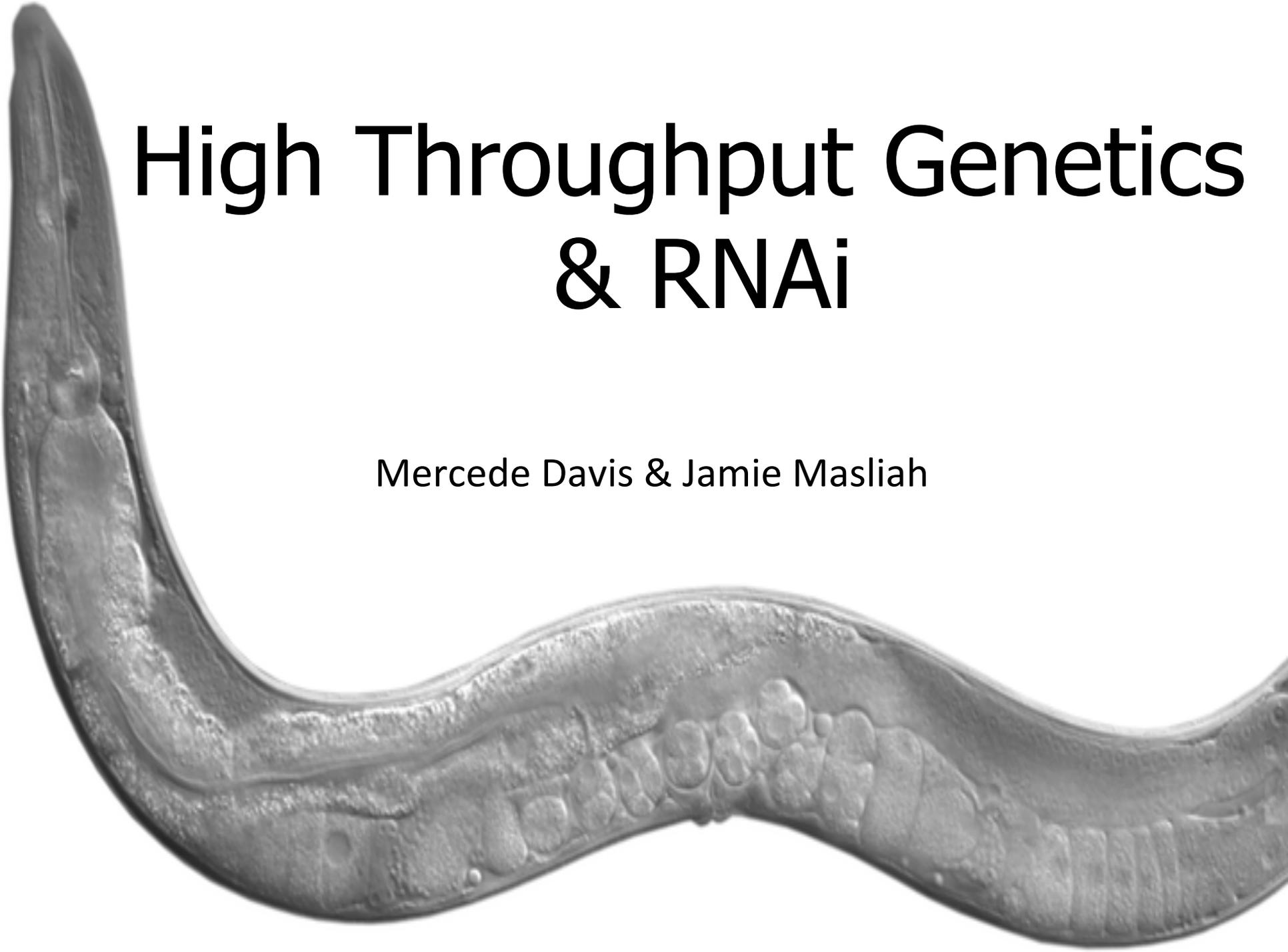


High Throughput Genetics & RNAi

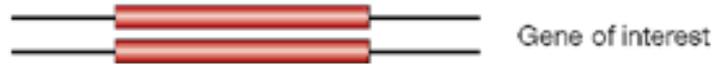
Mercede Davis & Jamie Masliah



What is RNAi?

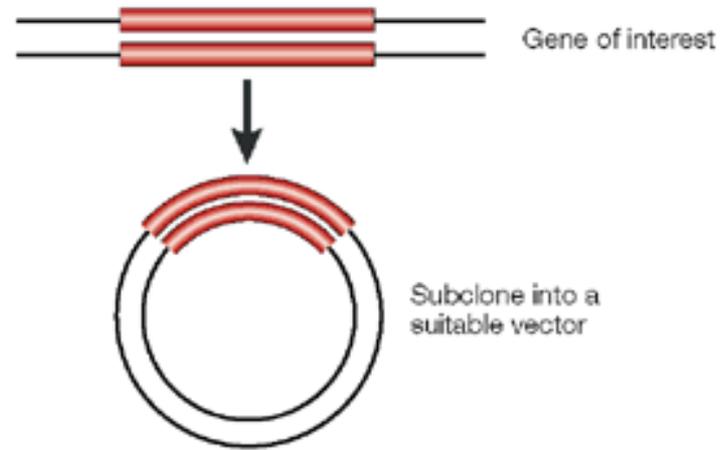
How is RNAi used in *C. elegans*?

a

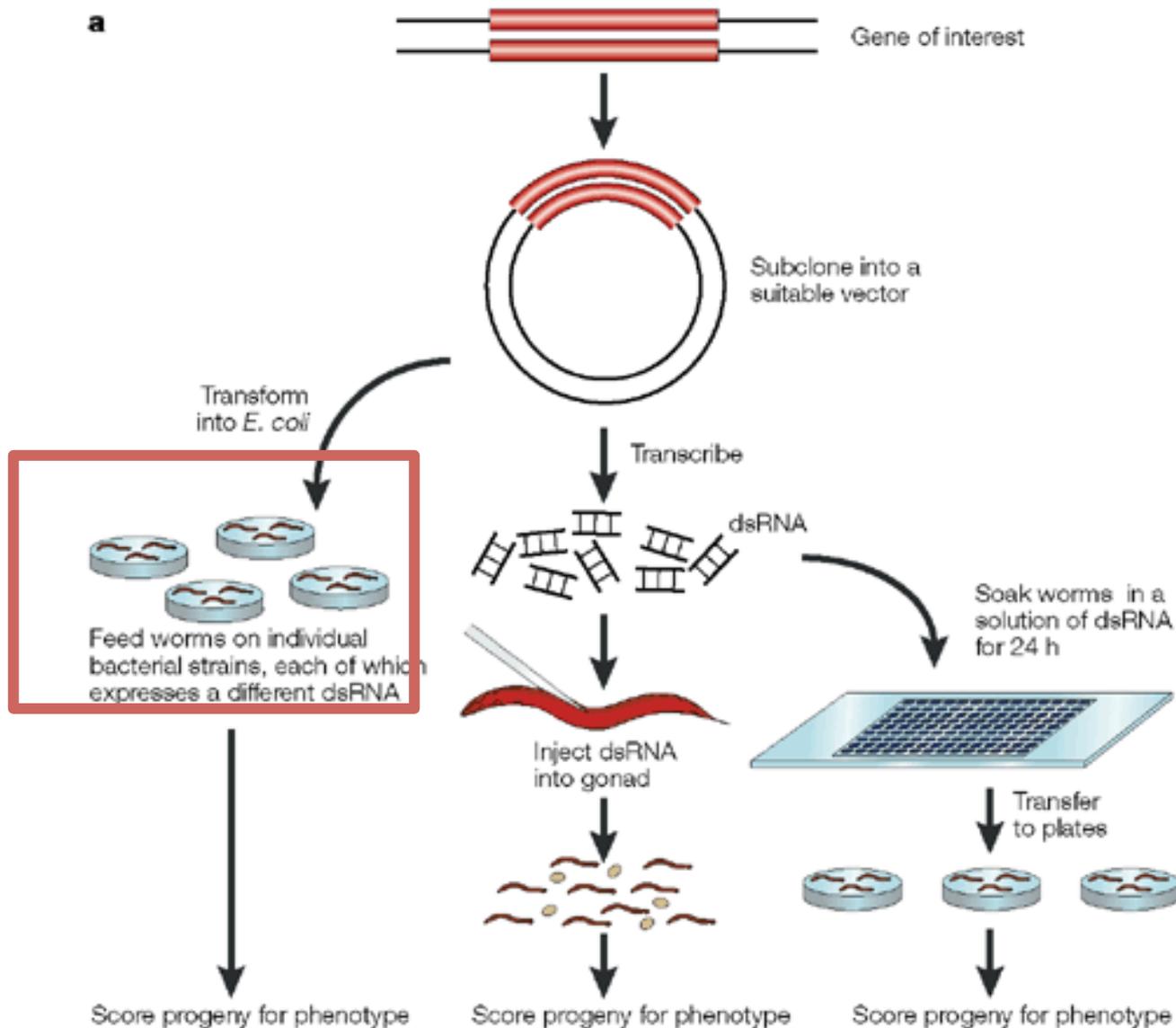


How is the gene of interest made into dsRNA?

