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Isolation of a rat liver Golgi mannosidase II clone by mixed oligonucleotide-primed amplification of cDNA

(polymerase chain reaction/glycosylation/deoxyinosine)

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ABSTRACT **A clone encoding Golgi mannosidase II (MII; GlcNAc-transferase I-dependent α1,3(α1,6) mannosidase), an enzyme involved in asparagine-linked oligosaccharide processing, was isolated from a rat liver λgt11 cDNA library by a method that employs a modification of the polymerase chain reaction. Specific oligonucleotide primers were designed from two regions of protein sequence and were combined in an amplification reaction with a single-stranded cDNA preparation derived from rat liver poly(A)$^+$ RNA. Based upon mapping of the protein sequences 42 kDa apart on the MII polypeptide, the procedure was expected to generate an ≈1150-base-pair amplification product representing a segment of the MII gene between the two primer regions. The size of the amplified product (1170 base pairs) was in close agreement with this predicted fragment size. The authenticity of the amplified fragment was confirmed by the agreement of the DNA sequence with additional protein sequence data. A 1474-base-pair clone was isolated from a cDNA library by plaque hybridization using the amplification fragment as a radiolabeled probe. The nucleotide sequence of this clone predicts a single continuous open reading frame and, based upon a polypeptide molecular mass of 117 kDa for the enzyme subunit, is consistent with the clone representing ≈50% of the coding sequence of MII. Both the clone and the amplification product hybridized to a rat liver mRNA of ≈8 kilobases, a message size ≈4.7 kilobases larger than the size of the predicted open reading frame. This extensive non-coding information on the MII message is a feature common to two other Golgi processing enzymes, both of which contain most of the non-coding information on the 3′ end of their messages. The function of these disproportionately large untranslated regions is not clear.**

Mannosidase II (MII; GlcNAc-transferase I-dependent α1,3(α1,6) mannosidase) is a resident enzyme of the Golgi complex where it catalyzes the committed step in the maturation of asparagine-linked oligosaccharides from oligomannose structures to exclusively complex type structures (1). Glycoproteins entering the Golgi complex contain oligomannose structures and can be acted upon by Golgi mannosidase I, which removes the α1,2-mannosyl residues yielding a $Man_5GlcNAc_2$ structure. After addition of a single GlcNAc by GlcNAc transferase I, the final hydrolytic step in the pathway is the specific removal of α1,3- and α1,6-linked mannosyl residues by MII to generate the $GlcNAcMan_3GlcNAc_2$ core. The oligosaccharides are then extended to mature complex structures by a variety of Golgi transferases (2). The essential role of MII in the oligosaccharide processing pathway has been demonstrated both *in vivo* and *in vitro* by the use of swainsonine, a specific inhibitor that causes the accumulation of hybrid type oligosaccharides in place of complex type structures (3).

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The enzyme has been purified and extensively characterized from rat liver where it resides in the Golgi complex anchored through an NH_2-terminal transmembrane domain (for review, see ref. 1). The catalytic center of the enzyme faces the lumen of the Golgi complex but can be released in a soluble form from permeabilized membranes after digestion with chymotrypsin (4). This soluble 110-kDa digestion product is catalytically similar to the intact enzyme purified from Golgi membranes but differs in NH_2-terminal protein sequence and lacks the hydrophobic character, and presumably the membrane-spanning domain, of the intact enzyme (5).

To determine the mechanism by which MII is selectively maintained within the Golgi complex, we have isolated a clone encoding rat liver MII by a modification of the method originally developed by Lee *et al.* (6). This method employs mixed oligonucleotide primers derived from protein sequence data and the polymerase chain reaction (PCR) to generate a double-stranded cDNA probe from a heterogeneous single-stranded preparation of cDNAs. The probe has been subsequently used to isolate a partial clone for MII. Hybridization studies with rat mRNA have also revealed several aspects about the message size and organization* that may be common to two other previously cloned Golgi enzymes (11, 17).

