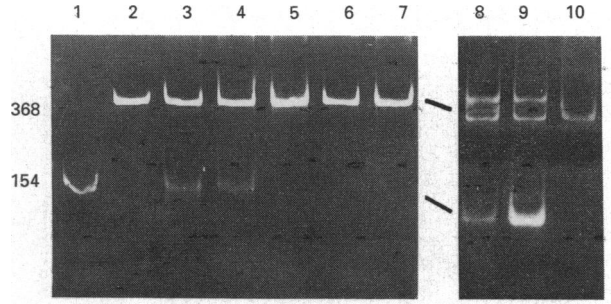

Fig. 1. Electrophoretic separation (ethidium-bromide-stained 6% polyacrylamide non-denaturing gel) of amplified and re-amplified hepatitis B virus (HBV) X-gene DNA

DNA depicted in lanes 1 and 2 represents the control size markers with their base lengths indicated on the left of the Figure. The band in lane 2 was prepared by standard symmetric amplification of an SP64 X-gene plasmid using primers 1 and 2 (HBV nucleotide sequences 1420–1443 and 1788–1765 respectively). Lane 1 represents DNA which was amplified using primer 2 and primer 3 (sense sequence 1634–1648, 5′ AGATCCTGCCCAAGG 3′). An aliquot (2%) from a PCR amplification reaction of DNA prepared as for lane 2 was re-amplified asymmetrically for 15 cycles using only primer 3 (normal sequence) and annealing temperatures of 50 °C (lane 3) and 60 °C (lane 4). Similar re-amplification with primer 3 sequences mismatched at the eighth base (C, A or T instead of G) is resolved in lanes 5–7. DNA which was produced after symmetric re-amplification using a mismatched primer (similar to that used in lane 5) and normal primer 2 at annealing temperatures of 50 °C and 60 °C is depicted in lanes 8 and 9 respectively. The DNA in lane 10 depicts separation of DNA re-amplified with the primer 3 sequence mismatched at the 3′ end (C instead of G). Two similar gels run independently (lanes 1–7 and 8–10) are depicted in the Figure.

mismatch is not always sufficient to decrease DNA synthesis significantly. More than one mismatch at the 3′ end of a primer is often required to detect a mutation [6].

Asymmetric PCR provides an alternative approach to the detection of small mutations. Here we have shown that linear re-amplification using only one primer improves the distinction between amplification from mismatched and normal primers. Using this procedure, incorporation of mismatched oligonucleotides and their complementary sequences into the template DNA does not occur. The data in Fig. 1 on the amplification of an X-gene fragment of the hepatitis B virus demonstrate this. In this set of experiments, a 368 bp X-gene fragment, amplified using a standard PCR protocol, was used as template source for reamplification from a set of five internal primers (see legend to Fig. 1 for details). After linear asymmetric amplification from a perfectly matched internal primer at a concentration of 0.25 μM (lanes 3 and 4), there is production of easily detectable extended product after 15 cycles—the 154 base fragment. This band was detectable when using an annealing temperature as high as 60 °C (lane 4). All three mismatches at the eighth base of the primer resulted in a failure to detect extension (lanes 5–7), as did a primer which had a 3′ mismatch (lane 10). In each of the experiments using mismatched primers the annealing temperature was 50 °C. Symmetric re-amplification with two primers (one mismatched) at equal concentrations does result in significant amplification of the smaller fragment (lanes 8 and 9 of Fig. 1). The extent of symmetric re-amplification was similar when using the fully complementary or any of the other mismatched primers. The additional larger DNA band seen in lanes 8 and 9 is an artefact which was often produced during symmetric logarithmic PCR using the conditions described here.

Decreased melting temperature or conformational changes at the site of extension by *Taq* polymerase are probably responsible for the observed differences between symmetric and asymmetric re-amplification. The inhibitory effect of single base mismatches on primer extension during asymmetric re-amplification is a useful property which can be exploited to assess oligonucleotide and template DNA sequence complementarity.

This work was supported financially by the H. E. Griffin Cancer Research Trust, the Poliomyelitis Research Foundation, the National Cancer Association and the Medical Research Council.

Patrick ARBUTHNOT and Wyn FITSCHEN

Department of Medical Biochemistry, University of the Witwatersrand Medical School, 7 York Road, Parktown 2193, South Africa

1. Bonner, T. I., Brenner, D. J., Neufield, B. R. & Britten, R. J. (1973) J. Mol. Biol. **81**, 123–135
2. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) in Molecular Cloning—A Laboratory Manual, 2nd edn., pp. 11.1–11.63, Cold Spring Harbor Laboratory Press, New York
3. Okayama, H., Curiel, D. T., Brantly, M. L., Holmes, M. D. & Crystal, R. G. (1989) J. Lab. Clin. Med. **114**, 105–113
4. Kwok, S., Kellogg, D. E., McKinney, N., Spasic, D. D., Goda, L., Levenson, C. & Sninsky, J. J. (1990) Nucleic Acids Res. **18**, 999–1005
5. Neubauer, A., Neubauer, B. & Liu, E. (1990) Nucleic Acids Res. **18**, 993–998
6. Main, B. F., Jones, P. J. H., MacGillivray, R. T. A. & Banfield, D. K. (1991) in PCR Topics, pp. 99–102, Springer-Verlag, Berlin

Received 18 August 1992

Mitochondrial cyclophilins

The cyclophilins, a family of proteins with peptidylprolyl *cis–trans* isomerase activity, were first identified as abundant cytoplasmic proteins, with about 165 amino acid residues and very strong sequence conservation. Bovine and porcine cyclophilin-A are identical, while the human enzyme differs at only three residues [1–3]. There are seven conservative differences between human and rat cyclophilin-A, plus one additional amino acid at the *C*-terminal end of the human protein [4]. Proteins with strong homology are present in the cytoplasm of all eukaryotic organisms studied and in *Escherichia coli*.

