The Role of the Leader Sequence Coding Region in Expression and Assembly of Bacteriorhodopsin*

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Bacteriorhodopsin is made as a precursor in Halobacterium halobium, which has 13 additional residues at the amino terminus. The codons for these residues have been proposed to form a hairpin structure in the mRNA and play a role in ribosomal binding; the leader peptide sequence also has been proposed to have a role in membrane insertion of bacteriorhodopsin (BR). We have made mutations in the leader gene region coding for the leader sequence and expressed the mutant genes in an H. halobium mutant lacking wild-type BR. The leader sequence coding region was found to be important for the stability of the mRNA and for its efficient translation. Single base substitutions in this region that did not affect the amino acid sequence caused significant reductions in protein expression. Deletion of the leader region resulted in unstable mRNA and almost no BR production. Introduction of a new ribosome-binding sequence within the coding region of the mature protein restored mRNA stability and some protein expression. Protein made without the leader peptide was properly assembled in the membrane.

Bacteriorhodopsin (BR) is a light-driven proton pump in the membrane of the archaeon Halobacterium halobium (also known as Halobacterium salinarum). Bacterio-opsin (BO), the apoprotein without the retinal chromophore, is made as a precursor that has 13 additional residues at the amino terminus. These residues, as well as one at the carboxyl terminus, are removed after membrane assembly. BR, the leader sequence, does not have the positive amino-terminal and hydrophobic domain characteristic of both prokaryotic and eukaryotic signal sequences and is also too short to span the bilayer. Nevertheless, it has been proposed to have a role in the insertion of BR into the halobacterial membrane. Mature BR lacking this sequence inserts spontaneously into phospholipid bilayers in vitro (5–9), and BO synthesized in vitro in a wheat germ system is integrated into dog pancreas microsomes equally well both with and without the leader sequence (10). These processes may, however, be different from what occurs in the H. halobium cell. The mRNA transcribed from the bop gene coding for BO, as well as mRNAs for some other H. halobium proteins, starts very close to the initiation codon and has been proposed to form a stem and loop secondary structure containing a sequence complementary to the H. halobium 16S rRNA (Fig. 1) that could act as a ribosome-binding site within the coding region for the protein (1, 11, 12). Our experiments have confirmed this role for the 5′ end of the bop mRNA that codes for the leader sequence, but we have found no requirement for the leader sequence itself in membrane insertion or assembly of BR.

EXPERIMENTAL PROCEDURES
Materials—Lovastatin (also known as mevinolin) was a gift from Dr. A. W. Alberts (Merck Sharp and Dohme). Nspl (Nsp7524 I) was obtained from Amersham Corp. Recombinant DNA procedures were carried out as described in Ref. 13 or 14 using enzymes and kits available from various commercial sources except as described below.

Plasmid Constructions—The gene from H. halobium strain ET1001 (provided by Dr. R. Bogomolni, UC Santa Cruz) was cloned by probing a library of size-selected BamHI–NotI genomic fragments in pBS/KS+ (Stratagene, La Jolla, CA) with the oligonucleotide 5′-CGCGCATCCCTTTTCCAGG. One of the positive clones was sequenced, and the bope coding region was found to be identical to the previously published sequence from an unidentified strain (1). The BamHI–NotI fragment containing the bope gene was excised from the vector and ligated to the BamHI and HindIII sites of plUC19 (15) using the adapter oligonucleotides 5′-GGCGCGACGCGGACTGATCTAGA and 5′-GAGCTTCTAGAAGTGCCCGCATG were used to amplify the region of the leader sequence and the first few amino acids of the mature protein with synthetic linkers. The oligonucleotides 5′-CCGGTGATCTGGGCCTGCATG were used to make pXU10 (see Fig. 2), and pXU10A was made using 5′-AAGTCTCGAGTACGGAGGCATG and 5′-CCGGTACCTGGAAGCGTTCATG. Single base substitutions in the leader sequence coding region were made by amplifying the region of the bope gene from base 356 to 878 (numbering according to GenBank entry HBA0, where base 361 is the A of the ATG initiation codon) using the polymerase chain reaction with a mutagenic primer for the coding strand and the primer 5′-GCTTGGAGTACGGAGCCCG for the complementary strand. The sense strand primers were 5′-GTTGAGTACGGAGTTATGC (Gp7)

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‡ The abbreviations used are: BR, bacteriorhodopsin; BO, bacterio-opsin; kb, kilobase pair(s); PAGE, polyacrylamide gel electrophoresis.

