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Identification and primary sequence of an unspliced human urokinase poly(A)$^+$ RNA

(plasminogen activator/cDNA/splicing/oligonucleotide)

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ABSTRACT **Human urokinase cDNA clones have been identified from a cDNA library prepared from total RNA of human fibroblasts transformed by simian virus 40 [Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* 3, 280–289]. Synthetic oligonucleotides, corresponding to urokinase protein sequence, were used as probes. The cloned cDNA covers most of the coding sequence and the entire 3′ untranslated region. The nucleotide sequence of one of the clones identifies this as a copy of a partially spliced polyadenylylated precursor to urokinase mRNA. The introns separate functionally different domains of the enzyme. Human urokinase mRNA has been identified by RNA blot and its size was estimated at 2500 nucleotides.**

Urokinase is a plasminogen activator isolated from human urine (1) and used in therapy as a physiological thrombolytic agent in deep-vein thromboses, coronary obstructions, etc. (2). Plasminogen activators convert the inactive zymogen plasminogen to the active serine protease, plasmin, thus initiating and finely controlling extracellular proteolysis (for review, see ref. 3). Besides and beyond its interest as a therapeutic tool, urokinase appears to play an important role in tissue formation and degradation (3) and in the metastatic activity of human tumors (4). The level of urokinase mRNA appears to be controlled by a variety of physiological and experimental effectors, including hormones (5), neoplastic transformation (6, 7), growth factors (8, 9), and tumor promoters (10).

Two different active forms of urinary urokinase have been isolated: a high- and a low-molecular-weight form (M_r, 54,000 and 33,000, respectively). High-molecular-weight urokinase is split by reduction into the A and B chains of M_r 20,000 and M_r 30,000, respectively (11). The low-molecular-weight form is made up by the same B chain and by a 21 amino acid long A1 chain (12). The A, A1, and B chains have been sequenced (11–13) and the A1 chain has been shown to be the COOH terminus of the A chain. The sequence data suggest that the A chain is the NH_2- and the B chain is the COOH-terminal portion of a single chain pro-urokinase precursor (12). We have previously shown that monoclonal antibodies directed toward either the A or B chain of urinary urokinase immunoprecipitate the same *in vitro* product of urokinase mRNA (14), thus showing that the two chains are encoded by the same gene. We now report the nucleotide sequence of most of the urokinase cDNA.

MATERIALS AND METHODS

Materials. Reagents were of the highest purity grade. Enzymes were purchased from Bethesda Research Laboratories, New England Biolabs, or P-L Biochemicals. Oligonucleotide 3 (see Fig. 1) was synthesized according to a published technique (15) and was purified through a Partisyl Sax 25 × 0.8 cm HPLC column, with a linear gradient (1–300 mM) of potassium phosphate (pH 6.5) in 30% ethanol. Oligonucleotides 1, 2, and 4 (see Fig. 1) were a gift of G. Cassani (Lepetit Spa, Milan, Italy) and were purchased from Creative Biomolecules (South San Francisco). The DNA sequence of all four oligonucleotide mixtures was checked.

Cells and Culture Conditions. The human epidermoid carcinoma cell line A431 (ref. 16; obtained from I. Pastan), human kidney carcinoma line A1251 (from S. Aaronson), normal human fibroblasts F13 (obtained from A. Fusco), and the HFS10 cells (17) were cultured in Dulbecco's modified minimal essential medium supplemented with 10% fetal calf serum.

RNA Extraction, Electrophoresis, and Blotting. RNA was extracted from cultured cells by the guanidine hydrochloride method (18), or by the procedure of Favaloro *et al.* (19). Poly(A)$^+$ RNA was purified from an oligo(dT) cellulose column (20). RNA blots were carried out on RNA electrophoresed in agarose formaldehyde gels (21) blotted onto a Schleicher & Schuell BA85 filter (22).

