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FREEZE-FRACTURE STUDY OF THE SUBSURFACE CISTERNA IN THE MURINE HEPATOCYTE

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SUMMARY: Subsurface cisternae, broad flattened sacs with varying numbers of fenestrae lying along the lateral plasma membrane, in murine hepatocytes were studied in thin sections and freeze-fracture replicas. Subsurface cisternae extended from pericanalicular to perisinusoidal areas of the cytoplasm of closely adjoining hepatocytes. Subsurface cisterna was made up of paired membranes, an inner cisternal membrane facing the cell interior and an outer cisternal membrane facing the lateral plasma membrane. The inner cisternal membrane was continuous in some locations with the endoplasmic reticulum. Subsurface cisterna was fenestra-rich at its portions underneath gap junctions. Subsurface cisternae appeared as anastomosing tubules at pericanalicular and perisinusoidal ends and joined with tubulovesicle networks seen in these cytoplasmic areas.

INTRODUCTION

Subsurface cisternae have been seen along the lateral surfaces of murine and rat hepatocytes in thin sections (9,15). Similar structures have been found in cerebral pyramidal and granule cells (3, 6), testicular Sertoli cells (8), cochlear outer hair cells (13), and retinal photoreceptor cells (4). Multilayered subsurface cisternae in cochlear outer hair cells have been shown in freeze-fracture replicas (13). However, subsurface cisterna in hepatocytes has not been identified in freeze-fracture replicas.

The present freeze-fracture study has shown the spatial extensions of subsurface cisternae locating along the lateral plasma membranes in murine hepatocytes.

MATERIALS and METHODS

Twenty adults dd-N strain mice of both sexes, fed laboratory chow and water ad libitum, were used in this study. The animals were killed by decapitation. Excised liver blocks were cut into small pieces and immersed in a fixative mixture of 2.5% glutaraldehyde, 2% paraformaldehyde, and 2% acrylaldehyde in 0.1M cacodylate buffer containing 5mM calcium chloride, pH 7.4 for 3h. Fixed tissue pieces were washed several times with

cacodylate-buffered 7.5% sucrose.

Thin-section electron microscopy

Tissue pieces were immersed in 1% osmium tetroxide in 0.1M phosphate buffer containing 0.5% glucose for 3h, dehydrated with graded series of ethanol, embedded in Epoxy resin and sectioned with a diamond knife equipped in an ultramicrotome (LKB 8800). Some tissue pieces were immersed in 1% osmium tetroxide in 0.1M cacodylate buffer containing 0.8% potassium ferricyanide (16). Thin sections were stained with uranyl acetate and lead hydroxide.

Freeze-fracture electron microscopy

Other tissue pieces were soaked in cacodylate-buffered 30% glycerol for about 12h. Small tissue pieces placed in small pits in copper plates were frozen on a brass-block apparatus (Hitachi HFZ-1) cooled in liquid nitrogen, fractured with the brass-block apparatus equipped in a vacuum evaporator (Hitachi HUS-5GB), shadowed with platinum-carbon, and backed with carbon. Replicated tissue pieces were immersed in a household bleach to remove the organic materials. Replicas were washed in several changes of 50% ethanol, and mounted on copper grids.

Freeze-fracture replicas and thin sections were examined in electron microscopes (JEOL JEM-100C and JEM-100CXII).

RESULTS

Thin-section electron microscopy

The hepatocyte had three surfaces: the bile-canalicular, lateral, and perisinusoidal surfaces. On both the bile-canalicular and perisinusoidal surfaces there were numerous microvilli. Smooth tubulovesicles were seen underneath the pericanalicular ectoplasm and often continuous with endoplasmic reticulum. The lateral surface was even and faced adjacent hepatocytes across the narrow intercellular space. At places, an intercellular space between contiguous hepatocytes was dilated. Tight junctions and desmosomes were seen in the lateral surface. Subsurface cisterna with narrow cisternal lumina was seen along the lateral plasma membranes of two contiguous hepatocytes (Fig.1). The lumen of each subsurface cisterna was bounded by typical membrane: an inner cisternal membrane facing the cell interior and an outer cisternal membrane facing the lateral plasma membrane. The inner cisternal membrane was often continuous with the outer cisternal membrane at fenestrae, giving rise to interrupted paired membranes. Therefore, some cross-sectioned subsurface cisternae were seen as rows of tubulovisicles. A band of cytosol was interposed between the outer cisternal membrane and the lateral plasma membrane.

