A reliable protocol for direct detection of lectin binding sites on the plasma membrane of a single living sperm cell in maize

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ORIGINAL ARTICLE

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Fluorophore-conjugated lectin labeling of the cell surface of isolated male and female gametes, central cells and synergids before and after fertilization in maize

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Abstract Three fluorescein isothiocyanate (FITC)-conjugated lectins, *Canavalia ensiformis* agglutinin (Con A), *Triticum vulgaris* agglutinin (WGA) and *Phaseolus vulgaris* erythroagglutinin (PHA-E), were used as probes to localize sugar moieties of glycoconjugates on the cell surface of isolated maize sperm, egg, central, antipodal cells, synergids, and in vitro- and in vivo-fertilized zygotes. Fluorescence signals on the surface of the cells were due to specific binding. Calcium was necessary for WGA and PHA-E binding and enhanced Con A labeling. Differences in glycoconjugate composition of the membranes of gametes and other embryo sac component cells were found. FITC-Con A strongly labeled egg and central cells, but labeled sperm only weakly. FITC-WGA binding sites were detected on egg, but not sperm cells. Con A and WGA binding sites were equally distributed around egg and central cell protoplasts. FITC-PHA-E binding sites were not found on sperm and egg cells before fertilization. Binding sites of these lectins were located on synergids, especially on their filiform apparatus. Interestingly, WGA binding to egg cells was enhanced after fertilization, whereas PHA-E binding to egg cell membranes could only be detected after fertilization. These results suggest the occurrence of fertilization-induced changes in glycoconjugate composition of the maize egg cell membrane. An increase in the number of WGA and PHA-E binding sites was also observed on newly formed cell walls of cultured two-celled embryos derived from in vitro-produced zygotes.

Keywords Maize · Lectin · Gamete · Zygote · Fertilization

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Introduction

Lectins are proteins that bind reversibly to specific mono- or oligosaccharides. These lectin-binding molecules are referred to as lectin receptors due to the structural complexity of the ligands. In animals, lectin receptors – mainly glycoproteins – on the surface of gametes play an essential role in fertilization, with respect to both adhesion and fusion (Florman and Wassarman 1985; Bleil and Wassarman 1988; Boldt et al. 1989; Wassarman 1992; Dhume and Lennarz 1995; Focarelli and Rosati 1995; Freeman 1996; Dell et al. 1999). In vitro fertilization experiments revealed that carbohydrates, as competing inhibitors, can prevent penetration of zona-free hamster and mouse eggs (Dravland and Mortimer 1988; Okabe et al. 1989; Ponce et al. 1994). In *Chlamydomonas*, lectins responsible for gamete adhesion have been identified and characterized on flagellar membranes (Musgrave et al. 1981; Pijst et al. 1984; Collin-Osdoby and Adair 1985; Klis et al. 1985; Samson et al. 1987). Wheat-germ agglutinin (WGA; *Triticum vulgaris* agglutinin), a lectin that binds to flagellar surfaces, induced all mating responses and increased intracellular cAMP in *Chlamydomonas eugametos*, suggesting an important function in gamete fusion and sexual signaling (Kooijman et al. 1990). In red algae, *Canavalia ensiformis* agglutinin (Con A) might be involved in gamete recognition (Kim and Kim 1999). In higher plants, lectin-receptor interaction might be involved in pollen-stigma recognition and pollen tube growth (Southworth 1975; Clarke et al. 1979). Binding of several lectins to somatic plant protoplasts derived from suspension cultured rose cells or

from maize root cortex has been reported (Walko et al. 1987). However, there is no information on whether lectins and their receptors are involved in fertilization and embryogenesis in higher plants and whether glycoproteins can be identified on the surface of egg cell membranes.

The present study was undertaken to identify lectin-fluorophore conjugates that would be useful as cytochemical markers for the plasma membrane of isolated gametic cells. Another purpose of this investigation was to examine whether there are fertilization-induced changes in glycoconjugate composition on the higher plant egg membrane.

