Rotation of the ε Subunit during Catalysis by *Escherichia coli* 
F₀F₁-ATP Synthase*  

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We report evidence for catalysis-dependent rotation of the single ε subunit relative to the three catalytic β subunits of functionally coupled F₀F₁-ATP synthase. Cysteines substituted at β380 and ε108 allowed rapid formation of a specific β-ε disulfide cross-link upon oxidation. Consistent with a need for ε to rotate during catalysis, tethering ε to one of the β subunits resulted in the inhibition of both ATP synthesis and hydrolysis. These activities were fully restored upon reduction of the β-ε cross-link. As a more critical test for rotation, a subunit dissociation/reassociation procedure was used to prepare a β-ε cross-linked hybrid F₁ having epitope-tagged βD380C subunits (βᵣ₃₈₀) exclusively in the two noncross-linked positions. This allowed the β subunit originally aligned with ε to form the cross-link to be distinguished from the other two βs. The cross-linked hybrid was reconstituted with F₀ in F₁-depleted membranes. After reduction of the β-ε cross-link and a brief period of catalytic turnover, reoxidation resulted in a significant amount of βᵣ₃₈₀ in the β-ε cross-linked product. In contrast, exposure to ligands that resulted in a significant amount of ε. The rotary aspect of the binding change mechanism remained a popular but speculative idea for a number of years until a critical test became possible following the publication of a high resolution structure for F₁ (4). Focussing on a βγ inter-subunit point of contact identified in the structure, we introduced a Cys into the β subunit at a position (β380) that would place it in close proximity to a naturally occurring Cys on the γ subunit (γC87). When the resultant βD380C-F₁ was exposed to an oxidant, a rapid and specific βD380C-γC87 disulfide cross-link was formed (9, 11). Using a subunit dissociation/reassociation approach with the β-γ cross-linked enzyme, we incorporated radioisotope- or epitope-labeled β subunits into the two noncross-linked β subunit positions. Following reduction of the cross-link and a short burst of ATP hydrolysis (9, 12) or synthesis (13), labeled and unlabeled β subunits in the hybrid F₁ showed a similar capacity to form a disulfide bond with the γ subunit indicating that γ had rotated relative to the three β subunits during catalysis. Subsequently, additional evidence for subunit rotation during ATP hydrolysis was obtained using immobilized chloroplast F₁ with a spectroscopic probe attached near the C terminus of the γ subunit. Recovery of polarized absorption after photobleaching was used to monitor the rotational motion of γ during ATP hydrolysis by the tethered F₁ on a time-resolved basis (14). Finally, in a dramatic visual demonstration, a fluorescent actin filament attached to one end of the γ subunit of immobilized bacterial F₁ was seen by fluorescence microscopy to undergo multiple unidirectional rotations during ATP hydrolysis (15).
Subunit Rotation in F_{6}F_{1}-ATP Synthase

EXPERIMENTAL PROCEDURES

Materials—NADH, ATP, ADP, phosphonopyruvate, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), N,N'-dicyclohexylcarbodiimide (DCCD), 5,5'-dithiobis(2-nitrobenzoate) (DTNB) were supplied by Sigma; pyruvate kinase and lactate dehydrogenase were supplied by Boehringer Mannheim; 5,5'-dithiobis(2-nitrobenzoate) (DTNB) was supplied by Aldrich; and dithiothreitol (DTT) was supplied by American Bioanalytical (Natick, MA). Oligonucleotides for site-directed mutagenesis were synthesized by Life Technologies, Inc. Pfu DNA polymerase I was from Stratagene, and all restriction endonucleases were from New England Biolabs. Anti-Flag M2 antibody was obtained from Eastman Kodak, 125I-labeled anti-mouse antibody was from Amersham Pharmacia Biotech, and [32P]Pi was from ICN. Other reagents and chemicals were the highest grade available.

