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Megakaryocytic irreversible P-TEFb activation

Kamaleldin E Elagib and Adam N Goldfarb

Department of Pathology; University of Virginia School of Medicine; Charlottesville, VA USA

Megakaryocyte development is particularly characterized by cellular enlargement and nuclear polyploidization, while erythroid development is characterized by cellular and nuclear atrophy, although both lineages originate from a common bipotential progenitor (MEP). The process of megakaryocyte enlargement and polyploidization, i.e., morphogenesis, is complex and controlled by tight regulation of transcriptional and signaling pathways. This process resembles cardiac hypertrophy and shares with it a sustained and global activation of P-TEFb kinase (a complex of Cdk9 and cyclin T1). Megakaryocyte morphogenesis is of physiological and clinical relevance, as it ultimately determines the number of platelets generated per cell. Disruption of the normal program can result in aberrant neoplastic megakaryopoiesis and in defective platelet production.

The majority of P-TEFb is sequestered in a large inactive complex known as the 7SK snRNP, which contains the 7SK small nuclear RNA and an array of associated nucleoproteins. The mechanisms of release of active P-TEFb form from the 7SK snRNP are not completely understood. Emerging evidence has started to shed light on some of the pathways for P-TEFb activation in a variety of model systems. For example, Liu et al. recently showed that recruitment of a BRD4/JMJD6 to distal "anti-pause" enhancers mediates the coordinated, local removal both of a repressive $H4R3me^{2(S)}$ chromatin mark and of the stabilizing methyl-cap on 7SK snRNA, causing localized release of P-TEFb from the 7SK snRNP at associated "paused" promoters.[1] Other pathways for P-TEFb release have included shifting of the splicing factor SRSF2 from the 7SK snRNP to binding sites on paused nascent mRNA,[2] induction of HEXIM1 phosphorylation by PI3K/AKT or PKC,[3] induction of cyclin T1 acetylation, and cooperative disruption of 7SK snRNP by Ca^{2+} activation of PP2B and PP1α phosphatases.[4]

The pathways described above provide reversible mechanisms for P-TEFb activation, usually restricted to specific promoters. We have recently discovered a distinct megakaryocytic pathway leading to global irreversible activation of P-TEFb that controls morphogenesis.[5] This pathway is triggered by the upregulation and activation of a calcium-dependent protease, calpain 2, early in megakaryopoiesis. Calpain 2 then directly cleaves MePCE, the 7SK snRNA methyl-capping enzyme and stabilizing factor. Concurrently, LARP7, another 7SK snRNA stabilizing protein, undergoes downregulation by a calpain 2-independent pathway. The combined loss of these factors completely destabilizes the 7SK snRNP, leading to a global release of active P-TEFb. The sustained nature of MePCE and LARP7 downregulation results in irreversible activation of P-TEFb.

Calpain 2 activation requires high, possibly millimolar, levels of calcium. The mechanism for its sustained activation during megakaryopoiesis is unknown. Intriguingly, the calcium-binding chaperone calreticulin is highly expressed in megakaryocytes while minimally expressed in other marrow lineages,[6] suggesting that megakaryocytes might have a dedicated pool of ER calcium to release for calpain 2 activation during development. Recently somatic mutations in *calreticulin* exon 9 were identified in a subset of patients with essential thrombocythemia and primary myelofibrosis, both myeloproliferativeneoplasms (MPN) characterized by increases in marrow megakaryocytes. The mutant calreticulin is also preferentially expressed in megakaryocytes,[6] but how it contributes to the phenotype of these MPN remains unclear.

Calpain 2 upregulation during normal megakaryopoiesis is dependent on the transcription factor GATA1 and is disrupted by the somatic GATA1s truncating mutation associated with megakaryocytic neoplasia in Down syndrome (i.e., DS-TMD and DS-AMKL).[5] Restoration of calpain 2 expression in mouse fetal liver progenitors with the GATA1s mutation corrects the megakaryocyte defects in development and growth arrest.[5] Thus, defects in calpain 2-driven P-TEFb activation may contribute to megakaryoblastic neoplasia in Down syndrome. The ectopic expression of erythroid genes in DS-TMD and DS-AMKL megakaryoblasts may additionally reflect a deficiency in activation of P-TEFb, which normally contributes to erythroid suppression during megakaryopoiesis.

The sustained, global activation of P-TEFb promotes high-level expression of a cohort of cytoskeletal remodeling factors (including filamin A, Mkl1, α-actinin1, and Hic5) that participate in megakaryocyte morphogenesis, i.e., enlargement and polyploidization.[5] In vivo roles of filamin A (*FLNA*) and Mkl1 in megakaryopoiesis have been validated in mouse knockout models, and human germline *FLNA* mutations associate with macrothrombocytopenia and dysmegakaryopoiesis.[7] More recently, mutations in α-actinin1 gene (*ACTN1*) have been linked to human autosomal dominant macrothrombocytopenia and dysmegakaryopoiesis, further supporting the role of this factor in megakaryocyte

*Correspondence to: Kamaleldin E Elagib; Email: ka8b@virginia.edu; Adam N Goldfarb; Email: ang3x@virginia.edu
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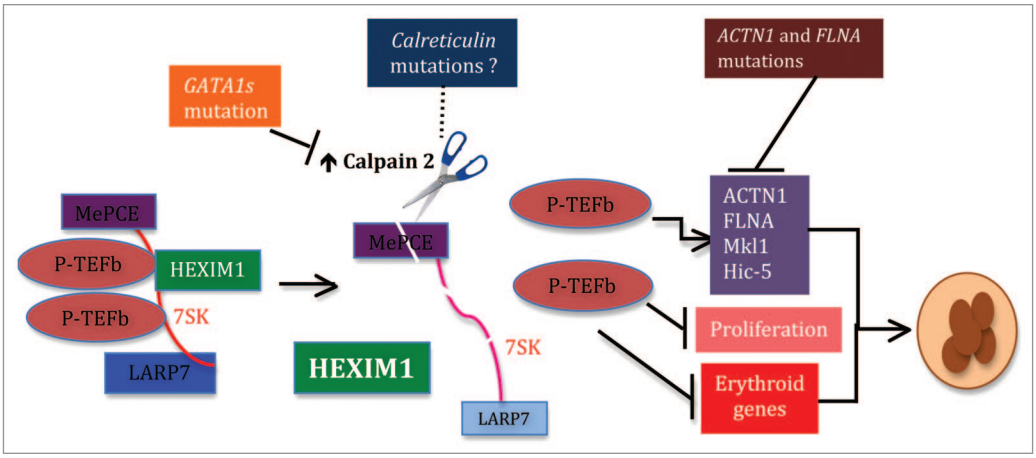

Figure 1. Cartoon depicting the irreversible and global P-TEFb activation pathway, leading to expression of cytoskeletal remodeling factors, suppression of proliferation, and suppression of erythroid genes expression during normal megakaryopoiesis. The pathway is disrupted by the acquired *GATA1s* mutation in Down syndrome patients. Germline mutations of *ACTN1* and *FLNA* contribute to congenital macrothrombocytopenia.

morphogenesis.[8] The role of Hic5 in megakaryocytic development remains to be investigated.

Additional defects in the megakaryocytic calpain 2-P-TEFb activation pathway might also account for alterations in morphogenesis seen during infancy or after cord blood transplant (small hypolobulated megakaryocytes), or in essential thrombocythemia (large hyperlobulated megakaryocytes). Future studies will address these questions, as this pathway could provide an entree for therapeutic modulation of aberrant megakaryopoiesis. (**Fig. 1**)

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