U-cadherin in *Xenopus* oogenesis and oocyte maturation

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Summary

U-cadherin is a member of the cadherin family in *Xenopus* that participates in interblastomere adhesion in the early embryo from the first cleavage onwards. Though a maternal pool of U-cadherin is available in the egg, it is not present on the egg membrane (Angres et al., 1991. Development 111, 829-844). To assess the origin of this unexpected distribution in the egg, the accumulation and localization of the cadherin during oogenesis and oocyte maturation were investigated.

We report here that U-cadherin is present in *Xenopus* oocytes throughout oogenesis. It is localized at the oocyte-follicle cell contacts suggesting that it functions in the adhesion of the two cell types.

When oocytes mature and the contacts to the follicle cells break, U-cadherin disappears from the oocyte surface. Evidence for a translocation of U-cadherin from the membrane to the inside of the oocyte was obtained when the fate of membrane-bound U-cadherin, which was labelled on the surface of oocytes prior to maturation, was followed through maturation.

The total U-cadherin content of the oocyte increases during maturation. Metabolic labelling experiments indicate that at maturation the translation of U-cadherin is elevated well above the level that one would expect from the general increase in protein synthesis occurring during maturation. This enhanced synthesis is presumably the main source of the maternal pool of U-cadherin in the egg.

Key words: cadherin, oocyte maturation, follicle cells, *Xenopus*.

Introduction

*Xenopus* oocytes are adhesive cells throughout oogenesis. At stage I of oogenesis, follicle cells become attached to the smooth surface of the oocytes by means of localized contacts of a poorly defined nature. The adjacent membranes are separated by a wide intermembrane space at these contact sites, and they have accumulated conspicuous electron-dense material on their cytoplasmic side (Browne and Werner, 1984).

With the emergence of the vitelline envelope at stage II-III of oogenesis, the oocyte-follicle cell border develops its final structure. Microvilli are now frequent on the oocyte surface extending into the space between the oocyte and the vitelline membrane. Follicle cells form a dense non-epithelial monolayer on the outer surface of the vitelline membrane. Macrovilli of the follicle cells penetrate the vitelline membrane through tunnels and contact the oocyte surface and its microvilli (Dumont and Brummett, 1978). In contrast to the earlier stages, gap junctions are now a major element in these contacts and the intermembrane space has narrowed accordingly (Browne et al., 1979; Browne and Werner, 1984). Beyond this, these contacts are not well characterized and no information is available regarding the presence and nature of cell-adhesion molecules.

The structural and biochemical changes through which the oocyte passes when it is transformed into the egg during maturation have recently been reviewed extensively (Bement and Capco, 1990; Smith, 1989). During this period, the oocyte membrane and the underlying cortex become fundamentally reorganized.

In the initial phase, the oocyte membrane responds to the maturation-inducing hormone progesterone with a decrease of the density of intramembranous particles (Bluemnck et al., 1983). An increase of endocytic activity is indicated by the appearance of coated pits and vesicles in the oocyte periphery (Bement and Capco, 1989; Dersch et al., 1991). After germinal vesicle breakdown (GVBD) has occurred, the oocyte-follicle cell contacts are broken and the oocyte microvilli decrease in size. A cortical endoplasmic reticulum develops (Campanella et al., 1984). The pathway of excretory and membrane proteins from the trans-Golgi compartment to the plasma membrane is blocked (Colman et al., 1985; Ceriotti and Colman, 1989; Leaf et al., 1990). The transport of metabolites ceases and pumping of alkaline ions decreases (Richter et al., 1984).
The egg plasma membrane that has formed during maturation is non-adhesive. This property is transmitted to the membrane domains on the embryonic surface, whereas the newly formed plasma membranes inside the embryo develop strong cell-cell adhesion (Roberson et al., 1980). This polarized adhesiveness of the cells on the embryonic surface is maintained throughout early development far into the gastrula stages. One may assume that the capacity of these cells to seal the surface of the embryo by epithelial functional complexes derives from this polarity (Müller, 1989). These considerations show that the properties of the egg membrane have far reaching consequences for embryogenesis and that processes controlled by these properties implicate the participation of cell-adhesion molecules.

