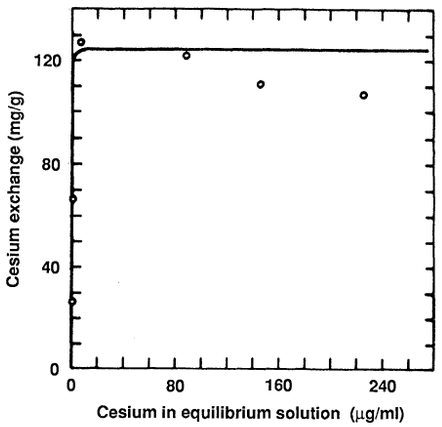

Fig. 2. Cesium exchange isotherm of K-depleted phlogopite mica with constant solid-solution ratio but increasing amounts of cesium.

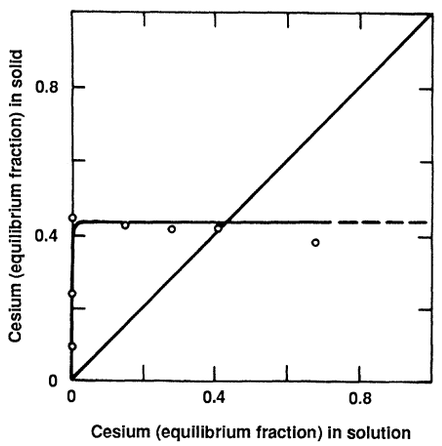

Fig. 3. Na ⇌ Cs exchange isotherm of K-depleted phlogopite mica with constant amounts of solid, water, and total cations, but variable proportions of Na^+ and Cs^+ cations.

above the diagonal line initially, which shows that Cs^+ is highly preferred over Na^+ initially. However, exchange does not go to completion, as indicated by the cesium exchange capacity of 91.4 meq/100 g, because of the interlayer collapsing and cesium ion trapping effect as described above. Cesium preference over sodium at the initial stages was extremely high, as indicated by the data points of the isotherm falling well above the diagonal line. The extreme preference of cesium over sodium ions in the K-depleted phlogopite mica is further attested by the fact that the total cesium exchange capacity is approximately the same whether sodium is present (91.4 meq of cesium per 100 g) or absent (93.7 meq of cesium per 100 g) in the equilibrating solution. The interlayer spacing of 2.89 Å is ideal for the diffusion of less hydrated cesium ions, just as in the case of γ-zirconium phosphate, which has an interlayer spacing of 2.85 Å (*6*).

A lower interlayer spacing would restrict the diffusion of all ions including cesium, whereas an increase in interlayer spacing would allow much better access to other hydrated ions and thus limit the preference of cesium ions. The ideal interlayer spacing coupled with the high charge density of the layers is instrumental in the selective uptake and trapping of the cesium ions. Partially K-depleted biotite mica has been shown earlier to specifically exchange cesium (*13*), but fully K-depleted phlogopite mica has not been previously studied. Full potassium depletion results in a 12.23 Å *c*-axis *d*(001) spacing that is essential for obtaining the highest capacity and selectivity for cesium exchange.

The selectivity of the K-depleted phlogopite mica for cesium ions in the presence of excess Na^+ and Ca^{2+} ions is compared with some of the cation exchangers that are presently used (Table 1). These results show that K-depleted phlogopite mica is by far the best material for selectively exchanging cesium from concentrated solutions containing Na^+ or Ca^{2+}, these two ions being dominant in natural waters.

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Generation of cDNA Probes Directed by Amino Acid Sequence: Cloning of Urate Oxidase

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Urate oxidase (E.C. 1.7.3.3) catalyzes the oxidation of uric acid to allantoin in most mammals except humans and certain primates. The amino-terminal amino acid sequence for porcine urate oxidase was determined and used in a novel procedure for generating complementary DNA (cDNA) probes to this amino acid sequence. The procedure is based on the polymerase chain reaction and utilizes mixed oligonucleotide primers complementary to the reverse translation products of an amino acid sequence. This rapid and simple cDNA cloning procedure is generally applicable and requires only a partial amino acid sequence. A cDNA probe developed by this procedure was used to isolate a full-length porcine urate oxidase cDNA and to demonstrate the presence of homologous genomic sequences in humans.