cDNA Library Screening. One microgram of DNA from a cDNA library of simian virus 40-transformed human fibroblasts (23) was used to transform strain HB101 (22); 4×10^6 clones were obtained and screened by the high-density screening procedure (24). Duplicate filters (20,000–30,000 colonies each) were hybridized to ^{32}P end-labeled oligonucleotides in 5× standard saline citrate (NaCl/Cit)/10× Denhardt's solution/250 μg of *E. coli* transfer RNA carrier per ml/0.5% sodium dodecyl sulfate (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate; 1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone) (25). Filters were incubated at 55°C and the temperature was allowed to drop to 32°C (oligonucleotides 1 and 2) or to 37°C (oligonucleotides 3 and 4) in 2 hr. The hybridization was henceforth continued for 14 more hr at the indicated temperature. Filters were washed in 4× NaCl/Cit/0.1% sodium dodecyl sulfate at 40°C (oligonucleotides 1 and 2) or at 45°C (oligonucleotides 3 and 4).

The same cDNA library was rescreened using as a probe the ≈1300-base-pair (bp) *Bgl* II/*Bam*HI fragment of pHUK-1 (see Fig. 2). In this case, standard hybridization conditions were adopted (22).

DNA Sequencing. DNA sequencing was carried out on 5′ labeled (22) restriction fragments according to Maxam and Gilbert (26).

RESULTS

To identify a cDNA clone containing sequences of urokinase, we have used oligonucleotide probes synthesized on the basis of the amino acid sequence of the human urinary protein (11–13). Fig. 1 shows a scheme of pro-urokinase and

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Abbreviations: NaCl/Cit, standard saline citrate; bp, base pair(s).

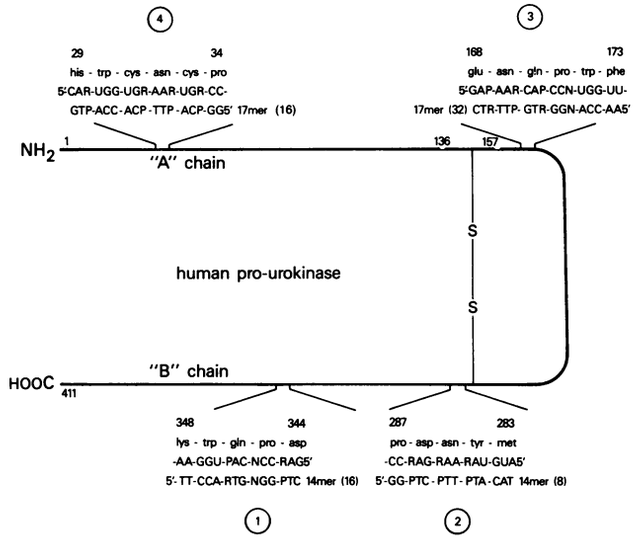

FIG. 1. Schematic drawing of human pro-urokinase and sequence of the four oligonucleotide mixtures used in the screening of the cDNA library. Numbers indicate amino acid residues. "A" chain (residues 1–157) and "B" chain (residues 159–411) are joined in this scheme by residue 158 (not shown). This residue was found in the sequence of pro-urokinase cDNA and brings the number of amino acid residues of pro-urokinase to a total of 411. For the oligonucleotide sequence, R indicates a pyrimidine, P a purine, and N any of the four bases. The first line below the amino acid sequence is the possible RNA sequence; the second line shows the sequence of the synthetic DNA.

the sequence of the four oligonucleotide mixtures.

Isolation of Urokinase cDNA Clones. 700,000 recombinant colonies were hybridized in duplicate to the 5′ ^{32}P-labeled oligonucleotide 1 (the one closest to the COOH terminus) (see Fig. 1); 30 positive clones were purified and rehybridized to oligonucleotides 2, 3, and 4 (Fig. 1). One clone, pHUK-1, was found to be positive with both probes 2 and 3, but not with probe 4; the other clones were negative with all three. pHUK-1 DNA was purified, a restriction map was constructed, and its nucleotide sequence was determined. Fig. 2 shows the restriction map of pHUK-1 DNA and the strategy used for determining the DNA sequence, which is reported in Fig. 3.