We have recently reported on a new member of the cadherin family in Xenopus, termed U-cadherin (Angres et al., 1991). It is present in the egg cytoplasmin but not on the egg plasma membrane. During cleavage, U-cadherin is inserted into the new membranes as they form in the interior of the embryo and participates there in interblastomere adhesion. The absence of U-cadherin from the plasma membrane domains on the surface of the embryo agrees with their non-adhesive property. Thus, U-cadherin fulfills the requirements for being a key molecule in the embryonic processes mentioned above. This communication deals with the question of how the distribution of U-cadherin in the egg, i.e. the absence from the plasma membrane in the presence of an internal pool, arises during oogenesis and oocyte maturation.

The observations made in this study relate to the more general questions of how oocytes are released from the investing ovarian tissue during spawning, how the non-adhesive property of the egg membrane and of the outer membrane domains of the early embryo is generated and how the intracellular maternal pool of U-cadherin required in the early embryonic stages is established.

Materials and methods

Preparation of oocytes

Adult females of Xenopus laevis were obtained from African Xenopus Facility C.C. (South Africa). Ovaries were removed from decapitated animals and stored in MBS-H Medium (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 0.82 mM MgSO₄, 0.41 mM CaCl₂, 0.33 mM Ca(NO₃)₂, 10 mM Hepes pH 7.4 and antibiotics). Oocytes invested in their follicle cell layer were obtained by incubating pieces of ovary with 2 mg/ml collagenase (Serva) in OR-2 medium (82.5 mM NaCl, 2.5 mM KCl, 1 mM Na₂HPO₄, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM Hepes pH 7.8 and antibiotics; Wallace et al., 1978) lacking Ca²⁺ at 28°C with gentle agitation. The presence of follicle cells was monitored by staining with 4’,6-diamidine-2-phenylindol-dihydro-chloride (DAPI) at various times during the digest. Removal of the follicle cell layer was completed after 4 to 5 hours. Oocytes were then washed several times in OR-2 minus Ca²⁺ before culturing them in agarose-coated Petri dishes containing OR-2 plus Ca²⁺ at 23°C. Oocytes were staged according to Dumont (1972).

In vitro maturation

Females were injected with 50 units gonadotropin (Sigma; from pregnant mare’s serum) 24 hours prior to the preparation of the oocytes. This pretreatment of the animals tends to synchronize the maturation response of the oocytes to progesterone stimulation in vitro. Stage VI oocytes were incubated in OR-2 containing 2 μg/ml progesterone (Serva). Control oocytes were cultured in the absence of the hormone. The percentage of oocytes that had undergone GVBD was assessed by the appearance of the maturation spot. GVBD₉₀ (the time when 50% of the population had undergone GVBD) varied between 2 and 4 hours of incubation in different batches of oocytes.

Antibodies

For the characterization of the monoclonal antibody (mAb) SD5 directed against U-cadherin see Angres et al. (1991). Diluted ascites fluid was used for immunohistology and for the immunoblot procedure. For immunoprecipitation, IgG was isolated from the ascites fluid by means of a protein A column. P₃, an inert IgG, was used as a control. All secondary antibodies were purchased from Dianova (FRG).

Immunohistological procedures

Whole-mount staining of oocytes was performed with fluorescent antibodies as described previously (Angres et al., 1991) and the specimens were embedded in glycolmethacrylate (Technovit 7100 from Kulzer, FRG). 5 μm sections were prepared for observation with a Zeiss Axioplan equipped with epifluorescence optics.

Alternatively, immunostained oocytes were mounted in Mowiol (Hoechst, FRG) on slides and gently squeezed with a coverslip. In this way an even surface area of the oocytes was obtained, which was analyzed directly with a BioRad MRC 500 confocal laserscan microscope.

Preparation of detergent extracts

Oocytes were sonicated at 4°C in lysis buffer (2% NP-40, 1 mM CaCl₂, 150 mM NaCl, 10 mM Tris pH 7.4 supplemented with the following protease inhibitors: 2 μM aprotinin, 2 μM pepstatin, 2 μM leupeptin, 2 mM iodoacetamide, 1 mM N-ethylmaleimide (NEM) and 2 mM phenylmethylsulfonyl-fluoride (PMSF). 10 μl of lysis buffer per oocyte were used.

Yolk was sedimented by centrifugation (1 minute at 14 000 revs/minute). The supernatant was then extracted with an equal volume of 1,1,2-trichlor-trifluoroethane and centrifuged. To avoid proteolytic degradation, the clear extract was immediately heated to 95°C in 2% SDS for 10 minutes and then stored at −70°C for later use in electrophoresis.

