

Role of Coelomocytes in Stress Response and Fertility in *Caenorhabditis elegans*

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Coelomocytes are specialized cells that continually and nonspecifically scavenge fluid from the body cavity through endocytosis in *Caenorhabditis elegans*. Our previous study revealed that coelomocytes were specifically required for dietary-restriction-induced longevity in *C. elegans*. In the present study, we examined the effect of coelomocyte ablation on the response to environmental stressors and reproduction in *C. elegans*. Coelomocytes were ablated using diphtheria toxin specifically expressed in coelomocytes. After exposing worms to 20 J/cm²/min of ultraviolet irradiation *in vivo*, the survival of the worms was monitored daily. To examine their response to heat stress, their survival after 10 h of 35° C heat shock was measured. Oxidative stress was induced using paraquat, and the susceptibility to oxidative stress was compared between wild-type control and coelomocyte-ablated worms. The total number of progeny produced was counted, and the time-course distribution of the progeny was determined. The worms with ablated coelomocytes showed reduced resistance to ultraviolet irradiation, but the ablation of coelomocytes had no effect on their response to heat or oxidative stress. The number of progeny produced during the gravid period was significantly decreased in the coelomocyte-ablated worms. These findings suggest that coelomocytes specifically modulate the response to ultraviolet irradiation and are required for normal reproduction in *C. elegans*. The findings could contribute to understanding of the mechanisms underlying dietary-restriction-induced longevity.

Key words : *C. elegans*, coelomocyte, dietary restriction, fertility, UV irradiation

Introduction

Coelomocytes are macrophage-like cells located in the body cavity of many invertebrates [20]. In *C. elegans*, there are six coelomocytes in adult hermaphrodites and five coelomocytes in adult males [23]. Biological function of coelomocytes in *C. elegans* is not fully understood yet. *C. elegans* have no adaptive, clonal immune system, but show primitive immune function [21]. Recent study suggests that coelomocytes may function as primitive immune cells in *C. elegans* [4]. It is suggested that coelomocytes may be involved in detoxification of deleterious substances ingested [4]. Coelomocytes are also scavenger cells that endocytose fluids from pseudocoelomic cavity [18]. Cells use endocytosis to uptake extracellular medium, nutrients, and recycling of membrane

components from outside of cells. Coelomocyte-specific ligand-gated ion channel, CUP-4, is essential for endocytosis of fluid from body cavity in *C. elegans* [18]. CUP-5 is a *C. elegans* homolog of human mucolipin-1, and involved in uptake of fluid in the body cavity and degradation of proteins through lysosomes [5]. Human *MCOLN1* is a causing gene for mucopolipidosis type IV disease which shows developmental neuropathology [1]. Mutation in *cup-5* leads to defective lysosomal biogenesis and transport in *C. elegans* [2].

Recent studies revealed that coelomocytes are involved in dietary-restriction-induced longevity in *C. elegans*. Dietary restriction is a well-known lifespan-extending intervention that shows a significant increase in both mean and maximum lifespan in various model organisms, from yeast to mice [6]. In *C. elegans*, dietary restriction using bacterial dilution exhibits significant increase in lifespan and loss of *cup-4* gene markedly suppresses dietary-restriction-induced longevity phenotype [17]. Other genes involved in endocytosis of coelomocytes also significantly inhibit lifespan-extending effect of dietary restriction. CUP-5 is a protein required for lysosome biogenesis and RNAi knockdown of *cup-5* specifically reduces lifespan of *eat-2*, a long-lived genetic mutant

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showing reduced food intake. RNAi of *cup-5* does not affect the long lifespan resulted from reduced insulin/IGF-1-like signal or mitochondrial dysfunction [17]. Inhibition of *lgc-26* encoding coelomocyte-specific ion channel also suppresses lifespan-extension through dietary restriction [17]. Previous study in our laboratory reported that coelomocyte ablation completely abolished longevity effect by dietary restriction [3]. Dietary restriction in both solid and liquid media extends lifespan in wild-type, but coelomocyte-ablated animals do not respond to dietary restriction. RNAi of *cco-1*, which encodes a cytochrome C oxidase, increases lifespan of both wild-type and coelomocyte-ablated animals, which suggests that coelomocytes are specifically required for dietary-restriction-induced longevity, but not related with lifespan extension by mitochondrial dysfunction [3].

