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**Review**

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# Uncommon and parallel developmental patterns of thymidylate synthase expression and localization in *Trichinella spiralis* and *Caenorhabditis elegans*

**Abstract:** *Trichinella spiralis* is a parasitic nematode causing trichinellosis, a serious disease, and *Caenorhabditis elegans* is a free-living nematode, which is used as a model in parasitological studies. High levels of thymidylate synthase (EC 2.1.1.45; ThyA) and certain other enzymes involved in thymidylate biosynthesis were found throughout *T. spiralis* and *C. elegans* developmental cycles, including developmentally arrested forms, that is, *T. spiralis* muscle larva and *C. elegans* dauer larva. As ThyA activity is characteristic for cells that left the $G_0$ phase of the cell cycle, an exceptional regulation of the cell cycle in nematodes is suggested, manifested by a global cell cycle arrest in developmentally arrested larvae of the two species. ThyA immunolocalization during development of *T. spiralis* and *C. elegans* revealed the presence of high enzyme levels not only in the developing embryos, where it was expected, but also in gonad primordia, egg and sperm cells, nerve ring and secretory cells, opening to *T. spiralis* esophagus and *C. elegans* pharynx, where it may point to those cell populations remaining cell cycle arrested.

**Keywords:** *Caenorhabditis elegans*; thymidylate synthase; *Trichinella spiralis*.

**Enzymes:** thymidylate synthase (EC 2.1.1.45); dihydrofolate reductase (EC 1.5.1.3); dUTP-ase (EC 3.6.1.23); ribonucleotide reductase (EC 1.17.4.1).

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## Introduction

*Trichinella spiralis* is a parasitic nematode causing trichinellosis, a serious disease. Mating of adult worms (developing from infective larvae, deriving from digested infected undercooked meat) occurs in a nonmembrane-bound section of columnar epithelium of the small intestine of the host. Fertilized females enter the intestinal wall and release newborn larvae to the lymph and bloodstream. Each of these penetrates skeletal muscle cell of the host and lives its section, modified in response to the presence of the larva, surrounded by a collagen capsule around which a circulatory rete develops, called the "nurse cell" (Figure 1). Nurse cell development within the muscle cell of the host, initiated by *T. spiralis* infection, is associated with a variety of changes, including cell cycle re-entry and induction of DNA synthesis, followed by apparent cell cycle arrest, suggested earlier to be of $G_2$/M type [1], but recently identified as a hypermitogenic $G_1$-like arrest [2]. In the nurse cell, the larva will grow and develop, reaching the stage of the infectious form after 15 days [3].

*Caenorhabditis elegans* is a free-living nematode, widely applied as a model in genetic, developmental and biochemical studies, also suggested to be used as such with respect to parasitic nematodes [4]. This small organism, its adult form being self-fertilizing hermaphrodite or male, is characterized by fast development (approx. 3 days at 25°C), involving four larval stages separated by molts, preceding adulthood (Figure 1). Certain conditions, for example, of poorer food supply, will cause formation of dauer larvae, a developmental stage that

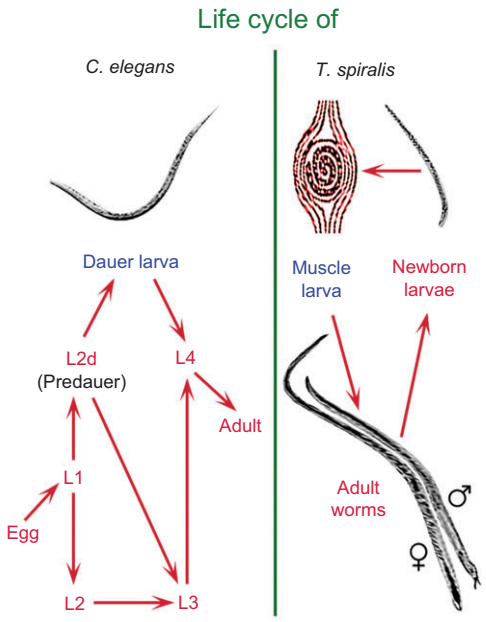


**Figure 1** Life cycle of *Caenorhabditis elegans* (left) and *Trichinella spiralis* (right). *C. elegans* larval forms are marked L1–4. *C. elegans* dauer (German verb "dauern" means "to endure") larva and *T. spiralis* muscle larva are developmentally arrested forms. Whereas the former is an optional long-time survival stage of the life cycle, the latter is an infective form, being an obligatory stage of the life cycle.

corresponds to *T. spiralis* muscle larvae, but, unlike that, is not obligatory [4].

