Worms on steroids: Using ligand binding domains for drug-inducible protein activation

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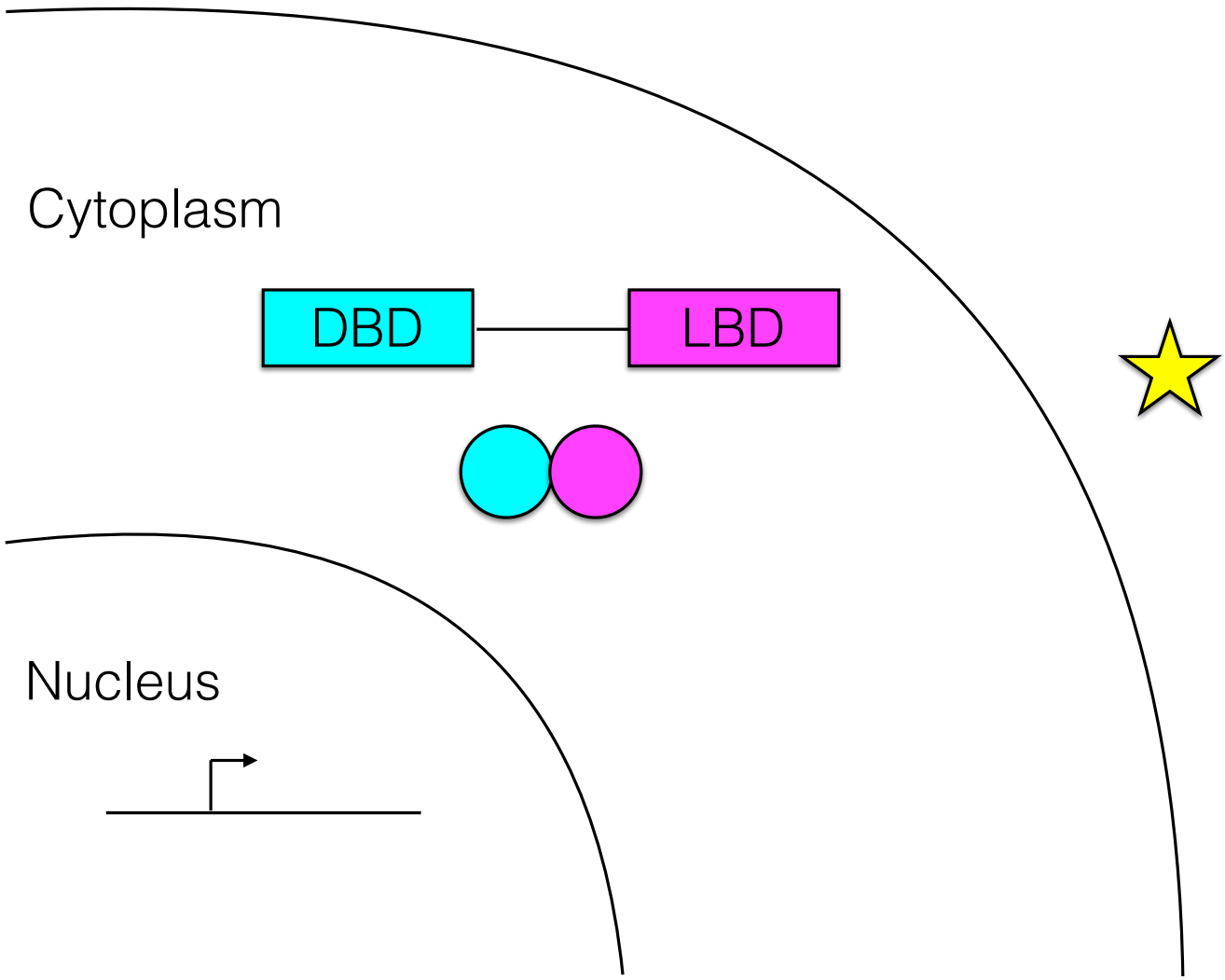
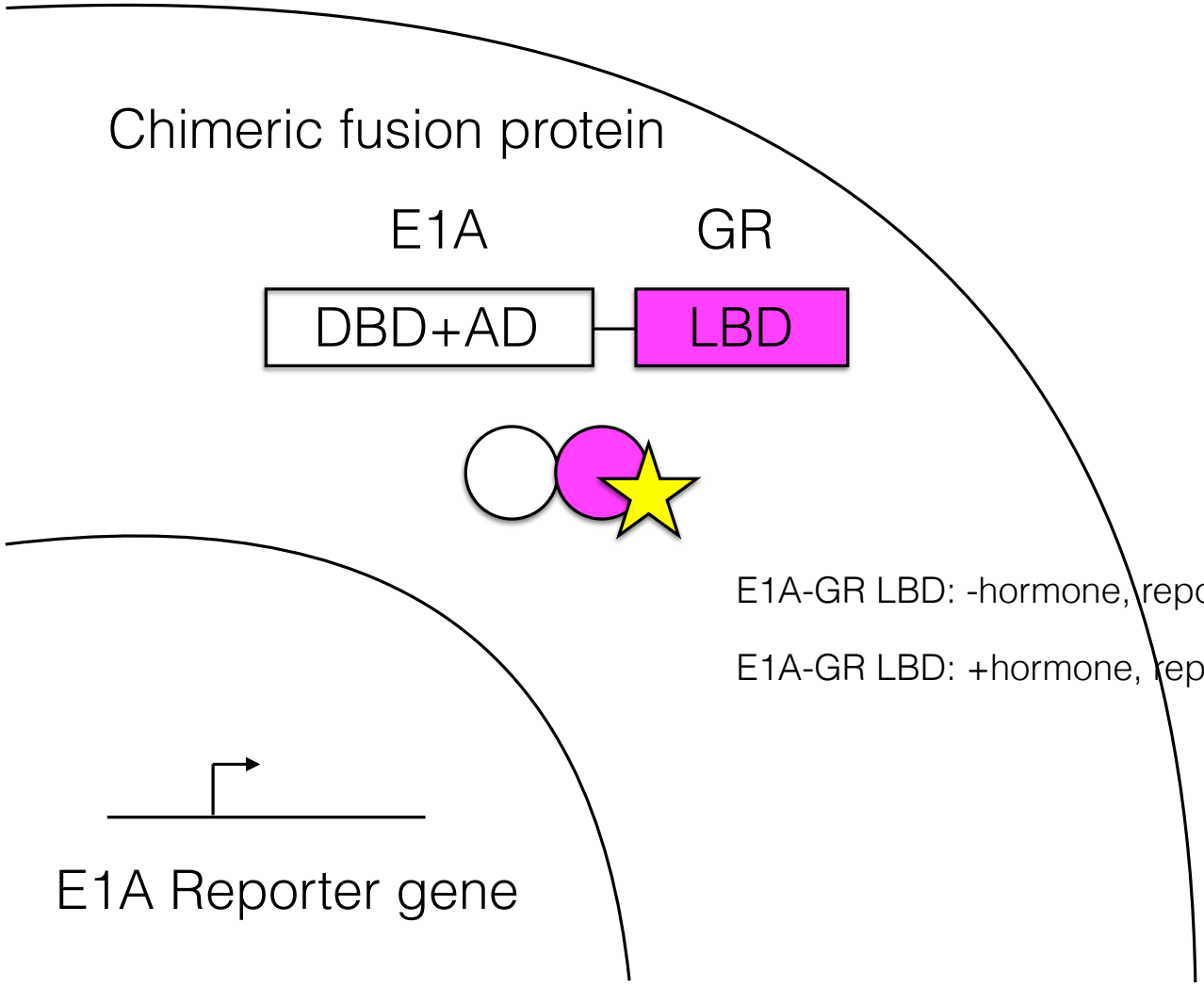
How do steroids get into cells?