SDS-PAGE and immunoblotting

The detergent-soluble material of five oocyte equivalents per lane was analyzed on a 7% SDS-PAGE (Laemmli, 1970). Proteins were transferred electrophoretically onto a nitrocellulose membrane. Immunostaining of the blots was performed as described elsewhere (Fey and Hausen, 1990). Antibody binding was detected using the ECL western blot detection system (Amersham).

Biotinylation of oocytes and isolation of biotinylated proteins by affinity to streptavidin

Surface labelling of defolliculated oocytes was carried out according to the method of von Boxberg et al. (1990). Oocytes were biotinylated and then incubated in OR-2 containing 2 μg/ml progesterone (Serva) before isolating the biotinylated proteins.
were cultured overnight in OR-2 medium prior to the labelling. 100 oocytes were placed in an agarose-coated Petri dish containing 2 ml of OR-2 medium deficient of antibiotics. A stock solution of 4 mg of Biotin-X-NHS (biotinylaminocaproicacid N-hydroxy-succinimideester; Calbiochem) in 20 µl DMSO was carefully added along the periphery of the dish. After 10 minutes, the reaction was terminated by addition of 1 ml of 500 mM glycine pH 7.4. The oocytes were washed 5 times with OR-2 and lysed as described above. The extracts were dialyzed against streptavidin (SAv) buffer (0.3% NP-40, 500 mM NaCl, 10 mM Tris, pH 8, and the protease inhibitors of the lysis buffer) and centrifuged for 30 minutes at 14 000 revs/minute.

SAv-agarose from 75 µl of the suspension (Sigma) was washed 2 times with SAv-buffer. The extracts were incubated overnight with SAv-agarose at 4°C. After incubation, the beads were washed 3 times by centrifugation and resuspension. Proteins were eluted by heating the pellet to 95°C for 10 minutes with SDS sample buffer (Laemmli, 1970) and used for immunoblotting. For controls either unlabelled extracts were processed or the SAv-agarose was preabsorbed with 1 mM d-biotin for 1 hour before affinity chromatography.

**Labelling of oocytes with [35S]methionine**

Prior to labelling, the timing of the maturation process for a given batch of oocytes was determined by observing oocytes that had been defolliculated and stimulated with progesterone 1 hour in advance of the experiment. 0.5 mCi L-[35S]methionine (cell labelling grade; Amersham) were added to 140 oocytes in 3 ml of OR-2 medium one hour before GVBD was expected to occur. After 1.5 hours of labelling, the oocytes were washed 4 times with OR-2 and lysed in immunoprecipitation buffer (RIPA) (0.1% SDS, 0.5% sodium deoxycholate, 1% NP-40, 150 mM NaCl, 1 mM CaCl2, 20 mM L-methionine, 50 mM Tris pH 7.4 and the protease inhibitors of the lysis buffer). The extracts were centrifuged and stored in liquid nitrogen.

To determine total radioactivity in the extracts, 10 µl were added to 10 µl of 1% BSA, spotted onto glass fibre filters, dried and counted on a Packard Tri Carb Scintillation counter.

**Immunoprecipitation**

To minimize unspecific absorption of labelled protein, the protein G sepharose used in these experiments was pretreated with unlabelled oocyte extract and the labelled extracts were precleared with protein G Sepharose loaded with an unspecific IgG. For these purposes, 15 µl of protein G Sepharose suspension (Pharmacia) was prewashed with RIPA and then preabsorbed with unlabelled oocyte extracts for 1 hour followed by a washing of 10 µg 6D5 IgG to these beads. For preclearing, P3 protein G Sepharose was prepared by binding 20 µg P3 IgG to 40 µl protein G Sepharose suspension.

The extracts of labelled oocytes were centrifuged for 1 hour at 25 000 revs/minute in a TL 100 ultracentrifuge (Beckman) using the the TLA 100.2 rotor. The supernatants were precleared twice by incubating them with 40 µl of P3 protein G beads for 1.5 hours followed by 5 minutes centrifugation at 14 000 revs/minute. The supernatants were incubated with 6D5 protein G beads for 2 hours. The beads were sedimented by low speed centrifugation and washed three times with RIPA. Proteins were removed from the beads by heating them to 95°C for 10 minutes in SDS sample buffer. After SDS-PAGE the gels were stained with R-250 Coomassie blue, processed for fluorography by impregnation in amplify TM (Amersham), dried and exposed to Cronex TM X-ray films (Du Pont de Nemours).