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spheroplasting solution; mixing was accomplished by gently tilting the tube back and forth. After 20 min of additional incubation at room temperature, 1 ml of regeneration salt solution was added. Cells were pelleted in a microcentrifuge at 4,000 rpm for 5 min, then resuspended in 1 ml of complex media (19) containing 15% sucrose, and incubated 18 h at 37°C. 100-μl samples were spread on plates containing complex media and 15% sucrose without drug and incubated for 5 days at 40°C. The bacteria were then harvested from the plates and spread on plates containing complex media and 16 μg/ml lovastatin. Alternatively, the transformed cells were incubated 2-3 days in complex media with sucrose and then spread directly on plates with drug. After about 10 days of incubation at 40°C, colonies were picked and grown in complex media with 16 μg/ml lovastatin, and their DNA and proteins were analyzed.

Southern Blots—Chromosomal DNA was isolated from H. halobium by a procedure similar to methods used for bacteria (13, 14) involving lysis in 0.25 M Tris, 0.05 M EDTA, pH 8, followed by digestion with RNase A followed by digestion with proteinase K in the presence of 1% SDS, extractions with phenol, phenol/CHCl₃, CHCl₃, and ether, and two ethanol precipitations. H. halobium DNAs were digested with either Smal or BamHI + Notl and run on 0.8% agarose gels in TAE buffer (13). The separated fragments were transferred to nitrocellulose or nylon membranes by Southern blotting (13), and the DNA was fixed to the membranes by microwaving the dried membranes for 2 min on "high". Blots were hybridized first with a probe made from the BamHI-Xbal fragment containing the bop gene (see above) by nick translation or random priming using [α-32P]dCTP. Following autoradiography the blots were stripped and then reprobed with the synthetic dinucleotides that had been used to make the mutation, which were labeled using [γ-32P]ATP and T4 polynucleotide kinase. The second hybridization was carried out at 50–60°C, and the washes that followed were in 6× SSC at 37°C. Under these stringent conditions, the probe did not bind to wild-type bop DNA sequences on control lanes of the blot.

Northern Blots—H. halobium mRNA was isolated by a procedure recommended for Gram-positive bacteria (13). RNAs were separated on 1% agarose gels containing formaldehyde and transferred to nitrocellulose by Northern blotting (13). The blots were probed with the same nick-translated or random-primed bop gene probes used for the Southern blots.

Quantitation of BR—BR makes up a substantial fraction of the membrane protein in the H. halobium strains used in this study when they have a functional copy of the wild-type bop gene; the yield of BR upon purification by standard procedures (19) is dependent on the level of BR expression, because the formation of purple membrane patches requires a high density of BR in the membrane. We therefore quantitated the amounts of BR in the membranes of various mutant strains by looking at the amount of BR relative to other proteins and chromophores in crude membrane preparations. H. halobium strains were grown for 3 days after reaching stationary phase, and equivalent numbers of cells were used to prepare membranes as follows. Cell suspensions (25-50 ml) were centrifuged at 3000 × g for 10 min; the cells were then lysed by resuspending the pellet in 2 ml of H₂O and 0.1 mg DNase I and sonicating for 1–2 min using a cup horn. Membranes were pelleted by centrifugation for 1 h at 180,000 × g; the membrane pellet was resuspended in 2 ml of distilled H₂O by sonication, centrifuged twice, and then resuspended in 0.5–1 ml of H₂O for spectrophotometric analysis.

Spectra were obtained on a Varian Cary 3 at room temperature; the absorbance at 410 nm due to cytochromes in the membranes served as an internal standard to adjust the concentration of membranes used in each sample. Aliquots were also analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and staining with Coomassie Blue or silver. In some cases proteins were transferred to polyvinylidene difluoride membranes by Western blotting and probed with rabbit antibodies to BR, followed by goat anti-rabbit Ig conjugated to HRP and then color development using 4-chloronaphthol and H₂O₂. The soluble fraction of the cell lysate was also examined by these methods.

