

# mRNA translation during oocyte maturation plays a key role in development of primordial germ cells in *Xenopus* embryos

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It is believed that cytoplasmic localization in the egg is necessary for development of primordial germ cells (PGCs) in *Xenopus* embryos. In this study, we sought to determine if translation of maternal mRNA during oocyte maturation is involved in the development of PGCs. Donor oocytes were collected from both stimulated (those who receive gonadotropin) and unstimulated females, artificially matured and fertilized using a host transfer technique. Using chloramphenicol (50  $\mu$ M and 500  $\mu$ M RNA), RNA translation was inhibited during oocyte maturation. Our results showed that in unstimulated embryos treated with 50  $\mu$ M chloramphenicol, there was a significant reduction in the number of PGCs reaching genital ridges. In stimulated embryos, however, the number of PGCs was unchanged unless a higher concentration (500  $\mu$ M) of chloramphenicol was used. From these results it is suggested that maternal mRNA translation during oocyte maturation plays a key role in development of PGCs.

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## 1. Introduction

Germ cells arise from primordial germ cells (PGCs) during the earliest stage of embryogenesis. PGCs are the descendants of the blastomeres containing the germ plasm (Ressom and Dixon 1988). In most species, germ cells then migrate to join the somatic cells of developing gonad. In early stage *Xenopus* embryo, PGCs lie among vegetal cells (future endodermal cells) and can be recognized histologically by their contents of cytoplasmic determinants, germ plasm. Later in development, just before the young tadpoles begin to feed, PGCs migrate out of endoderm into genital ridges (reviewed by Wylie 2000).

It is well established that localized maternal mRNA and proteins are important factors directing early embryonic development in many species. In *Xenopus*, it is believed that these cytoplasmic factors, collectively referred to as the germ plasm, are key elements in differentiation of the germ cells. Germ plasm contains mitochondria, electron-dense germinal granules, coding and noncoding RNA molecules surrounded by fibrillar matrix (Kobayashi *et al* 1998; Kloc *et al* 2000; reviewed by Houston and King

2000a). It was shown that some of these RNAs are necessary for development of PGCs (Houston and King 2000a,b).

While large amount of works have been focused on the role of maternal RNAs after fertilization, we have focused prior to fertilization, during oocyte maturation, to see if translation of maternal mRNA and therefore producing maternal protein is necessary for development of PGC in this period. To do this, translation of maternal mRNA was inhibited using chloramphenicol, an antibiotic known to block RNA translation. Counting the number of PGCs that have reached the genital ridges, we have shown that in chloramphenicol treated embryos, the mean number of PGCs was decreased compared to that in control embryos, suggesting that maternal proteins produced during oocyte maturation is necessary for proper development of PGC.

## 2. Materials and methods

Adult male and female *Xenopus laevis* (South African clawed toad) were kept in plastic tanks of filtered tap water and fed twice weekly on chopped liver and trout pellets.

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Host female toads were first injected with 20 international units (IU) of serum gonadotropin (Folligon, Intervet), at least 48 h before an injection of 350 IU chorionic gonadotropin (Chorulon, Intervet). Male toads were given a single injection of 150 IU Chorulon. Stimulated donor female toads were injected with 20 IU Folligon at least 48 h prior to the egg removal. Unstimulated females have not been stimulated with Chorulon and Folligon at least for 10 weeks.

### 2.1 Isolation and maturation of oocytes

Stimulated and unstimulated donor toads were anesthetized with 2 g MS222 per liter after which they were put on ice for surgery. One or two lobes of ovary were removed through a small incision in the ventral body wall. The incision was repaired by suturing the skin and the body wall after which the toads were allowed to recover for 15 min at 22°C tap water.

The ovary was placed in 100% modified mammalian ringer (MMR, 100 mM NaCl, 2.0 mM KCl, 1.0 mM MgSO<sub>4</sub>, 2.0 mM CaCl<sub>2</sub> and 5.0 mM HEPES, pH 7.6 (Kirschner and Hara 1980). Using a fine pairs of watchmaker's forceps, the full-grown oocytes (stage VI) were manually defolliculated. In order to mature the oocytes, progesterone (0.02%) was added to the MMR for 6–8 h. To inhibit mRNA translation, chloramphenicol with final concentrations of 50 µM and 500 µM was also added to the culture medium at the same time. To distinguish donor from host eggs, mature oocytes (showing germinal vesicle break down, GVBD, at the top) were stained with vital stain (nile blue sulphate 0.0005%, neutral red 0.025%) (Heasman *et al* 1991).