Structure of pHUK-1 DNA. The insert cloned in pHUK-1 DNA is ≈2900 nucleotides long, of which ≈160 are the poly(A) tail at the 3′ end. Comparison of the restriction map with that predicted by the amino acid sequence of human urinary urokinase (11–13), indicates the presence of unaccounted for regions (data not shown), which were confirmed by the nucleotide sequence (Fig. 3). Analysis of this sequence (Fig. 3) suggests that these inserts probably represent unspliced intervening sequences. Table 1 lists the positions and the sequence at the exon–intron junctions. The first 159 nucleotides of pHUK-1 DNA very well match (>95%) the sequence of the right monomer of a canonical *Alu* sequence (27). The coding region starts at nucleotide 217, corresponding to amino acid 46 of pro-urokinase (Fig. 1). The first 216 nucleotides, therefore, presumably represent the 3′ end of a truncated intron. The coding region continues through nucleotide 1694 (the COOH terminus) and is followed by a termination triplet TGA. As indicated above, the coding sequence is interrupted at the positions listed in Table 1. A 933-bp long 3′ untranslated sequence follows the coding region; at its 3′ end, a long T-rich stretch with two poly(A) addition sites precedes an ≈160-bp-long poly(A) tract, starting at nucleotide 2629 (not shown in Fig. 3).

On the basis of the data of Fig. 3, the complete sequence of the mature urokinase mRNA can be reconstructed and the amino acid sequence derived, starting from the codon for amino acid 46. The derived primary structure totally agrees with the published sequence of the A and B chains (11–13) (data not shown). One extra residue was found (lysine 158) connecting the A and B chain, which is evidently lost during the proteolytic activation of the zymogen.

Four other cDNA clones were isolated from the same library, using as a probe a 1300-bp nick-translated *Bgl* II/*Bam*HI fragment of pHUK-1 DNA (Fig. 2). Unfortunately, all these clones carried rather short recombinant sequences and were thus not too informative. In all cases, however, the 3′ untranslated region was found to be identical to that of pHUK-1 DNA, the only exception being the length of the poly(A) tract.

Identification and Size of Urokinase mRNA. Total RNA extracted from a urokinase-producing human cell line (A1251) was electrophoresed, RNA blotted, and hybridized (22) to a nick-translated ≈1300-bp *Bgl* II/*Bam*HI fragment of pHUK-1 DNA (see Fig. 2). Fig. 4 shows that a band, which from this and several other experiments can be estimated to

