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Genes Expressed in the Mouse Pituitary Corticotrope AtT-20/D-16v Tumor Cell Line

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Abstract. **The pituitary corticotrope AtT-20 stable cell line has been used as a model system to study peptide secretion, glucocorticoid regulation, and several other processes. In order to better understand this model cell line, a phage cDNA library was generated from AtT-20/D-16v cell mRNA and cDNA sequences were obtained for 317 clones representing 203 known genes and 48 novel cDNAs. The sequencing results revealed the prevalence of the mouse leukemia virus in this cell line and also identified a number of putatively secreted molecules that were not previously recognized as being secreted from AtT-20/D-16v cells or pituitary corticotropes. Nine completely novel cDNAs and 39 cDNAs homologous to known ESTs were also identified. A listing of other genes known to be expressed in AtT-20/D-16v cells is also provided.**

Keywords. **AtT-20/D-16v, cDNA library, corticotrope, EST, DNA sequencing, pituitary**

Introduction

About 200 types of cells comprise the tissues of mammalian organisms. Each cell type expresses a subset of the genes that are encoded in the genomic DNA. The specific genes expressed in each cell presumably encode the proteins that are necessary for the effective functioning of the particular tissue. In designing and interpreting experiments, it is therefore important to know what genes are expressed in a specific cell type or a model cell line. Thus, the information gained from the random sequencing of expressed genes in tissues (ESTs) has become a valuable resource for scientists. Whereas RNA expression data are available electronically through several databases including the nonredundant nucleotide and dbEST, a mechanism for easy access to all known sequences expressed in a particular cell has not been established. This paper reporting and reviewing genes expressed in the mouse pituitary corticotrope AtT-20/D-16v cell line is intended to serve as an initial summary of gene expression for those investigators who use this cell line as a model system.

The AtT-20/D-16v cell line had its origins in the work of Furth and colleagues, who examined the effects on mice of ionizing radiation from atomic bomb detonation [1,2]. They found that within 2 years of exposure a small percentage of the mice developed pituitary tumors. Three of these tumors were ACTH-secreting and were propagated by serial transplantation into LAF_1 mice [2]. One mouse developed an adrenocorticotropin hormone (ACTH)- secreting adenocarcinoma in the anterior pituitary; this tumor was designated AtT-20 ("AtT") is derived from adrenotrophic and "−20" comes from the tumor strain number [3]. Multiple passages of these AtT-20 tumor cells between culture and mice yielded the stable AtT-20 cell line [4]. From this cell line, a clonal cell line was obtained by plating single cells; only the cells growing in suspension above the monolayer were found to secrete ACTH [5]. This cell line growing as a suspension (AtT-20/D-1) is available from the American Type Culture Collection (CCL-89). Selection for ACTH-secreting cells growing as a monolayer produced three cloneal cell lines, AtT-20/D-12v, AtT-20/D-16v and AtT-20/D-18v [5]. The AtT-20/D-16v cell line (ATCC #CRL1795) has become one of the most abundantly used models for studying the biosynthesis and secretion of peptide hormones, transcriptional regulation by glucocorticoids, and several other processes.

To provide an initial gene expression summary as a resource to those researchers who use AtT-20/D-16v cells as a model system, I now report a number of genes that are expressed in these cells and also summarize previous reports of genes expressed in AtT-20/D-16v cells. The list of proteins that are apparently expressed by these cells has been expanded and the expression of several putatively secreted proteins is noted. These findings should enhance our knowledge of AtT-20/D-16v cells and pituitary corticotropes.

Materials and Methods

Cell culture

AtT-20/D16-v cells were grown in DMEM:F12 medium containing 10% fetal calf serum (Collaborative Re-

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search), and 10% NuSerum (HyClone Laboratories). Cells were fed every other day and passaged weekly.

Preparation of AtT-20/D-16v cDNA library

Total RNA was prepared from two- ~90% confluent 100-mm dishes of AtT-20/D-16v cells using a triazole/phenol reagent (Stat-60) according to the manufacturer's protocol (Tel Test 'B'). The RNA was examined on an ethidium bromide-stained denaturing gel for the presence of intact 18S and 28S ribosomal RNAs. PolyA$^+$ mRNA was prepared from the purified total RNA by annealing to oligo(dT) immobilized on magnetic beads, as described by the manufacturer (Promega; PolyAtract System IV). PolyA$^+$ RNA was concentrated by ethanol precipitation after addition of 10 μg of glycogen (Ambion).

The λZAP2 cDNA library was prepared with several modifications of the manufacturer's protocol (Stratagene): PolyA$^+$ RNA (6 μg) was denatured by incubation for 1 min in the presence of 93 mM β-mercaptoethanol and 9.1 mM methyl mercury. Single-stranded cDNA (ss-cDNA) was synthesized from the denatured RNA by annealing to an oligo(dT-*Xho*I) oligomer (Stratagene) and incubating at 42°C with Moloney murine leukemia virus reverse transcriptase (50 units) and Superscript reverse transcriptase (Life Technologies, 800 units) in 1st strand synthesis buffer (Stratagene) containing 0.3 mM methyl-dCTP and 0.6 mM dATP, dTTP, and dGTP. For these reactions 1 μg of RNA was split and used for first-strand synthesis in a separate reaction that also contained 30 μCi of [α-^{32}P]-dCTP. The remainder of the protocol was carried out as described by the manufacturer.

The recombinant phage DNAs were packaged using GigaPack Gold III (Stratagene) and the resulting primary library was titered. The library was amplified once by plating 2.1×10^6 plaque forming units (pfu) and the phage were recovered by incubating with 200 ml of PSM buffer (0.1 mM NaCl, 10 mM $MgSO_4$, 0.01% gelatin (w/v), 50 mM Tris-HCl, pH 7.5) overnight.

