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42. We thank W. Terry for his continued support, C. Puckett for brain mRNA, and N. Gravely for expert and congenial secretarial assistance. Supported in part by grants HL23348 and AG04807 to T.M. and HL35627 to T.M. and W.N.D.

5 March 1986; accepted 13 May 1986

Nucleotide Sequence of a Bovine Clone Encoding the Angiogenic Protein, Basic Fibroblast Growth Factor

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Basic and acidic fibroblast growth factors (FGF's) are potent mitogens for capillary endothelial cells in vitro, stimulate angiogenesis in vivo, and may participate in tissue repair. An oligonucleotide probe for bovine basic FGF was designed from the nucleotide sequence of the amino-terminal exon of bovine acidic FGF, taking into account the 55 percent amino acid sequence homology between the two factors. With this oligonucleotide probe, a full length complementary DNA for basic FGF was isolated from bovine pituitary. Basic FGF in bovine hypothalamus was shown to be encoded by a single 5.0-kilobase messenger RNA; in a human hepatoma cell line, both 4.6- and 2.2-kilobase basic FGF messenger RNA's were present. Both growth factors seem to be synthesized with short amino-terminal extensions that are not found on the isolated forms for which the amino acid sequences have been determined. Neither basic nor acidic FGF has a classic signal peptide.

BASIC FIBROBLAST GROWTH FACTOR (FGF) stimulates the proliferation in vitro of a wide range of mesoderm-derived cells and, in particular, is a potent mitogen for vascular endothelial cells (*1*). It has been purified from various tissues, including pituitary, brain, hypothalamus, retina, adrenal gland, corpus luteum, and kidney (*2*). A second endothelial cell mitogen, acidic FGF, is structurally and functionally related to basic FGF but has only been detected in neural tissues such as brain, hypothalamus, and retina (*2*). Both of these growth factors have a high affinity for the glycosaminoglycan heparin (*3*) and are probably identical to several other heparin-binding endothelial cell growth factors (*4*).

In addition to being mitogenic for the same types of mesoderm-derived cells, both basic and acidic FGF also stimulate new capillary growth (angiogenesis) in the chick chorioallantoic membrane and the rabbit cornea (*3, 5, 6*). Although the precise physiological role of the FGF's has not been established, they could promote the angiogenesis that accompanies the development of the corpus luteum, placenta, and fetus and may be involved in tissue repair. Also, the probable identity of basic FGF with tumor angiogenesis factor (*3*) implies an involvement with the neovascularization associated with the rapid growth of solid tumors.

The complete amino acid sequences of the bovine basic (*7*) and acidic (*8*) FGF's have been established. Basic FGF has 146 amino acids, although a form lacking the amino-terminal 15 amino acids has full mitogenic activity (*2*). Acidic FGF has 140 amino acids, and a form lacking the amino-terminal 6 amino acids is also biologically active. The two FGF's have a 55 percent amino acid sequence homology; slight homologies have also been detected between the FGF's and interleukin-1, and between a region of acidic FGF and neuropeptides such as neuromedin C, bombesin, substance P, and substance K (*6, 8*).

To clone the genes encoding acidic and basic FGF, we initially isolated a DNA fragment encoding part of the acidic FGF protein. Unique sequence oligonucleotide hybridization probes corresponding to amino acids 1 to 16 and 18 to 34 of bovine acidic FGF (*8*) were designed (Fig. 1), taking into account (i) the codons found in other sequenced bovine genes and (ii) the underrepresentation of the dinucleotide CpG in mammalian DNA (*9*). A bovine genomic library was screened in duplicate with these probes, and phages that hybridized to both probes were rescreened with a short, degenerate oligonucleotide probe corresponding to amino acids 7 to 12. Two phages, λBA2 and λBA3, hybridized to all three probes; these phages were purified and shown by restriction enzyme analysis to contain overlapping genomic sequences (*10*). A 250–base pair (bp) Alu I fragment was subcloned from λBA2, and its nucleotide sequence was determined (Fig. 1). An open translational reading frame, encoding the first 41 amino acids of bovine acidic FGF, could be identified within this sequence. Codon 42 is interrupted by an intron (*10*).

