Effects of High Pressure on Early Development of *Xenopus* Embryos

Takumi KAMATA, Shigeyuki TERADA and Takeo YAMAGUCHI

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Abstract

Effects of high pressures (20, 40, 60, and 80 MPa) and ultraviolet (UV) irradiation on *Xenopus laevis* development were examined by using embryos at 8-cell (stage 4), blastula (stages 7-8), and tailbud (stages 29-30). *Xenopus* embryos at all stages examined here were stable to a pressure of 20 MPa. However, the development of embryos at 8 cells or blastula was affected by pressures of 40 MPa or over, whereas the embryos at tailbud stage showed normal development against 80 MPa. On the other hand, UV-irradiated embryos at 8-cell and blastula stages developed normally but the embryos at tailbud stage were sensitive to UV irradiation. These results suggest that high pressures of 40 MPa or over affect the development of early embryos and that high pressure as well as UV is also a useful means to examine the development of embryos.

Key words: embryo, development, high pressure, UV, *Xenopus*

INTRODUCTION

A new organism is produced by proliferation and development of a fertilized egg. The frog *Xenopus laevis* is very useful because of breeding by hormonal injection, production of hundreds of embryos per mating, and rapid development during embryonic stages. In the early development of the *Xenopus* embryo, the single large egg cell subdivides into many smaller cells without any change in embryo's volume. Such cell divisions are characterized by the very high rate of DNA replication and mitosis, and by the cell cycle without G_1 and G_2 phases [1]. Therefore, the early development of embryos is dependent on reserved materials such as RNAs and proteins. During a midblastula transition after the twelfth cleavage, the cell cycle slows down by the synthesis of new mRNAs and the intervention of G_1 and G_2 phases between the S and M phases [2]. In previous reports about the high-pressure effect on proliferation of mammalian cells, we demonstrated that S-phase cells are more sensitive to high pressure [3]. So, it is of interest to examine whether the early development of embryos is affected by pressure. In the present work, we show that *Xenopus* embryos at 8-cell stage than tailbud stage are more sensitive to a pressure of 40 MPa.

MATERIALS AND METHODS

Adult males and females of *Xenopus laevis* were injected with 300 and 600 IU of human chorionic gonadotropin, respectively. Fer-

Department of Chemistry, Faculty of Science, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka, 814-0180, Japan

tilized eggs from mating pair of *Xenopus laevis* were suspended in a 1/5 DeBoer solution (DeBoer solution: 110 mM NaCl, 1.3 mM KCl, 1.3 mM $CaCl_2$, 5 mM Tris, pH 7.4) containing penicillin (0.1 U/ml) at 23 ℃. Embryos were staged according to Nieuwkoop and Faber [4]. To compress embryos, samples in 1/5 DeBoer solution were put into a syringe-type cell with a piston. The sample cell was placed in a pressure bomb made of stainless steel and incubated for 10 min at 23 ℃ and various pressures (20 ~ 80 MPa) [5]. For UV irradiation, embryos in 1/5 DeBoer solution were exposed to irradiation dose of 50 or 100 J/cm^2 at 254 nm. In embryos at early stages, the animal pole was irradiated. The development at atmospheric pressure and 23 ℃ of pressure- or UV-treated embryos was monitored under a light microscope.

RESULTS

Effects of high pressure (20 ~ 80 MPa) on *Xenopus* development up to stage 45 were examined by using embryos at 8-cell (stage 4), blastula (stage 7-8), and tailbud (stage 29-30) (Fig. 1A). When the embryos at all stages were exposed to a pressure of 20 MPa and then cultured at atmospheric pressure, all of them were developed normally up to stage 45. Complete inhibition of embryo's development by pressure was observed at 40 MPa for embryos at the 8-cell stage and 60 MPa for them at the blastula stage. Interestingly, the embryos at tailbud stage were insensitive to pressures up to 80 MPa, *i.e.*, these pressure-treated embryos showed normal development. On the other hand, the response of embryos to UV irradiation on development was different from that to high pressure. For UV irradiation, the embryos at 8-cell and blastula stages were insensitive but those at tailbud stage were sensitive (Fig. 1B).