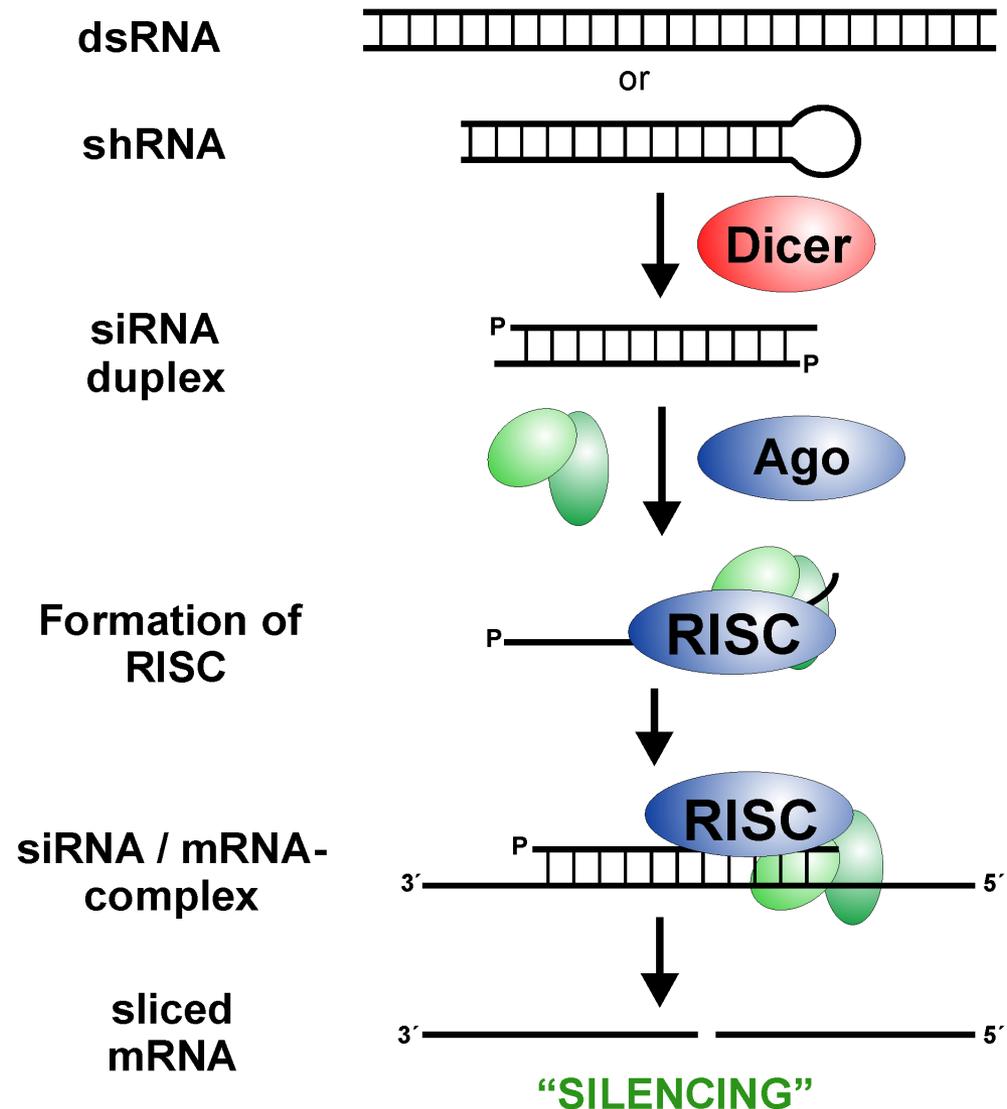
a



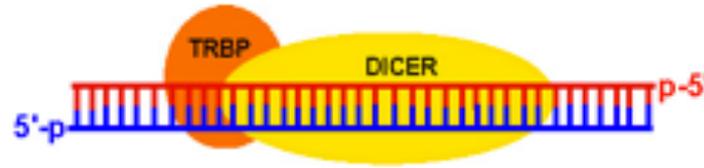
How is dsRNA taken up by *C. elegans*?



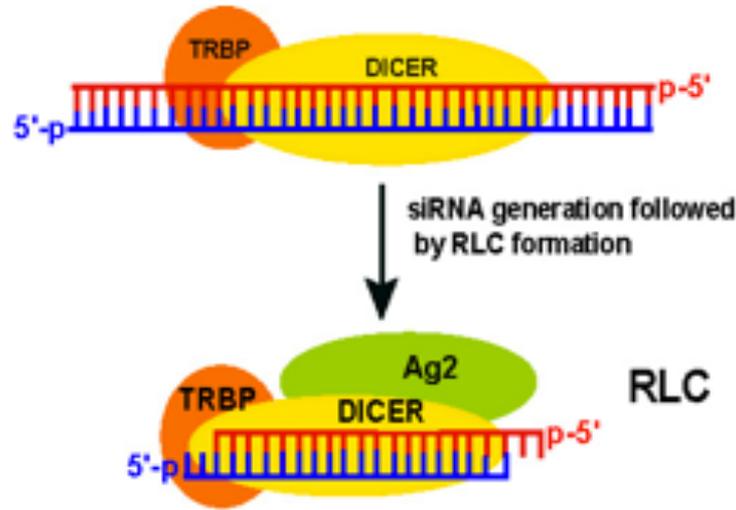
What is the mechanism for RNAi silencing?



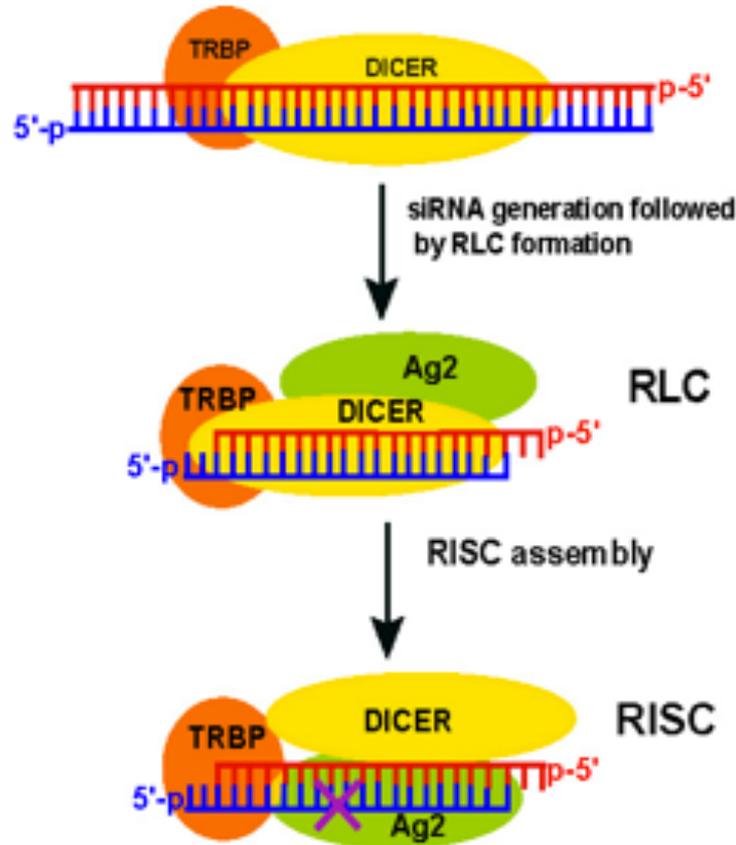
How does RISC form?



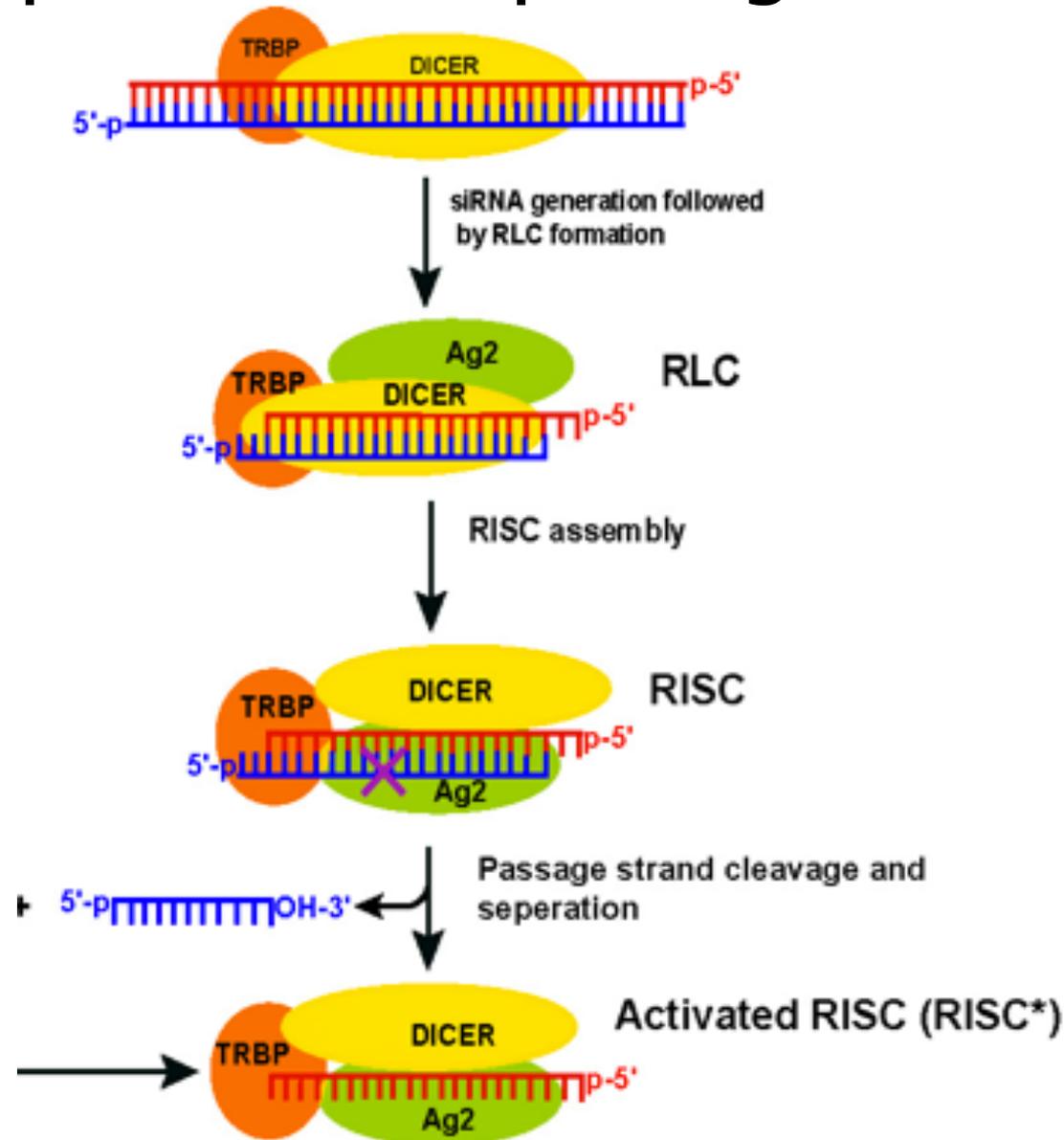
What happens after DICER cuts the dsRNA?



What happens once argonaute binds?