Here, we examined the role of coelomocytes on response to environmental stressors and fertility in worms whose coelomocytes are ablated using coelomocyte-specific expression of diphtheria toxin. Survival of worms under different environmental stressors, including ultraviolet (UV) irradiation, heat shock, and oxidative stress, were compared between wild-type control and coelomocyte-ablated worms. In addition, reproductive ability was examined by monitoring number of progeny produced during gravid period.

Materials and Methods

Worm strain and culture

Wild-type N2 worms were purchased from *C. elegans* Genetics Center (CGC, Minneapolis, USA) and used as a control for all experiments. NP717 is the strain whose coelomocytes are ablated by diphtheria toxin and provided by Dr. Hanna Fares from Columbia University. The genotype of NP717 is (*unc-119 (ed3); arIs37; cdlIs32[pcc1::DT-A (E148D); unc-19-pmyo-2::GFP*). Worms were grown on NGM agar plate (1.7% agar, 2.5 mg/ml peptone, 25 mM NaCl, 50 mM KH₂PO₄ pH6.0, 5 µg/ml cholesterol, 1 mM CaCl₂, and 1 mM MgSO₄). *E. coli* OP50 was added to solid NGM plate as a food source for *C. elegans*. Worms were cultured at 20°C for all experiments unless specific temperature condition was indicated.

UV resistance

Five 3-day-old young adult hermaphrodites were placed on new NGM plate and let lay eggs for 4 hr at 20°C. Then adult worms were removed from the plate and the eggs

were incubated at 20°C for 3 days to get age-synchronized worms. Sixty age-synchronized worms were exposed to UV (20 J/cm²/min) for 1 min in a 254 nm-UV crosslinker (BLX-254; Vilber Lourmat, France). Thereafter, dead worms were counted every day until all worms were dead. Resistance to UV irradiation was compared between wild-type N2 and NP717 using the log-rank test [19].

Thermotolerance

To determine the effect of coelomocyte ablation on thermotolerance, we monitored survival of worms after heat shock stress. Sixty age-synchronized worms were shifted to 35°C for 10 hr. The survival of worms after 10 hr of heat shock was measured under microscope. Four independent replicative experiments were performed. We calculated p-value using standard two-tailed student t-test.

Resistance to oxidative stress

Sixty age-synchronized worms were transferred to NGM plates containing 12.5 mg/l of 5-Fluoro-2'-deoxyuridine (Sigma-Aldrich, St. Louis, USA) to prevent internal hatching and 20 mM paraquat (methyl viologen dichloride hydrate, Sigma-Aldrich, St. Louis, USA) to induce oxidative stress in worms. The alive and dead worms were scored three times per day until all worms were dead. Worms not responding to mechanical stimuli by a worm picker were regarded as dead. We replicated three independent experiments.

Fertility assay

Five young-adult worms were let lay eggs on NGM plates 4 hr at 20°C. After incubating 48 hr at 20°C, we transferred single worm to new NGM plate and let lay eggs. Next day, adult worm was transferred to fresh NGM plate. Adult worms were transferred to new NGM plates every day until they don't produce progeny. The NGM plates containing eggs were incubated at 20°C for another 48 hr and number of progeny was counted for each adult worm tested. The fertility of ten different worms was monitored for wild-type N2 and NP717.

Statistical analysis

The statistical significance of resistance to oxidative stress and survival after UV irradiation was analyzed using the log-rank test. The log-rank test, also known as Mantel-Cox test, is a non-parametric test widely used for the comparison of survival curve of two groups [19]. We measured mean

survival time of each group and calculated p-value for each comparison.

