ISOLATION OF CILIATED OR UNCILIATED BASAL BODIES FROM THE RABBIT OVIDUCT

RICHARD G. W. ANDERSON

From the Oregon Regional Primate Research Center, Beaverton, Oregon 97005. Dr. Anderson's present address is the Department of Cell Biology, University of Texas Southwestern Medical School, Dallas, Texas 75235.

ABSTRACT

Techniques have been developed for the isolation of basal bodies with cilia attached or for the isolation of only basal bodies from the rabbit oviduct. Oviducts are removed, cut open, and placed in an extraction medium composed of 0.25 M sucrose, 0.001 M EDTA, 0.025 M KCl, 0.02 M Hepes buffer pH 7.5, and 0.05% Triton X-100. After the oviduct is agitated in this medium on a Vortex mixer for 15 min, the lumenal cortex of each ciliated cell, containing 200–300 basal bodies with cilia attached, is released as a unit. The cortices and the intact nuclei, which are also released from the disrupted cells, form a pellet when the extraction medium is centrifuged at 600 g for 10 min. When cortices which contain only basal bodies are to be isolated, the oviduct is subjected to conditions which remove the cilia prior to being processed as above. The cilia are removed when the oviduct is placed in a medium of 0.25 M sucrose, 0.01 M CaCl₂, 0.02 M Pipes buffer pH 5.5, and 0.05% Triton X-100 and continuously agitated for 15 min on a Vortex mixer. The low pH and Ca++ solubilize the transition region of the cilium and also prevent the cell from being disrupted. The cortices can be partially purified if the 600-g pellet is resuspended in 2.2 M sucrose pH 6.5 and centrifuged at 40,000 g for 2 h. Under these conditions, 85% of the nuclei form a pellet and the cortices float to the surface of the sucrose. In addition to the basal bodies or basal bodies with cilia, the cortices contain some adherent cytoplasm, a few fibers, and a few vesicles which may be remnants of mitochondria or endoplasmic reticulum. The structure of the cilia and the basal bodies isolated with either procedure is normal.

INTRODUCTION

The recent progress in mitochondria and chloroplast biochemistry has depended on satisfactory methods with which to isolate these organelles for in vitro analysis. Suitable techniques have been developed for isolating cilia from various organisms (5, 18, 19); these techniques have been directly responsible for much of our present knowledge of the chemistry and function of cilia (18). In contrast, the isolation of large quantities of intact, pure basal bodies has been only partially successful (3, 7, 10–12, 14–16, 20).

Thus far, the isolated oral apparatus from Tetrahymena pyriformis has provided the best preparation of basal bodies (14, 20, 21). These basal bodies seem to have a fairly normal structure, no mitochondria contamination, and very little cytoplas-
mic contamination. However, the isolated oral apparatus also contains an extensive collection of subpellicular microtubules, a ribbed wall with microtubule components, and structures composed of fine filaments (14). In addition, the rather severe conditions for isolating the basal body subject the cells to homogenization or to hypertonic media (14, 20, 21). As a result, the nuclei are disrupted and nuclear contamination of the basal bodies can ensue. Moreover, important but as yet undetected constituents of the basal body, e.g. a specific basal body enzyme system, may be removed.

Ciliated protozoa are a potentially unlimited source of basal bodies, but their structure is responsible for the various types of contamination associated with the isolated basal bodies. Therefore, the ciliated epithelium of the rabbit oviduct was chosen as a new source of basal bodies. These cells are structurally simple, the basal body-cilium complex being the only major microtubular structure. In addition, this basal body differs structurally from the kinetosome and is more representative of metazoan basal body and centriole structure (1, 17). Since the basal body can be isolated with the cilium attached, basal body-cilium interactions can be studied in vitro.

In the ciliated cell of the rabbit oviduct, 200-300 basal bodies arranged in rows beneath the cell membrane are held in position by basal body attachments to the cell membrane (1) and by a fibrillar interbasal body matrix. Under the proper conditions, the basal bodies are released from the cell without any nuclei being broken and with minimal trauma to other cell constituents. The organelles can be purified for analysis.

This report describes the requisite conditions for isolating basal bodies and basal bodies with cilia attached. The structure of the isolated organelles determined by both transmission and scanning electron microscopy is also described. Finally, the potential usefulness of this technique for studying the chemistry and function of the basal body is evaluated.