Freeze-fracture electron microscopy

At the borders of the lateral surface near the bile-canalicular and sinusoidal surfaces, subsurface cisternae turned inward and joined tubulovesicle networks (Figs. 1). The E faces of the outer cisternal membranes were seen through gaping holes in the P faces of

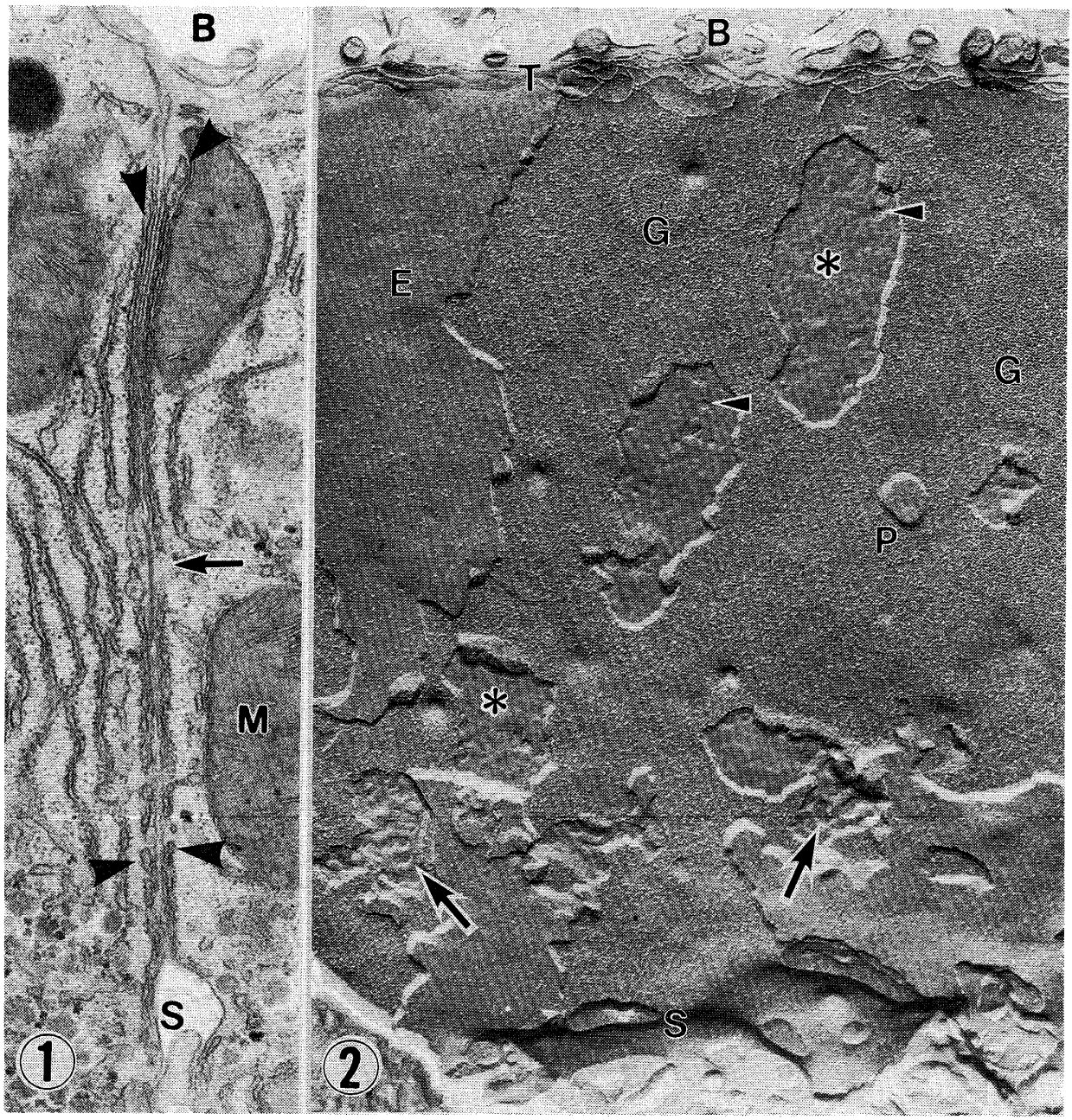

Fig. 1. A thin-section electron micrograph of contiguous hepatocytes. Subsurface cisternae (arrowheads) are seen along the lateral plasma membranes. Note the presence of fenestrae, or rows of tubulovesicles in subsurface cisternae. An arrow points to a gap junction. bile capillary (B), sinusoidal surface (S), mitochondria (M). × 28,000