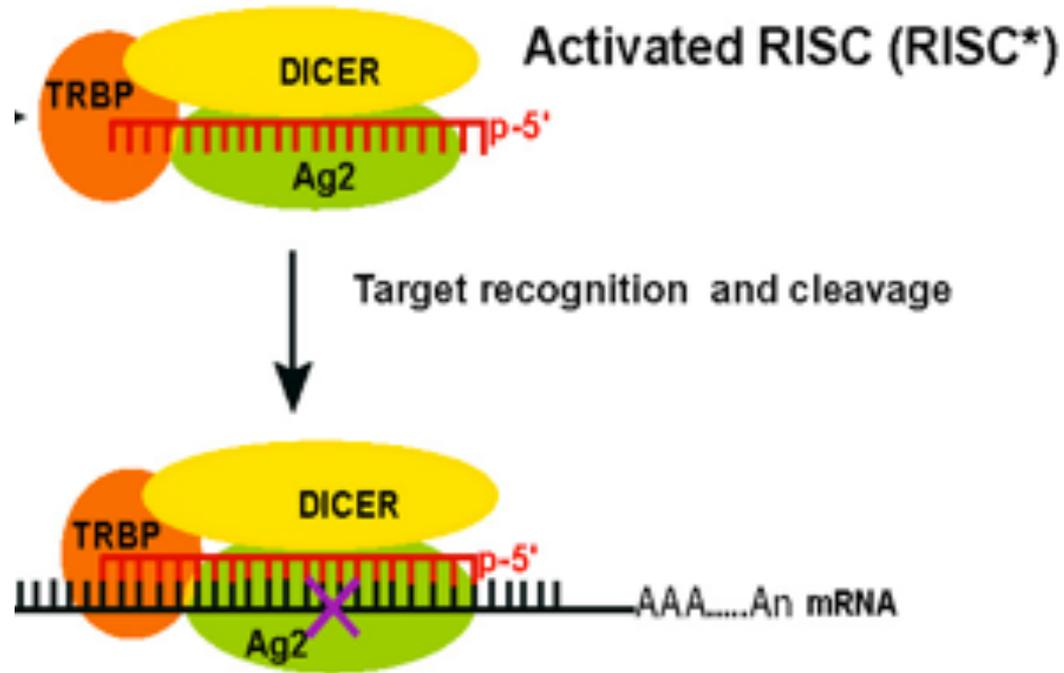
What happens to the passage RNA strand?



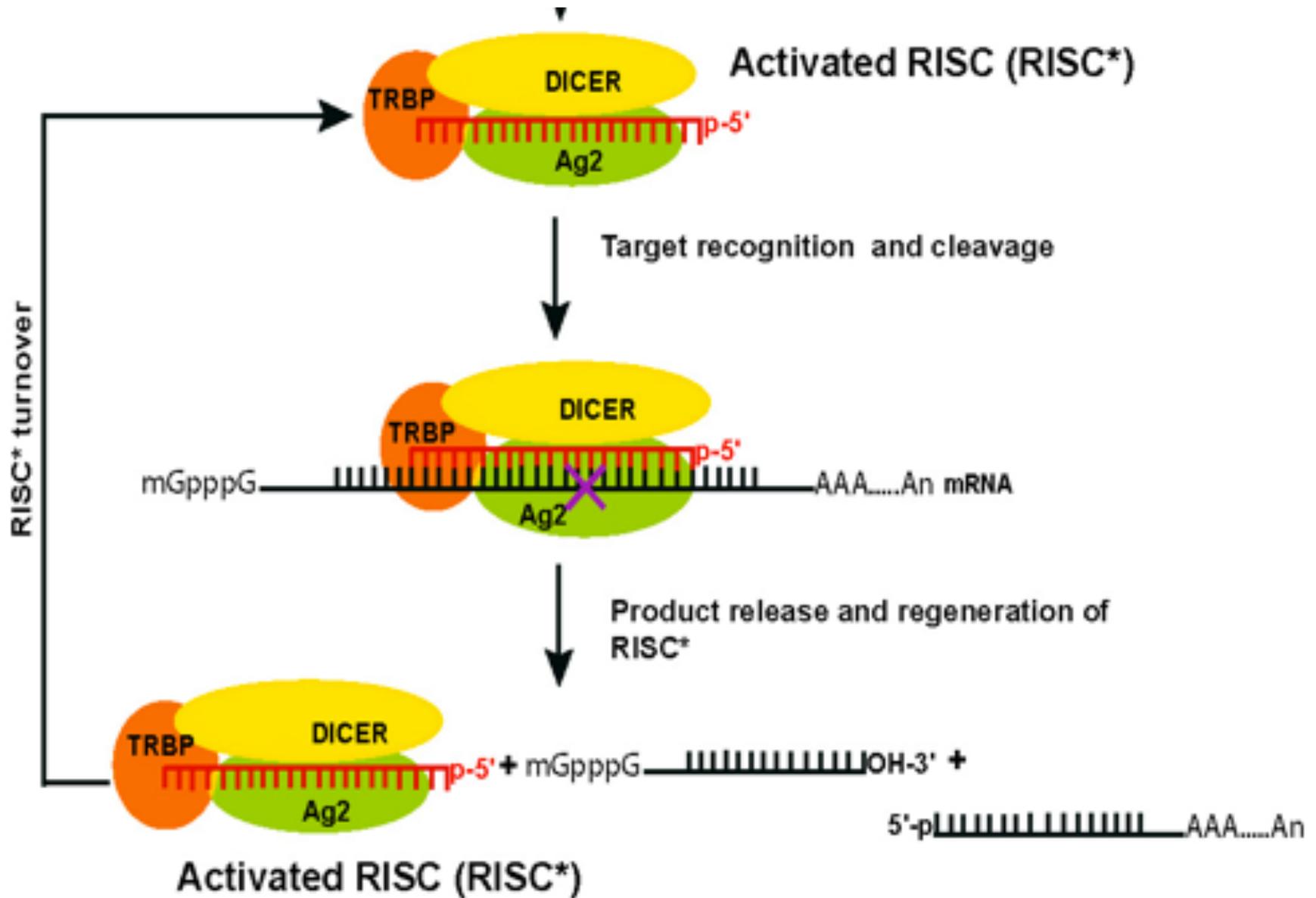
How does RISC degrade mRNA?

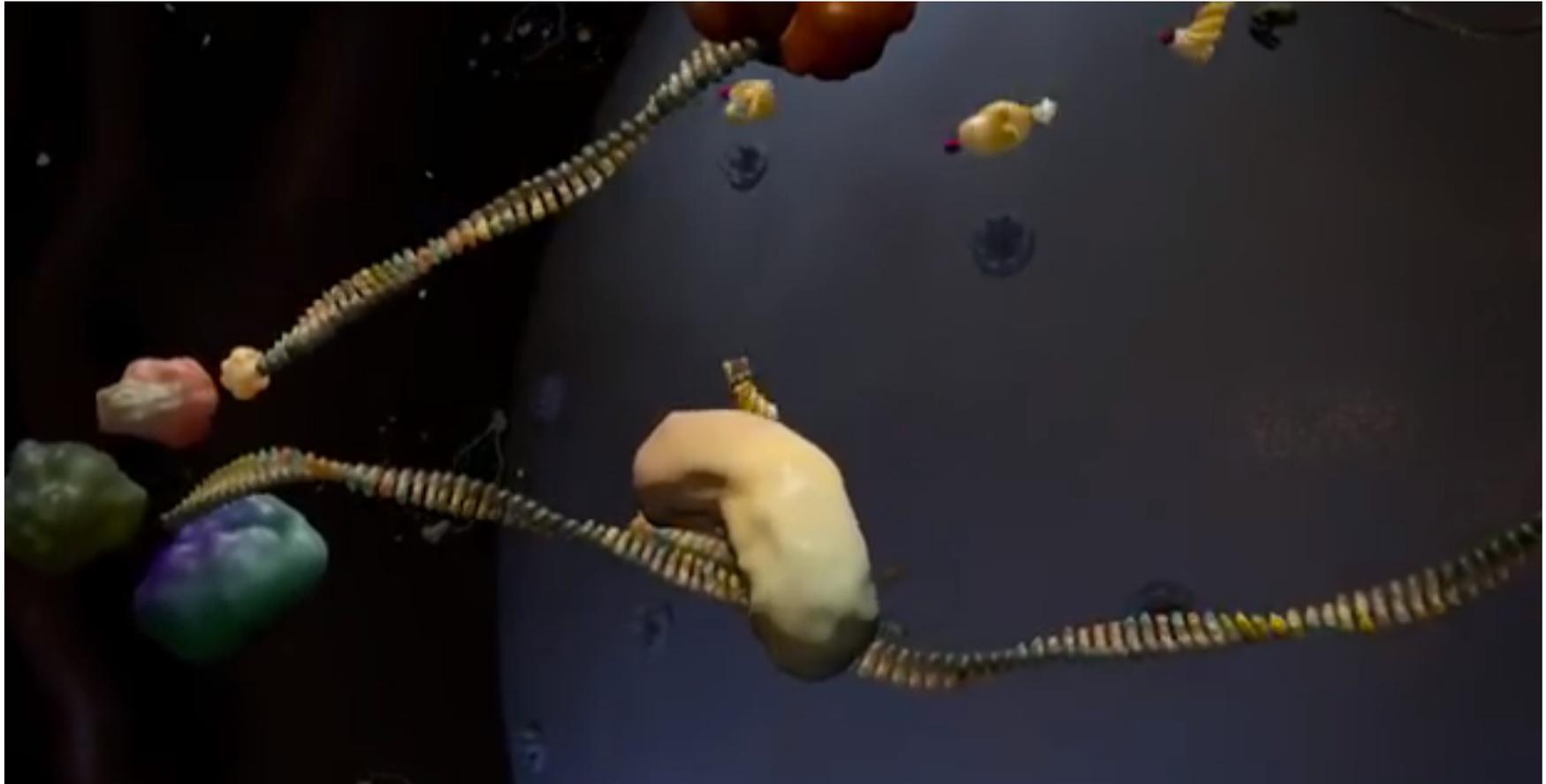


How does RISC find the correct mRNA?



What happens after RISC binds to mRNA?

