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EMBRYO GENOME TRANSCRIPTS CONTRIBUTE TO THE INCREASE IN ORNITHINE DECARBOXYLASE ACTIVITY REQUIRED FOR GASTRULATION

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SUMMARY

During early embryonic development of the polychaete *Ophryotrocha labronica* ornithine decarboxylase (L-ornithine carboxy-lyase; EC 4.1.1.17), the rate-limiting enzyme in polyamine synthesis, shows a distinct peak activity just before gastrulation. Prevention of embryo genome transcription during cleavage (by cordycepin or α-amanitin treatment) results in a 50 % decrease in peak ornithine decarboxylase activity. This indicates that the pregastrular increase in ornithine decarboxylase activity is depending not only on maternal message, but also on new embryo genome transcripts. This early mobilization of the embryo genome seems unique in that it contributes significantly to a pregastrular event (increased ornithine decarboxylase activity) that is indispensable for the successful accomplishment of gastrulation.

INTRODUCTION

In the polychaete *Ophryotrocha labronica* a marked temporary increase in putrescine synthesis is essential for gastrulation (1). This conclusion was arrived at in experiments with DL-α-methylornithine, a competitive inhibitor of ornithine decarboxylase (L-ornithine carboxy-lyase; EC 4.1.1.17) (2), the enzyme responsible for the formation of putrescine. DL-α-Methylornithine treatment prevents the pregastrular accumulation of putrescine and blocks development at gastrulation (1). The arrest of development at gastrulation in the presence of the ornithine decarboxylase inhibitor is probably effected by interference with nucleolar formation as evidenced by ultrastructural analysis (3). Nucleoli are only sparsely present following DL-α-methylornithine treatment and show an atypical scattered appearance (3).

In an attempt to study the regulation of putrescine synthesis during early embryonic development of the polychaete we have analyzed the extent

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to which an increased ornithine decarboxylase activity depends on new embryo genome transcripts and on long-lived maternal messages. A central question of developmental biology concerns the onset of participation of the embryonic genome in the events of embryogenesis. During cleavage invertebrate embryos are considered to express mainly maternal message (4). Embryonic message is synthesized during this period but remains largely quiescent until after the inception of gastrulation, i.e. when organogenesis begins (4).

MATERIALS AND METHODS

Cultivation of the polychaete *Ophryotrocha labronica* LaGreca and Bacci, and collection of developing eggs have been previously described (1). Cycloheximide, actinomycin D, cordycepin or α-amanitin were added to cultures of fertilized polychaete eggs at the times and concentrations indicated. Since all eggs in an egg-pack are synchronous in development, one half of the egg-pack was untreated and served as control in each experiment.

Eggs, frozen at -70°C, were sonicated and assayed for their ornithine decarboxylase activity as previously described (5,6) in a medium containing 100 mM glycyl-glycine buffer (pH 7.2), 5 mM dithiothreitol and 0.2 mM pyridoxal 5'-phosphate (about 1500 embryos in 1.00 ml). The ornithine decarboxylase activity of treated and untreated embryos was determined 48 hr after fertilization by measuring the release of $^{14}CO_2$ from DL-ornithine-1-^{14}C over a 1 hr period (37°C) in the presence of a saturating concentration (1 mM) of L-ornithine. The reaction was started by adding the substrate (specific activity, 18.5 MBq/mmole).

RESULTS AND DISCUSSION

The pattern of ornithine decarboxylase activity in early development of the polychaete embryo reveals a peak activity during cleavage just before gastrulation (Fig. 1). Considering the possibility that early embryos may possess a store of inactive ornithine decarboxylase, the dependence on protein synthesis for the increased enzyme activity was determined. Cycloheximide completely blocked the increase in ornithine decarboxylase activity (Table 1), thus excluding the possibility that this increase in enzyme activity is due to mobilization of stored, inactive material. Accordingly, the increased ornithine decarboxylase activity is a result of translation from a messenger which could be of either maternal or embryonic origin.