Mutagenesis and Plasmid Construction—Mutant constructs p3U-D380C/C87S and p3U-D380C/S108C were described previously, and these combined mutations have minimal effects on the F_{1} function in vivo (normal phenotypic growth on succinate) or on ATPase activity of purified F_{1} (11, 12). The eS108C mutation, originally created by Anggeler et al. (21), also has only minimal effects on the function of F_{1} in vivo or of purified F_{1}. Here a polymerase chain reaction-based, site-directed method (29) (Pfu DNA polymerase I) was used to introduce eS108C into p3U, which expresses all 8 F_{OF1} subunits. The antisense mutagenic primer 5'-TTTACAGCGTCGTCGCGCCAC-3' was a single-base change that generates the eS108C mutation and creates a Pvu II restriction site. The product was digested with Kpn I and Nde I and cloned into the corresponding sites of the p3U-D380C/C87S vector to produce p3U-D380C/C87S/eS108C. The mutated region of uncC was sequenced to confirm the presence of eS108C and absence of any additional mutations. To express mutant F_{1}, each construct was transformed into strain AN1460, which blocks the insertion of the chromosomal unc operon (30). Expression of p3U-D380C/C87S/eS108C yields membranes with normal levels of DCCD-sensitive ATPase and purified F_{1} with ATPase activity (35 μmol·min^{-1}·mg^{-1}) comparable with that of wild-type F_{1}.

Preparation of E. coli Membranes and Soluble F_{1}—Membranes were isolated and washed (31, 32), and soluble F_{1} was purified as described (9). Membranes prepared from strain AN1460 (33) were depleted of F_{1} with two additional washes with 10 mM Tris acid, 1 mM EDTA, pH 8.0. Washed membranes were resuspended in TM buffer (50 mM Tris·Cl, 5 mM MgSO_{4}, pH 7.5), quickly frozen, and stored at -70 °C.

Preparation of Hybrid F_{1} and Reconstitution with F_{1}-depleted Membranes—Aliquots of F_{1} stock solutions were passed through centrifuge columns containing Sephadex G-50-50 (34) equilibrated with TM buffer (20 mM Mops-Tris, 50 mM KCl, pH 8.0). For measurement of membrane-bound F_{1} from cross-linked membranes, [32P]Pi was used to label the presence of a transmembrane proton gradient and to block NADH oxidation by the respiratory chain. The synthesis of ATP by E. coli membranes was determined by a coupled enzyme assay as described (13). Protein concentrations were determined by a modified Lowry assay (41).

RESULTS

Disulfide Bond Formation between βD380C and eS108C in the Triple Mutant, βD380C/C87S/eS108C-F_{1}—Our approach in testing for rotation of the e subunit requires the reversible formation of a specific covalent linkage between a β subunit and the single copy of e. Guided by previous cross-linking studies (24, 42), we combined the eS108C and βD380C mutations in a single construct. To prevent cross-linking between β and γ subunits, the γC87S mutation was also included (11). As shown by nonreducing SDS-PAGE (Fig. 1), a rapid and near complete disappearance of e is accompanied by the appearance of a new band at 67 kDa when F_{1} (lane 2 versus lane 1) or membrane-bound F_{1} (lane 7 versus lane 6) containing these mutations is oxidized by DTNB. The high yield of cross-linked product correlates to a 90–95% loss of ATPase activity, and the same results were obtained when samples were oxidized in the presence of Mg^{2+}, MgATP, or MgADP/azide (data not shown). The apparent size of the cross-linked product (67 kDa) is consistent with the predicted molecular mass of 65 kDa for a 1:1 complex between β and e. Furthermore, immunoblotting confirmed the presence of both e and β in the 67-kDa band (data not shown). As expected for a disulfide linkage, cross-linking and inactivation are fully reversed by brief exposure to dithiothreitol (lane 3). Finally, oxidation of F_{1} lacking the eS108C mutation shows no 67-kDa band nor does the e band disappear (lane 4). We conclude that the 67-kDa product results from formation of a specific disulfide cross-link between βD380C and eS108C.

