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MAJOR PROTEINS IN PLANT AND ANIMAL EGGS

TAKASHI OKAMOTO[1] AND ERHARD KRANZ[2]

[1]*Department of Biological Sciences, Tokyo Metropolitan University, Minami-osawa 1-1, Hachioji, 192-0397 Japan*
[2]*Biozentrum Klein Flottbek und Botanischer Garten, Entwicklungsbiologie und Biotechnologie, Universität Hamburg, Ohnhorststr 18, 22609 Hamburg, Germany*

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In most higher plants, the female gametophyte is deeply embedded in the ovule. In an earlier work we isolated egg cells from maize ovule tissues and analyzed egg cell lysates by polyacrylamide gel electrophoresis and mass spectrometry-based proteomics technology, and identified the major protein components expressed in these cells. The identified proteins included three cytosolic enzymes for the glycolytic pathway (glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, triosephosphate isomerase), two mitochondrial proteins (ATP synthase β-subunit and adenine nucleotide transporter), and annexin p35. Our data indicate that the plant egg cell is rich in an enzyme subset for energy metabolism. This article provides a short overview of major proteins in animal eggs, reports on major protein components of maize egg cells, and compares the major proteins between animal and plant eggs.

Key words: Fertilization, egg cell, zygote, protein, polyacrylamide gel electrophoresis, mass spectrometry-based proteomics, maize.

INTRODUCTION

The functioning of processes in a cell is generally determined by controlled interactions of a variety of proteins. The composition of cellular proteins differs depending on the cell type, and the major proteins of highly differentiated cells reflect the biological function of the cell. For example, mesophyll cells have a large amount of ribulose-1,5-bisphosphate carboxylase/oxygenase for fixation of carbon dioxide, while the cotyledon cells of non-endospermic seeds such as legume seeds contain abundant storage globulins and albumins, which supply the nutrient source for growth of the hypocotyl during seed germination, and for growth of the seedling (Bewley and Black, 1994).

Egg cells of higher plants are highly differentiated haploid cells which, after fertilization by sperm cells, undergo early embryogenesis. Identification of the major protein components in egg cells will provide basic knowledge of their constituents and will provide cues for analyzing the mechanisms of female gametogenesis, fertilization and early embryogenesis in higher plants. However, unlike in animals and lower plants, egg cells of higher plants are located in embryo sacs embedded deeply in ovular tissue. To overcome the difficulties of direct observation and analysis of these cells, methods have been developed for isolating embryo sacs and egg cells in a wide range of higher plant species (for review: Theunis et al., 1991; Kranz and Kumlehn, 1999). In maize, 20–40 egg cells can be routinely isolated by one experienced person per day (Fig. 1a) and, under optimal conditions, up to 60 egg cells can be obtained (Kranz, 1999). This is a relatively small amount of plant material, but recent advances in proteomics technologies provide the possibility to identify proteins in such cells. We conducted biochemical analyses using maize egg cells to determine abundant proteins in egg cells, detected traceable amounts of proteins in a small number of egg cells by minimizing the size of the gels for one- and two-dimensional polyacrylamide gel electrophoresis, and identified major protein components by highly sensitive LC-MS/MS technology. In this paper we present a short overview of recent advances in the study of major proteins in animal eggs, report on the major protein components of maize egg cells, and finally compare the major proteins between animal and plant eggs.

MAJOR PROTEINS IN ANIMAL EGG CELLS

Early embryonic development is largely dependent on maternal gene products synthesized during oogenesis. Conceptually, animal eggs are divided into two groups on the basis of strategies for delivering nutrients to the developing embryo, called catering and box-lunch