**Results**

**Identification of U-cadherin in oocytes and its localization at the contact sites between oocytes and follicle cells**

The presence of U-cadherin in *Xenopus* oocytes was established by use of mAb 6D5 described recently by Angres et al. (1991). This antibody specifically recognizes U-cadherin and does not cross-react with two other known members of the cadherin family in the *Xenopus* embryo, E-cadherin (Angres et al., 1991) and N-cadherin (T. O. Joos and P. Hausen, unpublished data). Detergent extracts of stage VI oocytes that had been defolliculated with collagenase were analyzed by immunoblotting. A single immunoreactive band at 125 × 10^3 relative molecular mass (Mr), indicative of U-cadherin, was found (see Fig. 7).

For immunohistology, manually isolated follicles were whole-mount stained with mAb 6D5, plastic embedded and sectioned. In follicles of young (late stage I) oocytes, which are not yet surrounded by a vitelline membrane, U-cadherin is recognized by a smooth and even staining of the oocyte membrane (Fig. 1).

The pattern of staining changes in follicles of advanced oocytes. U-cadherin now forms a conspicuous punctuate pattern at the oocyte-follicle cell border. Prominent staining is also observed at the transversally sectioned contacts between the follicle cells (Fig. 2A, arrows). The controls in which an unspecific IgG was used as a first antibody do not show any significant staining (Fig. 2B).

We interpret the punctuate pattern at the follicle cell-oocyte border as an indication that the localization of U-cadherin is restricted to the sites where macrovilli of...
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Fig. 2. Localization of U-cadherin at intercellular contacts of oocytes and follicle cells. Follicles of stage VI oocytes were manually dissected from the ovary and immunostained with mAb 6D5 (A,C and D) or the inert IgG P3 as a control (B). Whole mounts were either embedded in glycolmethacrylate and sectioned (A and B) or analyzed directly with the confocal microscope (C and D). The arrows in A indicate transversally sectioned cell contacts between adjacent follicle cells. The intercellular contacts of the follicle cells are in focus in C and the oocyte surface in D. Bars represent 25 μm.

the follicle cells penetrate the vitelline membrane and contact the oocyte surface.

This interpretation was corroborated when the surface of whole-mount-stained specimens were inspected with a confocal laser scan microscope. At the level of the follicle cells, the cell boundaries are clearly marked by the antibody (Fig. 2C). 2.5 μm deeper at the level of the macrovillar-oocyte contacts, the punctuate pattern of antigen distribution is seen (Fig. 2D).

The oocyte-follicle cell border during maturation

When maturation is induced by progesterone in manually isolated follicles, the punctuate pattern of U-cadherin staining at the oocyte-follicle cell border disappears at around the time of GVBD (Fig. 3). Noticeably, the membrane contacts between the follicle cells are not visibly affected by the hormone. This observation might suggest that the follicle cells are not the primary target for progesterone when the system responds with a change in U-cadherin distribution during maturation. The staining pattern in oocytes that did not receive the hormone remained unimpaired during the time of incubation (not shown).

U-cadherin in defolliculated oocytes

The previous observations related to the U-cadherin distribution at the follicle cell-oocyte border and its changes during maturation. In order to study exclusively the response of oocyte U-cadherin to maturation, it was necessary to remove the follicle cells from the oocyte surface.

Fragments of ovary were incubated with collagenase to release the oocytes from their investments and free them from follicle cells. When these oocytes were assayed for U-cadherin on immunoblots, the signal recorded was rather weak, but it became stronger when the oocytes were further cultured in protease-free OR-2 medium (data not shown, but compare lanes b and d of Fig. 7).

Likewise, in immunostainings, U-cadherin was not found on the oocyte membrane immediately after the collagenase treatment, but it reappeared on the membranes within 2 hours when the oocytes were further cultured in protease-free medium (Fig. 4A). The pattern of labelling differs markedly from that seen at the oocyte-follicle cell border. In sections, it appears smoother and more even, though a grained, finely punctuated distribution may indicate a more intense labelling of microvilli on the oocyte surface (Fig. 4B). Direct observations of whole mounts support this view (not shown). The staining was found to be strongest in the animal pole region decreasing in intensity towards the vegetal pole (Fig. 4C).

Thus, the procedure of collagenase treatment followed by culture in protease-free medium produces oocytes free of follicle cells but with abundant cadherin on their surface. These oocytes were used in the following experiments.