We demonstrate lectin binding sites on single plant gametes, zygotes and related reproductive cells that are not readily accessible in quantities suitable for standard test tube-based labeling methods. Lectin binding sites were labeled directly on isolated single cells by means of single cell manipulation. Three FITC-conjugated lectins were used as probes to localize their specific binding sites on both male and female gametic membranes, other protoplasts of component cells, the embryo sac and protoplasts of isolated zygotes. These lectins were also used to localize their specific binding sites on cell walls of the embryo sac and newly formed cell walls of in vitro cultured zygotes. A comparative study was undertaken to investigate possible changes of specific lectin binding sites on the egg surface before and after fertilization in maize.

Materials and methods

Plant material

All cells were isolated from maize (*Zea mays* L.) plants, inbred line A 188, which were grown under standard conditions in the greenhouse of the Institute of General Botany of the University of Hamburg.

Isolation of protoplasts from sexual cells, somatic cells and zygotes

Sperm, egg and central cells, synergids, groups of antipodal cells and nucellar protoplasts were individually isolated and manipulated according to Kranz et al. (1991). Enzymatic maceration was prolonged to 1.5 h for nucellar cell protoplast isolation. To be sure that the cell wall was completely digested after treatment with cell-wall-degrading enzymes, selected protoplasts were treated with calcofluor white ST, which labels cell wall material (Kranz et al. 1995).

To examine whether there is a different lectin labeling pattern between the two sperm cells from one pollen grain, sperm cells were individually isolated from each pollen grain and collected.

Single protoplasts of both sexual and somatic cells were collected and stored in a droplet of 2,000 nl mannitol solution on a coverslip that was covered with a layer of mineral oil. To test whether binding of sugar residues is modified after enzyme treatment, egg cells and synergids were additionally isolated without the use of cell-wall-degrading enzymes.

In vivo zygotes were isolated 13 h after pollination using the procedures for ear segment pollination under controlled conditions (Kranz and Brown 1992) and for egg and central cell isolation according to Kranz et al. (1991).

In vitro gamete fusion and zygote culture

Single pairs of an isolated egg and a sperm cell were fused electrically according to Kranz et al. (1991). In vitro-produced zygotes were collected and stored in mannitol solution for lectin labeling or transferred into a Millicell-insert for culture. Culture techniques were as described by Kranz et al. (1991). After culturing for 1–3 days, zygotes were washed in mannitol solution three times before lectin labeling.

Lectin labeling procedure

Three kinds of FITC-conjugated lectins were used as probes: Con A (specificity: α-D-mannose and α-D-glucose), WGA [specificity: $(D\text{-GlcNAc})_2$ and NeuNAc] and *Phaseolus vulgaris* erythroagglutinin (PHA-E, specificity: complex oligosaccharides) purchased from Sigma-Aldrich. Unless noted otherwise, the labeling procedure was as follows. Protoplasts were washed three times in mannitol (680 mosmol for sperm cells, 650 mosmol for other protoplasts). After washing, protoplasts were placed in a 300 nl droplet of mannitol containing FITC-lectin and incubated for 30–40 min at 22°C. To investigate the effect of lectin concentration on binding, cells were treated with lectins at concentrations from 0 to 500 µg/ml for 10–60 min (Tables 1 –4).

To test the effect of calcium on lectin binding, protoplasts were treated with mannitol solutions containing lectin and 0.5 mM calcium. After treatment, protoplasts were washed by gradually diluting and finally replacing the initial incubation medium with mannitol. In all other experiments, protoplasts were treated with lectin without calcium, followed by addition of an equal amount of a mannitol solution containing 5 mM calcium to the same incubation droplet. After 10 min incubation, cells were subsequently transferred into another droplet of mannitol for washing. This procedure avoids protoplasts sticking to the glass surface and bursting. Control protoplasts were washed in mannitol solution without calcium.

Selected sperm cell pairs, isolated from single pollen grains, were treated with FITC-Con A, FITC-WGA and FITC-PHA-E following the procedure described by Sun et al. (2002).

Binding specificity test

Controls were made to test lectin binding specificity. Before incubation in lectin solutions, protoplasts were pretreated for at least 30 min in media containing 0.025, 0.05, 0.1 and 0.2 M monosaccharides. D-Mannose was used as a competitor for Con A and D-*N*-acetyl-glucosamine as a competitor for WGA; both were dissolved in mannitol solution (osmolality 680 mosmol for sperm and 650 mosmol for other protoplasts).

