Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 7548–7552, August 1994
Biochemistry

Characterization of the promoter region of the gene for the rat neutral and basic amino acid transporter and chromosomal localization of the human gene

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ABSTRACT **The promoter region of the rat kidney neutral and basic amino acid transporter (NBAT) gene has been isolated and sequenced. The major transcription initiation site was mapped by primer extension. The entire promoter region and a set of 5′ deletions within it were expressed at a high level in LLC-PK1 cells using the luciferase indicator gene. Positive and negative regulatory elements in the promoter region were observed. A human genomic clone of the transporter was also obtained and was used to localize the NBAT gene at the p21 region of chromosome 2.**

The cDNA for a rat kidney protein has been cloned that, when expressed in *Xenopus* oocytes, mediates sodium-independent transport of neutral amino acids, cystine, and dibasic amino acids (1, 2). The protein appears to be a broad spectrum neutral and basic amino acid transporter (NBAT) resembling the $b^{0,+}$ transporter (3). Additional cDNA clones, highly homologous to NBAT, were isolated from rabbit and human kidney (4–6). Previous studies on the distribution of mRNA had shown that NBAT is present in only a limited number of rat tissues (7). Immunocytochemical localization studies, using site-directed antisera raised against two different synthetic peptides representing epitopes in NBAT, showed this transporter to be localized to microvilli of epithelial cells lining renal proximal tubules and small intestinal epithelia of the rat (8, 9). Surprisingly, in the small intestine intense staining was seen within enteroendocrine cells and submucosal neurons (9). More recently, NBAT was shown to be present in rat brain and rat adrenal medulla. The material in the brain is localized in central autonomic nuclei (M. J. Nirenberg and V. M. Pickel, personal communication). The cellular localization of NBAT in renal and intestinal microvilli is in accord with its role as a transporter of amino acids across epithelial cells in these tissues. Its presence in both the central and peripheral nervous systems as well as in adrenal medulla suggests that these tissues have high requirements for amino acids transported by NBAT or that NBAT, or a closely related protein, is involved in neuronal functions other than transport. The limited distribution of NBAT mRNA in rat tissues suggests that the tissue-specific expression of NBAT may be regulated transcriptionally. To help answer some of these questions we have cloned, sequenced, and characterized the promoter region of the rat NBAT gene.‖ We also determined the position of the NBAT gene in the human chromosomal map.

MATERIALS AND METHODS

Materials. [α-^{32}P]dCTP and [γ-^{32}P]ATP were from Amersham; cDNA probes were radiolabeled by using an oligolabeling kit from Pharmacia; nitrocellulose filters were from Schleicher & Schuell; genomic DNA blots (Zoo-Blot and rat Geno-Blot) were obtained from Clontech. The gene light reporter vectors pGL2-Basic and pGL2-Control were from Promega; luciferase assay reagents and the Erase-a-Base system were from Promega; restriction enzymes were purchased from different commercial sources; superscript reverse transcriptase was from BRL; cell lines were from American Type Culture Collection; the $CaHPO_4$ transfection kit was from 5 Prime → 3 Prime, Inc.

Isolation of Rat Genomic Clones. Approximately 2×10^6 bacteriophage plaques from an adult rat liver genomic library in EMBL3 SP6/T7 (Clontech RL1022j) were screened by hybridization with a ^{32}P-labeled NBAT full-length cDNA. Hybridizations were performed at 65°C for 24 h in 6× SSC (15 mM sodium citrate, pH 7.0/0.15 M NaCl) containing 0.25% nonfat dry milk and the radiolabeled probe. Filters were washed with 1× SSC/0.1% SDS at 56°C for 1 h. Subsequent screenings of the genomic library with a 403-bp fragment that corresponds to the nucleotide sequence 1–403 of NBAT cDNA (5′ probe) were performed under the same stringency. The genomic fragments from positive clones were released from EMBL3 by *Bam*HI and subcloned into the Bluescript plasmid vector. A series of 5′ unidirectional deletions of the genomic fragments using the Erase-a-Base System was made and these truncated constructs were sequenced by the dideoxynucleotide chain-termination method (10). Sequencing for each construct was performed on both strands. DNA sequence analysis was carried out on an Applied Biosystems DNA sequencing system (model 373A). Sequence comparisons were made using GenBank and the European Molecular Biology Laboratory nucleotide data bases.

