



**Figure 8.6.** The principle of FRAP. A typical FRAP curve is illustrated in (A), where  $F_i$  is the pre-bleach fluorescence intensity,  $F_\infty$  is the post-bleach steady-state fluorescence intensity, and  $F_0$  is the fluorescence intensity after photobleaching. The half time is measured at time  $\tau_{1/2}$  such that  $F(\tau_{1/2}) = (F_\infty - F_0)/2 + F_0 = (F_\infty + F_0)/2$ . The FRAP curve is usually normalized as  $f(t) = [F(t) - F_0] / [F_\infty - F_0]$ , which sets the post-bleach fluorescence intensity and post-bleach steady-state fluorescence intensity as 0 and 1, respectively. The fraction difference between pre- and post-bleach steady states is defined as the immobile fraction, which is computed as  $([F_i - F_\infty] / [F_i - F_0])$ . When there are no immobile fluorescent molecules in the ROI and the size of the ROI is small, full recovery occurs (inset of (A)). (C) FRAP data for a palmitoylation mutant of HRas tagged with GFP. The pool of fluorescent protein associated with the Golgi complex was bleached in this experiment. (D) Schematic depiction of an FRAP experiment. See text for further details.