Observation and imaging

Cells were observed using an inverted microscope (Axiovert 35 M, Zeiss). Images were viewed by a 3CCD (Sony XC003P) with enhanced sensitivity, and collected and processed using Pro-Plus software.

Fluorescence signals of individual protoplasts were usually integrated for 1 s. To compare the intensities of fluorescence from different protoplasts or of different lectin labeling experiments, integration times from 0.5 to 2 s were applied.

The intensity of labeling of the plasma membrane was scored on a qualitative scale: ++++, very strong labeling, fluorescence signals could be clearly visualized by an integration time of 0.5 s; +++, strong labeling, the same signal visualized by 1.0 s of integration; ++, good labeling, 1.5 s was needed; +, weak labeling, 2 s was needed; – no labeling; no fluorescence signals were detected, even with longer integration time (2.5 s).

Table 1 The effect of lectin concentrations on binding to cell membranes of protoplasts of maize gametes. The gametes were incubated for 30 min in *Canavalia ensiformis* agglutinin (Con A) solution, or 45 min in *Triticum vulgaris* agglutinin (WGA) or *Phaseolus vulgaris* erythroagglutinin (PHA-E) at 22°C. After lectin treatment without calcium, an equal amount of a mannitol solution containing 5 mM calcium was added to the same droplet for a further 10 min incubation. For treatment at each concentration of the three lectins, the number of cells used was 30

Lectin	Concentration (μg/ml)	Labeling on gamete membrane	
		Egg cell	Sperm cell
Con A	250	+++[a]	+
	125	+++	+
	65	++	–
WGA	500	++	–
	250	++	–
	125	+	–
PHA-E	500	–	–
	250	–	–
	125	–	–

[a] Qualitative scale: ++++ very strong labeling, fluorescence signals could be clearly visualized by an integration time of 0.5 s; +++ strong labeling, the same signal visualized by 1.0 s of integration; ++ good labeling, 1.5 s was needed; + weak labeling, 2 s was needed; – no labeling, no fluorescence signals were detected, even with longer integration time (2.5 s)

Results

Conditions for lectin labeling

To control for the effect of enzyme treatment on lectin binding sites, egg and synergid protoplasts were isolated by dissection without cell-wall-degrading enzymes. We found no differences in fluorescence intensities and patterns of lectin binding site distribution when comparing labeling of enzymatically or mechanically isolated sexual cells.

Suitable concentrations for labeling differed for each lectin (Table 1). Strong fluorescence on cell membranes of egg cells was observed at 125 μg/ml for Con A and 250 μg/ml for WGA. With PHA-E (up to 500 μg/ml) no fluorescence signals were detected on egg or sperm cell membranes.

The duration of treatment of egg cells with probes needed for optimal binding of different lectins was determined. For Con A binding, 25 min was sufficient for optimal binding, while 40 min was necessary for WGA (Table 2). Even after an incubation time of 60 min, no fluorescence signals of PHA-E were detected on egg cell membranes. Changes of pH within the range 5.6–7.6 did not notably influence labeling.

In contrast to egg and central cells, sperm cells were more sensitive to lectin-FITC treatment. As soon as they were treated with lectin solutions (600–650 mosmol) they became sticky and easily stuck on the cover slip. Therefore, they burst easily during manipulation. In order to keep them intact, it was necessary to label and wash sperm cells in a solution with an osmolality of 750 mosmol. Egg cell protoplasts were incubated in solutions with osmolalities of 600–650 mosmol.

Table 2 Minimum time for lectin binding on the cell membrane of maize egg cells. Experiments were carried out at 22°C. After lectin treatment without calcium an equal amount of a mannitol solution containing 5 mM calcium was added to the same droplet for a further 10 min incubation. The number of the cells for each treatment was 20

Lectin	Concentration (μg/ml)	Incubation time (min)	Labeling on egg cell[a]
Con A	125	5	–
		10	+
		20	+++
		40	+++
WGA	250	10	–
		20	+
		40	++
		60	++
PHA-E	500	10	–
		20	–
		40	–
		60	–

[a] Scale as in Table 1

Table 3 The effect of calcium on lectin binding on the cell membrane of maize gametes. The concentration of Con A was 125 μg/ml, WGA and PHA-E were 250 μg/ml. Experiments were carried out at 22°C. The total number of the cells used for each treatment was 30

