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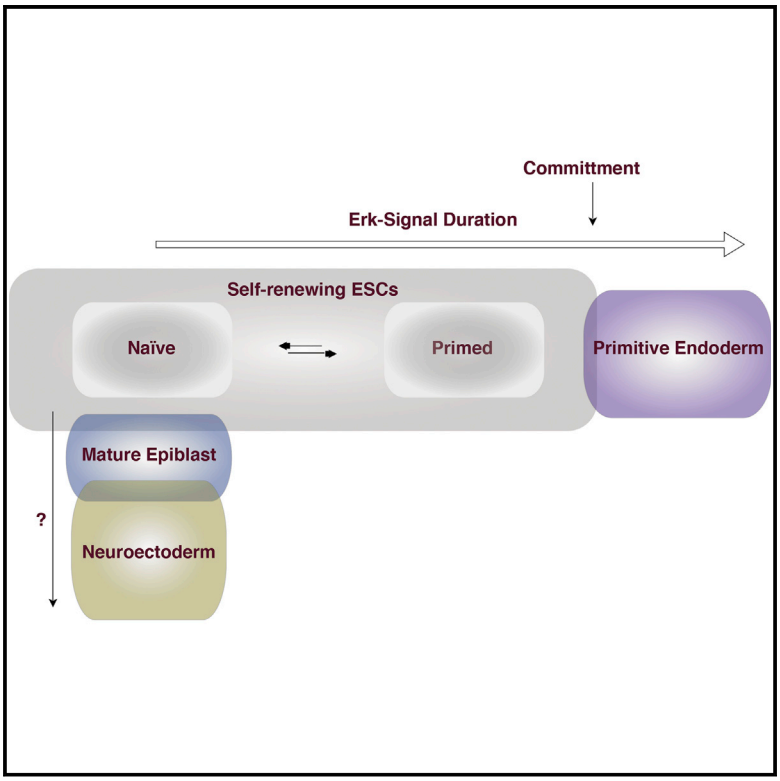
Erk Signaling Suppresses Embryonic Stem Cell Self-Renewal to Specify Endoderm

Graphical Abstract



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In Brief

Hamilton and Brickman show that Erk activity is not required for epiblast nor neural differentiation but promotes primitive endoderm priming and differentiation through suppression of a subset of the ESC gene regulatory network. The duration of Erk signaling determines the difference between reversible priming and differentiation.

Highlights

- Erk activity is dispensable for exit from pluripotency and neural differentiation
- Erk activity suppresses pluripotency gene expression to induce endoderm specification
- Erk signaling duration determines the difference between priming and differentiation
- Nanog blocks Gata6 induction but not the inhibition of pluripotency by Erk

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Erk Signaling Suppresses Embryonic Stem Cell Self-Renewal to Specify Endoderm

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SUMMARY

Fgf signaling via Erk activation has been associated with both neural induction and the generation of a primed state for the differentiation of embryonic stem cells (ESCs) to all somatic lineages. To dissect the role of Erk in both ESC self-renewal and lineage specification, we explored the requirements for this pathway in various in vitro differentiation settings. A combination of pharmacological inhibition of Erk signaling and genetic loss of function reveal a role for Erk signaling in endodermal, but not neural differentiation. Neural differentiation occurs normally despite a complete block to Erk phosphorylation. In support of this, Erk activation in ESCs derepresses primitive endoderm (PrE) gene expression as a consequence of inhibiting the pluripotent/epiblast network. The early response to Erk activation correlates with functional PrE priming, whereas sustained Erk activity results in PrE differentiation. Taken together, our results suggest that Erk signaling suppresses pluripotent gene expression to enable endodermal differentiation.

INTRODUCTION

Mouse embryonic stem cells (ESCs) are immortal, karyotypically stable cell lines derived from the inner cell mass (ICM) or early epiblast of preimplantation embryos (Evans and Kaufman, 1981; Martin, 1981; Najm et al., 2011). They are capable of maintaining their differentiation potential through multiple rounds of division, of differentiating into all the lineages of the future conceptus when reintroduced into a developing embryo (Morgani et al., 2013; Robertson et al., 1986), and of undergoing directed differentiation in vitro. ESCs are defined by these functional properties, self-renewal, and pluri- (and in some cases toti-) potency, but they are also characterized by the expression of an array of genes, primarily transcription factors such as Nanog, Oct4, Rex1 (Chambers et al., 2003; Mitsui et al., 2003; Nichols et al., 1998; Takahashi and Yamanaka, 2006), and cell-surface markers including SSEA1 and PECAM1 (Canham et al., 2010; Rugg-Gunn et al., 2012).

The maintenance of ESCs in an undifferentiated state is dependent on external signals such as leukemia inhibitory factor (LIF) (Smith et al., 1988) and BMP4 (Ying et al., 2003a). It is thought that these signals converge on a core network of transcription factors that cooperate to maintain an undifferentiated state (Martello et al., 2012, 2013). Recently, it has been shown that ESCs can be maintained in a minimal defined culture system through the combinatorial inhibition of both Mek-Erk and Gsk3 signaling (Ying et al., 2008). This condition, known as 2i, was shown to be highly effective at supporting pluripotent ESCs and, when supplemented with LIF, contains a subpopulation of single cells that exhibit totipotency (Morgani et al., 2013). The rationale behind this culture system is that robust Erk activity (downstream of Fgf4) is essential for multilineage differentiation of ESCs (Kunath et al., 2007; Ying et al., 2008), and therefore inhibiting it promotes self-renewal. The suppression of Gsk3 activity was believed to enhance the viability of undifferentiated cells grown in these defined conditions (Ying et al., 2008). However, recent studies suggest that Gsk3 activity regulates a crucial axis of the pluripotency network and can regulate self-renewal independently of Erk inhibition or LIF-Stat3 activation (Wray et al., 2011).

Although it has been proposed that Erk activity is required for differentiation toward derivatives of all three germ layers in vitro (Kunath et al., 2007; Ying et al., 2008), in vivo studies into various mutants of the Fgf-Erk pathway indicate this pathway is required for early extraembryonic endoderm differentiation (Chazaud et al., 2006; Kang et al., 2013), in addition to playing a role in aiding the survival of differentiated epiblast derived tissues (Arman et al., 1998; Feldman et al., 1995; Wilder et al., 1997). It has also been suggested that Erk activity is required for differentiation toward neural tissues in vitro (Kunath et al., 2007; Stavridis et al., 2007), although the in vivo evidence for a requirement for Fgf-Erk activity in the early stages of neural specification is controversial (Di-Gregorio et al., 2007). Experiments in chick and *Xenopus* disagree as to whether naive ectoderm can undergo neural induction merely as a result of BMP antagonism, the default model, or whether neural induction is mediated via an Fgf-Erk signal (Linker and Stern, 2004). Data from ESCs were seen to support such a role for Fgf-Erk in neural induction, although it was recently shown that Erk2, the primary Erk isozyme expressed in ESCs, is entirely dispensable for their multilineage differentiation (Hamilton et al., 2013). Moreover, although the inhibition of Erk activity enhances the differentiation of certain neural lineages when differentiated from mouse epiblast stem cells (EpiSCs) (Jaeger et al., 2011), Erk activity is still thought to be required for the progression of undifferentiated cells into a primed epiblast state, the first stage in neural differentiation.