

2 h at 4°C) to remove soluble proteins. The pellet was suspended in a Tris-buffer 0.1 M (pH 7.8) [B]. This suspension was incubated with phospholipase A<sub>2</sub> and 1.5% deoxycholate for 1 h at 35°C, and then centrifuged (105 000 g for 3 h at 4°C). The supernatant [C] contained 97% of the b-type cytochromes (Table 1). The b-type cytochromes were then precipitated with 20% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and the precipitate suspended in a Tris-buffer 50 mM (pH 7.8) [D]. The yield of cytochrome b after the precipitation step was 60% (Table 1), and the degree of purification 30-fold.

The cytochrome absorption spectra have been investigated. Fig. 1a shows a difference spectrum of cytochrome b in fraction [D]. There are three maxima: at 562, 530, and 430 nm ( $\alpha$ -,  $\beta$  and  $\gamma$ -bands), to be compared with those reported earlier for the membrane-bound cytochrome b<sub>561</sub> (561, 530 and 430 nm).<sup>1</sup> The heme content in the different fractions was calculated by the pyridine hemochromogen method.<sup>1</sup> Fig. 1b shows a pyridine hemochromogen difference spectrum of fraction [D]. The maximum at 556 nm is characteristic of protoheme,<sup>1</sup> which is the prosthetic group of b-type cytochromes.

*Acknowledgement.* This work was supported by grant No. K 2292-035 to H. Baltscheffsky from the Swedish Natural Science Research Council.

1. Cheah, K. S. *Biochim. Biophys. Acta* 205 (1970) 148.
2. Oesterhelt, D. and Stoekenius, W. *Methods Enzymol.* 31 (1974) 667.
3. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem.* 193 (1951) 265.

Received May 29, 1979.

## Regulation of the Ornithine Decarboxylase Activity by Concomitant Translational and Transcriptional Control during Early Embryonic Development \*

OLLE HEBY and HADAR EMANUELSSON

Department of Zoophysiology, University of Lund, Helgonavägen 3, S-223 62 Lund, Sweden

The polyamines putrescine, spermidine and spermine are implicated in the regulation of cell growth and proliferation. A dramatic (80-fold) increase in the activity of the pyridoxal 5'-phosphate-dependent enzyme ornithine decarboxylase (ODC; L-ornithine carboxy lyase; EC 4.1.1.17) precedes gastrulation in the polychete *Ophryotrocha labronica*. ODC catalyzes the initial and rate-limiting step in polyamine biosynthesis, i.e., the conversion of L-ornithine to putrescine. To evaluate the importance of the polyamines in early embryonic development, specific inhibitors of ODC have been used to block their synthesis. DL- $\alpha$ -Methylornithine, a competitive inhibitor of ODC,<sup>1</sup> as well as DL- $\alpha$ -difluoromethylornithine, an enzyme-activated irreversible inhibitor of ODC,<sup>2,3</sup> were found to effectively block pregastrular ODC activity. Experiments in which pregastrular ODC activity was selectively inhibited, strongly suggest that increased ODC activity is required for gastrulation inasmuch as development was blocked at this stage.<sup>4</sup> The block at gastrulation is probably due to interference with nucleolar formation, as indicated by the fact that inhibition of the ODC activity resulted in a marked reduction in the number of nucleoli; all showing an atypical scattered appearance.<sup>5</sup>

It is generally accepted that the embryo does not become genetically autonomous until the stage of gastrulation. Therefore, any pregastrular increase in enzyme activity, like that of ODC, would be expected to depend on stored maternal products, either inactive enzymes or messenger RNAs. However, the possibility remains that embryo genome transcripts are required, at least to some extent, for the initiation of pregastrular events.

Cycloheximide completely prevented development when added at the time of fertilization (0 h). Addition of cycloheximide at 42 h completely eradicated the 48 h ODC activity (Table 1), demonstrating its dependence on protein synthesis and its short biological half-life. Inhibition of protein synthesis completely blocked the increase in pregastrular ODC activity, thus excluding the possibility that the increased ODC

\* Communication at the Meeting of the Swedish Biochemical Society in Gothenburg, 7-8th June, 1979.

Table 1. ODC activity in early polychete embryos following treatment with RNA and protein synthesis inhibitors.

Inhibitor	Dose/ $\mu\text{g ml}^{-1}$	48-h ODC activity/ $\text{pmol } ^{14}\text{CO}_2 \text{ h}^{-1} \text{ embryo}^{-1}$ <sup>a</sup>	Inhibition/%
None	—	$1.252 \pm 0.074$	0
Cycloheximide	50	$0.039 \pm 0.031$	97
$\alpha$ -Amanitin	20	$0.630 \pm 0.066$	50

<sup>a</sup> Mean  $\pm$  S.D.,  $n=3-4$ .

activity is due to mobilization of stored inactive enzyme. Therefore, it appears that the increased ODC activity is a result of translation from a messenger which may be either stored maternal mRNA or newly transcribed embryonic mRNA.

If the increase in ODC activity is entirely dependent on a maternal message, one would expect it to be unaffected by inhibition of embryo genome transcription. Interestingly, treatment of the embryos with  $\alpha$ -amanitin, which inhibits transcription by interference with RNA polymerase II activity,<sup>6</sup> caused a 50% reduction in ODC activity.  $\alpha$ -Amanitin was added immediately after fertilization and enzyme activity was assayed 48 h later. This is the time during early embryonic development when the ODC activity culminates in a peak after a dramatic increase. In view of the fact that the  $\alpha$ -amanitin concentration (20  $\mu\text{g/ml}$ ) was the highest one that did not interfere with normal pregastrular development (treated and untreated embryos exhibited similar cell numbers at the 48 h assay), it seems probable that embryo genome transcription contributes to at least 50% of the pregastrular increase in ODC activity. This early mobilization of the embryo genome seems unique in that it demonstrates the commitment of the embryo genome in a developmental event (increased ODC activity) that precedes gastrulation.

**Experimental.** Embryos, frozen at  $-70^\circ\text{C}$ , were sonicated 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 ODC activity of treated and untreated embryos was determined 48 h after fertilization by measuring the release of  $^{14}\text{CO}_2$  from DL-ornithine-1- $^{14}\text{C}$  over a 1 h period ( $37^\circ\text{C}$ ) 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/mmol).

1. Mamont, P. S., Böhlen, P., McCann, P. P., Bey, P., Schuber, F. and Tardif, C. *Proc. Natl. Acad. Sci. U.S.A.* 73 (1976) 1626.
2. Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P. and Vevort, J. P. *J. Am. Chem. Soc.* 100 (1978) 2551.
3. Mamont, P. S., Bey, P. and Koch-Weser, J. In Gaugas, J. M., Ed., *Polyamines in Biomedical Research*, Raven, New York 1979. *In press*.
4. Emanuelsson, H. and Heby, O. *Proc. Natl. Acad. Sci. U.S.A.* 75 (1978) 1039.
5. Heby, O. and Emanuelsson, H. *Cell Tissue Res.* 194 (1978) 103.
6. Lindell, T. J., Weinberg, F., Morris, P. W., Roeder, R. G. and Rutter, W. J. *Science* 170 (1970) 447.

Received May 29, 1979.