prognostic value of TP53 mutation. However, it also shows that translation to more accessible procedures is possible in future clinical trials.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Monitoring granule traffic in megakaryocytes

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Heritable granule deficiencies have enabled the identification of proteins involved in dense granule biogenesis. Yet how these proteins are organized into pathways to create dense granules has remained unknown due to a lack of adequate cellular models to study platelet granule trafficking. Ambrosio et al now describe such a cell model and provide an unprecedented glimpse into the birth of dense granules.1

Dense granules belong to a highly specialized family of organelles termed lysosome-related organelles (LROs). They are distinguished from classical secretory granules in that they derive from the endosomal system rather than directly from the trans-Golgi network. Other LROs include melanosomes, cytotoxic T-cell granules, and basophilic and azurophilic granules. But by any standard, the dense granule is unique. It is only present in platelets. It has a calcium concentration of more than 2M, ADP and ATP concentrations of approximately 500μM, and high concentrations of polyphosphates, which chelate the calcium. Dense granules also contain bioactive amines such as serotonin and histamine, which accumulate as a result of the activity of vesicular pumps. The high concentrations of these components render these granules imperceptible to the beam of an electron microscope, making them clearly visible even in the absence of staining (hence the term “dense granule”).

Deficiency of dense granules occurs in several human diseases including Hermansky-Pudlak syndrome, Chediak-Hagashi syndrome, and Griscelli syndrome type II. Many mouse mutants identified on the basis of coat color defects (secondary to melanosome deficiency) were subsequently found to have dense granule defects as well. These experiments of nature led to the identification of a number of proteins including BLOC proteins, Rab isoforms, and adaptor protein (AP) isoforms that mediate membrane trafficking during dense granule biogenesis. Yet it is one thing to identify a protein involved in granule biogenesis and another thing entirely to determine what it does and where within the biogenesis pathway it acts. These issues have been particularly difficult to address in megakaryocytes because they are so challenging to culture in large quantities and so difficult to modify by standard molecular approaches.

To circumvent these issues, Ambrosio et al use a MEG-01 cell line to model dense granule biogenesis. They first develop a set of markers capable of identifying different components of the granule trafficking apparatus. They identify dense granules using the marker LAMP2 as well as the dense granule transporters MRP4 and VMAT2. Rab7 is used as a marker for multi-vesicular bodies (MVBS) and late endosomes (see “MVB/late endosome” in figure). They find that Rab7 associates with immature granules expressing dense granule markers. The MEG-01 model also enables more dynamic measurements to interrogate granule trafficking. The authors use live cell imaging and pulse-chase experiments with several fluid phase markers to demonstrate the involvement of the late endocytotic pathway in dense granule biogenesis (see “Endocytotic pathway” in figure). Based on these findings, they conclude that dense granules derive from MVB/late endosomes.

But if dense granules have an MVB/late endosomal origin, how do they acquire the transporters necessary for achieving such high concentrations of cargo? To address this question, Ambrosio et al use the serotonin transporter, VMAT2, as a model protein to evaluate how a maturing dense granule acquires such molecular pumps. They show that the adaptor protein AP-3, which localizes to an early endosomal compartment, recognizes a tyrosine- and dileucine-based sorting motif on the cytoplasmic tail of VMAT2 (see “AP-3” in figure). They also demonstrate a role for Rab32 and Rab38 in transferring VMAT2 and MRP4, the putative adenine nucleotide transporter,2 to maturing dense granules (see “Rab32/38” in figure). Further evidence that Rab32/38 associate with immature dense granules that subsequently lose these Rab proteins and concentrate ADP is obtained by isolation and characterization of dense granules at different stages of development. Immature dense granules have higher concentrations of Rab32/38, while more mature dense granules lose their Rab32/38 and concentration ADP.

The major breakthrough of this work by Ambrosio and colleagues is the establishment...
of a model to evaluate platelet granule trafficking. Granule trafficking is notoriously difficult to study in megakaryocytes. The fact that these studies were performed in MEG-01 cells, a megakaryoblastic leukemia cell line, will raise questions of their validity in bona fide megakaryocytes. The authors anticipate this concern and, to their credit, take great pains to compare the MEG-01 cells with megakaryocytes. They show that morphologically, dense granule formation in MEG-01 cells resembles that of immature megakaryocytes. They recapitulate key aspects of their model in megakaryocytes. Thus, there is good reason to believe that the MEG-01 model is highly relevant to megakaryocytes.

A manipulable megakaryocytoid granule trafficking model could help answer several recalcitrant questions in platelet granule biology. The majority of known genetic defects of granule formation result in either loss of dense granules with complete preservation of α-granules or vice versa. This observation implies marked early divergence of dense granule and α-granule synthetic pathways. However, both dense granules and α-granules appear to derive from an MVB/late endosomal compartment. The MEG-01 model could help resolve this issue, demonstrating how factors involved in dense granule synthesis (BLOC proteins, specific Rabs, VSP33a) and factors involved in α-granule synthesis (NBEAL2, VSP33b) enable granules to segregate from one another and mature along different pathways. The MEG-01 model will be useful in identifying the role of each of these proteins and ordering them in a pathway. Applying this model to α-granules could also help resolve the controversy of whether α-granules represent a single homogeneous population or rather a heterogeneous population of granules carrying distinct cargos. By overcoming a major roadblock in our ability to map the pathways involved in granule trafficking, Ambrosio et al open new avenues into the study of platelet granule biogenesis.

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**THROMBOSIS & HEMOSTASIS**

Comment on Langhauser et al, page 4082

**Contact with stroke**

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In this issue of *Blood*, Langhauser and colleagues report that kininogen knockout mice (KNG<sup>−/−</sup>) are protected from stroke induced by transient mechanical occlusion of the middle artery (MCAO).<sup>3</sup> KNG<sup>−/−</sup> mice develop smaller infarcts, less neurologic impairment, and exhibit lower mortality than wild-type (WT) mice studied at 24 hours and beyond. Benefit was retained in elderly mice and was conferred through reduction in microvascular thrombosis, preservation of blood-brain-barrier function, and attenuation of inflammation. These are important findings because they provide insight into the pathophysiology of stroke and identify potential novel targets for intervention.

Ischemic stroke remains one of the most common causes of mortality and protracted morbidity. It is estimated that approximately 750 000 new patients a year in the United States are affected. Notwithstanding extensive investigation, thrombolysis by tissue-type plasminogen activator (tPA) remains the sole US Food and Drug Administration–approved
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