MATERIALS AND METHODS

Protease Digestion and Protein Sequence Analysis. The 110-kDa MII chymotrypsin digestion product was purified from rat liver Golgi membranes by phase separaton in Triton X-114 followed by cation-exchange chromatography as described (5). The homogeneous enzyme preparation (125 μg) in 20 mM Tris·HCl (pH 7.7) was digested with 1 μg of endoproteinase Lys-C (Boehringer Mannheim) in a volume of 200 μl for 30 min at 37°C. The reaction was terminated by heating to 65°C for 10 min in $NaDodSO_4$/gel electrophoresis sample buffer (4). Processing of the digestion products and protein sequencing was carried out essentially as described by Matsudaira (7) by resolving the digestion products on an $NaDodSO_4$/11% polyacrylamide gel, transferring the gel to a polyvinylidene difluoride membrane (Immobilon transfer membrane, Millipore), and sequencing the immobilized peptides on an Applied Biosystems model 470 sequencer. An undigested MII sample (20 μg) was also processed for sequencing as above.

Oligonucleotides and cDNA Synthesis. Oligonucleotides were synthesized by the phosphoramidite method on a Milligen model 6500 oligonucleotide synthesizer or purchased from Operon Technologies. Total RNA was isolated from Sprague–Dawley rat liver by the method of Chirgwin *et al.*

Abbreviations: MII, mannosidase II; PCR, polymerase chain reaction; Taq, *Thermus aquaticus*; MOPAC, mixed oligonucleotide-primed amplification of cDNA.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M24353).

(8). Purification of poly(A)$^+$ RNA by oligo(dT) chromatography was performed as described (9). For some experiments total RNA was fractionated on sucrose gradients (10) prior to oligo(dT) chromatography to remove RNA species <3 kilobases (kb). cDNA synthesis was performed in a 100-μl reaction volume containing 200 units of murine leukemia virus reverse transcriptase (BRL), the enzyme buffer (as supplied by BRL), 5 μg of poly(A)$^+$ RNA, 40 units of RNasin (Promega), each dNTP at 1 mM, and either random hexanucleotide primers (Amersham) at 15 μg/ml or 0.05 A_{260} unit of oligo(dT) primers (Amersham) at 37°C for 1.5 hr. The reaction mixture was extracted with phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol), and desalted over a Sephadex G-50 column (Nick column, Pharmacia) equilibrated in 100 mM KCl/10 mM Tris·HCl, pH 8.0. This desalted preparation was used directly in the PCR amplification.

Amplification and Subcloning of the PCR Products. The amplification of MII was performed in a 100-μl reaction volume containing 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 1.5 mM $MgCl_2$, and 0.01% gelatin plus each dNTP at 200 μM, 0.5 μM 3′ and 5′ primers, 5 μl of either the desalted cDNA synthesis products or λDNA, 2.5 units of *Thermus aquaticus* (Taq) polymerase (Perkin–Elmer/Cetus), and 100 μl of mineral oil. The samples were placed in an automated heating/cooling block (DNA Thermal Cycler, Perkin–Elmer) programmed for a temperature-step cycle of 92°C (2 min), 50°C (2 min), and 72°C (8 min). This cycle was repeated for a total of 40 cycles with a 7-min extension at 72°C after the final cycle. The final reaction products were resolved on a 1% agarose gel containing ethidium bromide (0.5 μg/ml). Control reactions employed 30-mer exact-match oligonucleotide primers specific for rat α2,6-sialyltransferase at sequence positions 1–30 and 1183–1212, as described in figure 5 of ref. 11. The amplified fragment (PCR-1) was prepared for subcloning and sequencing after a fill-in reaction with T4 polymerase (12). The resulting blunt-ended cDNA fragment was either subcloned into the *Hinc*II site of pUC or M13 directly or digested at the internal *Bam*HI or *Pst* I sites of PCR-1 and subcloned into the corresponding sites in pUC and M13.