## Thymidylate synthase

Thymidylate synthase (EC 2.1.1.45; ThyA) catalyzes the reductive methylation of deoxyuridine monophosphate (dUMP) by $N^{5,10}$-methylenetetrahydrofolate (meTHF) to generate thymidylate (dTMP) and dihydrofolate. The reaction provides the sole intracellular de novo source of dTMP, making the enzyme essential for regulating the balanced supply of DNA precursors required for DNA replication, and is consequently also a key target for antitumor, antiviral, antifungal and antiprotozoan chemotherapy [5, 6].

High specific activity has been found of ThyA and certain other enzymes engaged in thymidylate biosynthesis, including dihydrofolate reductase (EC 1.5.1.3) and dUTP-ase (EC 3.6.1.23), not only in adult forms but also in muscle larvae of the parasitic nematode, *T. spiralis*, persisting in the larvae for 2 years after infection [7, 8]. Whereas the presence of ThyA and dUTP-ase in adult worms might be expected (female worms filled with young larvae), expression of the two enzymes in developmentally arrested muscle larvae was surprising. The constant presence of high level of ThyA activity in the larvae, accompanied by enzyme mRNA level similar to that found in newborn larvae and adult worms [9], is an interesting phenomenon, especially as the enzyme protein is suspected of being responsible not only for the catalysis of the key step of thymidylate biosynthesis and regulation of its own translation but also for regulation of other cellular genes [10], and high cellular level of its protein has been ascribed an oncogene-like activity [11]. In particular, a possibility of playing additional, besides catalytic, roles is indicated by a high level of enzyme expression in muscle larvae found later than 38 days after infection (Table 1), that is, at the time of documented lowered thymidine incorporation into larval DNA [15].

Moreover, further studies demonstrated a similar phenomenon occurring in the life cycle of the free-living

**Table 1** Specific activities of selected enzymes involved in thymidylate de novo biosynthesis in *T. spiralis* and *T. pseudospiralis* muscle larvae, *T. spiralis* adult forms, *C. elegans* adult forms, and L1, L3 and dauer larvae[a].

| Developmental stage (the time between infection and parasite isolation is given) | Enzyme specific activity, nmol/min/mg protein | | | |
|---|---|---|---|---|
| | Thymidylate synthase | Dihydrofolate reductase | dUTPase | Ribonucleotide reductase |
| *T. spiralis* | | | | |
| Adult forms | | | | |
| 6 days | 0.058 | 36.0 | NT[b] | NT |
| Muscle larvae | | | | |
| 30 days | 0.092[c] | 7.60 | 0.25 | 0.003 |
| 24 months | 0.088[c] | NT | 0.23 | NT |
| *C. elegans* | | | | |
| Adult forms | 0.12 | 7.4 | 3.9 | 0.012 |
| L1 larvae | 0.11 | 13.2 | 2.7 | NT |
| L3 larvae | 0.40 | 7.8 | 4.2 | NT |
| Dauer larvae | 0.10[c] | 14.0 | 0.83 | 0.008 |

[a]For both *Trichinella* species time between infection and isolation is indicated [8, 12, 13]; [b]not tested; [c]cf. specific activity of 0.1 nmol/min/mg protein found in regenerating rat liver extracts [14].

nematode *C. elegans*. The developmentally arrested *C. elegans* dauer larvae correspond to the developmentally arrested infective forms, such as *Trichinella* muscle larvae, of parasitic nematodes [4]. Those studies showed high specific activities of ThyA and other enzymes involved in thymidylate biosynthesis, dUTP-ase and dihydrofolate reductase and ribonucleotide reductase (EC 1.17.4.1) to be present (Table 1) in all developmental *C. elegans* forms (both adult and larval, including developmentally arrested dauer), as had been found for parasitic nematodes *T. spiralis*. High levels of ThyA mRNA were found throughout nematode development [12].