DISCUSSION

In the present work, we have demonstrated that the development of early embryos as seen at 8-cell stage is inhibited by a pressure of 40 MPa, but the embryos at tailbud stage are

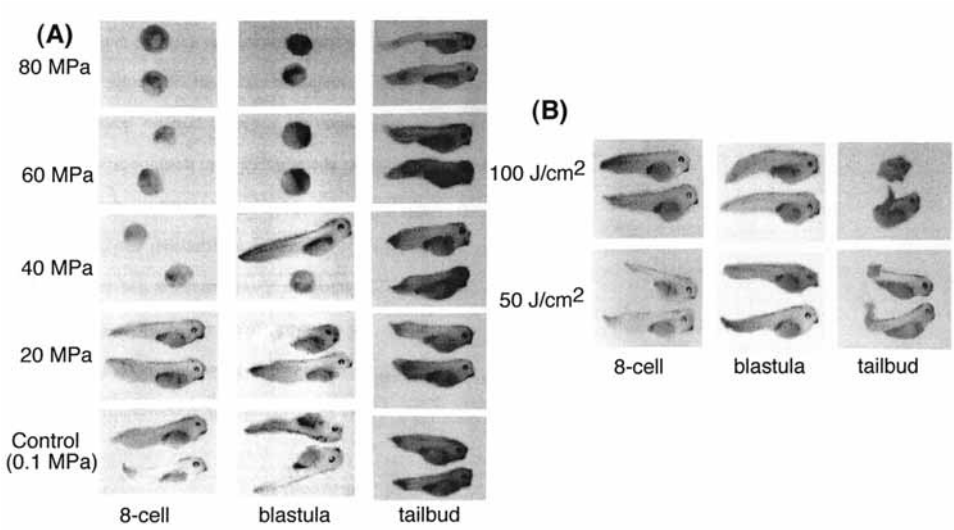

Fig. 1. Development of pressure- or UV-treated embryos. (A) The embryos of 8-cell, blastula, and tailbud stages were exposed to pressures (A) and UV (B), and then cultured at atmospheric pressure. Control, pressure- and UV-untreated embryos.

stable against a pressure of 80 MPa. Here, it is of interest to examine the response of a biological system to high pressure. For instance, the reversal of anesthesia in tadpoles occurs at ~ 10 MPa [6]. Rounding of CHO cells spread to fibronectin-coated glass is observed at ~ 40 MPa [7]. The inhibition of cell adhesion to the substratum begins to occur at ~ 60 MPa [7]. Cell proliferation is suppressed at 80 MPa [3]. Of cell cycles, S-phase cells are most sensitive to a pressure [3]. The S-phase cells exposed to a pressure of 80 MPa arrest in G_2 phase [3]. In 100 MPa-treated cells, apoptosis is induced [8, 9]. The structural changes in the plasma membrane are observed at higher pressures. Hemolysis and vesiculation of human erythrocytes occur at 130 ~ 140 MPa [10, 11]. Thus, reversal changes in cellular functions are observed at pressures below ~ 40 MPa, whereas cellular damages such as apoptosis and membrane disruption are induced around 100 MPa. The present work shows that *Xenopus* embryos at early stages are more sensitive to high pressure. Single large fertilized egg subdivides rapidly around the cell cycle of S and M phases. However, the rate of cell division after about 12 cycles of cleavage slows down by the appearance of G_1 and G_2 phases. Thus, the blastomeres of 8-cell and blastula (stages 7-8) progress around the cell cycles of S and M phases, whereas the cell cycle in tailbud-stage tadpoles is G_1, S, G_2, and M phases. Previously, we examined the pressure effect on DNA replication using a *Xenopus* cell-free system. The DNA replication occurs upon incubating 80 MPa-treated sperm nuclei in the pressure-untreated egg extracts, indicating the stability of DNA against high pressure [12]. On the other hand, when pressure-untreated sperm nuclei are added into 80 MPa-treated extracts, no DNA replication is observed [12]. Thus, egg extracts are unstable against a pressure of 80 MPa. Furthermore, from pressure effects on cell-cycle progression of sperm nuclei in a *Xenopus* cell-free system, we found that the cell cycle of sperm nuclei progresses normally in 40 MPa-treated extracts. The present work demonstrates that the embryo development at 8-cell stage is inhibited by a pressure of 40 MPa. These results suggest a possibility that the replication fork on DNA strands in early embryos is more sensitive to a pressure of 40 MPa, compared to DNA replication components in extracts. However, the effects of pressure on M-phase cells remain to be clarified. Surprisingly, tailbud-stage tadpoles subjected to a pressure of 80 MPa are able to develop to advanced stages. Previous work showed that the cells in G_1 and G_2 phases are stable to a pressure of 80 MPa, but the cells in S phase are sensitive to that pressure [3]. Therefore, further study is necessary concerning the insensitivity of tailbud-stage tadpoles to a pressure of 80 MPa. On the other hand, tailbud-stage tadpoles are sensitive to UV irradiation. In this case, all cells irrespective of cell cycles are damaged by UV irradiation. Insensitivity of embryos at 8-cell and blastula stages to UV irradiation from the animal side may be due to the brown pigment distributed primarily in the animal hemisphere of the embryos.