- Gabi Monsalve's postdoc project in Yamamoto lab
- Can we use glucorticoid receptor (GR) ligand-binding domain to control protein function in worms?
- Currently a dearth of inducible promoters in *C. elegans*

Steroid receptor signaling



Steroid receptor LBD inactivation function



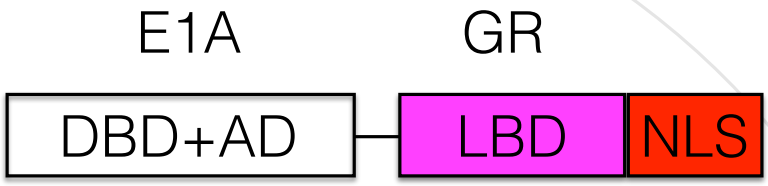
E1A-GR LBD: -hormone, reporter off

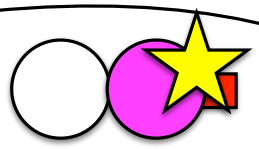
E1A-GR LBD: +hormone, reporter on

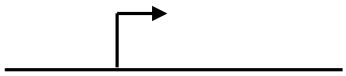
Picard et al., 1988

Steroid receptor LBD inactivation function

Chimeric fusion protein







E1A Reporter gene

E1A-GR LBD: -hormone, reporter off

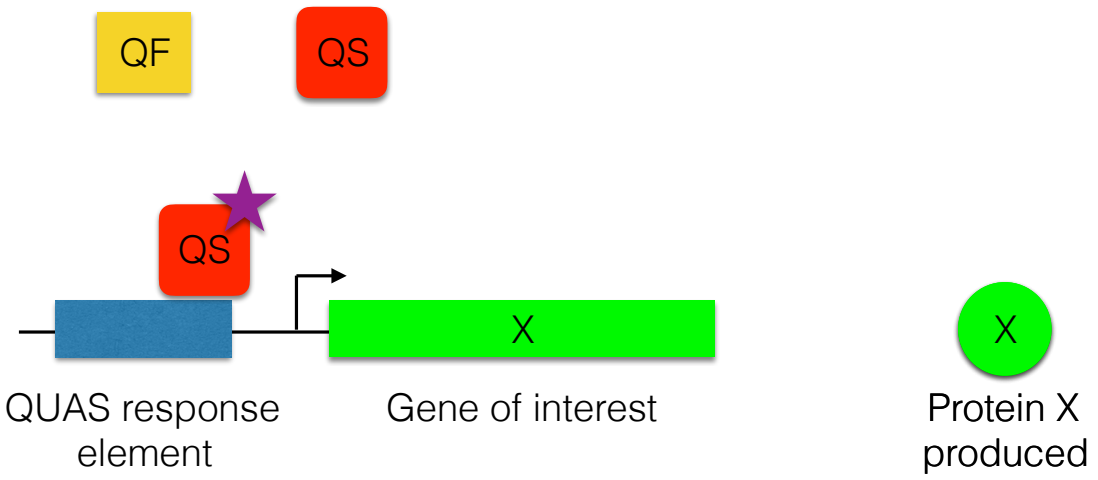
E1A-GR LBD: +hormone, reporter on

E1A-GR LBD-NLS: -hormone, reporter off

E1A-GR LBD-NLS: +hormone, reporter on

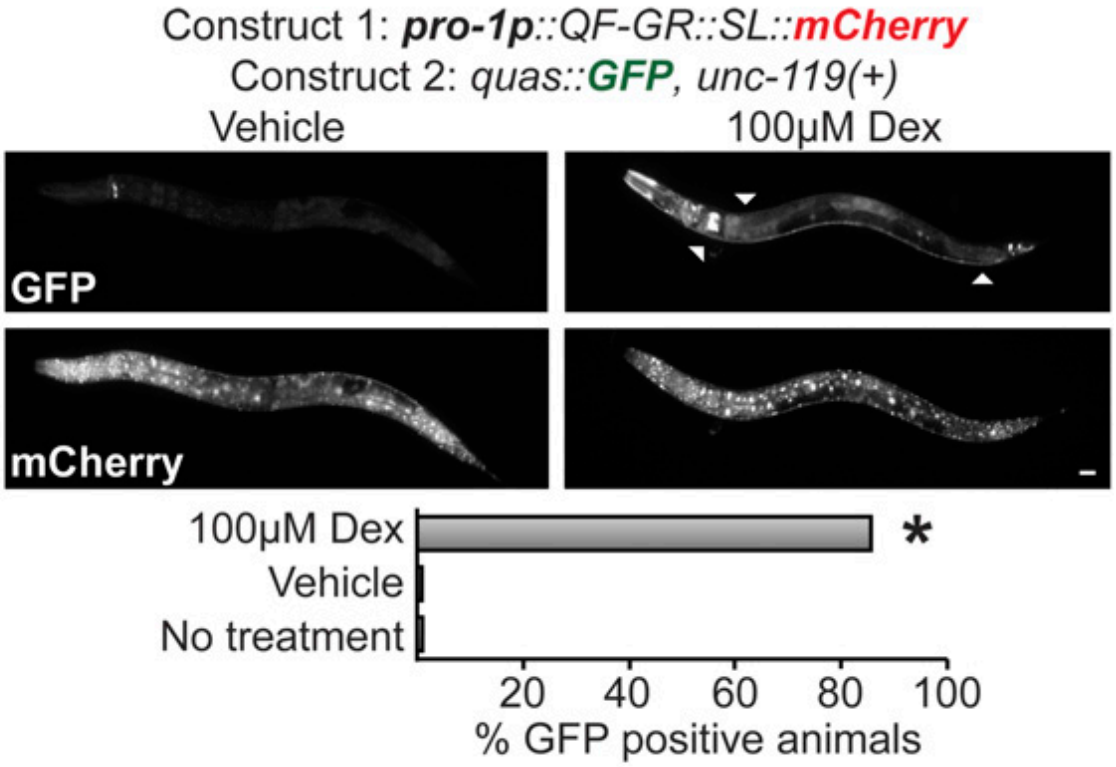
Picard et al., 1988

The Q bipartite gene expression system



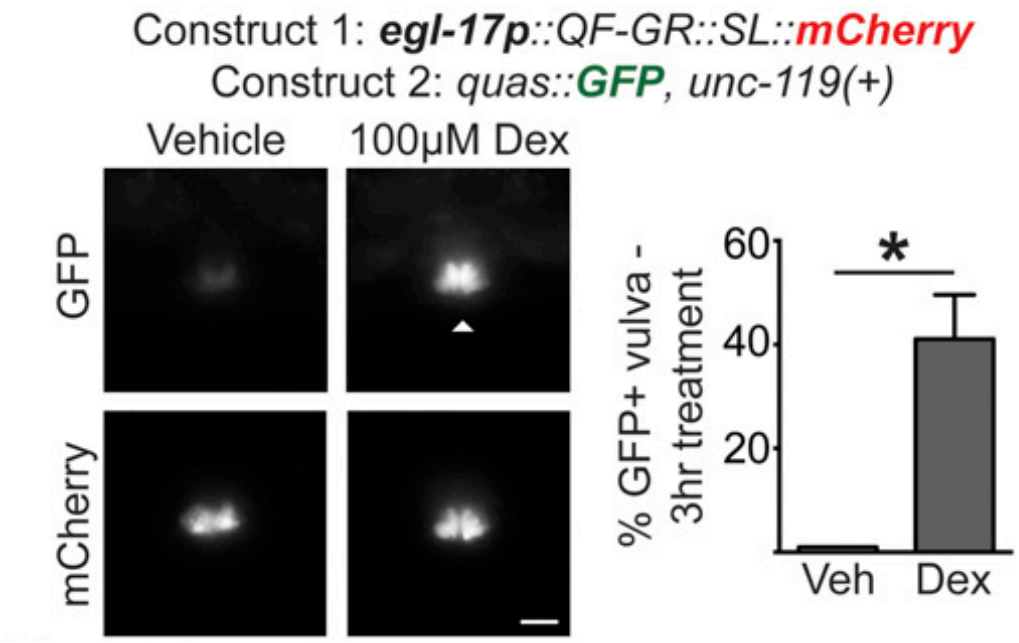
Wei et al., 2012

Q system is now ligand-inducible



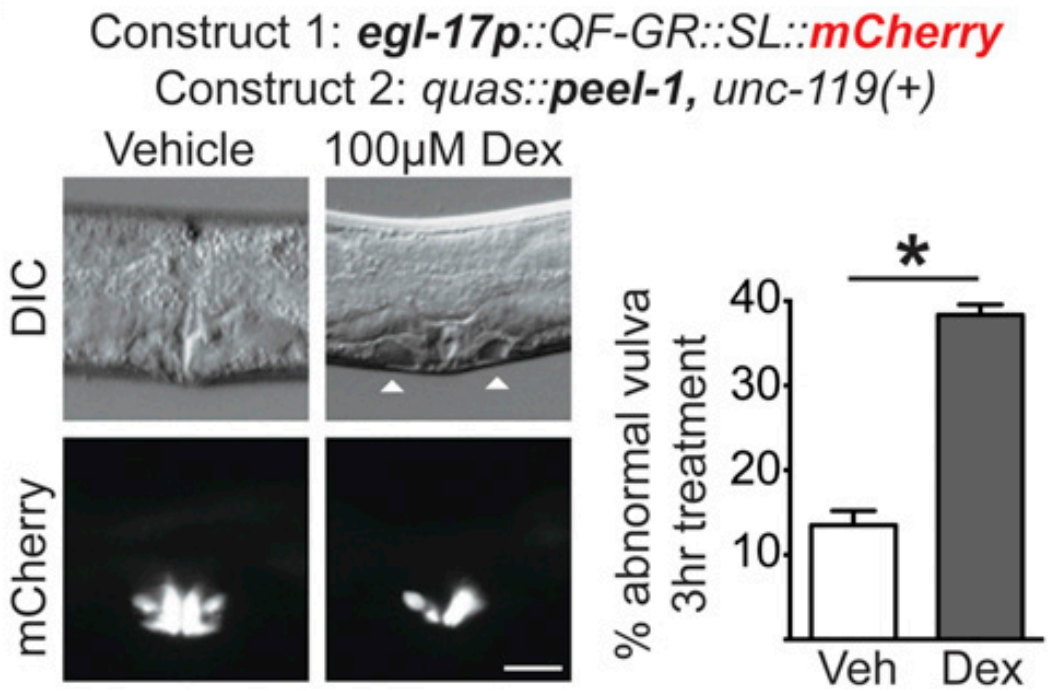
~ 2 hours till GFP is observed, plateaus at 8 hours

