Cloning of Human cDNAs Encoding Mitochondrial and Cytosolic Serine Hydroxymethyltransferases and Chromosomal Localization*

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From the †Department of Nutritional Sciences, University of California, Berkeley, California 94720, the ‡Department of Pediatrics, McGill University, Montreal, H3H 1P3 Quebec, Canada, and the ∥Ahmanson Department of Pediatrics and Medical Birth Defects Center, Cedars-Sinai Medical Center, UCLA, Los Angeles, California 90048

Human cDNAs for cytosolic and mitochondrial serine hydroxymethyltransferase (SHMT) were cloned by functional complementation of an Escherichia coli glyA mutant with a human cDNA library. The cDNA for the cytosolic enzyme encodes a 483-residue protein of M₂, 53,020. The cDNA for the mitochondrial enzyme encodes a mature protein of 474 residues of M₂, 52,400. The deduced protein sequences share a high degree of sequence identity to each other (83%), and the individual isoforms are highly homologous to the analogous rabbit liver cytosolic (92% identity) and mitochondrial (97% identity) SHMT isoforms (Martini, F., Angelaccio, S., Pancarella, S., Barra, D., Bossa, F., and Schirch, V. (1987) J. Biol. Chem. 262, 5499–5508; Martini, F., Maras, B., Tanci, P., Angelaccio, S., Pancarella, S., Barra, D., Bossa, F., and Schirch, V. (1989) J. Biol. Chem. 264, 8509–8519). SHMT is a highly conserved protein with the human isoforms retaining about 43% sequence identity with the E. coli protein. The human cytosolic and mitochondrial SHMT genes were localized to chromosome regions 17p11.2 and 12q13, respectively. The high degree of nucleotide sequence identity between the two isoforms, and the presence of keratin genes in both chromosomal regions, is consistent with these regions of chromosome 12 and 17 arising by a duplication event.

Folate coenzymes act as donors and acceptors of one-carbon units in a variety of reactions involved in one-carbon metabolism. In mammalian tissues, they function as substrates in a series of interconnected metabolic cycles involving thymidine, de novo purine biosynthesis, methionine biosynthesis and methyl group catabolism, serine and glycine interconversion and catabolism, and the catabolism of histidine and formate (1). The major one-carbon donor for folate-dependent one-carbon metabolism is the β-carbon of serine. Serine hydroxymethyltransferase (SHMT),1 a pyridoxal phosphate-containing enzyme, catalyzes the reversible conversion of serine and tetrahydrofolate to glycine and 5,10-methylene tetrahydrofolate (2,3). Incorporation of the β-carbon of serine into DNA and SHMT activity are increased when cells are stimulated to proliferate and during the S phase of the cell cycle (4,5), and SHMT activity is elevated in a variety of tumor tissues (6,7).

Some eukaryotic cells contain both cytosolic and mitochondrial forms of SHMT (3,8), and mammalian cells that lack mitochondrial SHMT activity are auxotrophic for glycine. It has been suggested that glycine synthesis from serine occurs in the mitochondria, whereas cytosolic SHMT may catalyze the conversion of glycine to serine (9), although direct evidence for this proposal is lacking. The cytosolic and mitochondrial SHMT isoforms from rabbit liver have been purified to homogeneity and compared with respect to reaction and substrate specificity (10–16). Each isoform is a tetramer of identical subunits, and both have isoelectric points near 7.2 (10). A study of cysteine-containing peptides from tryptic digests demonstrated that the two isoforms had different primary structures (13–15). Recently, the complete primary structure of the rabbit cytosolic (12) and mitochondrial (17) isoforms were determined by sequencing the proteins, confirming these differences. The primary structures of several bacterial SHMT proteins have been deduced from the sequence of their genes (18,20,21).

We are interested in the role of subcellular compartmentation in the regulation of mammalian one-carbon metabolism, the role of the different SHMT isoforms in this process, and the potential of SHMT as a target for anti-proliferation agents. These studies require a mammalian SHMT cDNA for studying SHMT expression and the effects of modulation of enzyme activity in specific subcellular compartments. As no mammalian or eukaryotic SHMT cDNA or gene had been isolated, we attempted to isolate a human SHMT cDNA by its ability to complement an Escherichia coli glyA (SHMT) mutant (22). This report presents the cDNA and deduced protein sequences of human cytosolic and mitochondrial SHMT and the localization of their genes to separate chromosomes.

