Table 1. Biological activities of hybrid proteins in crude *E. coli* cell extracts. Cultures of transformed *E. coli* HB101 and their cell extracts were made as described (*15*). Antiviral activity was assayed on human WISH cells challenged with EMC virus or VSV (*16*). All IFN titers were calibrated against the NIH standard IFN samples. TNF activity was measured on L929 cells without pretreatment with actinomycin D or mitomycin (*17*). Both IFN and TNF assay have been performed at least five times. The data shown are the results of representative experiments.

Clone	IFN activity (log_{10} IU/liter)	TNF activity (log_{10} U/liter)
pγTNF-β1	7.11	4.48
pγ143	7.41	0
pγTNF-β2	6.51	4.48
pTNF-β	0	6.28
pBR322	0	0

these bands correspond to molecular sizes of 15, 30, and 35 kD. These results together with the purification data indicate that pγTNF-β1 expresses a true fusion protein, whereas the fusion protein in pγTNF-β2 has been processed. Purified recombinant IFN-γ always gives two bands in SDS-polyacrylamide gels, one of 17 kD and another of 35 kD. This probably reflects the formation of covalent dimers. TNF-β migrates with a molecular size of 15.5 kD.

We further tested the anticellular activity of the fusion protein IFN-γ–TNF-β1 on ME-180, a human cervical cancer cell line. This cell line is sensitive to both IFN-γ and TNF and hypersensitive to a combination of these lymphokines. The anticellular effect of the hybrid IFN-γ–TNF-β1 protein is greater than that of either lymphokine by itself or the combined effect of both lymphokines at lower levels (Fig. 3).

In both pγTNF-β1 and pγTNF-β2, the first 23 amino acids of TNF were removed. However, in pγTNF-β2 the whole sequence of IFN-γ was retained, whereas in pγTNF-β1 the last nine amino acids were deleted. Natural human IFN-γ is processed at the carboxyl terminus (*11*). Six different termini have been identified and all have at least nine residues deleted. Thus this region of the IFN may form a sensitive site for proteolytic cleavage in the bacterial cell. This region contains two tandem arginine residues, and it is possible that this is a target for *E. coli* proteolytic enzymes (*12*).

Combined treatment with IFNs and TNFs results in a synergistic anticellular effect in many tumor cell lines (*1–5*). In ME-180 cells, this synergism may be related to upregulation of the number of receptors for TNFs by IFN-γ (*6, 13*). Epidermal growth factor and recombinant human transforming growth factor–β interfered specifically with the antiproliferation effects of TNFs but not with those of IFN-γ on ME-180 cells; neither growth factor had a substantial protective effect on the synergistic cytotoxicity of TNF and IFN-γ, implying that IFN-γ and TNFs inhibit tumor cell growth by distinct mechanism (*14*). We observed that very low levels of the hybrid IFN-γ–TNF-β protein caused an enhanced cytotoxic effect on ME-180 cells. This intramolecular synergism between the IFN-γ domain and the TNF-β1 domain within the hybrid protein may be different from the synergism between these two separate lymphokines, since the antiproliferative activity of IFN-γ–TNF-β was even higher than that of a combination of IFN-γ and TNF-β at lower levels (Fig. 3). At this time we do not know the exact mechanism whereby the hybrid molecule induces this enhanced antiproliferative activity, although this is possibly due to the formation of an unusual complex between the hybrid molecule and the receptors for TNF and IFN-γ on the cell surface. However this enhanced activity suggests a potential for clinical application of the hybrid protein.

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Hormone-Sensitive Lipase: Sequence, Expression, and Chromosomal Localization to 19 cent-q13.3

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Hormone-sensitive lipase, a key enzyme in fatty acid mobilization, overall energy homeostasis, and possibly steroidogenesis, is acutely controlled through reversible phosphorylation by catecholamines and insulin. The 757–amino acid sequence predicted from a cloned rat adipocyte complementary DNA showed no homology with any other known lipase or protein. The activity-controlling phosphorylation site was localized to Ser^{563} in a markedly hydrophilic domain, and a lipid-binding consensus site was tentatively identified. One or several messenger RNA species (3.3, 3.5, or 3.9 kilobases) were expressed in adipose and steroidogenic tissues and heart and skeletal muscle. The human hormone-sensitive lipase gene mapped to chromosome 19 cent-q13.3.

