near the Fermi level (*11*), the bright spots located above the Cu–S bonds in the anion images (Fig. 3) provide direct evidence of an additional modulation in the spatial dependence of the electron density of states. What makes this result surprising is the fact that, although the environment of both Cu atoms that appear within the unit cell is the same (equivalent positions), only one of these S–Cu bonds appears highlighted in the STM images. The presence of an additional modulation in the STM images is indicative of a fluctuation in the atomic arrangement of the unit cell. The nature of this modulation is unknown. Other experimental results that may suggest a possible interpretation are examined below.

Analysis (*12*) of the Shubnikov–de Hass effect on κ-$[ET]^+[Cu(NCS)_2]^-$ (*13*) suggests that the anisotropy of the in-plane conductivity implies the existence of nested states in the Fermi surface and the presence of a charge density wave (CDW). The strongest manifestation of such structural anisotropy in the *bc* plane would come from the anions because the $[(ET)_2]$ dimers arrange perpendicular to one another to form a 2-D network, and therefore an anisotropy in this plane would come from small structural deviations of the 2-D pattern. The polymeric chains formed by the anions, on the other hand, provide a natural 1-D character to the structure and could indeed be responsible for the anisotropy of the electrical conductivity in the *bc* plane. This would give the anions a more important role in the normal conductivity of the material than previously thought. X-ray absorption fine structure results have corroborated this conclusion (*14*), which may be manifested in the fact that the anions are so well resolved in the STM images. If the anions were simply insulating spacers between the ET conduction planes, they would not contribute to the density of states near the Fermi level and the images would be the result of fluctuations in the tunneling barrier height. Because the anion images reflect height corrugations on the order of 1 Å, this would imply barrier height fluctuations of ~1 eV or more (depending on the tip to sample distance) over the $[Cu(NCS)_2]$ atomic distances (*15*).

The existence of a CDW in the material would not be consistent with a modulation that has the same period as the undistorted lattice. We speculate that, if the additional modulation present in the anion images was due to the presence of a CDW commensurate with the lattice as observed in other low-dimensional organic systems (*16*), there should be an additional modulation (with longer period) that the current study does not resolve.

The presence of a CDW in the material with an incommensurate to commensurate transition at 240 K would explain the opening of a gap in the electron density of states near the Fermi level and the transition in the resistance versus temperature to a temperature-activated regime. As the temperature is decreased, the material is known to experience a pronounced contraction in the *c* direction and an expansion in the *a* direction (*3*, *17*). The contraction in the *c* direction would bring the polymeric chains closer together, increasing their interaction and diminishing the 1-D character of the material. These circumstances may cause the projection of the Fermi surface along the *bc* plane to become more circular, the nested states and the CDW to disappear, the gap to close, and the material to become metallic. This model would be consistent with the reported suppression of the anomalous temperature versus resistance behavior by the application of pressure (*18*) and the enhancement of this behavior by the application of tension along the *b* direction (*19*). Low-temperature STM studies are needed to test this hypothesis.

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Interaction Cloning: Identification of a Helix-Loop-Helix Zipper Protein That Interacts with c-Fos

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A facile method for isolating genes that encode interacting proteins has been developed with a polypeptide probe that contains an amino-terminal extension with recognition sites for a monoclonal antibody, a specific endopeptidase, and a site-specific protein kinase. This probe, containing the basic region–leucine zipper dimerization motif of c-Fos, was used to screen a complementary DNA library. A complementary DNA that encoded a member of the basic-helix-loop-helix–zipper (bHLH-Zip) family of proteins was isolated. The complementary DNA–encoded polypeptide FIP (Fos interacting protein) bound to oligonucleotide probes that contained DNA binding motifs for other HLH proteins. When cotransfected with c-Fos, FIP stimulated transcription of an AP-1–responsive promoter.

Many cellular processes involve specific protein interactions. Identification of the interacting proteins can be a prodigious task if one uses conventional biochemical approaches. We have developed a convenient method, involving molecular cloning, that facilitates the detection and isolation of the interacting entity. This method has been used to study the interactions of transcription factors.

