INVolvement of bristle coat structures
in surface membrane formations and membrane
interactions during coenocytotomic cleavage
in caps of acetabularia mediterranea

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ABSTRACT
In the multinucleate cap rays of the green alga Acetabularia mediterranea the cell
surface increases dramatically within a short time period during the final stages of
coenocytotomic cleavage. In early stages of cyst formation the cytoplast is tra-
versed by numerous large and prolate cleavage vesicles which are characterized by
typical columellar or spinous coat structures. The cleavage vesicles are closely
associated with the surface of plastids and, to a lesser degree, of mitochondria.
This intimate association seems to be mediated by regularly spaced, densely
stained intermembranous cross-bridge structures and is maintained throughout
cleavage. These cleavage vesicles contain a finely fibrillar material structurally
similar to the hyaline layer of mucilage that fills the space between the plasma
membrane and cell wall. They line up with invaginations of the plasmalemma and
vacuole membranes and, together with smaller vesicles interspersed, constitute
preformed “perforation lines” for the final separation of the coenoblast portions.
Equidistantly spaced plaques of attachment of such vesicles with surface mem-
brane are described. We hypothesize (a) that the cleavage vesicle membrane is the
immediate precursor to the new postcoenocytotomic surface membrane, (b) that
the cleavage vesicle coat structures are integrated into the subsurface coat of the
plasma membrane, (c) that growth of the laterally attached cleavage vesicles by
intussusception of small fuzzy-coated vesicles is confined to their “free ends,” (d)
that the intermembranous cross-bridge elements are related to bristle coat struc-
tures and play a role in the establishment of the cleavage lines, and (e) that the
coenocytotomic cleavage process is organized so that adjacent plastids are sepa-
rated in a way that guarantees the inclusion of several plastids in each cyst.

Current concepts and hypotheses on the mecha-
nisms of cell surface membrane formation provide
a series of arguments for and against the incorpo-
ration and transformation of intracellular mem-
branes into plasma membrane (for references, see
9, 12, 21, 23). It is obvious that in this discussion
on the modes of surface membrane formation the
study of the cytoplasmic cleavages (coenocyto-
tomy) of multinucleate cell systems is especially valuable since they represent stages in which large areas of cell surface appear, usually within a rather short time, such as in the formation of spores, cysts, “spherules,” and gametes (for references, see 2, 3, 14, 29, 31). The fine structural organization of the (polyenergid) coenoblast of cyst-forming caps of *Acetabularia* has been described in detail in various articles (e.g., references 1, 13, 34, 35). The onset of cyst formation is most typically recognized at the stage “Weiβer Fleck” (“pale spot”; for detailed descriptions, see references 28, 29, 33). This stage is characterized by a rather regular pattern of plastid-free patches, each of which contains a secondary nucleus and a portion of perinuclear cytoplasm, including basophilic “heavy bodies” and many microtubules (1, 13, 34, 35). Thereafter, the mononucleate (monoenergid) portions are dissected out by a sequence of coenocytotomic cleavages within less than 24 h (28). The resulting coenomers then form a special cyst wall (for references, see 33) and develop into coenoblastic gametangia (29). The present study is confined to the morphology of early stages of coenoblast cleavage and focuses on the involvement of membrane-associated bristle coat structures in processes of surface membrane formation and organelle distribution.

MATERIAL AND METHODS

Algae were cultivated, fixed, and processed for embedding as described (1, 11, 13), using both simultaneous and sequential fixations with glutaraldehyde and osmium tetroxide. Ultrathin sections were sequentially stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop 101 or a Zeiss EM 10.