REFERENCES

1. Newport, J. W. and Kirschner, M. W. (1984) Regulation of the cell cycle during early Xenopus development. *Cell* **37**, 731-742
2. Newport, J. and Kirschner, M. (1982) A major developmental transition in early Xenopus embryos: II. Control of the onset of transcription. *Cell* **30**, 687-696
3. Matsumoto, M., Yamaguchi, T., Fukumaki, Y., Yasunaga, R., and Terada, S. (1998)

High pressure induces G_2 arrest in murine erythroleukemia cells. *J. Biochem.* **123**, 87-93

4. Nieuwkoop, P. D. and Faber, J. (1967) Normal table of *Xenopus laevis* (Daudin), North-Holland, Amsterdam.
5. Yamaguchi, T., Kawamura, H., Kimoto, E., and Tanaka, M. (1989) Effects of temperature and pH on hemoglobin release from hydrostatic pressure-treated erythrocytes. *J. Biochem.* **106**, 1080-1085
6. Johnson, F. H. and Flagler, E. A. (1950) Hydrostatic pressure reversal of narcosis in tadpoles. *Science* **112**, 91-92
7. Hashiguchi, T., Yamaguchi, T., and Terada, S. (1999) Cell-substratum adhesion is suppressed by high pressure. *Jpn. J. Physiol.* **49**, 107-112
8. Take, J., Yamaguchi, T., Mine, N., and Terada, S. (2001) Caspase activation in high-pressure-induced apoptosis of murine erythroleukemia cells. *Jpn. J. Physiol.* **51**, 193-199
9. Takano, K.J., Takano, T., Yamanouchi, Y., and Satou, T. (1997) Pressure-induced apoptosis in human lymphoblasts. *Exp. Cell Res.* **235**, 155-160
10. Yamaguchi, T., Kajikawa, T., and Kimoto, E. (1991) Vesiculation induced by hydrostatic pressure in human erythrocytes. *J. Biochem.* **110**, 355-359
11. Kitajima, H., Yamaguchi, T., and Kimoto, E. (1990) Hemolysis of human erythrocytes under hydrostatic pressure is suppressed by cross-linking of membrane proteins. *J. Biochem.* **108**, 1057-1062
12. Takahashi, H., Yamaguchi, T., Koga, M., Kageura, H., and Terada, S. (2004) DNA replication reaction in *Xenopus* cell-free system is suppressed by high pressure. *Cell. Mol. Biol. Lett.* **9**, 423-427