Sequencing of cDNA library

The AtT-20/D-16v λ-ZAP2 cDNA library was used to make a pBS plasmid library by mass excision, using *E. coli* XL1-Blue MRF′, ExAssist helper phage, and *E. coli* SOLR cells, according to the manufacturer's protocol. The library was transformed and individual colonies were robotically arrayed into 384-well microtiter plates by the I.M.A.G.E. Consortium at Lawrence Livermore National Laboratory [6]. Plasmid DNAs were prepared and sequenced by automated dye-termination sequencing using the –40 RP primer (5′ end; Life Technologies) at the Washington University Sequencing Center. Sequences were deposited in the EST database at the National Center for Biotechnology Information (dbEST).

Results

AtT-20/D-16v cells are model neuroendocrine secretory cells that grow anchored on plastic dishes (Fig. 1). The cells are typically 15 μm long and most cells form bi- or tridentate processes that resemble the growth cones of neurons. To identify the genes that are most abundantly expressed in these cells, an oligo(dT)-primed directional cDNA library was prepared and 378 randomly selected clones were sequenced.

Characterization of the cDNA library

In preparation of the library, several controls were performed to ensure that cDNAs of the appropriate size were represented (Fig. 2). After the synthesis of the first and second cDNA strands, an aliquot was removed and fractionated on an agarose gel, the gel was dried, and exposed to film. The autoradiogram showed a broad smear ranging from 100 bp to 12 kb in the double strand cDNA sample, indicating that large-sized transcripts had been synthesized (Fig. 2A). After processing the cDNA by blunt-ending the cDNA termini, ligating *EcoR1* adapters to the cDNA, and digesting with *Xho1*, the larger-length transcripts were selected by size-exclusion chromatography on cDNA sizing columns (Clontech, Capfinder kit). The radiolabeled cDNAs in each column fraction were quantitated by Cherenkov counting, and aliquots of the near-peak fractions were analyzed by fractionation on an agarose gel and exposure to X-ray film (Fig. 2B). Fractions 7-11 containing transcripts ranging from 0.5 to 12 kb were then used to prepare the cDNA library as described.

The characteristics of the library were then examined. The primary AtT-20/D-16v library contained 1.9×10^7 pfu. The library was amplified one time using 2.1

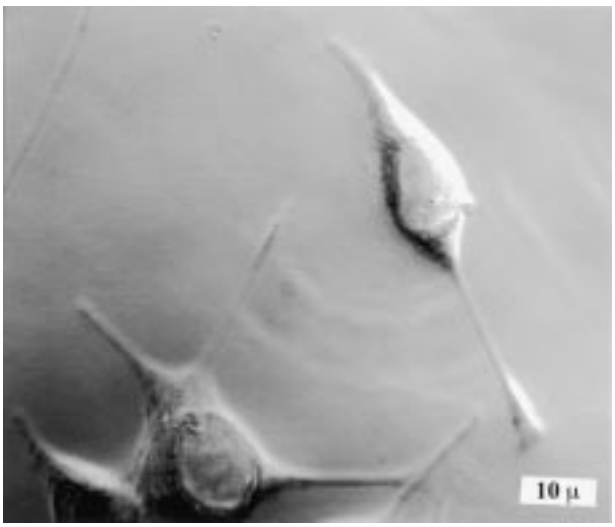

Fig. 1. *Photomicrograph of AtT-20/D-16v cells. Phase contrast photograph of AtT-20/D-16v cells taken with Normarski phase-contrast optics (X400 magnification).*

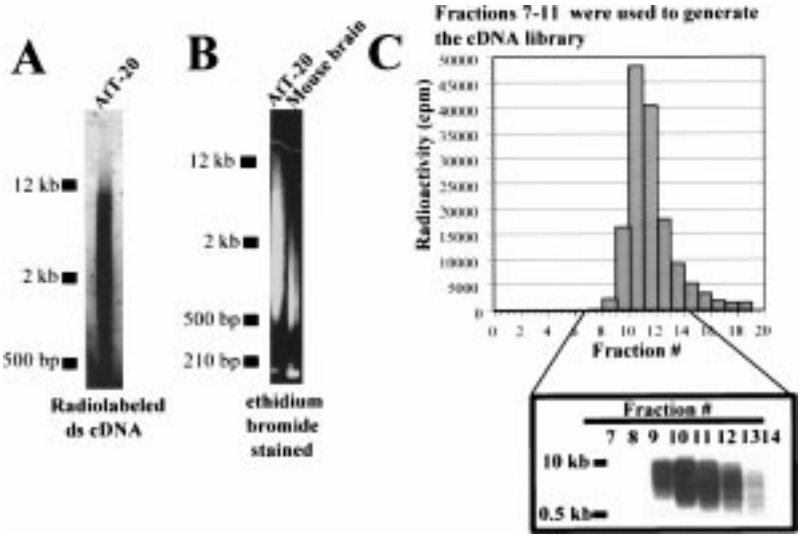

Fig. 2. *Controls for generation and preliminary characterization of the AtT-20/D-16v cDNA library.* **A.** *Analysis of double stranded cDNA used to make the AtT-20/D-16v cDNA library. Synthesis of single-stranded cDNA started with 1 μg of poly A⁺ RNA and radiolabeled dCTP, which was then pooled with a reaction containing 5 μg of poly A⁺ RNA and dCTP (no radioactivity). A sample of the pooled reaction mixture (1/100 of the total) was fractionated on a 1% agarose gel and the gel was dried and exposed to autoradiographic film (left panel). The migration of standards are indicated.* **B.** *Analysis of insert sizes in the amplified AtT-20/D-16v cDNA library. The cDNA inserts from the amplified AtT-20/D-16v and mouse brain (Stratagene) libraries were amplified by PCR using T3 and T7 primers, and 10% of the reaction was fractionated on a 1% agarose gel and photographed.* C. *Size selection of double stranded cDNA. The labeled cDNAs were fractionated on sizing columns and 1 drop fractions collected. Each fraction was quantitated by Cherenkov counting and 1/100 was analyzed by agarose gel electrophoresis. Fractions 7–11 were used to generate the cDNA library.*