Surface-bound U-cadherin during maturation

Defolliculated oocytes were allowed to restore U-cadherin on the plasma membrane in culture and were then incubated with progesterone. Immunohistological analysis revealed that U-cadherin disappears from the
Fig. 3. Absence of U-cadherin from the intercellular contacts of oocytes and follicle cells after in vitro maturation. Isolated follicles were treated with progesterone (2 μg/ml) and immunostained with mAB 6D5 after oocyte maturation. In A, the oocyte periphery in the equatorial region is shown. Arrows indicate intercellular contacts of the follicle cells. B and C show a pair of confocal scanning images in different focus planes. The follicle cell contacts are depicted in B and the oocytes surface in C. Bars represent 25 μm.

We have attempted to assess the timing of the disappearance of U-cadherin from the membrane more accurately. A population of synchronous oocytes was selected from one batch using external criteria of the maturation process (Huchon et al., 1981). From this group, samples were taken at different times. Immunohistological inspection of these oocytes revealed that U-cadherin is removed from the membrane within a period of 15-30 minutes after the beginning of GVBD.

Internalization of U-cadherin by the oocyte during maturation

Biotin succinimide ester, added to whole cells, labels proteins on the cell surface but not internal proteins, which are protected from labelling because the cell membrane is impermeable to this reagent (von Boxberg et al., 1990). This method was applied to defolliculated oocytes to discriminate between surface-bound and internal U-cadherin. The labelled oocytes were detergent extracted, biotin-labelled proteins were isolated by absorption on streptavidin-sepharose beads, and the U-cadherin in this fraction was assayed for in immunoblots.

Biotinylated U-cadherin was readily detected when the labelling reagent was applied to the oocytes before maturation (Fig. 6, lane a). No label was found when the reagent was applied after maturation (Fig. 6, lane e). These results corroborate the immunohistological observation that U-cadherin is removed from the cell surface at maturation. At the same time, the result proves the reliability of the method in showing that in fact internal U-cadherin does not become labelled.

When oocytes were prelabelled with biotin and then allowed to mature, the labelled U-cadherin persisted through maturation (Fig. 6, lane d). Since U-cadherin is not detectable on the oocyte surface after maturation, this result demonstrates that the labelled U-cadherin must have been internalized by the oocyte during maturation. The prolonged and multistep processing of the samples required in this type of experiment resulted
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Fig. 4. Restoration of U-Cadherin on the plasma membrane of oocytes defolliculated by collagenase treatment. Oocytes were defolliculated with collagenase and cultured in OR-2 medium. Oocytes were fixed at 0 hour (A), 12 hours (B) and 24 hours (C) of culture and immunostained with mAb 6D5. The equatorial regions of oocytes are depicted in A and B. A transversal section from the animal to the vegetal pole is shown in C. Bars represent 50 μm in A and B, 100 μm in C. The method of whole mount staining applied here causes an autofluorescence of the yolk platelets which varies between different specimens. This autofluorescence seems to depend in an uncontrollable way on the degree of dehydration of the specimen. Due to its different colour, the autofluorescence is easily distinguished from FITC label. Only the plasma membranes and some granular structures in the subcortical region exhibit true FITC staining.

in a certain variability of the yields of antigen. The difference in intensity of the bands in Fig. 6 lane a and lane d are a consequence of the procedure and was not consistent in different experiments. As a further control to this experiment, the disappearance of U-cadherin from the membrane of biotinylated oocytes was studied by immunohistology. No adverse effect of the treatment of oocytes with biotin succinimide ester was observed (data not shown).

U-cadherin synthesis during maturation

Oocytes were incubated with progesterone after defolliculation until 50% of the population had undergone GVBD. Controls were incubated likewise but in the absence of the hormone. Extracts were prepared and analyzed by immunoblotting. For comparison, extracts from whole follicles and from non-induced oocytes taken immediately after the defolliculation procedure were also included in the analysis.

The data show that the amount of U-cadherin has increased in the maturing oocytes as compared to the controls and exceeds even that of the whole follicles in which the U-cadherin of the follicle cells adds to the total amount (Fig. 7).

To determine whether this increase in U-cadherin results from a stimulation of its synthesis during maturation, pulse labelling experiments were performed.

Defolliculated oocytes were prepared and in a preliminary experiment the timing of the response to progesterone was determined for this particular batch of oocytes. [35S]methionine was added to hormonally induced oocytes at 1 hour before the maturation spot was expected to appear. The oocytes were washed after 1.5 hours with OR-2 medium containing unlabelled methionine and were extracted for immunoprecipitation as detailed under Material and Methods. Non-induced controls were treated likewise.

