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14. The *hb* binding sites are clustered in tight pairs. Deletion of the different pairs results in varying degrees of ectopic expression in the anterior region, suggesting that the different clusters may function with varying degrees of efficiency in their ability to effect repression. The 5′ most cluster seems to be essential for repression because its deletion results in almost complete ectopic expression in the anterior region (construct KP/NR); however, this cluster is not sufficient to provide complete repression (construct KT). The middle cluster appears to increase the efficiency of repression, because the presence of both 5′ and middle clusters (construct KC) results in almost total repression of ectopic gene expression in the anterior region; consistent with this, deletion of the middle cluster (KN/CR) results in some ectopic anterior expression. These results also indicate that the 3′ most cluster functions least effectively because its deletion has almost no effect (construct KC), but, in conjunction with the 5′ cluster, can provide some repression function (construct KN/CR). Taken together, these results suggest that the different *hb* sequences are required to efficiently repress *kni* expression, perhaps by redundant use of regulatory elements [G. Schaffner, S. Schirm, B. Müller-Baden, F. Weber, W. Schaffner, *J. Mol. Biol.* **201**, 81 (1988)].
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21. This mechanism must of course be repressed in the nuclei or cells that will give rise to nonabdominal parts of the body. This could be accomplished during syncytial blastoderm by factors, perhaps functional homologs of *hb* and *tll*, in the anterior and terminal regions of the embryo. The major conceptual requirement would be that at the time of cellularization, at least one cell forms which is not repressed by these factors. This would then give rise to the future abdominal segments through cell division.
22. M. Rothe and H. Jäckle, unpublished results.
23. The constructs *kni*1.8*lacZ* and kni0.5*lacZ* were generated from the previously described construct *kni*4.4*lacZ* (*10*). All three are derived from the parent vector pCaSpeR AUGβ-gal [C. Thummel, A. Boulet, H. Lipshitz, *Gene* **74**, 445 (1988)]. For the constructs with the heterologous heat shock–basal promoter, fragments were first cloned into pBluescript (Stratagene) and then moved into the vector HZ50PL [Y. Hiromi and W. Gehring, *Cell* **50**, 963 (1987)]. The construct KFn was made by use of polymerase chain reaction (PCR) primers.
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29. We thank D. Tautz and C. Thummel for vectors, M. Rothe for communicating the *kni* rescue results before publication, and G. Brönner, G. Jürgens, M. Rothe, R. Sommer, and D. Tautz for comments and discussions. The *tll* expression vector was provided by E. Steingrimsson and J. Lengyel before publication. Supported by grants from the Deutsche Forschungs Gemeinschaft.

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The *fms*-Like Tyrosine Kinase, a Receptor for Vascular Endothelial Growth Factor

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The *fms*-like tyrosine kinase (Flt) is a transmembrane receptor in the tyrosine kinase family. Expression of *flt* complementary DNA in COS cells conferred specific, high-affinity binding of vascular endothelial growth factor, also known as vascular permeability factor (VEGF-VPF), a factor that induces vascular permeability when injected in the guinea pig skin and stimulates endothelial cell proliferation. Expression of Flt in *Xenopus laevis* oocytes caused the oocytes to release calcium in response to VEGF-VPF. These findings show that *flt* encodes a receptor for VEGF-VPF.

VASCULAR PERMEABILITY FACTOR (VPF) was originally purified from guinea pig ascites and tumor cell culture medium as an agent that increases blood vessel permeability (*1, 2*). VPF also stimulates migration of monocytes across an endothelial cell monolayer (*3*). Independently, a mitogen specific for endothelial cells, termed vascular endothelial growth factor (VEGF), was purified from the conditioned medium of bovine pituitary folliculo stellate cells (*4, 5*). The amino acid sequences of VPF and VEGF are identical as predicted by their cDNA sequences, indicating that these different activities are embodied by the same molecule (*6–9*). VEGF-VPF is composed of two presumably identical chains held together by disulfide bonds. The amino acid sequence of VEGF-VPF is distantly related (18% overall identity) to the sequence of platelet-derived growth factor (PDGF), which is also a disulfide-linked dimer. The eight cysteines involved in intra- and interchain disulfide bridges in PDGF are identical in VEGF-VPF (*9, 11*). In contrast to other endothelial growth factors such as fibroblast growth factor (FGF) and platelet-derived endothelial cell growth factor (PD-ECGF), which do not have signal sequences for secretion by the endoplasmic reticulum pathway, VEGF-VPF has these sequences and is a secreted ligand (*12*).