$\times 10^6$ pfu, to yield a final titer of 2.1×10^9 pfu/ml. An aliquot of the library was transformed into NM522 *E. coli*, and plasmid DNA was prepared from 18 separate clones. These plasmids were digested with *EcoR1* and *Xho1* to release the inserts and analyzed on an agarose gel. Insert sizes ranged from 0.5 to 11 kb, with a median size of 1.3 kb (not shown).

In order to further evaluate the sizes of the inserts in the cDNA library, aliquots of the AtT-20/D-16v library and of a mouse brain library (Life Technologies) were amplified by PCR using T3 and T7 primers; 3-minute extension reactions were used to allow synthesis of longer-length cDNAs. Fractionation of the fragments on agarose gels (Fig. 2A) showed that, like the ds-cDNA, the amplified AtT-20/D-16v library contained a wide range of transcripts, whereas the commercially prepared library contained a lower amount of the larger-sized transcripts. Both libraries had contained a band of approximately 104 bp, which is the size of the fragment that would be amplified if no insert were present. The amplified AtT-20/D-16v library contained a lower amount of this band, and only 2 of the first 189 sequences obtained from this library had no insert.

Sequencing of cDNA library

Clones from the cDNA library were arrayed and sequenced from the 5′ end using the -40 RP primer. The sequencing results are summarized in Table 1. Of the 380 clones analyzed, 345 clones provided good-quality sequence, while 21 clones gave poor sequence, 6 clones did not contain an insert in the phage DNA, and 8 sequences were from *E. coli*. For the clones having good-quality sequence, 269 sequences were homologous to known genes, 48 sequences had not been previously identified or identified only in sequencing of other cDNA libraries, and 28 sequences were homologous to mouse mitochondrial DNA.

The accession numbers of the sequences identified in AtT-20/16v cells, the accession number of the known genes, the number of times each sequence was observed, the species to which the gene homologues were identified, and the names of the genes are shown in Table 2; because the accession numbers are provided in this table the publications characterizing the known

Table 1. *Profile of sequencing results from cDNA library*

	Number of sequences	Percentage of total
Clones sequenced	380	100
Clones with good sequence	345	90.8
Previously known genes	269	70.8
Previously unknown cDNAs	48	12.6
Mouse mitochondrial	28	7.4
Clones with poor sequence	21	5.5
No inserts	6	1.6
E. coli sequence	8	2.1