Screening of the λgt11 cDNA Library. An isolation procedure using the amplification protocol as a diagnostic test of λ phage library pools was used to enrich for the clones containing the PCR-1 fragment. Briefly, 120 minilysates were prepared, each containing ≈20,000 plaque-forming units of a rat liver λgt11 cDNA library (RL1001b, Clontech) and ≈4 × 10^7 Y1090 host cells in 1 ml of LB broth containing ampicillin (50 μg/ml; LB/amp). The cultures were grown at 42°C for 5 hr with constant agitation, and culture supernatants, containing phage titers of ≈3 × 10^9 plaque-forming units/ml, were used as source material to generate phage pools for amplification. Aliquots of the minilysates, representing 2.5 × 10^6 plaque-forming units each, were pooled in groups of 10 and used to generate a pool lysate from a culture containing ≈10^9 Y1090 host cells in 30 ml of LB/amp. The phage DNA was isolated by adsorption to immobilized anti-λ phage antibody (LambdaSorb, Promega) as described by the manufacturer. The DNA was resuspended in water and was used directly for PCR amplification using the primers shown in Fig. 2. The amplification products were resolved on an agarose gel and three pools were identified as positive for the amplification of the MII-specific product PCR-1. Phage in other aliquots from the original individual minilysates that had contributed to the three positive lysate pools were individually grown and the phage DNA was isolated as above. Three of the resulting minilysates were positive for amplification of PCR-1. These three lysates were plated on Y1090 host cells and screened by plaque hybridization by standard procedures (13) with a PCR-1 probe labeled by nick-translation.

DNA Sequencing. DNA sequencing was performed in phage M13 by the dideoxynucleotide chain-termination method using deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate and Sequenase (United States Biochemical), as described by the manufacturer. The sequence data were obtained from successive deletions in M13 using T4 polymerase (Cyclone I Biosystem, International Biotechnologies), subcloning the appropriate restriction fragments into M13, or sequencing from synthetic oligonucleotide primers. All sequences presented in Fig. 4 were determined at least once in each strand.

RNA Hybridization. RNA samples were denatured in a 50% (vol/vol) formamide/6% (vol/vol) formaldehyde buffer at 65°C, resolved on a 1% agarose gel containing 6% (vol/vol) formaldehyde, and transferred to a GeneScreen *Plus* nylon membrane (DuPont), and the filters were prehybridized and hybridized as described by the manufacturer. Both the PCR-1 and λMII-1 were labeled by nick-translation to give a specific activity of ≈8 × 10^8 cpm/μg.

RESULTS

Cloning Strategy and Amplification. Since our original attempts to clone MII by antibody screening of an expression library or by oligonucleotide probing of cDNA or genomic libraries were unsuccessful, we decided to employ a modification of the method of Lee *et al.* (6) for the isolation of a MII fragment by mixed oligonucleotide-primed amplification of cDNA (MOPAC). The 110-kDa chymotrypsin digestion product of the MII polypeptide was purified (5) and further digested under nondenaturing conditions by endoproteinase Lys-C into two major fragments of 70 and 42 kDa (data not shown). Each of these fragments, as well as the original 110-kDa form, was subjected to Edman degradation after $NaDodSO_4$/gel electrophoresis and transfer to a polyvinylidene difluoride membrane. The sequence and the map of the proteolytic digestions are shown in Fig. 1. Two regions of protein sequence were used to design the oligonucleotide primers shown in Fig. 2. These sequences bracket the 42-kDa fragment and would be expected to produce an ≈1150-base-pair (bp) fragment upon amplification.