We screened a cDNA library from human placenta with an oligonucleotide encoding a sequence common to many tyrosine kinases (*13*) and identified a cDNA clone, clone 9, which is almost identical to the published *fms*-like tyrosine kinase (*flt*) sequence (*14, 15*). Clone 9 and *flt* cDNAs encode proteins that are similar to the receptors in the PDGF receptor family, which include the CSF-1 receptor, the protein encoded by c-*kit*, and the PDGF α-type and β-type receptor (*16*). The similarities between the ligands VEGF-VPF and PDGF and between clone 9-*flt* and the PDGF receptor suggested that clone 9-*flt* might encode a receptor for VEGF-VPF.

The protein predicted by the *flt* cDNA would have seven immunoglobin-like domains in its extracellular region (compared to five immunoglobin-like domains in the other members of this receptor family), a single transmembrane spanning region, and a tyrosine kinase sequence that is interrupted by a kinase insert

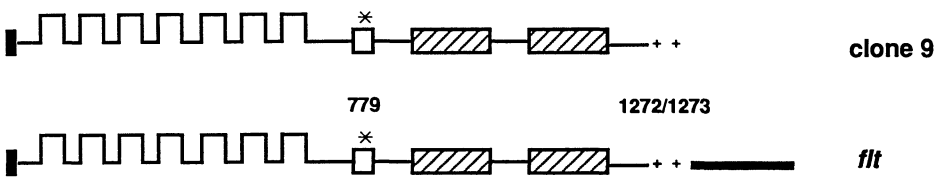

Fig. 1. Schematic representation of the structure of the proteins encoded by clone 9 and *flt*. The following structural features are identified: (■) hydrophobic leader sequence, immunoglobin-like sequence, (□) transmembrane membrane domain, (▨) kinase domains. The asterisk indicates the position in the transmembrane domain where the published *flt* sequence has Leu^{779} (*15*) and where our isolates of clone 9 and *flt* have Phe^{779}. The position in the COOH-terminal region at which clone 9 has Glu^{1272}-Val^{1273} and Flt has Asp^{1272}-Leu^{1273} is indicated (++). The thick line depicts the 65 additional amino acids at the COOH-terminus of Flt that are not present in clone 9.

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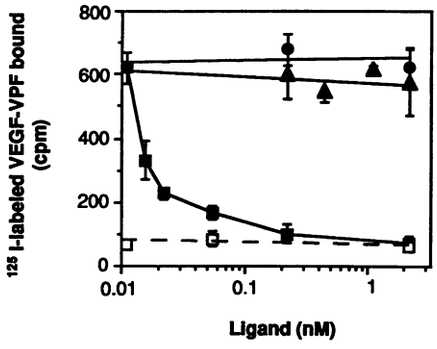

Fig. 2. Specific binding of ^{125}I–VEGF-VPF to COS cells expressing Flt. COS cells were transiently transfected (*19*) with the expression vector pSV7d (P. Luciw, Chiron Corporation, Emeryville, California) containing the *flt* cDNA (solid lines) or the PDGF receptor cDNA (dashed line). On the third day after transfection the cells were washed twice with binding medium [DMEM, 25 mM Hepes, bovine serum albumin (1%, w/v)] and binding was performed at 4°C for 2 hours in a total volume of 1 ml containing 15,000 cpm ^{125}I–VEGF-VPF (*29*) and the indicated concentrations of unlabeled ligand. The cells were then washed three times with cold binding medium and lysed with 0.5 ml of lysis buffer (*30*). The soluble lysates were counted in a gamma counter. The binding of ^{125}I–VEGF-VPF to COS cells expressing Flt at the indicated concentrations of unlabeled VEGF-VPF (closed squares), PDGF-BB (closed triangles), and basic FGF (closed circles) is shown. Binding of ^{125}I–VEGF-VPF to COS cells expressing PDGF β-type receptor is also shown (open squares). Each value represents the ± SEM of three determinations.