Table 2. *Summary of sequences of clones from the AtT-20 cell cDNA library*

AtT-20 accession no.	Known gene accession no.	No. of observ-ations	Gene name
Metabolism			
AI317074	L21027	1	A10 D-PHOSPHOGLYCERATE DEHYDROGENASE (MOUSE)
AI527589	D90228	1	ACETYL-COA ACETYLTRANSFERASE PRECURSOR, MITOCHONDRIAL (HUMAN)
AI876275	P41227	1	ACETYLTRANSFERASE; N-TERMINAL ARD1 SUBUNIT HOMOLOG (HUMAN)
AI876338	X70847	2	ADENINE NUCLEOTIDE TRANSLOCASE (MOUSE)
AI876387	D12780	1	S-ADENOSYLMETHIONINE DECARBOXYLASE (MOUSE)
AI527596	J04794	3	ALCOHOL DEHYDROGENASE (HUMAN)
AI317122	CE16861	1	ALCOHOL DEHYDROGENASE (HUMAN)
AI317121	U29152	1	ALDOLASE REDUCTASE (MOUSE)
AI317105	M14328	1	α-ENOLASE (HUMAN)
AI317092	P00848	2	ATP SYNTHASE A CHAIN (MOUSE)
AI316125	X04327	1	BISPHOSPHOGLYCERATE MUTASE (HUMAN)
AI876345	CE05783	1	LACTATE DEHYDROGENASE
AI876339	M22877	1	CYTOCHROME C (HUMAN)
AI317090	L08441	2	CYTOCHROME C OXIDASE POLYPEPTIDE III (HUMAN)
AI317087	P48659	3	CYTOCHROME C OXIDASE POLYPEPTIDE I (HORSE)
AI876411	O61538	1	GERANYLGERANYL PYROPHOSPHATE SYNTHASE
AI876289	K03195	1	GLUCOSE TRANSPORTER TYPE 1, ERYTHROCYTE/BRAIN (HUMAN)
AI876277	X01677	2	GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE(HUMAN)
AI876290	P70404	1	ISOCITRATE DEHYDROGENASE [NAD] MITOCHONDRIAL GAMMA (MOUSE)
AI876345	CE05783	1	LACTATE DEHYDROGENASE
AI527591	X60036	1	MITOCHONDRIAL PHOSPHATE CARRIER PROTEIN PRECURSOR (HUMAN)
AI317097	CE00241	1	4-NITROPHENYLPHOSPHATASE (MOUSE)
AI316115	O01591	1	4-NITROPHENYLPHOSPHATASE (SCHIZOSACCHAROMYCES POMBE)
AI317079	X55762	1	N4-(β-N-ACETYLGLUCOSAMINYL)-L-ASPARAGINASE (HUMAN)
AI317062	Q02369	2	NADH-UBIQUINONE OXIDOREDUCTASE B22 SUBUNIT (BOVINE)
AI317060	P73245	1	OXYGEN-INDEPENDENT COPROPORPHYRINOGEN III OXIDASE
AI876407	X60036	1	PHOSPHATE CARRIER PROTEIN PRECURSOR; MITOCHONDRIAL (HUMAN)
AI317094	Q16822	2	PHOSPHOENOLPYRUVATE CARBOXYKINASE (MOUSE)
AI876350	V00572	1	PHOSPHOGLYCERATE KINASE 1 (HUMAN)
AI876406	X16646	1	SODIUM, POTASSIUM ATPASE β SUBUNIT (MOUSE)
AI316134	X53333	1	TRIOSEPHOSPHATE ISOMERASE (MOUSE)
AI316138	P34666	1	UBIQUINONE BIOSYNTHESIS METHLYTRANSFERASE (MOUSE)
Translation and protein folding			
AI317061	X15267	1	ACIDIC RIBOSOMAL PHOSPHOPROTEIN PO (MOUSE)
AI317086	X14926	1	CALRETICULIN (MOUSE)
AI317043	Z31555	1	CHAPERONIN CONTAINING TCP-1 ε SUBUNIT (MOUSE)
AI317067	X16869	2	ELONGATION FACTOR 1-ALPHA 1 (HUMAN)
AI317081	D13748	1	EUKARYOTIC INITIATION FACTOR 4A-I (HUMAN)
AI317077	Z21507	1	ELONGATION FACTOR 1-DELTA (HUMAN)
AI316093	S65791	1	FRAGILE X MENTAL RETARDATION 1 PROTEIN (HUMAN)
AI876369	M19141	1	HEAT SHOCK PROTEIN 70 (MOUSE)
AI316098	L20868	1	L4 RIBOSOMAL PROTEIN; 60S (HUMAN)
AI876363	M29015	1	L7 RIBOSOMAL PROTEIN (MOUSE)
AI876293	D14531	1	L9 RIBOSOMAL PROTEIN, 60S (HUMAN)
AI527580	P46778	1	L21 60S RIBOSOMAL PROTEIN (HUMAN)
AI527588	X05021	1	L29 RIBOSOMAL PROTEIN (YEAST)
AI316109	M17887	1	P2 RIBOSOMAL PROTEIN; 60S ACIDIC (HUMAN)
AI876395	X76772	2	S3 RIBOSOMAL PROTEIN (MOUSE)
AI876248	M84711	1	S3A RIBOSOMAL PROTEIN; 40S (HUMAN)
AI876367	P29314	1	S9 RIBOSOMAL PROTEIN; 40S (RAT)
AI317031	M11408	1	S16 RIBOSOMAL PROTEIN (MOUSE)
AI527586	Z50159	2	SUI-1 (MOUSE)
AI317078	P16967	1	TRANSLOCON-ASSOCIATED PROTEIN, ALPHA SUBUNIT PRECURSOR
AI317065	P41252	1	TRNA SYNTHETASE, ISOLEUCYL, CYTOPLASMIC (HUMAN)
AI316124	NM 004990	1	TRNA SYNTHETASE, METHIONINE (HUMAN)
AI876265	P49589	1	TRNA SYNTHETASE, CYSTEINYL (HUMAN)
AI876308	X54326	1	TRNA SYNTHETASE, AMINOACYL, MULTIFUNCTIONAL (HUMAN)

