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Short Article

A Switch from Low to High Shh Activity Regulates Establishment of Limb Progenitors and Signaling Centers

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SUMMARY

The patterning and growth of the embryonic vertebrate limb is dependent on Sonic hedgehog (Shh), a morphogen that regulates the activity of Gli transcription factors. However, *Shh* expression is not observed during the first 12 hr of limb development. During this phase, the limb bud is prepatterned into anterior and posterior regions through the antagonistic actions of transcription factors *Gli3* and *Hand2*. We demonstrate that precocious activation of Shh signaling during this early phase interferes with the Gli3-dependent specification of anterior progenitors, disturbing establishment of signaling centers and normal outgrowth of the limb. Our findings illustrate that limb development requires a sweet spot in the level and timing of pathway activation that allows for the Shh-dependent expansion of posterior progenitors without interfering with early prepatterning functions of *Gli3*/Gli3R or specification of anterior progenitors.

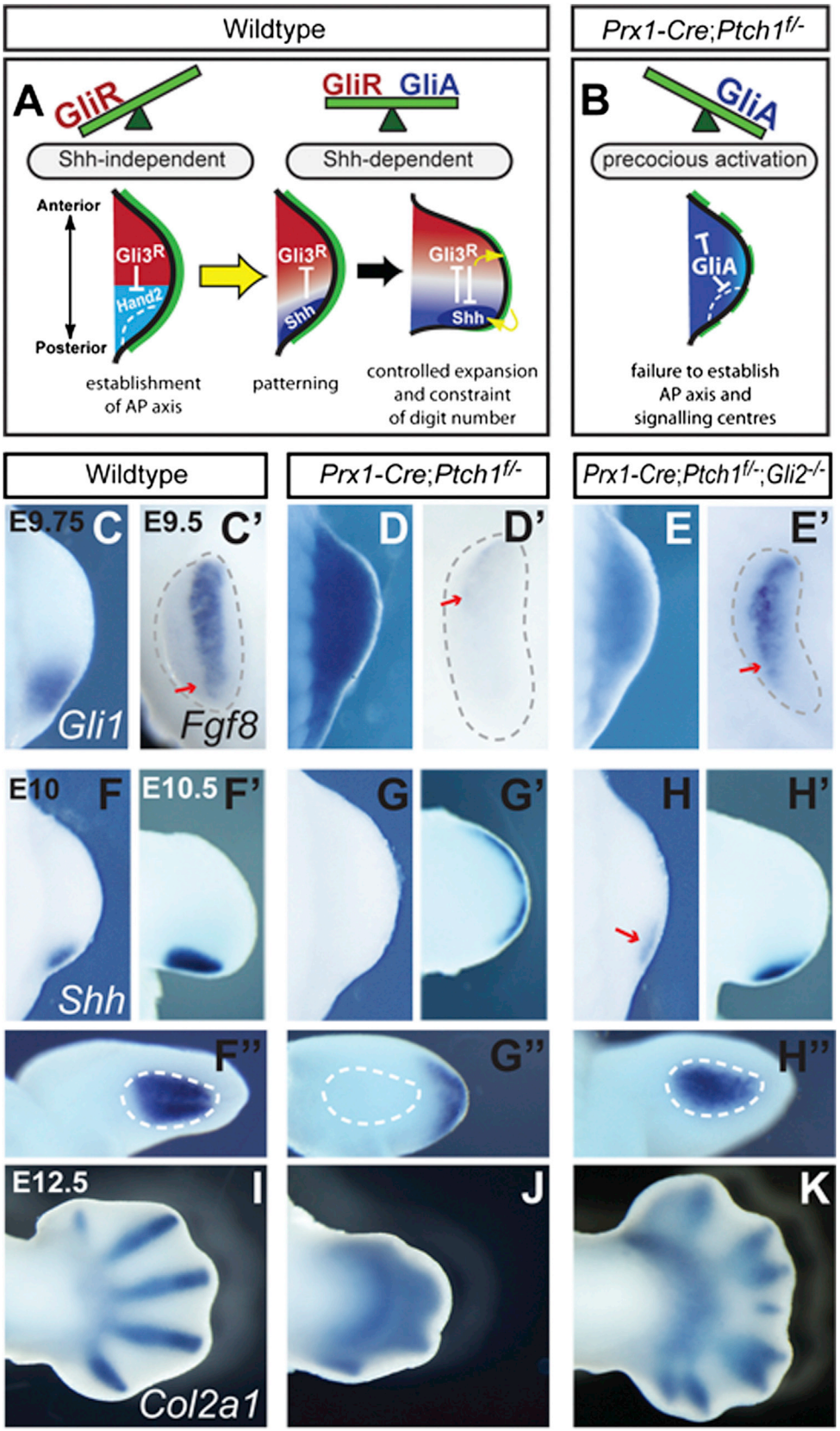
INTRODUCTION

Vertebrate digit patterning progresses through two phases, both dependent on the activity of *Gli3*. During the first phase, *Gli3* polarizes the limb into anterior and posterior (AP) regions through antagonism with the transcription factor *Hand2* (Chiang et al., 2001; Galli et al., 2010; te Welscher et al., 2002a; Vokes et al., 2008) (Figure 1A). Polarization of the limb during this prepatterning phase also positions the zone of polarizing activity (ZPA) in the posterior mesenchyme (Galli et al., 2010; Masuya et al., 1997; te Welscher et al., 2002a). The ZPA is a source of Sonic hedgehog (Shh), a morphogen responsible for digit patterning in the chick and the mouse (Riddle et al., 1993; Tickle et al., 1975). Shh mediates patterning by regulating Gli2 and Gli3, which act as full-length transcriptional activators (GliA) in the presence of Shh and are cleaved into truncated repressors (GliR) in its absence (Zhulyn and Hui, 2012). Although Gli2 contributes to posterior patterning and expansion of limb progenitors, the AP digit pattern results primarily from