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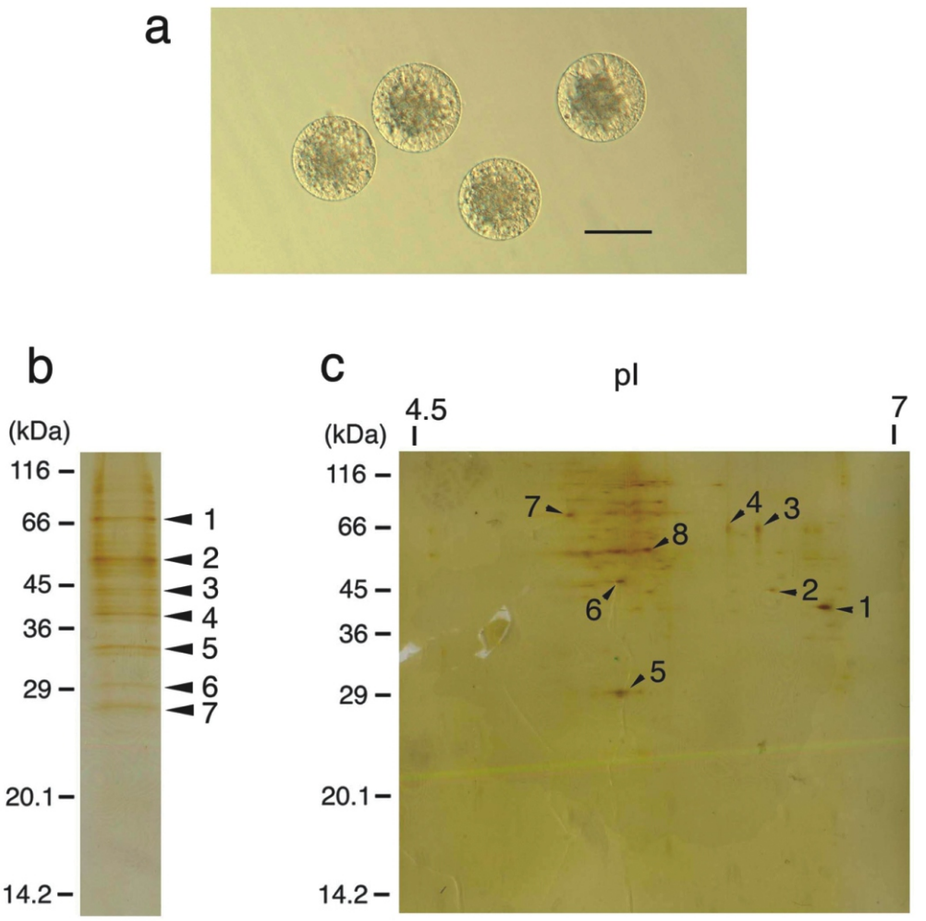

Fig. 1. SDS-PAGE and 2D-PAGE of proteins from maize egg cells. (**a**) Isolated egg cells. Bar = 50 µm, (**b**) Crude proteins from 75 egg cells were separated by SDS-PAGE, and the gel was stained by a silver-staining procedure modified for further in-gel triptic digestion and LC-MS/MS analysis. Numbers to the right of the arrowheads indicate the major protein identified by in-gel tryptic digestion and subsequent LC-MS/MS. The numbers are identical to those of protein bands in Table 1, (**c**) Crude proteins from 180 egg cells were separated by 2D-PAGE followed by modified silver-staining. Numbers around the arrowheads indicate the major protein identified by in-gel tryptic digestion and subsequent LC-MS/MS analysis. The numbers are identical to those of the protein spot in Table 2. The figure is modified from Okamoto et al. (2004).

strategies (Postlethwait and Giorgi, 1985). The former is common in mammals; the embryo stays inside the mother, and she supplies nutrients continuously via the blood stream until the embryo becomes mature enough to develop itself. In the latter, the mother packages nutrients into the eggs, and the embryo uses the yolk proteins during embryogenesis. This box-lunch strategy is employed by more than ~70% of animal species, including most invertebrates and many vertebrates (Izumi et al., 1994). The most common and abundant yolk proteins are vitellins, which are found in the frog, chicken, nematode, fish and many insects. Vitellins are synthesized as precursors, termed vitellogenins, in hepatic cells of vertebrates or in fat body cells of insects (Pan et al., 1969; Wyatt and Pan, 1978), and the precursors are cleaved into vitellins through post-translational processing by a subtilisin-like proteinase (Barr, 1991). The stored vitellins in the yolk are thought to provide a nutritional store for utilization during embryogenesis. Imschenetzky et al. (1999) reviewed the post-translational modifications of target proteins as mechanisms modulating events during fertilization, and proteolysis events relevant to egg-sperm interaction and chromatin remodeling. A series of biochemical events in the animal egg are triggered by the sperm to activate the egg metabolism, for example via

TABLE 1. Major proteins of maize egg cells identified by SDS-PAGE and subsequent tandem mass spectrometry

Band number	Protein	Accession number (GI)
2	mitochondrial ATP synthase β-chain	114420
3	cytosolic 3-phosphoglycerate kinase	28172915
4	cytosolic glyceraldehyde-3-phosphate dehydrogenase	6016075
5	annexin P35	7441507
6	mitochondrial adenine nucleotide translocator	22166

Band numbers are identical to those in Figure 1b. Table modified from Okamoto et al. (2004)

TABLE 2. Major proteins of maize egg cells identified by 2D-PAGE and subsequent tandem mass spectrometry

Spot number	Protein	Accession number (GI)
1	cytosolic glyceraldehyde-3-phosphate dehydrogenase	6016075
5	cytosolic triosephosphate isomerase	136063
6	cytosolic 3-phosphoglycerate kinase	28172915

Spot numbers are identical to those in Figure 1c. Table modified from Okamoto et al. (2004)

activation of protein phosphorylation cascades involving serine-threonine as well as tyrosine protein kinase (Abassi et al., 2000; Wu and Kinsey, 2000).