Results and Discussion

Requirement of coelomocytes for resistance to UV irradiation

To determine the role of coelomocytes in response to UV irradiation, we compared survival after UV irradiation between wild-type N2 and NP717 whose coelomocytes are ablated by diphtheria toxin. Survival of worms after UV irradiation was significantly decreased by ablation of coelomocytes (Fig. 1). Mean survival time of wild-type N2 was 4.86 d and that of NP717 was reduced down to 2.85d ($p < 0.001$). There was a 41.4% decrease in mean survival time by coelomocyte ablation. Independent repeated experiment also showed significant decrease in resistance to UV irradiation in NP717: mean survival times of wild-type N2 and NP717 were 5.22 and 4.51 d, respectively ($p = 0.009$) and % decrease by coelomocyte ablation was 13.6% (data not shown). Genetic mutants conferring increased lifespan accompany increased resistance to UV irradiation in *C. elegans* [14]. Our previous study showed that coelomocytes are required for dietary-restriction-induced longevity in *C. elegans* [3]. Lifespan-extending dietary restriction also retards UV-induced DNA damage in rodents [22]. Our findings indicate that coelomocytes are required for adaptive response to UV and could be involved in lifespan extension by dietary restriction in *C. elegans*. Further studies regarding the role of coelomocytes in alteration of UV-induced DNA repair by aging and dietary restriction will provide a critical evidence

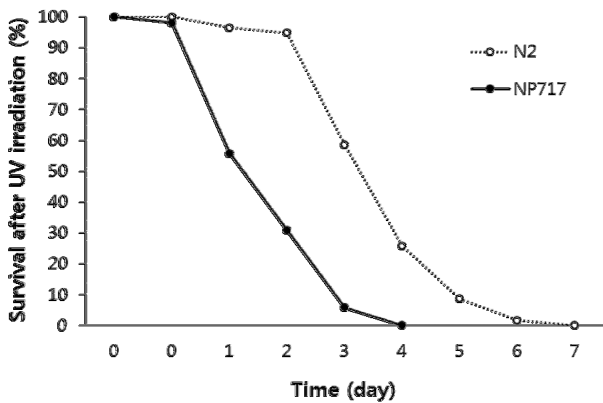


Fig. 1. Comparison of survival after UV irradiation between N2 and NP717. Survival of worms after UV irradiation was monitored every day. Survival of NP717 was significantly decreased compared to that of wild-type N2.

for the understanding of cellular mechanisms of dietary-restriction-induced longevity.

Effect of coelomocyte ablation on thermotolerance

Next, we asked whether coelomocytes are involved in response to heat stress in addition to response to UV irradiation. We measured the survival of worms after 10 hr of 35°C heat shock in wild-type N2 and NP717. Thermotolerance was slightly decreased by coelomocyte ablation, but not significantly different ($p > 0.05$) (Fig. 2). In wild-type N2, 37.5±9.19% (mean of 4 independent experiments ± Standard error of mean (SEM)) of worms were survived after heat stress. 28.4±6.46% of NP717 worms were survived after heat stress. Some long-lived genetic or nutritional interventions show increased thermotolerance. For example, *age-1*, the first genetic mutant reported for having increased lifespan in *C. elegans*, shows increased resistance to heat stress and dietary supplementation of *Acanthopanax sessiliflorus* extracts increases both thermotolerance and lifespan [10, 11, 15]. Interestingly, worms grown in media prepared with electrolyzed-reduced water confers increased resistance to heat stress and longevity phenotype [16, 24]. Therefore, there seems to be a positive correlation between thermotolerance and longevity in many long-lived *C. elegans*. However, we did not observe any change in response to heat shock by coelomocyte ablation in this study. Our data suggest that although coelomocytes are required for dietary-restriction-induced longevity phenotype, it is not involved in response to heat stress. The other possible explanation could be that dietary-restriction-induced longevity modulated by coelomocytes does not accompany increased

