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Short communication

Fluorodeoxyuridine enhances the heat shock response and decreases polyglutamine aggregation in an HSF-1-dependent manner in *Caenorhabditis elegans*

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ABSTRACT

The heat shock response (HSR) protects cells from protein-denaturing stress through the induction of chaperones. The HSR is conserved in all organisms and is mediated by the transcription factor HSF-1. We show here that a compound commonly used to prevent larval development in *Caenorhabditis elegans*, 5-fluoro-2′-deoxyuridine (FUdR), can enhance heat shock induction of *hsp* mRNA in an HSF-1-dependent manner. Treatment with FUdR can also decrease age-dependent polyglutamine aggregation in a Huntington's disease model, and this effect depends on HSF-1 as well. Therefore, FUdR treatment can modulate the HSR and proteostasis, and should be used with caution when used to inhibit reproduction.

The model organism *Caenorhabditis elegans* is frequently used in aging studies due to its rapid lifecycle, short lifespan, and ability to easily obtain a synchronous population. However, rapid progeny development makes it difficult to separate the parental generation from offspring during aging experiments. For this reason, the DNA synthesis inhibitor 5-fluoro-2′-deoxyuridine (FUdR) is commonly employed to maintain a synchronous population of nematodes for aging studies (Gandhi et al., 1980; Hosono, 1978; Mitchell et al., 1979). *C. elegans* embryos undergo rapid cellular divisions, requiring continual DNA synthesis. FUdR is able to inhibit DNA synthesis after it is metabolized into FdUMP (5-fluoro-2′-deoxyuridine 5′-monophosphate) by thymidine kinase. FdUMP subsequently inhibits thymidylate synthase, an enzyme that is essential for pyrimidine biosynthesis (Bijnsdorp et al., 2007). Therefore, treatment with FUdR can inhibit embryonic development and is used as a convenient inhibitor of reproduction. As adult nematodes undergo minimal cellular divisions, the standard practice for inhibition of reproduction is to treat synchronous populations of nematodes with FUdR around the L4/young adult (YA) life stage, right before progeny production occurs. This is thought to have a minimal impact on adult nematodes, while inhibiting the development of progeny.

Recent studies have suggested that FUdR may enhance some types of stress resistance. For instance, it was found that FUdR can increase the lifespan of the *gas-1* mitochondrial mutant and the *tub-1* fat storage mutant, and that it can also affect the metabolism of wildtype nematodes (Aitlhadj and Sturzenbaum, 2010; Davies et al., 2012; Van Raamsdonk and Hekimi, 2011). These studies have highlighted the importance of testing the effects of FUdR on the particular conditions that are going to be used in an experiment prior to its use. With this in mind, we sought to determine if any changes in heat shock protein (HSP) mRNA expression would occur upon treatment of *C. elegans* with FUdR. HSPs include protein chaperones that are regulated by the highly conserved heat shock response (HSR). The HSR enhances protein folding by producing HSPs, such as HSP70, after a cell encounters protein-denaturing stress. In *C. elegans*, this process is regulated by the transcription factor heat shock factor 1 (HSF-1).

To determine if the HSR is affected by treatment with the standard doses of 100 μM or 200 μM of FUdR given to L4/YA

Abbreviations: FUdR, fluorodeoxyuridine; HSR, heat shock response; HS, heat shock; HSP, heat shock protein; HSF-1, heat shock factor 1; L4, larval stage 4; L1, larval stage 1; YA, young adult; D3, day 3 of adulthood; RNAi, RNA interference; qRT-PCR, quantitative real-time polymerase chain reaction; Q35::YFP, 35 polyglutamine aggregates fused to YFP.