RESULTS

The typical aspect of the cytoplasmic layer which surrounds the large central vacuole is presented in Fig. 1. At the beginning of light microscopically identifiable cleavage (the stage designated “Felderrung“ in reference 28) the coenoblast has already detached in large surface areas from the cell wall proper, and the interspace between the wall and the plasmalemma is filled with loosely packed, hyaline material (Fig. 1). This hyaline material reveals a characteristic finely fibrillar texture and most probably represents slime material newly secreted at this stage, similar to what has been described in corresponding stages in some fungi and in various other algae (4, 15, 16, 26), and has also been demonstrated during gnetetogenesis of *Acetabularia* cysts (34). Similar finely fibrillar material is recognized also in the cleavage vesicles (Figs. 1 and 5) and, occasionally, in vesicles located at the distal poles of dictyosomes (e.g., Fig. 2; see reference 13), which strongly suggests an origin of this material in the Golgi apparatus (see references 15 and 26 for similar observations and speculations as to the possible function of this secretion in cytoplasm contraction and spore release). Most of the somewhat ellipsoidal plastids are oriented with their long axis towards the cell wall (Figs. 1 and 2). The coenoblast surface is rounded off and smooth, except for some invaginations that seem to be involved in the cleavage processes (e.g., Fig. 1). Occasionally, limited surface regions are noted to be covered by an external fuzzy coat (inset in Fig. 1). The inner membrane that delimits the central vacuolar space is more irregularly shaped and seems to curve around the cytoplasmic protrusions that contain the plastids (Figs. 1 and 2). Some particularly deep invaginations of the tonoplast membrane might also participate in cleavage phenomena and thus represent furrows (e.g., arrows in Fig. 2). At this stage of early cyst formation the cytoplasm is characterized by the occurrence of numerous small and large cleavage vesicles (Figs. 1 and 2). The latter represent a particular class and are usually more or less prolate (long axes of up to 3 μm) and interspersed between the plastids (Fig. 2). The most conspicuous feature of the large and prolate cleavage vesicles and cleavage furrows (see above) is their close association with the outer membranes of the plastids which is apparently mediated by the numerous regularly oriented cross-bridge structures (see also reference 11). Not infrequently, one cleavage vesicle is associated with three or even more plastids and thus reveals more than two “free”, i.e. nonassociated, ends (Fig. 3).

Bristle coat-like structures are commonly observed on the cytoplasmic surfaces of both size classes of vesicles, the small spherical ones and the large cleavage vesicles (Figs. 3–5), covering either the entire vesicle or parts of it. The details of the bristle coat organization are most clearly revealed at the “free edges” of the large, plastid-associated cleavage vesicle (Fig. 3) and closely resemble the structures described for typical coated vesicles in a great variety of animal and plant cells (for references, see 8, 12, 24). The membrane regions occupied by bristle coat are frequently continuous with those occupied by the intermembrane cross-
Figure 1. Survey electron micrograph showing the periphery of the cap content of a cyst-forming *Acetabularia mediterranea* during early stages of coenotomic cleavage. The multinucleate cytoplast body has already shrunken and retracted from the cap wall (*W*) and is separated from it by an extensive hyaline layer (*HL*) with a finely fibrillar texture. The plasma membrane of the cytoplast body reveals invaginations which in some regions extend rather deeply into the cytoplasm (arrow) and shows small areas set with an external fuzzy coat (arrow in the inset) that is unrelated to the internal plasmalemma coat. The cytoplasm contains numerous rather densely packed plastids (*P*), mitochondria, and dictyosomes (*D*) and surrounds a large central vacuole (*LV*). Among the various cytoplasmic vesicles, two types of vesicles, both of which are partly or completely "coated," are especially abundant: (a) small vesicles that are particularly frequent in the very periphery of the cell (some are denoted by the triple arrow in the inset), and (b) larger, usually prolate "cleavage vesicles" ("cytotomic vesicles," *CV*) that are mostly radially oriented and closely associated with plastidal surfaces. Many of these cleavage vesicles contain fibrillar structures similar to those of the hyaline layer, e.g., inset. Scales denote 2 μm and 1 μm (inset). Magnification, × 9,000; × 38,000.