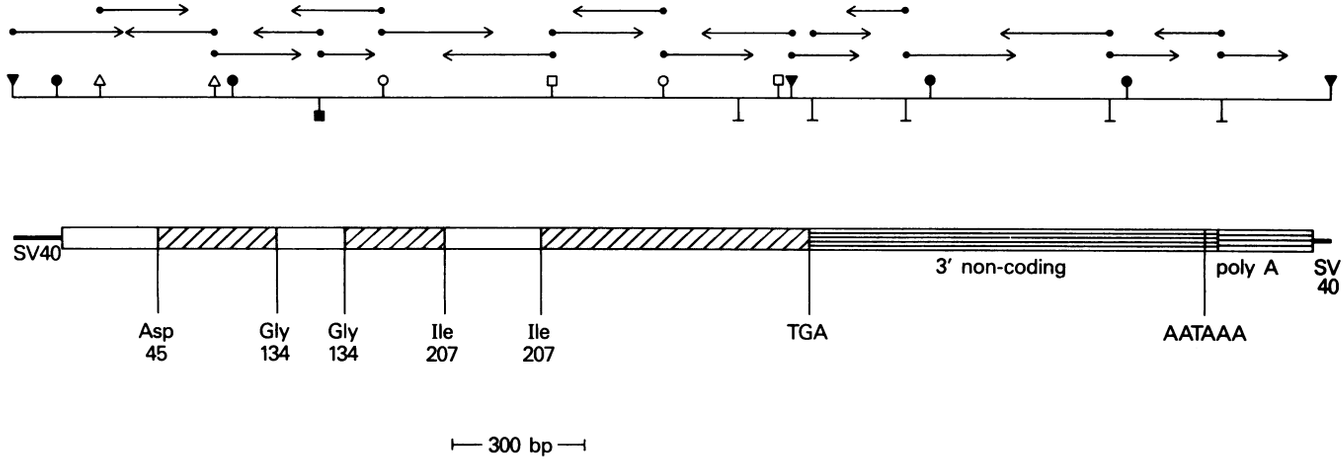

FIG. 2. Functional and restriction map of pHUK-1 cDNA insert. Empty boxes indicate unspliced intervening sequences; the horizontally striped boxes indicate the 3′ untranslated region and the poly(A) tail; the diagonal stripes show the coding sequences. The location of the poly(A) addition site (A-A-T-A-A-A) and the poly(A) tail are indicated. The amino acid residue numbers identify the position of the intron-coding region junctions within the pro-urokinase sequence. The arrows above the restriction map show the strategy adopted in the determination of the nucleotide sequence. The 1300-bp *Bgl* II/*Bam*HI fragment referred to in the text spans from the second *Bgl* II site to the middle *Bam*HI site. ▼, *Bam*HI; ♀, *Eco*RI; ♤, *Bgl* II; ●, *Pst* I; ♀, *Acc* I; ▲, *Ava* I; ⊥, *Ava* II.

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TCATGGTGGTGGATGCCTGTAGTCCCAGCTACTTGGGAGGCTGAGGGAGGAGAATTGCTTGAACATGGGAGGCCAGATCTCAGTGAGCTGAGATCACACC  100

ACTGCACTCCAACTGGGCGACAGAGCAAGACTCCATCTCAAAAAAAAAAAAAAAATAAAAGTTAGTTGGAATGTTCTTCTCTTTCTCATATTCTCTCATCC  200

TCCTGTCCCCTTGTAGATAAGTCAAAAACCTGCTATGAGGGGAATGGTCACTTTTACCGAGGAAAGGCCAGCACTGACACCATGGGCCGGCCCTGCCTGC  300
                    LysSerLysThrCysTyrGluGlyAsnGlyHisPheTyrArgGlyLysAlaSerThrAspThrMetGlyArgProCysLeuP

CCTGGAACTCTGCCACTGTCCTTCAGCAAACGTACCATGCCCACAGATCTGATGCTCTTCAGCTGGGCCTGGGGAAACATAATTACTGCAGGAACCCAGA  400
roTrpAsnSerAlaThrValLeuGlnGlnThrTyrHisAlaHisArgSerAspAlaLeuGlnLeuGlyLeuGlyLysHisAsnTyrCysArgAsnProAs

CAACCGGAGGCGACCCTGGTGCTATGTGCAGGTGGGCCTAAAGCCGCTTGTCCAAGAGTGCATGGTGCATGACTGCGCAGATGGTGAGCATCACTGACCT  500
pAsnArgArgArgProTrpCysTyrValGlnValGlyLeuLysProLeuValGlnGluCysMetValHisAspCysAlaAspG

GCTGATGACAGGTGGGTGGAAGGGGACAAACTTACATGTCCCCTTATTCCATCACAGGAGGACTGAGGAGGTGGGGGGTGCCCGAGAGGGATGCTTTCTC  600

CTACCTGCCTCCCTAAGACATCCCTCTGTTTGTCCTCCAGGAAAAAAGCCCTCCTCTCCTCCAGAAGAATTAAAATTTCAGTGTGGCCAAAAGACTCTGA  700
                                                    lyLysLysProSerSerProProGluGluLeuLysPheGlnCysGlyGlnLysThrLeuA

GGCCCCGCTTTAAGATTATTGGGGGAGAATTCACCACCATCGAGAACCAGCCCTGGTTTGCGGCCATCTACAGGAGGCACCGGGGGGGGCTCTGTCACCTA  800
rgProArgPheLysIleIleGlyGlyGluPheThrThrIleGluAsnGlnProTrpPheAlaAlaIleTyrArgArgHisArgGlyGlySerValThrTy

CGTGTGTGGAGGCAGCCTCATGAGCCCTTGCTGGGTGATCAGCGCCACACACTGCTTCATGTACGGCCCTGGGTTTCTCCTCTTCGACTCTTCTGCCCCA  900
rValCysGlyGlySerLeuMetSerProCysTrpValIleSerAlaThrHisCysPheIl