The leucine zipper (Zip) and the helix-loop-helix (HLH) dimerization motifs are found in eukaryotic transcription factors that are members of the basic region–Zip (bZip) and bHLH families, respectively. The Zip and HLH domains permit the formation of homodimers or heterodimeric combinations among family members. The Zip domain is characterized by a heptad repeat of leucine residues that form an amphipathic α-helical structure (*1*). When

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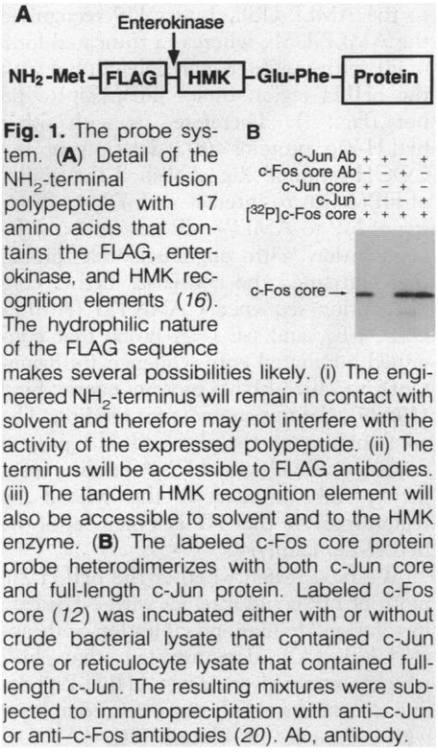

Fig. 1. The probe system. (**A**) Detail of the NH_2-terminal fusion polypeptide with 17 amino acids that contains the FLAG, enterokinase, and HMK recognition elements (*16*). The hydrophilic nature of the FLAG sequence makes several possibilities likely. (i) The engineered NH_2-terminus will remain in contact with solvent and therefore may not interfere with the activity of the expressed polypeptide. (ii) The terminus will be accessible to FLAG antibodies. (iii) The tandem HMK recognition element will also be accessible to solvent and to the HMK enzyme. (**B**) The labeled c-Fos core protein probe heterodimerizes with both c-Jun core and full-length c-Jun protein. Labeled c-Fos core (*12*) was incubated either with or without crude bacterial lysate that contained c-Jun core or reticulocyte lysate that contained full-length c-Jun. The resulting mixtures were subjected to immunoprecipitation with anti–c-Jun or anti–c-Fos antibodies (*20*). Ab, antibody.

aligned in parallel, the hydrophobic faces of two complementary helices form a coiled coil (*2*). When basic amino acid residues located NH_2-terminal to the α helices are juxtaposed, a functional DNA binding element is formed only in the dimer. For example, the mammalian transcription factor AP-1 is a dimer composed of the protein products of the *jun* and *fos* gene families (*3*). Fos-Jun heterodimers bind to a cognate AP-1 site with much greater affinity than Jun homodimers. The c-Fos protein is unable to homodimerize or to bind DNA on its own (*4, 5*).

The interaction motif of the bHLH proteins is composed of two amphipathic α helices separated by a variable loop of amino acids (6). Interactions between hydrophobic residues along faces of these helices are believed to mediate specific interactions between dimer partners. DNA binding by the bHLH proteins requires HLH-dependent dimerization and involves a region of basic amino acids located NH_2-terminal to the first helix in each of the dimer partners (*7*). The bHLH proteins recognize variations on the consensus core sequence CANNTG (N = C, T, A, or G).

An additional class of proteins, termed bHLH-Zip, has a bHLH motif combined with a COOH-terminal leucine zipper. Originally defined by the *myc* family, this class includes both bona fide and putative transcription factors TFE3, USF (upstream stimulatory factor), TFEB, AP-4, and Max (*8, 9*). With the exception of the specific Max-Myc association, intact proteins of this class fail to heterodimerize with other bHLH, bZip, or bHLH-Zip proteins.

We investigated the interaction repertoire of the bZip core of c-Fos. The probe we used included a cleavable isolation element and a detection element (Fig. 1A). In frame with the initiator methionine codon is a recognition site (FLAG) for a commercially available monoclonal antibody (anti-FLAG) and a recognition site for the specific endopeptidase enterokinase (*10*). In frame with the FLAG site is a five–amino acid recognition element for the catalytic domain of heart muscle kinase (HMK) (*11*). Insertion of the cDNA of interest occurs at a unique in frame Eco RI site, which generates the final product Met-[FLAG(enterokinase)]-(HMK)-(protein); these elements facilitate the rapid production, purification, and specific labeling of the expressed polypeptide. After purification, the FLAG sequence can be removed from the remainder of the protein by enzymatic cleavage. Labeling at the HMK site has several advantages over other labeling methods (*12*). The small size of these additions (17 amino acids) makes it likely that this combination should have only a minimal effect on the biochemical activities of the expressed polypeptide.