Figure 2. The cytoplasmic rim that surrounds the large central vacuolar cavity (*LV*) of the cap of *Acetabularia* is shown during the stage of coenotomic cleavage at higher magnification. Note that the surface membrane (*PM*) is rather smooth and rounded, except for the cytotomic furrows (see Fig. 1), whereas the inner membrane of the cytoplasmic layer, which delimits the vacuole and thus is equivalent to a tonoplast, is highly serrated and follows the protrusions made by the innermost plastids (*P*). It is not clear whether some especially deep tonoplast invaginations (thick arrows in the left part) participate as "tonoplast furrows" in the cleavage process. Coenocytotomic vesicles (*CV*) of various sizes (some small ones are designated *cv*) are very frequent, and the largest ones are closely applied to plastidal surfaces and separate adjacent chloroplasts. Note a similar close association between cleavage vesicles and mitochondria (*Mi*) and the fibrillar contents of these vesicles. It is possible that the very small vesicles (thin arrows), which often also reveal a "coated" aspect, are precursors to cytotomic vesicles and fuse with them. Such small coated vesicles are also frequently seen at the distal pole of dictyosomes (*D*). *S*, starch granules; *ER*, special cisternae of the endoplasmic reticulum which preferentially lie opposite to the proximal (forming) face of dictyosomes. Bar represents 1 μm. × 24,000.
FIGURE 3a–d Typical survey of the close association between a cleavage vesicle (CV) and several plastids (P₁, P₂, P₃). The relative electron-translucent regions within the plastics represent starch deposits (see Fig. 2), and the osmiophilic globules are plastoglobuli. Note the appearance of a fuzzy coat layer on the free (growing?) ends of the cleavage vesicles (arrowheads). The relationship of the bristle coatlike structures on the free ends of these cleavage vesicles to the intermembrane cross-link bridges that connect the vesicle membrane to the outer plastidal membrane is illustrated in b–d in which the individual membrane-associated elements are denoted by bars. These membrane-to-membrane cross-bridge elements appear either in a rather regular pattern (b and c) or in a less distinct and laterally aggregated form (as illustrated in the region denoted by the brackets in d). Bars represent 0.2 (a) and 0.1 μm (a) × 63,000; (b–d) × 174,000.
bridge elements, without perceptible transition or interruption (Figs. 3-5). This suggests but does not prove an ontogenetic relationship between these two structures. It should be noted, however, that the intermembranous bridge elements are usually somewhat columnella shaped and more massive (up to 18-nm thick and 23-nm long) and intensely stained than the typical bristle elements. In some areas these plastid-cleavage vesicle intermembrane bridges are very closely spaced and may even appear as a fused, indistinct dense layer (e.g., Figs. 3b-d). Similar associations of cleavage vesicles (as with plastids) are sometimes found with mitochondria (e.g., Fig. 2 and references 11 and 13).

Often, small fuzzy coat-bearing vesicles are recognized in the vicinity of the free ends of the cleavage vesicles (e.g., Fig. 4). Their frequency, together with the observations of distinct bristle-coated outpocketings on cleavage vesicles (e.g., Figs. 4a-b and 5b), suggests that the large cleavage vesicles grow, primarily at their free “tips,” by fusion with smaller coat-bearing vesicles. Similar fuzzy coat structures are also seen on invaginations of the plasma membrane (Fig. 4c), and very conspicuous are also alignments of large cleavage vesicles, small coat-bearing vesicles, and plasmamemmal invaginations, each series of which seems to represent a “perforation line” (cf. reference 14) for the cleavage events. Some of the fuzzy coat-bearing vesicles that are associated with the plasmalemma and/or cleavage vesicles also reveal typical equidistant “attachment plaques” (Fig. 5) in which densely stained intermembranous material is identified. Such a vesicle-to-surface membrane attachment is very reminiscent of the “preexocytotic attachment plaques” recently described in the secretory vesicles for casein in the rat mammary gland (12) and is readily distinguished from the subsurface cisternae of endoplasmic reticulum-like character as, for example, by their greater membrane thickness and pronounced unit membrane pattern distinctiveness (Figs. 4 and 5; cf. references 21 and 22). Intimate membrane-to-membrane associations were also noted between large cleavage vesicles and plasmalemma (Figs. 4c and 5c), either without an identifiable interspace, suggesting direct membrane contact (Fig. 5c), or with a rather equidistant interspace that contains some densely stained material (Fig. 5a-b).

With progressive coenocytotomy the cleavage vesicles become larger and less numerous, presumably through fusion processes. The final stage of the monoenergid segregation which separates the coenomeres was not observed in an adequately fixed state. This process, in which also some anucleate cytoplast portions (as to their occurrence see also reference 33) are segregated out along the preformed perforation lines, occurs so rapidly (cf. reference 28) that it is hardly recordable. The intimate lateral association of growing furrows and cleavage vesicles with the plastids was retained throughout cleavage and was further expressed in the nascent cysts as distinct plasma membrane-plastid associations.