Primer Extension. A 27-base oligonucleotide complementary to bases 22–48 in the 5′ region of the rat NBAT cDNA was end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Primer extension was performed as described (11). The labeled primer (10^6 cpm) was coprecipitated with either 50 μg of total RNA or 4 μg of mRNA at 30°C for 18 h, and the precipitate was resuspended in a buffer containing 40 mM Pipes (pH 6.4), 400 mM NaOAc, 1 mM EDTA, and 80% formamide. Extension was performed with 40 units of superscript reverse transcriptase for 90 min at 42°C in a solution containing 50 mM Tris·HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM each deoxynucleotide, and 1 unit of RNasin per ml. RNA templates were degraded with

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Abbreviations: NBAT, neutral and basic amino acid transporter; CMV, cytomegalovirus; β-Gal, β-galactosidase; FISH, fluorescence *in situ* hybridization.

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. U10110).

1 μg of RNase A. Extension products were extracted with phenol/chloroform, precipitated with ethanol, and then separated on an 8% acrylamide/urea gel. Sequencing of the NBAT genomic clone, utilizing the 27-base primer, was carried out for calibration.

Luciferase Plasmid Construction. The 3.6-kb *Bam*HI/*Msp* I genomic fragment corresponding to nucleotides −3633 to +63 of the rat NBAT gene was subcloned in front of the luciferase gene into the *Xho* I site of the pGL2-Basic vector. A series of 5′ unidirectional deletions of the constructed plasmid was created and sequenced as described above. As a positive control for transfection efficiency, we used the CMV–β-Gal (β-galactosidase) vector that contains the cytomegalovirus promoter and the *Escherichia coli lacZ* indicator gene.

Transient Transfection. LLC-PK1 cells (derived from pig kidney), OK cells (derived from opposum kidney proximal tubules), and CHO cells were cultured according to the American Type Culture Collection recommendations. At 20 h before transfection, cells were seeded at a density of ≈3 × 10^5 cells per 3.5-mm well in a six-well plate. To each well 4 μg of plasmid was added and transfection was carried out by the $CaHPO_4$ precipitation technique with a transfection kit (5 Prime → 3 Prime, Inc.). No glycerol shocking procedure was used. For cotransfection experiments, 0.1 μg of the reporter CMV–β-Gal was mixed with 4 μg of each construct. All transfections were carried out in duplicate. At 48–72 h after transfection, cells were rinsed with PBS and then lysed with 100 μl (per well) of lysis buffer (Promega kit for luciferase assay). For enzymatic reactions, 20 μl of each lysate was mixed with 100 μl of luciferase assay reagent (Promega) at 25°C. Luciferase activity was measured for 10 sec using a luminometer (Monolight 2010; Analytical Luminescence Laboratory, San Diego). Results are expressed as relative luciferase activity (light units) per mg of protein.

Isolation of Human Genomic Clones. A human genomic library in EMBL3 SP6/T7 (Clontech) was initially screened with the rat NBAT full-length cDNA (as described above). Positive clones, when rescreened with the rat NBAT 5′ cDNA probe, yielded three positive clones. Genomic fragments were released from the vector by *Bam*HI, subcloned into Bluescript, and partially sequenced. One clone was obtained that contained a 2.2-kb fragment, which included the nucleotide sequence 150–476 of human NBAT cDNA (6). This fragment was used for chromosomal mapping.

Southern Analysis of a Somatic Cell Hybrid Panel. The 2.2-kb genomic insert of the human NBAT was labeled with [α-^{32}P]dCTP and then used in Southern analysis following standard methods. The somatic cell hybrid DNA panels were derived from 35 hybrid cell lines consisting of various combinations of human chromosomes against a hamster background (Bios, New Haven, CT).

Fluorescence *in Situ* Hybridization (FISH). The human NBAT genomic insert was labeled with biotin-11-dUTP using the bio-nick labeling system (Life Technologies, Grand Island, NY) for chromosomal mapping. Metaphase chromosome preparations from phytohemagglutinin-stimulated and 5-bromodeoxyuridine-synchronized lymphocyte cultures, hybridization of biotin-labeled probe, and detection of signals by indirect immunofluorescence were performed as described (12, 13).