IN MOST MAMMALS, URATE OXIDASE IS present in the liver, with little or undetectable activity in other tissues. It is associated with the peroxisome and exists as a tetramer with an apparent subunit size of 32,000 daltons (*1*). Humans and certain primates lack this enzyme activity (*2*). Overproduction or elevated serum uric acid levels in man can lead to gouty arthritis. The recent identification of mice with complete hypoxanthine–guanine phosphoribosyl transferase (HPRT) deficiency that do not display any of the symptoms of Lesch-Nyhan's syndrome has raised the possibility that the absence of urate oxidase activity in

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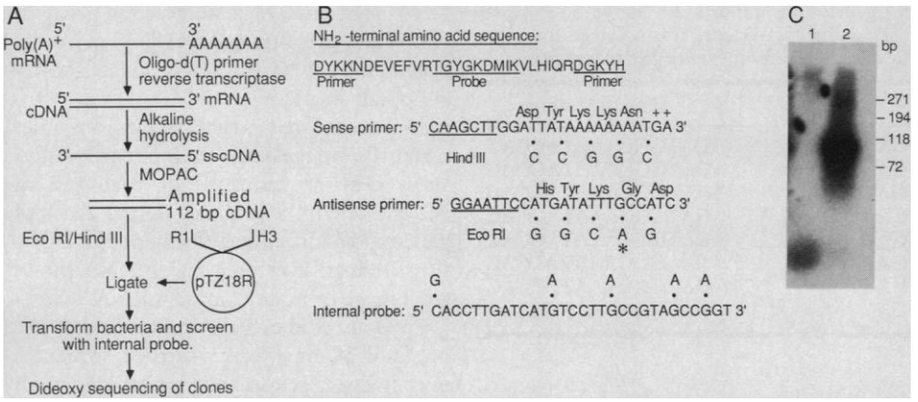

Fig. 1. The strategy for MOPAC cloning, the selection of primers, and probe and product analysis. (**A**) Schematic steps in cloning cDNA based on amino acid sequence and the MOPAC procedure. (**B**) The NH_2-terminal amino acid sequence for porcine urate oxidase and the selection of primers and probe for MOPAC. The sense primer was synthesized to the amino acid sequence 1 to 5. The inclusion of the next two nucleotides from the sixth amino acid is indicated by (++). The antisense primers were synthesized to the amino acid sequence 28 to 32. For both primers, every codon degeneracy was included except for the amino acid glycine, where (*) indicates the selected codon degeneracy. The selection of different restriction enzyme linkers (Eco RI/Hind III) is to facilitate the rescue of amplified cDNA. An internal oligonucleotide probe in the antisense orientation was synthesized for monitoring progress of the MOPAC procedure. (**C**) Autoradiograph of internal probe hybridized to the amplification reaction; lane 1, 10 μl of preamplified reaction mixed; lane 2, 10 μl of postamplified reaction mixed. The annealing and DNA amplification were carried out at 28°C (*18*). The cDNA was separated from primers on a 4% Nusieve agarose gel and transfered by Southern blotting to Zetabind membrane. The probe hybridization was as described (*19*).

Fig. 2. MOPAC cloned pPUO1 cDNA and the urate oxidase NH_2-terminal amino acid sequence. (**A**) DNA sequence of pPUO1. (**B**) The porcine urate oxidase NH_2-terminal sequence corresponding to the 5′ open reading frame sequence of a 2.2- kb cDNA. The sequence which is underlined or represented in bold letters corresponds to that obtained by peptide sequencing of the porcine and murine urate oxidases, respectively.