## Thymidylate synthase and cell proliferation

ThyA [16] induction is known to be associated with cell proliferation. A good illustration of the latter is the

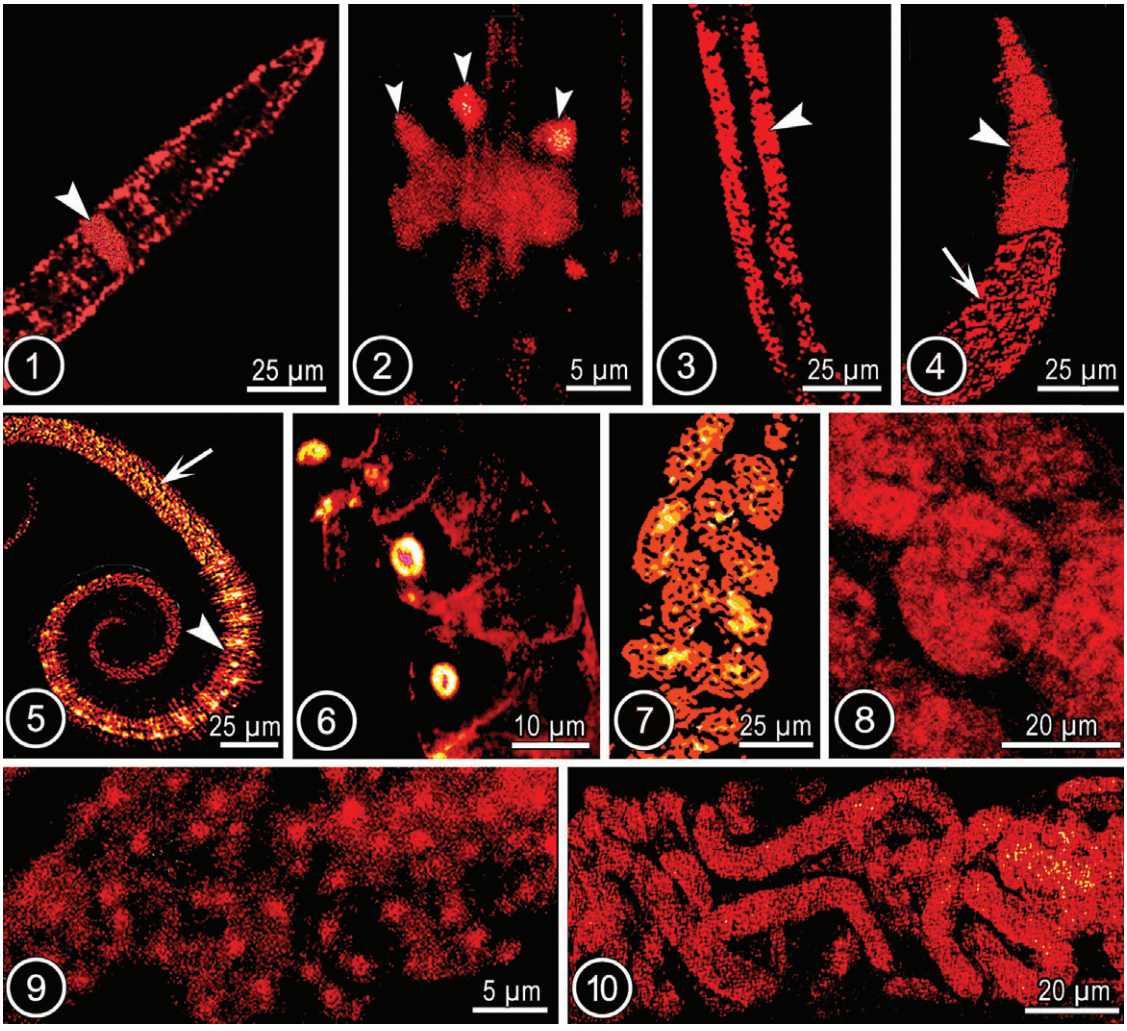


**Figure 2** Thymidylate synthase immunolocalization (reflected by fluorescence signal) during *Caenorhabditis elegans* (1, 3 and 7) and *Trichinella spiralis* (2, 4–6, 8–10) development [21]. (1) Distinct fluorescence signal in the nerve ring (arrowhead) of a 465 µm-long *C. elegans* L3 larva. (2) An 800 µm-long premature *T. spiralis* muscle larva: magnification of the nerve ring showing strong fluorescence. (3) Fluorescence signal in the gonad primordia (along the body wall) of the larva shown in (1). (4) Clear fluorescence signal in the uterus primordium (arrowhead) and ovary primordium (arrow) of a female *T. spiralis* muscle larva. (5) High fluorescence signal in or around the nuclei of the excretory-secretory organ stichosome (arrowhead) and in gonad primordium (arrow) of a male *T. spiralis* muscle larva. (6) Stichosome of an adult female *T. spiralis*, with high fluorescence signal in the nuclear region (the arrows indicate positions of nuclei laying out of focus). (7) An enlarged image of strong fluorescence signal in embryos developing in an adult *C. elegans* hermaphrodite. (8) A magnified image of embryos developing in the uterus of an adult *T. spiralis* female and showing a distinct signal. (9) An *in silico* magnified image of the seminal vesicle with a clear fluorescence signal from secondary spermatocytes of a male *T. spiralis* adult. (10) Strong signals demonstrated by *T. spiralis* larvae before birth.

enzyme specific activity in normal rat liver extracts of 0.003 nmol/min/mg protein [17], thus barely detectable, increasing to 0.1 nmol/min/mg protein [14] in extracts from rat liver regenerating following partial hepatectomy. Of note is that liver regeneration is a model of fast proliferating tissue.

Careful comparison of ThyA levels in proliferating and confluent cells showed the absence of detectable enzyme in the latter [18], indicating its induction in cells to be due to leaving quiescence (cell cycle $G_0$ phase). Besides, in mammalian cells ThyA mRNA level is very low in the $G_0$ phase, increases by 10- to 20-fold when cells enter S phase [19, 20], again becoming lower in the course of differentiation [21]. Comparison of different cells and tissues showed ThyA mRNA level to vary, reflecting the differences in proliferation rate [22]. Therefore, the presence of high level of the enzyme