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MEGAKARYOCYTE MOTILITY AND PLATELET FORMATION*

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Abstract

The mechanism of platelet formation is reviewed with special emphasis on the role of the cytoskeleton. The three major theories for platelet formation are by cytoplasmic budding, cytoplasmic dissolution or pseudopod formation. Most evidence indicates that platelets form as fragments of megakaryocyte pseudopodia. Pseudopodia formation is stimulated in vitro by thrombocytopenic rabbit plasma. It is inhibited by vincristine and altered by taxol. Cytochalasins cause pseudopodia to form in isolated megakaryocytes. Therefore, normal pseudopodia formation may depend on a combination of microfilament disorganization and microtubule elongation.

KEY WORDS: Megakaryocytes, morphology, cytoskeleton, platelet formation

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space (5) further supports this idea. The similarity of the DMS and plasma membrane was first implied by the observation that virus particles in megakaryocytes bud only from the plasma membrane and DMS (17) but not other membranes. More recent studies using freeze fracture and immunological techniques (8,41,74,75,97) have provided conflicting evidence on the similarity of the DMS and megakaryocyte plasma membrane. Tavassoli (78) and Bentfeld-Barker and Bainton (8) did not observe any difference in the intramembrane particle distribution in platelet and megakaryocyte plasma membranes. Zucker-Franklin, however, found that the distribution of intramembranous particles is similar in the DMS and the platelet plasma membrane, but different from the distribution of these particles in the megakaryocyte plasma membrane (97). In addition it was found that certain antiplatelet antisera do not react with megakaryocytes (75), implying a biochemical difference between the platelet and megakaryocyte plasma membrane. Based on these studies two conclusions are possible. If the DMS originates from the megakaryocyte plasma membrane, structural and biochemical changes occur which distinguish these two membranes. The second possible conclusion is that the megakaryocyte demarcation membrane does not arise from the plasma membrane but becomes continuous with it during maturation. The precise relationship between the DMS and the platelet plasma membrane remains to be fully understood as well as the role of the DMS in delineating future platelets. Although it has generally been assumed that the DMS delineates individual platelets this function of the DMS has not been universally accepted. Murata (59) has proposed that platelets form by blebs or pseudopodia which are delineated by the megakaryocyte plasma membrane and the DMS remains within the platelet as the surface connected canalicular system. This is not supported by Zucker-Franklin's finding as discussed above. It has also been postulated that the DMS may provide a source of stored membrane used to form long pseudopodia (67,68).

Mechanisms of Platelet Formation

Fragmentation of megakaryocytes into platelets has been proposed to occur by three mechanisms, pseudopod formation, cytoplasmic dissolution, and budding. The formation of pseudopodia, first described by Wright has been observed by others (1,6,10,18,23,26,27,42,59,64,74). It was noted that small pseudopodia, or blebs, extend through the endothelium as an extension of the peripheral cytoplasmic zone and contain no organelles (64,78). Consequently, their role in platelet formation was unclear. Micromotograph images of bone marrow in situ could not confirm a role for pseudopodia formation in the fragmentation of megakaryocytes (43). It was observed that intact megakaryocytes traversed the endothelium, but no pseudopodia were seen. Large pseudopodia extending through the endothelium have also been described (26,27,64). In these studies it was noted that pseudopodia containing a core region with a full complement of cytoplasmic organelles could give rise to platelets upon release from the main cell mass. Similar observations (6,42,59,65) have confirmed the existence of pseudopodia containing platelet organelles. Several scanning electron microscope studies have shown long cytoplasmic processes with regularly spaced constrictions extending from extravascular megakaryocytes into venous sinoids (4,29,32,67). Since megakaryocytes can migrate intact across the vessel wall (43,79,96), it is possible that pseudopodia formation represents the initial movement of megakaryocytes into venous sinoids rather than an intermediate step in platelet formation.