Proteomic analyses of mammal eggs are making progress. Calvert et al. (2003) revealed that mouse eggs contain abundant heat shock proteins (HSP70, HSP90a) and molecular chaperons (GRP94, GRP78), oxygen-regulated protein 150, calreticulin, calnexin and protein disulfide isomerase (PDI). The molecular chaperons are known to play a variety of roles in diverse cellular processes. It has been suggested that *HSP*70 is one of the first genes to be expressed following zygotic gene activation in the two-cell mouse embryo (Manejwala et al., 1991; Christians et al., 1995), and it appears to play a role in developmental processes in a number of species (Angelier et al., 1996). HSP90 has also been implicated in developmental regulation in the mouse (Loones et al., 2000). It has been proposed that members of the HSP family are involved in regulation of apoptosis, since HSP70 and GRP78 show anti-apoptotic activity, while HSP90 might have both pro-apoptotic and anti-apoptotic activity, depending on the specific stimuli (for review: Garrido et al., 2001). Moreover, early bovine embryos cultured in the presence of HSP70 antibodies exhibit increased apoptosis and reduced embryo viability (Matwee et al., 2001). However, the functions of egg HSPs and chaperons remain unclear, and the question of whether the unfertilized egg is actively apoptotic or undergoes necrosis in the reproductive tract is still controversial.

New post-genomic approaches in reproductive biology will focus on methods for functional analysis of genes collected from gametes and zygotes in order to understand the molecular mechanisms of egg development, fertilization and embryo formation, using model systems such as the African clawed frog *Xenopus laevis* (Sato et al., 2002) and mouse (Coonrod et al., 2002, 2004).

MAJOR PROTEINS IN PLANT EGG CELLS

Plant proteomics opens up new perspectives for analyzing complex functions of whole plants, specific tissues, cells and organelles (for review: Canovas et al., 2004). The proteomic approach is an area of research that analyzes protein expression by resolving, identifying, quantifying, and characterizing proteins through the use of a series of techniques including two-dimensional polyacrylamide gel electrophoresis (2D), tandem mass spectrometry, and computer analyses (Celis et al., 1998).

Tandem mass spectrometric analysis of proteomes allows amino acid microsequences to be rapidly obtained from a protein spot cored from a 2D gel. Microsequence data can then be compared against protein databases to establish whether a particular protein is known or novel. We employed the technology for identification of major proteins in maize egg cells. First we determined how many cells were needed to visualize protein bands and spots in gels of SDS-PAGE and 2D-PAGE, respectively. When a small gel mold ($50 \times 60 \times 1$ mm) was used, 15 and 45 cells were enough to detect silver-stained protein bands or spots in SDS-PAGE and 2D-PAGE, respectively, and the amount of protein in an egg cell was roughly estimated to be 100–200 pg (Okamoto et al., 2004). For in-gel triptic digestion and subsequent LC-MS/MS analyses, egg cell lysates from 75 and 180 cells were used for SDS-PAGE and 2D-PAGE, respectively, since the silver-staining methods were modified for in-gel triptic digestion and

subsequent LC-MS/MS analyses (Taoka et al., 2000). The gel images of maize egg proteins are shown in Figure 1b and 1c. We selected 7 protein bands and 8 protein spots from the gels of SDS-PAGE and 2D-PAGE, respectively, for further proteomic analyses. The identified major egg proteins are listed in Tables 1 and 2. Three cytosolic enzymes for the glycolytic pathway (glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase and triosephosphate isomerase), two mitochondrial proteins, (ATPase β-subunit and adenine nucleotide transporter), and annexin p35 were identified as major proteins in maize egg cells.