RESULTS AND DISCUSSION

Cloning of the Rat NBAT Promoter Region. NBAT cDNA was cloned from rat kidney (1, 2) and the corresponding mRNA was shown to be present in kidneys from rat, mouse, and rabbit as well as in rat intestine, brain, heart, and human colon carcinoma (CaCO-2) cells (7). Southern blot analysis of genomic DNAs from several eukaryotic species using rat NBAT cDNA as the probe has now revealed that the NBAT gene is present in rat, mouse, rabbit, chicken, cow, dog, monkey, and human (data not shown). We have used Southern analysis of rat genomic DNA to show that the NBAT gene is >20 kb and that it is a single copy gene (data not shown).

To isolate genomic clones corresponding to the 5′ end of the NBAT gene, we used full-length NBAT cDNA as a hybridization probe to screen a rat liver genomic library. Twelve positive clones were isolated and rescreened with the NBAT 5′ cDNA probe. Two positive clones were isolated, sequenced, and found to be identical. One of them (T6-3), containing a 5.1-kb insert, was selected for further studies. Its restriction map is shown in Fig. 1*A*. Sequencing of T6-3 showed it to contain exon 1 and a portion of the first intron. In exon 1, the NBAT sequence +33 to +492 (Fig. 1*B*) corresponds to positions 1–459 of the previously published cDNA sequence (1). Exon 1 from position +72 (ATG) encodes the first 140 amino acid residues of NBAT and contains a typical transmembrane domain (1, 2). The first intron starts at position +493 with a GT consensus splicing donor sequence (14).

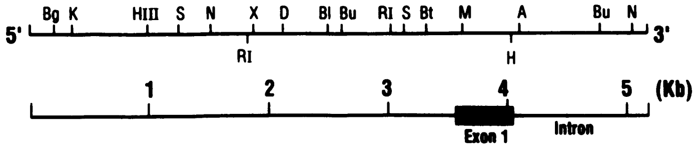
Sequence Analysis of the T6-3 Promoter Region. Fig. 1*B* shows the nucleotide sequence of the 5′ flanking region of the rat NBAT gene. This sequence contains a typical TATA box (positions −31 to −26) that is known to modulate the efficiency of transcription by RNA polymerase II (15). However, there is no CCAAT box. The 3.6-kb region upstream of exon 1 also contains consensus sequences for the binding of many transcription factors. However, the functions of these putative regulatory elements in the expression of the NBAT gene remain to be determined. Fig. 1*B* also shows some DNA motifs that are repeated a few times in the NBAT promoter region. The TGGCA motif serves as a basal transcription factor for viral and cellular enhancer/promoter elements (16). The LBP-1 site (T/ACTGG) is known to bind the leader-binding protein (17) and the CACCC site is present in the 5′ flanking region of many other genes (18). None of the DNA motifs that were found in the NBAT promoter region are known to be kidney-specific regulatory elements (19, 20).

A search for sequence homology between the NBAT promoter region and other genes, using GenBank and European Molecular Biology Laboratory data bases, showed that the sequence −1471 to −1171 is highly homologous (80%) to type 2 *Alu*-like repetitive elements (21). These elements are present at ≈10^5 copies in the rodent genome (21) and have been claimed to be gene markers for brain, embryonic, and oncogenic proteins (21).

Determination of Transcription Start Sites. The transcription start site of the rat NBAT gene was determined by primer-extension analysis. A synthetic oligonucleotide, corresponding to the NBAT cDNA sequence 22–48, was used as primer. As shown in Fig. 2, primer extension against rat kidney RNA revealed one major extension product of 81 nt and smaller amounts of two larger products (asterisks). Traces of additional start positions, upstream and downstream of the major site, may represent premature terminations during the reverse transcriptase reaction or artifactual hybridization of the primer.

Functional Analysis of the NBAT Promoter. The promoter activity of the cloned 5′ end of the NBAT gene was studied by transient expression assays with the luciferase gene as a reporter. Initially, the *Bam*HI/*Msp* I fragment that contains 3570 nt of upstream sequences and 63 nt of exon 1 [T6-3/(−3633)] was cloned in front of the luciferase gene in the plasmid pGL2-Basic. This construct and a series of deletion constructs were introduced by calcium phosphate-mediated transfection into three different cell lines, and the luciferase activity in the cell extracts was analyzed. Two different plasmids, pGL2-Control for luciferase activity and CMV–β-Gal for transfection efficiency, were used as positive con-