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                    AGCTGCTGAGCCATGGCTGAAGGAGAAACCACGACCTTCACGGCCCTGACTGAGAAG
                    AluI
TTT AAC CTG CCT CTA GGC AAT TAC AAG AAG CCC AAG CTC CTC TAC TGC AGC AAC
Phe Asn Leu Pro Leu Gly Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser Asn
 1                                   10
GGG GGC TAC TTC CTG AGA ATC CTC CCA GAT GGC ACA GTG GAT GGG ACG AAG GAC
Gly Gly Tyr Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr Lys Asp
      20                                  30
AGG AGC GAC CAG CAC A GTAAGCACCCATCTCACATTTCTGGTATCTTCCTTACTCAGGGACAGGA
Arg Ser Asp Gln His
               40
GAAGGGAGAATAGGGAGAATAGCT
                        AluI
```

Fig. 1. Nucleotide sequence of the 250-bp Alu I fragment of bovine genomic DNA that encodes amino acids 1 to 41 of acidic FGF. The GT of the consensus donor splice site within codon 42 is underlined, as are the TGA and ATG codons discussed in the text. Two codon choice hybridization probes, (i) 5′-GCAGTACAGCAGCTTGGGCTTCTTGTAGTTGCCCAGGGGCAGGTTGAA-3′ and (ii) 5′-GGTGCCATCCACAGTGCCATCAGGCAGGATGCTCAGGAAGTAGCAGCCATT-3′, were designed that corresponded to the complement of the sequence encoding amino acids 1 to 16 and 18 to 34, respectively. The oligonucleotides were labeled with ^{32}P with polynucleotide kinase and used to screen a bovine genomic library (*18*), constructed as a Sau 3A partial digest in bacteriophage λ Charon 28. Following hybridization under conditions previously described (*19*), the filters were washed twice at room temperature in 1× standard sodium citrate (SSC) and 0.1% sodium dodecyl sulfate (SDS), and once for 10 minutes in the same buffer at 55°C. Recombinant phages that hybridized to probes (i) and (ii) were rescreened with a ^{32}P-labeled probe (iii) 5′-TTNGCCTTCTTGTAGTT-3′, a 64-fold degenerate, 17-nucleotide sequence corresponding to the complement of the sequence encoding amino acids 7 to 12. The tetramethylammonium chloride wash procedure (*20*) was used with a final wash temperature of 50°C. DNA from phage λBA2 that hybridized to all three probes was digested with Alu I and "shotgun-cloned" into an Sma I–digested M13 vector (*21*). Recombinants that hybridized to probes (ii) and (iii) were sequenced by the chain termination method (*22*).

To clone basic FGF, we used the homology between amino acids 18 to 31 of basic FGF and amino acids 9 to 22 of acidic FGF (*7, 8*) to design a new 40-base oligonucleotide probe (Fig. 2A). Where the two amino acid sequences are identical, the probe contains the same codon choice as that found in the acidic FGF genomic fragment; at positions where the sequences differ, the probe incorporates the minimum possible number of nucleotide changes needed to allow the amino acid change.

We expected that this oligonucleotide would hybridize to both acidic and basic FGF sequences, but that, at conditions of higher stringency, it would be specific for basic FGF. The appropriate hybridization conditions to obtain this specificity were established through Southern blots of bovine genomic DNA that were washed at increasing stringencies (Fig. 2B). At both 50° and 55°C wash temperatures, a number of hybridizing fragments were detected, including those known from an analysis of clones λBA2 and λBA3 to correspond to acidic FGF (*10*). At a 65°C wash temperature, these acidic FGF fragments were no longer detectable, and the remaining 3.5-kilobase (kb) Pst I and 10.0-kb Eco RI fragments presumably corresponded to basic FGF.