Pulse-Chase Experiments—50-ml cultures of H. halobium were grown to an A₆₀₀ of 1.0 under fluorescent lights in complex media and 4 g/l lovastatin. The cells were pelleted at 3,000 × g for 10 min and resuspended in 10 ml of basal salts (medium without peptide; Ref. 19) and 2.5 mg/ml l-alanine in a 15-ml conical tube. The tubes were rotated at 40°C under fluorescent lights for 2 h; then 2 μC of [35S]-methionine (800 Ci/mmol) was added. After 2 h of incubation, a 2-ml aliquot was removed (pulse) and unlabelled l-methionine was added to the remaining 8 ml at a final concentration of 5 mg/ml. Incubation was continued and additional 2-ml samples were removed from 2 to 48 h after addition of the chase. Each aliquot removed was immediately spun down at 5,000 × g for 5 min; the cells were lysed by

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**FIG. 1.** Secondary structures and ribosome-binding sites that have been proposed at the 5' end of halobacterial mRNAs. bop, bacterio-opsin (1); brp, bacterio-opsin related protein (12); hop, halo-opsin (11).

**A**

Wild-type: 5'-ACUGUGGAGGAGGGUA----3' A) for pXU10, and 5'-GTGGCTATGGGAATTTGCCC (G³⁶⁶ → A) for pXU14, and 5'-GTGGCTATGGGAATTTGCCC (G³⁶⁶ → A) for pXU15 (see Fig. 5). Amplifications were done for 30 cycles (1 min at 94°C, 1 min at 55°C, and 2 min at 72°C) using Pfu Polymerase (Stratagene) and pBO6 as a template in a variety of different thermal cyclers. The polymerase chain reaction amplification products were cut with KpnI and BamHI–XbaI fragments of pXU15 together with the KpnI–NspI and KpnI–XbaI fragments of the wild-type bop gene in pXU3. Mutations were confirmed by sequencing the plasmids prior to transformation of H. halobium.

Transformation—H. halobium strain SD9 and SD16 (17), which have a 1-kb ISH1 and a 0.5-kb ISH2 insertion, respectively, in the coding region of the bop gene, were transformed using the plasmids described above. The transformation procedure was essentially as described in Ref. 18. Briefly, 20 ml of log phase cells were spun down and resuspended in 2 ml of spheroplasting solution; 0.2-ml aliquots were added to 20 μl of 0.5 M EDTA in spheroplasting solution and gently agitated; this converted the cells to spheroplasts in less than 10 min for the strains we used. After 15 min, 5–10 μg of DNA in 20 μl was added, followed 5 min later by 240 μl of 60% polyethylene glycol 600 in

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**FIG. 2.** A, DNA and encoded protein sequences for the wild-type bop gene and the leader sequence deletion mutants genes in pXU10 and pXU10A. Bold characters show the sequence of the mature protein. B, proposed secondary structure for the 5' end of the mRNA in mutant XU10A.
addition of 0.5 ml of\( \text{H}_2\text{O} \) and 0.1 ml 2.5 mg/ml DNase I and sonicated for 1 min at 0°C in a cup horn. Membranes were pelleted by centrifugation at 180,000 \( \times g \) for 30 min at 4°C. Pellets were resuspended in 50 \( \mu\text{l} \) of \( \text{H}_2\text{O} \); 125 \( \mu\text{l} \) of acetone (−20°C) were added, mixed, and centrifuged at 16,000 \( \times g \) for 30 min at 4°C. The final pellet was resuspended and boiled for 5 min in 10 \( \mu\text{l} \) of gel loading buffer containing 2% SDS. Samples were electrophoresed on 17.5% acrylamide gels, which were fixed or stained and dried, and then exposed to a PhosphorImager cassette for 6–15 days. The phosphor screens were scanned in a Molecular Dynamics 400Е PhosphorImager and analyzed using ImageQuant software.

**RESULTS**

Transformation of \( \text{H. halobium} \) Strains—In order to determine the role of the leader sequence in BR expression and membrane assembly, mutant bop genes had to be expressed in a \( \text{H. halobium} \) strain that did not make wild-type BR. Strains SD16 and SD9 (17) were chosen for this purpose. SD16 has a \( \text{H. halobium} \) membrane assembly, mutant mine the role of the leader sequence in BR expression and (Fig. 3). The blot was probed with a random-primed bop gene fragment, B, the same blot was stripped and re-probed with an oligonucleotide specific for the mutant sequence in pXU10A. DNA was from wild-type cells transformed with the following plasmids: Lanes 1, pXU3 (wild-type bop gene); lanes 2, pWL102 (vector alone); lanes 3, pXU10; lanes 4 and 5, pXU10A (1.2-kb band).