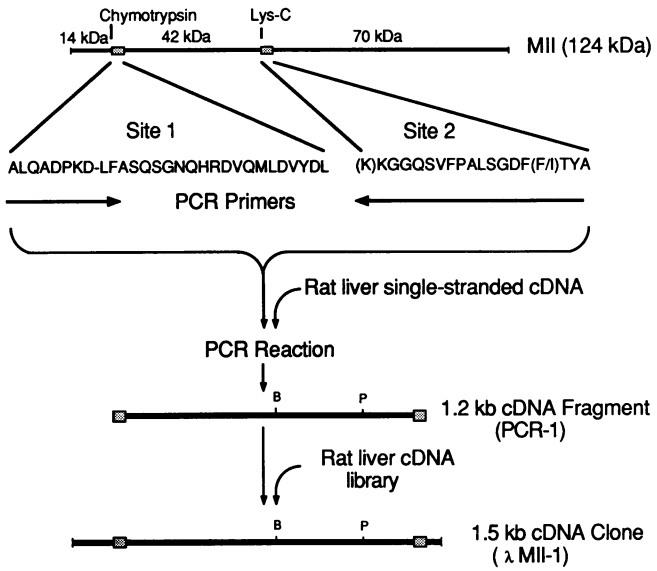

FIG. 1. Strategy for the amplification of MII from rat liver poly(A)$^+$ RNA. A map of the proteolytic cleavage and protein sequence data is shown at the top of the figure. The orientation of the two major digestion products was deduced by comparison with the sequence of the 110-kDa chymotrypsin digestion product. B, *Bam*HI; P, *Pst* I. The single-letter amino acid code is used.

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MII Protein          Ala Leu Gln Ala Asp Pro Lys Asp(Cys)Leu Phe Ala Ser Gln Ser...
Sequence (Site 1)

5' PCR   5'-GGAATTC GCI CTI CAG GCI GAC CCI AAG GAC TGC CTI TTC GC-3'
Primer  EcoRI Linker                    T           T   T       T


MII Protein         (Lys)Lys Gly Gly Gln Ser Val Phe Pro Ala Leu Ser Gly Asp Phe Ile Thr Tyr Ala
Sequence (Site 2)                                                          (Phe)

3' PCR Primer    3'-TTC TTC CCI CCI GTC III CAI AAA GGI CGI GAI III CCI CTA AAA TAI TGI ATA CG CTTAAGG-5'
                                                 G                     G   G         G
                                                                                      EcoRI Linker
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FIG. 2. Oligonucleotide primer design. The sequences for the 5′ and 3′ primers are derived from the site 1 and site 2 protein sequences, respectively (Fig. 1). The cysteine residue in position 9 of site 1 was inferred from the lack of recovery of a phenylthiohydantoin derivative on this cycle and the lysine residue at position 1 of site 2 was inferred from the specificity of endoproteinase Lys-C. The significance of the trace recovery of phenylalanine at position 16 of site 2 is discussed in *Results*.

The guidelines used in the MII primer design (Fig. 2) were arbitrarily determined based upon strategies used in oligonucleotide probe design. Deoxyinosine was substituted in positions where the codon degeneracy was >2. This included two serine residues in the site 2 protein sequence that were substituted with deoxyinosine at all three positions of the triplet codon. Mixed pairs of bases were used at positions where there was no preference between two triplet codons (<70% codon preference from a rat codon usage table). The site 1 and site 2 primers each had four positions with mixed bases resulting in a 16-fold mixture of sequences for each primer. Positions where a single triplet codon was preferred >70% based upon codon usage were synthesized as a single triplet codon.

Several cDNA synthesis conditions and RNA preparations were tested as templates for the primer-directed amplification of the predicted 1150-bp fragment. Under all conditions, a primer-dependent fragment (PCR-1) of the predicted size was produced (Fig. 3), although quantitative differences among the template preparations were found. cDNA preparations generated by random primers were ≈10-fold more effective as templates for amplification than oligo(dT)-primed cDNA preparations (data not shown). When the original RNA preparation was enriched for mRNA >3 kb, the magnitude of this difference was considerably diminished (Fig. 3, lanes 2 and 3). Similar results were also obtained in control reactions using exact-match primers specific for α2,6-sialyltransferase (data not shown). The difference in priming efficiency presumably results from the fact that the cDNA synthesis from oligo(dT)-primed reactions must extend ≈7 kb for MII and ≈4 kb for sialyltransferase to reach the 5′ primer sites (see *Discussion*). Removal of the more abundant low molecular mass RNA species would presumably reduce the competition for reverse transcriptase and improve the apparent extension efficiency of the oligo(dT)-primed cDNA synthesis reactions.