FREE FATTY ACIDS (FFA) DERIVED from adipose tissue triglycerides are the most important fuel in mammals and provide more than half of the caloric needs when dietary energy substrates are lacking. Hormone-sensitive lipase (HSL) has a vital role in the mobilization of FFA from adipose tissue by controlling the rate of lipolysis of the stored triglycerides. Like glycogen phosphorylase, the corresponding enzyme in carbohydrate metabolism, HSL is under acute neuronal and hormonal control.

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In both cases activation by catecholamines occurs through the cyclic adenosine 3′,5′-monophosphate (cAMP)–mediated phosphorylation of a single serine residue (*1*). The dephosphorylation of HSL by insulin is responsible for the antilipolytic effect of this hormone (*1*), one of its most important actions. In addition to its major role in FFA mobilization and in the control of brown adipose tissue thermogenesis (*1*), HSL presumably has an important role in steroidogenesis, since the cholesteryl ester function of this enzyme occurs in steroidogenic tissues (*2*). Furthermore, HSL has been noted in heart and skeletal muscle (*2*), but its role in these tissues is less clear. Thus, although originally believed to reside only in adipose tissue, it has become clear that HSL is a widespread tissue acylglycerol and cholesteryl ester hydrolase that confers hormone sensitivity on this function in many tissues.

The structures of other lipases, including lipoprotein lipase, hepatic lipase, and pancreatic lipase, with key roles in lipoprotein and lipid metabolism, have recently been determined through cloning and sequencing of cDNA from several sources (*3*). These enzymes were all found to belong to the same gene family (*3*). We cloned and sequenced HSL cDNA from rat adipocytes to find out if this was true also for HSL. We also wanted to obtain further insight into the structural, genetic, and regulatory features of HSL, especially with regard to the activity-controlling phosphorylation site, and to examine the possible role of the enzyme in obesity. In addition, we used the cDNA to analyze the expression of HSL mRNA in a variety of rat tissues and to map the human HSL gene to chromosome 19 cent-q13.3.

Rat adipose tissue cDNA for HSL was isolated with two complementary strategies, the first involving antibody screening of an expression library and the second involving amino acid sequencing of bovine HSL followed by construction of an oligonucleotide probe. An amino acid sequence from 13 bovine HSL tryptic peptides was obtained (Fig. 1). These peptides were between 6 and 20 amino acids in length and comprised a total of 126 residues. Based on a 17-residue sequence from one of the peptides (the most NH_2-terminal in Fig. 1) and codon usage tables for mammalian genes (*4*), an oligonucleotide probe of 51 nucleotides (nt) was synthesized. In parallel, an antibody against rat HSL (*5*) was used to screen about 700,000 recombinant phage from a rat adipocyte λgt11 cDNA library. The cDNA from three overlapping clones was subcloned into M13 phage vectors for sequence analysis (*6*). These clones comprised a total of 1.5 kb and did not cross-hybridize with the 51-nt probe. They contained nucleotide sequences coding for three peptides that are between 67 and 82% homologous to three of the bovine peptides (the three most COOH-terminal in Fig. 1), thus verifying the identity of these cDNA clones. In addition, these clones contained sequence coding for a stretch of 27 consecutive amino acids, which is highly homologous to a recently reported amino acid sequence surrounding the regulatory phosphorylation site of bovine adipose tissue HSL (*7*) (Fig. 1). The remainder of the HSL cDNA was obtained by rescreening 800,000 recombinant phage from the same library with the 51-nt probe. Two overlapping clones containing a sequence from cDNAs identified with the antibody plus additional sequence were obtained. The amino acid sequence of rat HSL predicted from the cDNA sequence (*8*) is shown in Fig. 1. All but one of the 13 bovine HSL tryptic peptides were located within the predicted rat HSL amino acid sequence and showed an overall sequence identity of 80%.