In order to maximize the odds of obtaining transformants with functional mutant genes and inactivated wild-type genes, transformations with mutant bop genes were done using strain SD9. Because the ISH1 insertion is near the location of all the mutations introduced into the bop gene on the plasmids, recombination between the plasmid and the chromosome both upstream of the mutation (in the 5′-flanking region) or downstream of the mutation (and of the ISH1 insertion in the chromosome) would result in the mutant gene remaining intact. SD9 cells transformed with the various plasmids containing wild-type and mutant bop genes were analyzed using Southern blots of BamHI + NotI digests that were first probed with a random-primed bop gene and then stripped and reprobed with an oligonucleotide specific for the mutant sequence. In each case the smaller (1.2 kb) band containing the uninterrupted bop gene hybridized to the mutant probe, whereas the larger (2.3 kb) band containing the ISH1 insertion did not. This is shown for two transformants with pXU10A in Fig. 3. Transformations of SD16 with mutant bop genes, on the other hand, frequently resulted in regeneration of a wild-type bop gene.

The Leader Sequence Coding Region Is Required for BR Expression—SD16 and SD9 cells transformed with pXU3 (the latter is referred to as strain XU3 below) produced BR at levels comparable to that of strain S9, the BR-overproducing strain from which SD9 and SD16 had been derived (17). However, SD9 cells transformed with pXU10 (strain XU10), the leader sequence deletion mutant (Fig. 2), did not produce BR at levels detectable by SDS-PAGE using Comassie Blue or silver staining or by examination of the visible spectrum of membranes from XU10 cells (not shown). Western blots of XU10 membranes showed extremely faint bands that co-migrated with BR, which could not be reproduced photographically.

In order to determine whether this lack of BR expression was due to lack of transcription of the bop gene with the deletion or lack of translation of the mRNA, RNA was isolated from XU10 and XU3 cells and analyzed by Northern blotting (Fig. 4). The bop mRNA in XU10 cells was found to be degraded to low molecular weight fragments that nevertheless still hybridized to the bop probe; the XU3 cells were always found to have only intact bop mRNA with the expected molecular weight as shown in Fig. 4.

Point Mutations in the Proposed Ribosome-binding Site Affect BR Expression—In order to test the theory that the bases coding for the leader peptide at the 5′ end of the bop mRNA also act as the ribosome-binding site and form a hairpin secondary structure, three point mutations were introduced in this part of the sequence (Fig. 5). In each case a single G in pXU3 was
Northern blots showed that the bop mRNA in XU13, XU14, and XU15 was the same size as in XU3 and was not degraded like that of XU10 (Fig. 4). The variations in intensity between the bands were probably due to differences in yield during RNA isolation and were reflected in the intensity of the total RNA staining with ethidium bromide in each lane on the gel used for the Northern blot (not shown).

Introduction of a New Ribosome-binding Site in the Leader Deletion Mutant Restores mRNA Stability and Some BR Expression—In order to test further the hypothesis that the coding region for the leader sequence at the 5′ end of the mRNA functions as a ribosome-binding site and is important for mRNA stability, we constructed a new mutant, designated XU10A. This mutant had the same deletion of the codons for the leader sequence as XU10 and additional mutations in the region coding for the first four residues of the mature protein that resulted in the 5′ end of the mRNA again having complementarity to the 16S rRNA and the potential to make a stem and loop secondary structure (see Fig. 2). These were either silent mutations or conservative substitutions (Gln → Asn, Ile → Val, Ala → Val) in order to minimize any effects on protein folding or stability. This amino-terminal region of the protein protrudes on the exterior side of the membrane in H. halobium and is not resolved in the three-dimensional structure of wild-type BR (20).

XU10A cells were pink, in contrast to the orange SD9 and XU10 or the purple XU3 cultures. Northern blots showed no degradation for XU10A mRNA (Fig. 4). The spectrum of XU10A membranes showed a peak at 570 nm characteristic of BR, which appeared as a shoulder on the carotenoid peak similar to that in XU14 membranes (Fig. 6). A faint BR band could also be detected on SDS-PAGE gels (Fig. 7). The relative amounts of polypeptide detected on gels are consistent with the amounts of the chromophore detected in spectra, indicating that most or all of the BO produced had bound retinal to form BR, which produces the characteristic absorption spectrum. No BR could be detected in spectra nor gels run on the soluble fraction of XU10A cells (not shown).