We constructed a bovine pituitary complementary DNA (cDNA) library of approximately 10^6 independent recombinants in bacteriophage λgt10 (*11*), and screened it with the probe shown in Fig. 2 using a 65°C wash temperature. A single hybridizing recombinant, λBB2, was isolated and shown by DNA sequence analysis to be a cDNA clone of 2122 bp encoding bovine basic FGF (Fig. 3).

The nucleotide sequence corresponding to the published 146 amino acid sequence of bovine basic FGF can be identified in λBB2. Basic FGF does not appear to be synthesized initially as a precursor with an extended carboxyl terminus because a termination codon directly follows the codon for amino acid 146. The 3′-untranslated region has 1554 nucleotides and contains neither a polyadenylation recognition signal nor a polyadenylate tail, suggesting that the cDNA clone is incomplete at the 3′ end.

The nucleotide sequence of λBB2 has an open reading frame extending upstream from the codon for the amino-terminal proline of mature basic FGF, and continuing to the end of the cDNA clone. Thus, basic FGF may be synthesized initially as a precursor with a long amino-terminal extension, and the initiator methionine may not be encoded by λBB2. Immunoreactive material with a considerably larger molecular weight has in fact been detected by antibodies to FGF in rat tissues (*12*), but because these assays were performed under nondenaturing conditions the higher molecular weight forms may simply represent aggregates between basic FGF-like material and carrier proteins.

Despite the open reading frame, features present in this upstream region suggest that it may be partly a noncoding sequence. First, an ATG (methionine) codon is found nine amino acids upstream from the proline at position +1 and is in the same translational reading frame as the mature protein (Fig. 3). The sequence surrounding the ATG (GGGCCATGG) is similar to the consensus sequence (*13*) proposed for the start of eukaryotic translation (CCACCATGG). Second, the sequence upstream from the ATG is extremely GC-rich (88 percent of the 103 nucleotides are GC). Such a sequence would encode a very unusual protein, rich in the amino acids alanine, glycine, proline, and arginine, and is, in fact, very reminiscent of the highly GC-rich 5′-untranslated regions found in the transforming growth factor-β, (TGF-β), insulin-like growth factor II (IGFII), platelet-derived growth factor (PDGF) β chain, and c-*myc* cDNA's (*14*).

Additional support for the suggestion that the sequences upstream from this ATG are noncoding sequences comes from a comparison of this region with the corresponding region of the bovine acidic FGF geno-

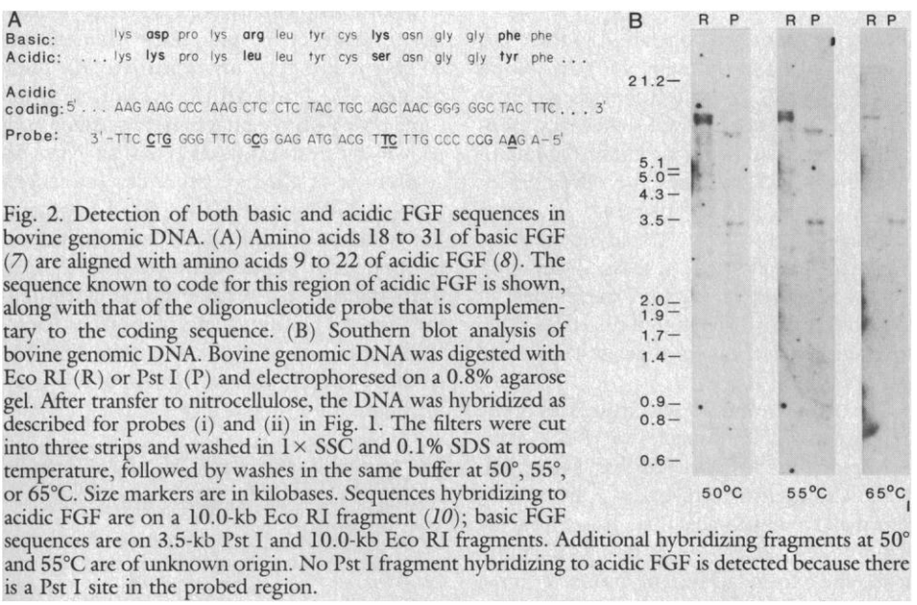

Fig. 2. Detection of both basic and acidic FGF sequences in bovine genomic DNA. (A) Amino acids 18 to 31 of basic FGF (*7*) are aligned with amino acids 9 to 22 of acidic FGF (*8*). The sequence known to code for this region of acidic FGF is shown, along with that of the oligonucleotide probe that is complementary to the coding sequence. (B) Southern blot analysis of bovine genomic DNA. Bovine genomic DNA was digested with Eco RI (R) or Pst I (P) and electrophoresed on a 0.8% agarose gel. After transfer to nitrocellulose, the DNA was hybridized as described for probes (i) and (ii) in Fig. 1. The filters were cut into three strips and washed in 1× SSC and 0.1% SDS at room temperature, followed by washes in the same buffer at 50°, 55°, or 65°C. Size markers are in kilobases. Sequences hybridizing to acidic FGF are on a 10.0-kb Eco RI fragment (*10*); basic FGF sequences are on 3.5-kb Pst I and 10.0-kb Eco RI fragments. Additional hybridizing fragments at 50° and 55°C are of unknown origin. No Pst I fragment hybridizing to acidic FGF is detected because there is a Pst I site in the probed region.