The cDNA size of 3225 nt is in agreement with the 3300-nt rat adipose tissue mRNA derived from RNA blot analysis (Fig. 2). Thus, it is likely that this represents the full-length HSL cDNA sequence. We propose that the ATG starting at nt 616 is the translation initiation codon, because it en-

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1   Met Asp Leu Arg Thr Met Thr Gln Ser Leu Val Ala Leu Ala Glu Asp Asn Met Ala Phe Phe Ser Ser Gln Gly Pro Gly Glu Thr Ala
31  Arg Arg Leu Ser Asn Val Phe Ala Gly Val Arg Glu Gln Ala Leu Gly Leu Glu Pro Thr Leu Gly Gln Leu Leu Gly Val Ala His His
61  Phe Asp Leu Asp Thr Glu Thr Pro Ala Asn Gly Tyr Arg Ser Leu Val His Thr Ala Arg Cys Cys Leu Ala His Leu Leu His Lys Ser
91  Arg Tyr Val Ala Ser Asn Arg Arg Ser Ile Phe Phe Arg Ala Ser His Asn Leu Ala Glu Leu Glu Ala Tyr Leu Ala Ala Leu Thr Gln
121 Leu Arg Ala Leu Ala Tyr Tyr Ala Gln Arg Leu Leu Thr Ile Asn Arg Pro Gly Val Leu Phe Phe Glu Gly Asp Glu Gly Leu Ser Ala
151 Asp Phe Leu Gln Asp Tyr Val Thr Leu His Lys Gly Cys Phe Tyr Gly Arg Cys Leu Gly Phe Gln Phe Thr Pro Ala Ile Arg Pro Phe
181 Leu Gln Thr Leu Ser Ile Gly Leu Val Ser Phe Gly Glu His Tyr Lys Arg Asn Glu Thr Gly Leu Ser Val Thr Ala Ser Ser Leu Phe
211 Thr Gly Gly Arg Phe Ala Ile Asp Pro Glu Leu Arg Gly Ala Glu Phe Glu Arg Ile Ile Gln Asn Leu Asp Val His Phe Trp Lys Ala
241 Phe Trp Asn Ile Thr Glu Ile Glu Val Leu Ser Ser Leu Ala Asn Met Ala Ser Thr Thr Val Arg Val Ser Arg Leu Leu Ser Leu Pro
271 Pro Glu Ala Phe Glu Met Pro Leu Thr Ser Asp Pro Lys Leu Thr Val Thr Ile Ser Pro Pro Leu Ala His Thr Gly Pro Gly Pro Val
301 Leu Ala Arg Leu Ile Ser Tyr Asp Leu Arg Glu Gly Gln Asp Ser Lys Met Leu Asn Ser Leu Ala Lys Ser Glu Gly Pro Arg Leu Glu
331 Leu Arg Pro Arg Pro Gln Gln Ala Pro Arg Ser Arg Ala Leu Val Val His Ile His Gly Gly Gly Phe Val Ala Gln Thr Ser Lys Ser
361 His Glu Pro Tyr Leu Lys Asn Trp Ala Gln Glu Leu Gly Val Pro Ile Ile Ser Ile Asp Tyr Ser Leu Ala Pro Glu Ala Pro Phe Pro
391 Arg Ala Leu Glu Glu Cys Phe Phe Ala Tyr Cys Trp Ala Val Lys His Cys Glu Leu Leu Gly Ser Thr Gly Glu Arg Ile Cys Leu Ala
421 Gly Asp Ser Ala Gly Gly Asn Leu Cys Ile Thr Val Ser Leu Arg Ala Ala Ala Tyr Gly Val Arg Val Pro Asp Gly Ile Met Ala Ala
451 Tyr Pro Val Thr Thr Leu Gln Ser Ser Ala Ser Pro Ser Arg Leu Leu Ser Leu Met Asp Pro Leu Leu Pro Leu Ser Val Leu Ser Lys
481 Cys Val Ser Ala Tyr Ser Gly Thr Glu Thr Glu Asp His Phe Asp Ser Asp Gln Lys Ala Leu Gly Val Met Gly Leu Val Gln Arg Asp
511 Thr Ser Leu Phe Leu Arg Asp Leu Arg Leu Gly Ala Ser Ser Trp Leu Asn Ser Phe Leu Glu Leu Ser Gly Arg Lys Pro His Lys Thr
541 Pro Val Ala Cys Asn Arg Asp Thr Ala Pro His Gly Phe Trp Ala Leu Thr Glu Ser Met Arg Arg Ser Val Ser Glu Ala Ala Leu Ala
571 Gln Pro Glu Gly Leu Leu Gly Thr Asp Ser Leu Lys Lys Leu Thr Ile Lys Asp Leu Ser Phe Lys Gly Asn Ser Glu Pro Ser Asp Ser