More recently a second family of cyclophilins with an *N*-terminal signal sequence has been reported in species ranging from *E. coli* to man [5–7]. The *E. coli* cyclophilin-B is retained in the periplasm, while the mammalian proteins in this group have a *C*-terminal decapeptide which may direct them to an endoplasmic reticulum-associated compartment [8]. These cyclophilins-B have a lower level of similarity to the cytoplasmic cyclophilins-A. In man there are 59 amino acid differences, many not conservative, in addition to the *N*- and *C*-terminal extensions of cyclophilin-B.

A mitochondrial form of cyclophilin has been reported in *Neurospora crassa*, encoded by the same genes as the cytoplasmic enzyme and arising by alternative splicing [9]. There is much evidence for cyclosporin-sensitive peptidylprolyl isomerases in mammalian mitochondria (see [10]), but no evidence for additional exons available for alternative splicing in the human cyclophilin-A gene [11]. Now Connern & Halestrap [10] have reported the *N*-terminal amino acid sequence of a rat mitochondrial cyclosporin-sensitive peptidylprolyl isomerase which shows clear homology with the known rat and human cyclophilins but has seven amino acid differences from rat cyclophilin-A and 12 differences from rat cyclophilin-B in the first 16 amino acids. However, this partial rat mitochondrial sequence shows

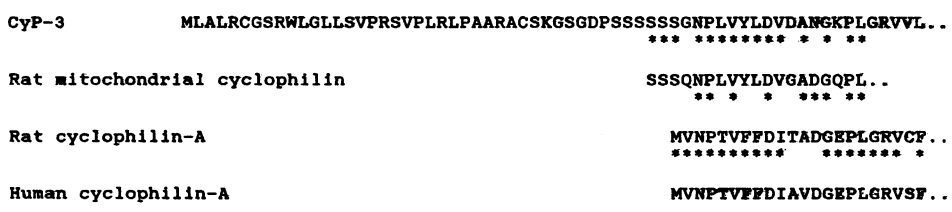

Fig. 1. Alignment of the N-terminal amino acid sequences of human CyP-3 [12], rat mitochondrial cyclophilin [10], rat cyclophilin-A [4] and human cyclophilin-A [3]

greater similarity to the amino acid sequence of the corresponding region of a third human cyclophilin cDNA recently reported by Bergsma *et al.* [12] and named CyP-3 (Fig. 1). This similarity includes a run of three serine residues at the *N*-terminal end of the mature protein, immediately following the cleavage site identified by Connern & Halestrap [10].

CyP-3 has 75% identity with human cyclophilin-A and over 60% identity with the corresponding region of cyclophilin-B. Bergsma *et al.* also noted that CyP-3 encoded a putative leader sequence and that the mature protein was associated with a particulate fraction containing membranes and organelles obtained by centrifugation of a post-nuclear fraction at 150000 *g* for 90 min. The *N*-terminal hydrophobic leader sequence of CyP-3 includes several basic residues, which are not found in the cyclophilin-B leader sequences but are characteristic of leader sequences targetting proteins into organelles such as mitochondria [13]. The CyP-3 sequence reported by Bergsma *et al.* [12] thus probably encodes a human mitochondrial cyclophilin homologous to the rat enzyme studied by Connern & Halestrap [10].

John E. KAY

Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Brighton BN1 9QG, U.K.

1. Harding, M. W., Handschumacher, R. E. & Speicher, D. W. (1986) J. Biol. Chem. **261**, 8547–8555
2. Takahashi, N., Hayano, T. & Suzuki, M. (1989) Nature (London) **337**, 473–475
3. Haendler, B., Hofer-Warbinek, R. & Hofer, E. (1987) EMBO J. **6**, 947–950
4. Danielson, P. E., Forss-Petter, S., Brow, M. A., Calavetta, L., Douglass, J., Milner, R. J. & Sutcliffe, J. G. (1988) DNA **7**, 261–267
5. Iwai, N. & Inagami, T. (1990) Kidney Int. **37**, 1460–1465
6. Price, E. R., Zydowsky, L. D., Jin, M., Baker, C. H., McKeon, F. D. & Walsh, C. T. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 1903–1907
7. Kawamukai, M., Matsuda, H., Fujii, W., Utsumi, R. & Komano, T. (1989) J. Bacteriol. **171**, 4525–4529
8. Arber, S., Krause, K.-H. & Caroni, P. (1992) J. Cell Biol. **116**, 113–125
9. Tropschug, M., Nicholson, D. W., Hartl, F.-H., Kohler, H., Pfanner, N., Wachter, E. & Neupert, W. (1988) J. Biol. Chem. **263**, 14433–14440
10. Connern, C. P. & Halestrap, A. P. (1992) Biochem. J. **284**, 381–385
11. Haendler, B. & Hofer, E. (1990) Eur. J. Biochem. **190**, 477–482
12. Bergsma, D. J., Eder, C., Gross, M., Kersten, H., Sylvester, D., Appelbaum, E., Cusimano, D., Livi, G. P., McLaughlin, M. M., Kasyan, K., Porter, T. G., Silverman, C., Dunnington, D., Hand, A., Prichett, W. P., Bossard, M. J., Brandt, M. & Levy, M. A. (1991) J. Biol. Chem. **266**, 23204–23214
13. Gavel, Y. & von Heijne, G. (1990) Protein Eng. **4**, 33–37

Received 27 August 1992