*Biochimica et Biophysica Acta*, 1152 (1993) 192–196


BBAMEM 70731

**Short Sequence-Paper**

# Isolation and characterization of cDNA clones and a genomic clone encoding rat mitochondrial adenine nucleotide translocator

Yasuo Shinohara, Makio Kamida, Naoshi Yamazaki and Hiroshi Terada *

*Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi-1, Tokushima 770 (Japan)*

(Received 24 June 1993)

Key words: ADP/ATP carrier; Mitochondrion; Solute carrier family; Primary structure; Gene structure; (Rat)

Two cDNA clones encoding rat mitochondrial adenine nucleotide translocator were isolated from libraries constructed from mRNAs of heart and liver. These two clones corresponded to the heart-skeletal muscle type (ANT1) and fibroblast type (ANT2), respectively. A genomic clone encoding rat ANT1 was also isolated and characterized.

The adenine nucleotide translocator (ANT or ADP/ATP carrier), present in the inner mitochondrial membrane, catalyzes the exchange of the adenine nucleotides ADP and ATP between the matrix and cytosol. There have been extensive studies on its structure in relation to its transport function, especially on that of bovine heart mitochondrial ANT [1–4]. Recently, genetic analyses have indicated the presence of ANT isoforms in yeast [5–7], maize [8], potato [9], bovine [10] and human [11–19] tissues. Rat liver mitochondria are most commonly used in studies on oxidative phosphorylation. However, little is known about rat mitochondrial ANT, such as its primary structure and the tissue distribution of its isoforms. Therefore, we performed molecular cloning and characterization of recombinant clones encoding rat mitochondrial ANT.

Libraries of heart and liver cDNAs of Sprague-Dawley rats were purchased from Clontech Laboratories (Palo Alto, CA, USA). A rat genomic library, which was constructed from genomic DNA extracted from the liver of a male Wistar rat, was a gift from Dr. Takiguchi (Institute for Medical Genetics, Kumamoto University Medical School). pUC19 and pBluescript were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan) and Stratagene (La Jolla, CA, USA), respectively.

Screening, isolation of clones, restriction mapping and sequencing of the cDNA and genomic clones were carried out essentially by standard methods [20]. Complementary DNA encoding human ANT1 obtained from a cDNA library of human heart was radiolabeled by the multi-priming method [20] and used as a probe for screening rat cDNA libraries. Northern blotting was carried out essentially as described previously [21]. Briefly, 5.0-$\mu$g samples of total RNAs purified by the guanidium thiocyanate method [20] were separated by electrophoresis in gel containing formaldehyde and transferred to a nitrocellulose membrane. Hybridization was carried out at 42°C in the presence of 50% formamide and then the membrane was washed with a solution of 300 mM NaCl and 30 mM sodium citrate (2 × SSC) containing 0.1% SDS for 10 min at room temperature. This treatment was repeated 3-times and then the membrane was washed twice with a solution of 1 × SSC containing 0.1% SDS at 65°C for 30 min (high-stringency condition). The 5′-terminal cDNA fragments of each clone (about 350 bp each) obtained by digestion with *Eco*RI and *Bgl*II were radiolabeled by the multi-priming method and used as probes.

On screening cDNA libraries of rat heart and liver with $^{32}$P-labeled human ANT1 cDNA under low-stringency hybridization conditions, we obtained more than 50 and 18 positive clones from $1.3 \cdot 10^6$ and $6.8 \cdot 10^5$ recombinants, respectively. We purified and analyzed $\lambda$ DNAs from one recombinant clone from each of these sources. The nucleotide sequences and deduced amino-acid sequences of these two clone are shown in Fig. 1. By comparison of the deduced amino-acid sequences of the clones with those of human ANT isoforms, we concluded that the cDNA clones obtained

* Corresponding author. Fax: +81 886 335195.

The nucleotide sequence data reported in this paper have been submitted to the DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession numbers D12770, D12771 and X61667, respectively.

