proton transfer. In this case, the pentavalent intermediate would be stabilized by hydrogen bonds to the side-chain amide of Gln 200, with an orientation opposite to that in $G_{t\alpha} \cdot GDP \cdot AlF_4^- \cdot H_2O$. In both mechanisms, the γ-phosphate is the ultimate general base, as proposed for $p21^{ras}$ (refs 11 and 12, and A. Wittinghofer, personal communication). Arg 174 facilitates production of the leaving group[4], and Gln 200 stabilizes the pentavalent intermediate as proposed for $p21^{ras}$ (ref. 13) and subsequently for $G_{i\alpha 1}$ (ref. 14). Finally, many of the critical interactions involved in the catalytic models presented here are facilitated by rearrangements within regions known to affect GTPase-activating protein (GAP) binding and activity[15–18]. We speculate that GAPs accelerate GTPase rates by reorienting these regions in order to stabilize the pentavalent intermediate in a manner similar to that described here.

Mutational analysis is consistent with these mechanisms. Substitution of Gln 61 in $p21^{ras}$ (Gln 200 in $G_{t\alpha}$) is a frequent mutation[19], and 17 different substitutions of Gln 61 lead to a similar ~10-fold reduction in the intrinsic GTPase rate[20]. Substitution of the homologous Gln 227 in $G_{s\alpha}$ also reduces the intrinsic rate of GTP hydrolysis and is associated with thyroid[21] and pituitary tumours[22]. Substitution of Gln 61 with either a glutamic acid residue[12] or an unnatural amino acid containing a nitro group[23] in place of the amide has little effect on the intrinsic or GAP-stimulated hydrolysis rate. These groups could partially substitute for the amide by hydrogen-bonding to a protonated phosphate. Substitutions of Gly 12 in $p21^{ras}$ (Gly 38 in $G_{t\alpha}$), conserved throughout the GTPase superfamily and the site of the most frequent oncogenic mutation in $p21^{ras}$ (ref. 19), also disrupt intrinsic GTPase activity. Like the GTP-bound formsof $p21^{ras}$ and $G_{t\alpha}$, Gly 38 in $G_{t\alpha} \cdot GDP \cdot AlF_4^- \cdot H_2O$ does not have unusual ϕ–ψ angles. However, the structure of $G_{t\alpha} \cdot GDP \cdot AlF_4^- \cdot H_2O$ shows that a β-substituent would weaken the stabilization of the pentavalent intermediate by sterically hindering the interaction of Gln 200 with a protonated, equatorial phosphate oxygen as well as the water-mediated hydrogen bond between Gln 200 and Glu 39. Other residues described as interacting with $GDP \cdot AlF_4^- \cdot H_2O$ (Gly 199 for example) are also conserved in G-protein families and affect GTPase rates[23]. In general, the ability of aluminium fluoride to affect numerous enzymes[24,25] that catalyse the hydrolysis of nucleotides, including tubulin, myosin, F_1-ATPase, hexokinase, and actin suggests that our conclusions may be applicable to many other systems. □

◀ FIG. 3 Stereochemistry of the GTPase mechanism. *a*, Interactions involving GTPγS or $GDP \cdot AlF_4^- \cdot H_2O$ at the active site of $G_{t\alpha}$ immediately relevant to the GTPase mechanism. $GDP \cdot AlF_4^- \cdot H_2O$ mimics the pentavalent intermediate; that is, the square planar arrangement of fluoride ions coordinating the Al^{3+} ion is replaced by a triangular planar arrangement of oxygen atoms surrounding the γ-phosphorus. The positively charged guanidinium group of Arg 174 would increase the transfer of negative charge from the attacking hydroxyl to the β–γ bridging oxygen, facilitating the collapse of the pentavalent intermediate to a terminal β-phosphate and a free orthophosphate. Omitted for clarity are supporting ligands found in similar conformations in both $G_{t\alpha} \cdot GTP\gamma S$ and $G_{t\alpha}GDP \cdot AlF_4 \cdot H_2O$. *b*, Concerted mechanism for GTP hydrolysis in which transfer of a proton to the γ-phosphate is coupled to the deprotonation of the attacking water. Tautomerization of Gln 200 enhances the nucleophilicity of the attacking water, reduces the negative charge of the γ-phosphate and stabilizes the pentavalent intermediate through two hydrogen bonds. *c*, Alternative mechanism for GTP hydrolysis involving direct transfer of a proton from the attacking water molecule to the γ-phosphate oxygen. This transfer is aided by the peptide NH of Gln 200 which is polarized by its contact with Arg 204. Here the pentavalent intermediate is stabilized by hydrogen bonds to a reorientated Gln 200 side-chain amide.

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ERRATUM

Interaction between two homeodomain proteins is specified by a short C-terminal tail

Martha R. Stark & Alexander D. Johnson

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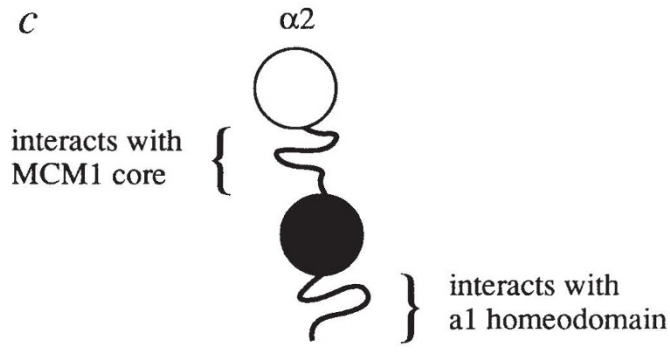

PART *c* of Fig. 1 of this Letter was accidentally omitted and is now shown here. □

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