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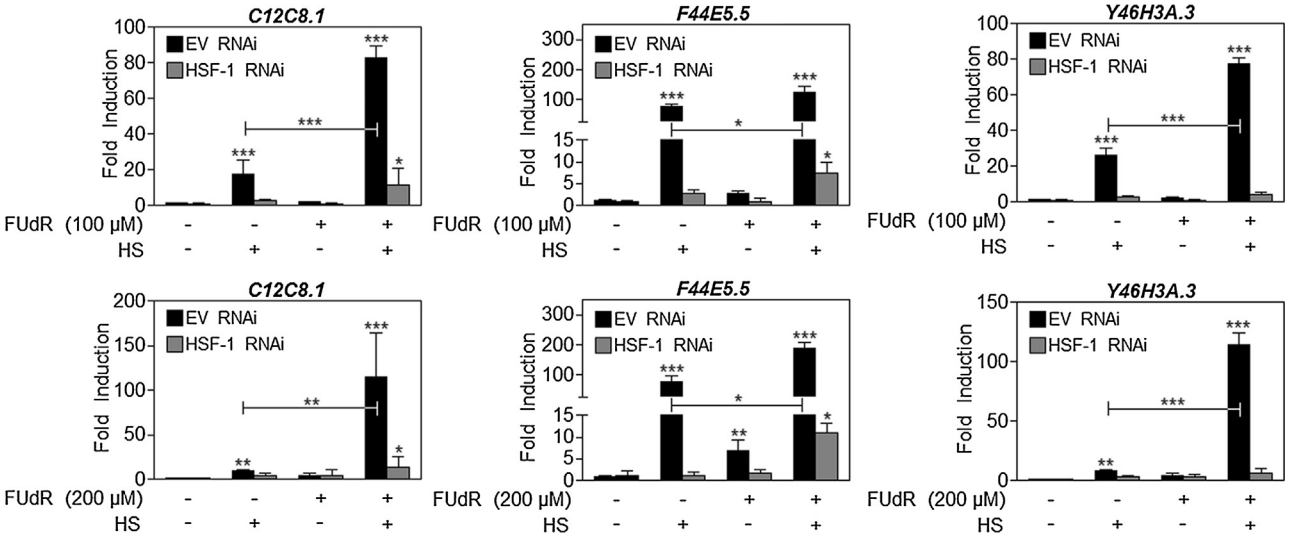

Fig. 1. Treatment with 100 μM or 200 μM of FUdR from the L4/YA stage enhances HS induction of *hsp70* and *hsp16.2* mRNA expression in *C. elegans* in an HSF-1-dependent manner. Synchronized nematodes were grown until the L4/YA stage at 24 °C on standard NGM plates supplemented with 100 μg/mL ampicillin and 1 mM IPTG and were fed empty vector (EV, black bars) or HSF-1 RNAi (grey bars) as indicated. After developing to the point just before progeny production, worms were either picked to new plates daily, or transferred to plates containing 100 μM or 200 μM of FUdR to avoid progeny contamination until collection at day 3 of adulthood. Worms were heat-shocked at 33 °C for 30 min and allowed a 15 min recovery before collection. mRNA levels were quantified for the *hsp70* genes *C12C8.1* and *F44E5.5*, and the *hsp16.2* gene Y46H3A.3 via qRT-PCR. Results are representative of averaged technical duplicates from independent biological triplicates. Statistical analysis was performed using ANOVA followed by Bonferroni's comparison test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Analysis revealed a significant F statistic for the interactions between both the RNAi and treatment variables ($F > 50$). A detailed description of the methods can be found in the online supplementary information.

worms, transcript levels of the *hsp70* genes *C12C8.1* and *F44E5.5* and the *hsp16.2* gene *Y46H3A.3* were analyzed via qRT-PCR with or without heat shock (HS) and with or without HSF-1 RNAi as indicated (Fig. 1). As expected, HS induced *C12C8.1*, *F44E5.5*, and *Y46H3A.3* mRNA expression in an HSF-1-dependent manner. Interestingly, 200 μM of FUdR on its own was able to activate expression of F44E5.5 mRNA. In addition, treatment with either 100 μM or 200 μM of FUdR enhanced HS induction of this gene and of the *C12C8.1* and *Y46H3A.3* mRNAs. This data indicates that standard FUdR treatment strategies used to inhibit progeny can enhance the HSR and would thus likely affect experimental results.

