

# Towards an Understanding of Megakaryocyte and Platelet Production

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With the recent cloning of thrombopoietin (TPO), the primary regulator of platelet production, many insights into the cellular and molecular mechanisms of megakaryocyte development have been realized. Thrombopoietin was cloned based on its binding to the proto-oncogene receptor c-Mpl. Using the recombinant hormone, multiple investigators have shown that TPO affects all aspects of megakaryocyte development, from hematopoietic stem cells to mature blood platelets. However, additional cytokines must also play important roles in megakaryocyte development, as numerous protein growth factors, including interleukin (IL)-3, IL-6, IL-11, stromal cell derived factor (SDF) and steel factor (SF), have been shown to work alone and in combination with TPO to augment megakaryopoiesis, and genetic elimination of TPO or its receptor substantially reduces but does not eliminate platelet production. Many of the molecular pathways that mediate the action of TPO and the other megakaryopoietic cytokines have been explored. Most signaling pathways are overlapping, but the finding that each of the cytokines appears to play a unique, albeit selected role in hematopoietic development suggests that some of the signal transduction mechanisms are non-redundant. Following binding of TPO, the Mpl receptor homodimerizes and activates members of the Janus family of cytoplasmic tyrosine kinases (JAKs). Once active, JAK2 phosphorylates the receptor, generating docking sites for a number of second messengers that ultimately affect multiple downstream signaling pathways. Affected pathways

include those utilizing signal transduction and activators of transcription (STATs), mitogen-activated protein (MAP) kinases, and phosphoinositol (PI)-3 kinase. Of interest, a virtually identical set of signaling intermediaries is induced by IL-3 in both cell lines that bear the IL-3 and Mpl receptors and in primary megakaryocytes. Clearly, TPO-specific signals have not yet been identified, and whether such signals exist is debated. Nevertheless, ultimately, cellular anti-apoptotic mechanisms are initiated, as is endomitosis, a mechanism peculiar to megakaryocytes that uncouples DNA synthesis from nuclear and cytoplasmic division, generating polyploid cells. Insights into this enigmatic pathway have also been obtained. Although several cell lines become polyploid as the result of loss of either the M phase kinase cdc2, or its regulatory subunit cyclin B, neither appears to be the case for normal endomitotic megakaryocytes. Rather, the departure from normal diploid cell behavior occurs in mid anaphase, when the chromosomes usually migrate to opposite ends of the cell. Instead, megakaryocytes form a circular spindle, and although the chromosomes separate, they do not migrate, the nucleus reforms around the entire chromosomal mass, and the cytoplasm fails to divide. The molecular explanations for these findings are under intense study. Finally, as the net result of TPO action is an expansion of cells that give rise to mature platelets, the availability of the recombinant hormone has provided new opportunities to manipulate blood cell development for therapeutic benefit.