The formation of pseudopodia by megakaryocytes can also be observed in vitro. Several studies (1,30,37,51,67,80) have shown that outside the confines of the bone marrow, megakaryocytes are still capable of forming pseudopodia. Megakaryocytes in bone marrow suspension were observed to spontaneously form multiple pseudopodia which became beaded in appearance, but fragmentation of the pseudopodia into smaller fragments was not observed in any of these studies. We have recently found that both guinea pig and rat megakaryocytes isolated from bone marrow are induced to form pseudopodia in vitro by the plasma from thrombocytopenic rabbits (50); a source of thrombopoietin activity (22) (Fig. 1a). Unlike previous studies, it was common to find small fragments of detached cytoplasm similar in appearance to proplatelets (4) or stress platelets of thrombocytopenic animals (82) (Fig. 1b.c). In addition, the fragments contained microtubule rings (50), providing evidence that the cytoplasm in the fragments is organized as in normal platelets.

Megakaryocytes in vitro have also been observed to undergo a dissolution into small fragments without pseudopodia formation (1,66,97), and it has been postulated that this may occur only in 8N megakaryocytes (65). If this occurs in vivo it implies either large or small fragments of megakaryocyte cytoplasm must migrate independently across the endothelium into the circulation. There is evidence that platelets have migratory capability (54,85), therefore it is possible that megakaryocyte fragments are also capable of migration, although direct evidence is lacking.

Another mechanism that has been postulated is the budding of platelets from the surface of megakaryocytes. Several investigators (3,13,20,36,59,84) have observed blebs on the surface of megakaryocytes by scanning electron-microscopy. Yet, accompanying transmission electron micrographs have failed to show platelet organelles within the blebs (20,59). Therefore, there is no compelling evidence to indicate platelets form in this manner. It may be that blebs are transient structures which form as the surface of the megakaryocyte alters, but do not separate from the megakaryocyte as platelets.

One area which should be mentioned but cannot be covered in depth in this review is the role of pulmonary megakaryocytes in platelet formation. Megakaryocytes were first observed in the pulmonary circulation by Aschoff (2). Howell and Donohue (34) first postulated that platelet formation may occur in the lungs by megakaryocytes which become trapped in the pulmonary capillaries. Since this time, studies showing a lower platelet count in pulmonary arterial blood than pulmonary venous blood have suggested that platelet formation may occur in the lung (16,39,81,83). Work from Barnhart's laboratory has shown pulmonary megakaryocytes which appeared to be forming platelets, based on the appearance of surface blebs and delineation of platelet areas by the DMS. Yet no actual fragmentation was observed and the precise mechanism of cytoplasmic fragmentation could not be determined (3,88,89,90). It seems likely that megakaryocytes normally occur in the pulmonary capillary bed, but the extent to which these megakaryocytes contribute to the maintenance of the circulating platelet population remains controversial.

Megakaryocyte Cytoskeleton

The cytoskeleton refers to structures based on the three cellular filamentous systems: microtubules, microfilaments and intermediate filaments. The distribution of microtubules in megakaryocytes has been described as random throughout the cytoplasm (6,10,25,47,72) (Fig. 2). Microtubules have also been seen forming aligned bundles in pseudopodia of megakaryocytes both in vivo (6,10) and in vitro (50,67). Studies using vincristine show that this drug causes pseudopodia to retract, indicating that they are supported by microtubules (68) (Fig. 3). The role of microtubules in supporting pseudopodia can also be demonstrated using the drug taxol. We have found that taxol, as in other cells, causes bundling of microtubules into large aggregates (Fig. 4). Under conditions for pseudopodia formation in vitro, taxol causes megakaryocytes to form a small number of very thick pseudopodia rather than several fine pseudopodia (Fig. 5). Consequently, the organization of microtubules is a determinant of pseudopodia formation. Immunofluorescence studies have confirmed that most microtubules are randomly oriented (47), however 17% of