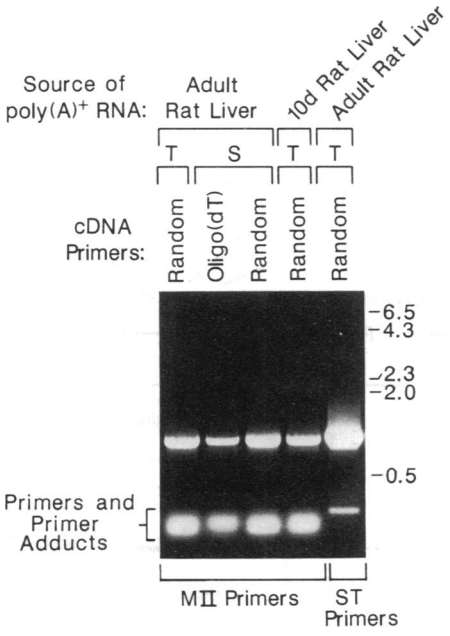

FIG. 3. Effect of RNA source and cDNA synthesis conditions on the amplification of MII or α2,6-sialyltransferase. PCR amplification of MII and the α2,6-sialyltransferase (ST) control reaction were performed as described except that the source of RNA was as follows: T, total poly(A)+ RNA; S, size-selected poly(A)+ RNA prepared on sucrose gradients to remove RNA <3 kb. Liver RNA was from adult or 10-day-old (10 d) rats as indicated. cDNA was synthesized with random hexamer primers or oligo(dT). Size standards are derived from a *Hin*dIII digest of λ phage DNA (in kb).

Subcloning and Sequencing of the Amplification Product. Attempts to subclone PCR-1 directly into pUC or M13 vectors as a blunt-ended fragment or using the restriction endonuclease sites designed into the 5′ ends of the primers were unsuccessful until the ends of the purified fragment were filled in with T4 polymerase prior to subcloning. The resulting subclones were variable in length along the primer region. It is our hypothesis that while Taq polymerase efficiently uses deoxyinosine-containing oligonucleotides as primers, it does not use deoxyinosine-containing primers efficiently as templates. As a result, the amplified fragment represents a collection of incomplete extension products across the primer region. Although this hypothesis has not been tested, the subcloning problem is readily resolved with the T4 polymerase reaction to create a blunt-ended cDNA fragment.

Only a portion of the protein sequence from site 1 was used in the primer design. As shown in Fig. 1, the remainder should be within the amplified region and could be used as confirmation that PCR-1 is a MII-specific amplification product. These additional 18 amino acids from site 1 were found to be in complete agreement with the sequence derived from PCR-1 (Fig. 4). The size of the amplification product (1170 bp) was also in close agreement with the expected size based on the protein sequence mapping.

Among the several subclones that were sequenced in the primer region, there was variability in recovery of the primer region, as discussed above. In addition, there was also an occasional variability in the DNA sequence among the several subclones (average deviation from the consensus of 0.5 bp/100 bp). This value is consistent with the previously characterized misincorporation rate of Taq polymerase during multiple rounds of amplification (14, 15). A comparison of the PCR-1 consensus sequence with a λgt11 clone spanning the entire PCR-1 region (λMII-1, see below) revealed only two differences in the 1080-bp region between the two primers, reflecting a high degree of fidelity for the overall amplification. These two differences presumably reflect a misincorporation by Taq polymerase early in the amplification process.

Isolation and Sequencing of a Partial MII cDNA Clone. Initial experiments suggested that the abundance of clones containing the PCR-1 fragment was quite low in all of the cDNA libraries tested. To isolate clones containing the PCR-1 fragment, we performed an enrichment procedure of phage pools using the amplification of the PCR-1 fragment as