**MATERIALS AND METHODS**

Adult female rabbits were anesthetized with 4% Halothane (Ayerst Laboratories, New York) or Nembutal (Abbott Laboratories, South Pasadena, Calif.) and laparotomy was performed. The freshly excised oviduct was placed in Hanks' balanced salt solution and dissected under a stereomicroscope. After the excess fat and connective tissue had been trimmed away, the oviduct was cut open from fimбриae to isthmus to expose the ciliated epithelium and cut into small pieces and placed in a vial containing the extraction media.

**Procedure I**

When basal bodies with cilia attached are to be isolated, the extraction solution should consist of 0.25 M sucrose, 0.02 M Hepes pH 7.5, 0.001 M EDTA, 0.025 M KCl, and 0.05% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.). The vials are agitated for 2 min on a Vortex mixer (Scientific Industries, Inc., Queens Village, N. Y.) every 5 min for 30 min. The tissue is removed from the vial and the solution is centrifuged at 600 g for 10 min. The pellet contains basal bodies with cilia, nuclei, and a little adherent cytoplasm.

**Procedure II**

When unciliated basal bodies are to be isolated, the oviduct must be exposed to conditions which will remove the cilia from the cell. The pieces of oviduct are placed in a media composed of 0.25 M sucrose, 0.02 M Pipes pH 5.5, 0.02 M CaCl₂, and 0.05% Triton X-100. The vials are agitated continuously for 15 min. The cilia can be collected if the solution is centrifuged at 20,000 g for 30 min. After the deciliation process, the basal bodies are isolated by the same process as in procedure I.

To partially purify the basal bodies or basal bodies with cilia attached, the 600-g pellet is resuspended in 3 ml of 2.2 M sucrose buffered with 0.02 M Hepes pH 6.5. This mixture is placed in a 5-ml centrifuge tube, and a solution of 0.25 M sucrose, 0.02 M Hepes pH 6.5, 0.01 M CaCl₂, and 0.025 M KCl is layered over the sucrose to fill the tube, which is then centrifuged at 40,000 g for 1.5 h. Under these conditions, the nuclei form a pellet and the cortices which contain the basal bodies collect at the interface of the two solutions. The purified cortices are collected and centrifuged at 600 g for 10 min. Approximately 5-7 mg dry weight of unciliated cortices per gram wet weight of oviduct can be isolated with this technique.

For transmission electron microscopy procedures, the pellets were fixed in 0.02 M collidine, 0.05 M Hepes buffered 0.38% glutaraldehyde pH 7.2 for 10 min. After being washed in 1% Na cacodylate, the pellets were postfixed with 1% osmium tetroxide and embedded in Araldite (Ciba Products Co., Summit, N. J.). Sections were cut on a Porter-Blum MT-2B and viewed with a Philips 200 electron microscope.

The fixation procedure was the same for scanning electron microscopy, except that the fixed specimens were then dehydrated in ethanol and passed through various concentrations of Freon TF (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) and finally into pure Freon. They were then critical point dried.
in a Bomar SPC-900 (The Bomar Co., Tacoma, Wash.), coated with gold-platinum, and viewed with a Cambridge scanning electron microscope.

RESULTS

General Description

The lumen of the rabbit oviduct is lined by a single layer of columnar epithelial cells. At the luminal surface, the cells are attached to each other by typical tight junctions. Desmosomes attach the cells at various points throughout the length of the opposing cell membranes. The basal surface of each cell is irregularly folded and attached to the basement membrane.

Depending on the region of the oviduct, the luminal surface of 40–70% of these cells is lined with 200–300 cilia. The corresponding basal bodies of each cell are embedded in a 0.5 µm thick cortical region (Fig. 1a). The cortex is devoid of mitochondria but contains numerous 100-Å diameter filaments which are not organized into any regular pattern. Occasional bundles of filaments run between basal bodies, from basal body to basal body, and from basal body to cell membrane. Filaments often intermingle with similar filaments from the junctional complex (Fig. 1b). Occasionally, microtubules are attached to the basal foot of a basal body.

