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Comparative expression analysis of multiple PDK genes in *Xenopus laevis* during oogenesis, maturation, fertilization, and early embryogenesis

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ABSTRACT

The complete family of expressed pyruvate dehydrogenase kinase (PDK) genes in the tissues of the African clawed frog, *Xenopus laevis*, consists of four members. Our previous study [Terazawa, Y., Tokmakov, A., Shirouzu, M., Yokoyama, S., 2005. Molecular cloning and expression analysis of PDK family genes in *Xenopus laevis* reveal oocyte-specific PDK isoform. Biochem. Biophys. Res. Commun. 338, 1798–1804] revealed that expression patterns of PDK genes differ greatly in the oocytes and somatic tissues of the adult frog. In the present work, using quantitative reverse-transcriptase PCR analysis, we demonstrate that the major transition from the oocyte-specific to somatic tissue-specific xPDK expression pattern occurs at the late stages of *Xenopus* embryogenesis after mid-blastula transition (MBT). Also, we show that the content of mRNA for xPDKo3, which is the predominant PDK isoform in oocytes and eggs, increases by about 3-fold during maturation. Other PDK family genes are down-regulated during oogenesis, thus being at their lowest expression levels in the grown-up oocytes, matured eggs, and early embryos. The expression of all PDK genes increases several-fold in the embryogenesis following MBT. Analysis of protein expression using an antibody raised against C-terminal of xPDKo3 confirmed isoform-specific up-regulation of xPDKo3 late in maturation and revealed cytoplasmic and mitochondrial localization of this protein. Bioinformatics and mass-spectrometric analyses allowed identification of an N-terminal mitochondrial targeting signal and a peptide cleavage site in xPDKo3 molecule.

Catalytic activity of the multimeric mitochondrial pyruvate dehydrogenase complex (PDC), which catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA, is regulated by reversible phosphorylation (Holness and Sugden, 2003; Patel and Korotchkina, 2006). Dedicated protein kinases, pyruvate dehydrogenase kinases (PDKs), inactivate PDC by the phosphorylation of three seryl residues in the pyruvate decarboxylase subunit, E1 (Yeaman et al., 1978; Sale and Randle, 1981), whereas pyruvate dehydrogenase phosphatases (PDPs) catalyze the dephosphorylation and reactivation of the E1 subunit (Teague et al., 1982; Huang et al., 1998). Recently, some isozymes of the PDK family were found to be induced in diabetes and starvation, making them a potential target for diabetes treatment (Wu et al., 1999, 2000; Sugden et al., 2000).

Four genetically and biochemically distinct PDK family isozymes (PDK1, 2, 3, and 4) have been identified in humans (Gudi et al., 1995; Rowles et al., 1996). They have diverse tissue-specific distributions and specificities (Bowker-Kinley et al., 1998; Korotchkina and Patel, 2001). Recently, we have demonstrated that the complete family of expressed PDK genes in the tissues of the African clawed frog, *Xenopus laevis*, also consists of four members, divided into two evolutionary groups (Terazawa et al., 2005). One of the identified *Xenopus* PDK genes (xPDKo3) was predominant in oocytes, suggesting its oocyte-specific function. A striking difference in xPDK expression patterns in the oocytes and somatic tissues of the adult frog has been detected.

In the present study we sought to investigate the dynamics of transition from the oocyte-specific to somatic tissue-specific PDK expression patterns. For this purpose, we studied the expression patterns of multiple xPDK genes in *Xenopus* oocytes, eggs, and early embryos. This biological model provides the opportunity to analyze cells at various stages of the cell cycle, at different steps of development and differentiation (Fig. 1). At present, there are no reliable data presented about PDK expression in oocytes, eggs, and embryos of other biological species due to the difficulties of sample collection.

We demonstrate here that the unique oocyte-specific pattern of xPDK expression is maintained at all studied stages of development and differentiation, preceding MBT. Expression of the

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oocyte-specific xPDKo3 isoform increases by about 3-fold in the process of progesterone-induced meiotic maturation and changes little during oogenesis, fertilization, and early embryogenesis. Other xPDK family genes are down-regulated during oogenesis. The major transition from the oocyte-specific to somatic tissue-specific xPDK expression patterns occurs at the late stages of *Xenopus* embryogenesis, after MBT. Expression of all PDK genes increases several-fold, and relative abundances of PDK mRNAs change dramatically at that time. Isoform-specific up-regulation of xPDKo3 late in maturation was also confirmed using an antibody raised against C-terminal of this protein. Bioinformatics and mass-spectrometric identification of the N-terminal mitochondrial targeting signal allowed mapping the peptide cleavage site between residues 20 and 21 of xPDKo3 molecule. Up-regulations of PDK genes during maturation and late in embryogenesis should be indicative of the essential metabolic changes that occur in development and differentiation. Notably, the changes in PDK expression coincide with the major changes in the nutritional environment during differentiation and development.