CCCCAAGCACATCCCTTTCTCCTTCCCAGCAAAGTGTTCCGCCTCATTTCTCCCTCATCTGCCCCTGTCCATGCGCCCATGGCCTTGGGGACAAGTCGTG 1000

CTTTGAGGCCTCTAGGGAGGGAAGGAAGAAGTGGCATGATTTCATGGGACTAAGCTGTTTGATGGGTATCTTCTTCCACAGTGATTACCCAAAGAAGGAG 1100
                                                                                    eAspTyrProLysLysGlu

GACTACATCGTCTACCTGGGTCGCTCAAGGCTTAACTCCAACACGCAAGGGGAGATGAAGTTTGAGGTGGAAAACCTCATCCTACACAAGGACTACAGCG 1200
AspTyrIleValTyrLeuGlyArgSerArgLeuAsnSerAsnThrGlnGlyGluMetLysPheGluValGluAsnLeuIleLeuHisLysAspTyrSerA

CTGACACGCTTGCTCACCACAACGACATTGCCTTGCTGAAGATCCGTTCCAAGGAGGGCAGGTGTGCGCAGCCATCCCGGACTATACAGACCATCTGCCT 1300
laAspThrLeuAlaHisHisAsnAspIleAlaLeuLeuLysIleArgSerLysGluGlyArgCysAlaGlnProSerArgThrIleGlnThrIleCysLe

GCCCTCGATGTATAACGATCCCCAGTTTGGCACAAGCTGTGAGATCACTGGCTTTGGAAAAGAGAATTCTACCGACTATCTCTATCCGGAGCAGCTGAAA 1400
uProSerMetTyrAsnAspProGlnPheGlyThrSerCysGluIleThrGlyPheGlyLysGluAsnSerThrAspTyrLeuTyrProGluGlnLeuLys

ATGACTGTTGTGAAGCTGATTTCCCACCGGGAGTGTCAGCAGCCCCACTACTACGGCTCTGAAGTCACCACCAAAATGCTATGTGCTGCTGACCCCCAAT 1500
MetThrValValLysLeuIleSerHisArgGluCysGlnGlnProHisTyrTyrGlySerGluValThrThrLysMetLeuCysAlaAlaAspProGlnT

GGAAAACAGATTCCTGCCAGGGAGACTCAGGGGGACCCCTCGTCTGTTCCCTCCAAGGCCGCATGACTTTGACTGGAATTGTGAGCTGGGGCCGTGGATG 1600
rpLysThrAspSerCysGlnGlyAspSerGlyGlyProLeuValCysSerLeuGlnGlyArgMetThrLeuThrGlyIleValSerTrpGlyArgGlyCy

TGCCCTGAAGGACAAGCCAGGCGTCTACACGAGAGTCTCACACTTCTTACCCTGGATCCGCAGTCACACCAAGGAAGAGAATGGCCTGGCCCTCTGAGGG 1700
sAlaLeuLysAspLysProGlyValTyrThrArgValSerHisPheLeuProTrpIleArgSerHisThrLysGluGluAsnGlyLeuAlaLeuSTP