The DNA segments that encode amino acids 120 to 206 of the rat c-Fos protein (*13*) (c-Fos core) and amino acids 206 to 340 of the human c-Jun protein (*14*) (c-Jun core) each contain the bZip region and retain their ability to heterodimerize and bind to an AP-1 site (*15*). Bacterially produced c-Fos core and c-Jun core proteins (*16, 17*) were detected in the soluble fraction of extracts by anti-FLAG in immunoblot analyses (*10, 18, 19*). The c-Fos core protein that contained the HMK domain could be specifically radiolabeled and immunoprecipitated by antibodies to c-Jun (anti-Jun) in the presence of c-Jun protein (Fig. 1B) (*20, 21*). When incubated with nonradioactive adenosine triphosphate (ATP) and HMK, c-Fos core protein cooperated with c-Jun for DNA binding (*19*).

The ^{32}P-labeled c-Fos core probe was

A

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FIP     -RTPRDERRRAQHNEVERRRRDKINNWIVQLSKIIP----DCNADNSKTGA----SKGGILSKACDYIRELRQTNQRMQETFKEAERLQMDNELLRQQIEELKNENALLRAQLQQHNLEMVGEGTRQ
USF     -RTTRDEKRRAQHNEVERRRRDKINNWIVQLSKIIP----DCSMESTKSGQ----SKGGILSKACDYIQELRQSNHRLSEELQGLDQLQLDNDVLRQQVEDLKNKNLLLRAQLRHHGLEVVIKNDSN
TFE3    -ALLKERQKKDNHNLIERRRRFNINDRIKELGTLIP----KSSDPEMRWN------KGTILKASVDYIRKLQKEQQRSKD-------LESRQRSLEQANRSLQLRIQELELQAQIHGLPVPGTPGLL
TFEB    -ALAKERQKKDNHNLIERRRRFNINDRIKELGMLIP----KANDLDVRWN------KGTILKASVDYIRRMQKDLQKSRE-------LENHSRRLEMTNKQLWLRIQELEMQARVHGLPTTSPSGMN
Max     -EVESDADKRAHHNALERKRRDHIKDSFHSLRDSVP----SLQG--------EKASRAQILDKATEYIQYMRRKNHT----------HQQDIDDLKRQNALLEQQVRALGKARSSAQLQTNYPSSDN
c-Myc   -SDTEENVKRRTHNVLERQRRNELKRSFFALRDQIP----ELEN-------NEKAPKVVILKKATAYILSVQAEEQK----------LISEEDLLRKRREQLKHKLEQLRNSCA
N-Myc   -SDSEDSERRRNHNILERQRRNDLRSSFLTLRDHVP----ELVK-------NEKAAKVVILKKATEYVHSLQAEEHQ----------LLLEKEKLQARQQQLKKIEHARTC
L-Myc   -SDTEDVTKRKNHNFLERKRRNDLRSRFLALRDQVP----TLAS-------CSKAPKVVILSKALEYLQALVGAEKR----------MATEKRQLRCRQQQLQKRIAYLSGY
AP-4    -RDQERRIRREIANSNERRRMQSINAGFQSLKTLIP----HTDG--------EKLSKAAILQQTAEYIFS----------------LEQEKTRLLQQNTQLKRFIQELSGSSPKRRRAEDKDEGIG
HLH     --------RR-----fER-R---fN--w--LK--f----------------K--K--fL--Af-Yf--f
                KK      R K      K       R  T               R     T   TT
                                 R         C
```

Basic region | Helix I | Loop | Helix II | Leucine zipper

B