Pulse-Chase Experiments Indicate That There Is No Increased Turnover of BR in XU10A—XU3, XU10A, and XU14 cells were pulse-labeled with [35S]methionine followed by a chase with unlabeled methionine, and the labeled proteins examined by SDS-PAGE and PhosphorImager analysis (Fig. 8). In all three strains no significant turnover of BR in the membranes could be detected over a period of 48 h after dilution of the label. The amount of label incorporated was lowest in XU10A and highest in XU3, reflecting the amounts of BR found in the strains cultured in rich media (see above and Figs. 6 and 7). No labeled BR was ever found in the soluble fractions of XU3, XU14, or XU10A cells.

**DISCUSSION**

Transformation of H. halobium SD16 and SD9 with the wild-type bop gene fully restored expression of BR; this was not surprising, because the wild-type gene had been inserted back into the chromosome at the bop locus. Efficient expression of the wild-type bop gene in strain L33 (which has an ISH2 insertion in the bop coding region like SD16) using a similar plasmid has been reported previously by Needlemen’s group (21). Their plasmid also contained the brp gene upstream of bop and was usually, but not always, found integrated into the chromosome (21). The reason we never found transformants with plasmids containing a bop gene that had not integrated into the chromosome may be a loss of unintegrated plasmids during the prolonged incubation period without drug selection.

The lack of BR production in the leader sequence deletion mutant XU10 was most likely caused by the degradation of the
mRNA and perhaps lack of translation of any remaining message due to deletion of the putative ribosome-binding site, which is in the region coding for the leader sequence (see Figs. 1 and 2). This ribosome-binding site is downstream of the initiation codon, unlike those of eubacteria, which are upstream of the initiation codon but similarly complementary to the 3' end of the 16S rRNA. Secondary structure and ribosome binding at the 5' end of the bop mRNA apparently enhance the efficiency of translation by an unknown mechanism but does not interfere with the binding of complementary tRNAs to these bases when translation is initiated.

Strains XU14 and XU15 have only silent mutations and produce wild-type BR; their bop mRNA was produced in normal amounts and was not found degraded, so the reduced level of BR expression in these strains must be due to decreased translation of bop mRNA. In the case of XU13, the presence of an amino acid substitution that created a major difference in the net charge of the leader peptide did not cause any additional reduction in the amount of BR found compared with XU15. Both XU13 and XU15 have mRNA sequences that are predicted to have reduced binding to the 16S rRNA as well as reduced stability of the mRNA secondary structure. This perturbation of the hairpin secondary structure would not occur in XU14, but the level of BR in this mutant was on average comparable with that of XU13 and XU15. This suggests that the ribosome-binding sequence determines the level of translation and the exact secondary structure of the hairpin may not be critical for translation. The presence of a hairpin may, however, be required to prevent rapid mRNA degradation.

The results obtained with the modified leader sequence deletion mutant XU10A indicate that the presence of a ribosome-binding sequence and/or hairpin loop at the 5' end of the bop mRNA in XU10A was sufficient to stabilize the mRNA and facilitate a modest level of translation. The reason that the level of BR expression was significantly less than that in XU3

**Fig. 6.** Spectra of membranes from *H. halobium* mutants. The relative absorbance at 568 nm (due to bacteriorhodopsin) gives an indication of the relative amounts of BR produced in each strain. Absorbances at 410 nm due to cytochromes and at 470–550 nm due to carotenoids are also visible. Spectra of membranes from strains XU3, XU15, XU13, XU14, XU10A, and SD9 (the parent strain) transformed with the vector pWL102 are shown.

**Fig. 7.** SDS-PAGE gel of membranes from *H. halobium* mutants stained with Coomassie Blue. Lane 1, SD9 transformed with pWL102; lane 2, XU3; lane 3, XU13; lane 4, XU14; lane 5, XU10A.

**Fig. 8.** Pulse-chase of *H. halobium* strains with [35S]methionine. SDS-PAGE gel and PhosphorImager analysis of membranes from strains XU3, XU10A, and XU14. Cells were pulsed for 2 h with [35S]methionine and then chased for 0, 2, 18, 26, and 42 h with unlabeled methionine.
may be because the new ribosome-binding site and secondary structure are not very close replicas of those in the wild-type bop gene. In fact, the structure of XU10A mRNA is in some ways more similar to that of the hop gene mRNA (see Fig. 1). This gene, coding for the protein halorhodopsin, is expressed at much lower levels than bop (22). Consistent with this interpretation is the observation that hop-bop fusions in which the 5′ untranslated region (including the promoter) of the bop gene is fused to the hop coding region do not significantly increase the level of hop expression, but fusions that include a portion of the bop coding region (and therefore the bop ribosome-binding site) result in a high level of production of halorhodopsin (23). Similarly, fusion of the bop promoter to the sopl (sensory opsin I) gene does not significantly increase expression of sopl, but fusions that also include the first 13 or 21 codons of the bop gene result in much higher expression levels (24).