DISCUSSION

Cytotomic and coenocytotomic cleavages can occur (a) by centripetal furrowing of the plasma membrane, (b) by coalescence of intracellular vesicles along certain but usually not morphologically conspicuous fusion planes, or (c) by concerted contributions of both types of processes. While furrowing is the predominant form of cleavage in animal cells, all three mechanisms may be generally involved in formations of gametes, spores, and cysts in fungi and plants. The specific mechanism may even vary in different phases of the life cycle of the same organism (for references, see 4, 14, 15, 18, 19, 26, 27, 31). Among the green algae, all three mechanisms have been described (e.g., references 4, 5, 7, 16, 18). The cleavage process described here for *Acetabularia* cyst formation involves simultaneously both invagination of the plasma membrane and fusion of files of vesicles. Apparently, the latter process makes the quantitatively more important contribution. Whether or not the deeply invaginated sinus of the tonoplast (see Figs. 1 and 2) actively participate in the coenoblast cleavage, i.e. as “centrifugal furrows,” remains to be clarified. The novel feature of the cleavage in the *Acetabularia* caps is the stable orientation of the cleavage vesicles relative to the plastids. This is mediated by the zipper-like membrane-to-membrane attachment structures, the intermembranous cross-bridges (for references on other examples of intermembrane cross-bridges, see 10 and 11). This mechanism necessarily leads to the segregation of adjacent plastids and illustrates that the cleavage planes here are not primarily ordered with respect to the nuclei (for a different type of pattern in some lower fungi, see reference 14) but dissect the coenochytoplasm along the plasmoidal surfaces. This pattern of cleavage indicates that it is the subsequent selection and survival of nucleate portions that results in the...
final formation of mononucleate cysts. Such a cleavage pattern also guarantees the inclusion of several plastids per cyst and also prevents the accidental formation of chlorotic cyst portions.

The total cell surface area within the Acetabularia cap increases several thousandfold within less than 1 h during the final stages of coenoblast cleavage (cf. reference 28). From our micrographs, it is clear that this rapid and dramatic increase involves the rapid and oriented fusion of intracellular vesicles with each other and with the preexisting plasmalemma and tonoplast membranes. Thus, the cleavage vesicle membrane is an immediate precursor to the plasma membrane of the cleavage products. Because the mucilage contents of the cleavage vesicles of Acetabularia and other algae are similar to the hyaline wall layer components (for references, see 13, 15, and 26) this fusion might also be regarded as a special secretory process which results in the abrupt release of polysaccharides. The observations that small cleavage vesicles occur at the distal poles of dictyosomes indicate derivation from the Golgi apparatus. This origin has been hypothesized for the cleavage vesicles described in other cells (e.g., references 14, 19, 27, 31).

In most eucaryotic cells the cell surface complex consists of not only the plasma membrane proper but also an intimately associated internal coat and skeleton which contains knob- and filament-like structures, including the dense “terminal web” and the “filamentous network” of some cells (for references, see 12 and 20). Consequently, formation of new surface membrane requires production of membrane material and a balanced and coordinated production and assembly of the associated coat elements, including the actomyosin complex, etc. Therefore, one has to postulate that such coat structures either are assembled on the newly incorporated membrane elements or are already associated with the precursor membranes, i.e., the cleavage vesicles of cyst-forming Acetabularia. Most of the cleavage vesicle surface is covered by distinct coat material, either as a fuzzy coat or in typical regular arrangements. These observations suggest that the cleavage vesicle coat is transformed, partly or completely, into the plasma membrane coat. Contributions of vesicle coat structures to surface membrane formation have also been discussed in other situations such as cell plate formation (for references, see 6 and 8), the regeneration of apical cell membrane in lactating mammary cells (12), and the formation of junctional complexes (30). Bristle coatlike structures similar to those described in this study have been described in the plasmalemma invaginations and associated vesicles during the “inversion” process of Volvox colonies (25). Bristle coatlike structures are also common on vesicles of the water expulsion vacuoles of some protozoa and protophyta (e.g., Fig. 7 in reference 21; 32) which eventually become incorporated, at least temporarily, as a part of the cell surface. It remains to be clarified, however, whether these bristle-coat structures which are so strikingly similar among the diverse cell systems all contain the polypeptide “clathrin” (180,000 daltons mol wt) recently described for the small coated vesicles from porcine brain (24).

