



**FIGURE 19.22** The descendants of Shh-secreting cells form digits 4 and 5 and contribute to the specification of digits 2 and 3 in the mouse limb. (A) In the early mouse hindlimb bud, the progenitors of digit 4 (green dot) and the progenitors of digit 5 (red dot) are both in the ZPA and express Sonic hedgehog (light green shading). (B) At later stages of limb development, the cells forming digit 5 are still expressing Shh in the ZPA, but the cells that form digit 4 no longer do. (C) When the digits form, the cells in digit 5 will have seen high levels of Shh protein for a longer time than the cells in digit 4. (D) Schematic by which digits 4 and 5 are specified by the amount of time they were exposed to Shh in an autocrine fashion; digit 3 is specified by the amount of time the cells were exposed to Shh both in an autocrine and paracrine fashion. Digit 2 is specified by the concentration of Shh its cells received by paracrine diffusion, and digit 1 is specified independently of Shh. (After Harfe et al. 2004.)

mouse limb, the resulting limbs have numerous digits, but the digits have no obvious specificity (Litngtung et al. 2002; Ros et al. 2003; Scherz et al. 2007). Vargas and Fallon (2005) propose that digit 1 is specified by *Hoxd13* in the absence of *Hoxd12*. Forced expression of *Hoxd12* throughout the digital primordia leads to the transformation of digit 1 into a more posterior digit (Knezevic et al. 1997).

By using conditional knockouts of the mouse *Shh* gene (i.e., researchers could stop Shh expression at different times during mouse development), Zhu and Mackem (2011) found that Sonic hedgehog works by two temporally distinct mechanisms. The first phase involves the specification of digit identity (from the posterior pinky to the anterior thumb). In this phase, Shh acts as a morphogen, with the digit identities being specified first by the concentration of Shh in that region of the limb bud, and then by the duration of exposure to Shh. In the second phase, Shh works as a mitogen to stimulate the proliferation and expansion of the limb bud mesenchyme, thus helping shape the limb bud.

The mechanism by which Sonic hedgehog establishes a digit's identity may involve cell-cycle regulation and the BMP pathway. The time and concentration dependent actions of Shh lead to the graded activation of the downstream transcriptional effector Gli3. These targets include the genes for the BMP antagonist Gremlin, the cell cycle regulator Cdk6, and the genes that synthesize hyaluronic acid (a component of cell adhesion). Shh (through Gli3) restricts the proliferation of cartilage progenitor cells (by downregulating Cdk6) and promotes their BMP-stimulated differentiation into cartilage by inhibiting the BMP antagonist Gremlin and by upregulating hyaluronic acid synthase 2 (Vokes et al. 2008; Liu et al. 2012; Lopez-Rios et al. 2012).

Shh initiates and sustains a gradient of BMP proteins across the limb bud, and this BMP gradient can specify the digits (Laufer et al. 1994; Kawakami et al. 1996; Drosopoulos et al. 2000). Identity is not specified directly in each digit primordium, however. Rather, the identity of each digit is determined by the *interdigital* mesoderm—that is, by the webbing between the digits (the region of mesenchyme that will shortly undergo apoptosis).

The interdigital tissue specifies the identity of the digit forming anteriorly to it (i.e., toward the thumb or big toe). Thus, when Dahn and Fallon (2000) removed the webbing