and its mRNA throughout the development of each nematode, and especially its persistence in developmentally arrested forms, that is, *T. spiralis* muscle larva and *C. elegans* dauer larva, is particularly unexpected, as it suggests the presence of large populations of cycling cells. With respect to the latter, it should be noted that extracts from those developmentally arrested forms show ThyA specific activity similar to that found in regenerating rat liver extracts (Table 1). To explain this, at least in the case of the parasite, the muscle larva cell population may be assumed to be undergoing cell cycle arrest through the lifetime of the host [7, 8].

## Global cell cycle arrest in nematodes

In accordance with foregoing ThyA immunolocalization during development of *T. spiralis* and *C. elegans* with the use of confocal microscopy revealed the presence of high enzyme levels in developing embryos, gonad primordia, egg and sperm cells, nerve ring and secretory cells, opening to *T. spiralis* esophagus (Figure 2) and *C. elegans* pharynx [23]. With the embryos, such distribution of the enzyme, known to be associated with proliferating tissues, is not unexpected. As high levels of ThyA are also known to characterize certain cell cycle-arrested biological systems, for example, unfertilized eggs [24–26], with animal oocytes shown to undergo cell cycle arrest before fertilization [27], its presence in the egg and sperm cells, nerve ring, as well as in secretory cells of both species points to those cell populations remaining cell cycle arrested.

The results suggest unusual regulation of the cell cycle in nematodes, manifested by a global cell cycle arrest in developmentally arrested larvae, such as *T. spiralis* muscle larvae and *C. elegans* dauer; the latter interpretation also being supported by Hong et al. [28] who drew a similar conclusion about global cell cycle arrest in dauer larvae based on different symptoms.

It should be mentioned that the above presented phenomenon of global cell cycle arrest in the developmentally arrested larvae, such as *T. spiralis* muscle larvae and *C. elegans* dauer larvae, may be potentially exploitable as a target for selective chemotherapy aimed against parasitic nematodes. Especially as ThyA protein may play some regulatory roles. Learning a possibility of selective influence on this protein in nematode cells (e.g., via change of conformation resulting from inhibitor binding) should enable interference with those functions.

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