Figure 4a–d. Details of the “free ends” of the large cleavage vesicles (CV) that are closely attached to plastidal (P, P1, P2) surfaces as revealed at higher magnification. Fig. 4a presents a flattened cleavage vesicle which seems to protrude and grow in the direction indicated by the arrow (in the bottom). This vesicle is still relatively loosely associated with the adjacent plastids as suggested by the few membrane-to-membrane attachment knobs. The membrane of the somewhat rounded-off end of this “cleavage cis- terna” (arrowhead) bears a typical coat (see text). Note that small vesicles (cv), which are also partly coated and may contribute to cleavage processes by fusion with the larger cleavage vesicles (CV), are frequent in such regions, particularly in the vicinity of the “growing tips” of the cleavage vesicles. Fig. 4b shows a bristle-coated evagination of the surface of a cleavage vesicle (arrowheads). This situation suggests fusion of coated vesicles with the free ends of vesicles which are closely attached to the plastidal surface. Fig. 4c presents a bristle-coat covered invagination of the plasma membrane (cv) that is in close contact (left arrow) with one of the larger cleavage vesicles (CV). A similar close and aligned association (arrowheads) between the coated edge of a large cleavage vesicle (CV) and one of the smaller coated vesicles (cv) and the edge of an invagination of the large central vacuole (LV) is shown in Fig. 4d. The three small arrowheads in the right of Fig. 4c denote small membranous vesicles and sacs in the very cortical zone of the coenocytoblast. P, P1, and P2 indicate cleavage vesicle-attached plastids. Bars indicate 0.2 μm; (a) × 100,000; (b) × 50,000; (c) × 85,000; and (d) × 110,000.
Figure 5a–c  Details of the interaction of the cleavage vesicle (CV) membrane with the plasma membrane (PM) and special subsurface cisternae of endoplasmic reticulum character (thin arrowheads in Fig. 5c) in the cortical zone of the cytoplasm of cyst-forming Acetabularia mediterranea caps. In some regions, the membrane of the large cleavage vesicles (CV) is intimately associated (Fig. 5a–c), if not already fused (thick arrow in the right of Fig. 5c), with the plasma membrane. In other regions, these two membranes seem to constitute attachment plaques (as indicated by the arrowheads in Fig. 5a and by the brackets in Fig. 5b) in which they are equidistantly separated by an about 15-nm thick layer of densely stained, cytoplasmic material. Similar associations can be observed with some of the small cleavage vesicles (cv; for example, Fig. 5a). Note also the coated evaginations of some of the large cleavage vesicles (Fig. 5b, arrowheads), which suggest local fusion with, or formation of, a smaller vesicle, and the regular bristle coat arrangements on confined regions of the rounded surfaces of such vesicles, e.g., between the two lower thick arrows in Fig. 5c). Fig. 5a presents an intimate association between a small coated vesicle (cv) and both the plasma membrane (PM) and a large cleavage vesicle (CV), an arrangement that perhaps constitutes a preformed cytotomic cleavage rupture line. Note also the close associations between the subsurface cisterna and both the plasma membrane and cleavage vesicles (Fig. 5c). The cleavage vesicle presented in Fig. 5c reveals a loosely arranged fibrillar content. HL, hyaline layer between the cell surface and the wall (cf. Fig. 1). Bars denote 0.3 μm (c) and 0.2 μm (a and b). (a) × 75,000; (b) × 83,000; and (c) × 94,000.
Another significant cytological observation in the present study is the phenomenon of "tip growth" of an extended and prolate vesicle. To the extent that growth of the cleavage vesicles takes place by fusion of small vesicles, this must occur on the ends of the large cleavage vesicles because these are the only accessible, i.e. not plastid-associated, regions, in concurrence with the observations of bristle-coated blebs in these regions.

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