Abbreviation: ANT, adenine nucleotide translocator.

from the heart and liver cDNA libraries corresponded to ANT1 and ANT2, respectively. We found that the structural features observed in the ANT isoforms of several mammals, such as an unusual amino-acid sequence RRRMMM and four cysteine residues at 56, 128, 159 and 256, are also conserved in the rat ANT isoforms. Because the N-terminal methionine of both isoforms of rat ANT is expected to be removed post-translationally, we omitted the first methionine residue in numbering the amino-acid sequences.

Next, we determined the steady-state transcript levels of the two isoforms in various tissues (Fig. 2A). Cross-hybridizations of these two probes were less than 5% under the present high-stringency conditions (Fig. 2B). As shown in Fig. 2A, when RNA samples were hybridized with the probe of ANT1, a strong band of transcript was observed in samples from heart and skeletal muscle, and a weak band of transcript in those from brain and kidney, but no transcript was detected in liver. A faint band of a transcript of lower mobility

A)

```
GGTGCGGTGCCTGGCCGGGCGTAGGCAAGAGCAAAAGAGCGGCTCCTTGCAGACTGTGCGCGCCCGCGTTTCAGCATGGGGGATCAGGCT
                                                                    MetGlyAspGlnAla

TTGAGCTTCCTTAAGGACTTCCTGGCAGGTGGCATCGCCGCCGCCGTCTCCAAGACCGCGGTCGCCCCGATCGAGAGGGTCAAACTGCTG
LeuSerPheLeuLysAspPheLeuAlaGlyGlyIleAlaAlaAlaValSerLysThrAlaValAlaProIleGluArgValLysLeuLeu

CTGCAGGTCCAGCATGCCAGCAAACAGATCAGTGCAGAGAAACAGTACAAAGGCATCATTGATTGTGTCGTGAGAATCCCCAAGGAGCAG
LeuGlnValGlnHisAlaSerLysGlnIleSerAlaGluLysGlnTyrLysGlyIleIleAspCysValValArgIleProLysGluGln

GGCTTTCTCTCCTTCTGGAGGGGTAACCTGGCCAACGTGATCCGGTACTTCCCCACCCAAGCTCTCAACTTCGCCTTCAAGGACAAGTAC
GlyPheLeuSerPheTrpArgGlyAsnLeuAlaAsnValIleArgTyrPheProThrGlnAlaLeuAsnPheAlaPheLysAspLysTyr

AAGCAGATCTTCCTGGGAGGTGTGGATCGTCATAAGCAGTTCTGGCGCTACTTCGCTGGTAACCTGGCCTCTGGTGGGGCAGCTGGGGCT
LysGlnIlePheLeuGlyGlyValAspArgHisLysGlnPheTrpArgTyrPheAlaGlyAsnLeuAlaSerGlyGlyAlaAlaGlyAla

ACCTCCCTCTGCTTCGTCTACCCACTGGACTTTGCTAGGACCAGGCTGGCTGCCGACGTGGGCAAGGGATCTTCCCAGCGTGAGTTCAAT
ThrSerLeuCysPheValTyrProLeuAspPheAlaArgThrArgLeuAlaAlaAspValGlyLysGlySerSerGlnArgGluPheAsn

GGGCTGGGTGACTGTCTCACCAAGATCTTCAAGTCTGATGGCCTGAAGGGTCTCTACCAGGGTTTCAGTGTCTCTGTGCAGGGCATCATC
GlyLeuGlyAspCysLeuThrLysIlePheLysSerAspGlyLeuLysGlyLeuTyrGlnGlyPheSerValSerValGlnGlyIleIle

ATCTACAGAGCTGCCTACTTCGGAGTCTATGACACTGCCAAGGGGATGCTGCCAGACCCCAAGAATGTGCACATTATTGTGAGCTGGATG
IleTyrArgAlaAlaTyrPheGlyValTyrAspThrAlaLysGlyMetLeuProAspProLysAsnValHisIleIleValSerTrpMet

ATTGCCCAGAGTGTGACAGCCGTGGCGGGGCTGGTGTCCTATCCATTTGACACTGTCCGTCGTAGGATGATGATGCAGTCTGGCCGGAAA
IleAlaGlnSerValThrAlaValAlaGlyLeuValSerTyrProPheAspThrValArgArgArgMetMetMetGlnSerGlyArgLys

GGGGCTGATATTATGTACACGGGGACAGTTGACTGCTGGAGGAAGATTGCAAAAGATGAAGGACGCAAAGCTTTCTTCAAAGGTGCTTGG
GlyAlaAspIleMetTyrThrGlyThrValAspCysTrpArgLysIleAlaLysAspGluGlyArgLysAlaPhePheLysGlyAlaTrp

TCCAACGTACTGAGAGGCATGGGGGGTGCTTTTGTATTGGTATTGTATGATGAGATCAAAAAATATGTGTAATGCTCAAGTTCACAGGTT
SerAsnValLeuArgGlyMetGlyGlyAlaPheValLeuValLeuTyrAspGluIleLysLysTyrVal***

CACAGATCCATTGTGTGGTTTAACAGACTATTCTTAAGGAAATAAAAAAAGACAGATCATGGATAAAACCAGACCATAAGGAATACCTCA
GAAAAATGCTTCATTGAGTATTCATTTAACCACAAAAGTATTTTGTATTTATTTTACATTTAGATTCCCACAGCAAACAGAAGATAGCTT
ATCATACTTGTTCAATTAATTAACTG
```