The BR produced in XU10A was assembled with the retinal chromophore, and it was all found integrated into the H. halobium membrane. We were unable to extract it from the other membrane components by high salt washes or density gradient centrifugation. Therefore, the leader peptide must not be required for proper folding or membrane insertion. It may, however, facilitate these processes in wild-type cells (i.e. the leader peptide may have a role in the kinetics of protein folding or membrane insertion). In either case, the absence of the leader may result in misfolding or improper targeting of a substantial fraction of the newly synthesized polypeptide. Ribosomes translating the bop gene have been found bound to membranes and to a 7S RNA with a proposed signal recognition particle (SRP)-like function (4); these interactions may involve the leader sequence and enhance the efficiency of membrane insertion. An altered amino-terminal sequence might also result in increased turnover of the BR in the membrane, but this was not observed.

The pulse-chase results suggest that the level of BR expression is determined by the rate of synthesis. Unfortunately, the rate of incorporation of labeled methionine into BR in XU10A cells was too slow to allow pulses shorter than 2 h. Therefore one cannot rule out the possibility that much of the BO polypeptide in this strain is rapidly degraded before it has a chance to be integrated into the membrane, because it is inefficiently translocated or misfolds in the absence of the leader sequence.

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REFERENCES
1. Dunn, R., McCoy, J., Simsek, M., Majumdar, A., Chang, S. H., RajBhandary, U. L., and Khorana, H. G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6744–6748
2. Seehra, J. S., and Khorana, H. G. (1984) J. Biol. Chem. 259, 4187–4193
3. Von Heljne, G., and Segrest, J. P. (1987) FEBS Lett. 213, 238–240
4. Gropp, R., Gropp, F., and Betlach, M. C. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1204–1208
5. Scotto, A. W., and Zakim, D. (1985) Biochemistry 24, 4066–4075
6. Dencher, N. A. (1986) Biochemistry 25, 1195–1200
7. Scotto, A. W., and Zakim, D. (1988) J. Biol. Chem. 263, 18500–18506
8. Zakim, D., and Scotto, A. W. (1989) Methods Enzymol. 171, 253–264
9. Scotto, A. W., and Gonopper, M. E. (1990) Biochemistry 29, 7244–7251
10. Bauer, U., Hildebrandt, V., Dencher, N. A., and Wrede, P. (1992) Biochem. Biophys. Res. Commun. 187, 1480–1485
11. Blanck, A., and Oesterhelt, D. (1987) EMBO J. 6, 265–273
12. Betlach, M., Friedman, J., Boyer, H. W., and Pfeifer, F. (1984) Nucleic Acids Res. 12, 7794–7799
13. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1989) Current Protocols in Molecular Biology, Wiley Interscience, New York
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
15. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene (Amst.) 33, 103–119
16. Lam, W. L., and Doolittle, W. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5478–5482
17. DasSarma, S., RajBhandary, U. L., and Khorana, G. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2201–2205
18. Cline, S. W., Lam, W. L., Charlebois, R. L., Schalkwyk, L. C., and Doolittle, W. F. (1989) Can. J. Microbiol. 35, 148–152
19. Oesterhelt, D., and Stoeckenius, W. (1974) Methods Enzymol. 31, 667–678
20. Henderson, R., Baldwin, J. M., Ceska, T. A., Zemlin, F., Beckmann, E., and Downing, K. H. (1990) J. Mol. Biol. 213, 899–929
21. Ni, B., Chang, M., Duschi, A., Lanly, J., and Needleman, R. (1990) Gene (Amst.) 90, 169–172
22. Steiner, M., and Oesterhelt, D. (1983) EMBO J. 2, 1379–1385
23. Heymann, J. A. W., Hackett, J. C., and Doolittle, W. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5478–5482
24. Spudich, E. N., and Spudich, J. L. (1993) J. Biol. Chem. 268, 16995–16997

2 R. Needleman, personal communication.
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