



A mutant form of *ilvA*, with much decreased sensitivity to feedback inhibition by isoleucine, was overexpressed in the transformed plants. BtkB, PhbB, and PhbC then catalyzed the remaining reactions needed to convert propionyl-CoA and acetyl-CoA to P(3HB-*co*-3HV). As discussed earlier, the BtkB 3-ketothiolase has a high affinity for both propionyl-CoA and acetyl-CoA and efficiently synthesizes both 3-ketovaleryl-CoA and acetoacetyl-CoA (Figure 8.17).

In *A. thaliana*, BtkB, PhbB, and PhbC were targeted to the chloroplast. For polymer production in *B. napus* seeds, the enzymes were targeted to the leucoplast. In the latter case, all four genes were expressed from a single vector and driven by a promoter with seed-specific expression. Analysis of a number of transgenic *A. thaliana* lines produced P(3HB-*co*-3HV). The concentration of the copolymer ranged from 0.08% to 0.84% per dry weight of material in shoots with a copolyester content of 3-hydroxyvalerate of 4 to 17 mol%. The 3-hydroxyvalerate content was highest in the lines accumulating the lowest amounts of P(3HB-*co*-3HV). In *B. napus*, copolymer accumulated to 1.5% per dry weight of seeds with 3 mol% 3-hydroxyvalerate. No deleterious phenotypic characteristics were consistently seen in the transgenic

FIGURE 8.17
Pathway for the synthesis of 3HB-*co*-3HV in the chloroplasts of *A. thaliana* and the seeds of *Brassica napus* introduced into these plants by transformation with appropriate constructs of *E. coli* threonine deaminase gene (*ilvA*), of the *R. eutropha* genes, *phbA*, *phbB*, and *phbC*, encoding the 3-ketothiolase, acetoacetyl-CoA reductase, and PHB synthase genes, respectively. [Slater, S., et al. (1999). Metabolic engineering of *Arabidopsis* and *Brassica* for poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) copolymer production. *Nature Biotechnology*, 17, 1011–1016.]