A DNA sequence of pPUO1

5' GATTATAAGAAGAATGATGAGGTAGAGTTTGTCCGAACTGGCTATGGGAAG
GATATGATAAAAGTTCTCCATATTCAGCGAGATGGCAAATATCAC 3'

B NH_2-terminal amino acid sequence of porcine urate oxidase

MAHYRN**DYKKNDEVEFVRTGYGKDMIKVLHIQRDGKYHSIKEVATSVQLT**LSSKKDY
LHGDNSDVIPTDTIKNTVNVLAKFKGIKSIETFAVTICEHFLSSFKHVIRA

the purine metabolism pathway in man may contribute to the development of the neurological symptoms observed in human patients (*3*). For these reasons we have undertaken the molecular cloning of urate oxidase.

A major procedure in complementary DNA (cDNA) cloning involves the synthesis of oligonucleotide probes to a known peptide sequence. However, the degeneracy of the genetic code for all amino acids except methionine and tryptophan requires synthesis of oligonucleotide mixtures for use as hybridization probes (*4–6*). Developing the conditions to distinguish an authentic signal from spurious hybridization is difficult and time consuming with degenerate oligonucleotide probes (*6*). To overcome these limitations, we have developed a procedure, based on the polymerase chain reaction (PCR), for cDNA probe generation from amino acid sequences with highly degenerate codons.

The recently described PCR technique has been used to construct mutations in vitro and to amplify single copy sequences within complex DNA mixtures for facile cloning and analysis (*7*). We now show that specific cDNA probes can be rapidly generated by the PCR when mixed oligonucleotides derived from amino acid sequence are used as primers. The cDNA probe generated by mixed oligonucleotide primed amplification of cDNA (MOPAC) can be used for hybridization studies or for screening a cDNA library for a full-length clone.

Porcine liver urate oxidase was obtained commercially and purified to homogeneity (*8*). Automated Edman degradation of polypeptide (100 picomoles) allowed the determination for the sequence of the first 32 amino acids. Purification of murine urate oxidase to homogeneity was also carried out (*8*) and the amino acid sequence from a cyanogen bromide cleavage peptide was determined. A GeneBank library search (*9*) has revealed no sequence homology to previously cloned genes or peptide sequence.

The strategy used to generate a cDNA probe from this amino acid sequence is described in Fig. 1A. Polyadenylated [poly(A)$^+$] messenger RNA (mRNA) was purified from porcine liver and first strand cDNA was generated with reverse transcriptase from Moloney murine leukemia virus and oligo(dT) primers. After alkaline hydrolysis of the mRNA (*10*), MOPAC was carried out on the single strand cDNA (sscDNA) population. The mixed oligonucleotide primers used for amplification were selected as described in Fig. 1B. The sense and antisense priming regions represented amino acid sequences 1 to 5 and 28 to 32, respectively. The sense primers were synthesized with a Hind III linker including two nucleotides from the codon specifying the sixth amino acid, asparagine, since the addition of these two nucleotides does not alter primer degeneracy. The antisense primers were synthesized with an Eco RI linker: the primers included two out of the four possible codon degeneracies for glycine. For each of these five selected amino acids there are two codon degeneracies. Thus, for each primer mix there are 32 (that is, 2^5) different combinations. An internal oligonucleotide probe was synthesized to represent amino acid sequence 13 to 21 with the most frequently used codons (*11*). The expected size of a successful amplification product would be a cDNA of 112 bp including the restriction enzyme linkers.

After 21 cycles of amplification, the success of MOPAC was determined by Southern hybridization with the internal probe. This probe hybridized strongly to a product of the expected size (112 bp) in the amplified fraction, but not in the preamplified fraction (Fig. 1C). In the preamplified fraction, the signal observed in the region of the gel where the primers migrated suggests a low level of nonspecific hybridization to the primers. These results show that a product of the expected size has been amplified. To rescue this amplified cDNA, the entire amplification reaction was purified by phenol/chloroform extraction, digested with Eco RI and Hind III, and cloned into the Eco RI and Hind III sites of vector pTZ18R.