A



B

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GACCTTCCTA TCTAAGGTGG ACCCCCCCCC CCCATCCCCT AGGCTCTAGG ATTTATATTT GGGTTGGTAT TTAGGAAAGC TTTTCTACTC CAGGATTGTT -2571
TTTATGTATT TTTTAAAAAT CCTTAAATTT TACCTTTTTT TTACTTTTAA AATCGGTGTA CATAGAAATT TTTTTTTTTT AAGGAGTAAG GTTAGCTTTA -2471
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                                                   LBP-I      TGGCA
TGTCAATGTT TTTCAACCAT TGTGAAACAA CACTATTATA CCCACTTAAT AACTGGCTTG GTGGCAGTGT GTCTGCATTA TTGTAGTACC ACAATTGTCA -571

CGTCTCTAAA ACTCATCAAA ATCCCTAGCT AGAATTCTAT TCCATTGGCC AACACTTTCC CACAGTTCCT CCTAAACCCC CAACCACTGT TCTGTTCTGT -471

GCTTCTGTGA ACATAGATTC CTTCTCTAAG TGGAACCACA CAATATTTGT GTGGCTGTGG TTATTTTCCA CTCAGCGTAA TGCCCTTGAG GTCCATCCAT -371
                                                                                               LBP-I
CCTGTAGTGT GTGTCAGACT TGGCTTCCCT TTCAGGGAAT AATTTCTCCT GTACGTCCAT TTCACATTCA CATGATGATG GGTGCATCTC TGGGTCACCT -271

CCACCTTTGG TAGATTTGTG AATGGAATTA CTGTAGGCAG TTGGAACTCA CACAAGTAGC ATCTTACGGT GTTTGGAAGC CCGCCTCTTC TCCCCAGTCT -171
                                                              CACCC-binding protein
CCTCCCCCAT GTACTGTTAC TGTAGCTGAG TCAACCACCT GGTGTAAAGA TCATTTGTGC ACACACCCCC TCCCCCTGAC CCCCTTTACT AAACTTTACC -71
                                                                        +1
                                            TATA Box
CTTTCTTCCT TGGCTGGACT TTGTCCAGCC AGGATTATTT ATAAACCATT CAAAGCTCAT TAATAAACCA CAGCACCCAG CAAGCCACCT TTTCACTCTG 30

TCCCTACTGG AAAGCACTGG GAAGAGCTGC ACCGGATAGA CATGAATGAG GACAAAGACA AGAGAGACTC TATCCAGATG AGTATGAAGG GATGCCGGAC 130
                                            MetAsnGlu AspLysAspL ysArgAspSe rIleGlnMet SerMetLysG lyCysArgTh

GAATAACGGG TTTGTCCAAA ATGAAGACAT CCAGGAGCAG GACCCAGACT CCAGGGACAC TCCACAGTCC AACGCTGTTA GTATCCCTGC TCCAGAGGAG 230
rAsnAsnGly PheValGlnA snGluAspIl eGlnGluGln AspProAspS erArgAspTh rProGlnSer AsnAlaValS erIleProAl aProGluGlu

CCTCAACTAA AGGTGGTGCG GCCCTATGCA GGGATGCCCA AGGAGGTGCT GTTCCAGTTC TCTGGCCAGG CTCGCTACCG GGTGCCTCGG GAGATCCTCT 330
ProGlnLeuL ysValValAr gProTyrAla GlyMetProL ysGluValLe uPheGlnPhe SerGlyGlnA laArgTyrAr gValProArg GluIleLeuP

TCTGGCTCAC CGTGGTCTCC GTGTTCCTGC TCATCGGAGC CACCATAGCC ATCATCATCA TCTCTCCAAA ATGCCTTGAC TGGTGGCAGG CAGGTCCCAT 430
heTrpLeuTh rValValSer ValPheLeuL euIleGlyAl aThrIleAla IleIleIleI leSerProLy sCysLeuAsp TrpTrpGlnA laGlyProMe

GTACCAGATC TACCCGAGGT CTTTTAAGGA CAGTGACAAG GATGGGAATG GAGACCTGAA AGGTGGGTGT TCGGCGCTTA GGACCAGTGG AGGGTGGTTT 530
tTyrGlnIle TyrProArgS erPheLysAs pSerAspLys AspGlyAsnG lyAspLeuLy s