Tissue-specific, inducible gene expression



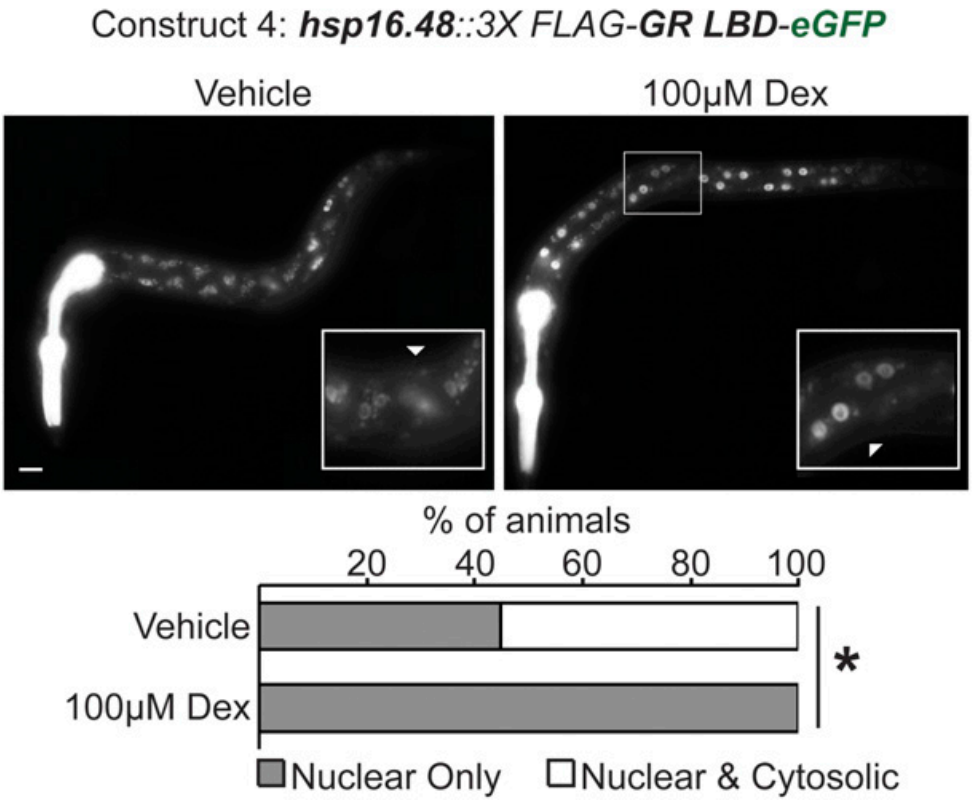
GFP in developing vulva

Inducible cell death by expressing a toxin

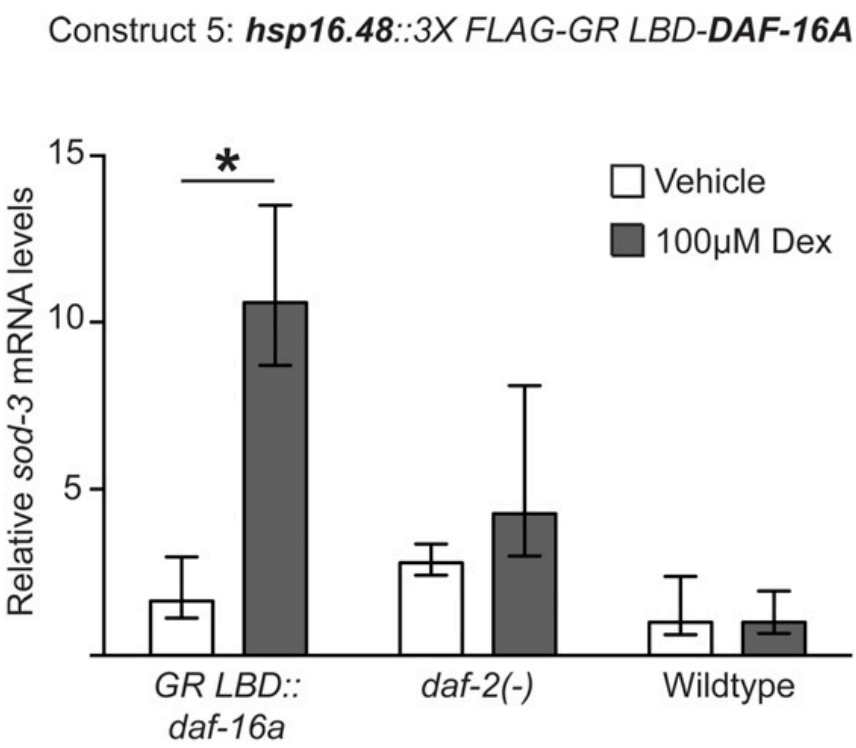


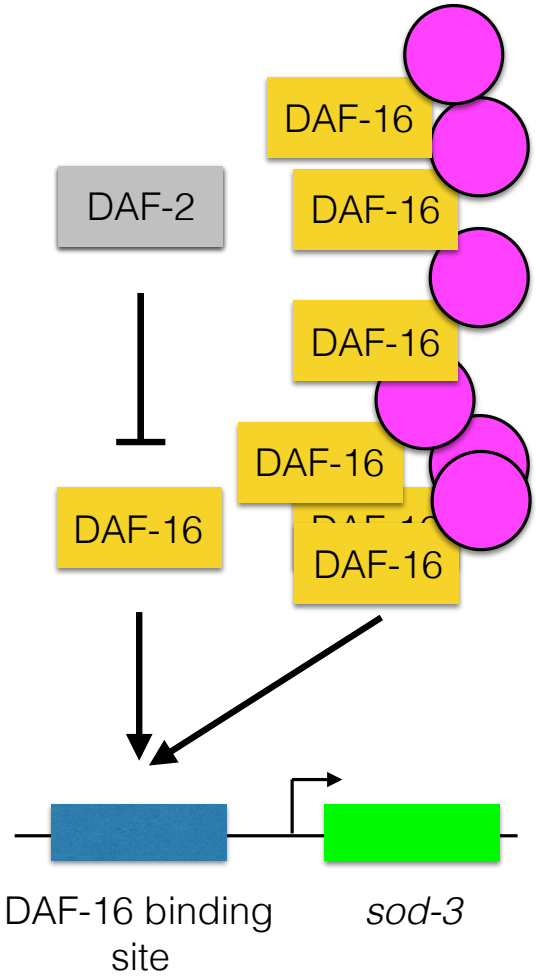
PEEL-1 is a sperm-derived toxin (selfish genetic element)

GR LBD works on other proteins



GR LBD renders DAF-16 ligand-inducible





Perspective

- makes Q system faster to activate
- useful for induction of transgenes when heat-shock is not ideal or for sustained expression
- need to continue optimization
- make other factors ligand-regulated?