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Figure 1. Rat megakaryocyte fragmentation in vitro. Isolated rat megakaryocytes incubated for 18 h with 5 mg/ml of the 60%-80% ammonium sulfate fraction of thrombocytopenic rabbit plasma.

a) Megakaryocyte in which the cytoplasm has almost entirely separated into long proplatelets or pseudopodia. Beading along the pseudopodia is evident.

b) Proplatelet fragments separating from the nucleus (large arrow). Many proplatelets have begun breaking into small platelet like fragments (small arrows).

c) Proplatelet fragments floating in the culture medium which are no longer associated with any cell nucleus. a, b and c, Bar = 100µm.

Figure 2. Megakaryocyte microtubules. Section of a guinea pig megakaryocyte in vitro showing multiple microtubules (arrows), in an apparently random orientation. Bar = 250 nm.

Recent studies have observed megakaryocytes contained microtubule rings in their cytoplasm. These rings are the same dimension as the platelet marginal microtubule coil (Fig. 6). Microtubule rings are also seen in megakaryocyte pseudopodia and proplatelets in vitro (50). Recently it has been demonstrated that the 210 K dalton microtubule associated protein (210 K MAP) is present in human platelets (69). Consequently, it is probable this protein is also in megakaryocytes. It has been postulated that this protein may link the microtubule coil to other elements of the cytoskeleton. The 210 K MAP may also play a role in the formation of the microtubule coil in megakaryocytes.

The presence of actomyosin in megakaryocytes was established using immunofluorescence microscopy (60) and shortly thereafter, microfilaments were observed in both the peripheral and intermediate zone of megakaryocytes (7, 10). Heavy meromyosin labeling in glycerinated cells confirmed the actin composition of the filaments (7). By use of immunofluorescent staining,

Figure 3. Vincristine inhibition of megakaryocyte fragmentation. A megakaryocyte which had begun to form several pseudopodia, as in Fig. 1a, was incubated with 10µm vincristine for 1 h. The pseudopodia have rounded up and retracted. Bar = 20µm.
Figure 4. Microtubule bundles induced by taxol. Megakaryocytes were treated with 10 µm adenosine diphosphate (ADP) to induce spreading; which allows better visualization of cytoskeletal structures. After 30 minutes exposure to ADP, 10 µm taxol was added for an additional 24 h prior to staining with anti-tubulin antibody. Large bundles of microtubules are seen. Bar = 20 µm.

Figure 5. Effect of taxol on megakaryocyte fragmentation. Isolated rat megakaryocytes in vitro, incubated with 5 mg/ml of the 60%–80% ammonium sulfate fraction of thrombocytopenic rabbit plasma and 10 µm taxol. Instead of numerous fine pseudopodia, a few very large pseudopodia form. Bar = 100 µm.

Figure 6. Microtubule rings in megakaryocytes. Isolated megakaryocyte, spread in response to 10 µm ADP followed by staining with anti-tubulin antibody. Several microtubule rings can be seen (arrows). These rings may represent newly forming platelet microtubule coils. Bar = 20 µm.

Figure 7. Intermediate filaments in megakaryocytes. Section of a megakaryocyte spread in culture after exposure to 10 µm ADP. An area with many intermediate filaments is shown. Bar = 250 nm.

Figure 8. Spread megakaryocyte fragments. Thin section of fragments from a megakaryocyte induced to spread with 10 µm ADP. The fragments are lacking granules, mitochondria and microtubules, and contain only peripheral zone cytoplasm. Bar = 1.0 µm.
myosin, filamin and alpha-actinin have also been demonstrated to be components of the megakaryocyte cytoskeleton (47). These proteins are arranged in a discontinuous fashion along actin based filaments, similar to stress fiber structure (46). Several actin associated regulatory proteins have been characterized in blood platelets (44,45,56,70,87). Two of these proteins, actin binding protein and alpha-actinin, are known to be in megakaryocytes (47), and the others most likely are as well. These proteins control the organization of actin in other cells but their function in megakaryocyte motility is not known. A possible role for these proteins in platelet formation is discussed below.