In an attempt to block transcription of new, i.e. embryonic, mRNA, the embryos were treated with a high dose (7) of actinomycin D. Surprisingly, we observed that actinomycin D caused superinduction of the ornithine decar-

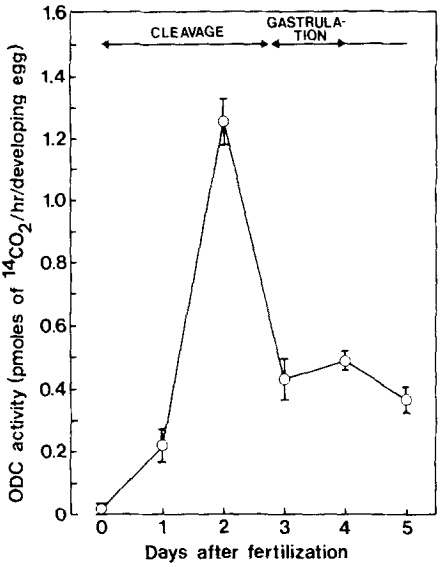

Fig. 1. Ornithine decarboxylase activity in developing eggs of the polychaete *Ophryotrocha labronica*. Values represent the mean ± S.D. (n = 3-4). ODC, ornithine decarboxylase.

Table 1. Pregastrular ornithine decarboxylase activity as affected by inhibitors of transcription and translation

Treatment*	Dose (µg/ml)	Developmental time (hr) at Addition	Developmental time (hr) at Assay	Ornithine decarboxylase activity** (pmoles of $^{14}CO_2$/hr/developing egg)
Cycloheximide	50	42	48	0.039 (3 %)
Actinomycin D	50	0	48	2.507 ± 0.137 (193 %)
Cordycepin	50	0	48	0.675 ± 0.057 (55 %)
	50	12	48	0.646 ± 0.024 (52 %)
	50	24	48	0.693 ± 0.042 (56 %)
α-Amanitin	20	0	48	0.630 ± 0.066 (55 %)

*Cycloheximide completely prevented development when added at the time of fertilization (0 hr). Addition of cycloheximide at 42 hr completely eradicated the 48-hr ornithine decarboxylase activity demonstrating its dependence on protein synthesis and its short biological half-life. Actinomycin D, cordycepin and α-amanitin, at the concentrations used, did not interfere with normal pregastrular development, as evidenced by the fact that treated and untreated embryos exhibited similar cell numbers at the 48 hr assay.
**Mean ± S.D. (n = 3-4). Within brackets are the enzyme activities in percent of untreated controls. All control values were within the same range as that shown for other 2-day eggs (Fig. 1).

boxylase activity (Table 1). Superinduction of this enzyme activity has been observed in other experimental systems (8,9) but since the mechanism involved remains controversial (10,11) and provides no information as to the possible dependence of the increased ornithine decarboxylase activity on new embryo genome transcripts, the present results prompted the use of another inhibitor of mRNA synthesis with a different mode of action. Thus, cordycepin (3'-deoxyadenosine), an inhibitor of messenger polyadenylation (12), was administered to the embryos during various periods of pregastrular development (Table 1). Cordycepin treatment inhibited the 48-hr ornithine decarboxylase activity by approximately 50 %. This was true for 0-48 hr, 12-48 hr and 24-48 hr treatments, suggesting that transcription of ornithine decarboxylase mRNA occurs between 24 and 48 hr of early development, i.e. well before gastrulation. Further support for this contention was obtained in experiments in which embryos were treated with cordycepin between 0 and 12 hr, and between 12 and 24 hr of development. No significant inhibition (less than 6 %) of the 48-hr ornithine decarboxylase activity was observed. It appears that maximally about 50 % of the 48-hr ornithine decarboxylase activity is due to translation of embryonic message since a 10-fold increase in the concentration of cordycepin (0.5 mg/ml) caused no further inhibition. Consequently, about 50 % of the ornithine decarboxylase activity should be due to the presence of maternal message.

The results of these experiments do not exclude the possibility that the ornithine decarboxylase-inhibitory effect of cordycepin is due to inhibition of polyadenylation of stored underadenylated maternal mRNA. However, α-amanitin, which inhibits transcription by interfering with RNA polymerase II action (13,14), blocked the ornithine decarboxylase activity to the same extent as did cordycepin (Table 1). Indeed, the fact that the α-amanitin concentration used was the highest possible that did not interfere with normal pregastrular development (as evidenced by the fact that treated and untreated embryos exhibited similar cell numbers at the 48-hr assay) makes it highly

probable that embryo genome transcripts are responsible for about 50 % of the increase in pregastrular ornithine decarboxylase activity.

In conclusion, the increased ornithine decarboxylase activity, which is a prerequisite for gastrulation, appears to be regulated by maternal as well as embryonic ornithine decarboxylase messenger. Besides ornithine decarboxylase, histones (4) appear to be the only proteins in the invertebrate embryo that are coded for in substantial amounts from embryonic messages already during cleavage. The early expression of the embryo genome coding for ornithine decarboxylase seems unique in that it contributes to the rapid and fundamental change in the pregastrular enzyme pattern essential for the successful accomplishment of gastrulation.

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