B)

```
GAATTCCGGCAGTCCTGTCCTGCAGTCGCCTGCCTCTTCTTTTTTGCTTTCAACATGACAGATGCCGCTGTGTCCTTCGCCAAGGACTTC
                                                    MetThrAspAlaAlaValSerPheAlaLysAspPhe

TTGGCTGGTGGAGTGGCCGCGGCCATCTCCAAGACGGCGGTAGCACCCATCGAGCGGGTCAAGCTGCTGCTGCAGGTGCAGCACGCCAGC
LeuAlaGlyGlyValAlaAlaAlaIleSerLysThrAlaValAlaProIleGluArgValLysLeuLeuLeuGlnValGlnHisAlaSer

AAGCAAATCACGGCAGATAAGCAATACAAGGGCATCATAGACTGCGTGGTTCGTATCCCCAAGGAACAGGGAGTCCTGTCCTTCTGGCGT
LysGlnIleThrAlaAspLysGlnTyrLysGlyIleIleAspCysValValArgIleProLysGluGlnGlyValLeuSerPheTrpArg

GGCAACCTGGCCAATGTCATCAGATACTTCCCCACCCAGGCTCTCAACTTTGCCTTCAAAGATAAATACAAGCAGATCTTTTTGGGTGGT
GlyAsnLeuAlaAsnValIleArgTyrPheProThrGlnAlaLeuAsnPheAlaPheLysAspLysTyrLysGlnIlePheLeuGlyGly

GTGGACAAGAGGACCCAGTTTTGGCGGTACTTTGCAGGGAACCTGGCATCAGGTGGTGCTGCTGGGGCCACATCCTTGTGCTTTGTGTAC
ValAspLysArgThrGlnPheTrpArgTyrPheAlaGlyAsnLeuAlaSerGlyGlyAlaAlaGlyAlaThrSerLeuCysPheValTyr

CCTCTTGATTTTGCCCGTACCCGTCTAGCAGCTGATGTGGGCAAAGCTGGAGCTGAAAGGGAATTCAAAGGCCTTGGTGACTGCCTGGTT
ProLeuAspPheAlaArgThrArgLeuAlaAlaAspValGlyLysAlaGlyAlaGluArgGluPheLysGlyLeuGlyAspCysLeuVal

AAGATCTACAAATCTGATGGGATTAAGGGCCTGTACCAAGGCTTTAATGTGTCAGTGCAGGGCATTATCATCTACCGTGCTGCCTACTTC
LysIleTyrLysSerAspGlyIleLysGlyLeuTyrGlnGlyPheAsnValSerValGlnGlyIleIleIleTyrArgAlaAlaTyrPhe

GGTATCTATGACACTGCAAAGGGAATGCTCCCGGATCCCAAGAATACTCACATCTTCATCAGCTGGATGATTGCACAGTCTGTCACTGCT
GlyIleTyrAspThrAlaLysGlyMetLeuProAspProLysAsnThrHisIlePheIleSerTrpMetIleAlaGlnSerValThrAla

GTTGCTGGCCTAACTTCTTATCCTTTTGACACGGTTCGCCGTCGTATGATGATGCAGTCTGGACGCAAAGGAACTGATATCATGTATACA
ValAlaGlyLeuThrSerTyrProPheAspThrValArgArgArgMetMetMetGlnSerGlyArgLysGlyThrAspIleMetTyrThr

GGCACGCTTGACTGCTGGCGGAAGATCGCTCGAGACGAAGGAGGCAAGGCCTTTTTCAAGGGTGCATGGTCCAACGTTCTCAGAGGCATG
GlyThrLeuAspCysTrpArgLysIleAlaArgAspGluGlyGlyLysAlaPhePheLysGlyAlaTrpSerAsnValLeuArgGlyMet

GGTGGTGCCTTTGTGCTTGTCTTGTATGATGAAATCAAGAAGTACACATAATTATGTCCTAGGTGTTCTCCCCGAGAACAGGCATGTTGT
GlyGlyAlaPheValLeuValLeuTyrAspGluIleLysLysTyrThr***

ATAATAGACCATATTCTTGAACATTCTGGACAGATTCTCTGGGCTTGTCTGTTTTATCAATGGCAACTATTTACCAGTTGAAAAGTGGAA
GCAATAATATTCATCTGACCAGTTTTCTCTTAAAGCCATTTCCATAATGACAATAATGATGGGACTCAATTATATTTTTTATTTCAGTCA
CTCCTGATAATAAAAAATTGAAGAAATAAAAATATCCCGGAATTC
```