Within each cell, the basal bodies are lined up approximately in rows, their basal feet all pointing in the same direction. Although deviations from this arrangement occur, especially near the margins of the cell (Fig. 1b), apparently no cortical force(s) exist, perhaps the cortical filament system, which induce the ordered arrangement. Because there is a correlation between the direction of ciliary beat and the orientation of the basal foot (6), the cortical maintenance of basal body orientation must be important for the proper coordinate function of the ciliated cell.

Structurally, the basal bodies of the rabbit and monkey oviduct are similar (1), i.e., the arrangement of the tubule systems and cylindrical morphology are the same. However, in the rabbit, the basal body does not have a rootlet accessory structure and the alar sheets are not well developed. In addition, part of the C tubule of each triplet is frequently missing in the upper region of the organelle (Fig. 1d). No attempt has been made to determine the direction and degree of triplet pitch or the amount of change in outside diameter between the base and the apex of this organelle (1).

Sequence of Cell Disruption

When a piece of opened oviduct is placed in an extraction solution, the detergent in the medium immediately dissolves the epithelial cell membranes, and, depending on the pH and the ion content of the solution, the cells are disrupted, the cell organelles are released, and the cytoplasm is dispersed. The nuclei and mitochondria are released free into the medium, but the basal bodies maintain their cortical organizational pattern after cell disruption (Figs. 2b, 4b). A few basal bodies are freed from the cortex and some local disruption of the rows of basal bodies occurs, but for the most part the cortical forces are strong enough to keep the basal bodies together during the extraction process. Because of this property of cell organization, the basal bodies, with or without cilia attached, can be collected along with the nuclei if the solution is centrifuged at 600 g for 10 min. The basal bodies can then be separated from the nuclei on a sucrose gradient.

The amount of cytoplasmic contamination of the cortical units, the presence or absence of cilia attached to the basal bodies, and the preservation of good basal body and cilia structure during isolation depend on the pH and the divalent cation content of the isolation medium. In general, low pH (5.5–6.5) favors good basal body and cilia structure but causes extensive cytoplasmic contamination of the cortices and tends to detach the cilia from the basal bodies. Likewise, 0.01 M CaCl₂ or 0.01 M MgCl₂ has similar effects. These conditions also stabilize the cell and tend to prevent the release of the cell organelles, including the cortical unit, into the extraction medium. Cortices isolated with media which contain 0.001 M EDTA and a pH 7.5 have very little cytoplasmic contamination, and the cilia remain attached to the basal body. In addition to good cilia and basal body structure, these conditions preserve the structure of the cilia transition region. 0.5 M sodium acetate has the same effects as low pH and divalent cations whereas media which contain 0.001% spermidine or do not have EDTA remove basal bodies with the cilia attached.

The properties of these various extraction solutions can be used to obtain clean cortical units with or without cilia attached. To obtain quantities of basal bodies with cilia attached, the EDTA medium is used. However, a two step procedure is required to isolate clean basal bodies free of their cilia. The oviduct is first treated with the
FIGURE 1 An arrangement of micrographs showing the *in situ* organization of basal bodies and cilia in the cortical region of the rabbit oviduct ciliated, epithelial cell. (a) Longitudinal section through several ciliated cells. The basal bodies and cilia are aligned at the luminal surface of the cell. The cortical region is defined as the portion of the cell that contains the basal bodies. Notice that no mitochondria are associated with the basal bodies. $\times 8,760$. Scale 5,000 Å. (b) A cross section through the cortex of a ciliated cell. The basal bodies are arranged approximately into rows with the basal feet all pointing in the same direction. The organization of the cortical fibers can be seen (see text). $\times 32,700$. Scale 2,500 Å. (c) A longitudinal section through a basal body and the transition region (between arrows) of a cilium. Notice that ciliary membranes are very straight in the transition region. Compare this region of the cilium to the same region in Fig. 4 d. $\times 34,400$. Scale 3,500 Å. (d) Transverse section through the upper base region (left center) and midregion (upper right) of two basal bodies. The C tubule of the triplet is sometimes unfolded (arrows) in the upper region of the basal body. $\times 65,500$. Scale 1,000 Å. (e) Transverse section through the transition region of two cilia. The short interdoublet linkers and the champagne glass-shaped radial linkers are clearly seen. The distal cup-shaped portion of the radial linkers seems to be attached to the ciliary membrane. However, see Fig. 4 g. $\times 65,500$. Scale 1,000 Å. (f) Transverse section through the midregion of several cilia. $\times 71,000$. Scale 1,000 Å.
CaCl₂, low pH mixture which removes the cilia but does not disrupt most of the cells. The oviduct is then exposed to the 0.001 M EDTA pH 7.5 solution, the unciliated cortical units are released, and the cell cytoplasm is dispersed. The chelation of both Ca⁺⁺ and Mg⁺⁺ is necessary to obtain clean preparations.