The total radioactivity incorporated was determined as a measure of the efficiency of methionine uptake into the oocytes under stimulated and non-stimulated conditions. In addition, the radioactivity incorporated into protein was determined. Assuming that the
endogenous pool of methionine remains constant during maturation, the radioactivity within the oocytes provides a measure of the specific activity of methionine available for translation, and the percentage of the internal methionine incorporated into protein gives an approximate measure of the general translational activity in the oocytes. As may be seen from Table 1, transport of methionine into maturing oocytes has decreased by a factor of 1.6 and the general translational activity has increased by a factor of 1.3. The estimates of the stimulation of translation ranged in different experiments from 1.3 to 2.5 depending on the batch of oocytes.

Aliquots of the extracts were adjusted to equal protein radioactivity and used for immunoprecipitation by mAb 6D5. The precipitated proteins were analyzed by electrophoresis followed by fluorography (Fig. 8).

Two major bands of radioactive proteins showed up in both the induced and in the non-induced samples. None of these bands was found in the control, in which the inert IgG P3 was applied in the immunoprecipitation procedure (lane c).

The band at $125 \times 10^3 M_r$ indicates the mature U-cadherin and the protein at $140 \times 10^3 M_r$ represents at all probability the cadherin precursor described by others for different members of the cadherin family. (Peyriéras et al., 1983; Hatta et al., 1988; Choi and Gumbiner, 1989).

Both protein bands are more heavily labelled in the samples from the hormonally induced oocytes (lane b) as compared to the non-induced controls (lane a). This indicates that indeed U-cadherin synthesis is stimulated at maturation. In particular, the heavier label of the short-lived cadherin precursor molecule supports this supposition.

When the radioactivity was measured in the corre-
Fig. 6. Immunoblot analysis of surface-bound U-cadherin during oocyte maturation. Defolliculated oocytes were prepared and cultured in OR-2 medium for 12 hours. Oocytes were induced to mature with progesterone and labelled with Biotin-X-NHS. Biotinylated proteins were precipitated with Streptavidin (SAv) agarose as described in Materials and methods and the presence of U-cadherin in the biotinylated protein fractions was analyzed by immunoblotting with mAb 6D5. The position (bars) and size ($M_t \times 10^3$) of molecular weight markers is indicated. Lane a, oocytes labelled and processed prior to maturation; lane b, unlabelled oocytes were processed; lane c, SAv was preabsorbed with biotin; lane d, oocytes labelled prior to maturation and processed after maturation; lane e, oocytes labelled and processed after maturation.

Corresponding gel regions, it was found that the label in the bands from the stimulated oocytes exceeds that of the controls by a factor of 5.6. Since the samples applied to the gel were adjusted to equal protein radioactivity, the factor of 5.6 indicates a stimulation of U-cadherin synthesis above the elevated level of general translational activity in the induced oocytes.

A third band at $100 \times 10^3 M_t$, appeared only in the induced sample. The nature of this protein is unclear. It possibly represents an as yet unknown degradation product, but it might as well represent an U-cadherin associated component that precipitates together with the cadherin.

**Discussion**

**U-cadherin at the oocyte-follicle cell contacts**

The presence of U-cadherin at the oocyte-follicle cell contacts suggests that it effects the adhesion of the two cell types or participates in it. The finding of Bement and Capeo (1990) that the follicle layer dissociates from the oocytes in calcium-free buffer, a result that agrees with our own observations (data not shown), corroborates this supposition.

The difference in distribution of U-cadherin at the oocyte-follicle cell border in young (stage I) and more advanced (stage V and VI) follicles reflects the difference in the nature of the contacts at these stages.

**Table 1. Pulse labelling of oocytes with $[^{35}S]$methionine**

<table>
<thead>
<tr>
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<th>Progesterone-induced oocytes</th>
<th>Control oocytes</th>
<th>Induced/controls</th>
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<tbody>
<tr>
<td>Radioactivity added per oocyte [cts minute$^{-1}$]</td>
<td>$3.9 \times 10^6$</td>
<td>$3.9 \times 10^6$</td>
<td></td>
</tr>
<tr>
<td>Total uptake of radioactivity per oocyte [cts minute$^{-1}$]</td>
<td>$4.8 \times 10^5$</td>
<td>$7.7 \times 10^5$</td>
<td>0.62</td>
</tr>
<tr>
<td>Radioactivity incorporation into protein per oocyte [cts minute$^{-1}$]</td>
<td>$1.3 \times 10^5$</td>
<td>$1.6 \times 10^5$</td>
<td></td>
</tr>
<tr>
<td>% of total radioactivity in protein</td>
<td>27</td>
<td>21</td>
<td>1.3</td>
</tr>
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</table>