countergradients of anterior Gli3R and posterior Shh (Figure 1A) (Bowers et al., 2012; Litingtung et al., 2002; Riddle et al., 1993; Wang et al., 2000; te Welscher et al., 2002b). Shh is activated approximately 12 hr after limb bud initiation occurs, and it is required for patterning and outgrowth of posterior limb elements. Although it is expressed in the limb from embryonic day 9.5 (E9.5) to E11.5, it appears that Shh is involved in patterning only during the first 12 hr (Zhu et al., 2008). In contrast, *Gli3* is required throughout limb development, establishing AP polarity during prepatterning and regulating digit number and progenitor proliferation during the Shh-dependent phase (Figure 1A) (Chiang et al., 2001; Galli et al., 2010; Litingtung et al., 2002; Lopez-Rios et al., 2012; te Welscher et al., 2002b). The transient role of Shh in the patterning process suggests that the timing of its activity as a regulator of GliA/R may be important. Notably, both *Shh*-expressing cells and the cells responding to the Shh signal physically contribute to the formation of posterior limb elements (Ahn and Joyner, 2004; Harfe et al., 2004; Towers et al., 2008). Conversely, anterior elements, including digit 1, are believed to be *Shh* independent (Cao et al., 2013; Chiang et al., 2001; Galli et al., 2010). In the accompanying paper in this issue of *Developmental Cell*, Li et al. (2014) demonstrate that these anterior elements are specified by homeodomain transcription factors *Irx3* and *Irx5* and undergo progressive determination, which is negatively regulated by Shh. This suggests that the limb comprises at least two progenitor pools with distinct signaling requirements: high Shh in the posterior and low Shh/high Gli3R in the anterior. We propose that the timing of Shh activity is constrained to allow the formation of AP progenitors and the establishment of the limb organizers, the apical ectodermal ridge (AER), and the ZPA, which govern their patterning and expansion.

RESULTS AND DISCUSSION

Precocious Shh Activation during Prepatterning Phase Inhibits Limb Outgrowth and Establishment of Signaling Centers

To assess the temporal requirement for Shh signaling, we examined the effects of precocious Shh pathway activation by using



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