```
1       CCGGGGCCGC GCCGCGGAGC GCGTCGGAGG CCGGGGCCGG GGCGCGGCGG CTCCCCGCGC GGCTCCAGGG GCTCGGGGAC CCCGCCAGGG CCTTGGTGGG GCC

        -9                              -1   1                                       10                                      20
        met ala ala gly ser ile thr thr leu PRO ALA LEU PRO GLU ASP GLY GLY SER GLY ALA PHE PRO PRO GLY HIS PHE LYS ASP PRO LYS
104     ATG GCC GCC GGG AGC ATC ACC ACG CTG CCA GCC CTG CCG GAG GAC GGC GGC AGC GGC GCT TTC CCG CCG GGC CAC TTC AAG GAC CCC AAG

                                        30                                      40                                      50
        ARG LEU TYR CYS LYS ASN GLY GLY PHE PHE LEU ARG ILE HIS PRO ASP GLY ARG VAL ASP GLY VAL ARG GLU LYS SER ASP PRO HIS ILE
194     CGG CTG TAC TGC AAG AAC GGG GGC TTC TTC CTG CGC ATC CAC CCC GAC GGC CGA GTG GAC GGG GTC CGC GAG AAG AGC GAC CCA CAC ATC

                                        60                                      70                                      80
        LYS LEU GLN LEU GLN ALA GLU GLU ARG GLY VAL VAL SER ILE LYS GLY VAL CYS ALA ASN ARG TYR LEU ALA MET LYS GLU ASP GLY ARG
284     AAA CTA CAA CTT CAA GCA GAA GAG AGA GGG GTT GTG TCT ATC AAA GGA GTG TGT GCA AAC CGT TAC CTT GCT ATG AAA GAA GAT GGA AGA

                                        90                                      100                                     110
        LEU LEU ALA SER LYS CYS VAL THR ASP GLU CYS PHE PHE PHE GLU ARG LEU GLU SER ASN ASN TYR ASN THR TYR ARG SER ARG LYS TYR
374     TTA CTA GCT TCT AAA TGT GTT ACA GAC GAG TGT TTC TTT TTT GAA CGA TTG GAG TCT AAT AAC TAC AAT ACT TAC CGG TCA AGG AAA TAC

                                    120                                     130                                     140
        SER SER TRP TYR VAL ALA LEU LYS ARG THR GLY GLN TYR LYS LEU GLY PRO LYS THR GLY PRO GLY GLN LYS ALA ILE LEU PHE LEU PRO
464     TCC AGT TGG TAT GTG GCA CTG AAA CGA ACT GGG CAG TAT AAA CTT GGA CCC AAA ACA GGA CCT GGG CAG AAA GCT ATA CTT TTT CTT CCA