GTGGGCCCAC TCCCAGCTTC CTGGAAACCA CTTTGTGCCT GGCTTGTGTG GTAAACACAT TCCAGGATGG CTGGCACCCG TTCCCCTGGG AGTTCATTCA 630
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TTTCTCCACA CTCTCCGTCT GGGGTGGATC 1630
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FIG. 1. Organization and nucleotide sequence of the 5′ end of the rat NBAT gene. (*A*) Restriction map of the 5.13-kb *Bam*HI fragment (clone T6-3) containing exon 1 and part of intron 1. Sites for restriction enzymes starting from the 3′ end are *Nde* I (N), *Bsu*36I (Bu), *Apa* I (A), *Hae* II (H), *Msp* I (M), *Bst*EII (Bt), *Ssp* I (S), *Eco*RI (RI), *Bcl* I (Bl), *Dra* I (D), *Xba* I (X), *Hin*dIII (HIII), *Kpn* I (K), and *Bgl* I (Bg). (*B*) Sequence of the 5.13-kb *Bam*HI fragment. Nucleotides are numbered with respect to the major transcription initiation site (+1); exon 1 is boxed and the translation initiation codon (ATG) is double underlined. Arrowhead indicates cytosine at the transcriptional start point. Consensus sequences for some DNA motifs—TATA, CACCC, LBP-1, and TGGCA—are enclosed in small boxes.

trols. Luciferase activity was normalized to β-Gal activity. All transfected cells gave positive results, with the lowest at ≈70 times above background (pGL2-Basic). Since the construct T6-3/(−3633)Luci yielded much higher levels of luciferase activity in LLC-PK1 cells than in OK or CHO cells (data not shown), we carried out analysis of the NBAT promoter in those cells. A series of 5′ deletions of T6-3/(−3633)Luci was made to search for regulatory elements and to determine the minimum sequence needed for promoter activity (Fig. 3). A marked increase in luciferase activity was observed when the sequence −2966 to −2783 was deleted, suggesting the presence of negative regulatory elements in this region. Further deletions between −2783 to −573 did not have much effect on the high level of luciferase activity. However, an additional deletion from −573 to −158 led to a marked decrease in luciferase activity, indicating the presence of positive regulatory elements within this sequence. It should be noted, however, that the activity of the T6-3/(−158)Luci construct was still ≈70 times higher than background. These findings indicate that the upstream sequence −573 to −2783 contains a highly efficient promoter. Furthermore, appreciable regulatory activity still resides in the proximal 5′ 158 nucleotides. Clearly, additional studies on structure–function relationships of upstream sequences in the NBAT gene are needed to evaluate their importance in tissue-specific expression.

Chromosomal Localization of the Human NBAT Gene. Mapping of the NBAT gene to a human chromosome may help in understanding the role of NBAT in amino acid transport both in normal and abnormal tissues and in human inherited disorders of transport such as cystinuria (22) and Hartnup disease (23). In this context, it should be noted that

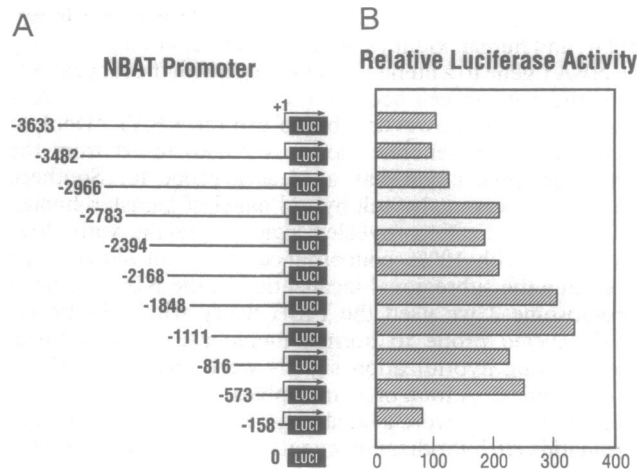

FIG. 3. Functional analysis of NBAT promoter in LLC-PK1 cells by 5′ deletions. (*A*) Schematic representation of the T6-3 deletions/Luci constructs. The 5′ deletion fragments were generated by an *Exo* III digestion and then linked to the luciferase gene. Number of nucleotides cloned in front of the luciferase gene is indicated. (*B*) Relative luciferase activity observed after transfection in LLC-PK1 cells. Data are expressed as the increase of luciferase activity above background per mg of protein and represent the mean of five experiments.

