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REVIEW

Platelet α-granules: Basic biology and clinical correlates

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SUMMARY

α-Granules are essential to normal platelet activity. These unusual secretory granules derive their cargo from both regulated secretory and endocytotic pathways in megakaryocytes. Rare, inheritable defects of α-granule formation in mice and man have enabled identification of proteins that mediate cargo trafficking and α-granule formation. In platelets, α-granules fuse with the plasma membrane upon activation, releasing their cargo and increasing platelet surface area. The mechanisms that control α-granule membrane fusion have begun to be elucidated at the molecular level. SNAREs and SNARE accessory proteins that control α-granule secretion have been identified. Proteomic studies demonstrate that hundreds of bioactive proteins are released from α-granules. This breadth of proteins implies a versatile functionality. While initially known primarily for their participation in thrombosis and hemostasis, the role of α-granules in inflammation, atherosclerosis, antimicrobial host defense, wound healing, angiogenesis, and malignancy has become increasingly appreciated as the function of platelets in the pathophysiology of these processes has been defined. This review will consider the formation, release, and physiologic roles of α-granules with special emphasis on work performed over the last decade.

Overview of platelet α-granules

Platelets are anucleate, discoid shaped blood cells that serve a critical function in hemostasis and other aspects of host defense. These cells are replete with secretory granules, which are critical to normal platelet function. Among the three types of platelet secretory granules – α-granules, dense granules, and lysosomes – the α-granule is the most abundant. There are approximately 50–80 α-granules per platelet, ranging in size from 200 to 500 nm.[1] They comprise roughly 10% of the platelet volume, 10-fold more than dense granules. The total α-granule membrane surface area per platelet is 14 μm^2, ~8-fold more than dense granules and approximately equal to that of the open canalicular system (OCS),[1] an elaborate system of tunneling invaginations of the cell membrane unique to the platelet.[2] The extra membrane provided by the OCS and α-granules enables the platelet to increase its surface area by 2–4-fold upon platelet stimulation and/or spreading.

Morphologic features observed by electron microscopy have historically defined α-granules. They include (1) the peripheral membrane of the granule, (2) an electron dense nucleoid that contains chemokines and proteoglycan, (3) a less electron dense area adjacent to the nucleoid that contains fibrinogen, and (4) a peripheral electronluscent zone that contains von Willebrand factor (vWf).[3] Not all zones, however, need to be observed in order to positively identify an α-granule. α-Granules have also been identified based on immunofluorescence studies. Staining of granule constituents such as P-selectin, vWf, and/or fibrinogen or other established markers identifies α-granules by this technique. However, α-granules appear to be heterogeneous with regard to cargo.[4,5] Absence of a particular α-granule marker does not preclude classification of a vesicular structure as an α-granule. Thus, the definition of α-granules may yet undergo further refinement as we learn more about their formation, structure, and content.

Formation of α-granules

Vesicle trafficking

The development of α-granules begins in the megakaryocyte, but continues in the circulating platelet. In the megakaryocyte, α-granules are derived in part from budding of small vesicles containing α-granule cargo from the trans-Golgi network (Fig. 1).[6,7] In other cell models, an orchestrated assemblage of coat proteins (e.g., clathrin, COPII), adaptor proteins (e.g., AP-1, AP-2, AP-3), fusion machinery (e.g., soluble NSF attachment protein receptors [SNAREs]), and monomeric GTPases (e.g., Rabs) mediate vesicle trafficking and maturation. Clathrin coat assembly likely functions, too, in trafficking of vesicles from the trans-Golgi network to α-granules in megakaryocytes. The clathrin-associated adaptor proteins AP-1, AP-2, and AP-3 are found in platelets[8,9] and are proposed to function in clathrin-mediated vesicle formation in

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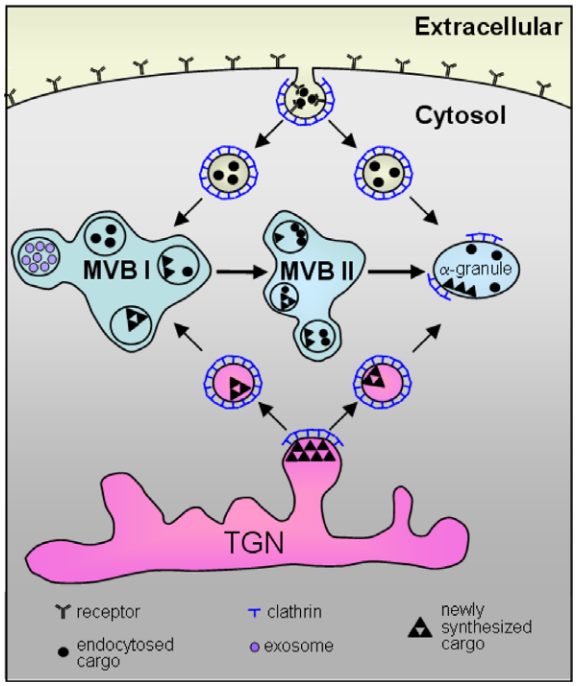

Fig. 1. Working model of α-granule formation in megakaryocytes. α-Granule cargo derives from budding of the trans-Golgi network (TGN) and endocytosis of the plasma membrane. Both processes are clathrin-mediated. Receptor-mediated endocytosis is depicted in this figure; however, pinocytosis of α-granule cargo can also occur. Vesicles can subsequently be delivered to multivesicular bodies (MVBs), where sorting of vesicles occurs. It is possible that vesicles may also be delivered directly to α-granules. Some vesicles within MVBs contain exosomes. MVBs can mature to produce α-granules.

platelets.[10] Mutations in the gene encoding AP-3, for example, results in impaired dense granule formation.[9] Clathrin-mediated endocytosis also functions in the delivery of plasma membrane into α-granules (Fig. 1). Vesicles budding off from either the trans-Golgi network or the plasma membrane can subsequently be directed to multivesicular bodies (MVBs).