```
EFHSWRRHVVSTAAFAGGQQAVTQVGVDGVAQRPGPAAAS  40
VPPGPAAPFPLAVIQNPFSNGGSPAAEAVSGEARFAYFPA  80
SSVGDTTAVSVQTTDQSLQAGGQFYVMMTPQDVLQTGTQR 120
TIAPRTHPYSPKIDGTRTPRDERRRAQHNEVERRRRDKIN 160
NWIVQLSKIIPDCNADNSKTGASKGGILSKACDYIRELRQ 200
TNQRMQETFKEAERLQMDNELLRQQIEELKNENALLRAQL 240
QQHNLEMVGEGTRQ                            254
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Fig. 2. (**A**) Comparison of the bHLH-Zip regions of FIP (amino acids 137 to 254) to the bHLH-Zip proteins USF (amino acids 193 to 310), TFE3 (amino acids 133 to 241), TFEB (amino acids 326 to 434), Max (amino acids 8 to 111), c-Myc (amino acids 348 to 439), N-Myc (amino acids 367 to 456), L-Myc (amino acids 275 to 364), and AP-4 (amino acids 25 to 121) (*8, 9*). Shaded regions identify conserved amino acid residues. Also shown is the derived consensus sequence for the bHLH protein family (*8*). f = L, I, V, and M; w = F, L, I, and Y (*36*). (**B**) Amino acid sequence of FIP (*25, 36*). The bHLH region is underlined. Leucine residues that make up the zipper region are underscored twice.

used in an expression library screen to identify genes that encoded protein domains that could interact specifically with the bZip dimerization domain. We screened ~250,000 plaques (*22*) of an amplified, random-primed HeLa cell cDNA library in λgt11 (*23*). Three positive plaques were identified, plaque-purified, and sequenced directly. Two of the phages contained cDNA sequences identical to human JunD (*24*) and were not characterized further. The fact that these JunD-encoding clones were isolated confirms the ability of the c-Fos core probe to interact with known partners in a complex mixture.

The third cDNA encoded a Zip domain in-frame with the λgt11 *lacZ* coding region and contained ~725 bp of DNA, of which 207 bp encoded protein. When isolated and expressed in vitro, the encoded 68–amino acid polypeptide (which contained only a Zip domain) formed stable complexes with c-Fos core in solution (*19*). The predicted amino acid sequence of a longer representative clone (*25*) of this Fos interacting protein (FIP) (Fig. 2) showed the presence of a bHLH domain NH_2-terminal to the Zip domain.

The FIP sequence fits the consensus for both the bHLH and the Zip domains (Fig. 2). The FIP is most similar to USF (~68% identity) in the segment that extends from the NH_2-terminal residue of the basic region to the COOH-terminus. The USF (also known as MLTF and UEF) is a cellular factor that binds to an upstream stimulatory element (USE) and regulates transcription in several tissue-specific and developmentally regulated genes (*26*, *27*). Although their overall identity is ~53%, there is no similarity between the two proteins in the 100 NH_2-terminal amino acids of FIP. In the basic region and helices of the bHLH motif, these two molecules are almost 100% identical at the amino acid level. This similarity suggested that FIP might bind a DNA recognition element similar or identical to the adenovirus major late promoter USE (AMLP-USE).

We used an electrophoretic mobility-shift assay (*28*) to test whether FIP bound to the AMLP-USE. Intact FIP recognized the AMLP-USE, whereas a truncated form of FIP with the Zip region deleted, but with the bHLH region intact [FIP(ΔZip)], did not (Fig. 3). Therefore, as with other bHLH-Zip proteins (*8*), deletion of the COOH-terminal Zip abolished the ability of FIP(ΔZip) to interact with DNA. Binding of FIP to AMLP-USE was inhibited by competition with oligonucleotide probes that contained the consensus bHLH core recognition sequence CANNTG (AMLP-USE, μE_3, and μE_4). A probe that contained a mutated core sequence (NIR-mutant), to which bHLH proteins cannot bind (*19*, *29*), did not compete for binding. The combination of the deduced FIP protein sequence, the observed DNA binding specificity, and the effect of the deleted Zip region indicate that FIP is a member of the bHLH-Zip family.