Exchange of e Subunits between Soluble F_{1} Complexes—A second requirement for our F_{1}-hybrid approach in testing for rotation of e is that e must not dissociate from F_{1} during the course of the experiment. If this occurred, e could rebind in a manner that would allow it to cross-link to a different β than the one with which it was originally aligned, thus giving a false indication of subunit rotation. This requirement presented a potential problem in using soluble F_{1} because e is known to undergo reversible dissociation from the E. coli enzyme (43). Hence, the experiment presented in Fig. 2 was conducted to...
determine the rate of $\epsilon$ subunit exchange between members of an F$_1$ population. The strategy behind this assay was to mix two different forms of the enzyme: one containing SC-F1 and the other containing epitope-tagged $\beta$ and wild-type $\epsilon$ (B$_{\text{wild}}$D380C/\gammaC87S-F$_1$). Because wild-type $\epsilon$ cannot cross-link to $\beta_{\text{flag}}$, the only way that a $\beta_{\text{flag}}^{-}\epsilon$ cross-link can form is if an SC-F1 subunit dissociates from the triple mutant enzyme and rebinds to a Flag-tagged double mutant F$_1$ that has released its own wild-type $\epsilon$. DTTN was added at the times indicated, and the Flag epitope in the cross-linked product was measured in the immunoblot shown in Fig. 2A. As expected, the amount of Flag in the $\beta$-band increased with time. The $t_{1/2}$ for the exchange of $\epsilon$ was found to be about 1 min (Fig. 2B). Because this is on the same time scale as our assay for subunit rotation, we conclude that the soluble E. coli enzyme is unsuitable for this test. In contrast, $\epsilon$ does not dissociate from F$_1$F$_0$ (as confirmed below in Fig. 3, lane 8) and in fact is required for binding F$_1$ to F$_0$ (44). Hence, tests for $\epsilon$ subunit rotation were conducted with reconstituted hybrid F$_1$F$_0$.

Rotation of $\epsilon$ Relative to the $\beta$ Subunits during ATP Hydrolysis by F$_1$F$_0$—Preliminary studies (not shown) confirmed that DTNB-treated B380C/\gammaC87S/eS108C-F$_1$ can be dissociated into subunits, reassembled, and reconstituted with F$_0$ in F$_1$-depleted membranes without breaking the B380C-eS108C disulfide bond. Membrane-bound, $\beta$-$\epsilon$ cross-linked F$_1$, is catalytically inactive. However, treatment with DTT to reduce the disulfide cross-link restores ATP hydrolysis (9.5 $\mu$mol min$^{-1}$ mg$^{-1}$) and synthesis (30 nmol min$^{-1}$ mg$^{-1}$) activities. Furthermore, when reconstituted membranes were preincubated with DCCD, the hydrolysis and synthesis of ATP were inhibited by 80% and >98%, respectively. These results demonstrate that cross-linked B380C/\gammaC87S/eS108C-F$_1$ can rebind to F$_0$ to form an F$_1$F$_0$ complex that is functionally coupled following reduction of the disulfide.

Using a subunit dissociation/association procedure developed previously (9), we formed a $\beta$-$\epsilon$ cross-linked hybrid F$_1$ containing $\beta_{\text{flag}}$D380C subunits exclusively in the two non-cross-linked $\beta$ positions. The resulting hybrid provides a means of distinguishing the $\beta$ subunit that is initially oriented to allow cross-linking to $\epsilon$ from the other two $\beta$ subunits. Hybrid F$_1$ was rebound to F$_1$-depleted membranes, and excess soluble F$_1$ was removed. To test for possible rotary movement of $\epsilon$, the reconstituted membranes were briefly reduced with DTT, exposed to various ligands, and reoxidized with DTNB. In the absence of rotation, eS108C would be expected to cross-link to the original unlabeled $\beta$ subunit. However, if $\epsilon$ has rotated, eS108C would be positioned to cross-link to Flag epitope-labeled $\beta$ in a significant fraction of the $\epsilon$ will be detected in the $\beta$-cross-linked band following reoxidation (Fig. 3, lane 4). In contrast, when reconstituted membranes were reduced and exposed to ligands that bind to catalytic sites but do not allow catalytic turnover, little Flag epitope was found in the $\beta$-bands (Fig. 3, lanes 5 and 7). The amount of epitope in the $\beta$-band was also low when membranes were reduced and reoxidized in the presence of MgADP but absence of azide (not shown).