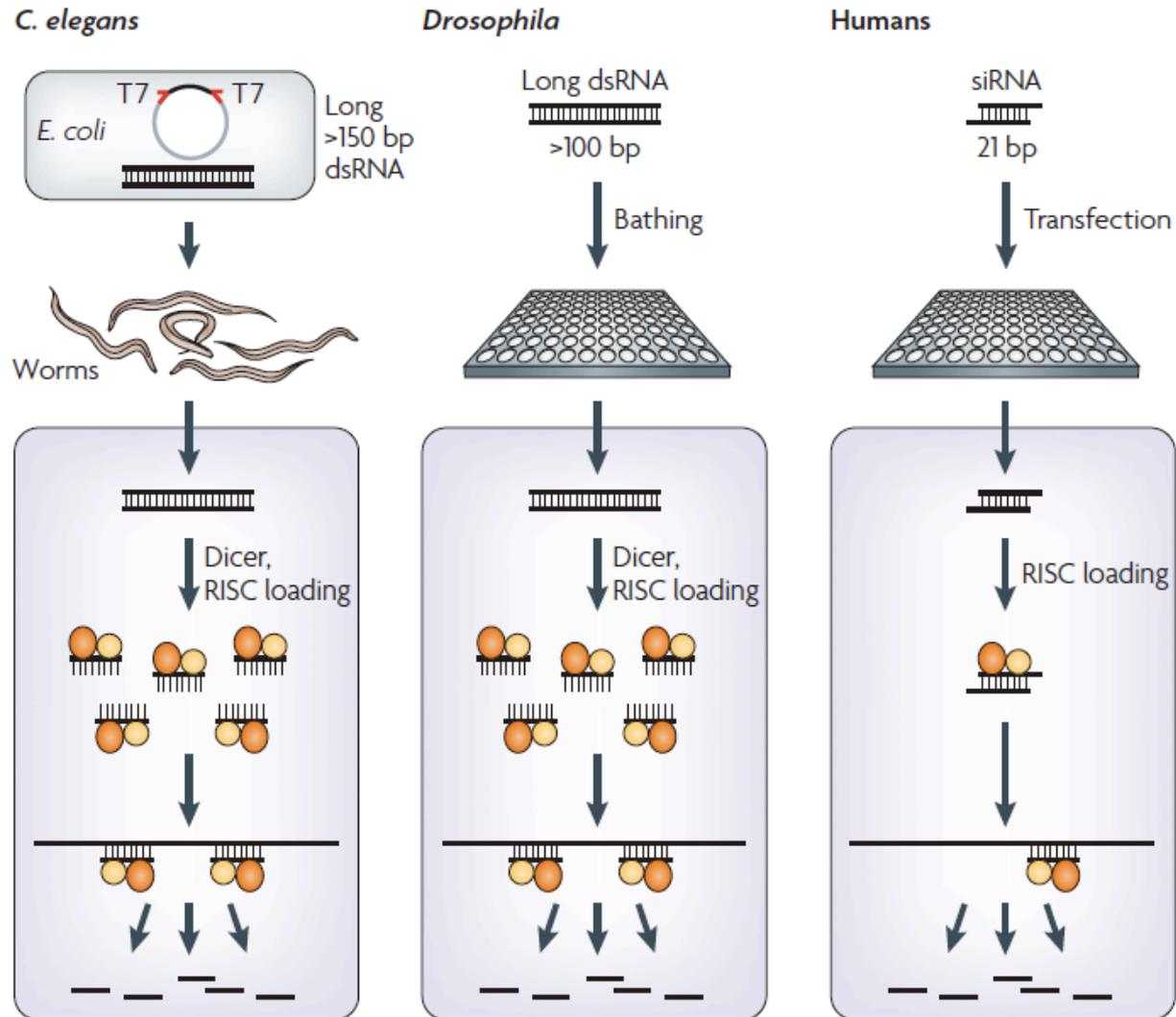




http://www.youtube.com/watch?v=cK-OGB1_ELE

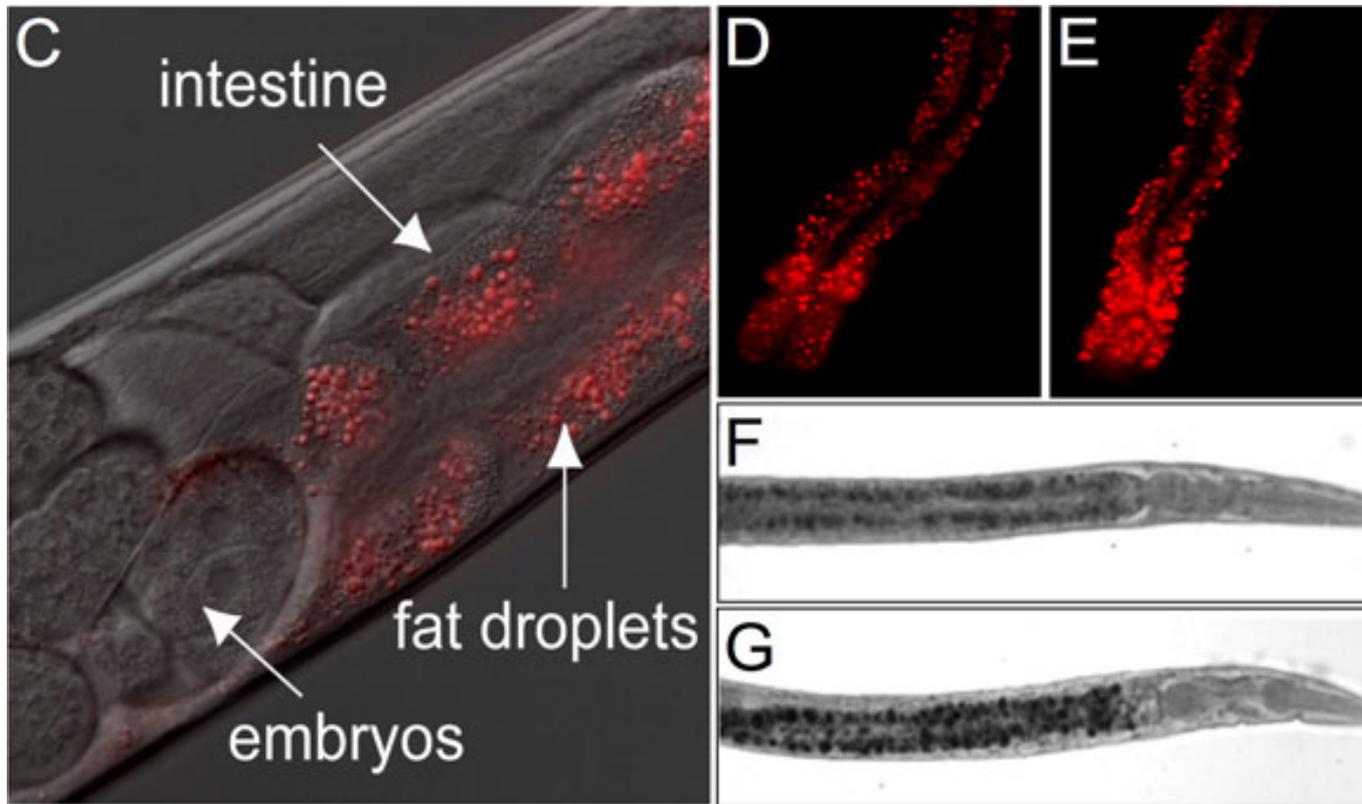
How is RNAi a useful tool in screens?

How is RNAi used in “genome-wide” screens?



Why is *C. elegans* a good model organism for RNAi screens?

Why is *C. elegans* a good model organism for RNAi screens?





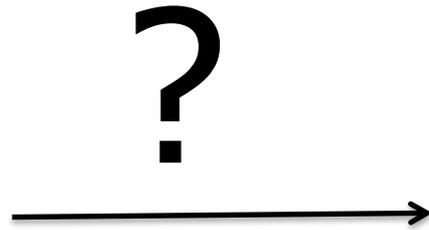
Genome-wide RNAi analysis of
Caenorhabditis elegans fat
regulatory genes

Ashrafi K., et al. (2003) Nature

Why is obesity a problem?



How can we study obesity?



Are there obesity genes in *C. elegans*?

Are there obesity genes in *C. elegans*?