We next tested whether the bHLH-Zip motif of FIP could interact with c-Fos core in solution. Immunoprecipitation with anti-FLAG (*30*) demonstrated that FIP, which contained an intact bHLH-Zip domain, formed stable heteromeric complexes with c-Fos core in solution (Fig. 4A). Deletion of the Zip domain [FIP(ΔZip)] abolished complex formation with c-Fos core (*19*). Immunoprecipitation with antisera to c-Fos (anti–c-Fos) demonstrated that FIP also formed stable heteromeric complexes with full-length c-Fos (Fig. 4B). Our preliminary data do not support direct interactions between FIP and c-Jun or USF (*19*). The FIP–c-Fos interaction suggests, therefore, that there may be a functional link between a member of the bHLH-Zip family and a member of the bZip family.

To test whether FIP affects c-Fos activity in vivo, we performed a series of transfection experiments in undifferentiated F9 em-

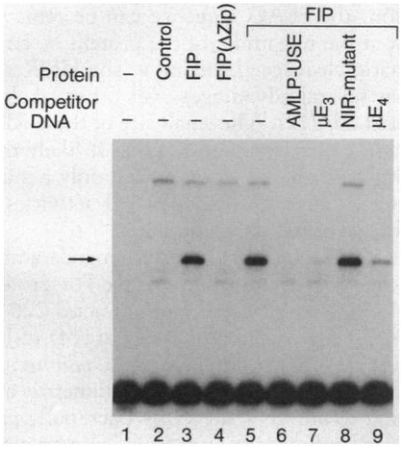
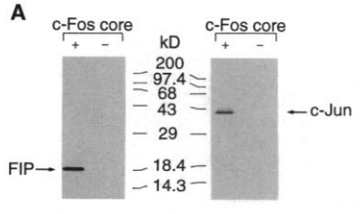


Fig. 3. FIP binds specifically to an oligonucleotide DNA probe that contains the conserved bHLH binding motif, CANNTG. Reticulocyte-produced FIP and FIP(ΔZip) proteins were analyzed by a gel electrophoresis DNA binding assay with a double-stranded AMLP-USE oligonucleotide probe (*28*). When added, competitor DNAs were in tenfold excess relative to the radiolabeled probe DNA. Control protein was unprogrammed rabbit reticulocyte lysate. Arrow indicates the specific complex formed on the DNA probe.



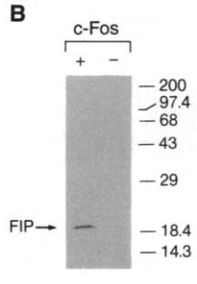


Fig. 4. FIP forms stable heterocomplexes with c-Fos core and full-length c-Fos. (**A**) [^{35}S]-methionine-labeled FIP or c-Jun proteins, produced in rabbit reticulocyte lysate, were incubated either in the presence or absence of c-Fos core (*17*) and subjected to immunoprecipitation with an anti-FLAG monoclonal antibody (*30*). (**B**) [^{35}S]methionine-labeled FIP protein was incubated either in the presence or in the absence of full-length c-Fos protein and subjected to immunoprecipitation with a rabbit antiserum specific for the NH_2-terminal region of c-Fos. Molecular weight markers indicated in kilodaltons.

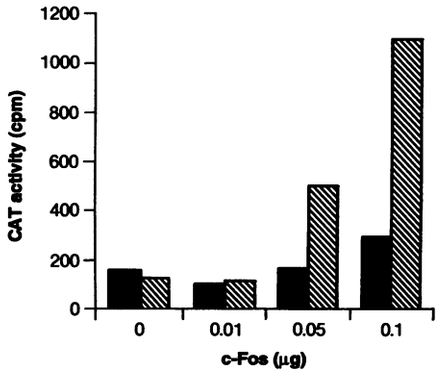


Fig. 5. Cotransfection of FIP and c-Fos stimulates transcription from an AP-1–responsive promoter in uninduced F9 embryonal carcinoma cells. Subconfluent monolayers of F9 cells were cotransfected with the calcium phosphate procedure (*37*) with 2.6 μg of either an FIP expression vector (+FIP; hatched bars) or the parent vector (−FIP; solid bars), 2 μg of A3-CAT reporter plasmid, 0.2 μg of β-galactosidase expression plasmid (pΔ6RL) as an internal control for transfection efficiency, and increased amounts of pRSV–c-Fos expression vector as indicated. Cells were incubated overnight with the calcium phosphate precipitates, then treated with 15% (v/v) glycerol in serum-free medium for 2 min and washed twice with phosphate-buffered saline, and fresh medium was added. After an additional 24 hours, cells were harvested and extracts were prepared. Heat-treated portions (5 min at 68°C) were subjected to a nonchromatographic CAT assay (*38*); a second portion, not heat-treated, was subjected to a β-galactosidase assay (*39*). CAT activities shown, normalized to β-galactosidase activity, are from a representative experiment. Average induction in the presence of FIP with c-Fos (0.10 μg) was 3.53-fold with a standard deviation of 0.99. Transfection experiments were repeated three or more times.