Plant mitochondria rarely respire fatty acids, in contrast to animal mitochondria which respire fatty acids and glycolytically derived pyruvate (for review: Plaxton, 1996). This indicates that glycolysis is of crucial importance in plants because it is the predominant pathway supplying fuels for plant respiration. Recently it was revealed that seven glycolytic enzymes, including glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase, are associated with the outer membranes of mitochondria, suggesting that such micro-compartmentation of glycolysis allows pyruvate to be provided directly into the mitochondrion (Giege et al., 2003). In mitochondria, ATPase synthesizes ATP, which is the principal energy source for the cells, via an H^+ gradient between the inner and outer membranes, and the resulting ATP is exchanged with cytosolic ADP by adenine nucleotide transporters (Vignais, 1976, Mozo et al., 1995). Five of the six major egg proteins identified in this study are thought to be involved in the cytosolic and mitochondrial energy production pathways, suggesting that the egg cell has sufficient enzymes and transporters to produce and transport an energy source. After in vitro fusion of the maize egg with a sperm cell, the majority of cytoplasmic organelles migrate towards the zygote nucleus, the cell wall is actively formed, and division of the nucleus occurs as part of the early cytological events in the zygote (Kranz et al., 1995). These energy-consuming serial zygotic events might explain why egg cells abundantly contain proteins for energy production.

When we analyzed the expression level of annexin p35 in the zygote, 2-celled embryo, central cells and cultured cells, it was indicated that annexin p35 was strongly expressed only in egg cells (Okamoto et al., 2004). Annexins are Ca^{2+}- and phospholipid-binding proteins. Extensive studies of these proteins of animal cells have shown their multifunctional roles in essential cellular processes such as membrane trafficking, ion transport, mitotic signaling, cytoskeleton rearrangement and DNA replication (for review: Gerke and Moss, 2002). Plant annexins share the basic properties of Ca^{2+}-dependent membrane-binding molecules, and are structurally similar to their animal counterparts (Pirck et al., 1994; Clark and Roux, 1995; Battey et al., 1996). Exocytosis and Golgi-mediated secretion of newly synthesized plasma membranes and cell wall materials have been reported as a function of annexin in plant cells (Carroll et al., 1998; Battey et al., 1999; Clark et al., 2001). It has been demonstrated that cell wall formation around the zygote starts 30 seconds after in vitro fusion of the egg with a sperm cell (Kranz et al., 1995). This rapid formation of the cell wall around the zygote suggests that cell wall materials are stored in the egg cells before fertilization and are secreted via possible exocytosis after fertilization. It is well known that Ca^{2+} exerts regulation of exocytosis in plant and animal cells (Bush, 1995; Battey et al., 1999). Carroll et al. (1998) reported that Ca^{2+}-stimulated exocytosis in root cap cells is enhanced by exogenous annexin p35, suggesting that annexin is involved in Ca^{2+}-stimulated exocytosis. In addition, concentrations of cytosolic Ca^{2+} in the maize egg cell/zygote appears to increase after fertilization (Digonnet et al., 1997), possibly via influxes of extracellular Ca^{2+} (Antoine et al., 2000). Annexin p35, abundant in egg cells, might play a role in exocytosis, which is stimulated by fertilization-induced increases of Ca^{2+} levels in the zygote, for rapid formation of cell wall around the zygote.

CONCLUSIONS AND PROSPECTS

Our analyses of major proteins in maize egg cells have shown that plant eggs, unlike mammal eggs, do not contain HSP or chaperons as major protein components. This means that the protein composition of the egg largely differs between plants and mammals. However, plant egg cells, like mammal eggs, might be classified as catering-type eggs, since storage proteins such as the yolk proteins found in box-lunch type animal eggs could not be identified as major proteins in maize eggs. These results are consistent with our knowledge that nutrients are supplied from the mother plant during embryogenesis and seed formation. Although there are only a few stored proteins in plant egg cells, storage proteins such as seed albumins and globulins are accumulated in embryos and endosperms during seed maturation, and are degraded and utilized for seed germination and seedling growth. Interestingly, papain-type cysteine proteases, which play major roles in degradation of storage proteins, are also involved in degradation of yolk proteins in box-lunch-type animal eggs/embryos. Plant seeds and box-lunch-type animal eggs are both disconnected from their mothers, and they appear to employ similar mechanisms for the breakdown and utilization of stored proteins until they can survive independently. In terms of their storage proteins, seeds of higher plants may correspond to box-lunch-type animal eggs.

Plant egg cells are highly polarized cells. As found in polar eggs of animals, maternal genetic systems

might be found which are responsible for the polarity of the plant egg, and subsequently for the first unequal division of the zygote. A challenge for future work will be to demonstrate the putative unequal localization of maternally expressed egg proteins. Their polar distribution may lead to the unequal division of the zygote. Besides dissection of asymmetric protein localization, isolation and functional analyses of the proteins whose expression is induced by fertilization is of importance for our understanding of early plant embryogenesis and endosperm development. Using our in vitro fertilization system, gametes can be isolated and individually fused, and the embryo and endosperm can develop independently under defined culture conditions. Micromanipulation techniques together with highly sensitive analytical methods such as LC-MS/MS are powerful tools to dissect the processes responsible for fertilization and early embryo development in higher plants.

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