Mammals

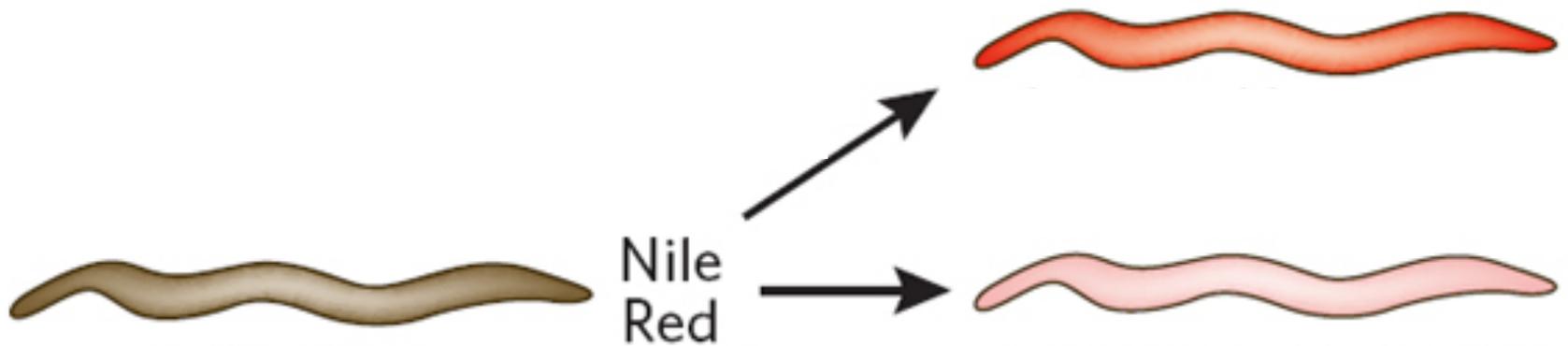
C. elegans

Tubby  *tub-1(nr2004)*

Serotonin receptors  *tph-1(mg280)*

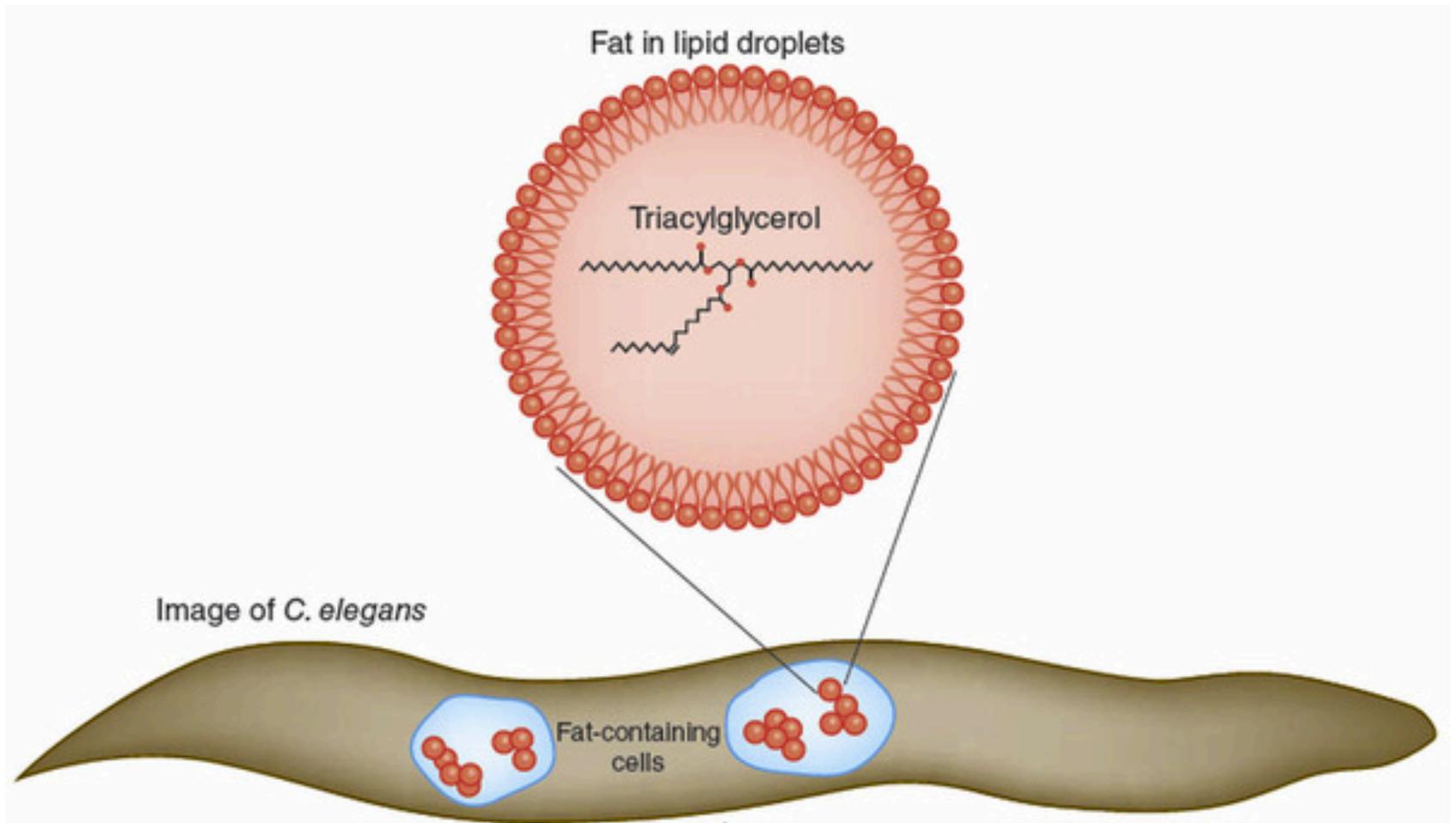
Insulin-like signals  *daf-2(e1370)*

What was used to visualize the phenotype?

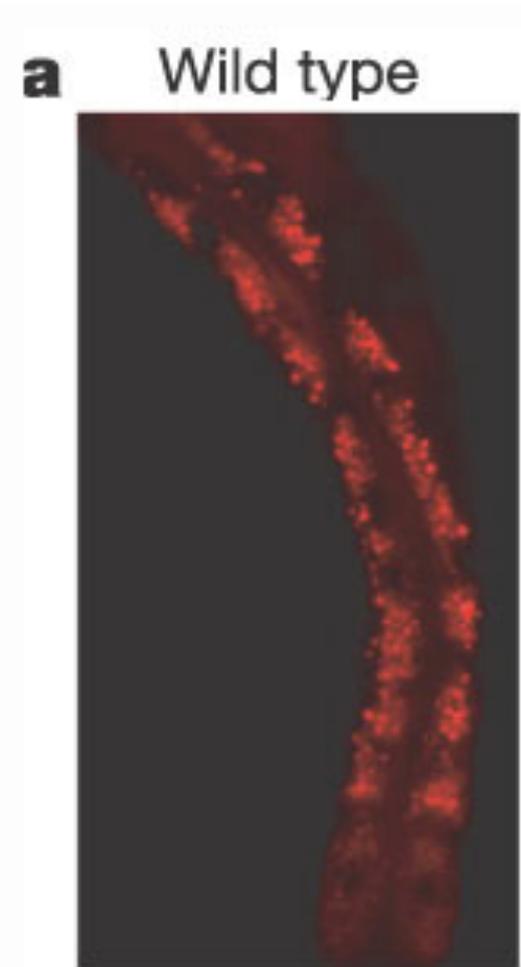


- Through feeding
- Localized to intestinal cells
- No effect on growth rate

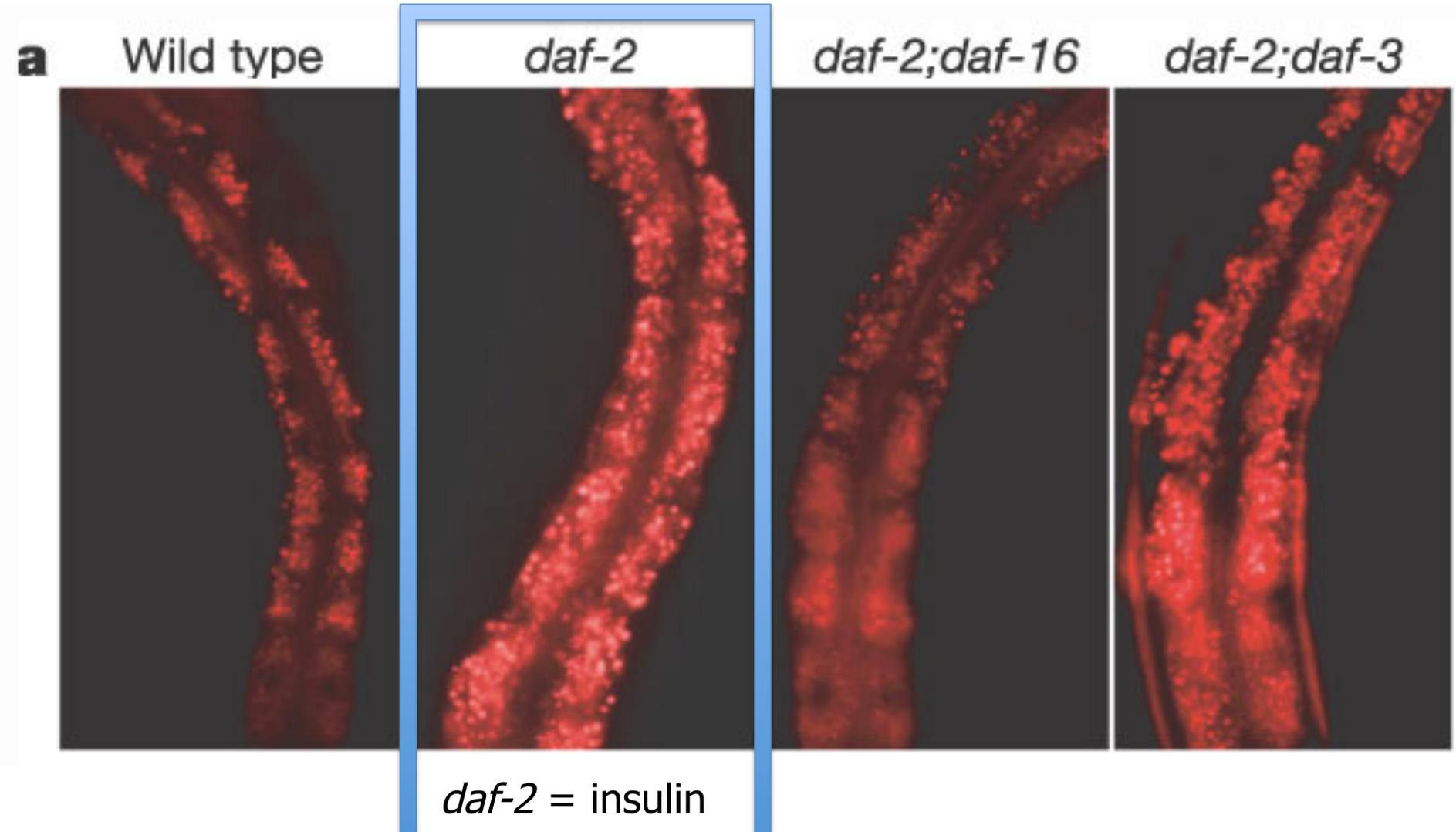
Where does Nile Red go?