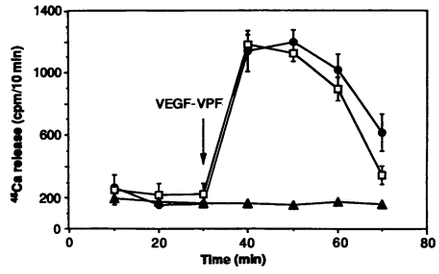

Fig. 4. Induction of calcium efflux by VEGF-VPF in *Xenopus* oocytes expressing Flt or clone 9. Oocytes were injected with 35 ng per oocyte of *flt* mRNA (closed circles), clone 9 mRNA (open squares), or water (closed triangles). Two days after injection the oocytes were incubated with $^{45}Ca^{2+}$, and the calcium efflux was measured at 10-min intervals as described (*31*). The addition of VEGF-VPF (20 ng/ml) is indicated by the arrow. The values ± SEM of three determinations are shown. For some points the error bars are smaller than the symbols.

domain (*15, 16*). The coding sequence of clone 9 is identical to the published sequence of *flt* except at residue 779 (Phe in clone 9 and Leu in *flt*) and at the 3′ ends; clone 9 has a stop codon at position 1274 preceded by codons for Glu^{1272} and Val^{1273}, whereas the published sequence of *flt* encodes Asp^{1272} and Leu^{1273} and continues for 65 amino acids before terminating (Fig. 1). Oligonucleotides from the *flt* sequence were used to amplify a full-length cDNA clone of *flt* by polymerase chain reactions (PCRs) with human placenta mRNA as template (*17*). Every *flt* sequence we isolated from the cDNA library and from a different source of placental RNA encoded Phe at codon 779 (*18*).

To test the hypothesis that Flt-clone 9 is a receptor for VEGF-VPF, we expressed the cDNAs encoding either clone 9 or Flt in COS cells (*19*). Transfectants that expressed Flt bound ^{125}I-labeled VEGF-VPF with high affinity, whereas parental COS cells did not bind ^{125}I–VEGF-VPF (Fig. 2). A concentration of 20 pM of unlabeled VEGF-VPF blocked half of the binding of the labeled ligand. This affinity is consistent with the affinity for VEGF-VPF binding to human umbilical vein endothelial cells that respond mitogenically to this factor (*20, 21*). The interaction of VEGF-VPF with the receptor on transfected cells is specific, because PDGF and basic FGF did not compete with binding of ^{125}I–VEGF-VPF (Fig. 2). Similarly, COS cells transiently expressing the PDGF β-type receptor bound ^{125}I–PDGF (Fig. 3) but did not bind ^{125}I–VEGF-VPF (Fig. 2). The same binding specificity was observed in COS cells expressing clone 9 (*22*), indicating that the intracellular COOH-terminal region of Flt (amino acids 1272 to 1338) is not required for high-affinity binding of VEGF-VPF to Flt.

To identify the VEGF-VPF receptor protein, we performed cross-linking studies with ^{125}I–VEGF-VPF and *flt* transfectants. Complexes with apparent molecular sizes of 205 kD and more than 300 kD were observed (Fig. 3). The high molecular size complex presumably represents a dimer of receptors cross-linked with VEGF-VPF. Subtraction of the molecular size of VEGF-VPF (46 kD) from the apparent size of the 205-kD complex yields an estimated molecular size for Flt of 160 kD, which is 10 kD larger than the molecular size of Flt as calculated from its amino acid composition (*15*). An excess of unlabeled VEGF-VPF blocked the formation of both complexes, whereas unlabeled PDGF did not affect cross-linking of ^{125}I–VEGF-VPF with the protein encoded by *flt* (Fig. 3). The complexes of ^{125}I–PDGF and PDGF β-type receptor had molecular sizes similar to those of the VEGF-VPF-Flt complexes (Fig. 3).

To test whether VEGF-VPF can induce biological activation of Flt, the receptor was expressed in *Xenopus laevis* oocytes and its activation was assessed by a calcium efflux assay that has been used to measure the activity of PDGF and FGF receptors (*23, 24*). Full-length *flt* cDNA was transcribed in vitro and the capped mRNA was injected into *Xenopus* oocytes (*25*). Addition of VEGF-VPF to the oocytes induced a fivefold increase in calcium efflux both in oocytes injected with *flt* mRNA or clone 9 mRNA (Fig. 4). Oocytes expressing Flt or clone 9 did not respond to PDGF-BB (*22*), and oocytes injected with water did not respond to VEGF-VPF or PDGF-BB. These findings show that VEGF-VPF can induce activation of both Flt and its truncated form clone 9, indicating that VEGF-VPF is a ligand for Flt and that the COOH-terminal portion of Flt (amino acids 1272 to 1338) is not required for this biological response.