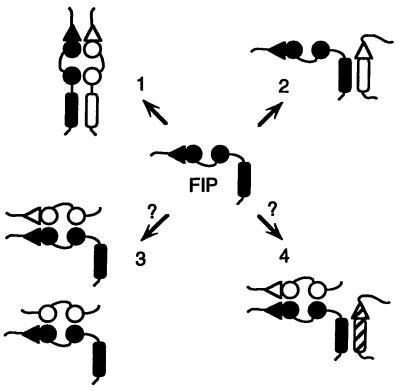


Fig. 6. A model of bHLH-Zip protein-protein interactions. At the center is a diagrammatic representation of the bHLH-Zip region of FIP. Some possible combinations of FIP-containing heteromers are: (1) with itself, or with other members of the bHLH-Zip family; (2) with c-Fos, or with other members of the bZip family; (3) with bHLH or HLH proteins; and (4) with two proteins, one each of the bHLH and bZip families. Triangles indicate basic domain; circles joined by connectors indicate the helix-loop-helix domain; and rectangles indicate zipper domain. Black indicates FIP; white or striped indicates any other protein.

bryonal carcinoma cells, which lack endogenous AP-1 activity (*4*, *31*) but may contain limited amounts of Jun (*32*). A reporter gene construct (A3-CAT) was used, in which three copies of a consensus AP-1 binding site were inserted upstream of a minimal promoter driving expression of a chloramphenicol acetyltransferase (CAT) reporter gene (*32*). Separately, neither FIP nor c-Fos activated A3-CAT expression (Fig. 5). However, when FIP and c-Fos were cotransfected, expression was enhanced three- to fivefold.

Because FIP and c-Fos interact in vitro, increased CAT activity in the transfected F9 cells may be explained by the FIP–c-Fos heteromeric complex binding to an AP-1 site and stimulating transcription directly. Expression was dependent on the presence of the AP-1 sites because no enhancement was observed in their absence (*19*). However, we have been unable to demonstrate that the FIP–c-Fos complex binds to an AP-1 site in vitro. This could be a result of the conditions of the binding assay or the lack of appropriate protein modifications, such as phosphorylation, on the bacterially or reticulocyte-produced proteins. An alternative explanation for the FIP-dependent activation in vivo could be that FIP titrates an endogenous repressor of c-Jun (or of Jun family members), which allows c-Jun to heterodimerize with c-Fos and which generates active AP-1. It is also possible that FIP may bind at another site in the vector and synergize with low amounts of active AP-1 that is generated upon transfection with c-Fos.

Diverse regulatory modes of transcription could be achieved by means of the formation of bHLH-Zip protein heterocomplexes (*8*). The observation that FIP and c-Fos can form a stable heterocomplex provides direct evidence that the HLH and Zip domains can act independently. Independent interaction of each of the dimerization domains in bHLH-Zip proteins would permit a large number of heteromeric complexes to be formed, possibly each with a distinct function (Fig. 6). Although we have not determined exhaustively the interaction repertoire of c-Fos, our method allows one to define this repertoire provided that the proteins are expressed in a form that interacts with the probe. Interaction cloning may be generally applicable to the identification and cloning of other interaction domains.