Experimental Procedures

Materials—L-[3-14C]serine (524 mCi/mmol) was obtained from Amersham Corp. Amino acids and vitamins were from GIBCO and

1 The abbreviations used are: SHMT, serine hydroxymethyltransferase; IPTG, isopropyl-1-thio-β-D-galactopyranoside; kb, kilobase pair(s).

2 Stauffer, G. V. (1989) GenBank submission. Accession number X15816.
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Sigma. Restriction endonucleases and T4 DNA ligase were from Boehringer Mannheim.

Bacterial, Bacteriophage, and Plasmid Strains—The E. coli glyA (SHMT) strain GS245 (MC4100, pheA905, thi, araD139, strA, ΔlacU169, ΔglyA::Mu; Ref. 22) was a gift from G. V. Stauffer (University of Iowa). Bacteriophage AYES-R, a human cDNA library in AYES-R and E. coli BNN132 containing 10 μg/ml ampicillin, and thi, was a gift from S. Elledge (Baylor College of Medicine). The construction of AKC and λYES-R and the phenotype of BNN132 have been described (23).

The cre gene on AKC allows automatic subcloning of plasmid pSE936, contained between lox sites on λYES-R, when E. coli is infected with λYES-R (23). The human cDNA library, containing EcoRI-XhoI-SfiI linkers, was made from mRNA derived from Epstein-Barr virus-transformed B-lymphocytes and was cloned into a unique EcoRI site located downstream from the hc promoter in the pSE936 region of λYES-R (23). AKC was rescued from BNN132 by mitomycin C induction and was used to infect GS245 (23).

Kanamycin-resistant colonies were tested for the glyA phenotype on VS agar plates containing kanamycin (50 μg/ml), phenylalanine (50 μg/ml), and thiamine (10 μg/ml), with or without glycine (50 μg/ml) supplementation. Single cells of GS245(AKC) were isolated.

Cloning of SHMT—GS245(AKC) was grown overnight in LB medium (2.5 ml) containing 0.2% maltose, 1 mM isopropyl β-D-thiogalactoside (IPTG), and 50 μg/ml kanamycin, resuspended in 10 mM MgSO4, and mixed with the human cDNA library in AYES-R (2.5 X 10^10 phage) and incubated at 30 °C for 30 min without agitation as described by Elledge et al. (23). VB medium (4 ml) containing phenylalanine (50 μg/ml), glycine (50 μg/ml), thiamine (10 μg/ml), mannitol (0.2%), and IPTG (100 μg/ml) was added and the culture incubated at 30 °C with shaking for 2 h. Twenty-five million cells were resuspended in medium lacking glycine, spread on 10 VB/2% agar plates containing 0.2% mannitol, 0.001% thiamine, 50 μg/ml ampicillin, and 50 μg/ml phenylalanine, and incubated at 30 °C. Control plates also contained glycine (50 μg/ml). In a second experiment, IPTG (1 mM) was added to the culture plates.

DNA Sequencing—EcoRI inserts of pSE936 plasmids which complemented GS245 were subcloned into pTZ19U and transformed into E. coli MV1910 (Bio-Rad). Single-stranded DNA, produced using helper phage M13K07, was sequenced by the method of Sanger et al. (24) using Sequenase 2 (United States Biochemical Corp.). Primers were synthesized by the Micro-Chemical Facility (University of California, Berkeley).

Assay of SHMT Activity—SHMT activity in E. coli extracts was assayed as described by Taylor and Weissbach (25) using [3-14C]serine and (6R,GS)-tetrahydrofolate as substrates.