601 Pro Glu Met Ser Gln Ser Met Glu Thr Leu Gly Pro Ser Thr Pro Ser Asp Val Asn Phe Phe Leu Arg Ser Gly Asn Ser Gln Glu Glu
631 Ala Glu Thr Arg Asp Asp Ile Ser Pro Met Asp Gly Ile Pro Arg Val Arg Ala Ala Phe Pro Asp Gly Phe His Pro Arg Arg Ser Ser
661 Gln Gly Val Leu His Met Pro Leu Tyr Ser Ser Pro Ile Val Lys Asn Pro Phe Met Ser Pro Leu Leu Ala Pro Asp Val Met Leu Lys
691 Thr Leu Pro Pro Val His Leu Val Ala Cys Ala Leu Asp Pro Met Leu Asp Asp Ser Val Met Phe Ala Arg Arg Leu Lys Asp Leu Gly
721 Gln Pro Val Thr Leu Lys Val Val Glu Asp Leu Pro His Gly Phe Leu Ser Leu Ala Ala Leu Cys Arg Glu Thr Gly Arg Pro Arg Ser
751 Cys Ala Cys Ser Ala Ser Gly
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Fig. 1. Amino acid sequence of rat hormone-sensitive lipase predicted from the cDNA sequence. The position of bovine HSL tryptic peptides, obtained by microsequencing, are designated by underlining. Each asterisk indicates identity between rat and bovine residues. The actual bovine peptide sequences, in the single letter code, follow in the order they occur in the figure: TMTQSLVTLAEDDMAFF; DQAFGLF; SLVHTA; YVA(E)X(R); (E)(S)(T)(N)(N)(A)(E)(L)(E)A(Y)L(A)ALTQQ; ALAYCAQR; LLTTNQPGR; NETGISVTAAS; (L)(L)(S)(L)(P)(P)(V)(A)FEMPLTSDPELT; LLSLMDPLL GA; ALGVMGVQR; GLGQPVTLLR (*17*). Those amino acid residues with questionable assignments in the protein sequencing are in parentheses. The regulatory phosphorylation region as described by Garton *et al.* (*7*) is boxed, and the serine phosphorylated by cAMP-dependent protein kinase is indicated by a closed circle. A possible lipid binding region is overlined. Bovine adipose tissue HSL was purified as described (*18*). Peptides obtained after trypsin-digestion and fractionation were subjected to automated chemical degradation on a gas-phase microsequencer (Edman) (*19*). A rat adipocyte λgt11 cDNA library (Clontech) was screened with an antibody against rat HSL (*5*) and an ^{125}I-labeled protein A. Additional HSL clones were obtained by screening with a 51-nt probe, synthesized on the basis of one of the larger bovine HSL tryptic peptides (most NH_2-terminal peptide) and codon usage tables for mammalian genes (*4*). Filters were hybridized in 0.75*M* NaCl, 75 m*M* trisodium citrate, 50 m*M* sodium phosphate (*p*H 7.0), 5× concentrated Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin), 0.1% SDS, and denatured salmon sperm DNA (150 μg/ml) at 45°C overnight and washed twice (20 min each wash) in 300 m*M* NaCl, 30 m*M* trisodium citrate, 0.1% SDS at 47°C, and exposed to x-ray film. DNA from positive clones was purified and subcloned into M13 phage vectors, and both strands were sequenced by the dideoxy–chain termination method (*6*). Shown is the predicted amino acid sequence derived from the sequence of this cDNA (*8*).