MVBs found in most cells are endosomal structures containing vesicles that form from the limiting membrane of the endosome.[11,12] They are typically transient structures involved in sorting vesicles containing endocytosed and newly synthesized proteins. In megakaryocytes, MVBs serve in an intermediate stage of granule production.[13] Both dense granules and α-granules are sorted by MVBs.[13,14] Vesicles budding from the trans-Golgi network may be delivered directly to MVBs (Fig. 1).[13] Kinetic studies in megakaryocytes have demonstrated that transport of endocytosed proteins proceeds from endosomes to immature MVBs (MVB I, with internal vesicles alone) to mature MVBs (MVB II, with internal vesicles and an electron dense matrix) to α-granules. α-Granules within MVBs contain 30–70 nm vesicles, termed exosomes.[13] Some exosomes persist in mature α-granules and are secreted following platelet activation.[15] Although it is unknown whether all or most vesicle trafficking to α-granules proceeds through MVB, these observations indicate that MVB represent a developmental stage in α-granule maturation.

Maturation of α-granules continues in circulating platelets by endocytosis of platelet plasma membranes.[16–18] A clathrin-dependent pathway leading to the delivery of plasma membrane to α-granules has been described, as has a clathrin-independent pathway that traffics vesicles to lysosomes.[18] Unlike other cells, coated vesicles in platelets retain their clathrin coat throughout trafficking and for a period following fusion with α-granules.[17] Platelet endocytosis appears to be a constitutive activity of resting platelets. The molecular control of endocytosis in platelets is not known, but may involve the Src family receptors Fyn, Fgr, Lck, and/or Lyn based on colocalization studies, their tyrosine-phosphorylation status,[19,20] and evidence of a role for Src family receptors in lymphocyte endocytosis.[21] Studies performed in dogs evaluating the accumulation of fibrinogen and immunoglobulin, which are endocytosed by circulating platelets, show that levels of endocytosed, but not endogenous, α-granule proteins increase as platelets age.[22] This observation confirms that constitutive trafficking to α-granules continues throughout the lifespan of the platelet.

Protein sorting

Many α-granule proteins are produced by megakaryocytes and sorted to α-granules via a regulated secretory pathway. These proteins are synthesized in the endoplasmic reticulum, exported to the Golgi for maturation, and subsequently sorted at the trans-Golgi network.[23] Trafficking of some well-known α-granule proteins synthesized in megakaryocytes, such as P-selectin, has been evaluated. Initial studies in heterologous cells indicated that the sorting sequence for P-selectin is contained within its cytoplasmic tail.[24–27] Subsequent studies, however, indicated that the cytoplasmic tail of P-selectin targets this adhesion molecule to storage granules in endothelial cells, but not in platelets.[25,28] This observation demonstrates that while some principles of protein sorting can be generalized among cell types, the mechanism of sorting of a particular protein can vary between cell types.

Trafficking of soluble proteins has also been evaluated. Study of the targeting of CXCL4 (also known as platelet factor 4) to α-granules has led to the identification of a signal sequence responsible for sorting chemokines into α-granules.[29,30] These experiments demonstrate that a four amino acid sequence within the exposed hydrophilic loop is required for sorting of CXCL4 into α-granules.[30] An analogous sequence was identified in the platelet chemokines RANTES and NAP-2.[30]

Soluble proteins must be incorporated into vesicles formed at the trans-Golgi network to become cargo within mature α-granules. A mechanism involving binding to glycosaminoglycans has been proposed for sorting small soluble chemokines. Mice that lack the dominant platelet glycosaminoglycan, serglycin, fail to store soluble proteins containing basically charged regions, such as CXCL4, PDGF, or NAP-2, in their α-granules.[31] This observation suggests that glycosaminoglycans may serve as a retention mechanism for chemokines possessing an exposed cationic region. A mechanism to incorporate larger soluble proteins into α-granules is by aggregation of protein monomers.[32] Although not formally proven to sort by aggregation, large, self-assembling proteins such as multimerin have been proposed to sort into immature vesicles by homoaggregation.[33] vWf self-assembles into large multivalent structures and is packaged into a discrete tubular structure within α-granules.[34,35] Heterologous expression of vWf can drive the formation of granules in cell lines possessing a regulated secretory pathway (e.g., AtT-20, HEK293, or RIN 5F cells), but not in cells lines that lack such a pathway (CHO, COS, or 3T3 cells).[36,37] While aggregation and glycosaminoglycan binding represent plausible mechanisms for sorting soluble proteins, alternative sorting receptors must exist for other endogenous α-granule proteins.

Plasma proteins are incorporated into α-granules via several distinct mechanisms of endocytosis. During receptor-mediated endocytosis, a plasma protein is bound to a platelet surface receptor and subsequently internalized via a clathrin-dependent process. The most well-studied example is the incorporation of fibrinogen via integrin αIIbβ3.[38–42] Plasma proteins such as immunoglobulins and albumin incorporate into α-granules via pinocytosis.[43] The endocytosis of factor V by megakaryocytes involves two receptors. Following initial binding to a specific factor V receptor,