Chromosomal Localization—SHMT cDNAs probes were labeled with biotin-11-dUTP by nick translation (26) and hybridized to metaphase chromosomes prepared from normal male peripheral blood lymphocytes by the bromodeoxyuridine synchronization method (27). In situ hybridization was performed by the method of Lichter et al. (28) with the following modifications. The hybridization solution contained probe DNA (400 ng), Cot 1 DNA (3 μg), and sonicated salmon sperm DNA (7 μg) per 10 μl of hybridization mix. After pre-annealing the probes for 10 min at 37 °C, the mixtures were applied to slides. Post-hybridization washes were carried out with 2 X SSC, 50% formamide

CTCA GGGCCCTGC AACGGTCA ATC AGC AGG ACA GCC CCC ACC AAC GAG GTT GCT GAC TGC TGG TCA

FIG. 1. Nucleotide sequence of human cytosolic SHMT cDNA and derived amino acid sequence. The numbered lines refer to the first ATG codon.

FIG. 1.
at 44 °C (four times) and 1 x SSC at 55 °C (three times). Hybridized DNAs were detected with avidin-conjugated fluorescein isothiocyanate (Vector Laboratories). Two amplifications were carried out using biotinylated anti-avidin. Metaphase chromosomes were counterstained with chromomycin A3 followed by distamycin A, by a modified cDNA library in AYES-R as described by Elledge et al. (29), to generate clear reverse bands. Images were photographed with a Zeiss Axiopt microscope equipped with filter set 5.

RESULTS

Cloning of Human SHMT by Complementation of GS245(KC)—GS245(KC) cells were infected with the human cDNA library in AYES-R as described by Elledge et al. (23) and cultured for 2 h at 30 °C in nonselective medium (plus glucose) containing 1 mM IPTG. Washed cells were then plated on selective agar plates. After 3 days at 30 °C, about 100 colonies were obtained and 30 were re-streaked onto selective plates. Only a few colonies were obtained in the absence of IPTG. Twenty-four of the colonies continued to grow without glycine supplementation. Plasmids were isolated from the transformants and used to transform GS245. Several plasmids retained the ability to complement the glyA phenotype.

Restriction enzyme digestion of EcoRI cDNA inserts in pSE936 plasmids that complemented GS245 indicated two classes of inserts of approximately 1.7 and 1.9 kb, respectively, with distinctly different restriction maps. One EcoRI insert was generated by using a different procedure of Magenis et al. (29), to generate clear reverse bands. Images were photographed with a Zeiss Axiopt microscope equipped with filter set 5.

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However, the open reading frame is out of frame with the ATG translation start sites in the pSE936 and pTZ19U vectors, and translation must have initiated at an internal ATG in the cDNA, even though the 5'-untranslated region in the cDNA lacks sequences resembling a bacterial Shine-Dalgarno sequence.

**Human Mitochondrial SHMT**—The cDNA sequence and the deduced protein sequence of human mitochondrial SHMT are shown in Fig. 2. The mitochondrial cDNA lacks 5' residues that would code for the start methionine and a mitochondrial import sequence but does contain an open reading frame of 1422 base pairs, which would code for a protein of 474 amino acid residues with an M, of 52,400. The 3' end of the cDNA import sequence but does contains an open reading frame of 1422 base pairs, which would code for the start methionine and a mitochondrial import sequence but does contain an open reading frame of 1422 base pairs, which would code for a protein of 474 amino acid residues with an M, of 52,400. The 3' end of the cDNA lacks sequences resembling a bacterial Shine-Dalgarno sequence. The mito-

Amino Acid Homology among SHMTs—The deduced amino acid sequences of human cytosolic and mitochondrial SHMT are compared with the corresponding rabbit liver proteins in Fig. 3. The primary structures of the rabbit enzymes were obtained by sequencing the purified rabbit liver proteins (12, 17). The cytosolic and mitochondrial isozymes show a very high degree of sequence identity. Ninety-seven percent of the amino acid residues with an M, of 52,400. The 3' end of the cDNA lacks sequences resembling a bacterial Shine-Dalgarno sequence.

![Fig. 3. Comparison of the amino acid sequences of eukaryotic and prokaryotic SHMTs.](image-url)
residues of the two mitochondrial proteins are identical, and about one-third of the remaining residues show conservative changes. About 92% of the residues of the two cytosolic proteins are identical, and about half of the remaining non-identical residues represent conservative substitutions. The two human SHMT proteins share about 63% sequence identity, and about one-third of the remaining residues show conservative changes. The mitochondrial and cytosolic isoforms diverge most at their N termini and, to a lesser extent, at their C termini.