Fig. 1. Nucleotide sequences and deduced amino-acid sequences of cDNA clones of ANT obtained from a heart library (A) and liver library (B) of Sprague-Dawley rats.

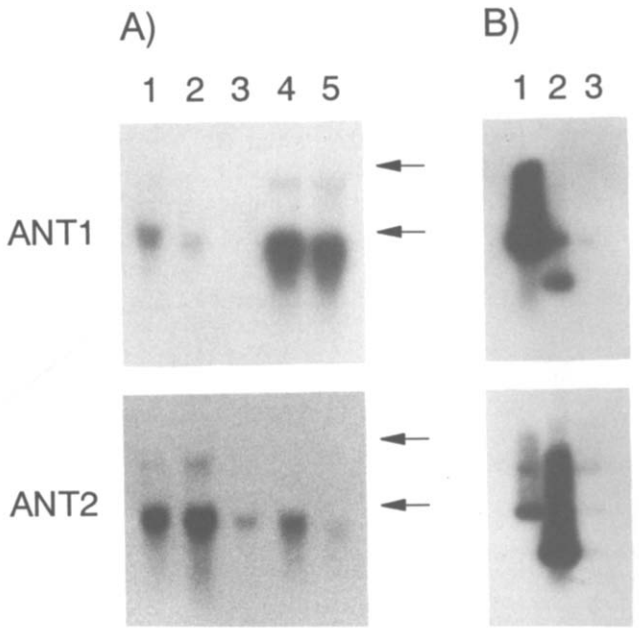


Fig. 2. Tissue distributions of rat ANT isoforms. (A), Total RNAs (5.0 $\mu$g) from brain (lane 1), kidney (lane 2), liver (lane 3), heart (lane 4) and skeletal muscle (lane 5) of Wistar rats were analyzed. The positions of 28 S and 18 S ribosomal RNAs are shown. (B), Cross-hybridizations of probes used for Northern blotting examined by Southern analysis. The cDNA of ANT1 (lane 1) and the two cDNA fragments of ANT2 (lanes 2 and 3) were separated in native agarose gel, transferred to a membrane, denatured and hybridized with probes of ANT1 and ANT2.

