as areas of early *Hox* gene expression. *Hoxc-6* has been identified in the area where the forelimb will appear in the zebra fish, the frog *Xenopus,* and the mouse (Oliver et al. 1988; Molven et al. 1990; De Robertis, Morita, and Cho 1991).

The **apical ectodermal ridge (AER)** develops at the tip of the limb bud as it emerges from the flank (see Fig. 10.15). It is formed from superficial ectoderm that is influenced by factors from the underlying lateral plate mesoderm. The ridge is essential to limb growth and differentiation; if it is removed, the limb bud regresses. Under normal conditions, the apical ectodermal ridge maintains the directly underlying mesenchyme in a state of cell proliferation and prevents the cells from forming cartilage. This region of cell division, the **progress zone**, determines the proximal–distal limb axis. The first cells to divide and leave the progress zone form proximal structures; those cells that have undergone numerous divisions within the progress zone become more distal structures. One may imagine the expression of each of the sequential *Hox* genes during the time of maximal cell proliferation: 10 A and D (i.e., *Hoxa-10* and *Hoxd-10*) as the cells that will form the humerus appear; 11 A and D with the precursors of the ulna, radius, tibia, fibula, and proximal carpals and tarsals; 12 A and D with the distal carpals and tarsals; and 13 A and D with the metapodials and digits (Davis et al. 1995).

The anterior–posterior axis of the limb is determined by the **zone of polarizing activity (ZPA)**, which is a group of cells on the posterior margin of the limb whose activity is initiated and maintained by the presence of the apical ectodermal ridge. The ZPA is apparent as early as the 16-somite stage, before the limb bud is even visible. The effect of this zone has been suggested as a result of the diffusion of some substance from the cells in this area, or as a result of successive cell–cell interactions.

Detailed analysis of the functional sequence of gene and structural activation in the ZPA was undertaken by Charité et al. (1994) and Niswander et al. (1994). In the mouse, *Hoxb-8* as well as *Hoxc-6* is expressed in the forelimb field. It is present for only a short period, but this may be sufficient to induce formation of the zone of polarizing activity. Three genes are activated in succession in response to the presence of the ZPA: *Sonic hedgehog (SHH), Fibroblast growth factor 4 (Fgf-4),* and *Hoxd-11. SHH* and *Fgf-4* maintain the activity of the ZPA as *Hoxb-8* expression is lost. *Sonic hedgehog* encodes a protein that is thought to be an intercellular signal molecule controlling anteroposterior patterning in the limb. This molecule may activate the bone morphogenetic proteins. The number of digits that result from transplants within the limb is proportional to the number of posterior cells implanted at the margin of the ZPA (Charité et al. 1994).

Transplantation or removal of the AER or ZPA, disruption or misexpression of *Hox* and other genes, and treatment of developing limbs with the putative morphogen retinoic acid all influence the development of digits, which may be reduced or increased in number, duplicated, or their orientation altered. An especially informative disruption is achieved by removing the cells from the limb bud, disaggregating them, and replacing them in the bud: Digits are formed, but unlike the results of manipulation of the AER and ZPA, the digits do not conform with the specific pattern of any particular, normal digits. This suggests that the capacity to form digits is determined separately from the capacity to establish specific digit identity.