Table 2. *Continued*

AtT-20 accession no.	Known gene accession no.	No. of observ-ations	Gene name
Secretion and Signaling			
AI317113	Z19599	4	14-3-3 ε (MOUSE)
AI317054	X14309	1	4F2 ANTIGEN HEAVY CHAIN, AMINO ACID TRANSPORTER (MOUSE)
AI317076	M57763	1	ADP-RIBOSYLATION FACTOR 6 (HUMAN)
AI317039	X06989	1	AMYLOID A4 PROTEIN PRECURSOR (HUMAN)
AI317055	CE14950	1	ADP-RIBOSYLATION FACTOR (MOUSE)
AI527581	AF133912	1	AIP-5 ADP RIBOSYLATION FACTOR
AI317027	P56213	1	AUGMENTER OF LIVER REGENERATION (MOUSE)
AI317044	X78683	1	B-CELL RECEPTOR ASSOCIATED PROTEIN (MOUSE)
AI316111	O08904	1	BRX PROTEIN,EPIDERMAL GROWTH FACTOR RECEPTOR KINASE SUBSTRATE
AI316095	O09007	1	BUB1 PROTEIN KINASE
AI876271	P73467	1	CELL DIVISION INHIBITOR
AI876375	O35457	1	β-CHEMOKINE RECEPTOR, PUTATIVE
AI876399	P53676	1	CLATHRIN COAT ASSEMBLY PROTEIN AP47 HOMOLOG 1 (RAT)
AI317051	P53620	1	COATOMER GAMMA SUBUNIT (BOVINE)
AI527582	M38297	15	CORTICOTROPIN-LIPOTROPIN PRECURSOR (POMC) (HUMAN)
AI316137	M64278	1	CROMOGRANIN A (MOUSE)
AI876276	X53028	1	CHROMOGRANIN B (MOUSE)
AI317103	X52803	2	CYCLOPHILIN (MOUSE)
AI876396	Q13352	1	β 3-ENDONEXIN
AI317124	Q15668	2	EPIDIDYMAL SECRETED PROTEIN PRECURSOR E1 (MOUSE)
AI317042	D37790	1	β-1,4 GALACTOSYLTRANSFERASE (HUMAN)
AI876393	P31421	1	GLUTAMATE RECEPTOR 2 PRECURSOR, METABOTROPIC (RAT)
AI317022	X56009	2	GUANINE NUCLEOTIDE-BINDING PROTEIN G(S), α SUBUNIT (HUMAN)
AI316097	X75313	2	GUANINE NUCLEOTIDE BINDING PROTEIN RELATED GENE (MOUSE)
AI876380	P94063	1	HAL3 HOMOLOG (YEAST)
AI876358	P46694	1	IEX-1 RADIATION-INDUCIBLE IMMEDIATE-EARLY GENE
AI876288	Q13418	1	INTEGRIN-LINKED KINASE
AI876348	O35563	1	KI ANTIGEN
AI317101	X57437	1	L-HISTIDINE DECARBOXYLASE (MOUSE)
AI317056	P49028	5	MAGO NASHI PROTEIN (DROSOPHILA)
AI876336	X06086	1	MAJOR EXCRETED PROTEIN (MOUSE)
AI527587	D10049	16	MELANOMA ANTIGEN (MOUSE)
AI876256	V00835	1	METALLOTHIONEIN-1 (MOUSE)
AI316116	X01838	1	β-MICROGLOBULIN (MOUSE)
AI876355	D63784	1	MIDA1 (MOUSE)
AI876386	Q62768	1	MUNC13-1 DIACYLGLYCEROL RECEPTOR(MOUSE)
AI317030	O18979	1	NEUROENDOCRINE SECRETORY PROTEIN 55
AI876270	O35684	1	NEUROSERPIN PRECURSOR (MOUSE)
AI876404	O54940	1	NIP2L
AI876306	O08658	1	NUCLEOPORIN NUP84
AI876398	P54369	1	ORNITHINE DECARBOXYLASE ANTIZYME (MOUSE)
AI876378	Z17804	1	P120 GENE (MOUSE)
AI876392	V00574	1	P21/H-RAS-1 (HUMAN)
AI317052	M20473	1	PROTEIN KINASE A, cAMP-DEPENDENT (MOUSE)
AI876262	L11285	1	PROTEIN KINASE KINASE 2; MITOGEN-ACTIVATED (HUMAN)
AI876397	O35708	1	PROTEIN PHOSPHATASE 2A B'α3 REGULATORY SUBUNIT
AI876352	M64241	1	QM PROTEIN (HUMAN)
AI316101	Q62145	1	RAB6/RAB5-ASSOCIATED PROTEIN
AI876251	L26528	1	RAB11B (MOUSE)
AI317047	O00194	1	RAS-RELATED PROTEIN RAB-27B (HUMAN)
AI317075	P97695	1	SEC7B (MOUSE)
AI317119	M31303	1	STATHMIN (HUMAN)
AI527595	Y00703	1	STIMULATORY GTP BINDING PROTEIN (MOUSE)
AI317114	X99337	1	SYNAPTIC MEMBRANE GLYCOPROTEIN 55 OR 65 (RAT)
AI317098	Q63754	1	β-SYNUCLEIN (RAT)
AI316107	Z14044	1	VALOSIN-CONTAINING PROTEIN, CDC45 HOMOLOG (MOUSE)
AI876302	Y00094	1	YPT1 RAS RELATED PROTEIN (MOUSE)