Seven clones containing the 112-bp insert were identified by in situ hybridization (*10*) of transformed bacterial colonies with the internal probe. One of these (pPUO1) was sequenced by the dideoxy procedure (*12*), which revealed an open reading frame sequence that corresponded to the NH_2-terminal amino acid sequence of porcine urate oxidase (Fig. 2A).

For isolating the full-length porcine urate oxidase cDNA, a porcine liver λ cDNA library was constructed (*13*). About 200,000 primary recombinant phage were screened and about 1 in every 10,000 phage plaques hybridized to the insert from pPUO1 and contained inserts ranging from 1.8 kb to 2.2 kb. Dideoxy sequencing of the 5′ region of a 2.2-kb cDNA gave an open reading frame amino acid sequence that corresponded to the NH_2-terminal sequence for both porcine and murine urate oxidase derived from peptide sequencing (Fig. 2B).

Table 1. Primers used in the synthesis of the pPUO clones. The primers are in the 5′-3′ sense orientation for the seven characterized pPUO clones. The authentic sequence was obtained from the 2.2-kb cDNA, and the underlined nucleotides show the variation from the authentic sequence.

Clone	Sense primer	Antisense primer
pPUO 1	GATTATAAGAAGAATGA	GATGGCAAATATCAC
pPUO 2	GATTATAAGAAGAATGA	GATGGCAAATACCAC
pPUO 3	GATTACAAGAAGAACGA	GACGGCAAATACCAC
pPUO 4	GACTATAAGAAGAATGA	GATGGCAAATACCAC
pPUO 5	GATTATAAGAAGAATGA	GATGGCAAATACCAC
pPUO 6	GACTATAAGAAGAATGA	GACGGCAAATATCAC
pPUO 7	GATTACAAGAAGAATGA	GACGGCAAATATCAC
Authentic	GACTACAAAAAGAATGA	GATGGAAAATATCAC

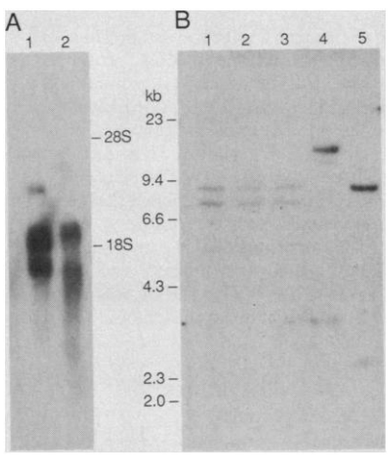

Fig. 3. Northern and genomic Southern analysis for urate oxidase. (**A**) Autoradiography on poly(A)$^+$ mRNA probed with the 2.2-kb cDNA probe: lane 1, 5 μg of murine liver mRNA; lane 2, 5 μg of porcine liver mRNA. (**B**) Autoradiograph of genomic DNA digested with Eco RI and probed with the 2.2-kb cDNA. Lanes 1, 2, and 3 are three unrelated humans; lane 4, mouse; lane 5, hamster. Probe hybridization was carried out at 42°C in the presence of 50% formamide (*19*). The blots were washed in 2× SSC, 0.1% SDS at 65°C.

Thus, the 2.2-kb cDNA is an authentic clone for the porcine urate oxidase.

The pPUO1 sequence is homologous to the 2.2-kb cDNA except for the region corresponding to the primers used for the MOPAC procedure. To determine whether any primers were preferred, six other pPUO clones obtained by the MOPAC procedure were sequenced. The results (Table 1) demonstrate that the generated sequences are identical to the authentic sequence but not the primers' sequence. Thus, the MOPAC procedure will generate authentic sequence even when there are base pair mismatches between the primers and the cDNA. Different primers were found in the pPUO clones although two particular sets of primers were found more frequently than others. There are no obvious rules governing these base pair mismatches, although a C-T mismatch seems more prevalent than a G-A mismatch. The authentic codon usage for glycine, which was not available in the primers, was accommodated by the selection of GGC as the preferred codon in the pPUO clones.