Intermediate filaments have recently been observed in blood platelets (77) and we have now observed intermediate filaments in megakaryocytes (Fig. 7). The function of intermediate filaments is still unresolved and their role in megakaryocytes and platelets is unclear.

**Megakaryocyte Motility**

Studies on pseudopod formation, as previously discussed clearly demonstrate the motile nature of megakaryocytes. Megakaryocytes in culture show a motile response to platelet activators by ruffling and spreading (24,38,47). Megakaryocyte spreading is associated with a change in microfilament organization and adherence of the cell. Platelet activators also alter the electrophysiological properties of the megakaryocyte membrane (48).

In addition to platelet activators, megakaryocyte morphology is altered by extracellular matrix components. Isolated megakaryocytes cultured on collagen and fibronectin coated dishes (47) or on endothelial cell extracellular matrix (52) spread the same as cells exposed to platelet activators. Megakaryocytes in vivo are in close contact with the endothelium (53,57,91). The close contact may be maintained by the strong affinity of megakaryocytes for extracellular matrix components as observed in vitro. Under certain conditions megakaryocytes which spread on extracellular matrix coated dishes break into platelet sized fragments (52). Our own studies of spread cells show that they occasionally fragment, but the fragments are devoid of organelles (Fig. 8). Therefore it is not clear to what extent this mode of fragmentation represents platelet formation.

**Regulation of Megakaryocyte Fragmentation**

and Model of Platelet Formation

There is limited evidence that the final stage of megakaryocyte maturation, the formation of platelets, is hormonally controlled. Odell et al. (61) showed that platelet size increased in thrombocytopenic rats within 18 h after the injection of antiplatelet antisera. This has recently been confirmed by Corash et al. (15). Since the time of transit through the final stage of megakaryocyte maturation is 24-42 h (21) these results can only be explained by an alteration of the most mature megakaryocytes in the thrombocytopenic state. This may be through the increase of circulating thrombopoietin activity, which occurs in thrombocytopenia (22). Also, Stranava et al. (76) have provided evidence that serum from aplastic patients may increase the rate of cytoplasmic maturation of isolated human megakaryocytes. Finally, our study of guinea pig megakaryocytes shows that thrombopoietin enriched rabbit plasma stimulates the fragmentation of megakaryocytes in vitro (49,50). Consequently, it appears that the morphological alterations which occur during platelet formation can be induced by a circulating agent which increases concentration during thrombocytopenia. The mechanism by which this agent triggers the intracellular events leading to fragmentation is a problem which requires further research.

Figure 9 summarizes our model of the role of cytoskeletal elements in pseudopod formation and platelet release from megakaryocytes. We propose that the initial stimulus to pseudopod formation is the binding of a circulating agent, which is increased during thrombocytopenia, to mature megakaryocytes. This may be facilitated by the extension of small cytoplasmic blebs across the endothelium where the megakaryocyte could directly detect a change in circulating thrombopoietin, as postulated by Tavassoli (78). The initial stage in pseudopod formation we propose to be the disassembly of actin filaments or filament networks in the peripheral zone nearest the endothelium causing the cytoplasm of this zone to become more liquid (solution), as described by Condeelis and Taylor (14). This could occur by a change in binding of actin binding protein, alpha-actinin or other actin associated proteins which influence the state of actin. We base our assumption of a change in the state of actin on our recent study with cytochalasin (50). We found that incubation of megakaryocytes in vitro with cytochalasin B or D caused pseudopod formation. Therefore, actin filament disassembly, the primary action of cytochalasin, is sufficient to initiate pseudopod formation. The weakened cytoplasm of the peripheral zone may actually break and fragment as appears to occur in the study by Zucker-Franklin (97). Concurrently, cytoplasmic microtubules form the core of pseudopodia which push through the weakened peripheral zone into the venous sinusoid either by reorganization or polymerization of the microtubules. The direction of pseudopod formation and the number of pseudopodia could be controlled by initial restriction of peripheral zone solution to small areas near the endothelium. Fusion of the DMS causes separation of the cytoplasm into long proplatelet structures which continue as part of the pseudopodia into the circulation. An alternate mechanism for the determination of pseudopod number has been proposed by Radley (67). Based on the observation of multiple centrioles in megakaryocytes (9,58) it was proposed that centrioles...
direct the location and direction of pseudopod growth. Since
cellular number may be related to ploidy, it is a consequence of the
model that the number of pseudopodia would be affected by the
ploidy.