codes the only methionine in an open reading frame before the first identified peptide. In addition, it is the only methionine codon upstream of the first peptide that is within the most optimal consensus sequence for initiation by eukaryotic ribosomes [AXX-ATGG (*9*)].

The predicted primary translation product is 757 amino acids in length and is 82,820 daltons in size. These data are consistent with the apparent size in SDS-polyacrylamide gels of 84,000 daltons (*1*). A computer search of the GenBank and the National Biomedical Research Foundation libraries failed to reveal any significant homology at either the nucleic acid or protein level between HSL and any other protein of known sequence. In particular, HSL shares no homology with either the members of the lipase gene family (lipoprotein lipase, hepatic lipase, and pancreatic lipase) (*3*) or any other sequenced lipase. This is despite the fact that HSL shares similar biochemical and functional features with at least some of these enzymes. For example, both HSL and lipoprotein lipase hydrolyze long-chain triacylglycerols, have similar positional specificity, are inhibited by serine-directed reagents, and probably exist as dimers (*1, 3*). On the other hand, HSL exhibits several unique properties. The most remarkable of these is its rapid activation on phosphorylation, which in vivo can be as much as a 50-fold increase, and its ability to hydrolyze cholesteryl esters (*1*).

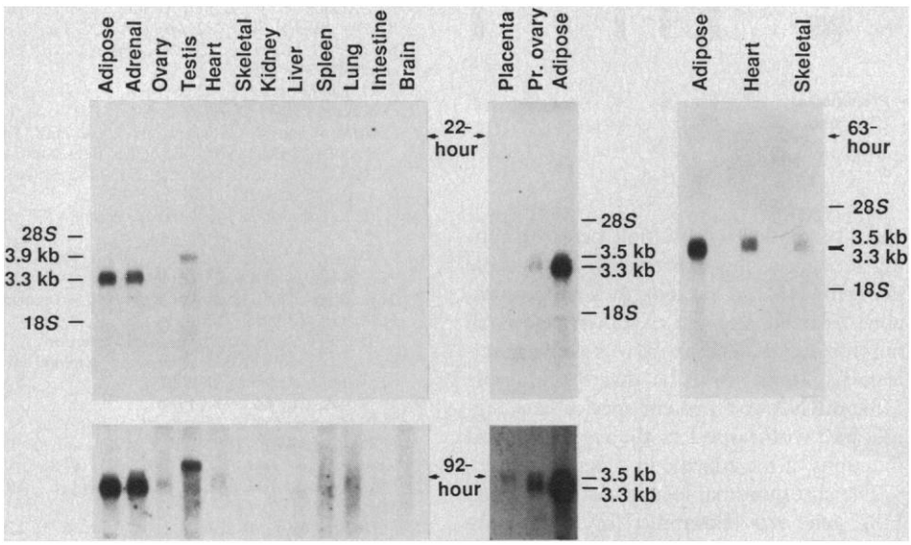

Fig. 2. RNA blot analysis of rat tissue RNAs. Total RNA (10 μg) from all tissues, except adrenal glands [1 μg poly(A)$^+$ RNA], and heart and skeletal muscle in right panel [5 μg polyadenylated poly(A)$^+$ RNA], was analyzed by electrophoresis in a 1% agarose, 2.2 *M* formaldehyde gel, transferred to nylon blots, and cross-linked by exposure to ultraviolet light (*20*) (left and middle panel) or transferred to nitrocellulose and immobilized by baking in vacuum at 80°C for 2 hours (right panel). Blots were probed with either a 1.1-kb (left panel) or a 2.0-kb (middle and right panels) ^{32}P-labeled HSL cDNA (approximately 10^9 cpm/μg) in 0.5*M* sodium phosphate (*p*H 7.0), 7% SDS, 1% bovine serum albumin, and 1 m*M* EDTA at 60°C (nylon blots), or in 0.75*M* NaCl, 75 m*M* trisodium citrate, 50 m*M* sodium phosphate (*p*H 7.0), 5× concentrated Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin), 50% formamide, denatured salmon sperm DNA (150 μg/ml) at 42°C (nitrocellulose blot). The filters were washed twice (20 min each wash) in 15 m*M* NaCl, 1.5 m*M* trisodium citrate, and 0.1% SDS at 60°C. RNA sources are as indicated; pr. ovaries are ovaries obtained from pregnant animals. The inserts at the bottom of left and middle panel show a portion of the same filters at a longer exposure in order to visualize weak signals. Exposure times are given on the figure.