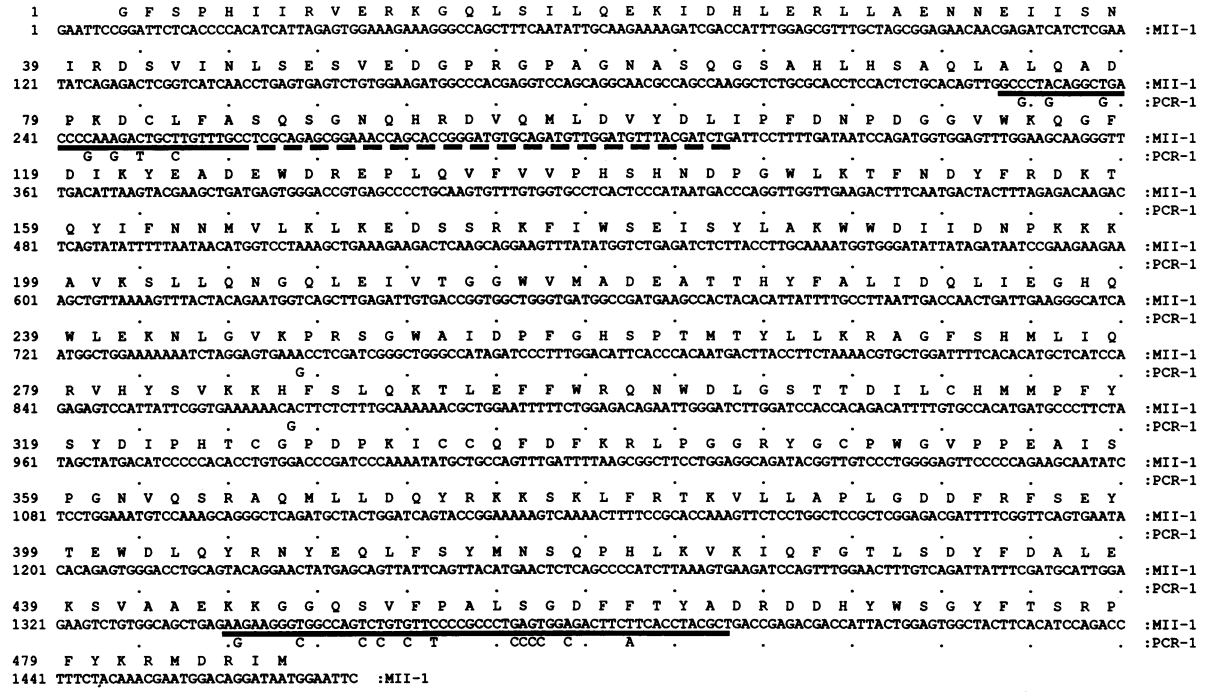

FIG. 4. Sequence of the PCR amplification product (PCR-1) and the MII λ clone (λMII-1). The continuous sequence from position 1 to position 1474 is λMII-1 and positions where the consensus sequence of PCR-1 differs from λMII-1 are shown underneath the corresponding λMII-1 sequence. The PCR-1 fragment starts at position 227 on λMII-1 and ends at position 1397. The solid line indicates the position of the PCR primers and the dashed line indicates the additional protein sequence from site 1 that is in agreement with the translation of λMII-1.

a diagnostic test. This procedure detected 3 pools out of 120 pools that were positive for PCR-1, a 40-fold enrichment prior to plate screening. Subsequent isolation of the clones from each of the phage pools by plaque hybridization using labeled PCR-1 as a probe revealed that all of the clones were identical. These results suggest that this cDNA library contains only one clone spanning the entire PCR-1 fragment length. This clone (λMII-1) was isolated, subcloned, and sequenced (Fig. 4). The sequence reveals a single, continuous open reading frame of 1474 bp extending the full length of the clone, including both protein sequence sites, and is consistent with this clone representing ≈50% of the coding region of the MII gene. No similarities were found between λMII-1 and any of the sequences in the GenBank (release 55.0) or the National Biomedical Research Foundation protein or nucleic acid sequence data bases (releases 15 and 32, respectively).

Comparison of the λMII-1 clone with the primer sequences revealed that two of the eight decisions based on codon usage had resulted in base-pair mismatches between the template and the primer. In addition, residue 16 in the protein sequence of site 2 was originally determined to be isoleucine, with a trace recovery of phenylalanine. The PCR-1 sequence was consistent with the isoleucine determination, but the λMII-1 sequence had a 1-bp mismatch yielding a phenylalanine at this position. This discrepancy may represent a natural polymorphism at this site since the enzyme, RNA, and the cDNA library were all derived from pooled livers of the same outbred strain of rat. Futher analysis will be necessary to confirm this point. After the subcloning of PCR-1, positions originally occupied by deoxyinosine in the PCR primers were found to be converted to deoxyguanosine residues (Fig. 4). Presumably this results from Taq polymerase matching deoxycytidine with the deoxyinosine during the PCR extension reaction.