Table 2. *Continued*

AtT-20 accession no.	Known gene accession no.	No. of observ-ations	Gene name
Structural			
AI876278	M21495	1	γ-ACTIN(MOUSE)
AI317085	M34175	1	β-ADAPTIN (HUMAN)
AI876257	P26444	1	β-CRYSTALLIN A2 (BOVINE)
AI316117	M15395	1	CELL SURFACE ADHESION GLYCOPROTEIN LFA-1 (HUMAN)
AI317053	L05670	1	CLUSTERIN (MOUSE)
AI317064	X64713	1	CYCLIN B1 (MOUSE)
AI316133	Q63618	1	ESPIN
AI527592	X60671	1	EZRIN (MOUSE)
AI317040	U37720	1	G25K,CDC42 HOMOLOG (MOUSE)
AI317089	U04443	1	NON-MUSCLE MYOSIN LIGHT CHAIN 3 (MOUSE)
AI317028	X15051	1	NCAM-140 and NCAM-180 (MOUSE)
AI317115	U35141	1	RETINOBLASTOMA BINDING PROTEIN (MOUSE)
AI317032	O35405	1	SCHWANNOMA-ASSOCIATED PROTEIN, PHOSPHOLIPASE D HOMOLOG
AI317099	X80339	1	SIX-1 HOMEOBODX PROTEIN (MOUSE)
AI317069	AF042379	1	SPINDLE POLE BODY PROTEIN GCP2 (HUMAN)
AI527593	P39447	1	TIGHT JUNCTION PROTEIN ZO-1 (MOUSE)
AI876384	M13445	1	α-TUBULIN ISOTYPE M-α-1 (MOUSE)
AI316130	M13446	1	α-TUBULIN ISOTYPE M-α-2 (MOUSE)
AI527578	X79535	1	TUBULIN β-2 CHAIN (HUMAN)
AI316113	J00314	1	TUBULIN β-1 CHAIN (HUMAN)
AI876299	X04663	1	β-TUBULIN ISOTYPE M-β-5 (MOUSE)
AI317021	AF067656	1	ZW10 INTERACTOR PROTEIN ZWINT (HUMAN)
Proteolysis			
AI27594	O09175	1	AMINOPEPTIDASE B (RAT)
AI316105	X52886	1	CATHEPSIN D (MOUSE)
AI316131	P00755	2	KALLIKREIN K6 PRECURSOR, GLANDULAR (MOUSE)
AI317033	CE00390	1	LEUCINE AMINOPEPTIDASE
AI876295	AF121856	1	NEXIN-6 (HUMAN)
AI317109	P70195	1	PROTEASOME Z SUBUNIT PRECURSOR
AI527590	X51703	5	UBIQUITIN (MOUSE)
AI317116	P07552	1	UBIQUINOL-CYTOCHROME C REDUCTASE COMPLEX 6.4 KD PROTEIN (BOVINE)
AI317111	P51966	3	UBIQUITIN-CONJUGATING ENZYME E2-18 KD UBCH7 (HUMAN)
AI876372	P55855	1	UBIQUITIN-LIKE PROTEIN SMT3B (HUMAN)
Nucleic Acids			
AI527585	Q60817	1	α NAC/1.9.2. TRANSCRIPTION FACTOR (MOUSE)
AI876410	O15298	1	BASIC LEUCINE ZIPPER NUCLEAR FACTOR 1-LIKE
AI317048	U20326	1	CELLULAR NUCLEIC ACID BINDING PROTEIN (MOUSE)
AI876389	Q13112	1	CHROMATIN ASSEMBLY FACTOR I P60 SUBUNIT (HUMAN)
AI317072	P52433	1	DNA-DIRECTED RNA POLYMERASE II 19 KD POLYPEPTIDE (HUMAN)
AI316100	P38447	1	ENDONUCLEASE G, MITOCHONDRIAL (BOVINE)
AI876263	O70132	1	ETS DOMAIN TRANSCRIPTION FACTOR PET-1.
AI876285	L10426	1	ETS-RELATED PROTEIN 81 (MOUSE)
AI876344	122593	1	FIBRILLARIN (MOUSE)
AI317120	P51991	1	HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN A3 (HUMAN)
AI876304	S74678	1	HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN K (HUMAN)
AI316126	X65488	1	HETEROGENOUS NUCLEAR RIBONUCLEOPROTEIN U (HUMAN)
AI876357	M37583	1	HISTONE H2A.Z (HUMAN)
AI876283	M11353	1	HISTONE H3.3 (HUMAN)
AI317110	X12944	2	HMG-17 NONHISTONE CHROMOSOMAL PROTEIN (MOUSE)
AI876282	M94087	2	MATF4 (MOUSE)
AI317068	CE03769	1	MITOCHONDRIAL RNA SPLICING MSR4
AI317045	X71327	1	MRE-BINDING TRANSCRIPTION FACTOR (MOUSE)
AI876353	O00567	1	NOP-56 NUCLEOLAR PROTEIN
AI876379	Q99848	1	NUCLEOLAR PROTEIN P40
AI316110	D12618	1	NUCLEOSOME ASSEMBLY PROTEIN-1 (MOUSE)
AI876361	X56135	1	PROTHYMOSIN α (MOUSE)

Table 2. *Continued*

AtT-20 accession no.	Known gene accession no.	No. of observ-ations	Gene name
AI317132	AF119121	1	RNA BINDING PROTEIN, PUTATIVE
AI876307	O89086	1	RNA-BINDING PROTEIN 3, PUTATIVE (MOUSE)
AI316132	NM 005872	1	SPLICEOSOME ASSOCIATED PROTEIN DAM1 (HUMAN)
AI876284	Q08170	1	SPLICING FACTOR ARGININE/SERINE-RICH 4 (HUMAN)
AI317108	L10911	1	SPLICING FACTOR (CC1.4) (HUMAN)
AI876268	O08950	1	TRANSCRIPTION INITIATION FACTOR IIA GAMMA CHAIN (RAT)
Unknown Function			
AI876383	X06407	2	21 KD PEPTIDE UNDER TRANSLATIONAL CONTROL (MOUSE)
AI317114	P17790	1	BASIGIN (CHICKEN)
AI527584	AF151806	1	CGI48 PROTEIN (HUMAN)
AI316096	X61450	1	E161 (MOUSE)
AI317133	Q61070	1	ETOPOSIDE INDUCED 2.4 KB MRNA
AI316123	Q08509	1	EPS8 PROTEIN
AI876255	O80775	1	F13P17.10 PROTEIN
AI876260	O60735	1	GA17 PROTEIN.
AI876373	CE06364	1	HYPOTHETICAL 52.8 KD PROTEIN
AI876360	P53990	1	HYPOTHETICAL PROTEIN KIAA0174 (HUMAN)
AI876279	P53223	1	HYPOTHETICAL 27.6 KD PROTEIN (YEAST)
AI876259	O75393	2	HYPOTHETICAL 33.8 KD PROTEIN
AI317100	Q57918	1	HYPOTHETICAL PROTEIN MJ495 PRECURSOR
AI317070	O15031	1	KIAA0315 BRAIN PROTEIN
AI317058	AF129332	1	MUM2 (HUMAN)
AI876292	Z31555	2	mRNA (MOUSE)
AI876267	L32751	1	mRNA (MOUSE)
AI876258	CE16032	1	mRNA (MOUSE)
AI317046	M38188	1	OVARIAN GRANULOSA CELL 13.0 KD PROTEIN HGR74 (HUMAN)
AI317125	Q94919	1	PAST-1 (MOUSE)
AI876342	Q63400	1	RAT ORF
AI317104	U04268	1	SCA-2 CELL SURFACE PROTEIN PRECURSOR (MOUSE)
AI316135	Z36851	1	XS99 MRNA (HUMAN)
Viral Bacterial			
AI316112	M11024	1	ENDOGENOUS MAMMARY TUMOR VIRUS (MOUSE)
AI317034	P03374	1	ENV POLYPROTEIN
AI316128	P70863	1	HI0412 PROTEIN (H. INFLUENZAE)
AI527583	X16670	1	INTRACISTERNAL APARTICLE, TYPE IIB (MOUSE)
AI527579	M87550	1	MURINE LEUKEMIA VIRUS REVERSE TRANSCRIPTASE (MOUSE)
AI316122	P31795	1	POL POLYPROTEIN
AI317020	L01991	1	TAFG-1-LIKE NEURONAL GLYCOPROTEIN (MOUSE)