140 oocytes were cultured in 1.5 ml OR-2 for 2.5 hours in the presence of progesterone. They were then labelled for 90 minutes with 0.5 mCi $[^{35}S]$methionine. After washing the oocytes with OR-2, detergent extracts were prepared. Total radioactivity in the extracts and the fraction of radioactivity incorporated into protein was determined in aliquots. 140 non-induced oocytes were treated likewise and served as controls.
Initially, the two cell types are joined by broad contacts of as yet undefined nature (Browne and Werner, 1984) and the distribution of U-cadherin along the intercellular border is smooth and even. With the appearance of the vitelline membrane between the two coupled cells, U-cadherin becomes reorganized along the oocyte-follicle cell border into a punctuate pattern that reflects at all probability the pattern of the follicle cell macrovilli as they penetrate the vitelline membrane and contact the oocyte. Membrane regions, which are prevented from contact by the vitelline membrane, seem to be devoid of cadherin.

The contacts between the macrovilli of the follicle cells and the oocyte membrane are of the gap junction type. It has been reported that the presence of cadherins on adjacent cells is a precondition for the formation of gap junctions (Matsuzaki et al., 1990). U-cadherin might have this function in the oocyte-follicle cell junctions. These junctions, however, show a special configuration. A region of narrow intermembrane space contains intermissions of wider intermembrane space and the individual gap junction connexons form an irregular network (van den Hoef et al., 1984). An ultrastructural analysis is required to assess the possible localization of U-cadherin in these specialized junctions.

Oocytes and follicle cells are apparently the only cell types in the ovary that express U-cadherin on their surface. It is remarkable that the two cells, which belong to two exceedingly different cell lineages, are joined by a cadherin. A more detailed analysis of the emergence of this expression pattern in the early ovary would be of considerable interest.

In this context, it remains to be clarified whether U-cadherin is the only cell adhesion molecule at the oocyte-follicle cell contacts. Observations made by others on cadherins in *Xenopus* oocytes may relate to this question.

Anti-XB-cadherin antibodies strongly stain the cytoplasm of oocytes but the oocyte-follicle cell contacts remain unstained (Herzberg et al., 1990). EP-cadherin is found in the cortical region of the unfertilized egg (Ginsberg et al., 1991). The question remains whether these observations, which differ from ours on U-cadherin, indicate that different cadherins are present in oocytes or whether they are the result of differences in the staining methods employed. No data are available on the localization of CLP, a further cadherin-related protein in oocytes (Choi et al., 1990).

**Defolliculated oocytes**

When ovarian oocytes are treated with collagenase in calcium-free buffer, U-cadherin is removed from the oocyte surface together with the surrounding tissue including the follicle cells. Most likely the U-cadherin is degraded by proteases present in the rather crude collagenase preparation. The observation that at the end of the collagenase treatment only small amounts of U-cadherin are detected in the oocytes indicates that most of the oocyte's U-cadherin is surface-bound and that the intracellular pool is small. However, the defolliculated oocytes quickly restore the cadherin on their surface upon incubation in protease-free buffer. An increase in the amount of U-cadherin per oocyte is also seen. This interesting regulatory event has not been investigated further.

We have used these defolliculated oocytes to investigate specifically the behaviour of oocyte owned U-cadherin during maturation. Conclusions from these experiments must take into account that the state of the cadherin on the membrane of these oocytes does not accurately reflect the condition in vivo where it is bound to the oocyte-follicle cell contacts and engaged in cell-cell adhesion.

**U-cadherin during oocyte maturation**

Two events regarding U-cadherin occur during maturation: (1) The total amount per oocyte increases and (2) U-cadherin disappears from the outer membrane of the oocyte.

In addition to the obvious assumption that the observed accumulation of U-cadherin results from a stimulation of its synthesis, other processes may be envisaged that could lead to the rise in U-cadherin in the oocyte extracts.

The hypothesis that the increased amount of U-cadherin in the extracts from stimulated oocytes is solely caused by a shift of preformed material into an extractable form can be dismissed on the basis of the finding that U-cadherin labelling is increased during a 1.5 hour pulse with $^{35}$S-methionine.