        MET SER ALA LYS SER  *
554     ATG TCT GCT AAG AGC TGA TCTTAATGGC AGCATCTGAT CTCATTTTAC ATGAAGAGGT ATATTTCAGA AATGTGTTAA TGAAAAAAGA AAAATGTGTA CAGTGAG
659     CTG CTCAGTTTGG GTAACTGTTC AGATAACCGT TTATCTAAGA GTAAAATATT TAACCATTGC CTTAGTTTTT TTTTAAAGAA AAAACACAAT AACAGCAAAA ATTCC
767     TGGAA AATGTATACA TTTCCACTTT TTATACAGCA TTTCCTTTTA TCCAGTGAAA CTTACTTAAA GCTACAATCT TTCATACAGT TGCTTCATTT GAAGAGGCTT TTA
875     AAATGTG TACAAACAAG TTTTCTTCAT GGAAATTATA GACATTAGAA AATTAAAGTC ATATTTAGTT ATTAACCCAA ATGTCCACTA CTTCCTATAA TATGGCACAC A
983     TTAATCTAC ATGTACAACT TACTTAAACA TGTACAACTT ACTTAAACAT TTTAAAAACA TGTAAATATG AATTTAATCC ATTCCTGTCA TAGTTTTGTA ATTGTCTGGC
1092    AGTTTCTTGT GATAGAGTTT ATAGAACAAG CCTGTGTAAA CTGCTGGCAG TTCTTCCATG GTCAGATCAA TTTTGTCAAA CCCTTCTTTG TACCCATACA GCAGCAGCC
1201    T TGCAACTCTG CTTGTTATGG GAGTCGTATT TTTAGTCTTG ACTAGATCGC TGAGATTCAT CCACTCACAC TTTAAGCATT CACGCTGGCA AAAATTTATG GTGAATG
1309    AAT ATGGCTTTAA GCGGCAGATA ATATACATAT CTGACTTCCC AAAAGCTCCT GGATGGGTGT GCTGTTGCCG AATACTCAGG AGGGATCTGA ATTCGGATTT TATAC
1417    CAGTC TCTTCAAAAA CTTCTCGAAC TGCTGTATCT CCTACATAAA AGAAAATGTA CAAATCAATA ACGATTATAC TTTTAGAAAT TTAATCAAAG ATTTTCAGAT AAG
1525    GAAGCAT TATTATGTAA AGATTCAAAA GGTAAAAATT TACCCTAAGA AAAGAAAGCT TTCCCTGTAA ACTCTGTCCT CTGGACATTC TGAAAAAACA AAGTATTTTC T
1633    TACCACTGT ATAGCTAAGA AGCTTTTGAA ATAATATTTC TTTGGCTTCT ACTTGCAAGC TTACCCATCT ATATATATGT ATTTTGGGAG TCACATATTT TTAAATTCTT
1742    CCTGCTTTAT TTCCCAAAAG TTAATATTCC TGTATATTTT TTCATTATTA TCTTGTTCCT GATTATCCAT TAAAACTGCC TAAACTGATA AACATTTGAA GTAAGAAAA
1851    A GTGATCCATT CTTCTTTACA AAAGTCTGTA GAGCTGCAGA ATATATAGAA CTAGGAAATG ATTCAAATCA TCCCTGGTCT CTCCTGGGAC TGTCAGGCCT CTGAAGT
1959    CAT AGGTCGGATT TCGTTATAAC CATTTTGTTA TGCTCTTCTA GTTATTCTGT CAGTGGAATC CCACCATGGT AATTTCTGGC ATTTTCTTTG TTTCTTGCTG TTTCA
2067    AAGAA CTTGGATTCA TTCTTCTAAC ACCAAAATGC TACAGTCATC AGAAGTTTAA A
```

Fig. 3. Nucleotide sequence of the bovine basic FGF cDNA clone, λBB2, and the amino acid sequence of the mature protein, numbered from 1 to 146. The amino acid sequence of the proposed amino-terminal extension (−9 to −1) is shown in lower case. The oligonucleotide described in Fig. 2A was labeled with ^{32}P with polynucleotide kinase and used to screen a cDNA library made in λgt10 from bovine pituitary mRNA. After hybridization under conditions previously described (*19*), the filters were washed twice at room temperature in 1× SSC and 0.1% SDS, and once for 10 minutes in the same buffer at 65°C. The single hybridizing recombinant, λBB2, was sequenced by the chain termination method (*22*) following subcloning of fragments into M13 vectors (*21*).

mic clone (Fig. 1). When the two encoded amino acid sequences are aligned, allowing a three amino acid gap in the acidic FGF sequence, the sequence homology between the two FGF's (*8*) extends upstream (Fig. 4). The potential initiator methionine in the basic FGF encoded sequence aligns exactly with a methionine in the acidic sequence; this latter ATG is also found within a sequence (GAGCCATGG) that is similar to the consensus sequence. In the case of acidic FGF, there is an in-frame termination codon (TGA) two codons upstream from the ATG (Fig. 1). Upstream from the ATG's, the two sequences diverge completely. Use of the proposed initiation codon in the basic FGF sequence would result in a primary translation product of 155 amino acids, which is consistent with the recent reports that a slightly higher molecular weight form of the protein has been isolated from a human hepatoma cell line (SK-HEP1) (*15*).