After completion of these studies, the sequences of two eukaryotic SHMT genes were reported: the *Neurospora crassa* cytosolic enzyme (30) and the pea mitochondrial enzyme (31). The deduced human sequences are also compared with these sequences and with four bacterial SHMT sequences in Fig. 3. The calculated evolutionary relationship between these proteins is shown in Fig. 4. The 10 proteins share a high degree of sequence identity. Twenty percent of the amino acid residues are common to all 10 proteins, and 39% are common to the six eukaryotic sequences (Fig. 3). The human cytosolic and mitochondrial isoforms are 57 and 60% identical, respectively, to the pea mitochondrial enzyme, 56% identical to the *Neurospora* cytosolic enzyme, and 42 and 43% identical to the *E. coli* protein. The major regions of sequence divergence are at the N and C termini. The eukaryotic sequences also contain three regions of amino acid insertions (4–14 residues) that are lacking from the prokaryotic sequences. The region around the pyridoxal phosphate binding site, Lys-257 in the consensus sequence (Fig. 3), is highly conserved in all the proteins. Lys-257 is preceded by a His residue and the active site His-Lys is preceded by 4 Thr/Ser residues and is followed by a Thr/Ser residue in all SHMTs (32, 33). The deduced evolutionary relationship between the SHMT proteins (Fig. 4) suggests that the cytosolic and mitochondrial isoforms diverged from each other probably by a single gene duplication event and that this occurred after the divergence of the bacterial and eukaryotic proteins.

**Nucleotide Homology between SHMT Isozyme cDNAs—**The cDNA sequences of human cytosolic and mitochondrial SHMT show a high degree of sequence identity (57%) and are 65% identical over the their protein coding regions, whereas there is no significant homology between the human and bacterial sequences.

**Expression of SHMT Activity in Transformants—**Complementation of GS245 under selective conditions was accompanied by restoration of SHMT activity. SHMT activity in crude extracts of GS245 transformants averaged 104 and 64% of wild type levels in transformants expressing the mitochondrial and cytosolic cDNAs, respectively, whereas no activity was detected in GS245 extracts. Although pTZ19U is a high copy number plasmid, none of the transformants displayed elevated SHMT activity. This probably reflected that the mitochondrial enzyme would have been synthesized as a fusion protein, whereas the cytosolic cDNA lacks a region with homology to the Shine-Dalgarno ribosome binding site and the translation efficiency of its mRNA in bacteria would be expected to be very poor.

**Localization of Human SHMT Genes—**The probe for the cytosolic SHMT gene was mapped to chromosome band 17p11.2 (Fig. 5, A and B). The mitochondrial SHMT cDNA probe was mapped to chromosome 12q13, most likely on sub-band 12q13.2 (Fig. 5, C and D). Two independent experiments were carried out, and over 400 metaphase cells were evaluated. For cytosolic SHMT, signals were noted on two chromatids of at least one chromosome 17p11.2-p12 in 45% of cells (n = 290) and the gene was finally localized to 17p11.2. For the mitochondrial SHMT probe, clear signals were noted on two chromatids of at least one chromosome 12q13 in 50% of cells (n = 290). No secondary signals on any chromosome band were noted in greater than 0.5% of cells.

**DISCUSSION**

This report describes the cDNA sequences of human cytosolic and mitochondrial SHMT and their deduced protein sequences. The relative ease with which these two cDNAs, which code for high abundance proteins, were cloned highlights the value of the λ-YES expression vector system (23). We have previously used this system to clone a cDNA for human folylpolyglutamate synthetase, an extremely low abundance protein (34). However, a limitation of this technique, at least when used for expression in bacteria, is the need for the expressed protein to be functionally active, and consequently full-length cDNAs for inactive pre-proteins, possessing N-terminal leader sequences, would not be isolated. The mitochondrial cDNA obtained in the current study lacked 5' non-coding sequences coding for the mitochondrial import signal, and the start ATG and the mitochondrial isozyme was expressed as a fusion protein from an ATG present in the λ-YES vector. Preliminary attempts at isolating the 5' region of the cDNA from the λ-YES library using polymerase chain reaction techniques were not successful. Although the possibility that the mitochondrial isozyme lacks a conventional leader sequence cannot be excluded, the presence of a leader sequence in the recently described pea mitochondrial isozyme (31) suggests this is unlikely. We have recently isolated genomic clones for both human SHMT isoforms, and sequence analysis of the mitochondrial clone should indicate the nature of the leader sequence.