Lectin	Calcium concentration (mM)	Labeling on gametes[a]	
		Egg cell	Sperm cell
Con A	0.5	+++	+
	0	++	–
WGA	0.5	+	–
	0	–	–
PHA-E	0.5	–	–
	0	–	–

[a] Scale as in Table 1

Effects of exogenous Ca^{++} and competing inhibitor

Calcium influenced lectin binding on the surface of both female and male gametes. Although addition of exogenous calcium was not necessary for Con A to bind to the membrane of egg cells, the addition of calcium enhanced its binding, indicated by stronger fluorescence signals. Egg cells could be labeled by WGA only in calcium-containing WGA solutions. Similarly, sperm cells could be labeled by Con A only in the presence of calcium (Table 3). These observations indicate that calcium is important for lectin binding on sexual cell protoplasts of maize. No differences in lectin labeling were found whether calcium was present in the lectin solution before cell transfer or whether it was added after cells were

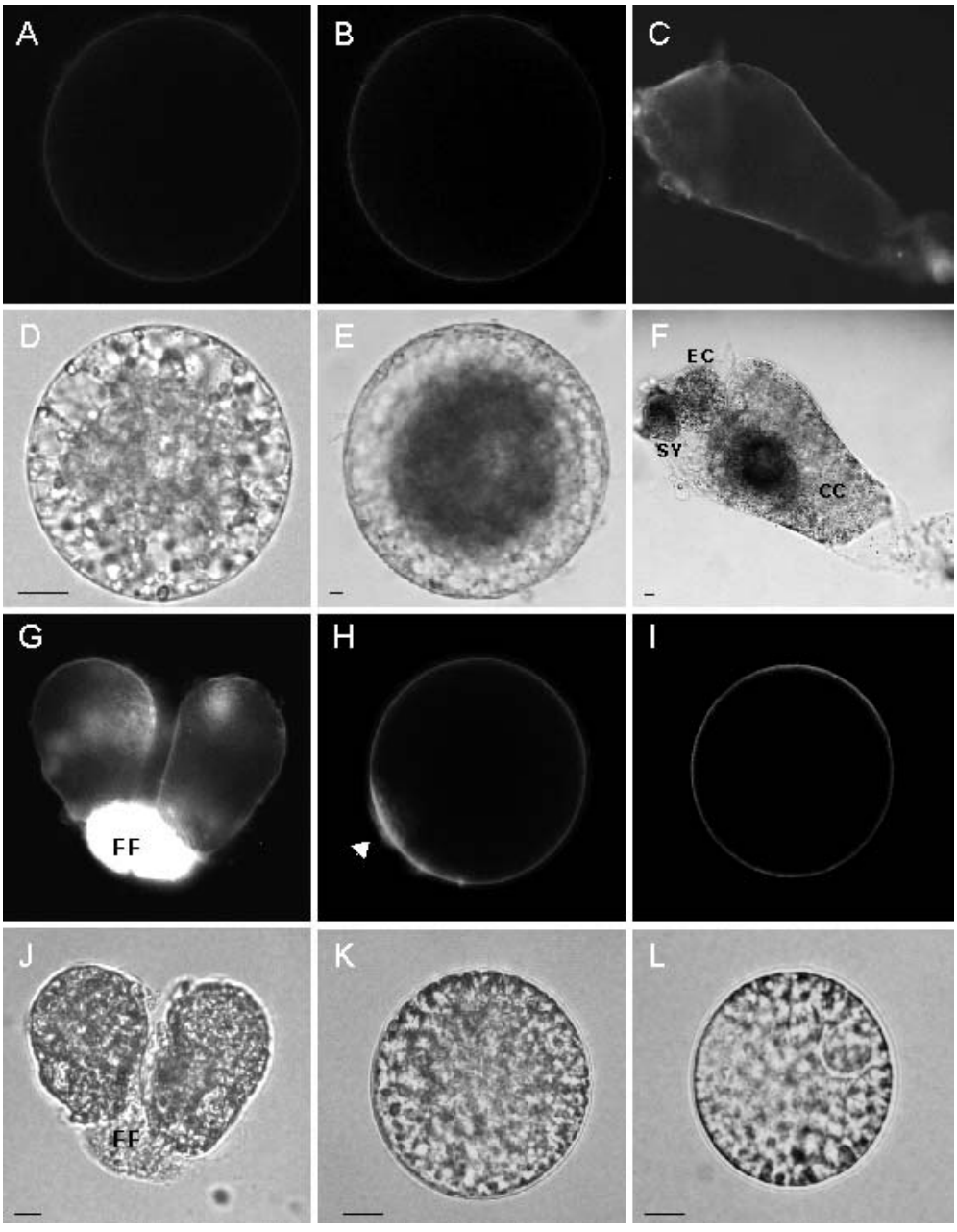

Fig. 1 **A**–**L** Distribution of lectin binding sites on sexual cells labeled by fluorescein isothiocyanate (FITC)-conjugated *Canavalia ensiformis* agglutinin (Con A) before fertilization in maize as an illustration of general pattern. **A**, **D** Egg cell. **A** Fluorescence image, **D** bright field image. **B**, **E** Central cell. **B** Fluorescence image, **E** bright field image. **C**, **F** Isolated embryo sac. **C** Fluorescence image, **F** bright field image. *EC* Egg cell, *SY* synergid, *CC* central cell. **G**, **J** A pair of synergids. **G** Fluorescence image [note the strongest fluorescence signal of the filiform part (*FF*)], **J** bright field image. **H**, **K** Synergid