**Structure of Isolated Basal Bodies and Cilia**

**Organelles isolated by procedure II:** Unciliated cortices isolated in this procedure contain rows or groups of basal bodies with some surrounding cytoplasm (Figs. 2 a-c). The cytoplasm contains filaments, a few membrane-bound vesicles which may be remnants of the endoplasmic reticulum, and an occasional mitochondrion. The cytoplasm has been severely extracted by the procedure. In addition, considerably fewer filaments are in the isolated cortical matrix than were originally present (compare Fig. 1 b and 2 c).

The size of an individual cortex varies. Three factors contribute to this size heterogeneity. (a) Not every ciliated cell within the oviduct has the same number of basal bodies or the same amount of surface area in the cortex. (b) Occasionally the cortices of several cells remain together during the isolation procedure, joined by tight junctions (Fig. 2 c). (c) Finally, some cortices are partially disrupted during isolation and a few of the basal bodies are lost.

Preparations of unciliated basal bodies also contain a few anomalous cortices. For example, a few cortices are present with the cilia incompletely detached from the basal bodies. Observations of the transition region of these ciliated basal bodies indicate that the tubules are only partially solubilized. Moreover, some apparent cell cortices do not contain any basal bodies. These units are usually found joined to a normal cortex by a tight junction. Most likely these are cortices from former secretory cells although no secretory granules have been identified.

Morphologically, the basal body appears to be unaltered by the isolation procedure (Figs. 2 d-g). The cilium has detached at the triplet-doublet junction, and the basal body appears very much like a recently synthesized organelle (2). The tubule system looks normal (Figs. 2 e-g) and the surrounding intertriplet matrix material has not been removed. The basal foot and alar sheets are still attached. The sheetlike morphology of the alar sheet is easily recognized in longitudinal sections of the isolated organelle (Fig. 2 d).

The cilia released during the deciliation procedure have a fairly normal structure (compare Figs. 1 f and 2 h). The central pair with filaments radiating to the outer nine doublets can be seen in transverse section. Often the tubule morphology is fuzzy, as if the lumen of each tubule had filled with some material.

The isolated cilium is missing the transition region or basal portion of the organelle. This part of the cilium dissolves in the extraction medium and causes the organelle to be released during the deciliation process. The 0.01 M CaCl₂, pH 5.5, and 0.05% Triton X-100 are the agents in the extraction medium which dissolve the transition region.

Several scanning electron micrographs of basal bodies which did not lose their cilia during procedure II extractions are seen in Figures 3 a-c. The ciliary membrane was dissolved by the detergent, and the individual peripheral doublets of the cilium are visible (Figs. 3 b, 3 c). However, the amorphous material that surrounds the basal body obscures the triplet tubules. In Figs. 3 a and b, the three-dimensional arrangement of the cilium in the cortex can be seen. Fig. 3 e shows a complete basal body-cilium complex in which the amorphous material is thinner in the base region of the basal body. In addition, the basal foot can be seen in the midregion and the alar sheets in the apical region of the organelle. These micrographs demonstrate again that the important structural components of the basal body and cilium remain intact during isolation.

**Organelles isolated by procedure I:** Cortices isolated in a medium containing 1 mM EDTA are similar to those described above except that the cilia are attached. Interbasal body organization is maintained, and the units have some adherent cytoplasm (Figs. 4 a-c). As with the unciliated cortices, the cortex size and the amount of cytoplasmic contamination vary.

The conditions for extracting ciliated cortices do not substantially alter the structure of the basal bodies or the cilia (Figs. 4 d-h). The EDTA prevents the cilium from being removed because it stabilizes the peripheral doublets in the transition region (Figs. 4 d, 4 g). In addition, the EDTA preserves the two types of microtubule linkers normally found in this region.