Northern Blot Analysis of Rat mRNA. The PCR-1 fragment (Fig. 5) and the λMII-1 clone (data not shown) were each used as probes to determine the size of the MII mRNA from rat liver. A single band of ≈8 kb was identified by both probes.

DISCUSSION

Previous studies have examined several aspects of MII structure and function including investigations into enzymology, purification, biosynthesis, immunocytochemistry, cell fractionation, topology, and membrane association (for review, see ref. 1). Extension of this work into areas of gene regulation and Golgi localization requires access to the structural gene for the enzyme. Unfortunately, the low abundance of the enzyme in mammalian tissues in combination with unusual aspects of gene organization (discussed below) have combined to make the enzyme resistant to standard molecular cloning techniques. This report describes the isolation of a 1474-bp clone encoding approximately half of the MII open reading frame by a modification of a method developed by Lee *et al.* (6). The main advantage of this MOPAC procedure is in the direct production of large cDNA fragments using oligonucleotide primers generated from protein sequence data. The sensitivity and specificity of the amplification procedure is demonstrated in Fig. 3 by the production of >1 μg of MII-specific products from ≈60 ng of poly(A)$^+$ RNA. These amplification fragments can then be used as probes in

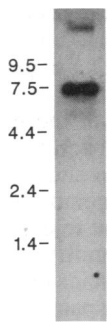

FIG. 5. RNA blots of rat liver MII. Rat liver poly(A)$^+$ RNA (10 μg) was resolved on a 1% agarose/formaldehyde gel, blotted onto a nylon membrane, and probed with radiolabeled PCR-1. Locations of size standards (RNA ladder, BRL) are as indicated (in kb).

the high-stringency screening of cDNA librarires (6) circumventing need for the standard low-stringency oligonucleotide probing methods routinely used in primary cDNA cloning. An additional advantage to this procedure is in the unambiguous confirmation of the authenticity of the fragment by comparison of the PCR fragment DNA sequence with adjacent protein sequence not used in the primer design.

Several modifications of the original protocol were introduced to allow the effective production of large amplification products that were derived from the two separate regions of protein sequence. Taq polymerase, rather than DNA polymerase I (Klenow fragment), was used to catalyze the amplification, allowing the high temperature conditions of annealing and elongation to generate unambiguous products >1 kb long (14). The amplification products were readily detected by ethidium bromide staining prior to subcloning, obviating the need for detection by Southern blotting. The primers were substantially longer than those used by Lee *et al.* (6) and ambiguous positions of codon degeneracy were extensively substituted with deoxyinosine (14 and 26%, respectively, for the 5′ and 3′ primers) to reduce primer degeneracy (16). Subsequent studies using analogous amplification conditions have demonstrated that primers containing as much as 30% deoxyinosine substitution (in a 30-mer primer) at each end of an amplification fragment can successfully generate specific amplification products (unpublished data). These results suggest that the MOPAC protocol is an effective general procedure for the production of specific amplification products from two non-contiguous protein sequences, each ≥8–10 amino acids long, assuming the gene segment between the two primer regions is not beyond the 2- to 3-kb limit of the amplification methodology. Amplification of the MII PCR product could be considered an appropriate test of the limit of this approach since the message abundance is expected to be quite low (≈0.05% of the total cell protein), the message size is large (≈8 kb), and the amplified fragment is >1 kb.

A lower recovery of amplification products was observed when oligo(dT) was used as a primer of the initial cDNA synthesis reactions instead of random hexamer primers. Since MII and sialyltransferase contain a significant amount of 3′ non-coding information on their messages (≈4.5 kb and 3.2 kb, respectively; see below), and the oligo(dT)-primed reactions must extend through this entire 3′ region as well as the entire coding region to reach the 5′ primer sites, the lower efficiency of these reactions relative to the random-primed syntheses would be expected.