protoplast. **H** Fluorescence image [note some wall material is still attached to the protoplast (*arrow*)], **K** bright field image. **I**, **L** Totally isolated synergid protoplast. Note that the synergid protoplast shows a stronger signal than the egg and central cell protoplast. **I** Fluorescence image, **L** bright field image. *Bars* 10 μm

already incubated in lectin-containing solution without calcium. The latter procedure avoided cells sticking to the coverslip and therefore allowed cell transfer for further manipulation.

To test lectin binding specificity, specific monosaccharides were used as competing inhibitors in the incubation medium. In the presence of inhibitors, lectins were not able to bind to the membranes of any of the protoplasts tested.

Cytological observations

All cells were intact and viable under the experimental conditions used. Synergids, egg and central cell protoplasts incubated in lectin containing solutions tended to adhere to the glass slides, but with gentle stirring using a glass needle, the cells could be kept suspended even after the addition of calcium-containing mannitol solution, enabling these protoplasts to be easily transferred into washing medium. No morphological changes to the cells were observed during any steps of the procedure.

Fluorescence signals emitted from labeled protoplasts were observed as a distinctive ring around the cell perimeters (Fig. 1 A, B, I), indicating intact membranes. Labeled cytoplasm was observed as an indication of damage to the cell membranes. Therefore, the absence of fluorescence signals in the cytoplasm was used as an indicator to confirm cell viability.

