The Mechanism of Platelet Release

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The mechanism of platelet release has been studied in mouse bone marrow, using scanning and transmission electron microscopy. Cytoplasmic processes extended into sinusoids by megakaryocytes were found to undergo attenuation, and at the distal end develop constrictions between segments of platelet size. Rupture at sites of constriction is thought to release platelets. Microtubules were present in the processes, orientated longitudinally, and became concentrated in the constrictions. The narrowest constrictions observed were about 0.2 μm in diameter and several microns in length. The structural similarity of the narrowest constrictions with those found in bridges separating midbody and cell in the terminal phase of cytokinesis suggests that platelets and cells share a common mechanism for release. The presence of a centriole in each cytoplasmic process was revealed by serial sectioning. It is suggested that the microtubules in each cytoplasmic process originate from an organizing center associated with the centriole, and that the number of cytoplasmic processes that emerge from a megakaryocyte is governed by its ploidy.

Platelets are formed upon fragmentation of cytoplasmic processes extended into the sinusoids of bone marrow by extravascularly located megakaryocytes. There is little evidence to indicate how release actually occurs. Platelets are thought to be partially delineated within the cytoplasm of megakaryocytes by the demarcation membrane system.

The present study. Three-month-old BALB/c x C57Be-F1 hybrid mice were injected intravenously with 5-fluorouracil (I 50 mg/kg). Administration of 5-fluorouracil to mice results in a marked increase of megakaryocytes in bone marrow after 7 days, with subsequent thrombocytosis. These effects were utilized to facilitate the present study. Three-month-old BALB/c x C57Be-F1 hybrid mice were injected intravenously with 5-fluorouracil (150 mg/kg body weight). Seven days later they were perfused for 10 min with a mixture of 2% paraformaldehyde, 2% glutaraldehyde in 0.08 M cacodylate buffer (pH 7.2). Humeri were removed and placed in fresh fixative. For scanning electron microscopy, bones were split open and exposed to fixative overnight at 4°C. They were then subjected to critical point drying by the routine procedure involving before examination. For transmission electron microscopy, humeri were fixed for 3 hr, decalcified in 12.5% EDTA (pH 7.0), postfixed for 1 hr in 1% osmium tetroxide in buffer, dehydrated, and then included in Spurr resin. Sections were stained with uranyl acetate and lead citrate. Samples from mice perfused at earlier times after 5-fluorouracil, and from untreated mice, were also examined.

RESULTS

Scanning electron microscopy of megakaryocyte cytoplasmic processes protruding into sinusies showed that they undergo attenuation and become constricted at irregular intervals along their lengths into segments of platelet size (Fig. 1). Transmission electron microscopic examination of attenuated processes revealed the presence of organelles normally found in platelets, viz. mitochondria, ribosomes, short lengths of rough endoplasmic reticulum, secretory granules, surface connected canalicular system, and dense tubular system. In addition, microtubules were found orientated in the direction of the long axis of the processes (Figs. 2 and 3). This was a consistent finding, having been observed in every one of several hundred sections through processes. In transverse sections the microtubules were seen to occur either isolated or in small clusters, and were generally located towards the periphery of a process (Fig. 4). Of particular interest was the finding that microtubules converged in a bundle at constriction sites, and in the narrowest (about 0.2 μm diameter) they were the predominant structure in the cytoplasm (Fig. 5). With narrowing, constrictions became longer, forming a bridge up to several microns in length between platelet sized segments of cytoplasm. On either side of constrictions microtubules splayed out towards the periphery of the cytoplasmic process and continued in the longitudinal direction. Individual microtubules up to 2 μm in length in a single section have been observed. Microfilaments

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were also present, particularly in the periphery of processes, and were orientated in the same direction as the microtubules (Figs. 3 and 4).

A search was undertaken to determine the location of centrioles during megakaryocyte development, and to see if they occurred in cytoplasmic processes. In young megakaryocytes they were found adjacent to the nucleus. However, at later stages of maturation when the demarcation membrane system was extensive and secretory granules abundant, centrioles were located singly near the cell periphery (Fig. 6). Serial sectioning was carried out to determine whether or not centrioles were present in cytoplasmic processes; in each of eight processes so far examined a single centriole was found (Fig. 7). Every centriole was located in the body of a process and not in the attenuated region. Microtubules were observed in the vicinity of the centrioles, but there was no evidence to suggest the latter became basal bodies.