was also detected in these samples, for some unknown reason. When RNA samples were hybridized with a probe of ANT2, a major transcript was observed in all tissues analyzed, its intensity in these tissues increasing in the order, skeletal muscle, liver, heart, brain, kidney. We did not compare the steady-state transcript levels of these two isoforms quantitatively. However, to obtain a similar intensity of transcripts of ANT2 to that of ANT1 transcripts after the same exposure time (25 h), it was necessary to use an intensifying screen, indicating that expression of ANT2 was much weaker than that of ANT1, as observed with the human ANT isoforms [14].

To characterize the genes encoding rat ANT isoforms, we tried to isolate genomic clones. On screening a $\lambda_{EMBL4}$ rat liver genomic library with $^{32}$P-labeled rat ANT1 and ANT2 cDNA fragments, 14 positive clones were obtained from among $1.2 \cdot 10^6$ recombinants. All the positive clones analyzed (10 clones) that hybridized with the cDNA fragment of ANT2 were found to contain pseudogenes, but one clone ($\lambda_{MK1}$) that hybridized with the cDNA fragment of the rat ANT1 clone was found to contain the rat ANT1 gene. Thus, we determined the restriction map of this clone (not shown) and the entire nucleotide sequence (Fig. 3). Transcription was found to be initiated at 107 bases and 109 bases upstream from the start site of the translation initiation codon ATG by the primer exten-

```
AATTCCCCGGATCAGCAAACATTTAAAAAGACTGCCTGCTTTGTCAGATGTCCAAACGAAGGTCAGCAACTGTGGGGAAAGAACAGAGTA      90
AGAGGCTTGAAGTTAGAAAACAAAAACAAAAACAAAACAAAACAAAAAACCAAAAAAAAAAAAACAAAAAACGAATTGCCCTCATTCTTGT     180
GGAAGAGACTGCAGTTTGGAAAATATGCAGTTATATTTGAACTGTCTTTAAAGATGTAAGGGAAACTAAACCTCACATCCATCTACTTTA     270
AAAAAATTAATTGCAGTTACTTTCCCGGGATTAGATAGCGCCTAGGCTCCCCAACCTCAAGCTGCCTATTCCAGAGGCAGACACAGCCAA     360
GGATTCGACCTTCCCGAAAGCTAAGGGCTCTGATCCTTAAGAAGCCACCGGAACCATTCTGCACAGAATATGGTTGAGATTCCATTAAGA     450
GCAGAGTCTTTCAAGCCCTTTAGAGGTCTGTCTACAAGAGAATCCCAGGAGCTGAGAGATAGATGAGGGGCTAATAAAGGAGTGTCTTTT     540
CTTTAGGAGTCCTCGGCTGTGTCCGTGTTACAGGCTGAGTTAACACATCCACCTAGCCACACATCCACATTCACTGCCAAAACGACTATC     630
CACTTAGCGTCCTTGCATTCCGCTTTTCCCGTTGATGGCGCAGAGTGCCGGTGCATAAATATTTCCTAACAGATTGAATGGTTCAAGGAT     720
TGAATGGAGAGAAGTCGGAGACAGGACCTTGCCCAATACTATGAGGCAATAGCGGCATAAAAAGATGTTAGTATGTATTGGGGAGGAGTA     810