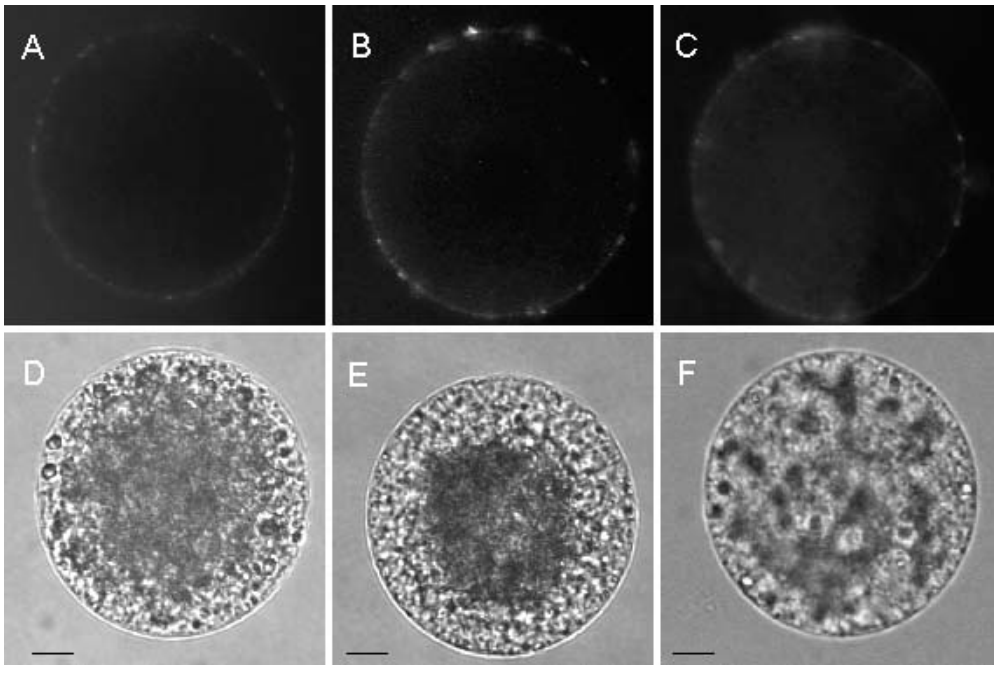

Fig. 2 **A**–**F** Localization of *Triticum vulgaris* agglutinin (WGA) and *Phaseolus vulgaris* erythroagglutinin (PHA-E) binding sites on membranes of protoplasts of an isolated egg cell and isolated zygotes, demonstrating fertilization-induced changes of lectin binding sites. **A**, **D** FITC-WGA labeled egg cell. **A** Fluorescence image [note the FITC-WGA labeled membrane always forms fluorescent patches and the signal is not as smooth as that of Con A labeling (see Fig. 1 A)], **D** bright field image. **B**, **E** FITC-WGA labeled zygote. **B** Fluorescence image, **E** bright field image. **C**, **F** FITC-PHA-E labeled zygote. **C** Fluorescence image, **F** bright field image. *Bars* 10 µm

Table 4 Comparison of lectin binding on cell membranes of protoplasts isolated from cells of the embryo sac of maize before and after fertilization, of sperm and nucellar cells and on cell walls of the embryo sac. Concentration of FITC-Con A, 125 µg/ml; FITC-WGA, 250 µg/ml; FITC-PHA-E, 500 µg/ml. Cells were incubated for 30–45 min at 22°C and then treated with 5 mM $CaCl_2$. *EC* Egg cell, *SP* sperm cell, SY_1 and SY_2 two synergids, *CC* central cell, *AP* antipodal cells, *ES* embryo sac, *NC* nucellar cell, *ZY* isolated zygote, *PS* persistent synergid, *DS* degenerated synergid, *PE* primary endosperm cell. Cells per treatment, 45; except AP, 20; ES, 10; PE, 10; and CC, 5

Lectin	Cell labeling[a]											
	EC	SP	SY1	SY2	CC	AP	ES	NC	ZY	PS	DS	PE
Con A	+++	++	++++	++++	+++	++	++++	+++	+++	++++	+++++	+
WGA	+	–	+	+	+	+	++	–	++	+	++	+
PHA-E	–	–	+	+	–	–	–	–	+	+	+	–

[a] Scale as in Table 1

Distribution of different lectin binding sites on membranes and cell walls of sexual cells

Isolated embryo sacs, synergids, and egg, central and nucellar cells could easily be labeled by Con A (Table 4, Fig. 1 A–C, G–I). Compared to labeled membranes of the egg (Fig. 1 A) and central cell (Fig. 1 B), the embryo sac wall showed a stronger fluorescence signal (Fig. 1 C). Lectin binding sites accumulated in the filiform apparatus of the synergids (Fig. 1 G). As protoplasts rounded off during treatment with cell-wall-degrading enzymes, the thick cell wall material of the filiform apparatus gradually disconnected from the synergids. Material from the filiform apparatus that occasionally remained attached was observed on the isolated synergid, as indicated by a bright signal of fluorescence (Fig. 1 H). Isolated synergid protoplasts, without attached cell wall material, showed a stronger labeling than egg and central cell protoplasts (Fig. 1 I). Antipodal cells were weakly labeled. Compared to Con A, WGA-labeled sexual cells showed much weaker fluorescence (Table 4, Fig. 2 A, B). WGA binding sites were equally distributed around egg and central cell protoplasts. Synergids were weakly labeled by PHA-E, and no signals were found on other embryo sac component cells before fertilization.

Sperm cells were labeled by Con A at a frequency of 60–80%. No differences were found in labeling of one of the two sperm cells of a pair (data not shown). Con A-treated nucellar cells showed intensities of signals similar to those of the egg cells. Under the same conditions used for gametic cells, nucellar protoplasts showed no signal when treated with WGA and PHA-E (Table 4). This result was the same with or without calcium.