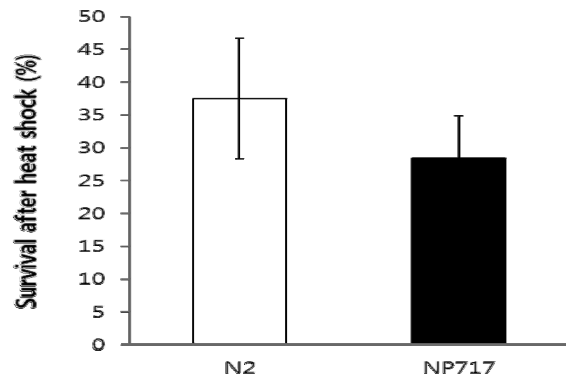


Fig. 2. Effect of coelomocyte ablation on thermotolerance. Survival after 10 hr of heat stress was compared between wild-type N2 and NP717. Data show the average of four independent experiments. Error bars indicate SEM.

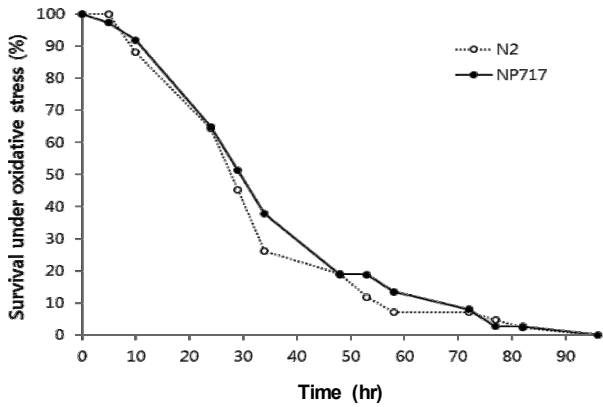


Fig. 3. Resistance to oxidative stress in N2 and NP717. Age-synchronized young adult worms were exposed to 20 mM paraquat to induce oxidative-stress and survival of worms was monitored three times per day until all worms were dead.

thermotolerance unlike other interventions previously mentioned. Further studies regarding involvement of coelomocytes in different methods of dietary restriction and effect of each method of dietary restriction on resistance to heat stress will provide more concrete explanation for the role of coelomocytes in response to heat stress and dietary restriction.

Role of coelomocytes on resistance to oxidative stress

Free radical theory of aging suggests that age-related accumulation of free-radical-induced cellular damages is one of major causing factors of aging [8]. Having observed role of dietary-restriction-induced longevity and response to UV irradiation of coelomocytes, we examined response to oxidative stress of NP717 [3]. Susceptibility to oxidative stress induced by paraquat was not altered by ablation of coelomocytes (Fig. 3). Mean survival times were 27.0 and 28.7 hr

in wild-type N2 and NP717, respectively. In the replicative experiments, there was no significant difference in resistance to oxidative stress between N2 and NP717 (data not shown). Taken together, coelomocytes are required for long lifespan induced by dietary restriction, and specifically involved in response to UV irradiation, but not to heat or oxidative stress. It seems that coelomocytes do not affect cellular generation or removal of free radicals and therefore cannot change response to oxidative stress. These findings suggest that increased resistance to oxidative stress observed in dietary-restricted animals is independent on coelomocytes.

Effect of coelomocyte ablation on reproduction

The disposable soma theory of aging suggests that cellular resources are allocated between reproduction and maintenance of soma and aging results from the accumulation of cellular damages which can be restored at the expense of reproductive effort [12]. In *C. elegans*, many genetic mutants having increased lifespan exhibit reduced or delayed progeny production [7, 9, 13]. We examined the effect of coelomocyte ablation on fertility. Interestingly, total number of progeny produced during gravid period in NP717 was significantly decreased compared with that in wild-type N2 (Fig. 4A). 338.9±19.26 (mean ± SEM) progeny were produced in wild-type N2, whereas only 112.8±14.85 progeny were produced in NP717 ($p<0.001$). Time-course distribution of progeny number also showed a marked difference between wild-type N2 and NP717 (Fig. 4B). NP717 worms produced less number of progeny on 3, 4, and 5 days after hatching than wild-type N2 worms. These data indicate that coelomocytes are required for normal fertility. However, in contrast to the hypothesis suggested by the disposable soma theory of aging, inhibition of dietary-restriction-induced longevity by coelomocyte ablation does not seem to be associated with