genes are not referenced. In the final compilation, 317 sequences represented 251 different cDNAs; 203 different known genes and 48 cDNA sequences had no match to known genes. Of the 203 known genes 32 were involved in metabolism, 24 in protein translation and protein folding, 57 were involved in secretion and signaling, 22 were related to cellular structure, 10 were related to proteolysis, 28 were related to nucleic acid modification or regulation, 23 had no known function, and 7 were previously observed only in bacteria or viruses.

Thirty-nine of the 48 novel cDNAs were homologous to previously identified ESTs and 9 cDNAs were completely novel. Of the 48 novel cDNAs four (AI317123, AI317129, AI876300, and AI876365) were observed twice in the sequencing results, suggesting that they are abundant in this cell line. By generating contigs from the novel cDNAs using other mouse ESTs, the cDNAs appear to represent 3′ and 5′ untranslated, and coding regions of mRNAs (not shown). These known and novel sequences can be found in the nonredundant nucleotide or EST database using "Schiller" and "AtT-20" as queries.

Discussion

A number of genes have previously been reported to be expressed in AtT-20 cells. A review of the literature

using 'PubMed' and AtT-20 as a search query was used to generate a listing of genes known to be expressed in AtT-20 cell (Table 3). Sequencing of clones from the AtT-20/D-16v library has expanded the number of genes known to be expressed in AtT-20 cells (Table 2). The number of times that a sequence was observed in sequencing of the library likely reflects the expression of that gene in AtT-20 cells [13]; however, such an interpretation is limited because of the the small number of sequences obtained relative to the total number of transcripts in the cell. In addition, smaller size transcripts may be underrepresented because the AtT-20/D-16v library was selected to remove a high percentage of smaller sized cDNAs. It is interesting to note that POMC, chromogranin A, G-protein α-subunit, glandular kallikrein, protein kinase A (type 1 regulatory subunit), and NCAM are the only proteins observed in this library that were previously studied as endogenous proteins in AtT-20 cells [7–12]. Thus, the results of this study provide the researchers using this cell line with new information regarding the genes expressed in AtT-20 cells.

Proopiomelanocortin, ubiquitin, mago nashi protein, melanoma antigen, alcohol dehydrogenase, cytochrome C oxidase subunit 1, 14-3-3 ε, and ubiquitin conjugating enzyme were the most abundantly observed sequences in the AtT-20/D-16v cell library. Proopiomelanocortin and melanoma antigens were highly represented genes encoding proteins that are secreted. Proopiomelanocortin is the prohormone precursor of several bioactive peptides, including adrenocorticotrophin, α-melanocyte stimulating hormone,

Table 3. *Published genes expressed in AtT-20 cells*

Gene	Reference
DNA binding proteins	
Brn3a POU	[30]
c-fos, fos B	[31]
jun B	[31]
c-jun	[31]
KIN17 zinc-finger DNA-binding protein	[32]
NNP-1 nuclear protein (NM 010925)	[33]
Ptx1 homeo box transcription factor	[34]
SOSC-3	[35]
Wnt-1	[36]
Zac1	[37]
Secreted proteins	
apolipoprotein AI	[38]
[a]Chromogranin A	[39]
Chromogranin B	[39]
Insulin growth factor binding protein −5	[40]
Insulin growth factor binding protein −4	[40]
interleukin-2	[41]
interleukin-10	[42]
interleukin-11	[43]
MIF macrophage migration inhibitory factor	[44]
pro-cholecystokinin	[45]
pro-opiomelanocortin	[46]
pro-vasopressin	[47]
Secretogranin II	[7]
storage granule protein-23	[48]
Receptors and channels	
K+ channel, Kir3.1G-protein gated	[49]
K+ channel, Kir3.21G-protein gated	[49]
Ca2+ channel α1E	[50]
Ca+-sensing receptor (CaR)	[51]
corticotrophin releasing hormone receptor 1	[52]
G-protein, heterotrimeric α subunits; Gsα, Gtα, Gqα, G11α, G12α, G13α, G14α, G15α, Gzα, Gi2α, Gi3α, and Goα	[12]
GABA receptor	[53]
glucocorticoid receptor (GR)	[54]
guanylate cyclase-linked GC-B natriuretic peptide receptor subtype	[55]
histamine H3 receptor	[22]
interleukin-1 receptor type II	[56]
interleukin-2 receptor-β	[57]
muscarinic acetylcholine receptor m4	[58]
NOR-1, neuron-derived orphan receptor	[59]
retinoic acid receptor-β	[60]
somatostatin receptor 1, 2A, 2B, 4, 5	[61]
sulfonylurea receptor	[62]
TNF receptor p60 and p80	[63]
Proteins related to the secretory pathway	
7B2	[64]
acetylcholinesterase	[65]
asppcr1 protease	[66]
carboxypeptidase E	[67]
cellubrevin	[68]
GalNAc-transferase	[69]
GGnM-4-sulfo-transferase	[69]
kallikrein, tissue	[70]
P-CIP2	[71]
PACE4	[72]
PC1	[73]
PC2	[73]
peptidylglycine α-amidating monooxygenase	[74]
protein kinase A	[75]
protein kinase C α, β, ε, and ζ	[76]
Rab3a	[68]
Rab3D	[77]
Rab18	[78]
RESP18	[79]
SNAP-25	[68]
synaptobrevin 2	[68]
synaptotagmin	[68]
synaptophysin	[68]
syntaxin 1	[68]
Other proteins	
LIM-3	[80]
NCAM 140 and 180	[11]
SAR1	[81]