1. Results

1.1. Expression of xPDK genes during oogenesis

Oocytes at different stages of oogenesis (stages II–VI, by the classification of Dumont) (Dumont, 1972) were manually collected from collagenase-treated frog ovaries. Total content of xPDK mRNAs in oocytes was measured with the use of the specially designed universal primers that could amplify simultaneously all of the xPDK isoforms (Terazawa et al., 2005). We found that the overall level of xPDK expression did not change statistically significantly during oogenesis (Fig. 2A). Furthermore, the relative abundances of PDK gene transcripts were determined by the quantitative real-time PCR, with the primer sets that specifically amplify a homologous sequence segment from each individual xPDK isoform (Fig. 3A). Concerning primer specificity, our data demonstrate that PDKk- and PDKe-specific primers could amplify only their cognate sequences (Fig. 3B and D), whereas PDKo1- and PDKo3-specific primers could amplify, in addition, the highly homologous PDKo3 and PDKo1 sequences, respectively, albeit with much lower efficiency (Fig. 3C and E). Using these specific PCR primers, we found that xPDKo3 was the major PDK isoform at all stages of oogenesis investigated (Fig. 4A). Its expression level, as well as that of xPDKo1, remained virtually unchanged during oogenesis. On the other hand, the expression of two minor isoforms, xPDKe and xPDKk, decreased several-fold over oogenesis stages II and III, reaching the lowest level in the oocytes of stages IV–VI (Fig. 4A). The obtained data on the relative abundances of xPDK gene transcripts in oogenesis agreed well with the data on the total content of xPDK mRNA (Fig. 2A).

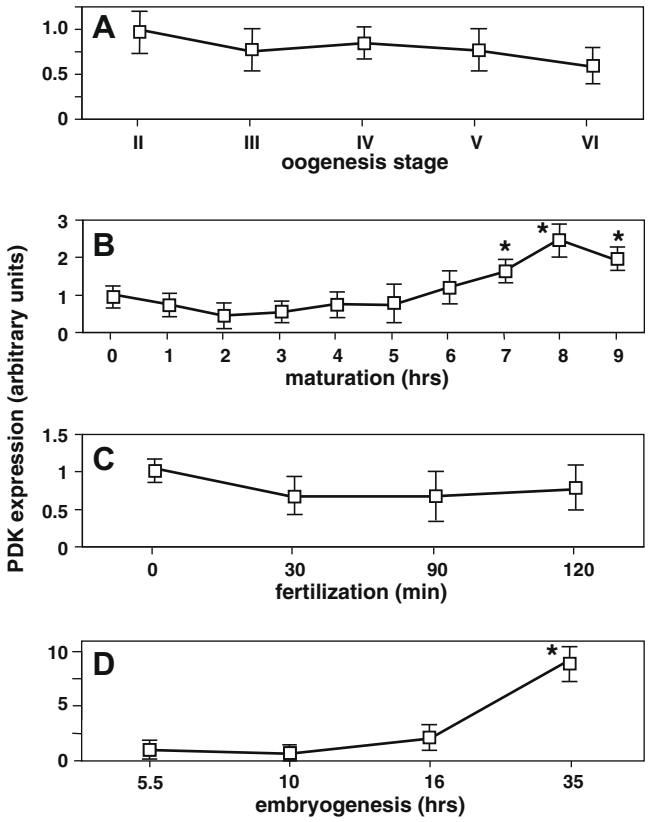

Fig. 2. Analysis of xPDK expression using universal quantitative PCR primers. The universal primers, xPDK-FWuni and xPDK-RVuni, that can amplify all of the xPDK isoforms simultaneously, were constructed as described previously by Terazawa et al. (2005). Panels (A)–(D) represent the total content of xPDK mRNAs during oogenesis, maturation, fertilization, and embryogenesis. The data are the means ± SD of four measurements, obtained in two independent experiments. Asterisk indicates a significant difference from the control ($P < 0.05$).

1.2. Expression of xPDK genes during meiotic maturation

Next we investigated changes in the expression of xPDK genes during progesterone-induced meiotic maturation of *Xenopus* oocytes. Using the universal primers that could amplify simultaneously all of the xPDK isoforms, we found that the overall level of xPDK expression changed statistically significantly by the end of maturation, in 7–9 h after progesterone administration (Fig. 2B). However, similarly to oogenesis, the expression pattern of xPDK isoforms did not change essentially during maturation (Fig. 4B). The major detected developmental change during this process involved the oocyte-specific PDK isoform, xPDKo3. Its

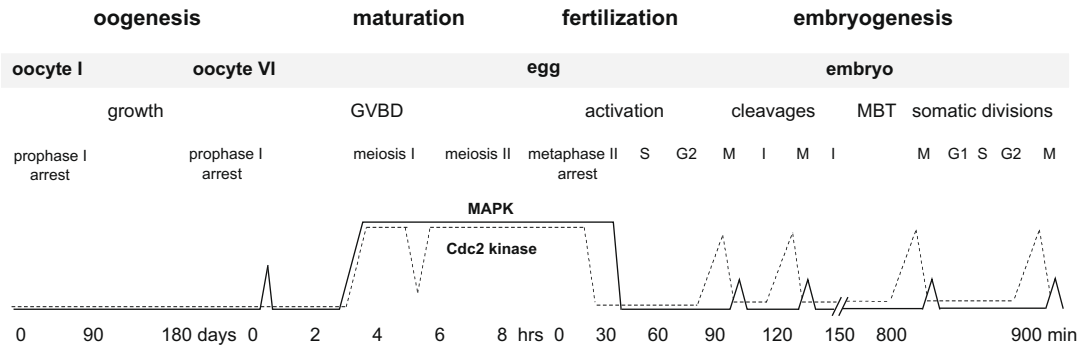

Fig. 1. *Xenopus* oocytes, eggs, and early embryos. Cell cycle progression during *Xenopus* oocyte oogenesis, egg maturation and fertilization, and embryogenesis is presented schematically along with the activities of the key cell cycle regulators, MAPK and Cdc2 kinase.