Colony hybridization with a homologous oligonucleotide probe derived from amino acid sequence 6 to 27 indicated that authentic pPUO clones are about 2% of the total transformed bacterial colonies. It is envisaged that with the introduction of a size fractionation step, the frequency for bona fide MOPAC clones would be increased to a level suitable for direct mini-plasmid analysis. Selection of MOPAC clones should be based on size of the expected cDNA product. Several clones having inserts of 70 bp or less were also characterized and found to contain predominantly primers. These appear to be nonspecific amplified products similar to those reported for PCR (*14*).

A Northern blot analysis of the porcine and murine poly(A)$^+$ mRNA with the 2.2-kb cDNA probe identifies mRNAs of about 1.8 and 2.2 kb (Fig. 3A). In the murine sample, a minor 3.0-kb mRNA is also observed. From the size of the mRNA, the 2.2-kb cDNA would represent an apparent full-length cDNA for the porcine urate oxidase. This has been confirmed by the presence of a similar 3′ sequence including a poly(A) tail and a 5′ sequence corresponding to the NH_2-terminal peptide sequence from different full length cDNA clones.

Human and certain primates have no detectable activity for urate oxidase. The total absence of enzyme activity is in keeping with the fact that uric acid is a major excretion product in these mammals (*2*). Surprisingly, a Southern blot analysis of human genomic DNA indicates the presence of restriction fragments that are homologous to porcine urate oxidase cDNA (Fig. 3B). When pPUO1 was used as the probe, a single restriction fragment was observed with human, mouse, and hamster genomic DNA suggestive of a single copy gene. In Old World primates and certain New World primates, a low level of urate oxidase activity is present in the liver and has low in vitro stability compared to the enzyme from other mammals suggesting evolutionary differences (*15*). In humans, the total loss of enzyme activity is probably due to a lack of gene expression since a Northern blot analysis of human liver poly(A)$^+$ mRNA shows an absence of urate oxidase mRNA.

Previous studies have indicated that the presence of imperfectly matched sequences in the same reaction may not interfere with the hybridization of the homologous sequence with its cDNA (*16*). This would suggest the MOPAC procedure will favor the homologous primer as compared to nonhomologous primers. Our results show that this need not be the case. The observation that the characterized pPUO clones do not always share the same primers and that none of these primers are homologous to the authentic sequence indicates that the Klenow fragment of DNA polymerase I (*17*) will efficiently catalyze polymerization using imperfectly matched primers. This is probably a reflection of the flexibility of Klenow polymerase rather than an aberration in DNA hybridization kinetics. This is supported by studies in which PCR has been used to generate mutations in vitro by mispriming (*14*). Mispriming can occur in the MOPAC procedure during the initial annealing or during the subsequent amplification cycles. Our results demonstrate that bona fide probe generation will occur even when there is a 20% base pair mismatch between the primer and the authentic cDNA. The tolerance for such a high level of base pair mismatch is an important advantage of this procedure, since codon degeneracy can vary with different amino acids. The maximum tolerable level of base pair mismatch is not clear although the maximum possible mismatch of 33% was not observed in the clones characterized. We are uncertain of the upper limit for the number of primer combinations although the flexibility in base pair mismatch may reduce the significance of this parameter.

This MOPAC procedure for cDNA cloning has advantages over library screening with degenerate oligonucleotide probes synthesized to the polypeptide sequence. The rapid confirmation of the MOPAC-generated probe by dideoxy sequencing allowed its use for the screening of a cDNA library for full-length clones at maximum hybridization stringency for efficient elimination of unauthentic clones. The MOPAC procedure should have general application to the cloning of genes for proteins whose amino acid sequence is known.

