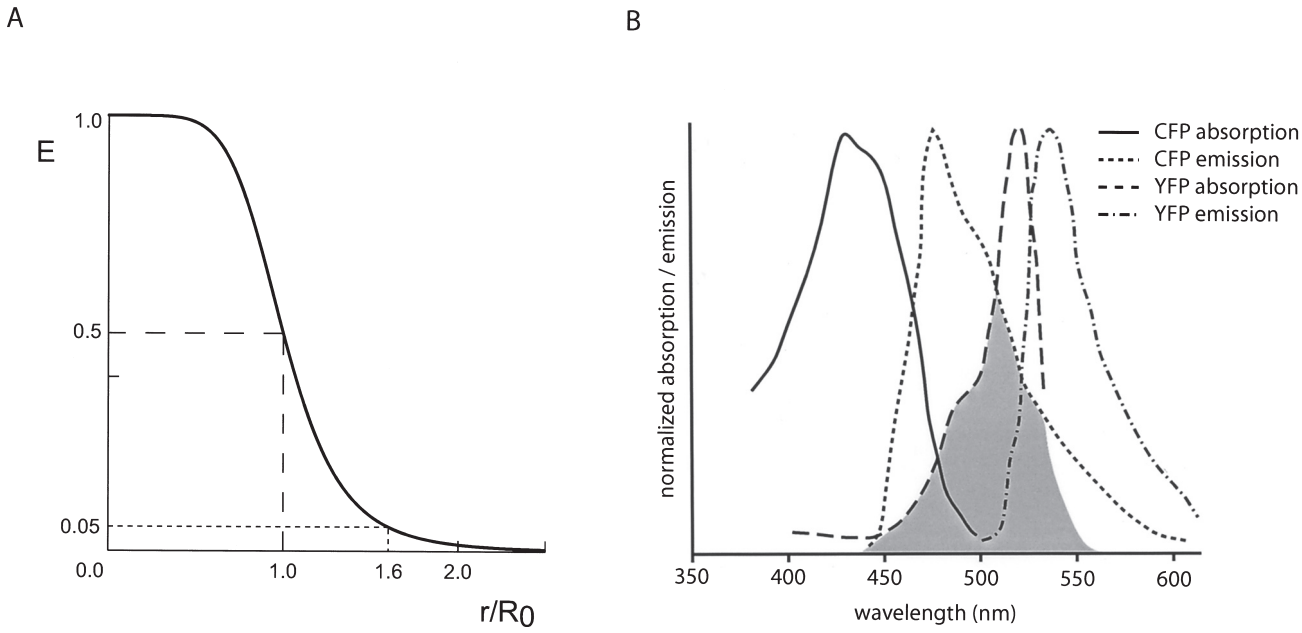

FIGURE 1. (*A*) FRET efficiency, *E*, as a function of interchromophore distance (*r*) in units of R_0 (i.e., r/R_0). Indicated are $E = 50\%$ at $r = R_0$, and $E \approx 5\%$ $r \approx 1.6 \times R_0$. (*B*) Absorption and emission spectra of CFP and YFP. The spectral overlap between the CFP emission and the YFP absorption is indicated as a shaded area. The absorption spectrum of YFP has a tail toward lower wavelength, therefore direct excitation may occur when CFP is excited. The absorption spectrum of CFP is, however, steep toward higher wavelengths, making it easy to specifically excite YFP. The fluorescence emission of CFP has a long tail at the higher wavelengths, which leads to bleedthrough when detecting the YFP emission at those higher wavelengths.