An important control (the "nonhybrid" control) is shown in Fig. 3, lane 8. For this experiment, cross-linked B380C/\gammaC87S/eS108C-F$_1$ and $\beta_{\text{flag}}$380C/\gammaC87S-F$_1$ remained separated during subunit dissociation/association and were mixed in a 1:1 ratio only after reconstituting F$_1$F$_0$. The resulting sample was then reduced, exposed to MgATP, and reoxidized. The absence of $\beta_{\text{flag}}$ in the $\beta$-band (lane 8) excludes the
were pretreated with DCCD to modify FO as described under "Experimental Procedures." Membranes were suspended at 4 mg of protein/ml in buffer containing 50 mM Tris acetate, 5 mM MgSO₄, 50 mM glucose, 5 µM FCCP, pH 7.5 (TMGF buffer) and subjected to the following treatments. Lane 5, membranes were incubated with 10 mM DTT for 30 s, passed through a centrifuge column equilibrated with TMGF buffer, and collected in a tube containing DTNB (50 µM final), and incubated for 10 min at 22 °C. Lanes 4 and 7, same as for lane 5 except that DTT was omitted. Lane 2, same as for lane 5 except that the column effluent was collected in the absence of DTNB. Lane 6, same as for lane 4 except that membranes were pretreated with DCCD to modify FO as described under "Experimental Procedures." Lane 3, same as for lane 4 except that FO/F₁ was reconstituted using F₁ that could have β₅₋₆ at all three β positions (see "Experimental Procedures"). Lane 8, as for lane 4 except that FO-depleted membranes were reconstituted separately with βD380C/γC87S/εS108C-F₁, or β₅₋₆D380C/γC87S-F₁, and then mixed in a 1:1 ratio. Hexokinase was included at 3 units/ml in samples 1, 2, 5, and 7 to prevent any potential ATP hydrolysis. The uncoupler FCCP was present in all samples to prevent formation of a transmembrane proton gradient. An aliquot of each sample containing 6 µg of membrane protein was subjected to SDS-PAGE under nonreducing conditions, and immunoblotting was performed as described under "Experimental Procedures." The results shown are typical of three replicate experiments.

To determine the maximal level of Flag epitope expected in the β-ε band if εS108C has an equal chance of reacting with any of the three β subunits following catalytic turnover, a noncross-linked control was run (Fig. 3, lane 3). This case, βD380C/γC87S/εS108C-F₁, was dissociated in the presence of a source of MgATP. Of notable significance is the fact that very little β₅₋₆ appeared in the β-ε band in the absence of catalytic turnover (Fig. 4, 5–7%)

FIG. 3. Reorientation of ε relative to the β subunits in FO/F₁ during catalytic turnover. The presence of Flag epitope in the β-ε band following various treatments was detected by immunoblotting. For lanes 1, 2, and 4–7, β-ε cross-linked hybrid F₁, having Flag-tagged β exclusively in the two noncross-linked β positions was prepared and reconstituted with FO-depleted membranes as described under "Experimental Procedures." Membranes were suspended at 4 mg of protein/ml in buffer containing 50 mM Tris acetate, 5 mM MgSO₄, 50 mM glucose, 5 µM FCCP, pH 7.5 (TMGF buffer) and subjected to the following treatments. Lane 5, membranes were incubated with 10 mM DTT for 30 s, passed through a centrifuge column equilibrated with TMGF buffer, and collected in a tube containing DTNB (50 µM final), and incubated for 10 min at 22 °C. Lanes 4 and 7, same as for lane 5 except that DTT was omitted. Lane 2, same as for lane 5 except that the column effluent was collected in the absence of DTNB. Lane 6, same as for lane 4 except that membranes were pretreated with DCCD to modify FO as described under "Experimental Procedures." Lane 3, same as for lane 4 except that FO/F₁ was reconstituted using F₁ that could have β₅₋₆ at all three β positions (see "Experimental Procedures"). Lane 8, as for lane 4 except that FO-depleted membranes were reconstituted separately with βD380C/γC87S/εS108C-F₁, or β₅₋₆D380C/γC87S-F₁, and then mixed in a 1:1 ratio. Hexokinase was included at 3 units/ml in samples 1, 2, 5, and 7 to prevent any potential ATP hydrolysis. The uncoupler FCCP was present in all samples to prevent formation of a transmembrane proton gradient. An aliquot of each sample containing 6 µg of membrane protein was subjected to SDS-PAGE under nonreducing conditions, and immunoblotting was performed as described under "Experimental Procedures." The results shown are typical of three replicate experiments.

