



**FIGURE 1.** (a) Time-resolved infrared absorbance difference spectra ( $\Delta A$ ) during the intrinsic Ras-catalyzed GTPase reaction. Global fit analysis (Hessling et al. 1993) provides a single exponential function and shows the change from Ras•GTP toward Ras•GDP as seen by the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -phosphate vibrations of Ras•GTP. Changes of amide groups are observed simultaneously. (b) Time-resolved infrared absorbance difference spectra during the GAP-catalyzed GTPase reaction of Ras. The global fit is a sum of three exponential functions, giving rise to two intermediates, seen at 1143  $\text{cm}^{-1}$  and 1114  $\text{cm}^{-1}$ , respectively. At 1143  $\text{cm}^{-1}$ , appearance of GTP from caged GTP and GTP hydrolysis is observed. At 1114  $\text{cm}^{-1}$ , the protein-bound  $P_i$  appears and is released in the rate-limiting step to the external bulk medium. Compared with the intrinsic reaction, it is several orders of magnitude faster. To the right are structural models of Ras (a) and GAP•Ras (b) bound to GTP (Wittinghofer and Pai 1991).

*Protein-Protein Interactions: A Molecular Cloning Manual*, 2nd Ed., © 2005 by Cold Spring Harbor Laboratory Press, Chapter 15, Figure 1.