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19. The internal probe was end-labeled in the presence of [^{32}P]ATP (3000 Ci/mmol) by T4 polynucleotide kinase (*10*). The probe hybridization was carried out at 42°C in 6× SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 0.1% Denhardt's solution, 50 m*M* tris-HCl, *p*H 7.5, and 50 μg/ml denatured herring sperm DNA. The blot was washed in 2× SSC, 0.1% SDS at 42°C.
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Aquatic Productivity and the Evolution of Diadromous Fish Migration

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Diadromous migration, in which some fish species migrate from freshwater and feed in the ocean (anadromous species) and others migrate from the ocean and feed in freshwater (catadromous), has long been perplexing. However, when the distribution of diadromous species is examined with respect to global patterns in aquatic productivity, this apparent paradox is resolved. The contrasting directions of migration can largely be explained by the relative availability of food resources in ocean and freshwater habitats. Oceans are more productive than freshwaters in temperate latitudes, and anadromous species predominate. In contrast, catadromous species generally occur in tropical latitudes where freshwater productivity exceeds that of the ocean.

LARGE-SCALE MOVEMENTS OF ANIMALS are found in many taxonomic groups (*1*). The migrations of diadromous fish species, those that migrate between the ocean and freshwater, are particularly enigmatic because this behavior necessitates physiological changes in osmoregulation (*2*). Diadromous fishes are found in 28 families and include two distinctly different groups: (i) 87 anadromous species, such as salmon (Salmonidae) and lamprey (Petromyzontidae), which are born in freshwater, migrate to the ocean, and return to freshwater to spawn; and (ii) 41 catadromous species, such as some eels (Anguillidae) and mullets (Mugilidae), which are born in the ocean, migrate to freshwater, and return to the ocean to spawn (*3*). The existence of these contrasting directions of migration has long been perplexing. Indeed, it has been described as a paradox in animal migration (*1*). We report that diadromous migrations may occur in fishes because of the differential availability of food resources in ocean and freshwater habitats. Moreover, it is because the relative productivity of oceans and freshwaters is not constant but changes with latitude that the contrasting directions of anadromous and catadromous migration can exist.

In theory (*4*), diadromous life histories will evolve through natural selection only when migration across the ocean-freshwater boundary provides a gain to individual fitness (lifetime reproductive success) that exceeds the costs of this behavior. These costs may include adjustments to physiology, allocation of energy for swimming, and increased probability of mortality during migration. Several authors (*1, 5–9*) have speculated on what factors might favor juvenile fishes deserting their habitat of birth for residency elsewhere. Among these have been decreased predation, decreased disease, decreased physiological stress, or increased food availability. To date, these hypotheses have not been tested quantitatively because of the logistic problems presented by animals that may travel several thousand kilometers and because of our limited knowledge of the life histories of many fish species.

McDowall's (*3*) findings on the global geographic distribution of diadromous species are shown in Fig. 1A. These data indicate latitudinal differences in the worldwide distribution of anadromous and catadromous fishes, with anadromy being more common in temperate (including arctic) latitudes and catadromy in the tropics. Therefore, any hypothesis for the evolution of diadromy must not only provide evidence for a substantial fitness benefit to a diadromous migrant, but must also account for the geographical distribution of diadromy. A hypothesis based on the differential availability of food in the oceans and freshwaters meets these criteria.

Let us first consider whether such a hypothesis can allow for significant fitness benefits through migration. The importance of food intake for body growth (*10*) and the contribution of growth to fitness through decreased mortality (*11*), increased fecundity (*12*), and improved male (*13*) and female (*14*) breeding success have been documented in many fishes. There are also well-studied cases of growth rates increasing with movement across the freshwater-ocean boundary. Juvenile anadromous Pacific salmon, for example, can experience a 10 to 50% increase in their daily growth rate during their first week of ocean life (*11*). In addition, a recent survey by Gross (*4*) of diadromous and nondiadromous populations within seven salmonid species showed that the only significant difference in major life history traits was that individuals in

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