[a]Underlined genes were also identified in sequencing the AtT-20 cDNA library.

and β-endorphin, and it was previously known to be highly expressed in AtT-20 cells [14]. Melanoma antigen is an endogenous mouse protein that is expressed only in transformed cells and encodes the envelope gene and long terminal repeat of the endogenous ecotropic murine leukemia provirus [15]. The observation of several genes related to the murine leukemia C-type virus is not surprising because this virus has previously been reported in AtT-20/D-1 cells [16]. Several other viral proteins were identified that may be related to transformation events involved in generating this cell line.

While AtT-20 cells are well known for the large quantities of endogenous proopiomelanocortin that they produce, several other identified molecules (histamine, neuroendocrine secretory protein 55, amyloid precursor protein, augmenter of liver regeneration, β-2-microglobulin, chromogranins A and B, epididymal secreted protein E1, major excreted protein, metallothionein 1, and neuroserpin) are predicted to be secreted from AtT-20 cells or catalyze the biosynthesis of secreted molecules from these cells. POMC and chromogranin A were previously known to be secreted from AtT-20 cells [7,14]. Neuroendocrine secretory protein 55 and chromogranin B are expressed in the anterior pituitary, and thus their expression in AtT-20 cells is not surprising [17–18]. Amyloid precursor protein is a membrane protein that is ubiquitously expressed; fragments of these proteins are concentrated in neuritic plaques in Alzheimers disease. Major excreted protein (MEP) is a mouse cathespsin L analog that is secreted from fibroblasts [19]. Metallothionein 1 is a metal chelating protein that is found in the blood, urine, and bile, indicating that this protein is secreted [20].

The identification of other secreted molecules may provide additional clues to their function or physiology. The presence of histidine decarboxylase, an enzyme catalyzing the synthesis of histamine, in AtT-20 cells is interesting, because corticotropes are responsive to histamine [21].

Furthermore, AtT-20 cells have previously been shown to express the histamine H3 receptor, a finding that suggests the presence of an autocrine loop [22]. On the other hand, histidine decarboxylase may be used in AtT-20 cells in some other metabolic capacity. Expression of the augmenter of liver regeneration gene is though to be restricted to the liver [23], and the epididymal secreted protein is only known to be associated with sperm in the epididymis [24]; the possible production of these proteins in the pituitary suggests a broader scope for their function. β-2-microglobulin is known to be secreted from hepatoma and B-cell after stimulation with cytokines, suggesting that this protein may also be secreted from AtT-20 cells [25–26]. Neuroserpin is a serine protease inhibitor that is secreted from the axons of neurons [27–28]. At present it is unknown whether these proteins are secreted through the constitutive or regulated secretory pathways, or secreted at all.

The sequencing results also indicated the expression of the putative β-chemokine receptor, metabotropic glutamate receptor 2, and the JMUNC13-1 diacylglycerol receptor, which were not previously known to be expressed in AtT-20 cells. The metabotropic glutamate receptor was previously reported in pituitary corticotropes [29]. The expression of these receptors in AtT-20 cells raises the possibility that other ligands may be mediators of stimulated peptide secretion in corticotropes, although further study will be required.

The identification of genes in AtT-20/D-16v cells in this study does not necessarily indicate that these genes are expressed in pituitary corticotropes. Altered expression of genes could have been caused by the radiation induction of the AtT-20 pituitary tumors, the manipulations required to generate the stable AtT-20/D-1 and D-16 cell lines, or the rapid rate of AtT-20 cells proliferation in cell culture when compared to the adult pituitary.

The identification of sequences in specific cell lines and cell types allows us to better understand the molecular events important to the functions of these cells. In this study I report 251 genes, most of which have been previously characterized but not known to be expressed in AtT-20 cells. This study was intended to provide more information about a model cell line that is used by numerous investigators from various fields. Several genes not previously known to be expressed in AtT-20 cells were identified. Such data can be used to generate new hypotheses concerning corticotrope function. For example, the identification of histidine decarboxylase suggests that AtT-20 cells produce histamine. The expression of a histamine receptor in AtT-20 cells [22] raises the possibility of a histamine autocrine loop in corticotropes.

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Notes

Accession numbers

Accession numbers for previously known genes are in Table 2. Accession numbers for cDNAs with no

matches in the nonredundant nucleotide database are below: AI317130, AI317129, AI317127, AI317126, AI317123, AI317117, AI317091, AI317096, AI317102, AI317088, AI317083, AI317071, AI317063, AI317059, AI317050, AI317038, AI317035, AI316118, AI316102, AI316099, AI316127, AI316132, AI317025, AI317023, AI876408, AI876403, AI876402, AI876400, AI876303, AI876301, AI876300, AI876249, AI876253, AI876252, AI876266, AI876381, AI876359, AI876356, AI876388, AI876371, AI876370, AI876365, AI876349, AI876286, AI876264, AI876274, AI876273, AI876272.

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