The integration of the peripheral doublets with
the two types of linkers is dramatically revealed in the isolated basal body-cilium complex. The short interdoublet linkers (Fig. 4 g) are preserved and seem to maintain the close juxtaposition of each doublet. Because of the short interdoublet spacing, the cilium diameter is smaller in this region than in the midregion. In the intact cilium (Fig. 1 c, e), the second set of linkers, the radial linkers, appears to be a system of fibers which connect the peripheral doublets to the ciliary membrane. However, it is clear in Fig. 4 d that these linkers are sheets of material which are attached to the doublets throughout the transition region. The champagne glass profile (9) of these sheets in cross section is preserved in isolated preparations. In addition, the cup-shaped end of each linker merges with a band of specialized amorphous material which encircles the cilium (Fig. 4 g).

In the intact cilium, this band of material appears to be part of the ciliary membrane (Fig. 1 e). The amorphous band with the interdigitated linkers appears to form a system which probably adds some rigidity to this region of the cilium.

**Purification**

Since the cortical units released during either of the isolation procedures sediment at 600 g along with the nuclei, the purification of the basal bodies requires the separation of the nuclei from the cortices. The most important criterion for the separation of the two components is that the nuclei be free of any cytoplasmic contamination. The EDTA step in both procedures is important for obtaining cytoplasm-free nuclei.

Roughly 80–90% of the nuclei can be removed if the 600-g pellet is resuspended in 2.2 M sucrose buffered with Hepes to pH 6.5 and centrifuged for 2 h at 40,000 g. Under these conditions, the nuclei form a pellet and the cortices float to the surface of the sucrose. The structures of the basal bodies and cilia are not altered during the purification procedure.

**DISCUSSION**

**Evaluation of Techniques**

The oviduct appears to be a unique source of basal bodies. The conditions for extracting the organelle are much milder than those for obtaining basal bodies from ciliated protozoa (14, 15, 20). The nuclei remain intact and can be separated from the basal bodies and cilia. In addition, there is relatively little cytoplasmic contamination of the basal bodies. No persistent structure, e.g. nonbasal body microtubules, mitochondria, etc., is associated with the cortices that would interfere with the experimental analysis of basal body function and biochemistry. Finally, by adjusting the extraction conditions, one can select cortices which have cilia attached to the basal bodies.

The structure of the basal body is maintained during the isolation procedure. Minor alterations in the accessory structures are sometimes seen but all of the components of the organelle are present.
Figure 3  Three scanning electron micrographs showing the three-dimensional organization of the ciliated cortex and the isolated basal body-cilium complex. (a) View of the isolated ciliated cortex. Numerous thick strands of cytoplasm seem to hold the cortex together. This aspect of cortical organization is not detectable in transmission electron micrographs. $\times 7,000$. Scale 5,000 Å. (b) High magnification view of the cilia and basal bodies in the isolated cortex. The longitudinally arranged light and dark regions seen in some of the cilia (arrows) are produced by the doublet and interdoublet spacings, respectively. The ability to recognize the doublet tubules in scanning electron micrographs means that the longitudinal arrangement of these tubules throughout an individual cilium can be studied. $\times 13,300$. Scale 5,000 Å. (c) Micrograph of an individual basal body-cilium complex. The organization of the basal body can be seen: A, the thick amorphous material which surrounds the base of the basal body; B, the basal foot; C, the alar sheets. Although the tubules in the cilium can be seen, the surrounding amorphous material obscures the triplet tubules in the basal body. $\times 14,000$. Scale 5,000 Å.
Therefore, the isolated basal body of the oviduct is a good model for studying basal body biochemistry. Because the basal body and the centriole have similar structures, information accruing from the study of this model can also apply to the centriole.

Isolated cortices with cilia attached to the basal bodies may prove useful for studying reactivated cilia. Cilia-basal body interactions, as well as basal body-to-basal body interactions, could be studied in reactivated cortices. Since a few isolated cortices always remain attached by tight junctions, the patterns of cilia interaction between adjacent cortical units could be investigated in reactivated preparations. Changes in the spatial relationships of the peripheral doublets during various phases of the beat could also be observed with the scanning electron microscope.