We then tested for an alternative FUdR treatment strategy that would still inhibit progeny formation while not activating the HSR or affecting worm growth. We found that FUdR doses lower than 100 μM given at the L4/YA stage were not as effective at preventing progeny development (data not shown). However, we found that a low-dose treatment of 25 μM of FUdR could effectively inhibit all progeny from hatching if given at the L1 stage instead of the L4/YA stage. Also, this early treatment with 25 μM of FUdR did not cause a delay in growth (Supplementary Fig. 1). Using this FUdR treatment condition, we again evaluated the transcript levels of *C12C8.1*, *F44E5.5*, and *Y46H3A.3* with or without HS and with or without HSF-1 RNAi (Fig. 2). However, we observed that treatment with this lower dose of FUdR could still affect the HSR. Interestingly, 25 μM of FUdR alone induced *C12C8.1*, *F44E5.5*, and *Y46H3A.3* expression in an HSF-1-dependent manner, and the induction of all three mRNAs were enhanced by HS. We were thus unable to find a FUdR treatment condition that could effectively prevent progeny formation while also not activating or enhancing the HSR.

As activation of the HSR can improve proteostasis, we next tested whether FUdR was able to affect protein aggregation using a *C. elegans* Huntington's disease model. This model expresses 35 polyglutamine tracts fused to YFP (Q35::YFP), where polyglutamine aggregates form in an age-dependent manner (Morley et al., 2002). We observed that treatment with 25 μM of FUdR from L1 to day 3, or 200 μM of FUdR from L4/YA to day 3, both decreased polyglutamine aggregation in an HSF-1-dependent manner, and that the effect was enhanced by HS (Fig. 3A and B). As a separate non-biased approach to aggregate counting, we also quantified the aggregate numbers using ImageJ software. The results indicate that blind analysis by hand counting aggregates,

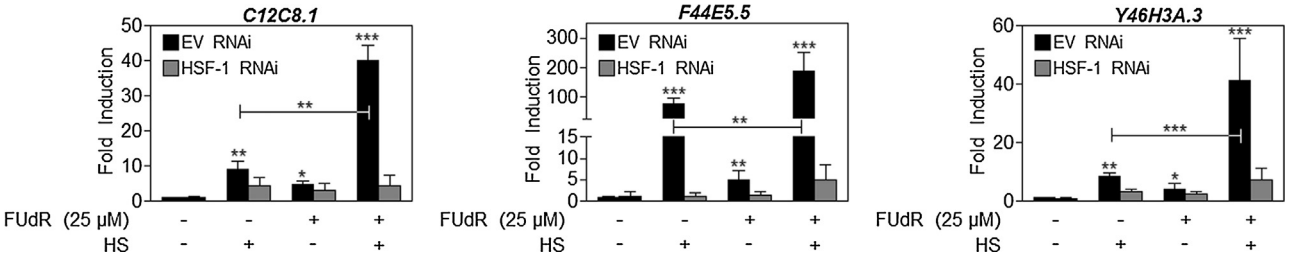

Fig. 2. Treatment with 25 μM of FUdR from the L1 stage enhances HS induction of *hsp70* and *hsp16.2* mRNA expression in *C. elegans* in an HSF-1-dependent manner. The same growth and experimental conditions were used as stated in Fig. 1, except nematodes were grown on 25 μM FUdR plates from the L1 stage until day 3 of adulthood. Results are representative of averaged technical duplicates from independent biological triplicates. Statistical analysis was performed using ANOVA followed by Bonferroni's comparison test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Analysis revealed a significant F statistic for the interactions between both the RNAi and treatment variables ($F > 20$). A detailed description of the methods can be found in the online supplementary information.