The budding of proplatelets (4), we believe, occurs as a result of
microtubule coil formation, a process which may begin prior to
pseudopod formation (Fig. 6). Microtubule coil formation may
therefore be a critical determinant of platelet size. The constric-
tion of cytoplasm between nascent platelets cannot occur by an
actomyosin contraction since fragmentation occurs in the presence
of cytochalasins. Although highly speculative, we propose that
interaction of the forming microtubule coil and the plasma mem-
brane possibly through other elements of the cytoskeleton may
draw the membrane into a beaded configuration. How the final
fragmentation between platelets occurs remains unknown.

This model attempts to explain one mechanism by which
platelets arise from megakaryocytes. Though incomplete, we
believe this model to be useful as a basis to direct future research.
We cannot exclude the possibility that platelets also form by a
rapid dissolution of the entire megakaryocyte cytoplasm, or by the
shedding of cytoplasmic blebs.

Though we are beginning to understand the role of the
cytoskeleton in pseudopod formation, we expect further studies on
the distribution of microfilament and microtubule associated pro-
teins will help explain how cytoskeletal rearrangements occur in
megakaryocytes. Of particular interest is how the microtubule coil
forms and what relationship may exist between microtubules and
the cell membrane. The recent development of an
in vitro system to study fragmentation of isolated megakaryocytes (50) will allow
for continued unraveling of the complex process of platelet forma-
tion.

References

1. Albrect M (1957) Studien zur Thrombocytobildung an
17, 160–168.

2. Aschoff L (1983) Veber capillare embolie von resekn-

3. Barnhart MI, Noonan, SM (1978) Fine structure and sur-
face features of platelets and megakaryocytes. Suppl. Thromb.
Haemost. 63, 3–36.

4. Becker RP, De Bruyn PPH (1976) The transmural pas-
sage of blood cells into myeloid sinusoids and the entry of plate-
telets into the sinusoidal circulation, a scanning electron micro-

5. Behnke O (1968) An electron microscope study of the
megakaryocyte of the rat bone marrow. I. The development of the
demarcation membrane system and the platelet surface coat.

megakaryocyte. II. Some aspects of platelet release and microtu-

7. Behnke O, Emmerson J (1972) Structural identification of
thrombosthenin in rat megakaryocytes. Scand. J. Haemat. 9,
130–137.

8. Bentfeld-Barker ME, Bainton DF (1977) Ultrastructure of
rat megakaryocytes after prolonged thrombocytopenia. J. Ultrastruc.
Res. 61, 201–214.

structure of megakaryocytes. Anat. Rec. 149, 361 (abstr.)

10. Breton-Gorius J (1973) Aspects ultrastructuraux de la
Fr. D’Hemat. 13, 504–514.

J. Exp. Med. 18, 278–286.

12. Bunting CH (1909) Blood-platelet and megakaryocyte
reactions in the rabbit. J. Exp. Med. 11, 541–552.

13. Chen S, Barnhart MI (1984) Scanning electron micro-

14. Condeels JS, Taylor DL (1977) The contractile basis of
ameboid movement. V. The control of gelation, solation, and
contraction in extracts from Dicystomulum discoidcum. J. Cell
Biol. 74, 901–927.

