dehydratase enzymes, this seems borne out in a convincing manner. The enzymatic *syn* addition-elimination of water with thioester substrates is not the most chemically efficient pathway but appears to depend instead on historical contingency.

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12. We have also examined the effect of solvent on these reactions. The deuteroxide-catalyzed H-D exchange of ethyl β-hydroxybutyrate in D_2O gives a 5.7:1 ratio of the 2*R**,3*R**:2*R**,3*S** diastereomers, in a pattern consistent with our other results. The 2*R**,3*R** notation refers to the absolute configurations at C-2 and C-3 and to the fact that this diastereomer is present as a racemic mixture.
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17. The extent of reaction and product diastereoselectivity were ascertained by multiple integrations of 500-MHz ^1H NMR (D_2O) and 76-MHz ^2H NMR (H_2O) spectra, respectively. At 100°C and 0.5 M KOD, ~10% H-D exchange of one α proton on malate required 30 min; the conjugate addition was one-eighth as fast under comparable conditions. All reactions were carried out to 2 to 5% addition or 2 to 15% exchange and were done in duplicate, with at least two samples from different times examined in each reaction.
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20. Because of concomitant hydrolysis, the pH of reaction mixtures dropped substantially from pH 13 to 9 over a 30-min period; in addition reactions, we added KOD to bring the pH back to 13 in order to produce sufficient adduct. Final purification of **4** was done on silica gel with a 3% methanol–97% ethyl acetate eluant. We separated the hydroxythioester and hydroxyacid from inorganic salts by extraction of the crude lyophilized product with methanol, followed by further extraction with CH_2Cl_2 to dissolve the thioester. After purification of **4** on silica gel, ^2H NMR analysis (61 MHz) was carried out in a 3:2 or 4:1 benzene-methanol mixture, which provided a baseline between the integrated peaks (δ 2.58 and δ 2.43; C_6D_6 = δ 7.15). Exchange ranged from 15 to 37%, averaging 30% of one proton (seven reactions). Addition data were averaged from five reactions.
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Interaction of Papillomavirus E6 Oncoproteins with a Putative Calcium-Binding Protein

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Human papillomaviruses (HPVs) are associated with the majority of cervical cancers and encode a transforming protein, E6, that interacts with the tumor suppressor protein p53. Because E6 has p53-independent transforming activity, the yeast two-hybrid system was used to search for other E6-binding proteins. One such protein, E6BP, interacted with cancer-associated HPV E6 and with bovine papillomavirus type 1 (BPV-1) E6. The transforming activity of BPV-1 E6 mutants correlated with their E6BP-binding ability. E6BP is identical to a putative calcium-binding protein, ERC-55, that appears to be localized in the endoplasmic reticulum.

Infection with "high-risk" HPV, such as types 16, 18, and 31, can lead to malignancy, the most common of which is cervical cancer. Two viral transforming genes, *E6* and *E7*, are selectively retained and expressed in these cancers. Other HPVs such as types 6 and 11 are referred to as "low-risk" viruses because these are generally limited to benign genital and cervical papillomas that rarely progress to cancer. The high-risk HPV E6 genes induce immortalization of primary human epithelial cells either alone or in cooperation with *E7* [reviewed in (*1*)]. High-risk HPV E6 proteins bind the cellular factor E6-AP in vitro, and together these proteins bind and promote the ubiquitination and degradation of p53 (*2*, *3*). In cultured cells the introduction of HPV-16 E6 leads to increased p53 turnover (*4*, *5*), inhibits p53-regulated transcription (*6*, *7*), and blocks p53-induced G_1 growth arrest (*4*, *8*).

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Several observations suggest that papillomavirus E6 genes encode p53-independent transformation functions. HPV-16 E6 transforms NIH 3T3 cells but trans-dominant p53 mutants did not (*9*). We have found that HPV-16 E6 induces anchorage-independent growth of p53-deficient cells (*10*). The E6 genes from HPV-5 and HPV-8, BPV-1, and cottontail rabbit PV have oncogenic properties, yet these E6 proteins do not interact with p53 (*11*). To identify additional cellular proteins that interact with HPV-16 E6, we screened (*12*) a HeLa cell complementary DNA (cDNA) library (*13*) using the yeast two-hybrid system (*14*). After screening ~10^6 colonies on X-Gal plates, we isolated a HeLa cDNA encoding a protein referred to as E6BP (E6-binding protein) that specifically interacts with HPV-16 E6 (*12*).

Sequence analysis of the E6BP cDNA revealed a 210–amino acid open reading frame encoding a protein with four potential calcium-binding motifs, the EF hand (*15*), and a putative endoplasmic reticulum (ER) retention peptide (HDEL) at the COOH-terminus. E6BP is identical in sequence to ERC-55, a protein recently isolated on the basis of its reactivity with human auto-immune antiserum (*16*). The E6BP cDNA encodes a truncated version of