Effect of fertilization on lectin labeling

After fertilization, no changes were observed in the pattern of Con A labeling on either in vitro or in vivo fertilized egg cells. However, after fertilization the degenerated synergid showed a stronger signal than the persistent synergid (Table 4). Even when the degenerated synergid

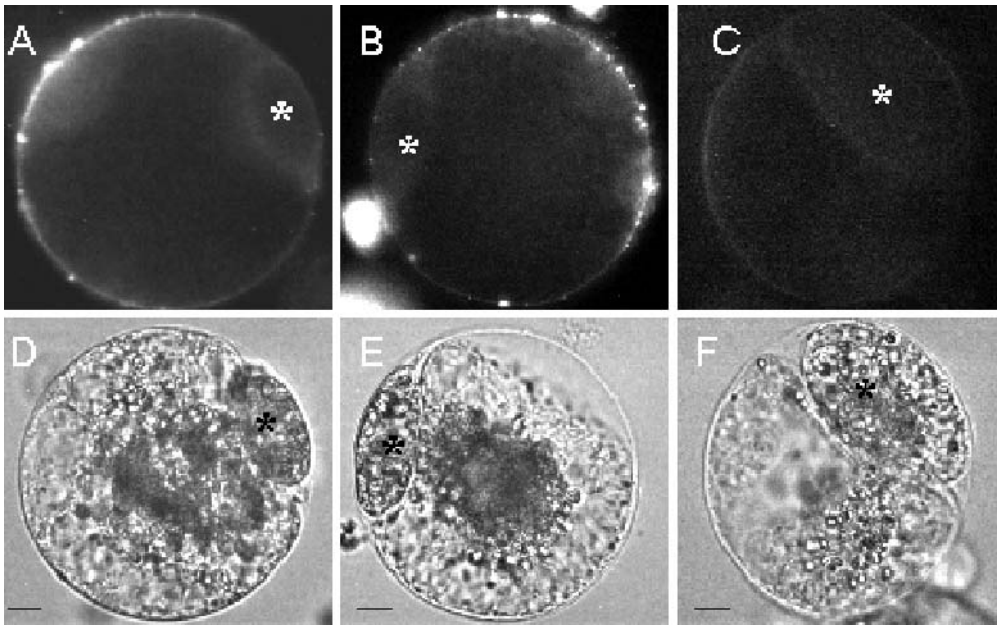

Fig. 3 **A**–**F** Lectin binding on cell walls of two-celled embryos derived from cultured, in vitro fertilized egg cells. Dividing zygotes were stained after 43 h in culture. Note the asymmetric divisions of the zygotes, producing a small cell (*) and a big cell. **A**, **D** FITC-Con A labeling. **A** Fluorescence image, **D** bright field image. **B**, **E** FITC-PHA-E labeling. **B** Fluorescence image, **E** bright field image. **C**, **F** FITC-WGA labeling. **C** Fluorescence image, **F** bright field image. *Bars* 10 µm

appeared to be intact with its original shape, its entire cytoplasm was labeled, indicating loss of its normal membrane structure (data not shown). In comparison, the persistent synergid was labeled only on the membrane surface. The structural disorganization of the membrane of the synergid can be considered as one of the earliest signs of synergid degeneration.

Binding sites of all three lectins were detected on the cell membrane of isolated zygotes. Protoplasts of isolated zygotes labeled by Con A showed fluorescence signal intensities similar to that of the egg cell membrane. In contrast, the cell membrane of the isolated zygote showed a stronger WGA signal than the egg cell membrane (Table 4; Fig. 2 B). Interestingly, the cell membrane of the unfertilized egg cell could not be labeled by PHA-E, whereas the cell membrane of the isolated zygote showed a weak signal (Table 4; Fig. 2 C).

All three lectins were detected on newly formed cell walls during zygote development and during the first embryonic cell division. In vitro-produced zygotes were labeled by Con A 24 h after fertilization; subsequent culture showed weaker fluorescence compared to egg cells. In contrast, in vitro-produced and cultured zygotes labeled by WGA exhibited stronger fluorescence than egg cells (data not shown). Lectin binding sites were also detected on newly formed cell wall of two-celled embryos, and were labeled by Con A (Fig. 3 A). An increase in signal was observed on both FITC-PHA-E (Fig. 3 B) and FITC-WGA-labeled two-cell embryos (Fig. 3 C).