TCCCCAGGGAGGAAACGGGCACCACCCGCTTTCTTGCTGGTTGTCATTTTTGCAGTAGAGTCATCTCCATCAGCTGTAAGAAGAGACTGGGAAGATAGGC 1800

TCTGCACAGATGGATTTGCCTGTGGCACCACCAGGGTGAACGACAATAGCTTTACCCTCAGGCCACAGGCCTGGCTGCCGGCTGCCCAGACCCCTCTGGC 1900

CAGGATGGAGGGGTGGTCCTGACTCAACATGTTACTGACCAGCAACTTGTCTTTTTCTGGACTGAAGCCTGCAGGAGTTAAAAAGGGCAGGGCATCTCCT 2000

GTGCATGGGTGAAGGGAGAGCCAGCTCCCCCGACCGGTGGGCATTTGTGAGGCCCATGGTTGAGAAATGAATAAATTTCCCAATTAGGAAGTGTAAGCAG 2100

CTGAGGTCTCTTGAGGGAGCTTAGCCAATGTGGGAGCCAGCGGTTTGGGGAGCAGAGACACTAACGACTTCAGGGCAGGGCTCTGATATTCCATGAATGTA 2200

TCAGGAAATATATATGTGTGTGTATGTTTGCACACTTGTTGTGTGGGCTGTGAGTGTAAGTGTGAGTAAGAGCTGGTGTCTGATTGTTAAGTCTAAATAT 2300

TTCCTTAAACTGTGTGGACTGTGATGCCACACAGAGTGGTCTTTCTGGAGAGGTTATAGGTCACTCCTGGGGCCTCTTGGGTCCCCCACGTGACAGTGCC 2400

TGGGAATGTACTTATTCTGCAGCATGACCTGTGACCAGCACTGTCTCAGTTTCACTTTCACATAGATGTCCCTTTCTTGGCCAGTTATCCCTTCCTTTTA 2500

GCCTAGTTCATCCAATCCTCACTGGGTGGGGTGAGGACCACTCCTTACACTGAATATTTATATTTCACTATTTTTATTTATATTTTTGTAATTTTAAAATA 2600

AAAGTGATCAATAAAATGTGATTTTTCTGA
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FIG. 3. Nucleotide sequence of pHUK-1 DNA and amino acid sequence of its coding regions. Two possible polyadenylylation sites are underlined.

Table 1. Position and nucleotide sequence of the exon–intron junctions in pHUK-1 DNA

Junction	Nucleotides	Sequence		
		Coding	Intron	Coding
Intron A–CR 2	216–217		T-G-T-A-G	A-T-A-A
CR 2–intron B	483–484	A-G-A-T-G	G-T-G	
Intron B–CR 3	640–641		T-C-C-A-G	G-A-A
CR 3–intron C	860–861	T-T-C-A-T	G-T-A	
Intron C–CR 4	1081–1082		C-A-C-A-G	T-G-A

CR represents coding regions of pHUK-1 DNA. The first coding region is identified as CR 2, because we know from the amino acid sequence that there must be at least one other CR preceding CR 2 (11). The symbol CR was preferred to the word exon, because the latter identifies genomic DNA that contains no intervening sequence. One CR, however, may correspond to more than one exon in the gene.

be ≈2500 nucleotides long, hybridizes with the radioactive probe.

The abundance of the 2500-nucleotide band varies widely in different cell lines. It is barely detectable with normal human fibroblast RNA, while simian virus 40-transformed fibroblasts HFS10, A431, and A1251 tumor cell lines each show a higher, yet variable, amount of it (data not shown).

DISCUSSION

The DNA sequence of the pHUK-1 insert (Fig. 3) suggests this to be a copy of a polyadenylylated unspliced precursor to urokinase mRNA. This is suggested by the interruptions of the coding sequence and by the observation of canonical splice junctions (Table 1); this interpretation agrees with the size of the urokinase mRNA estimated by RNA blot (see below). Preliminary data on the structure of genomic clones support this interpretation and show that, in fact, other introns are present in the gene in addition to those found in pHUK-1 DNA (data not shown). This clone therefore is a cDNA copy of an intermediate, only partially spliced, precursor to urokinase mRNA. It must be noted that pHUK-1 was isolated from a library prepared from total, not cytoplasmic, RNA (23) and might well contain nuclear precursor cDNA clones. Whether the isolation of pHUK-1 represents a fortuitous case or specifically reflects the source of RNA (simian virus 40-transformed cells) or unusual splicing properties of urokinase mRNA, remains to be determined.

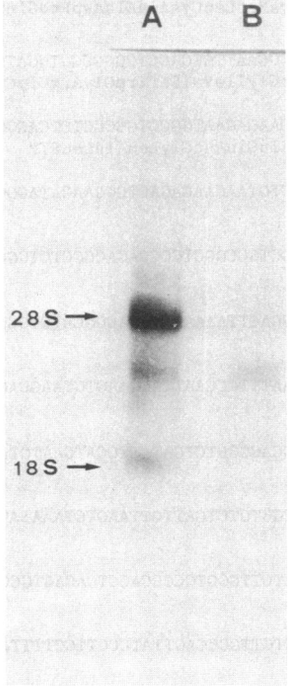

FIG. 4. RNA blot of urokinase mRNA. Total cytoplasmic RNA of A1251 cells was electrophoresed on a 1.5% agarose-formaldehyde gel, blotted onto nitrocellulose paper and hybridized (22) (lane B) to the 1300-bp *Bgl* II/*Bam*HI fragment of pHUK-1 DNA (see Fig. 2). After 2 weeks, the same filter was re-hybridized (lane A) to a human rDNA probe (courtesy of A. Simeone). Arrows indicate the migration of 18S and 28S human rRNA markers.

