Twitter Thread by Dr. Coleen Murphy, flaunting my PhD

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As promised, a thread explaining our newest BioRxiv paper, where we've discovered that C. elegans can transfer memories of pathogen avoidance to naïve individuals.

What if one worm could tell another that it has learned it is infected with a pathogen, and warn others? The idea of memory transfer has a checkered past, since the earliest reports of memory transfer in planaria.

We- @rebeccasmoore1 and Rachel Kaletsky, primarily- have been studying how C. elegans learns to avoid Pseudomonas after becoming sick. We previously found that they eat bacterial small RNAs, and one small RNA (P11) that is only around when the bacteria are pathogenic triggers...

...an avoidance response that happens not only in mothers, but is remembered by four generations of their progeny. We know that this process involves uptake and Dicer processing of small RNAs in the intestine, germline amplification, piRNAs,

and downregulation of a neuronal gene, maco-1, in the ASI neuron.

But how does the germline get a signal to the neurons once the worm has eaten bacterial small RNA? Do all C. elegans "read" pathogenic bacteria small RNAs and use it to avoid Pseudomonas, and pass it on to their progeny? These were our original questions.

But one day we also wondered, Is there something inside trained worms – or their progeny or grandprogeny – that could actually transmit this information to other, untrained worms?

So we homogenized (ground up) grandkids of P11-trained grandmothers, and exposed untrained worms to that lysate (worm juice) – and they acquired avoidance! They basically transferred their memories to the other worms. This was crazy to us.

Lysates from the F3 and F4, but not F5 (when worms lose avoidance) also conferred avoidance on untrained animals. Moreover, this lysate training, like Pseudomonas or P11 training, also induces memory for 4 generations, resetting in F5, and is specific to Pseudomonas.

So what is in that lysate that is so important? Here we were influenced by work done by @JasonSynaptic's lab, showing that the Ty3/Gypsy-related Arc protein can carry RNA between neurons.

Hypothesizing that a similar capsid-like protein might be responsible, Chen Lesnik used density ultracentrifugation to fraction the lysate, then tested these fractions. Only the heaviest – the one where capsids might be – induced avoidance behavior.

With help from Edith Blackman in @zgitai's lab, we did EM, and found vesicle-like particles (VLPs) in that fraction. Those capsids also protect RNA. (There wasn't quite enough to build sequencing libraries from, though, so we'll be working on finding out what's inside.)

Next, inspired by Jim Priess' work, we tested Cer1, a Ty3/Gypsy transposon that forms VLPs in the germline. https://t.co/37zRUD3wSI

Sure enough, loss of Cer1 eliminated the whole thing: small RNA-mediated pathogenic learning, transgenerational inheritance, daf-7 activation in the ASI neuron, and lysate induction of memory transfer were all absent.

When we knocked Cer1 down only in later generations, we can see that Cer1 is not required in the maintenance step, but more likely in the transmission of message from germline to neurons, since its pattern is more like daf-7 (neurons) than prg-1 (germline).

Finally, we came back to our original question, how conserved is the ability to learn to avoid Pseudomonas, and to pass it on to progeny? We found that some wild strains (JU1580) have this ability, but others (Hawaiian) do not.

@leonidkruglyak's group had already found that Cer1 is present in some wild strains, and not in others, so we tested them. You guessed it: wild strains that have Cer1 could do it, and without it they couldn't. And knocking it out of a wild strain prevents this ability.

Cer1 likely conveys learned pathogen avoidance by carrying RNA from the germline to neurons; breaking open the worms allows other worms to use this information. But would that ever happen in nature? (What's the physiological relevance, right, @OdedRechavi ?)

First, worms infected with Pseudomonas die quickly, and often lyse (break open) when they do, so it is possible for neighboring worms to ingest these particles.

2ndly, Pseudomonas-infected mothers often die of matricide (bagging), and those progeny might even eat these particles in the mother. Learning to avoid this pathogen that they are normally attracted to could offer a benefit, even though Cer1 is generally bad for uninfected worms.

Thus, memory transfer – passing on the wisdom of their experiences and subsequent learned pathogenic avoidance – both to their fellow C. elegans and to their descendants – might offer an advantage, even under pretty bad conditions.

Cer1 is kind of like a membership card, since the recipient worms need Cer1 in their genome in order to take advantage of this wisdom.

Three different lines of questions all led us toward Cer1's role in memory transfer: differences in wild strains' ability to learn small RNA-mediated avoidance, signaling from the germline to neurons, and parallels between Arc in mammals and Ty3/Gypsy in worms.

As always, I'd like to thank Rachel and @rebeccasmoore1 for a staggering amount of work that they did in record time, from the time we thought of it about a year ago, and before and after the lab was shut for 4 months.

Chen Lesnik contributed her biochemistry skills, Vanessa Cota did the beautiful germline IF, Edith Blackman in the Gitai lab did EM, and Lance Parsons helped with bioinformatics.

And of course this all grew out of many good discussions about crazy ideas with Rachel, Rebecca, and Zemer, and with others, including @carrie_adler and @jasonsynapic