This result also renders the argument unlikely that U-cadherin exhibits turnover in the oocyte and that the degradation process becomes blocked at maturation. To explain the 5.6-fold labelling of U-cadherin in the induced oocytes solely on the basis of a decreased...
turnover rate, it has to be assumed that the whole U-cadherin complement of non-induced oocytes turns over once every 16 min. This estimate gives only the minimum turnover rate since the effective pulse time in these large cells is much shorter than the time of exposure to the label and the specific activity of the methionine from which the cadherin is synthesized in the induced cells is lower by a factor of 1.6 as compared to the controls. Further, the $140 \times 10^3\, M_\text{r}$ precursor is a short-lived intermediate in U-cadherin synthesis because it is seen in labelling experiments only and not in the conventional immunoblots. In the case of a rapid turnover, one would expect a similar labelling of the precursor in both control and induced oocytes, but a large difference in labelling was found in the two states of the oocyte.

We therefore argue that U-cadherin synthesis is stimulated during oocyte maturation and that the observed accumulation of U-cadherin is a result of this synthesis. As the stimulation of U-cadherin synthesis lasts until after the time of GVBD, when transcription has already ceased (LaMarca et al., 1975), it is most likely effected on the translational level. The stimulation of total protein synthesis by a factor of about two during maturation (Wasserman et al., 1982, and our observations) is largely due to a general activation of the translational machinery since the pattern of proteins synthesized does not change significantly during maturation (Younglai et al., 1981; Richter et al., 1982). This rise in general protein synthesis can not account for the large increase in the total amount of U-cadherin. A selective recruitment of U-cadherin mRNA during maturation has to be postulated. Accumulation of the cadherin-like protein (CLP), which may be identical to U-cadherin, at maturation and labelling of the precursor molecule has been reported by Choi et al. (1990). These authors have not observed a stimulation of the rate of labelling of the cadherin comparable to our results, but they have used a different timing schedule for exposure of the oocytes to the label.

The U-cadherin produced at maturation is of normal size as can be judged from its electrophoretic mobility. As it is known that an inhibition of glycosylation by tunicamycin produces a molecule of lower molecular weight in tissue culture cells (Angres et al., 1991) and in oocytes (data not shown), we conclude that during maturation the newly synthesized cadherin is glycosylated to an appreciable degree.

This interpretation agrees with the finding of Ceriotti and Colman (1989) and of Leaf et al. (1990) that protein glycosylation proceeds normally in maturing oocytes in spite of the degeneration of Golgi structures, as defined by ultrastructural criteria (Colman et al., 1985). Studying the behaviour of foreign proteins translated from injected mRNAs, Leaf et al. (1990) concluded that in the mature oocyte the pathway of excretory and membrane proteins to the oocyte plasma membrane is blocked after the trans-Golgi compartment. We assume that this block is also effective for the genuine oocyte protein U-cadherin.

It appears, however, that blocking the insertion of U-cadherin into the plasma membrane is not sufficient to explain its rapid disappearance from the membrane, which is complete in 15-30 minutes during GVBD. An active removal of U-cadherin from the oocyte membrane has to be inferred. The biotinylation experiments indicate that most likely the molecule is internalized by the oocyte during maturation. When the surface of the oocyte is labelled prior to the removal of U-cadherin from the surface, the molecule persists in the oocyte though none of it is found on the surface after maturation.

The exact mechanism of how the internalization of U-cadherin is accomplished has yet to be elucidated. Similar to U-cadherin, membrane-bound Na/K ATPase is removed from the plasma membrane at around the time of GVBD (Richter et al., 1984; Schmalzling et al., 1990). The endocytic process postulated in this report may also be active in the removal of U-cadherin.

**General considerations**

As we have proposed recently, it may be a general phenomenon that membrane proteins that are required during cleavage are supplied from a maternal pool established before fertilization (Servetnik et al., 1990). U-cadherin and also $\beta_1$-integrin (V. Gawantka and P. Hausen, unpublished data) are specific examples for this notion.

As removal of U-cadherin from the oocyte membrane by protease treatment depletes the oocyte of most of its cadherin, it has to be assumed that the internal store of this molecule is small. The maternal pool must therefore be formed by translation during maturation from a store of mRNA, which was established during oogenesis. Whether in addition to newly synthesized U-cadherin, the internalized fraction from the membrane is also added to this pool and reused during early cleavage remains to be clarified.

The plasma membrane of the early cleavage blastomeres is divided into an apical and a basolateral domain. The apical domain derives from the egg membrane and its specific properties are maintained throughout cleavage. The characteristic non-adhesiveness of this domain is established by the removal of U-cadherin during maturation. It may be anticipated that the final constitution of the egg membrane arises by further changes in its protein outfit during oocyte maturation and egg activation.

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**References**


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