The sequence of human urokinase cDNA confirms or further supports the hypothesis (12) that activation of the secreted single-chain pro-urokinase (refs. 28 and 29; unpublished results) to the two-chain urinary form (11) is accompanied by the loss of the residue lysine-158 (Fig. 3).

The introns present on pHUK-1 DNA separate potential functionally different domains of urokinase. Intron A is located at the end of the "growth factor" region (before residue 46)—i.e., in the region having homology with the epidermal growth factor (11). The intron B is inserted within the triplet coding for glycine-134, the site of the cleavage that generates the A1 chain of the low-molecular-weight form of urokinase (11). This intron follows a coding sequence strongly homologous to one of the kringles of plasminogen and tissue plasminogen activator (11–13, 30) and separates it from the sequence that encodes the A1 chain. This peptide connects the A to the B chain in the pro-urokinase molecule. Intron C, located within the triplet coding for isoleucine-207, interrupts the coding sequence so as to separate the three residues required for the formation of a serine–protease catalytic site (30, 31) (histidine-204 from aspartic acid-255, and serine-356). Whether aspartic acid-255 and serine-356 are also separated from each other and from aspartic acid-344 (required for the hydrolysis of basic residues) (31), remains to be clarified and awaits the completion of the analysis of the genomic clones. These four crucial amino acids might be encoded by different exons, analogous to the structure of the mouse kallikrein gene (32).

Comparison of the sequence of urokinase cDNA with the DNA sequences available in the Los Alamos data bank has revealed the same kind of homologies already observed at the amino acid sequence level (11–13). Because of the lack of the 5′ terminal region of urokinase mRNA in pHUK-1, the search in the data bank could not be made for the region preceding amino acid 46—i.e., for the growth factor region. In view of the reported mitogenic activity of urokinase for chicken lymphocytes (33) and of the potential involvement of urokinase synthesis and secretion in human tumors (34), it will be interesting to establish the structure of this region.

The availability of a cDNA probe has allowed the size of the urokinase mRNA to be estimated (Fig. 4). This length (2500 nucleotides) agrees well with the size of urokinase mRNA as reconstructed from the pHUK-1 sequence. We have calculated the mRNA to be made up of >2367 nucleotides [933 of 3′ untranslated region; 160 of poly(A) tail; 1099 of coding sequences accounted for in pHUK-1; 135 coding for the missing 45 NH_2-terminal amino acids (11–13)]. To this number, in fact, a 5′ untranslated region, and presumably a signal peptide sequence, will have to be added.

The RNA blot of Fig. 4 was carried out on cytoplasmic RNA and has obviously shown one single band and no evidence of larger precursors. An analysis of the splicing pattern and kinetics of the urokinase gene can be carried out once the genomic structure is solved.

The abundance of urokinase mRNA varies in some tumor cell lines; it is high in the A431 epidermoid adenocarcinoma cell line, intermediate in the A1251 kidney adenocarcinoma line and in the simian virus 40-transformed human fibroblasts, and low in normal fibroblasts (unpublished data). These results suggest a transcriptional regulation of the

expression of the human urokinase gene or a different stability of the mRNA in different cells with a possible alteration of these controls in tumor cells.