### 2.2 Fertilization of the oocytes

To fertilize naturally, a host frog that has already started to lay her own eggs was anaesthetized and the donor oocytes were transferred (using a fire-polished Pasteur pipette) into abdominal cavity of the host through a small incision. After suturing, the host toad was allowed to recover for 15 min in 22°C tap water. Normal and experimental (coloured) fertilized eggs were transferred into 25% MMR and allowed to develop until sibling control embryos reached stage 46, when the number of PGC was examined in the genital ridges.

### 2.3 Histology and PGC counting

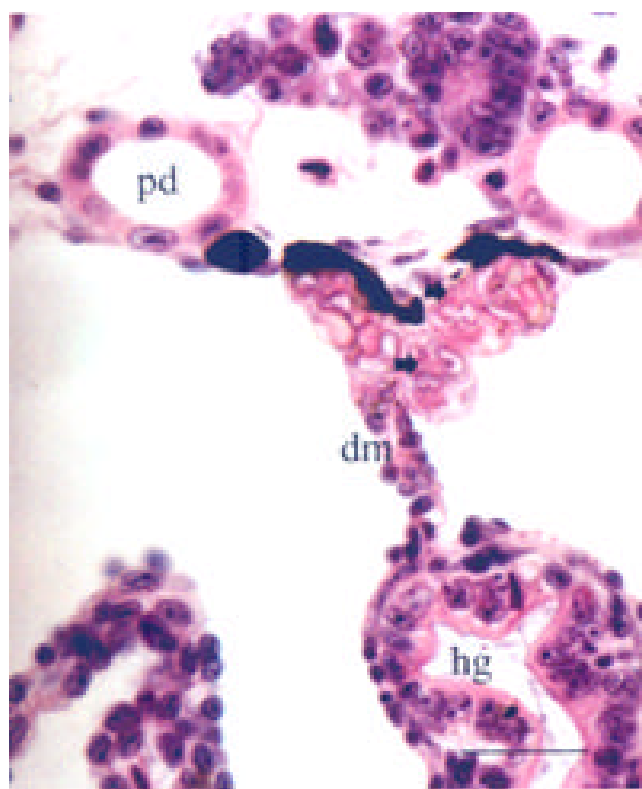
Stage 46 embryos were fixed in Smith's fixative, dehydrated, cleared in xylene and paraffin embedded. Section were cut at 7 µm paraffin and stained with haematoxylin

and eosin. PGCs were counted according to Cleine and Dixon (1985). The embryos were staged according to the normal table by Nieuwkoop and Faber (1967).

## 3. Results

In this study, the importance of mRNA translation during oocyte maturation in the development of PGC was investigated. To get large amount of eggs with good quality, we have used Folligon stimulated females as suggested elsewhere (Hedrick and Nishihara 1990). To consider the possibility of any effect by gonadotropin however, oocytes from both stimulated and unstimulated donor females were used. Chloramphenicol treated donor oocytes were fertilized using a host transfer technique and the embryos allowed to develop until stage 46, the time when they were examined for the total number of PGCs in genital ridges. At stage 46 embryos, PGC can be seen in the median genital ridges as a large spherical cell having yolk granules in its cytoplasm and a bean-shaped nucleus with 1–2 lobes (figure 1).

The results in figure 2 show the comparison between the mean number of PGCs in genital ridges of control and



**Figure 1.** Section through stage-46 *Xenopus* embryo showing lateral genital ridge either side of dorsal mesentery occupied by several PGCs (arrow). dm, dorsal mesentery; hg, hind gut; pd, pronephric duct. Scale bar: 42 µm.

chloramphenicol treated embryos. In unstimulated embryos treated with 50  $\mu$ M chloramphenicol, the number of PGCs was significantly decreased compared to that in control embryos. In stimulated embryos treated with 50  $\mu$ M chloramphenicol however, PGC numbers were unchanged. When higher concentration of chloramphenicol (500  $\mu$ M)