It is well known that covalent modification of one or more c subunits by DCCD blocks proton translocation through FO, and inhibits both ATP synthesis and hydrolysis by FO/F₁ (37). In addition, we recently reported that DCCD modification of FO prevents the catalysis-dependent rotation of the γ subunit in membrane-bound FO/F₁ (12, 13). A similar experiment was carried out here to test for functional coupling between ε rotation in F₁ and proton conduction through FO. For this purpose, membranes were treated with DCCD under conditions that selectively modify the c subunits of FO. As shown in Fig. 3 (lane 6) and Fig. 4 (+DCCD), exposure of DCCD-inhibited membranes to MgATP yielded only 3% of the calculated maximal amount of Flag epitope in the β-ε band. This emphasizes the tight functional coupling of subunit rotation in F₁ to proton translocation through FO.

DISCUSSION

The results presented demonstrate catalysis-dependent rotation of the ε subunit in functionally coupled membrane-bound FO/F₁. In view of earlier evidence for the rotation of γ in F₁ (9, 14, 15) and in FO, F₁ (12, 13), we conclude that γ and ε constitute part of the rotor that couples proton transport through FO to the required binding changes in F₁. The fact that DCCD modification of subunit c in FO prevents catalysis-dependent rotation of γ (12, 13) and ε (Fig. 3, lane 6) supports the possibility that subunit rotation in F₁ is coupled to subunit rotation in FO. However, it remains to be determined whether the ε subunit complex of FO constitutes the remaining portion of the rotor (8, 9, 14).

The intact E. coli FO/F₁ complex was used in these studies to avoid two potential problems that might have been encountered with soluble F₁. The first relates to the well established ability of ε to inhibit catalysis when F₁ is separated from FO (43). This could hinder attempts to detect catalysis-dependent subunit rotation, particularly if ε inhibits by preventing the rotation of γ. A second difficulty could arise from the fact that ε readily dissociates from F₁. As noted earlier, when monitoring the orientation of ε relative to the three β subunits as a means of detecting subunit rotation, it is essential to rule out dissociation and recombination of ε as an alternative cause for its reorientation. The use of FO/F₁ avoided both of these problems because in the native complex, ε does not inhibit activity (45) nor does it dissociate from the complex (Fig. 3, lane 8).
The $t_{1/2}$ for exchange of $\epsilon$ between $\beta$D380C/$\gamma$C87S/$\epsilon$S108C-$F_1$ and $\beta_{\text{mut}}$D380C/$\gamma$C87S-$F_1$ molecules was found to be about 1 min (Fig. 2), whereas at the same temperature, the $t_{1/2}$ for dissociation of $\epsilon$ from our wild-type $F_1$ is about 5 min. This suggests that these mutations weaken the interaction of $\epsilon$ with $F_1$ resulting in an increased dissociation rate. This is not surprising in view of the fact that $\beta$D380 is in close contact with $\gamma$C87 and $\epsilon$S108 as evidenced by the facility with which substituted cysteines can cross-link. The results imply that one or more of the native residues contributes to the stability of the interaction of $\epsilon$ with the rest of $F_1$.