Fig. 2. Parts of E face of the outer cisternal membrane (asterisks) are seen through the gaping holes in the P face of the lateral plasma membrane (P). Note the presence of some fenestrae (arrowheads). Near the sinusoidal surface (S), anastomosing tubular portions of a subsurface cisterna (arrows) can be seen. bile-canalicular surface (B), tight junction (T), gap junction (G), E face of the plasma membrane in an adjacent hepatocyte (E). × 25,000

the lateral plasma membranes (Fig. 2). The P face of the outer cisternal membranes was seen overlying the E faces of the lateral plasma membranes (Fig. 3). The inner cisternal membranes were often seen accompanying the fractured cytoplasmic faces (Figs. 4, 5) and were continuous in some locations with the endoplasmic reticulum (Fig. 4). Occasionally, mitochondria were closely apposed to subsurface cisternae. While some cisternae were fenestra-rich others were fenestra-poor. In the gap-junction areas the fracture planes often stepped from the plasma membranes to the cisternal membranes. Therefore, fragments of the cisternal membranes in the gap-junction areas appeared as islets on the E face of the lateral plasma membranes and were situated beneath holes in the P face of the lateral plasma membranes. These gap-junction cisternae were fenestra-rich and resembled the sieve plates in sinusoidal endothelial cells (Figs. 3). Sometimes gap-junction areas had dilated fenestrae, or appeared to be composed of anastomosing tubules. The distribution pattern of intramembranous particles on the inner cisternal membranes was similar to that on the outer cisternal membranes and closely resembled that of the endoplasmic reticulum, but the population density of intramembranous particles on the cisternal membranes was lower than on the plasma membrane.

On the basis of these findings, a schematic drawing of the hepatocyte subsurface cisterna is illustrated (Fig. 6).

DISCUSSION

In the present study, both fenestra-rich and fenestra-poor subsurface cisternae were found in murine hepatocytes. The significance of such structural differences remains unclear. However, it may reflect a functional diversity among hepatocytes. Anastomosing tubular profiles were seen in perisinusoidal and pericanalicular areas of subsurface cisternae. Similar regional differences of subsurface cisternae have been shown in cochlear outer hair cells by both thin-section and freeze-fracture studies (13) and in retinal photoreceptor cells by the OsFeCN staining method (16). It is well known that perisinusoidal and pericanalicular areas of the cytoplasm are involved in transporting endocytotic and/or secretory compartments - additives in and/or derivatives from membranous cellular organelles, and thus it is consistent that perisinusoidal and pericanalicular areas of subsurface cisternae consisted of anastomosing tubular structures. Tubuloreticular networks in the perisinusoidal and pericanalicular cytoplasm were joined with subsurface cisternae. Therefore, it is possible that subsurface cisterna serves as s transcellular transport pathway. In a previous thin-section study of subsurface cisternae of rat hepatocytes, a similar suggestion has been proposed (9).

In the present study, anastomosing tubular or dilated fenestra was seen in gap-junction areas of subsurface cisternae. However, such membranous structures underneath gap junctions have not been shown in previous thin-section studies (9, 15). In lutein cells of pregnant rats and mice, an anastomosing smooth endoplasmic reticulum has been seen beneath gap junctions (1). It has been thought that gap junctions allow an exchange of ions and metabolites between closely apposed cells and enable the synchronized motility of cell groups (11). Accordingly, fenestra-rich profiles of gap-junction subsurface cisternae are consistent with this function. Concerning the probable role of this gap-junction cisterna, it has been suggested that this serves as an excitation-contraction coupling site in smooth muscle cell (5). It can be suggested that gap-junction subsurface cisterna in murine hepatocytes may be implicated in functional cell coupling.