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1. Duckert, F. (1978) in *Fibrinolytics and Anti-Fibrinolytics*, ed. Markuardt, F. (Springer, Berlin), pp. 209–237.
2. White, W. F., Barlow, G. H. & Mozen, H. M. (1966) *Biochemistry* **5,** 2160–2169.
3. Reich, E. (1978) in *Molecular Basis of Biological Degradative Process*, eds. Berlin, R. D., Herrman, L., Lepow, I. H. & Tanzer, J. M. (Academic, New York), pp. 155–169.
4. Ossowski, L. & Reich, E. (1984) *Cell* **35,** 611–619.
5. Nagamine, Y., Sudol, M. & Reich, E. (1983) *Cell* **32,** 1181–1190.
6. Unkeless, J. C., Tobia, A., Ossowski, L., Quigley, J. P., Rifkin, D. B. & Reich, E. (1973) *J. Exp. Med.* **137,** 85–111.
7. Rifkin, D. B. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44,** 665–668.
8. Gordon, S., Unkeless, J. C. & Cohn, Z. A. (1974) *J. Exp. Med.* **140,** 995–1010.
9. Lee, L. S. & Weinstein, I. B. (1978) *Nature (London)* **274,** 696–697.
10. Wigler, M. & Weinstein, I. B. (1976) *Nature (London)* **256,** 232–233.
11. Günzler, W. A., Steffens, G. J., Ötting, F., Buse, G. & Flohé, L. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363,** 133–141.
12. Günzler, W. A., Steffens, G. J., Ötting, F., Kim, S. A., Frankus, E. & Flohé, L. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363,** 1155–1165.
13. Steffens, G. J., Günzler, W. A., Ötting, F., Frankus, E. & Flohé, L. (1982) *Hoppe-Seyler's Z. Physiol. Chem.* **363,** 1043–1058.
14. Salerno, G., Verde, P., Nolli, M. L., Corti, A., Szöts, H., Meo, T., Johnson, J., Bullock, S., Cassani, G. & Blasi, F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 110–114.
15. Duckworth, M. L., Gait, M. J., Goelet, P., Hong, G. F., Singh, M. & Titmas, R. C. (1981) *Nucleic Acids Res.* **9,** 1691–1701.
16. Fabricant, R. N., DeLarco, J. E. & Todaro, G. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 565–569.
17. Boast, S., La Mantia, G., Lania, L. & Blasi, F. (1983) *EMBO J.* **2,** 2327–2331.
18. Gordon, J. I., Deeley, R. G., Burns, A. T. H., Pakerson, B. M., Christman, J. L. & Goldberger, R. F. (1977) *J. Biol. Chem.* **252,** 8310–8319.
19. Favaloro, J., Treisman, R. & Kamen, R. (1980) *Methods Enzymol.* **65,** 718–749.
20. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
21. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, M. (1977) *Biochemistry* **16,** 4743–4751.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 203.
23. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3,** 280–289.
24. Hanahan, D. & Meselson, M. (1980) *Gene* **10,** 63–67.
25. Stoppelli, M. P., Verde, P., Galeffi, P., Locatelli, E. K. & Blasi, F. (1984) in *Peptide Hormones, Biomembranes and Cell Growth*, eds. Bolis, C. G., Frati, L. & Verna, R. (Plenum, New York), in press.
26. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–580.
27. Houck, C. M., Reinhard, F. G. & Schmid, C. W. (1979) *J. Mol. Biol.* **132,** 289–306.
28. Nielsen, L. S., Hansen, J. G., Skriver, L., Wilson, E. L., Kaltoft, K., Zeuthen, J. & Danø, K. (1982) *Biochemistry* **21,** 6410–6415.
29. Wun, T., Ossowski, L. & Reich, E. (1982) *J. Biol. Chem.* **257,** 7262–7268.
30. Pennica, D., Holmes, W. E., Kohr, W. J., Harkins, R. N., Vehar, G. A., Ward, C. A., Bennett, W. F., Yelverton, E., Seeburg, P. H., Heyneker, H. L., Goeddel, D. V. & Collen, D. (1983) *Nature (London)* **301,** 214–221.
31. Krieger, M., Kay, L. M. & Stroud, R. M. (1974) *J. Mol. Biol.* **83,** 209–230.
32. Mason, A. J., Evans, B. A., Cox, D. R., Shire, J. & Richards, R. I. (1983) *Nature (London)* **303,** 300–307.
33. Cohen, S. D., Israel, E., Spiess-Meier, B. & Wainberg, M. A. (1981) *J. Immunol.* **126,** 1415–1420.
34. Mullins, O. E. & Rohrlich, S. T. (1983) *Biochim. Biophys. Acta* **695,** 177–214.