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12. The labeling reaction was performed in aqueous conditions (*20*) at one defined location on the polypeptide. Such direct labeling at a unique site on the protein avoided the inherent problems of "random" surface labeling (by iodination or biotinylation) and secondary detection techniques (by antibody). Placement of the FLAG site at the NH_2-terminus should have minimized any steric effects when antibodies were used for identification, purification, or immunoprecipitation. The catalytic subunit of bovine HMK was obtained as a lyophilized powder (Sigma). Phosphorylation reaction mixtures contained 20 mM tris-HCl (pH 7.5), 1 mM dithiothreitol (DTT), 100 mM NaCl, 12 mM $MgCl_2$, 5 μl of [γ-^{32}P]ATP (Du Pont Biotechnology Systems NEG-035C), 20 to 50 ng of target protein, and 10 units of reconstituted HMK, in a total volume of 30 μl (*11*). The labeled protein was separated from unincorporated [γ-^{32}P]ATP by gel exclusion chromatography. Specific activity of labeled c-Fos core protein was estimated to be ~8 × 10^7 cpm per microgram.
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16. The expression vector pAR(ΔRI) [59/60] was derived from pAR3040 (also known as pET3a) (*33*). pAR3040 was digested with Eco RI, and the site was destroyed either by treatment with the Klenow fragment of DNA polymerase or by limited digestion with mung bean nuclease. This intermediate plasmid was termed pAR(ΔRI). A double-stranded DNA adaptor, which contained the oligonucleotides MAB 59, TATGGACTACAAAGACGATGACGATAAAGCAAGAAGAGCATCTGTGGAATTCCA, and MAB 60, TATGGAATTCCACAGATGCTCTTCTTGCTTTATCGTCATCGTCTTTGTAGTCCA, was ligated into the unique Nde I site of pAR(ΔRI) to generate pAR(ΔRI) [59/60]. pAR(ΔRI) [59/60]::c-Fos core contained DNA that encoded amino acids 120 through 206 of the rat c-Fos protein (*13*). The pAR(ΔRI) [59/60]::c-Jun core encodes residues 206 to 340 of the human c-Jun protein (*14*). The specific fragments used for cloning were generated by the polymerase chain reaction. All inserts and constructs were verified by DNA sequence analysis.
17. For production of proteins, plasmids were introduced into BL21 p*LysS* bacteria and induced with isopropyl-β-thiogalactopyranoside (IPTG) as described (*33*). For partial purification of the c-Fos core protein, bacterial sonicate was incubated at 4°C with ×0.3 packed volume of DEAE-Sephacel (Pharmacia). The resulting supernatant solution was applied to a 1-ml heparin-sepharose (Pharmacia) column, washed with buffer H [30 mM Hepes-KOH (pH 7.7), 1 mM EDTA, 10% glycerol, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium metabisulfite, and 1 mM DTT] supplemented with 0.1 M KCl, and eluted with 1 column volume each of buffer H supplemented with 0.2 to 1.0 M KCl (in 0.1 M increments). The majority of active c-Fos core was eluted with the 0.5 M and 0.6 M step fractions. The 0.5 M fraction was used in all subsequent experiments.
18. A crude bacterial extract that contained the c-Fos core could successfully heterodimerize and bind a cognate AP-1 DNA probe in combination with either a crude bacterial extract that contained c-Jun core or full-length c-Jun made in rabbit reticulocyte lysate. These complexes were specific for the AP-1 DNA probe as demonstrated by competition with various nonradioactive competitor DNAs.
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20. Proteins for immunoprecipitation were preincubated without DNA. The mixture was diluted 50-fold with radioimmunoprecipitation assay buffer (RIPA) [50 mM tris-HCl (pH 8.0), 150 mM NaCl, 1% deoxycholate, 1% Triton X-100, and 0.2% (SDS)] that contained 4 μg per microliter of bovine serum albumin, and five volumes of 10% Protein A–Sepharose (Sigma) in RIPA was added. After incubation, the supernatant solution was transferred to a fresh tube and antibody was

added. Five volumes of a 10% suspension of Protein A–Sepharose in RIPA was added, and this mixture was incubated with continuous gentle agitation overnight. The pellet was washed four times with 1 ml of RIPA and transferred to a fresh tube on the final wash. The resultant pellet was resuspended in (×1) SDS sample buffer, and the proteins were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography.