TAATTTATTTGCAGAAACGCAGAACACTGCAGAGGCCCGAATCCAGGCAGCGAGCTCTAGTTGGAAACGCCCTGACAACGGGGAAGGGGT     900
GGAAGCCTGAAACCTTCTTGGCCAAAAGCACAATGGTCAGGATCGAGGTCAAAGTGCTTCTTTTCTTGAAGCATCGGCCCGCTCTCTGTC     990
CCCAGGTCGTCCCAGGCTAAACCATCGTGAGCTATAAATACAACGGCCCACATGCAGCAGAGACATAGTGTTCCCTCGGCTCCCTGGGAC    1080
AGATAACATGAATTTGCCCTTTAAACGTCCCAAGCTGCAAGCACAGCCCCCAACCCACCCCAAGCTCCCGGAAGCGTCTTCGCCCCCAAT    1170
GCCCTCTGCAAAGAAGGGGGATGGAGAGGGGCTGTCCAGGGACCGGCCCAGCCAATGCGCGCTGGCACGCCGGGGTCCCTCCTCCTCTCG    1260
CGAGACGCGAGCCGGGGATATAAGGAGGGCTGCGGTCAGGCGGGCGGTCCCTTCGCGTCGTGCAGGGACGGAGACCGGGTGCGGTGCCTG    1350
GCCGGGCGTAGGCAAGAGCAAAAGAGCGGCTCCTTGCAGACTGTGCGCGCCCGCGTTTCAGCATGGGGGATCAGGCTTTGAGCTTCCTTA    1440
                                                                    MetGlyAspGlnAlaLeuSerPheLeuL

AGGACTTCCTGGCAGGTGGCATCGCCGCCGCCGTCTCCAAGACCGCGGTCGCCCCGATCGAGAGGGTCAAACTGCTGCTGCAGGTAAGGA    1530
ysAspPheLeuAlaGlyGlyIleAlaAlaAlaValSerLysThrAlaValAlaProIleGluArgValLysLeuLeuLeuGln

CGGCTGGGGGCGCGCGGGGCCGGCAGGGGGGGATGCGGGCGGGGCCCGGCAGGCAGGGATGCGGGCGGGGCCGGGATGCTGTGCACGGCC    1620
CGACGCTGCGGAAAATCTGTGCTAGGCCGCAAGCCCGGGGCCTACACGAAGGAGAAGGTGCCCTCTACGCAGATACAGGTCCAGCGTCAG    1710
TGACAGATTCCTGGTGTCGGGTGGCACCCGCGTTGGGGTGTCTATATATGGAAACCCACCCCGAAGCCGGTCTACCTCGAGCCAGATCCT    1800
GCGCCCGGTACAGCAAGCGGCGTTCCATCCAAGGCCTCTTAGACGGGCCTCACCCTTTGGCCACATCCTTTGCACCTTGTTATCTGCATC    1890
CACCTTTTACAATAGGAGCATCCGATTAATCATTGACTGGGGATCGCTCTGTGTCGCGTGCACTGCGCTGTAACTCGACATTGATTTCAT    1980
TCATCTTTCTATAACCTCTGCGAGATACGAATCAACCTTGGCCTGTATTCACGAATGGAGACACTGAGGCAGAAATTCAGAAACAATATT    2070
CAGGATAAATTCAGATCACCTTAGCCATCTTGTCCCCATGTAATTTTCTCCCTGGTCCTTGAAGGGCCAGTCCTCATCATCTGATCTGTC    2160
CCAAAGGCAAACGCTATTCAGTGGGGGCATAGTCATTTGGGCATTTAAACATCAACACGAGTGCTCCAACAGATTGGCCACAACAGTAGT    2250
AACTCAAAAATCGCTTACCTGAATTACCTGCTTGAAAAATTTCAGACACCTACTGCTAAGACAACCTTGTCTCAAGGGACATTAGAGAGA    2340
AGCAGGGGAATTGTCATCTGATCTCCATGTTAAGAGCCCTTGAGGACTTCCAGAAGCCTACAGGGAATTTCCCTCGAATACCCTCATAAT    2430
GACTAAAAATTGCATTGCTTTTTAGCTTAGCCAAGTAGCTAACATTTACTGGTGTTTTCTTGATAACTGATTTCCTGATACATTAAAAAA    2520
```

Fig. 3. Nucleotide sequence of the rat ANT1 gene. The deduced amino-acid sequence is shown below the nucleotide sequence of each exon. TATA and CCAAT boxes, Sp1 binding motifs and putative polyadenylation signals are underlined.