The cAMP-dependent protein kinase phosphorylation site (regulatory site) of HSL (*7*) is in the COOH-terminal third of the molecule, located at residue 563. This site is predicted to be within a hydrophilic α helix, according to the algorithms of Hopp and Woods and Chou and Fasman (*10*). This is consistent with a position at the surface of the molecule and thus accessible to the protein kinase. The presence of one or more functional sulfhydryl groups in the HSL molecule has previously been proposed based on inhibition of activity with cysteine-directed reagents (*1*). However, it is unclear which of the cysteine residues participate in the formation of disulfide bonds. It has been proposed that the disulfide linkages of pancreatic lipase are conserved in lipoprotein lipase and hepatic lipase (*3*). However, putative disulfide bonds in HSL do not show any similarity to this pattern. Analysis by the method of Kyte and Doolittle (*11*) predicts several relatively hydrophobic regions in HSL that could represent lipid binding regions. One of them includes a sequence that corresponds to a weak consensus sequence for lipid binding regions (G-X-S-X-G preceded by four hydrophobic residues), found in pancreatic lipase, lecithin-cholesterol acyltransferase, lingual lipase, gastric lipase (*12*), and hepatic

Table 1. Distribution of human HSL gene with human chromosomes in human-mouse cell hybrids. The first symbol within the parentheses indicates those hybrids that were either positive (+) or negative (−) for the HSL gene as determined by the presence or absence of the human band in a DNA blot analysis of hybrid genomic DNA. The second symbol within the parentheses indicates hybrids that contained (+) or lacked (−) the chromosome. A panel of 17 mouse-human somatic cell hybrids was derived from the fusion of thymidine kinase–deficient mouse cells and normal human male fibroblasts, and genomic DNA was isolated from these hybrid clones (*14*). Genomic DNA from the parental cell lines and hybrid clones was digested with the restriction enzyme Eco RI, and each sample (6 μg) was electrophoresed in 1% agarose gels, transferred to nylon, and cross-linked by exposure to ultraviolet light (*17*). HSL cDNA was labeled with ^{32}P to approximately 10^9 cpm per microgram of DNA by oligolabeling. Hybridization was perfomed in 0.5*M* sodium phosphate (*p*H 7.0), 7% SDS, 1% bovine serum albumin, and 1 m*M* EDTA at 65°C. The filters were washed twice in 15 m*M* NaCl, 1.5 m*M* trisodium citrate, and 0.1% SDS at 60°C. The human HSL gene sequences, present in fragments of 7.7 and 14 kb, could be easily distinguished from the mouse sequences, present in fragments of 2.9 and 6.2 kb.

	Chromosome number																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Concordant hybrids																								
(+/+)	5	4	5	6	4	7	6	7	0	5	2	3	5	7	6	2	7	5	7	6	2	5	1	2
(−/−)	10	8	4	4	5	3	4	3	10	6	5	1	7	4	6	9	0	2	10	3	3	7	9	9
Discordant hybrids																								
(+/−)	2	3	2	1	3	0	1	0	7	2	5	4	2	0	1	5	0	5	0	1	5	2	6	5
(−/+)	0	2	6	6	5	7	6	7	0	4	5	9	3	6	4	1	10	5	0	7	7	3	1	1
Discordancy (%)	12	29	47	41	47	41	41	41	41	35	59	76	29	35	29	35	59	59	0	47	71	29	41	35