The MII amplification product was confirmed as an authentic MII gene fragment both by correspondence with the predicted size and by the agreement of the DNA sequence with an additional 18 amino acids of protein sequence data. cDNA library screening resulted in the detection of only one clone that spans the entire PCR-1 segment. Unfortunately, this clone was not much larger than the PCR-1 fragment and did not extend beyond either end of the open reading frame. No similarities to the λMII-1 clone were detected in nucleic acid or protein sequence data bases.

One surprising feature of the sequence was an apparent absence of a membrane-spanning domain within the λMII-1 clone. Protease protection and topology studies (4, 5) have clearly indicated that the membrane-spanning domain resides within the 14-kDa fragment released from the intact 124-kDa MII polypeptide by chymotrypsin digestion (see Fig. 1). λMII-1 extends 73 amino acids into this fragment without any evidence of a membrane-spanning domain. These data suggest that the transmembrane domain resides within the first 50–60 NH_2-terminal amino acids of the protein. This NH_2-terminal anchor orientation appears to be common with two other Golgi enzymes, β1,4-galactosyltransferase (17, 18) and α2,6-sialyltransferase (11). All three enzymes also contain a proteolytically sensitive region on the lumenal face of the Golgi membrane allowing the release of soluble, catalytically active forms of the enzymes either *in vivo* or *in vitro* (4, 11, 17, 18).

The similarities among the three Golgi enzymes also extends to mRNA organization. While a MII message size of ≈8 kb is substantially larger than would be required for a polypeptide of 117 kDa, both galactosyltransferase and sialyltransferase are encoded by messages that are ≈3.4 times larger than their respective ≈1.2-kb open reading frames (11, 17). Both transferases contain >90% of the extra non-coding information on the 3′ end of their messages. Although the 3′ sequence of murine galactosyltransferase has been determined and has been shown to contain mouse repetitive elements, the significance of the disproportionately large message size for this enzyme, as well as the other two Golgi enzymes, is not clear (17). The similarities among these three enzymes suggest that the general features of orientation, topology, and gene organization may extend to the entire class of enzymes involved in Golgi modification events. A further test of this hypothesis will await the cloning and characterization of additional Golgi enzymes.

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1. Moremen, K. W. & Touster, O. (1988) in *Protein Transfer and Organelle Biogenesis*, eds. Das, R. C. & Robbins, P. W. (Academic, San Diego), pp. 209–240.
2. Kornfeld, R. & Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54**, 631–664.
3. Tulsiani, D. R. P. & Touster, O. (1983) *J. Biol. Chem.* **258**, 7578–7585.
4. Moremen, K. W. & Touster, O. (1986) *J. Biol. Chem.* **261**, 10945–10951.
5. Moremen, K. W. & Touster, O. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 1680 (abstr.).
6. Lee, C. C., Wu, X., Gibbs, R. A., Cook, R. G., Muzny, D. M. & Caskey, C. T. (1988) *Science* **239**, 1288–1291.
7. Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035–10038.
8. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
9. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
10. Kraus, J. P. & Rosenberg, L. E. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4015–4019.
11. Weinstein, J., Lee, E. U., McEntee, K., Lai, P.-H. & Paulson, J. C. (1987) *J. Biol. Chem.* **262**, 17735–17743.
12. Tabor, S. & Struhl, K. (1988) in *Current Protocols in Molecular Biology*, eds. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Wiley, New York), p. 3.5.10.
13. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
14. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J. & Higuchi, R. (1988) *Science* **239**, 487–491.
15. Tindall, K. R. & Kunkel, T. A. (1988) *Biochemistry* **27**, 6008–6013.
16. Takahashi, Y., Kato, K., Hayashizaki, Y., Wakabayashi, T., Ohtsuka, E., Matsuki, S., Ikehara, M. & Matsubara, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1931–1935.
17. Shaper, N. L., Hollis, G. F., Douglas, J. G., Kirsch, I. R. & Shaper, J. H. (1988) *J. Biol. Chem.* **263**, 10420–10428.
18. Shaper, N. L., Shaper, J. H., Meuth, J. L., Fox, L., Chang, H., Kirsch, I. R. & Hollis, G. F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1573–1577.