Acknowledgements

- Gabi Monsalve
- Keith Yamamoto
- Yamamoto lab
- Ward lab

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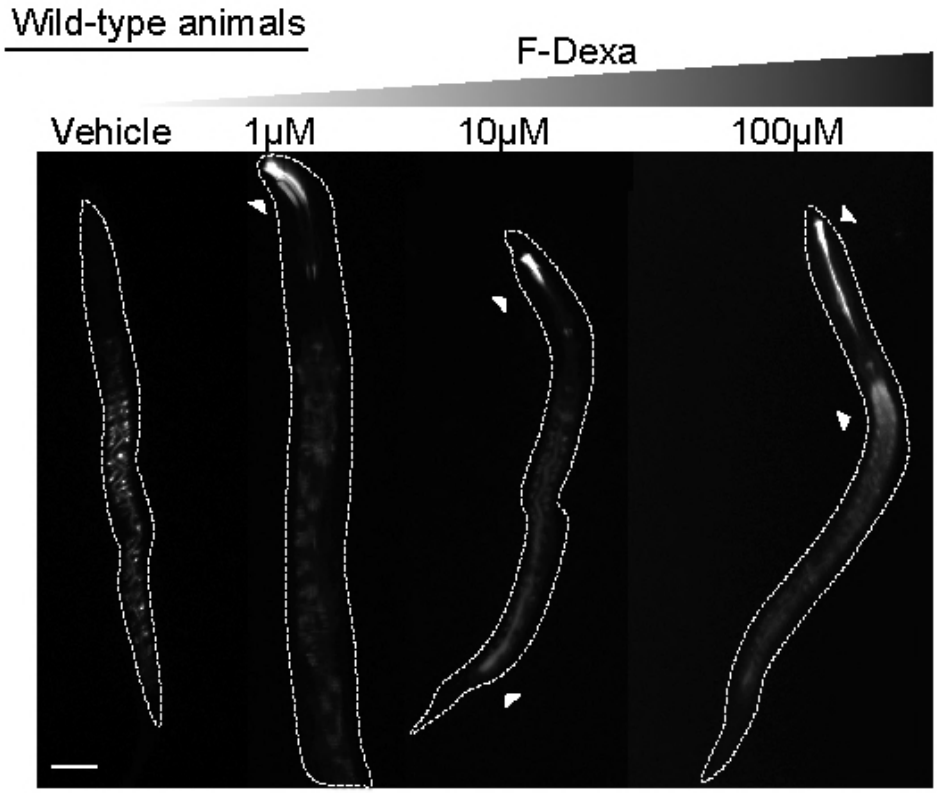
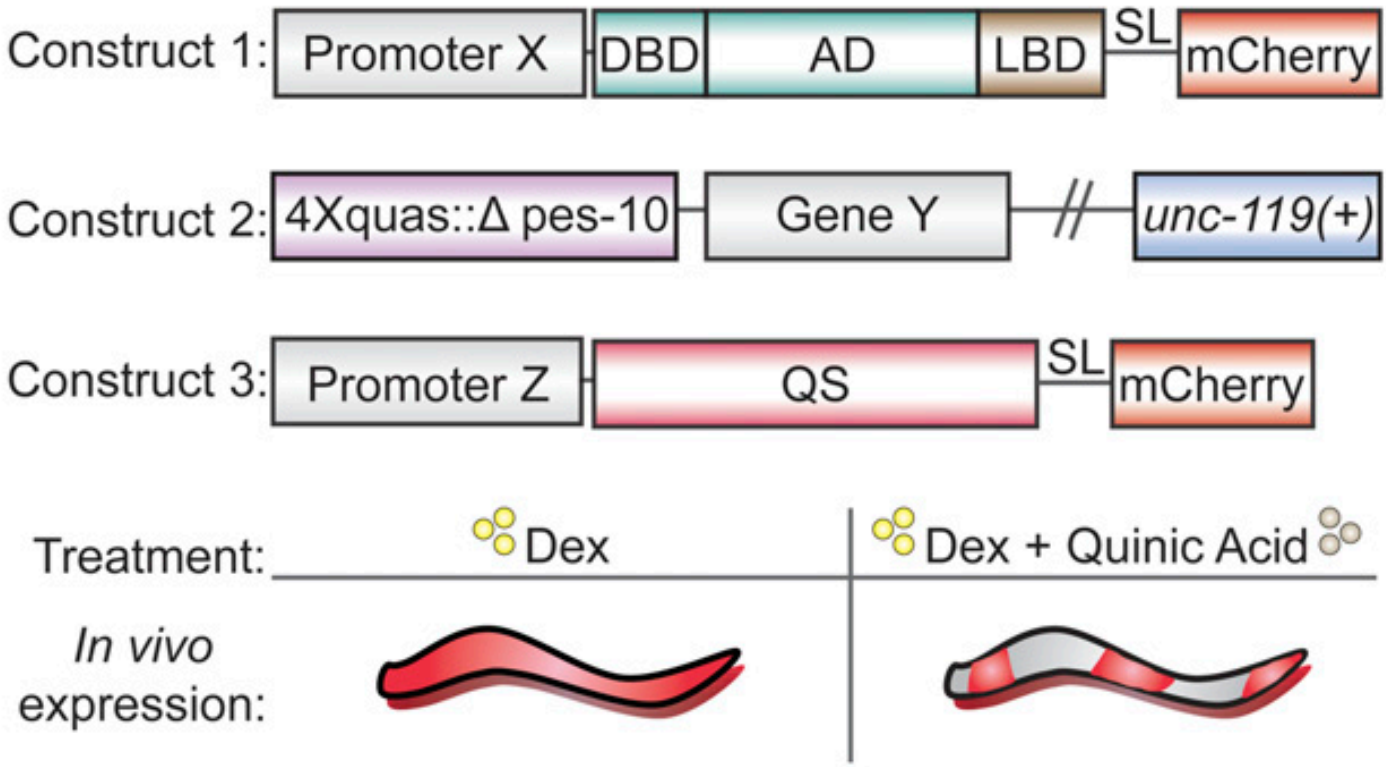
GR ligand dexamethasone (dex) gets into worms