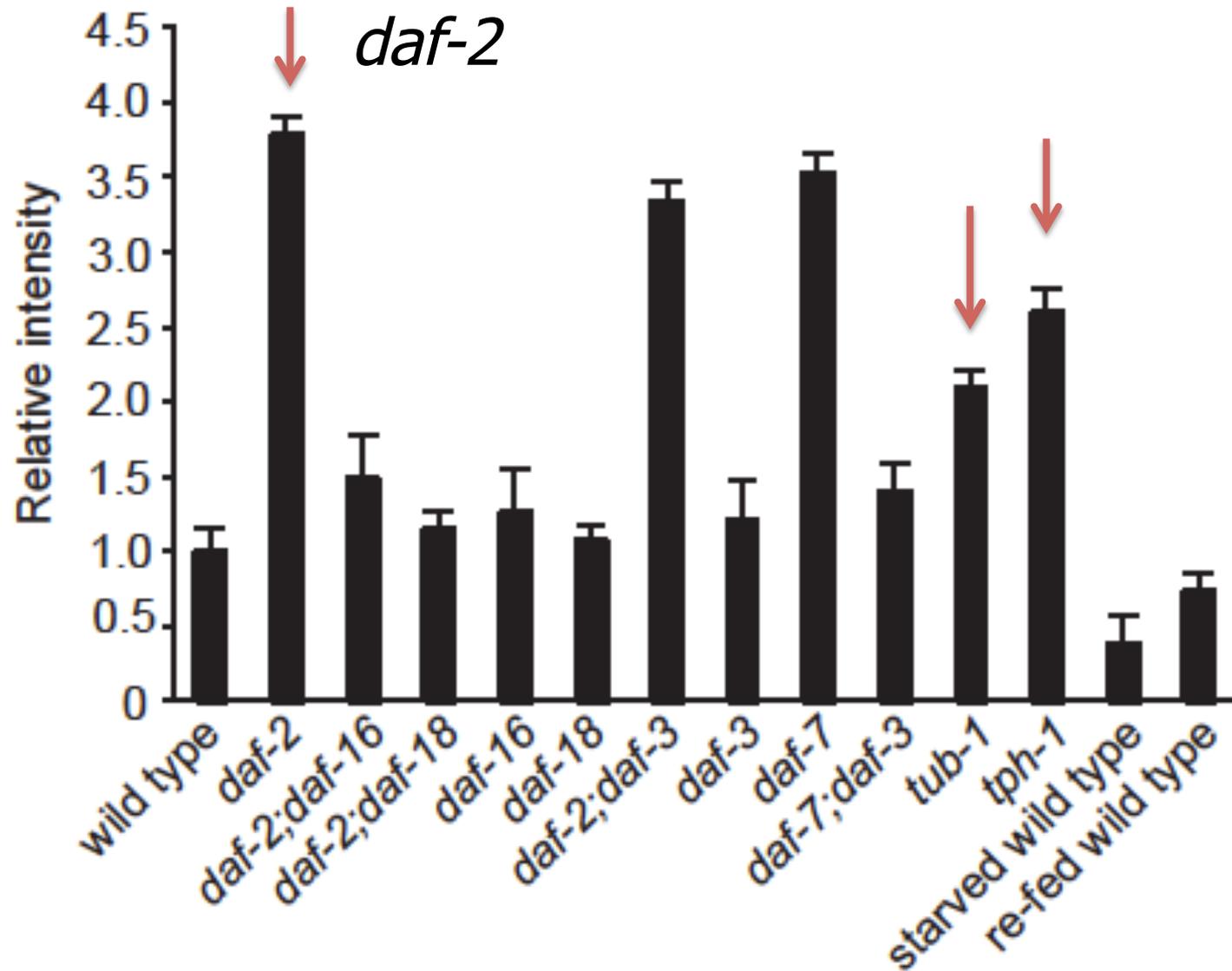
How do you visualize fat storage in *C. elegans*?



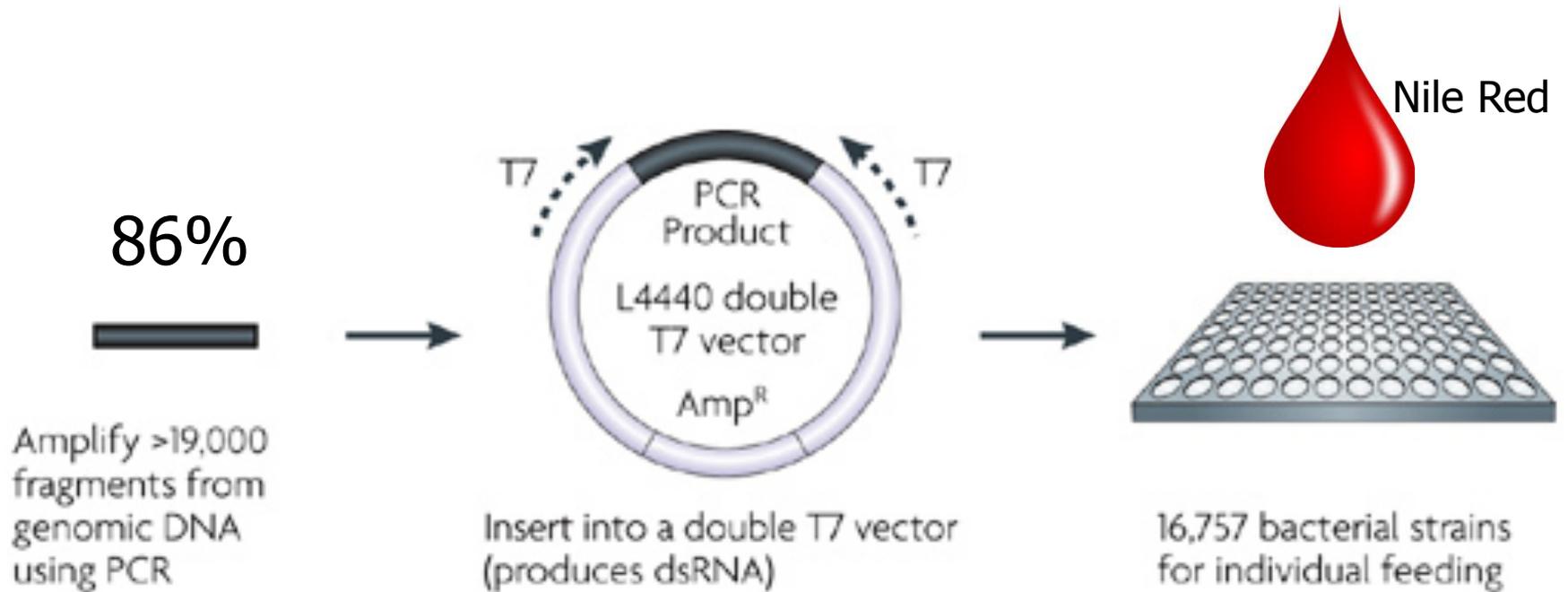
How do you visualize increased fat storage?



Can you quantify the Nile Red?



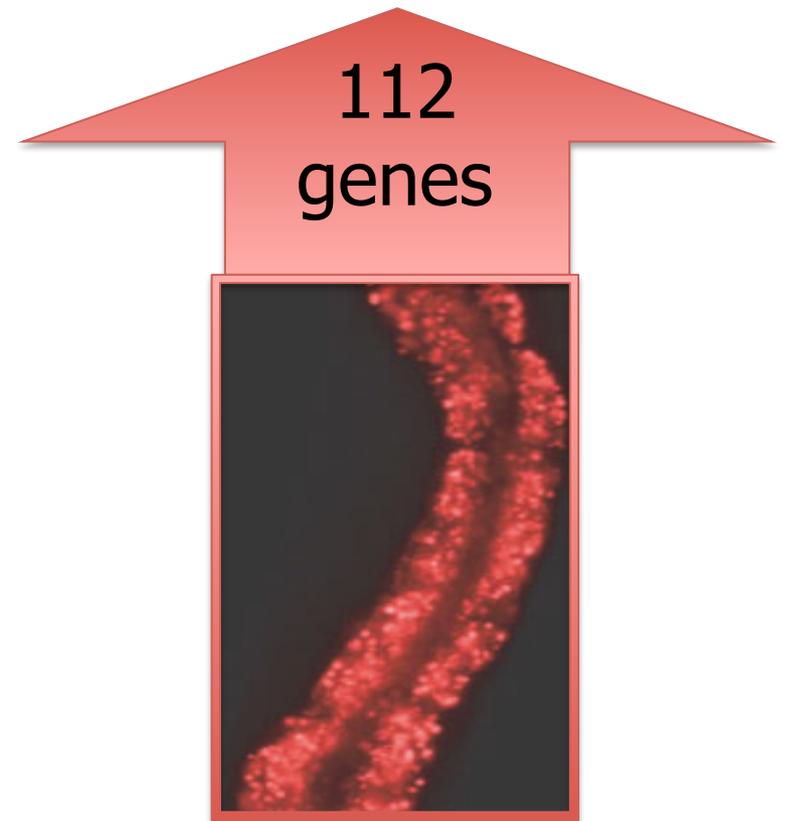
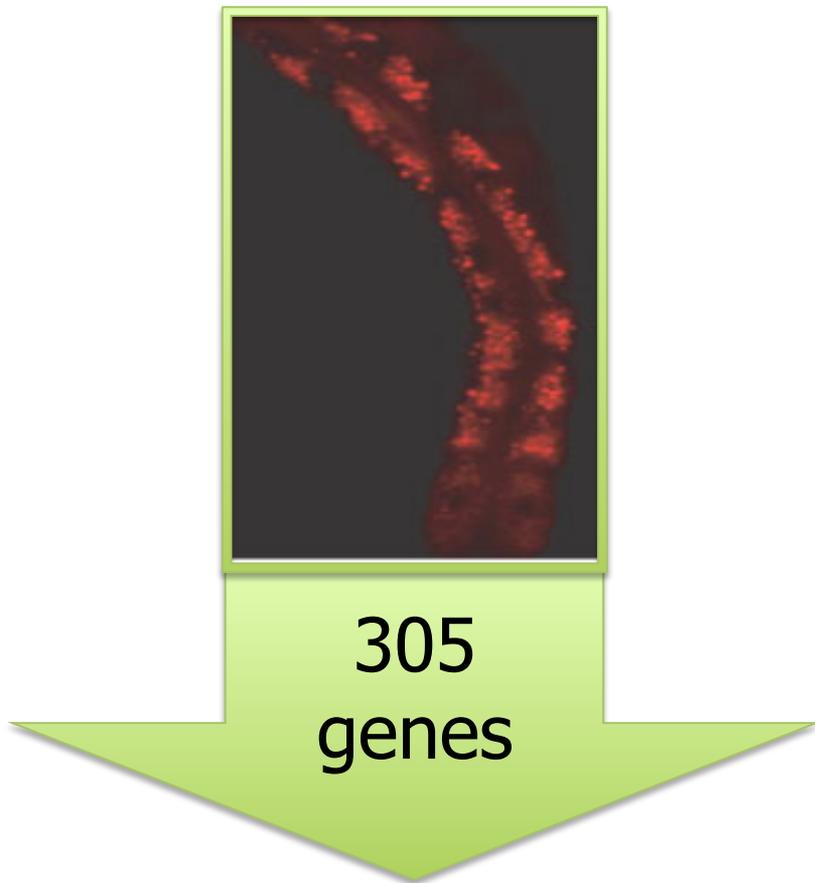
How was the RNAi screen done?