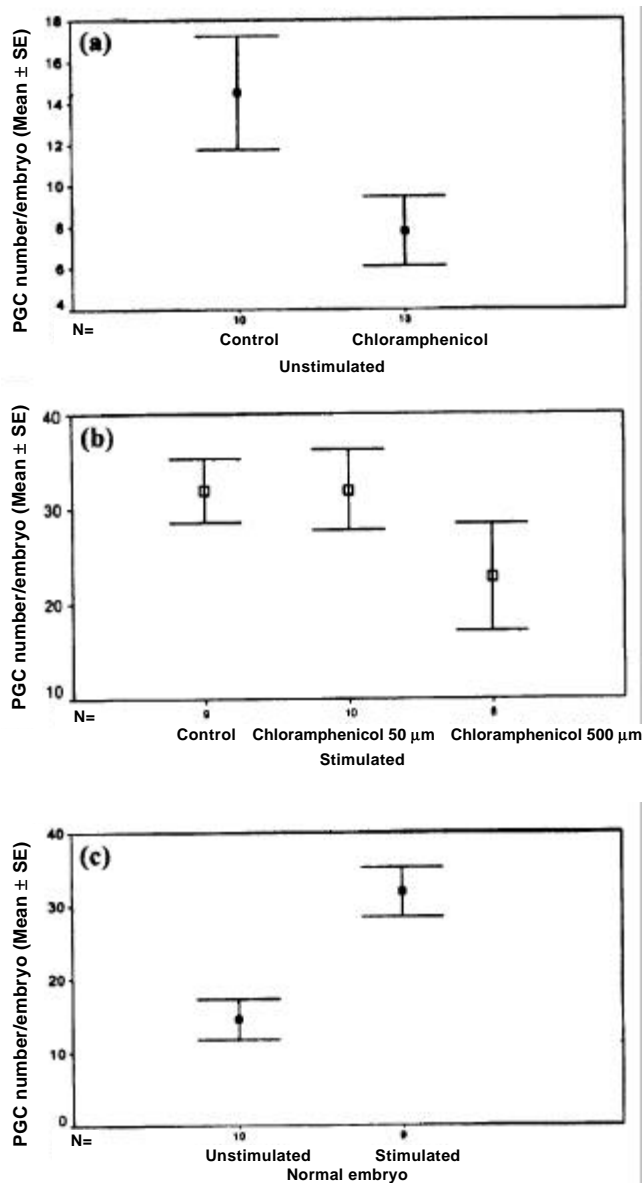
was used in these embryos a reduction not statistically significant was observed. The morphology and the size of the PGCs in chloramphenicol treated embryos were the same as those of the PGCs in control embryos. From these results, we suggest that inhibiting RNA translation during oocyte maturation can reduce the number of PGCs reaching genital ridges.

Apart from results of the effect caused by chloramphenicol, interestingly we found that gonadotropin treatment increases the number of PGCs; PGC number in stimulated embryos was twice as many as that in unstimulated control embryos. This result indicates that gonadotropin has a positive effect on PGC numbers.

#### 4. Discussion

It is generally believed that cytoplasmic localization of the egg such as maternal mRNAs is responsible for development of the PGCs in *Xenopus* embryos (reviewed by Dixon 1981; Wylie 2000). To find out whether translation of maternal mRNAs during oocyte maturation has any role in the development of PGCs, mRNA translation was inhibited using chloramphenicol. We have shown that in the chloramphenicol treated embryos the number of PGCs reached the genital ridges have significantly been decreased compared to that in control embryos. This result implicates that translation of maternal mRNA during oocytes maturation has a role in the formation and/or differentiation of the PGC.

Here we also present some evidence, which may lead to the conclusion that the number of PGCs reaching the genital ridges depends on the amount of determinant molecules (such as maternal proteins and coding RNAs). As indicated in the results, we found unexpectedly that the number of PGCs in gonadotropin stimulated embryos was almost twice as many as that in the unstimulated ones, suggesting that gonadotropin increases PGC numbers. As there are evidence (Wasserman *et al* 1982; Smith *et al* 1991) indicating that the rate of mRNA translation and therefore protein synthesis during oocyte maturation are elevated in stimulated oocytes, one can speculate that gonadotropin may increase PGC numbers through an increase in the amount of determinant. Moreover according to our data, gonadotropin stimulated embryos need higher amount of chloramphenicol, which may also explain the existence of higher amounts of determinants. Role of the amount of determinants in differentiation of PGC is also documented elsewhere. Kotani *et al* (1993) have reported that reduced number of germinal granules results in a reduction of PGC numbers. Further, evidence by Ephrussi and Lehmann (1992) showed that adding extra determinants (Oskar mRNA) in *Drosophila* oocyte leads to the formation of more pole cells, making us to speculate that the higher the amount of determinant the higher the requirement for RNA translation inhibition.



**Figure 2.** PGC numbers in genital ridges of stage 46 *Xenopus* embryos. (a) Unstimulated embryos treated with 50  $\mu$ M chloramphenicol show a significant decrease ( $P = 0.05$ ) in PGC numbers compared to that in control embryos. (b) Stimulated embryos treated with 50  $\mu$ M and 500  $\mu$ M chloramphenicol. PGC numbers in embryos treated with 500  $\mu$ M chloramphenicol show a decrease compared to that in control embryos, however it was not statistically significant. (c) Gonadotropin stimulated embryos show a significant increase ( $P < 0.001$ ) in PGC numbers compared to that in unstimulated embryos.  $P$  values derived from student's  $t$ -test.

In conclusion, we suggest that translation of maternal mRNAs in the oocyte has a key role in the formation and/or differentiation of PGC in early embryos, a process, which could be controlled by the amount of the determinants such as maternal mRNAs and proteins formed during oocyte maturation.

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