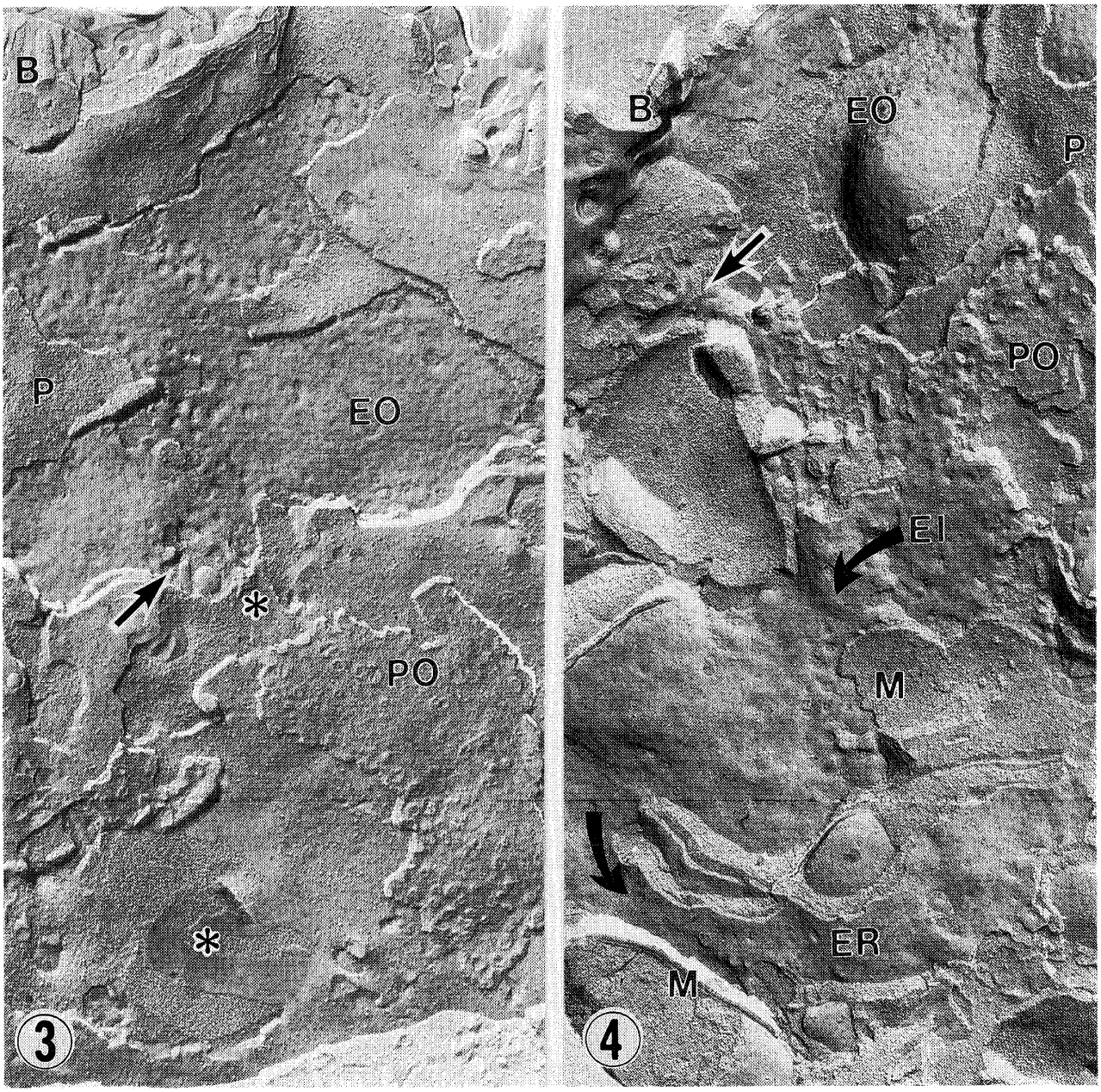

Fig. 3. P face (PO) and E face (EO) of the outer cisternal membranes in two adjoining hepatocytes. An arrow points to dilated fenestrae near a gap junction (asterisks). bile-canalicular surface (B), P face of the plasma membrane (P). × 26,000

Fig. 4. E face of the inner cisternal membrane (EI) is continuous with the endoplasmic reticulum (ER, curved arrows). Note the connection between an apical tubulovesicle network and a subsurface cisterna (straight arrow). bile-canalicular surface (B), mitochondria (M), E face of the outer cisternal membrane in an adjacent hepatocyte (EO), P face of the plasma membrane in an adjacent hepatocyte (EO). × 26,000

In fish retinal photoreceptor cells (double cones), characteristic subsurface cisterna has been shown (4). Functional cell coupling has been indicated between adjoining fish retinal photoreceptor cells provided with subsurface cisternae (7) but has not been detected in amphibian retinal photoreceptor cells lacking subsurface cisternae (2). The calcium-sequestration in subsurface cisternae, a similar function to the sarcoplasmic reticulum in skeletal muscle cells, has been shown in leech retinal photoreceptor cells (16, 17). In mole cochlea outer hair cells, it has been shown that subsurface cisterna is linked to the cytoskeletal components as well as to the plasma membrane (12). The intermittent contraction of has been observed by time-lapse cinematography (10, 14). It is possible that subsurface cistern, gap junctions, and a filament network are instrumental in performing peristaltic movement of the bile canaliculi. However, although this speculation is attractive, it remains to be confirmed experimentally.