Because of the localization of the cytosolic SHMT gene to chromosome region 17p11.2, it may be of interest to determine the relationship of SHMT to the duplication responsible for Charcot-Marie-Tooth neuropathy type 1A and the Smith-Magenis syndrome, as both are located in the same region (35). This region is located significantly centromeric to the region deleted in the Miller-Dieker syndrome and is relatively gene-rich, containing genes for ubiquitin B (UBB), muscle nicotinic cholinergic receptor β polypeptide (CHRN1), keratin 18 (KRT18), and numerous other keratin-related sequences.

A gene for SHMT was previously mapped to chromosome 12q12-q14 using a panel of somatic cell hybrids of the Chinese hamster ovary cell gY4A, which lacks mitochondrial SHMT.
activity (36). The mapping of the mitochondrial SHMT gene to chromosome region 12q13 suggests this is the same gene, as no additional signals were seen in this region suggestive of a third SHMT-related gene. The techniques used in the current study would have detected members of a multigene family sharing somewhat less than 90% homology over at least 1.2 kb. Chromosome region 12q13 is also relatively gene-rich, although the precise band assignments of many of these genes (12q12–14) are not known (37). Of interest here, however, may be the fragile site, folic acid type, rare frac(12q13.1) located within the same band, likely centromeric to SHMT (38, 39). Several neoplasia are also associated with translocations involving 12q13, although the relationship, if any, to SHMT is unknown. Two additional keratin genes (6A and 7) map to the region 12q12–21, which has suggested homology between this region and region 17p (37). The localization of the SHMT genes to these chromosomal regions and the high degree of nucleotide sequence identity between mitochondrial and cytosolic SHMT cDNAs are also consistent with this suggestion and suggest that the genes arose by a relatively recent duplication event. The deduced evolutionary relationship between SHMT proteins also suggests that the two isozymes arose from a gene duplication after the divergence of bacterial and eukaryotic proteins, and it is interesting to note that while some eukaryotic cells possess both cytosolic and mitochondrial SHMT isozymes, other eukaryotic cells express only the cytosolic or the mitochondrial activity.

The primary structure of SHMT has been very highly conserved through evolution, which may reflect a rigid requirement of residues for substrate binding and catalysis. It has been proposed that SHMT may be part of a multi-protein complex involved in purine or thymidylate synthesis in eukaryotic cells (19, 40), and the high degree of sequence conservation may also reflect conservation of residues or tertiary structure involved in protein-protein interactions. The availability of mammalian cDNAs for cytosolic and mitochondrial SHMT will allow an assessment of essential noncatalytic residues that may be involved in complex formation and will also allow studies on the regulation of SHMT in mammalian cells and on the physiological role of the cytosolic and mitochondrial isozymes.