Discussion

Female gametes of higher plants are deeply embedded in sporophytic tissues and can be obtained only in limited amounts. To detect lectin binding sites on the surface of these isolated gametes it was therefore necessary to work out a reliable procedure for single cell manipulation. We carried out the main steps of the labeling procedure in microdroplets, which were prepared in equal amounts using a micropump. By using a CCD camera, combined with image processing software, fluorescence signals from lectin labeling of different cells could be easily compared by adjusting the integration time. Concentrations of 125 µg/ml Con A, 250 µg/ml WGA and 500 µg/ml PHA-E were found to be optimal for lectin binding and viability of gametic cells.

Sugar specificity tests using competing inhibitors demonstrated that fluorescence labeling was due to specific binding on all protoplasts. For example, the presence of mannose in the medium prevented Con A binding to membranes of all kinds of gametic cells, as indicated by the absence of any fluorescent signal. The presence of fluorescence signals on sperm and egg cell membranes demonstrates that Con A had specifically bound to residues located on the surface of these cells.

Control experiments with sexual cells, which were isolated without the use of cell-wall-degrading enzymes, indicate that functional binding sites were not appreciably removed by enzymatic treatment during the isolation procedure. There are contradictory reports about the influence of enzymatic treatment on lectin labeling of cell membranes. Walko et al. (1987) reported that several cell-wall-degrading enzymes, including cellulysin and pectolyase Y-23, generally had no effect on lectin binding. One exception was driselase, which markedly reduced *Arachis hypogaea* agglutinin binding to protoplasts. However, Sun et al. (1992) showed that a combination of cellulysin and pectolyase Y-23 had an effect on *Arachis hypogaea* agglutinin. Our results revealed no modifications of sugar residues on the cell membrane of gametic cells after enzymatic treatment.

Calcium influenced lectin binding on the membrane surface of both female and male gametic protoplasts. Our observations indicate that calcium is important for lectin binding on sexual cell protoplasts of maize. Calcium was necessary for WGA binding on egg cells and for Con A binding on sperm cells, and it enhanced Con A binding on the membrane surface of egg protoplasts. Also, the binding of several lectins to somatic protoplasts derived from suspension cultured rose cells was enhanced by increasing the calcium level in the buffer. It was concluded that the effect of calcium and other divalent cations appeared to involve the plasma membrane binding sites of the somatic protoplasts rather than the lectins (Walko et al. 1987).

In fertilization in humans and other mammals, lectin-specific binding site interactions may play a crucial role in gamete recognition and fusion (Florman and Wassarman 1985; Wassarman 1992). In sea urchin, a sperm receptor found on egg cells (Dhume and Lennaz 1995) has been shown to be a 350 kDa glycoprotein with *O*-linked oligosaccharide chains that are involved in sperm binding. Although techniques for the isolation and in vitro gamete fusion are now available, so far there is little information available on gamete surface proteins of higher plants, particularly regarding the isolation and localization of proteins on the sperm membrane (Southworth and Kwiatkowski 1996; Xu and Tsao 1997).

The present study revealed Con A binding sites on the membrane of both male and female gametes. WGA binding sites were found only on the egg and central cell membrane, whereas PHA-E binding sites were not found on either male or female gametes. Synergids, sperm, egg and central cells showed differences in fluorescence signals indicating variations in the membrane composition of different gametes and embryo sac component cells.

More WGA binding sites were located on the membrane of the isolated zygote than on the egg cell membrane. Also, WGA labeled newly formed cell walls of the two-celled embryo more strongly than the egg cell membrane. PHA-E signals, indicating oligosaccharides, appeared on the cell membrane of fertilized egg cells. This observation indicates that the egg cell membrane underwent a modification in its composition after fertilization. Also, an increase of WGA and PHA-E signals occurred on newly formed cell walls of two-celled embryos derived from in vitro produced and cultured zygotes. Apparently, fertilization-induced changes in the composition of glycoconjugates of the egg cell surface occur. These might have implications in processes such as, for example, signal transduction during the transition from the sexual to the asexual generation.

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