Nature Reviews | **Genetics**

Non-biased RNAi feeding

What were the results of the screen?



What are the three GO categories?

Which genes showed a decrease?

Table 1 RNAi clones that reduce fat in wild type and high-fat mutants

GenePairs*	Description	WT	daf-2	tph-1	tub-1	GenePairs*	Description	WT	daf-2	tph-1	tub-1
Metabolic enzyme						Transporter					
F52B11.2	Phosphom	Y	Y			C37A5.1	Ion channel	Y	Y		
Y55F3C.c	Thioredoxi	Y	Y			ZK682.2	Sugar transporter	Y	Y		
Y49A3A.1	OE phosphotransferase	Y	Y			K05F1.6	OCT1 organic carrier	Y	Y		
C06E7.3	S-adenosylmethionine synthase	Y	Y	Y	Y	C13D9.7	Na ⁺ /Ca ²⁺ exchanger	Y	Y		Y
C36A4.9	Acetyl-CoA synthetase	Y	Y	Y	Y	C32C4.1	Potassium channel	Y	Y		Y
F08F8.2	HMG-CoA reductase	Y	Y	Y	Y	F15H10.4	Lysosomal transporter	Y	Y	Y	Y
K10B3.7	GAPDH	Y	Y	Y	Y	K04E7.2	PepT1 symporter	Y	Y	Y	Y
F11E6.5	Fatty acid elongase	Y	Y	Y	Y	C34G6.4	ABC transporter	Y	Y	Y	Y
F13D11.1	Lysosomal acid phosphatase	Y	Y	Y	Y	Energy metabolism					
K07C6.5	Cytochrome P450 2C2	Y	Y	Y	Y	F20D1.9	Uncoupling protein	Y	Y		
K09D9.2	Cytochrome P450	Y	Y	Y	Y	F28H6.2	Energy transfer protein	Y	Y		Y
Fat/lipid interacting						Vesicular transport					
R07B7.9	Brush border esterase/lipase	Y	Y			F11A5.3	RAB2	Y	Y		
F13D12.6	Esterase/lipase	Y	Y	Y	Y	R01H2.3	Sortilin				Y
Transcription factor						F58G6.1	Clathrin ada				Y
ZK686.4	Zinc-finger C ₂ H ₂ type	Y	Y			Protein degradation					
F11A1.3	NHR (dihydroxyvitamin D3)	Y	Y			Y55B4B10.e	Ubiquitin protein ligase	Y	Y		
T09F3.1	Zinc-finger C ₂ H ₂ type	Y	Y			F49E12.4	Ubiquitin-conjugating enzyme	Y	Y	Y	
K08A2.b	NHR (HNF-4α)	Y	Y			C49C3.3	Ubiquitin family	Y	Y	Y	Y
F22A3.5	Pre-B-cell leukaemia factor	Y	Y			Other					
Y47B3C.7	SREBP			Y	Y	Y77E11A	Collagen	Y	Y		
Signal transduction						C06G3.2	Kinesin motor domain	Y	Y	Y	
C23H5.17	D111/G-pa	Y	Y			R04A9.4	Translation initiation	Y	Y	Y	
T04D3.2	EF-hand family domain	Y	Y			No function assigned					
ZC302.1	Serine/threonine protein phosphatase	Y	Y			Y24D9A.b	Unknown	Y	Y		
ZC504.4	Tyrosine and serine/threonine kinase	Y	Y			B0034.2	Unknown	Y	Y		
T19D2.2	Dual specificity phosphatase	Y	Y	Y		C30G4.5	Unknown	Y	Y		
F46G11.3	Protein kinase	Y	Y	Y	Y	T10D4.1	Unknown	Y	Y		
M01B12.5	Tyrosine kinase	Y	Y	Y	Y	R08F11.2	Unknown	Y	Y		
ZK675.1	Patched	Y	Y	Y	Y	K12D12.4	Unknown	Y	Y		
Receptor						B0554.7	Unknown	Y	Y		
H09F14.1	GPCR	Y	Y			F46F5.10	Unknown	Y	Y		
C38C10.1	Neurokinin-type receptor	Y	Y			F10A3.11	Unknown	Y	Y		
F07C4.1	GPCR	Y	Y	Y	Y	B0554.6	Unknown	Y	Y	Y	
T07C12.5	GPCR	Y	Y	Y	Y	T01D3.4	Unknown	Y	Y		Y
T14E8.3	Dopamine receptor	Y	Y	Y	Y	C18E9.5	Unknown	Y	Y		Y
Y40H7A.7	SRA family chemoreceptor	Y	Y	Y	Y	K09H11.2	Unknown	Y	Y	Y	Y
Neuronal						ZK131.8	Unknown	Y	Y	Y	Y
H27A22.1	Glutaminyl cyclase	Y	Y			F52C6.12	Unknown	Y	Y	Y	Y
T19B4.7	DCC/axon guidance	Y	Y			F59F5.2	Unknown	Y	Y	Y	Y

Clones were tested on wild-type (WT), *daf-2(e1370)*, *tph-1(mg280)* and *tub-1(nr2004)* animals. 'Y' indicates that RNAi caused a reduced fat phenotype in the fat content of the test strain. ABC, ATP-binding cassette; NHR, nuclear hormone receptor; GPCR, G-protein-coupled receptor; DCC, deleted in colorectal cancer.

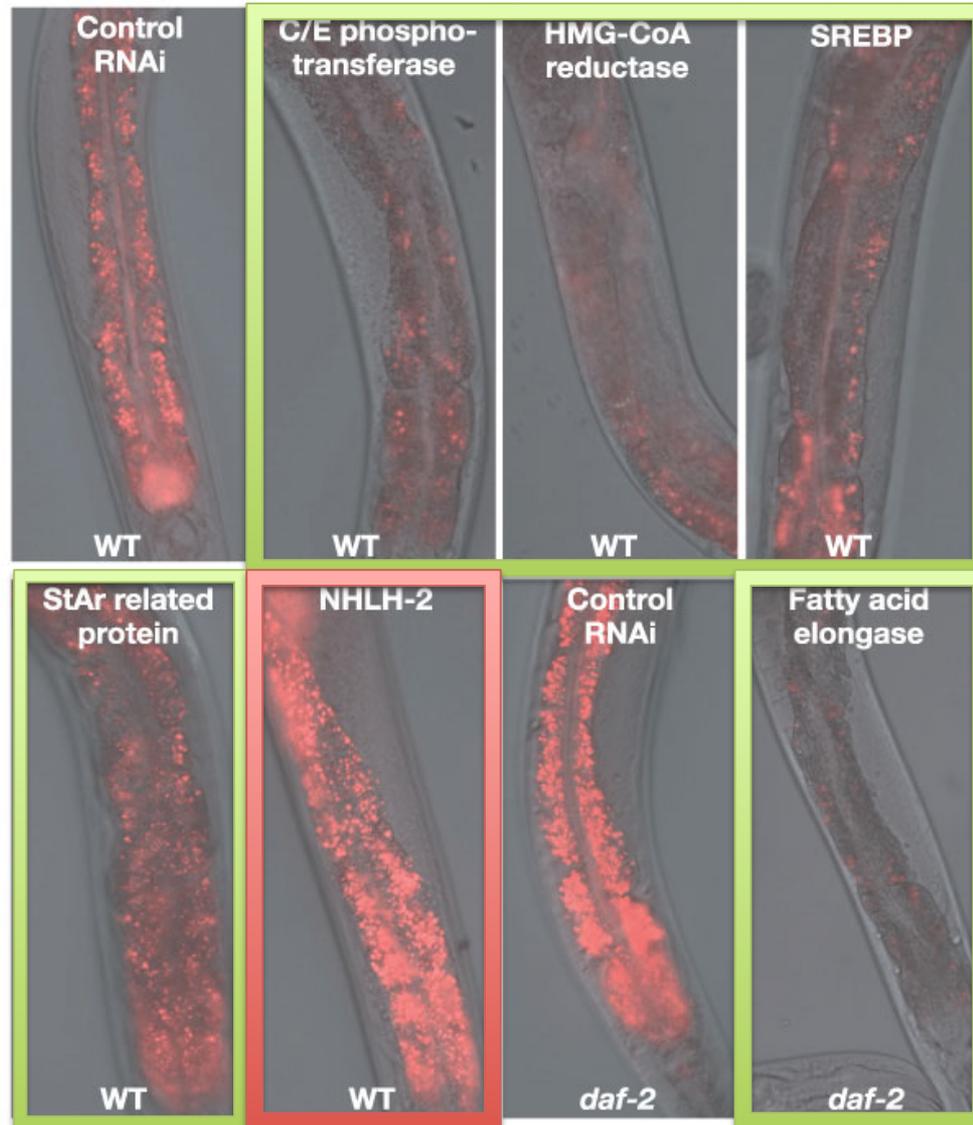
Which genes showed an increase?

Table 2 Partial list of RNAi targets that produce increased fat content

GenePairs*	Description	GenePairs*	Description
Metabolic enzyme (7)		Signal transduction (15)	
E04F6.3	Hydroxysteroid 17-β dehydrogenase	K08F8.1	Ribosomal S6 kinase
F28F8.2	Long-chain fatty-acid CoA ligase	F56H11.6	Casein/tau-tubulin kinase
ZC513.1	Phospholipid transfer protein	C04G2.2	Serine/threonine kinase
C33A12.6		F39B1.1	Phosphoinositide 3-kinase
VF12512L.1		W08D2.1	Wnt-1 family kinase
Transcription factors (9)	Transcription factors	W03A5.4	Guanylate kinase associated
C43H6.3	Nhlh2/Nscl-2	C24F3.2	Glucokinase-associated, phosphatase
K10C3.6	NHF-4 α subtype NHR	F46C5.6	Protein phosphatase PP2A
F33D4.1	Oestrogen-type NHR	T04C9.1	Oligophrenin-1 GTPase
C56C10.10	Aryl hydrocarbon NHR	Y11D7A.9	FGF receptor activating protein
C37F5.1	Elk-1	C18H9.7	RAPSN
R11H6.5	Interleukin enhancer factor 2	Channels/transporters (7)	Potassium channel
H12C20.3	C4-type steroid receptor	ZC410.4	
Receptors (6)		F52H2.2	
F56B6.5	Somatostatin receptor-type	F14E5.1	
C43H6.9	Glutamate receptor	Cell surface/structural (5)	
Y27F2A.g		C34F6.3	Collagen triple helix repeat
Y46H05_11.6		Y38F1A.9	Contactin 6/myopalladin
Vesicular transport (3)	Vesicular transport	No function assigned (62)	
F00H0.5	Cubilin/endocytic receptor		
C04G2.4	Vesicle associated protein		

*For each RNAi clone the Research Genetics GenePairs designation is provided. Genes corresponding to the RNAi clones were grouped into functional classes. Total number of genes assigned to each functional category appear in parenthesis. A full list including the extent of increase in fat content is given in the Supplementary Information. FGF, fibroblast, growth factor. NHR, nuclear hormone receptor; GPCR, G-protein-coupled receptor; RAPSN, acetylcholine receptor-associated protein.