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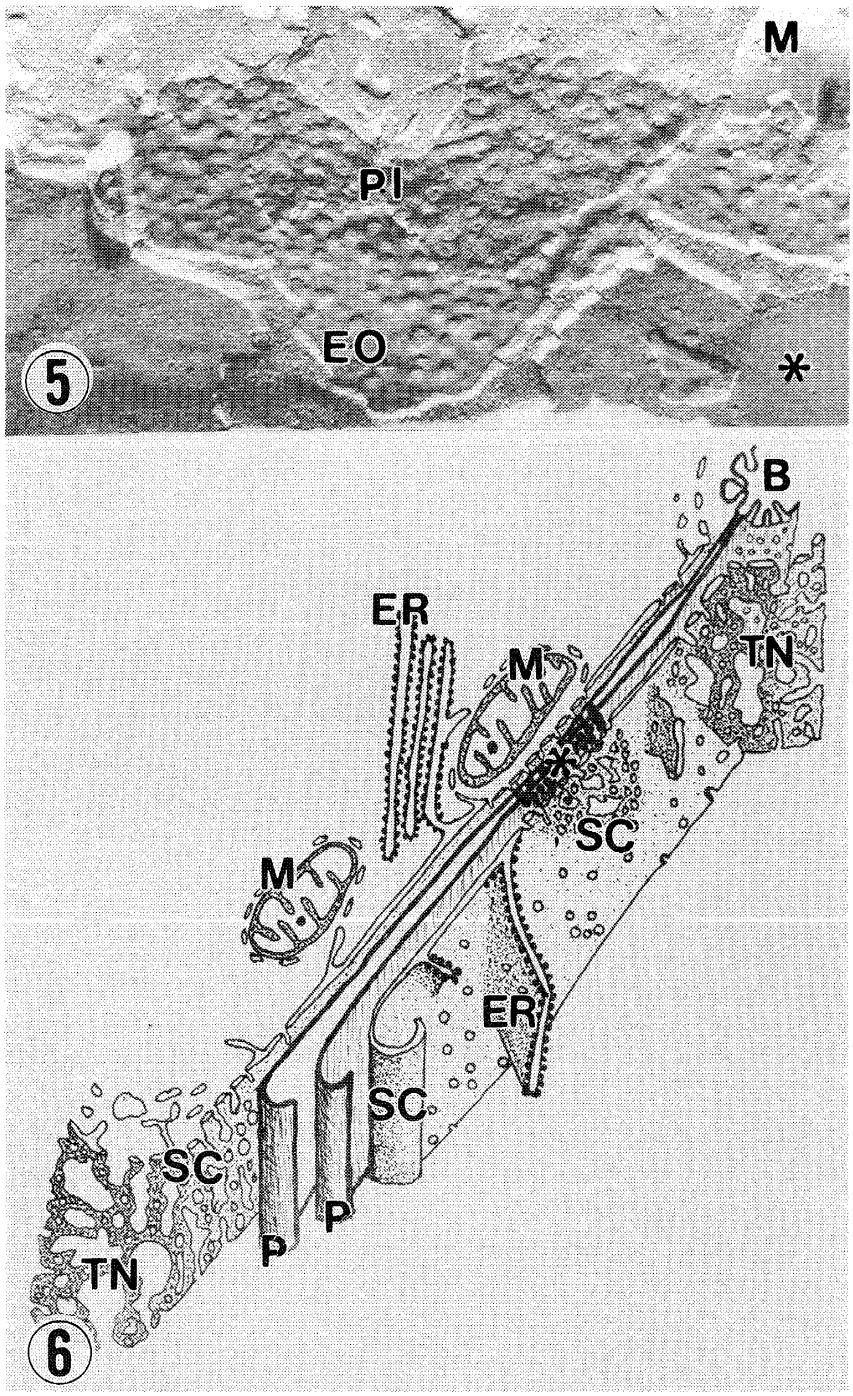

Fig. 5. P face of the inner cisternal membrane (PI) and E face of the outer cisternal membrane (EO) of a fenestra-rich subsurface cisterna. Note the close apposition between a subsurface cisterna and a mitochondrion (M). gap junction (Asterisks), E face of the plasma membrane in an adjacent hepatocyte (E). × 30,000

Fig. 6. A schematic drawing of the membranous structures in the border of contiguous hepatocytes. Subsurface cisternae (SC) lie along the lateral plasma membranes (P) and join tubulovesicle networks (TN) at both sinusoidal and bile-canalicular fronts. Subsurface cisterna is fenestra-rich at the area underneath the gap junction (asterisk). Arrows point to the intercellular space. mitochondria (M), bile capillary (B), endoplasmic reticulum (ER).