21. Full-length human c-Jun protein was produced from mRNA synthesized in vitro with a T7 expression vector that contained the coding region of human c-Jun.
22. For interaction cloning, recombinant λgt11 bacteriophage were plated and induced with IPTG (*34*). Nitrocellulose filters were processed through a denaturation-renaturation cycle as described (*35*). After renaturation, filters were incubated for 60 min in ×1 Hepes binding buffer (HBB) with 1 mM DTT, 5% Carnation nonfat powdered milk (milk), and 0.05% Nonidet P-40 (NP-40), followed by incubation for 30 min in ×1 HBB with 1 mM DTT, 1% milk, and 0.05% NP-40. Hybridization solution [Hyb(75)] consisted of 20 mM Hepes-KOH (pH 7.7), 75 mM KCl, 0.1 mM EDTA, 2.5 mM $MgCl_2$, 1% milk, 1 mM DTT, and 0.05% NP-40, to which was added HMK-phosphorylated probe protein to a final concentration of between 1×10^5 and 3.5×10^5 cpm per milliliter. After hybridization (~12 to 16 hours), filters were washed in three changes of Hyb(75) (without probe) for a total elapsed wash time not exceeding 30 min.
23. The HeLa cell library was derived from polyadenylated mRNA isolated from HeLa cells that had been treated with human γ-interferon (Immuneron, Biogen) for 48 hours. A random-primed, size-selected (>500 bp) cDNA library was prepared in λgt11 by Stratagene. The primary library size was 7×10^6 recombinants. Clones (1×10^6) from the primary library were amplified, which yielded a titer of $\sim 5.5 \times 10^{10}$ plaque-forming units per milliliter. The amplified library was used in all subsequent manipulations.
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28. An 879-bp DNA insert that encoded FIP was subcloned into a T7 promoter-directed expression vector. The plasmid was linearized by digestion with Not I and used for in vitro production of mRNA. For production of FIP(ΔZip), the plasmid vector was linearized by digestion with Sst I. For electrophoretic mobility-shift assays (*29*), reactions contained 10 mM Hepes-KOH (pH 7.8), 75 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 3% Ficoll 400, 0.5% polyvinyl alcohol, poly(dI-dC) · poly(dI-dC) (0.8 μg per microliter of solution), and ~0.1 ng of labeled oligonucleotide probe that contained AMLP-USE. The sequences of the probe and the competitor DNAs were: AMLP-USE, 5′-GATCCTAGGCCACGTGACCGG; μE_3, 5′-GATCCAGGTCATGTGGCAAGG; NIR-mutant, 5′-GATCCTAGGACTCGTTAGAGG; μE_4, 5′-GATCCTACCCAGGTGGTGTTG (and their complements).
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Fast Perceptual Learning in Visual Hyperacuity

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In many different spatial discrimination tasks, such as in determining the sign of the offset in a vernier stimulus, the human visual system exhibits hyperacuity by evaluating spatial relations with the precision of a fraction of a photoreceptor's diameter. It is proposed that this impressive performance depends in part on a fast learning process that uses relatively few examples and that occurs at an early processing stage in the visual pathway. This hypothesis is given support by the demonstration that it is possible to synthesize, from a small number of examples of a given task, a simple network that attains the required performance level. Psychophysical experiments agree with some of the key predictions of the model. In particular, fast stimulus-specific learning is found to take place in the human visual system, and this learning does not transfer between two slightly different hyperacuity tasks.

For any given visual task, it is tempting to propose a specific algorithm and a corresponding neural circuitry. It has been often implicitly assumed that this machinery may be hardwired in the brain. This extreme point of view, if taken seriously, may quickly lead to absurd consequences. Consider for instance the many different hyperacuity tasks (*1*), some of which are shown in Fig. 1. Computational analysis reveals that the photoreceptor spacing and the low-pass characteristics of the eye's optics satisfy (in the fovea) the constraints of the sampling theorem (*2*). Thus, the underlying reason for the spectacular performance of human subjects in hyperacuity tasks is that the signal sampled by the photoreceptors and relayed to the brain contains the information necessary for precise localization of image features. This observation, however, does not constitute an explanation of hyperacuity, because each task is different and, in principle, would require a different circuit for its solution. Note that the idea of a fine-grid reconstruction of the image in some layer of the cortex (*2*) does not address the problem, because it still requires yet another mechanism that looks at the reconstructed image and applies a different routine or circuitry for each specific hyperacuity task.

We have proposed instead (*3*) that the brain may be able to set up—possibly in the cortex—appropriate task-specific modules that receive input from retinotopic cells and learn to solve the task after a short training phase in which they are exposed to

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