Fig. 3 (continued).

sion method and S1 nuclease method, respectively (not shown). Just upstream of this site, there are TATA and CCAAT boxes at −135 bp and −191 bp, respectively, from the translation initiation site of the ATG codon. Moreover, Sp1 binding motifs (5′-GGGCGG) were observed in the 5′-terminus of the first intron, but not in the 5′-flanking region of this gene, as in the human ANT1 gene [12,19]. Although similarity was observed between the nucleotide sequences of the 5′-flanking regions of the rat and human ANT1 genes, the nucleotide sequence of the known heart-skeletal muscle specific transcriptional element (OXBOX) found in the human ANT1 gene [13] was not strictly conserved in the rat ANT1 gene.

## References


1 Klingenberg, M. (1985) in The Enzymes of Biological Membranes, Vol. 4 (Martonosi, A.N., ed.), pp. 511–553, Plenum, New York.

2 Klingenberg, M. (1989) Arch. Biochem. Biophys. 270, 1–14.

3 Vignais, P.V. (1976) Biochim. Biophys. Acta 456, 1–38.

4 Majima, E., Koike, H., Hong, Y.-M., Shinohara, Y. and Terada, H. (1993) J. Biol. Chem. 268, in press.

5 Adrian, G.S., McCammon, M.T., Montgomery, D.L. and Douglas, M.G. (1986) Mol. Cell. Biol. 6, 626–634.

6 Lawson, J.E. and Douglas, M.G. (1988) J. Biol. Chem. 263, 14812–14818.

7 Kolarov, J., Kolarova, N. and Nelson, N. (1990) J. Biol. Chem. 265, 12711–12716.

8 Bathgate, B., Baker, A. and Leaver, C.J. (1989) Eur. J. Biochem. 183, 303–310.

9 Emmermann, M., Braun, H.P. and Schmitz, U.K. (1991) Curr. Genet. 20, 405–410.

10 Powell, S.J., Medd, S.M., Runswick, M.J. and Walker, J.E. (1989) Biochemistry 28, 866–873.

11 Neckelmann, N., Li, K., Wade, R.P., Shuster, R. and Wallace, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 7580–7584.

12 Li, K., Warner, C.K., Hodge, J.A., Minoshima, S., Kondoh, J., Fukuyama, R., Maekawa, M., Shimizu, Y., Shimizu, N. and Wallace, D.C. (1989) J. Biol. Chem. 264, 13998–14004.

13 Li, K., Hodge, J.A. and Wallace, D.C. (1990) J. Biol. Chem. 265, 20585–20588.

14 Stepien, G., Torroni, A., Chung, A.B., Hodge, J.A. and Wallace, D.C. (1992) J. Biol. Chem. 267, 14592–14597.

15 Battini, R., Ferrari, S., Kaczmarek, L., Calabretta, B., Chen, S. and Baserga, R. (1987) J. Biol. Chem. 262, 4355–4359.
16 Ku, D.-H., Kagan, J., Chen, S.-T., Chang, C.-D., Baserga, R. and Wurzel, J. (1990) J. Biol. Chem. 265, 16060–16063.
17 Houldsworth, J. and Attardi, G. (1988) Proc. Natl. Acad. Sci. USA 85, 377–381.
18 Lunardi, J. and Attardi, G. (1991) J. Biol. Chem. 266, 16534–16540.
19 Cozens, A.L., Runswick, M.J. and Walker, J.E. (1989) J. Mol. Biol. 206, 261–280.
20 Maniatis, T., Fritsch, E.F. and Sambrook, J. (1989) Molecular Cloning: A Laboratory Mannual, Cold Spring Harbor Laboratory, Cold Spring Harbor.
21 Shinohara, Y., Ichihara, J. and Terada, H. (1991) FEBS Lett. 291, 55–57.