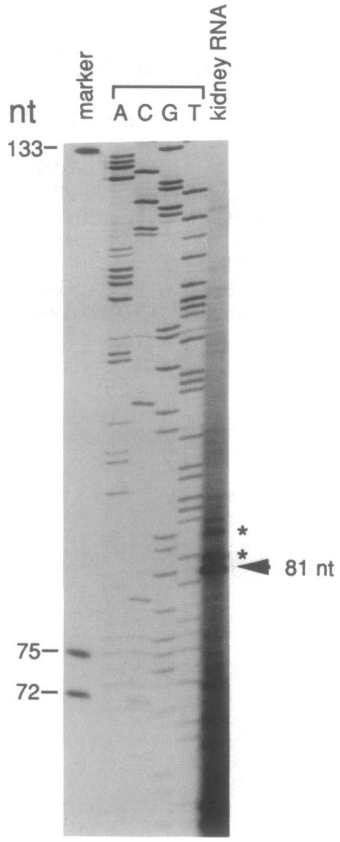

FIG. 2. Primer-extension analysis of NBAT RNA from rat kidney. Arrow indicates major extension product of 81 nt. Asterisks indicate additional possible extension products. Primer used was a 27-mer oligonucleotide located at +55 to +81 in exon 1. Size marker on the left was used to determine the size of the extended products. DNA sequence generated with the 27-mer oligonucleotide on clone T6-3 is shown for calibration.

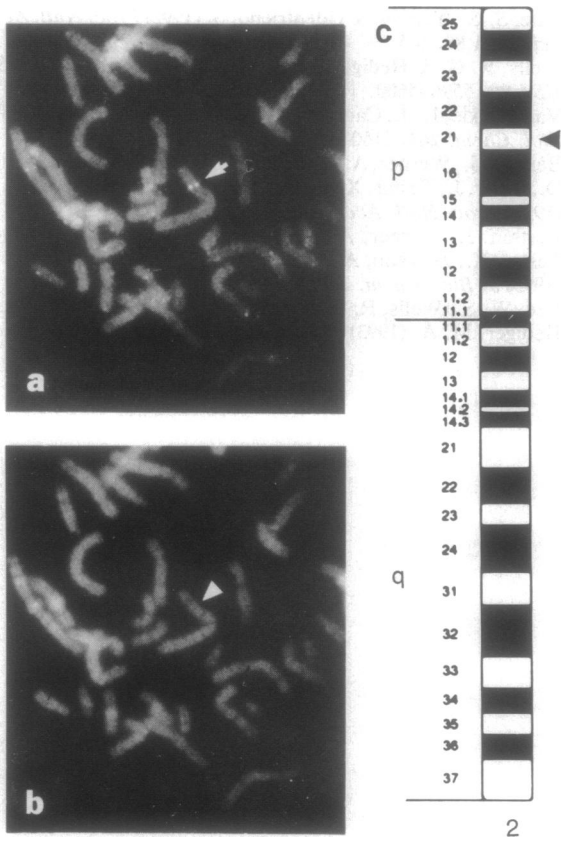

FIG. 4. Localization of human NBAT gene to chromosome 2p21. Partial metaphase showing propidium iodide staining (*a*) and 4′,6-diamidino-2-phenylindole (*b*) following hybridization of the probe. Arrow indicates hybridization signals and arrowhead shows band 2p21. (*c*) Idiogram of chromosome 2 showing hybridization signals at p21. Arrowhead represents 26 hybridization events with signals on both chromatids (doublets) and 10 hybridization events with signals on one chromatid.

NBAT is thought to be the major cystine transporter in rat, rabbit, and human kidneys (4–6). Our initial attempts to map the NBAT gene to a human chromosome with the rat genomic clone did not succeed because the signal obtained was very weak. We were, however, able to use rat NBAT cDNA to isolate a human genomic clone. A 2.2-kb insert from the human genomic clone was used as a probe for Southern analysis of the somatic cell hybrid panel of hamster–human DNAs. Hybridization revealed signals syntenic with chromosome 2 with 100% concordance (data not shown). To determine the subregional localization of the NBAT gene in chromosome 2 we used the FISH assay with a biotin-11-dUTP-labeled probe to normal metaphase chromosomes. The resulting hybridization signals were highly specific to chromosome 2. A total of 36 metaphase spreads with signals on chromosome 2 were available for analysis, either on both chromatids (26 hybridization events) or on one chromatid (10 hybridization events). All the hybridization signals clustered at the p21 region of chromosome 2 (Fig. 4).

