cells remains highly controversial, with different studies postulating an important function in HSCs, or no role whatsoever (Cerdan and Bhatia, 2010; Maillard et al., 2008). The role of wnt signaling in HSCs is equally unclear, with claims of a role in controlling hematopoietic stem and progenitor fates, or that it is dispensable in HSCs (Malhotra and Kincade, 2009; Cerdan and Bhatia, 2010). It will be interesting to see if these novel insights into the role of wnt signaling in HSC specification can also help to clarify potential roles in the complex niches of adult HSCs.

Although the current study includes some indications of the cellular source of the wnt16, precise identification of the specific wnt16-producing cell type, and of the molecular control of its expression, will be necessary to comprehensively unravel blood specification. The simultaneous requirement of two independent Notch ligands is puzzling and of wide interest. Part of the mechanisms behind this synergy has probably just been described in another recent publication (Wright et al., 2011). It will be important to determine whether the molecular mechanism described by Traver and colleagues also functions in mammalian development. This study very nicely confirms and illustrates the existence of specific, sequential windows of time at which defined combinatorial environmental cues ultimately lead to HSC specification. A similar signaling requirement that specifies cell fate choices—long after the signals themselves were active—was recently shown for BMP in the in vitro generation of blood cells from ESCs (Chiang and Wong, 2011). Molecular programs that propagate over time by either non-cell-autonomous relay signaling or cell-intrinsic deterministic mechanisms are not only highly interesting as models for molecular regulation. In combination with the required timed presence of many different cell types and their inductive signaling, they can also offer an explanation for the remaining difficulty in inducing HSCs from pluripotent cells (Cerdan and Bhatia, 2010). Hopefully, these novel insights will contribute to the comprehensive understanding of the required combinatorial timed signals (Ciau-Uitz et al., 2010) that will allow the efficient generation of unlimited, well-defined, and clinically applicable HSCs in vitro.

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Pluripotency without Max

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Myc/Max complexes are thought to be essential for maintaining pluripotency and self-renewal of embryonic stem cells (ESCs). In this issue of *Cell Stem Cell*, Hishida et al. (2011) provide genetic evidence that this requirement can be bypassed in well-defined culture conditions.

Due to its pervasive involvement in human tumorigenesis, the Myc oncoprotein and two of its cousins, N-Myc and L-Myc, have been under intense scrutiny for many years. More recently, endogenous c- and N-Myc proteins have been demonstrated to be individually or collectively essential for the self-renewal of embryonic and adult (e.g., hematopoietic) stem cells (Smith and Dalton, 2010). Importantly, deregulated expression of Myc, as is the hallmark of many human tumors, enhances the formation of induced pluripotent stem cells (iPSCs), suggesting that the oncogenic functions of Myc may be mechanistically related to its ability to confer self-renewal capacity to differentiated cells given the right genetic context (Takahashi and Yamanaka, 2006). As a result, the biochemical analysis of Myc function, particularly in ESCs, has begun to set paradigms for the study of Myc in human tumors.

Myc has an essential role in maintaining ESCs in a proliferative, self-renewing, and undifferentiated state. Together with a set of interacting proteins, Myc binds to a large set of promoters that are distinct from promoters bound by other factors involved in maintaining the pluripotency

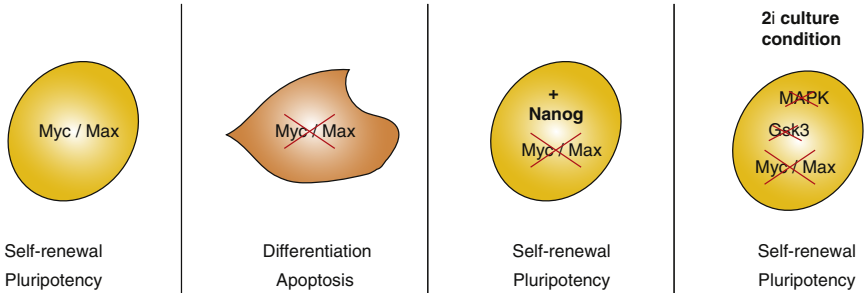

Figure 1. Myc/Max Complexes Can Be Dispensable for ESC Pluripotency
Both alleles of the *Max* gene are disrupted in the Max null ESCs, and a doxycycline-regulatable *Max* cDNA is expressed from the *ROSA26* locus. The lack of functional Myc/Max complexes induces the differentiation and subsequent apoptosis of Max null ESCs, unless cells express Nanog or are cultured in the presence of MAP kinase and GSK3 inhibitors.

of ESCs (''Myc module'' versus ''core module'' genes) (Kim et al., 2010). Expression of Myc module genes is downregulated during differentiation, concomitant with loss of Myc expression. Furthermore, expression of genes of this module is enhanced in human tumors, often correlating with their aggressiveness, underlining the similarity between oncogenic and stem cell functions of Myc (Ben-Porath et al., 2008). The observation that Myc is a global regulator of transcriptional elongation and phosphorylation of RNA polymerase II suggests a mechanistic basis for gene activation by Myc in mouse ESCs (mESCs) (Rahl et al., 2010). Taken together, the data support a model in which one key mechanism by which Myc acts to maintain ESC pluripotency is by enhancing the expression of a large set of genes that in some way collectively promote pluripotency.