The principal contaminants in the isolated basal body preparations are the nuclei and the cytoplasm which adheres to the cortices. About 85% of the nuclei can be removed if the 600-g pellet is resuspended in 2.2 M sucrose and centrifuged at 40,000 g for 2 h. Preliminary experiments with linear sucrose gradients indicate that more nuclei can be removed, but it is doubtful that 100% removal will be possible with this method. The development of other techniques, such as those used to purify brush borders (13) from intestinal epithelia, may be necessary to achieve complete purification. Likewise, techniques can be developed to remove more of the cortical cytoplasm if this contaminant interferes with biochemical analysis of the basal body.

**Observations on Ciliated Cell Organization**

To discover that the whole cortex of the cell, complete with ordered rows of basal bodies, remained intact during the isolation step was unexpected. This region of the cell does not contain any microtubule systems analogous to those in the cortex of ciliated protozoa (11, 14). Apparently, the numerous fibers that course randomly among the basal bodies, (the interbasal-body apparatus [8]), and the sol-gel properties of this region are specializations of the cell which maintain this order. It is strange, however, that most of the cytoplasm and fibers seem to be lost during the isolation without the loss of interbasal body order.

This behavior of the cell cortex indicates that in the intact cell this region is rigid. Probably the basal bodies are held in place by the high viscosity of the surrounding cytoplasm. Even though it is not well organized, the fiber system must create resistance to any type of basal body movement; this may be important to the proper form of the ciliary beat.

The high rigidity that probably exists in the cell cortex does not allow very much translational movement of the individual basal bodies during the beat. Therefore, these basal bodies must become aligned and oriented during differentiation before the cortex becomes rigid. Since the orientation of the basal body is related to the direction of the beat (6), it is possible that the coordinated direction of beat for each cell is set at the time of differentiation and does not alter for the life of the cell.

This study has demonstrated that the transition region of the cilium has some unique morphological and biochemical features. When the cortex of the ciliated cell is isolated in procedure I, the EDTA stabilized the doublets, interdoublet linkers, and radial linkers. Although the EDTA may have some intrinsic ability to preserve these structures, more than likely its effect is due to the removal of the divalent cations from the environment of this region. Transverse and longitudinal views (Figs. 4 d, 4 g) of the transition region in the isolated basal body-cilium complex show the radial linkers to be sheets of material which attach to the doublet throughout the length of the region. There seem to be two components to each sheet; (a) a flat sheet portion which extends 150 Å from the doublet and attaches to the convex side of a (b) half cylinder-shaped portion. The two edges of the half cylinder segment attach to an amorphous band of material which encircles the cilium in this region. These interpretations do not agree with the description of a similarly shaped structure in the transition region of *Elliptio* cilia (9). Gilula and Satir have presented several micrographs which seem to show that the radial linkers are champagne glass-shaped structures with the stem attached to the doublet and the cup attached to the ciliary membrane. Either the structure of this linker is different in the rabbit oviduct cilia, or the isolation procedure has caused the individual linkers to merge together in the longitudinal plane to create the sheetlike appearance.

Regardless of the shape of the radial linker, there seems to be no doubt that the distal segment of the structure attaches to some amorphous material which remains even when the cell membrane is removed. Presumably, this nondescript material is a
component which is important to the function of the radial linkers. Normally this amorphous material must be intimately associated with the ciliary membrane. It would be interesting to know whether this component of the radial linkers has any relationship to the specialized membrane particles, the ciliary necklace, found in this region of the ciliary membrane.

In many types of ciliated cells, under the proper conditions, the cilia will detach just above the basal body. The conditions differ for each cell type but include dilute acid, heating or cooling, mild shearing forces, and ethanol treatment. This study has shown that low pH, high Ca++, Triton X-100, and vigorous agitation are required to remove the oviduct cilia. Apparently the cilia are removed because the ions and the detergent solubilize the components of this region and the agitation shears off the weakened cilia from the basal body.

The effects of Ca++, pH, and detergent on the tubules in the transition region indicate that the molecular composition of these microtubules differs from that of the microtubules in the rest of the basal body-cilium complex. Most likely, the longitudinal heterogeneity in the molecular composition of microtubules, which are continuous from the proximal part of the basal body to the distal part of the cilium, has important implications for the mechanism of cilium formation and function. The ability to differentially solubilize the transition region could be the basis of a technique to investigate the comparative structure of tubules in the various regions of the basal body-cilium complex.

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