Recently, Lee *et al.* (6) were able to assign the human NBAT gene to chromosome 2 by somatic cell hybrid analysis. In the present studies, we have precisely assigned this gene to segment p21 on chromosome 2 by the FISH technique. The significance of this localization is still unclear. Additional genes that were mapped to 2p21 are the human luteinizing hormone/choriogonadotropin receptor gene (24), the forebrain cleavage gene (25), the human follicle-stimulating hormone receptor gene (26), the interferon-inducible double-stranded RNA-dependent protein kinase gene (27), and the cardiac sarcolemmal Na^+–Ca^{2+} exchanger gene (28).

1. Tate, S. S., Yan, N. & Udenfriend, S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1–5.
2. Wells, R. G. & Hediger, M. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5596–5600.
3. Van Winkle, L. J., Campione, A. L. & Gorman, J. M. (1988) *J. Biol. Chem.* **263**, 3150–3163.
4. Bertran, J., Werner, A., Moore, M. L., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacin, M. & Murer, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5601–5605.
5. Bertran, J., Werner, A., Chillaron, J., Nunes, V., Biber, J., Testar, X., Zorzano, A., Estivill, X., Murer, H. & Palacin, M. (1993) *J. Biol. Chem.* **268**, 14842–14849.
6. Lee, W.-S., Wells, R. G., Sabbag, R. V., Mohandas, T. K. & Hediger, M. A. (1993) *J. Clin. Invest.* **91**, 1959–1963.
7. Yan, N., Mosckovitz, R., Udenfriend, S. & Tate, S. S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9982–9985.
8. Mosckovitz, R., Yan, N., Heimer, E., Felix, A., Tate, S. S. & Udenfriend, S. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4022–4026.
9. Pickel, V. M., Nirenberg, M. J., Chan, J., Mosckovitz, R., Udenfriend, S. & Tate, S. S. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 7779–7783.
10. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
11. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
12. Pinkel, D., Straume, T. & Gray, J. W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2934–2938.
13. Mathew, S., Murty, V. V. V. S., Hunziker, W. & Chaganti, R. S. K. (1992) *Genomics* **14**, 775–779.
14. Mount, S. M. (1982) *Nucleic Acids Res.* **10**, 459–472.
15. Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50**, 349–383.
16. Rupp, R. A. W. & Sippel, A. E. (1987) *Nucleic Acids Res.* **15**, 9707–9726.
17. Jones, K. A., Luciw, P. A. & Duchange, N. (1988) *Genes Dev.* **2**, 1101–1114.
18. Schule, R., Muller, M., Otsuka-Murakami, H. & Reinkawit, R. (1988) *Nature (London)* **332**, 87–90.
19. Horiuchi, M., Nakamura, N., Tang, S.-S., Barrett, G. & Dzau, V. J. (1991) *J. Biol. Chem.* **266**, 16247–16254.
20. Fouser, L. & Avner, E. D. (1993) *Am. J. Kidney Dis.* **21**, 64–70.
21. Weiner, A. M. (1986) *Annu. Rev. Biochem.* **55**, 631–661.
22. Segal, S. & Thier, S. O. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), pp. 2479–2496.
23. Bergeron, M. & Scriver, C. R. (1992) in *The Kidney: Physiology and Pathophysiology*, eds. Seldin, D. W. & Giebisch, G. (Raven, New York), pp. 2947–2969.
24. Rousseau-Merk, M. F., Misrahi, M., Atger, M., Loosfelt, H., Milgrom, E. & Berger, R. (1990) *Cytogenet. Cell Genet.* **54**, 77–79.
25. Hecht, B. K., Hecht, F. & Munke, M. (1991) *Am. J. Med. Genet.* **40**, 130.
26. Rousseau-Merk, M. F., Atger, M., Loosfelt, H., Milgrom, E. & Berger, R. (1993) *Genomics* **15**, 222–224.
27. Hanash, S. M., Beretta, L., Barcroft, C. L., Sheldon, S., Glover, T. W., Ungar, D. & Sonenberg, N. (1993) *Genes Chromosomes Cancer* **8**, 34–37.
28. Shieh, B. H., Xia, Y., Sparkes, R. S., Klisak, I., Lusis, A. J., Nicoll, D. A. & Philipson, K. D. (1992) *Genomics* **12**, 616–617.