Although the above results were obtained using 5-fluorouracil treated mice, similar observations have been made in marrow from untreated animals.

**DISCUSSION**

The present study supports the view that platelets are released from megakaryocyte cytoplasmic processes after the latter have undergone elongation and attenuation. The presence of microtubules and their longitudinal orientation in the attenuated processes suggests they have a role either in the formation or maintenance of shape of such extensions. By contrast, microtubules have a disorderly arrangement in the unattenuated portion of a process. Judging from their number and parallel alignment in the narrow
constrictions that develop along the attenuated processes, microtubules would also appear to have a role during the separation of segments of cytoplasm resulting in their release into the circulation as platelets.

There is much published information relating to microtubule involvement in cell separation, but a detailed description of cytokinesis in D-98S cells by Mullins and Biesele is particularly relevant to the present study. Following development of the cleavage furrow each newly formed cell remains linked by a cytoplasmic bridge to the midbody, the latter apparently acting as an anchor for microtubules that traverse the bridge and penetrate the body of the cell. The microtubules themselves are thought to act in a cytoskeletal capacity, imparting rigidity to the bridge. Progression of cytokinesis is marked by the development of a constriction along part of the bridge. The narrowest constrictions have a diameter of about 0.2 μm and contain a closely packed parallel bundle of microtubules. These authors have predicted that narrowing will be found to be a characteristic feature of the completion of cytokinesis. The present observations show it is also a feature of the separation of putative platelets. The ultrastructural similarities of the narrowest constrictions found during the separation of platelets with those occurring during the terminal phase of cytokinesis suggests that the mechanism of release of platelets and cells are similar. The view that the demarcation membrane extends across a constriction to produce platelet release may thus be too simplistic. Cinemicrographic observations of cytokinesis have shown that the detachment of the cell from the mid-body occurs when stretching of the bridge at its thinnest part causes it to rupture, but in similar studies of megakaryocyte processes the spontaneous release of platelets has not yet been observed.

What determines the number and location of the constrictions that form in the attenuated cytoplasmic processes remains to be elucidated. It is obviously a matter of importance, since it dictates platelet number and size. Nor can it be said how and when the peripheral bundle of microtubules forms in the platelet, but it is probably after its release judging from the teardrop shape of some intrasinusoidal platelets. The finding of centrioles in cytoplasmic processes is of interest since it is well known that microtubule organizing centers are associated with these organelles. We assume that the centrioles are derived by migration from megakaryocytes in the extravascular compartment, rather than arising de novo, in view of their changing location during megakaryocyte development. It has recently been reported that at the conclusion of the endomitotic phase the centriolar complex of the megakaryocyte disappears, and that procentrioles mature and centrioles separate while retaining a juxtanuclear position. We have made similar observations, but in addition have found individual
centrioles deep within the cytoplasm late in the maturation of megakaryocytes. Centrioles were never found in the remnants of cytoplasm retained around senecent megakaryocyte nuclei in the extravascular compartment, although random sections through well over 100 of them have been examined. This latter observation suggests that each centriole must eventually be incorporated into one of the numerous platelets formed from the cytoplasmic process to which it migrates, and could account for the unusual sighting of a centriole in a platelet.17

It seems reasonable to assume that the centrioles within the cytoplasmic processes have a functional role as they are known to migrate towards the periphery of some types of cell to become basal bodies for the outgrowth of cilia.1819 This occurs when cells are out of cycle, a state that the postendomitotic megakaryocyte is permanently in. However, megakaryocyte centrioles, which are single when they migrate, do not become basal bodies, and if they are acting as foci for microtubules, it is likely that nucleation occurs in pericentriolar material. Microtubules were seen in the vicinity of centrioles but their continuity with those occurring in the more distal regions of processes remains to be established. Should the microtubules in cytoplasmic processes be found to originate at centriolar sites, it would imply a relationship between the number of processes formed by a megakaryocyte and its ploidy, since ploidy determines centriole number. However, since we cannot preclude that more than one centriole may be present in some processes the actual figure may in fact be lower. No functional significance has hitherto been ascribed to centrioles in platelet separation, even though eight or more may be present in the parent megakaryocyte.16

REFERENCES

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