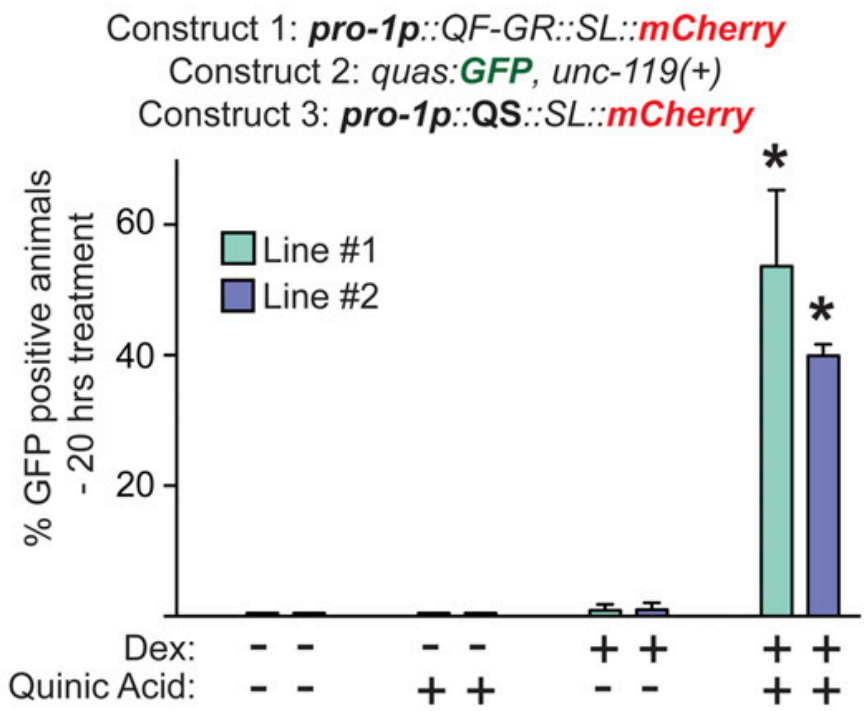


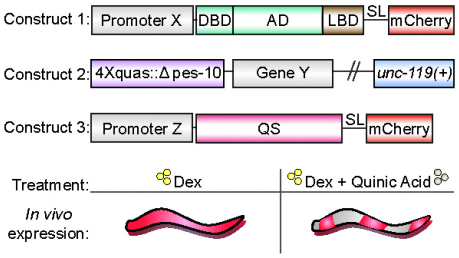
Reduce background with QS repressor



QS represses QF; quinic acid alleviates repression

Reduce background with QS repressor at a cost of slower response





Future directions

- did not see expression in germline, but were using arrays. Remove piRNA sites? Use PATC introns? Single copy?
- Where does leakiness come from (seen in flies too; promoter- and transgene-dependent)
- can make transcription factors drug-inducible (other nuclear proteins as well?)
- could add to other useful systems (cGal system, Flp and Cre recombinases, Cas9)

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