```
        -9       ↓+1              ↓+16
Basic   MAAGSITTLPALPEDGGSGAFPPGHFKDPK...
        ** *  **  ** *        * *  * **
Acidic  MAEGETTTFTALTEK___FNLPLGNYKKPK...
        -15               ↑+1    ↑+7
```

Fig. 4. Comparison of the amino-terminal sequences encoded by the bovine basic and acidic FGF clones. Two forms of basic FGF, with amino termini at amino acids +1 and +16, and two forms of acidic FGF, with amino termini at amino acids +1 and +7, have been purified from tissue and sequenced (*2, 7, 8*). Negative numbers refer to the proposed amino-terminal extensions discussed in the text.

Because both FGF's are growth factors that function extracellularly, they would be expected to have signal peptides to direct their secretion. The region of basic FGF from amino acids −9 to 4 (Fig. 4) has the appearance of a hydrophobic signal peptide core sequence (*16*), but there is no evidence from the sequences of the purified forms of basic FGF that signal peptidase cleavage at the appropriate position relative to this core actually occurs. The corresponding region of acidic FGF (Fig. 4) is not sufficiently hydrophobic to form a signal peptide core. In this respect, therefore, the two FGF's may be like the two forms of interleukin-1, which also have no clear signal peptide sequence (*17*).

Basic FGF has been isolated from several tissues, but its site of biosynthesis and the size of its messenger RNA (mRNA) have not been established. We therefore did Northern blot analysis of RNA isolated from one of the known tissue sources of basic FGF, bovine hypothalamus, using the bovine basic FGF cDNA as a hybridization probe (Fig. 5, lane 1). There was a single hybridizing mRNA of approximately 4.6 kb, indicating that one or more cell types within the hypothalamus synthesize basic FGF.

The similarity in heparin-binding affinity, amino acid composition, and mobility on reversed-phase high performance liquid chromatography between basic FGF and the endothelial cell mitogen purified from the human hepatoma cell line SK-HEP1 (*15*) suggests that these two factors are identical. If this suggestion is correct, then the SK-HEP1 cell line should synthesize basic FGF mRNA. In fact, mRNA complementary to the bovine basic FGF cDNA is synthesized in these cells (Fig. 5, lane 2). Two species of mRNA were detected—a 4.6-kb mRNA that comigrates with the mRNA from hypothalamus and an additional 2.2-kb mRNA. The availability of a cDNA clone for basic FGF will allow the relation between these two mRNA's to be determined and will also allow precise cellular localization of basic FGF synthesis in normal tissue.

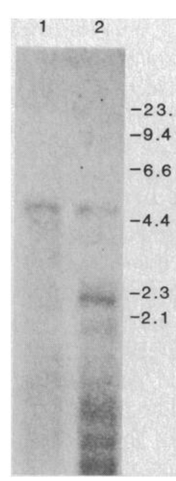

Fig. 5. Northern blot analysis of bovine hypothalamus (lane 1) and human SK-HEP1 (lane 2) mRNA's. Polyadenylated mRNA was isolated from both sources, and 10 μg per lane was fractionated on a 1.2% agarose-formaldehyde gel and transferred to nitrocellulose (*23*). The hybridization probe was a 1.4-kb Eco RI fragment of λBB2 (nucleotides 1 to 1401). Size markers (in kilobases are Hind III-digested λ DNA.

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24. We thank J. Iwasa and T. Scheuermann for synthesizing the oligonucleotides, F. Rottman (Case Western Reserve) for the bovine genomic library, J. Miller for help with the DNA sequencing, B. Greenberg for compiling the bovine codon choice table, E. Stoelting for the artwork, L. Fritz and K. Talmadge for their critical comments on the manuscript, and C. Remington, M. Matsumura, and E. Pond for preparing the manuscript.

10 March 1986; accepted 5 May 1986

