc-42p::zif-1 + cdc-42p::mCherry].

Acknowledgements

Nance lab

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