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REFERENCES

1. Shane, B. (1989) Vitam. Horm. 45, 263–335
2. Schirch, L. (1987) Adv. Enzymol. 53, 83–112
3. Schirch, L. (1984) in Pointers and Petrie (Blakley, R. L., and Benkovic, S. J., eds) Vol. 1, pp. 399–431, John Wiley & Sons, New York
4. Richter, H.-G., Hubbard, R., and Snell, K. (1982) Biochim. Biophys. Acta 701, 101–106
5. Snell, K., Natsumeda, Y., and Weber, G. (1987) Biochem. J. 245, 609–612
6. Snell, K., Natsumeda, Y., Eble, J. N., Glover, J. L., and Weber, G. (1988) Br. J. Cancer 57, 87–90
7. Snell, R. (1989) in Liver Cell Carcinoma (Bannasch, P., Keppler, D., and Weber, G., eds) pp. 375–387, Academic Press, New York
8. Chasin, L. A., Feldman, A., Konstam, M., and Urbach, G. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 718–722
9. Appling, D. R. (1991) FASEB J. 5, 2646–2651
10. Schirch, L. (1975) J. Biol. Chem. 250, 1939–1945
11. Schirch, L., and Peterson, D. (1980) J. Biol. Chem. 255, 7801–7806
12. Martini, F., Angelaccio, S., Pascarella, S., Barra, D., Bossa, F., and Schirch, V. (1987) J. Biol. Chem. 262, 5499–5504
13. Schirch, L., Slagel, S., Barra, D., Martini, F., and Bossa, F. (1980) J. Biol. Chem. 255, 2986–2990
14. Gavilanes, F., Peterson, D., and Schirch, L. (1982) J. Biol. Chem. 257, 11431–11436
15. Gavilanes, F., Peterson, D., Bullis, B., and Schirch, L. (1983) J. Biol. Chem. 258, 13155–13159
16. Shostak, K., and Schirch, V. (1988) Biochemistry 27, 8007–8014
17. Martini, F., Maras, B., Tanci, P., Angelaccio, S., Pascarella, S., Barra, D., Bossa, F., and Schirch, V. (1986) J. Biol. Chem. 261, 8509–8519
18. Plamann, M. D., Stauffer, L. T., Urbanowski, M. L., and Stauffer, G. V. (1983) Nucleic Acids Res. 11, 2065–2073
19. Caperellxi, C., Benkovic, P. A., Chettur, G., and Benkovic, S. J. (1980) J. Biol. Chem. 255, 1885–1890
20. Rossbach, S., and Hennecke, H. (1991) Mol. Microbiol. 5, 39–47
21. Chan, V. L., and Bingham, H. L. (1991) Gene (Amst.) 101, 51–58

4 J. Korenberg, unpublished data.
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22. Stauffer, G. V., Flamann, M. D., and Stauffer, L. T. (1981) Gene (Amst.) 14, 63-72
23. Elledge, S. J., Mulligan, J. T., Raser, S. W., Sportswood, M., and Davis, R. W. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1731-1735
24. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467
25. Taylor, R. T., and Weissbach, H. (1985) Anal. Biochem. 13, 80-84
26. Leuher-Safer, P. R., Levine, M., and Ward, D. C. (1982) Proc. Natl. Acad. Sci. U. S. A. 78, 4381-4386
27. Zabel, B. U., Naylor, S. L., Sakaguchi, A. Y., Bell, G. L., and Shows, T. B. (1985) Proc. Natl. Acad. Sci. U. S. A. 80, 6833-6836
28. Lichter, P., Chang-Tang, C. I., Call, K., Hermann, G., Evans, G. A., Houman, D., and Ward, D. C., (1990) Science 247, 64-69
29. Magenis, R. E., Donlon, T. A., and Tomar, D. R. (1985) Hum. Genet. 69, 300-303
30. McClung, C. R., Davis, C. R., Page, K. M., and Denome, S. A. (1992) Mol. Cell. Biol. 12, 1412-1421
31. Turner, S. R., Ireland, R., Morgan, C., and Rawsthorne, S. (1992) J. Biol. Chem. 267, 13828-13834
32. Vealer, G. L., and Snell, E. E. (1989) Biochemistry 28, 7306-7313
33. Angelaccio, S., Pascarella, S., Fattori, E., Bossa, F., Strong, W., and Schirch, V. (1992) Biochemistry 31, 155-162
34. Garrow, T. A., Admon, A., and Shane, B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9151-9155
35. Solomon, E., and Ledbetter, D. H. (1991) Cytogenet. Cell Genet. 58, 686-736
36. Law, M. L., and Kao, F. T. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 102-114
37. Craig, J. W., and McBride, O. W. (1991) Cytogenet. Cell Genet. 58, 555-576
38. Sutherland, G. R., and Hinton, L. (1981) Hum. Genet. 57, 217-219
39. Stetten, G., Storks, B., Norbury-Glaeser, M., and Corson, V. L. (1988) Am. J. Med. Genet. 31, 521-525
40. Reddy, G. P. V., and Pardee, A. B. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 3312-3316