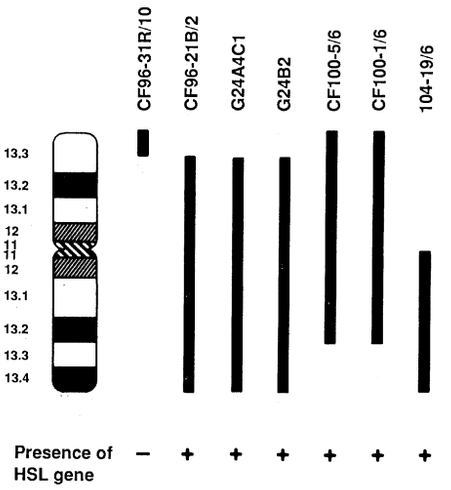

Fig. 3. Regional mapping of the HSL gene on human chromosome 19. The remaining part of the deleted chromosome 19 is noted for each of seven somatic cell hybrids used for regional mapping of HSL. The portions of chromosome 19 contained in the hybrids are: CF96-31R/10: pter-p13.3; CF96-21B/2: qter-p13.3; G24A4C1 and G24B2: qter-p13.3; CF100-5/6 and CF100-1/6: pter-q13.3; 104-19/6: cent-qter. The presence (+) or absence (–) of the human HSL gene in each hybrid, as determined by DNA blot analysis, is noted at the bottom. Clones CF100-5/6 and CF100-1/6 were derived from fusion of mouse A9 cells and human fibroblasts containing a chromosome X/19 translocation (GM 0089, Human Mutant Cell Repository, Camden, New Jersey). Clones G24A4C1 and G24B2 are chromosome X/19 translocations (*21*). Clones CF96-31R/10 and CF96-21B/2 are chromosome 17/19 translocations (*21*), and clone 104-19/6 is a chromosome 1/19 translocation (*14*). DNA from these clones was prepared and hybridization was performed as described in Table 1.

lipase (*3*). The HSL protein has a predicted α helix and β sheet content of 38 and 26% (*10*), respectively. However, none of the α helices or β sheets are of apparent amphipathic nature. On the basis of detergent studies, it has been speculated that HSL is an intrinsic membrane protein (*13*). However, its predicted amino acid sequence does not appear to contain a membrane-spanning region.

RNA blot analysis (Fig. 2) showed that HSL mRNA is present in a variety of tissues, including adipose tissue, adrenal glands, ovaries, testes, and placenta, and heart and skeletal muscle. Although it is not found in ovaries from nonpregnant rats, HSL mRNA was detected in ovaries from pregnant animals, presumably due to the presence of corpora lutea vera, which are highly active in the production of progesterone. Also, in heart and skeletal muscle HSL, mRNA was not detected in total RNA but a signal was visible in poly(A)$^+$ RNA. This is not surprising because the amount of HSL is low in muscle tissues (less than 2% of that in adipose tissue) (*2*). Three distinct mRNA species were observed in the tissues examined, and these species exhibited a tissue-specific distribution. Adipose tissue and adrenal glands express a single 3.3-kb species; heart muscle, skeletal muscle, and placenta express a 3.5-kb species; ovaries express a 3.3- and a 3.5-kb species; and testes express another mRNA species of 3.9 kb. The presence of a different size mRNA in testes is consistent with a previous study in which we observed three immunoreactive proteins of about 86, 110, and 130 kD in testes (*2*). Any or all of these three proteins could be coded for by a 3.9-kb mRNA. It is possible that the different size mRNAs arise either through alternative splicing, use of multiple polyadenylation signals, or multiple transcription start sites. On the other hand, they could be products of multiple genes exhibiting tissue-specific expression. If multiple genes for HSL are present, they are presumably clustered, because as shown below, all hybridizing sequences map to a single genetic locus. It may well be that the multiple HSL mRNA and protein species have significance with respect to the regulation and functions of the enzyme in different tissues.

The chromosomal location of the human HSL gene was determined by hybridizing DNA from a panel of mouse-human somatic cell hybrids to HSL cDNA. Restriction fragments corresponding to the human gene segregated with chromosome 19, while all other chromosomes exhibited multiple discordancies (Table 1), indicating that the HSL gene resides on chromosome 19. Examination of hybrid clones containing various translocations of human chromosome 19 by DNA blotting indicated that the HSL gene is located in the region from the centromere to band q13.3 (Fig. 3). No other lipases have been mapped to this location, including hepatic lipase (located on chromosome 15) and lipoprotein lipase (located on chromosome 8). Although several other genes for proteins involved in lipid metabolism reside on chromosome 19, including those for apolipoproteins CI, CII, and E (q12), the low-density lipoprotein receptor (p13.1-p13.2), and the insulin receptor (p13.2-p13.3) (*13*), this is unlikely to have any functional or regulatory significance. Although several familial disorders have been mapped to chromosome 19 (*14*), none appear likely to involve HSL. Variations in the expression or structure of HSL may be related to the development of obesity of metabolic origin, the rare syndrome of lipodystrophy (*15*), and lipomatosis, a disease characterized by formation of lipomas that are defective in adrenergic-stimulated lipolysis (*16*).

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17. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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