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REFERENCES

1. Boyer, P. D. (1997) Annu. Rev. Biochem. 66, 717–749
2. Fillingame, R. H. (1997) J. Exp. Biol. 200, 217–224
3. Fillingame, R. H., Jones, P. C., Jiang, W., Venkatesan, F. I. & Dmitriev, O. Y. (1998) Biochim. Biophys. Acta 1365, 135–142
4. Abrahams, J. P., Leslie, A. G., Lutter, R. & Walker, J. E. (1994) Nature 370, 621–628
5. Boyer, P. D., Cross, R. L. & Momsen, W. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2837–2839
6. Kayalar, C., Rosing, J. & Boyer, P. D. (1977) J. Biol. Chem. 252, 2486–2491
7. Boyer, P. D. & Kohlbrener, W. E. (1981) in Energy Coupling in Photosynthesis (Selman, B. & Selman-Reiner, S., eds) pp. 231–240, Elsevier/North-Holland, New York
8. Vik, S. B. & Antonio, B. J. (1994) J. Biol. Chem. 269, 30364–30369
9. Duncan, T. M., Bulygin, V. V., Zhou, Y., Hutcheon, M. L. & Cross, R. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10964–10968
10. Hatch, L. P., Cox, G. B. & Howitt, S. M. (1995) J. Biol. Chem. 270, 29407–29412
11. Duncan, T. M., Zhou, Y., Bulygin, V. V., Hutcheon, M. L. & Cross, R. L. (1995) Biochim. Biophys. Acta 1275, 96–100
12. Zhou, Y., Duncan, T. M., Bulygin, V. V., Hutcheon, M. L. & Cross, R. L. (1996) Biochim. Biophys. Acta 1275, 96–100
13. Zhou, Y., Duncan, T. M. & Cross, R. L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10583–10587
14. Sabbert, D., Engelbrecht, S. & Junge, W. (1996) Nature 381, 623–625
15. Noji, H., Yasuda, R., Yoshida, M. & Kinosita, K., Jr. (1997) Nature 386, 299–302
16. Junge, W., Lill, H. & Engelbrecht, S. (1997) Trends Biochem. Sci. 22 420–423
17. Dimroth, P., Ruim, G. & Matthey, U. (1998) Biochim. Biophys. Acta 1365, 87–92
18. Elston, T., Wang, H. & Oster, G. (1998) Nature 391, 510–513
19. Kinosita, K., Jr., Yasuda, R., Noji, H., Ishiwata, S. & Yoshida, M. (1998) Cell 93, 21–24
20. Dunn, S. D. (1992) J. Biol. Chem. 257, 7354–7359
21. Aggeler, R., Cherfas-Cruz, K., Cai, S.-X., Keana, J. F. W. & Capaldi, R. A. (1992) Biochemistry 31, 2956–2961
22. Watts, S. D., Tang, C. & Capaldi, R. A. (1996) J. Biol. Chem. 271, 28341–28347
23. Schulenberg, B., Wellner, F., Lill, H., Junge, W. & Engelbrecht, S. (1997) Eur. J. Biochem. 249, 134–141
24. Aggeler, R., Haughton, M. A. & Capaldi, R. A. (1995) J. Biol. Chem. 270, 9185–9191
25. Aggeler, R. & Capaldi, R. A. (1996) J. Biol. Chem. 271, 13888–13891
26. Aggeler, R., Ogilvie, I. & Capaldi, R. A. (1997) J. Biol. Chem. 272, 19621–19624
27. Hasler, K., Engelbrecht, S. & Junge, W. (1998) FEBS Lett. 426, 301–304
28. Kato-Yamada, Y., Noji, H., Yasuda, R., Kinosita, K., Jr. & Yoshida, M. (1998) J. Biol. Chem. 273, 19775–19777
29. Landt, O., Grunert, H.-P. & Hahn, U. (1990) Gene (Amst.) 96, 125–128
30. Gibson, F., Downie, J. A., Cox, G. B. & Radik, J. (1978) J. Bacteriol. 134, 728–736
31. Senior, A. E., Faye, D. R. H., Downie, J. A., Gibson, F. & Cox, G. B. (1979) Biochem. J. 180, 111–118
32. Wise, J. G. (1990) J. Biol. Chem. 265, 10403–10409
33. Downie, J. A., Langman, L., Cox, G. B., Yanofsky, C. & Gibson, F. (1980) J. Bacteriol. 143, 8–17
34. Penefsky, H. S. (1977) J. Biol. Chem. 252, 2891–2899
35. Vogel, G. & Steinhart, R. (1976) Biochemistry 15, 208–216
36. Tommasino, M. & Capaldi, R. A. (1985) Biochemistry 24, 3972–3976
37. Hermolin, J. & Fillingame, R. H. (1989) J. Biol. Chem. 264, 3896–3903
38. Laemmli, U. K. (1970) Nature 227, 680–685
39. Towbin, H., Staehelin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
40. Pullman, M. E., Penefsky, H. S., Datia, A. & Racker, E. (1960) J. Biol. Chem. 235, 3322–3329
41. Peterson, G. L. (1977) Anal. Biochem. 83, 346–356
42. Dallmann, H. G., Flynn, T. G. & Dunn, S. D. (1992) J. Biol. Chem. 267, 18693–18696
43. Smith, J. B. & Sternweis, P. C. (1977) Biochemistry 16, 306–311
44. Dunn, S. D. & Futai, M. (1980) J. Biol. Chem. 255, 111–118
45. Sternweis, P. C. & Smith, J. B. (1980) Biochemistry 19, 526–531

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