Serial studies of megakaryocyte DNA content and platelet volume
in response to variable degrees of thrombocytopenia. Blood
65, 157a. (abstr.)


An electron microscopic study of a series of murine lymphoid

18. De Bruyn PPH (1964) The fine structure of the
megakaryocyte of the bone marrow of the guinea pig. Zeit. fur
Zellforsch. 64, 111–118.

19. De Leval M (1967) Mise en evidence d’antigene pla-
quette dans le cytoplasme des megakaryocytes par la technique
des anticorps fluorescents. Application a l’étude de la maturat-
ion des megacaryocytes dan la moelle osseuse de cobaye. CR Soc.

observations on the mechanism of platelet release from


activity of fractions of rabbit plasma: studies in rabbits and mice.

sur la liberation des plaquettes par les megakaryocytes humains.
Blut 16, 57–64.

pig megakaryocytes. I. Uptake of H-serotonin by megakaryocytes
and their physiologic and morphologic response to stimuli for the

25. Fedorko ME (1978) Morphologic and functional obser-
vations on bone marrow megakaryocytes. In: The Year in Hema-
tology. Gordon AS, Silber R, LoBue J (eds.) Plenum, NY,
171–209.


27. Gautier A, Jean G, Probst M, Falcao L (1963) Ultra-
structure du megacaryocyte et problemes de plaquetogenese.

28. Geyer G, Schaaf P (1972) The megakaryocyte demarca-
tion membrane (DM)-endoplasmic or superficial origin? Acta.
Histochern. 44, 137–143.

29. Haller CJ, Radley JM (1983) Time-lapse cinematogra-
phy and scanning electron microscopy of platelet formation by

30. Ham AW Cormack DH (1979) Histology. J.B. Lippin-

31. Han SS, Baker BL (1964) The ultrastructure of
149, 251–268.

Scanning electron microscopic studies of megakaryocytes and
platelet formation in the dog and rat. Am. J. Vet. Res. 47,
2454–2460.

33. Heidenhan M (1894) Neve Ontersuchungen Uber die
zentralkoLrper und ihre Beziehungen zum kern-unl Zellerproto-

34. Heidenhan M (1954) Mise en evidence d’antigene pla-
quette dans le cytoplasme des megacaryocytes par la technique
des anticorps fluorescents. Application a l’étude de la maturat-
ion des megacaryocytes dan la moelle osseuse de cobaye. CR Soc.

An electron microscopic study of a series of murine lymphoid

36. De Bruyn PPH (1964) The fine structure of the
megakaryocyte of the bone marrow of the guinea pig. Zeit. fur
Zellforsch. 64, 111–118.

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the blebs may be a means by which the megakaryocytes probe the components of the basement membrane, collagen, fibronectin. Studies have shown (47, 52) that megakaryocytes are very adherent to the sinus wall in the bone marrow.

Author: I agree that the surface blebs may assist in attachment of megakaryocytes in the marrow, could these blebs serve as an "anchoring" mechanism whereby the cell is fixed to the endothelial wall? The increasing of calcium dependent platelet proteins that interact with actin. Cell 25, 637–649.


**Discussion with Reviewers**

M. Tavassoli: The blebs that are seen in SEM and are devoid of platelet organelles in TEM have been the subject of speculation for many years. In view of the perinodal position of megakaryocytes in the marrow, could these blebs serve as an "anchoring" mechanism whereby the cell is fixed to the endothelial wall? The fact that the cell is otherwise motile makes this possibility even more plausible.

Author: I agree that the surface blebs may assist in attachment of megakaryocytes to the sinus wall in the bone marrow. In vitro studies have shown (47, 52) that megakaryocytes are very adherent to components of the basement membrane, collagen, fibronectin and laminin. Unfortunately we still know very little about the in vivo activities of megakaryocytes. I would like to speculate that the blebs may be a means by which the megakaryocytes probe the sinus wall for sites which will best allow the passage of the megakaryocyte cytoplasmic extension across the endothelium into the circulation.