The current manuscript in this issue of *Cell Stem Cell* by Hishida et al. (2011) tests some of the predictions of this model by analyzing mESCs that in essence have an inducible knockout of the Max protein (Figure 1). Max is a partner protein of Myc that is required for all known functions of Myc proteins. The sole exception is Myc's function in promoting transcription by RNA polymerase III, which is mediated by a direct contact of Myc with TFIIIB, so the ability to promote RNA polymerase III-dependent transcription may be retained in Max-less ESCs (Gallant and Steiger, 2009).

In line with previous results, Hishida et al. find that loss of Max in mESCs leads to loss of self-renewal, inhibition of cell proliferation, differentiation, and, as an indirect consequence, apoptosis (Figure 1). Loss of the undifferentiated state is accompanied by upregulation of MAP kinase signaling, a known inducer of ESC differentiation. How loss of Max induces MAP kinase activity remains open. Also in line with previous work, loss of Max leads to a downregulation of genes of the Myc module. Most genes of the modules are only weakly affected, although the effects are stronger on a subset of 206 genes.

Surprisingly, however, loss of Max in mESCs has no effect on the expression of genes that were downregulated by the concomitant deletion of c- and N-Myc in hematopoietic stem cells, arguing that there is virtually no overlap in Myc/Max-regulated genes between both cell types—although in both cases Myc/Max complexes are required for maintaining self-renewal capacity. Even more surprisingly, expression of Nanog inhibits differentiation and permits long-term proliferation of Max-depleted mESCs (Figure 1). Nanog-rescued cells maintain expression of Myc-module genes in the absence of Max. So if Myc/Max complexes have a direct and critical function in upregulating Myc module genes, this function cannot be unique and can be quite easily substituted or bypassed by expression of Nanog or one of its downstream target genes.

Furthermore, previous work had demonstrated that mESCs can exist in a ''ground state,'' in which they self-renew and maintain pluripotency, independent of extrinsic stimuli such as leukemia inhibitory factor (LIF) and its intracellular target, Stat3 (Ying et al., 2008). This ground state can be induced by inhibiting MAP kinase signaling and by inhibiting GSK3 (resulting in the so called ''2i'' condition), thereby blocking differentiation and promoting the biosynthetic capacities of mESCs. Myc levels are very low in ground state mESCs, which prompted Hishida et al. to test whether the 2i condition drives self-renewal of mESCs without Myc/Max transcriptional complexes. Indeed, they found that Myc module genes show a marginal decrease in expression (on average a 10% decrease) when Max is deleted under 2i culture conditions, reinforcing the notion that loss of Myc/Max complexes can be largely dispensable for maintaining expression of Myc module genes.

A final surprise is contained in one of the Supplemental Figures. Based on the use of a small molecule inhibitor, 10058-F4, that disrupts heterodimerization of Myc and Max in vitro and inhibits cell proliferation, Young and colleagues concluded in a recent publication that a central function of Myc is to promote elongation by RNA polymerase II (Rahl et al., 2010). One piece of evidence is the finding that addition of high concentrations of 10058-F4 inhibits phosphorylation of RNA polymerase II at serine 2, a hallmark of elongating RNA polymerase. A prediction from this model is that the genetic ablation of Max should have the same effect, yet Hishida et al. show that this does not occur, arguing that the function of Myc/Max complexes in transcriptional elongation can be provided by the substitution of other factors.

Collectively, the findings that Myc/Max complexes can be dispensable for self-renewal and maintaining expression of Myc module genes (Figure 1) argue against a model in which Myc/Max complexes have an essential and mechanistically unique role in upregulating expression of a large group of downstream target genes to promote self-renewal. An alternative model suggesting that a smaller set of specific target genes mediates the effects of Myc on self-renewal is supported by recent studies showing that repression of the primitive endoderm master regulator GATA6 and regulation of miRNA expression contribute to Myc's ability to maintain pluripotency (Lin et al., 2009; Smith and Dalton, 2010). These findings are also reminiscent of observations in *Drosophila*, in which larval development can be completed in the absence of dMyc, provided its antagonist,

dMnt/dMad, is also deleted (Pierce et al., 2008). Analogously, it is possible that the conditions that render Max dispensable in mESCs affect the abundance and function of Mad/Mnt proteins. If so, the observations reported here suggest that the relative abundance of Myc/Max and Mad/Max complexes at a large number of ESC promoters reflects the need to alter expression of the bound genes—and subsequently cell growth and proliferation—in response to developmental signals, rather than a direct role of these genes as an integral part of establishing and maintaining pluripotency.

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