A Cusp Singularity in Surfaces That Minimize an Anisotropic Surface Energy

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A mathematical proof shows that a surface with a cusp-shaped singularity can arise from minimizing an anisotropic surface free energy for a portion of a crystal surface. Such cusps have been seen on crystal surfaces but usually have been interpreted as being the result of defects or nonequilibrium crystal growth. Our result predicts that they can occur as equilibrium or near-equilibrium phenomena. It also enriches the mathematical theory of minimal surfaces.

THE MATHEMATICAL MODELING OF shapes that minimize total crystal surface free energy has a long history. Initially, only isotropic fluids (as represented by soap films, for example) were considered (*1*). This led to the mathematical subject of minimal surfaces, which is currently very active (*2*). But from Gibbs (*3*) onward, it has been recognized that the surface free energy per unit area of the surface of a crystal of fixed orientation is a function of the unit normal directions of the surface (*4*). The anisotropy arises from the fact that the atomic structure of the surface can be very different in different unit normal directions.

Unlike liquid drops, crystals can have edges and corners as part of their equilibrium shapes. In our investigation of such singularities (*5*) and their evolution, we encountered a problem that suggested that a cusp-shaped singularity could occur in an energy-minimizing surface. The present report is the mathematical proof that it is so, together with some experimental evidence.

With the orientation of the solid phase or phases fixed in space, and with fixed temperature, pressure, and chemical potentials, the surface free energy per unit area, γ, is a function that maps unit vectors $\mathbf{n}$ to positive numbers. The normals $\mathbf{n}$ are chosen to point from the crystal to the other phase. The nature of both phases determines γ. The other phase can be vapor, liquid, or another crystal, perhaps of the same material but with another orientation. It is convenient to describe γ by means of its Wulff shape (also called its equilibrium crystal shape)

$$W = \{\mathbf{x} \in R^3: \mathbf{x} \cdot \mathbf{n} \leq \gamma(\mathbf{n}) \text{ for each unit vector } \mathbf{n}\} \quad (1)$$

That is, W is the set composed of all the vectors $\mathbf{x}$ in 3-space for which $\mathbf{x} \cdot \mathbf{n} \leq \gamma(\mathbf{n})$. W is the shape of least surface energy for the crystal of fixed volume entirely embedded in the other phase (*6–9*).

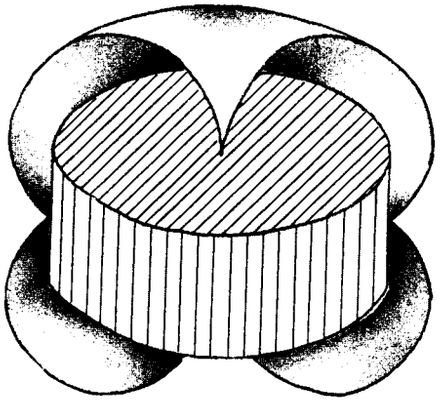

Fig. 1. A cylindrical W. Any γ that satisfies the conditions in expressions 2 through 4 will have this W as its Wulff shape. The back half of the polar plot of one such γ is drawn around W.

The surface free energy function γ that we will use for the examples and proofs below is any function whose Wulff shape is a vertical right circular cylinder (Fig. 1). Normalizing γ if necessary, we can and do assume that

$$\gamma(\mathbf{n}) = 1 \quad (2)$$

for each horizontal unit vector $\mathbf{n} = (n_1, n_2, 0)$ and

$$\gamma((0,0,1)) = \gamma((0,0,-1)) = \gamma_v \quad (3)$$

Since W is a cylinder,

$$\gamma(\mathbf{n}) \geq (1 - n_3^2)^{1/2} + \gamma_v |n_3| \quad (4)$$

for each of the other unit vectors $\mathbf{n} = (n_1, n_2, n_3)$.

Description of a cusp-shaped singularity. Consider a surface S, as in Fig. 2, which consists of a vertical cliff face with a horizon-

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