Examples of Nile Red increase and decrease

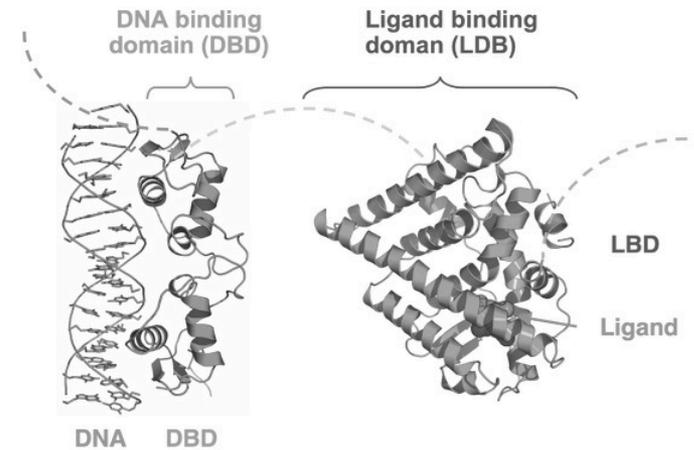
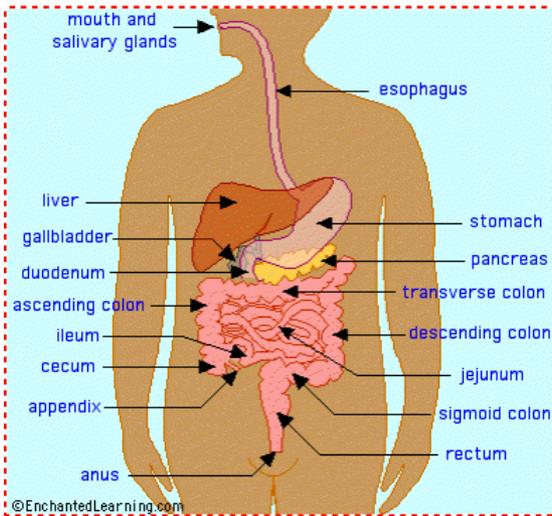


What were the main findings?

- Number of genes identified
- Variety of biological processes- GO terms
- Notable RNAi targets

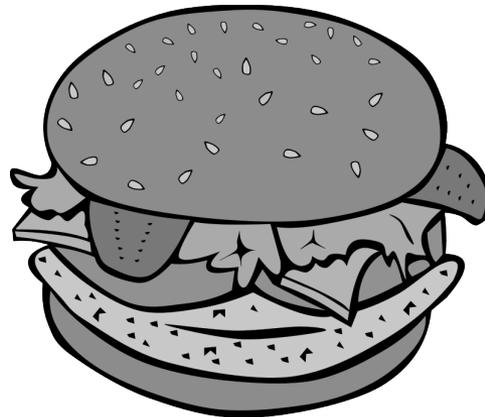


Which processes are significant?



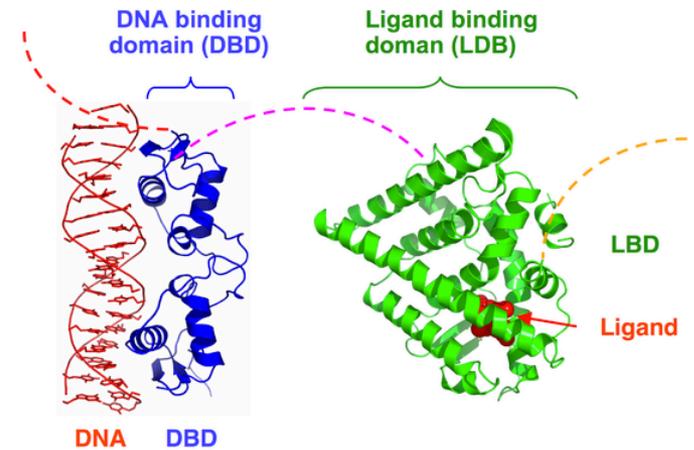
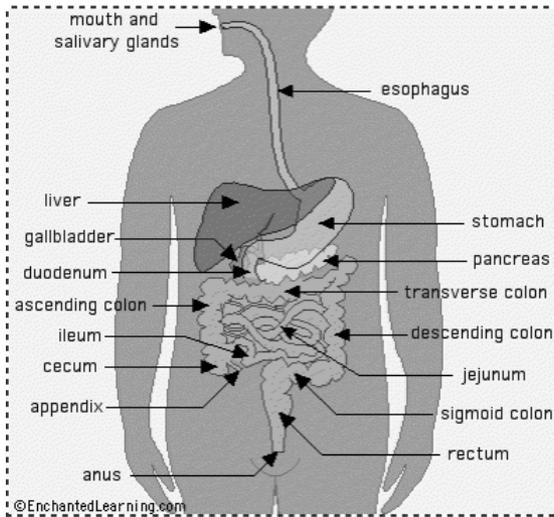
Glucose/ glycerol metabolism

Nuclear hormone receptors



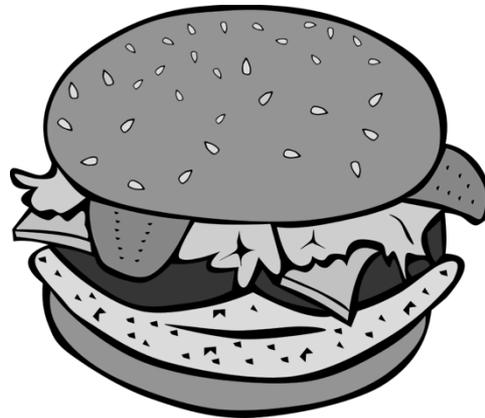
Food sensation

Which processes are significant?



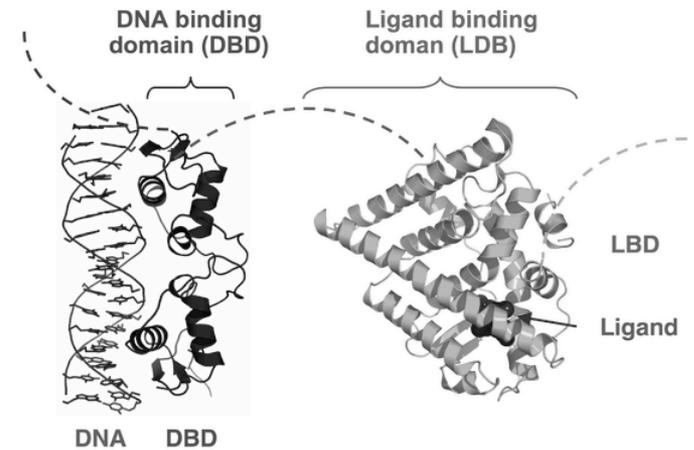
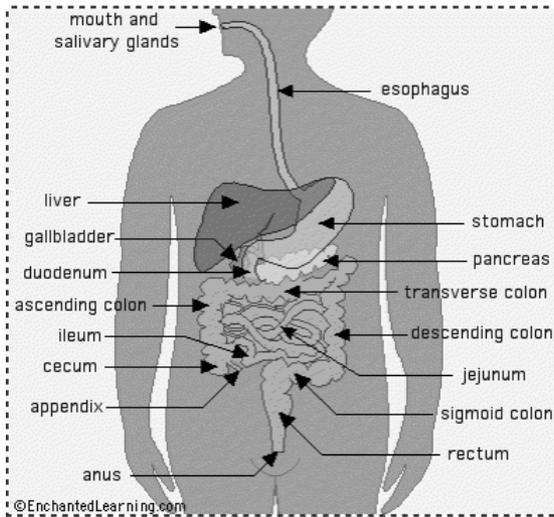
Glucose/ glycerol metabolism

Nuclear hormone receptors
HNF4- α



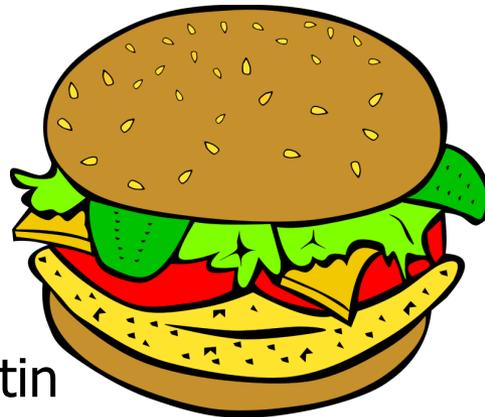
Food sensation

Which processes are significant?



Glucose/ glycerol metabolism

Nuclear hormone receptors



Somatostatin

Food sensation

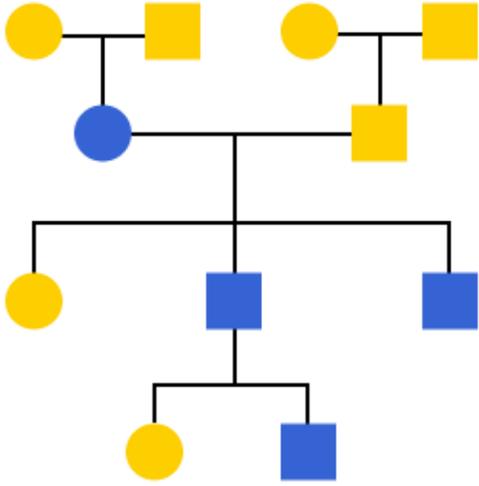
How complex is fat regulation?

- 11% in *Tub-1*, *tph-1*, *daf-2* and WT
- Cellular trafficking
- Mammalian homologues

What are the benefits of RNAi in this study?

What are the disadvantages RNAi?

Next steps



Questions?