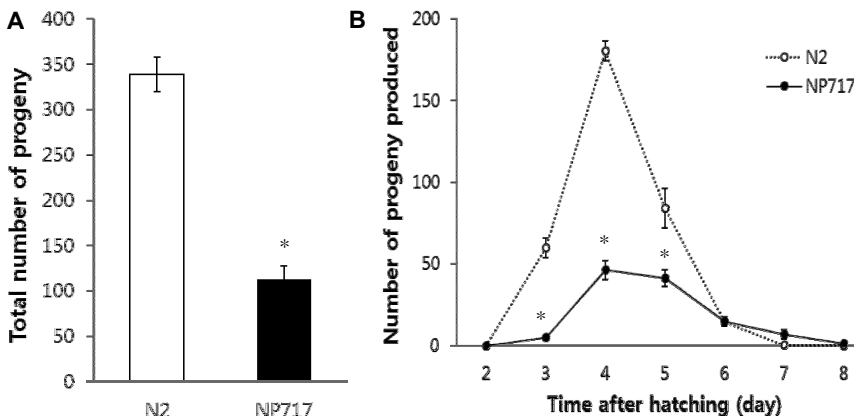


Fig. 4. Reduced fertility by coelomocyte ablation. (A) Total number of progeny produced during gravid period. (B) Time-course distribution of progeny counted every day until no progeny was hatched. Asterisk indicates a significant difference ($p<0.05$ by the Student's t test). Error bars indicate SEM.

a change in fertility. Role of coelomocytes in reproduction under different stress conditions and dietary restriction could deepen our knowledge of coelomocytes.

The exact biological functions of coelomocytes are still mainly unknown. The present study evaluated for the first time the role of coelomocytes in response to various environmental stressors and organism's reproductive ability. Ablation of coelomocytes specifically reduced resistance to UV irradiation among environmental stressors tested. In addition, coelomocyte-ablated worms produced significantly less number of progeny compared to wild-type N2. These finding will expand our understanding on biological functions of coelomocytes and provide a possible explanation regarding the role of coelomocytes in dietary-restriction-induced longevity.

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초록 : 꼬마선충의 coelomocyte 세포가 스트레스 저항성 및 번식력에 미치는 영향

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체강 소체는 꼬마 선충에서 내화과정을 통해 체강 내 액체를 세포 안으로 들여오는 특이적인 세포이다. 본 연구실에서의 최근 연구 결과에 의하면, 꼬마 선충에서 식이제한에 의한 수명연장에 체강 소체가 필수적임이 발견되었다. 본 연구에서는 꼬마 선충에서 체강 소체를 제거하였을 경우, 환경적 스트레스에 대한 저항성과 번식력을 개체 수준에서 연구하였다. 체강 소체는 체강 소체에만 특이적으로 발현하는 디프테리아 독소를 이용하여 제거하였다. 먼저 자외선에 대한 저항성은 20 J/cm²/min의 자외선을 조사한 후, 생존률의 변화를 매일 기록하였다. 또한 꼬마 선충을 35℃ 배양기에 10시간 동안 배양하여 열 스트레스를 가한 후, 생존률의 변화를 관찰하였다. 산화성 스트레스는 paraquat를 이용하여 생체 내 산화성 스트레스를 유도한 다음, 산화성 스트레스에 대한 저항성을 비교하였다. 번식력의 경우, 번식기간 동안의 총 자손의 수와 날짜 별 자손의 수를 비교 분석하였다. 그 결과, 체강 소체는 개체의 자외선 스트레스의 저항성에는 필수적이지만, 열과 산화성 스트레스 저항성에는 영향을 미치지 않는 것으로 보인다. 그리고 개체의 번식력에도 관여하는 것으로 나타났다. 본 연구 결과는 노화 및 식이제한에 의한 수명연장의 기전을 이해하는데 기여할 것으로 사료된다.