R.P. Becker: Neutrophils apparently can also undergo a segmentation of cytoplasm to form viable packets called "pseudoplatelets" Harker and Giamarra (100). Have you observed this phenomenon for neutrophils in your cultures?

Author: No I have not observed neutrophils responding in this manner in our cultures, but there are few granulocytes in the enriched megakaryocyte preparation.

M.E. Fedorko: Are you the sure the tissue culture conditions do not contribute to what you see in the in vitro system, such as the presence of silicon or glass, inhibitors or serum?

Author: I do not believe these factors are involved. We see the same morphological response on glass or plastic surfaces and at varying concentrations of inhibitors. Also, there are no siliconized surfaces in the cultures.

M.E. Fedorko: How are your in vitro preparations obtained and prepared?

Author: Guinea pig and rat megakaryocytes are obtained by slight modification (47) of the technique of Levine and Fedorko (101). The cells are maintained in liquid culture using Dubbecos modified Eagle medium and 10% fetal calf serum. Thrombocytopenic plasma or control plasma fractions are added at 1–10 mg/ml and cultures are incubated up to 45 hours. These conditions are described in detail in Leven and Yoc (50).

M.E. Fedorko: How do you explain the in vivo effect of vincristine which is used clinically to increase platelet levels?

Author: The increase in platelet count seen in vivo after vincristine injection is not inconsistent with our model. In response to vincristine it has been shown by Ebbe et al. (99) and by Choi et al. (98) that there is an initial decrease in both platelets and megakaryocytes. This is entirely consistent with our interpretation of platelet formation. Thrombocytosis occurs only several days later, apparently as an overcompensating response to the initial depletion of the megakaryocyte and platelet population.

D.B. Warheit: What are the major difficulties and drawbacks associated with describing mechanisms of platelet formation in vivo based upon studies carried out under culture conditions?

Author: The obvious problem is that one cannot reconstruct in vitro the complex in vivo environment of the bone marrow, though to do so would not necessarily be desirable. By analyzing a simple in vitro system it should be possible to determine the relative importance of different components of the marrow environment. These components include circulating factors, such as thrombopoietin, hematopoietic cells, stromal cells, endothelial cells and the endothelial basement membrane. The relationship to the basement membrane is of special interest due to the proximity of megakaryocytes to the marrow endothelium. As described in this paper megakaryocytes in vitro adhere to and spread on basement membrane components. It would be interesting to know if an in vivo counterpart to this activity exists. I believe that in vitro studies can best be used as a guide for what may occur in vivo, and to then test the in vitro models as best as possible in the intact animal.

D.B. Warheit: What experiments could be carried out to determine whether the demarcation membrane system is derived from the megakaryocyte plasma membrane?

Author: This is a particularly interesting and particularly difficult problem. At present I do not know of any experiment that would definitively answer this question. One approach would depend on
immunologic detection of distinct demarcation membrane antigens. One could then label the demarcation membranes and using tannic acid or ruthenium red, attempt to see if the demarcation membrane is or is not always continuous with the extracellular space.

D.B. Warheit: How convincing is the data that platelet formation occurs as a result of cytoplasmic dissolution? Based on the current data, what are the strengths and weaknesses of this proposed mechanism?

Author: The evidence that platelets could form in the marrow by rapid dissolution of the megakaryocyte into platelet sized fragments is limited. It is difficult to explain how these small fragments could cross the endothelium into the circulation. As far as I know, no ultrastructural evidence exists to show such a process occurs. In contrast, if platelet formation occurs in pulmonary capillaries it seems reasonable that a rapid dissolution of the megakaryocyte into platelets could occur since migration across the endothelium would not be necessary.

Additional References