The cytoplasmic region of Flt includes a sequence that is up to 60% identical (excluding the kinase insert region) to amino acid sequences of the tyrosine kinase domain of members of the PDGF receptor family (*15*). When stimulated by ligand binding, the receptors in this family undergo autophosphorylation of

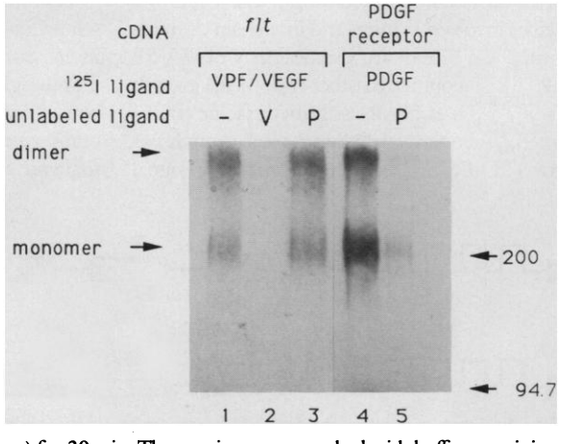

Fig. 3. Cross-linking of ^{125}I–VEGF-VPF and ^{125}I–PDGF-BB to COS cells expressing either Flt or PDGF β-type receptor. COS cells transiently expressing Flt were incubated with ^{125}I–VEGF-VPF (Fig. 2) in the absence (lane 1) or presence of unlabeled VEGF-VPF (0.8 nM; lane 2) or PDGF-BB (1.1 nM; lane 3). As a control, ^{125}I–PDGF-BB was bound to COS cells transfected with PDGF β-type receptor in the absence (lane 4) or in the presence (lane 5) of unlabeled PDGF-BB (1.1 nM). The cells were incubated with the ligands for 2 hours at 4°C, washed three times with phosphate-buffered saline, and then exposed to the cross-linking agent 3,3′-bis(sulfosuccinimyl)suberate (BS^3; 1 mM; Pierce) for 30 min. The reaction was quenched with buffer containing 10 mM tris, pH 8.0, and 0.1 M glycine for 10 min and the cells were lysed with lysis buffer (*30*). The lysates were partially purified by binding to wheat germ agglutinin Sepharose and analyzed by SDS–polyacrylamide gel electrophoresis (5% gel), followed by autoradiography. Molecular size markers are indicated in kilodaltons. Monomeric and putative dimeric forms are indicated on the left margin. V and P, unlabeled VEGF-VPF and PDGF, respectively.

cytoplasmic tyrosine residues. Stimulation of human umbilical vein endothelial cells with VEGF-VPF results in tyrosine phosphorylation of a protein with a molecular size of approximately 200 kD (*21*) and mobilization of calcium (*26*). However, we did not observe a ligand-dependent increase in tyrosine phosphorylation of Flt in oocytes (*22*). The *flt* protein expressed in *Xenopus* oocytes is constitutively phosphorylated on tyrosine residues, so it may be difficult to detect ligand-induced phosphorylation.

High-affinity binding sites for VEGF-VPF have a tissue distribution similar to that of *flt* mRNA (*15, 27*). The *flt* transcript was detected in human umbilical vein endothelial cells that respond to VEGF-VPF (*28*). However, it is not known whether Flt is the only receptor for VEGF-VPF or whether it mediates the change in endothelial cell growth and vascular permeability induced by VEGF-VPF. Other endothelial cell mitogens, such as FGF, do not affect endothelial cell permeability or monocyte migration, suggesting that there are unique signal transduction pathways activated by VEGF-VPF.

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17. Human placental polyadenylated RNA was specifically primed with an oligonucleotide hybridizing to the *flt* 3′ untranslated sequence (nucleotides 4288 to 4313). A Sal I restriction site was added to the oligonucleotide (5′GATGTCGACGGTATAAATA CACATGTGCTTCTAG). Subsequently, a PCR was performed with the same primer and an oligonucleotide containing the unique Bgl II site in *flt* (nucleotides 3233 to 3248) (5′CTATGGAAGATCTGATT-TCTTACAGT). The generated fragment was digested with Bgl II and Sal I and ligated with the 5′ fragment of clone 9 (Eco RI–Bgl II) in the expression vector pSV7d (Eco RI–Sal I).
18. The *flt* sequence encoding the transmembrane domain was isolated from human placental mRNA by priming of a reverse transcriptase reaction with an oligonucleotide complementary to nucleotides 2801 to 2828 (5′CACAGTCCGGCACGTAGGTGATT). To start the PCR, an oligonucleotide that bound upstream of the transmembrane encoding sequence (nucleotides 2411 to 2435) (5′GTCACAGAAGAGGAT-GAAGGTGTCTA) was added. The generated 417-bp fragment was purified from an agarose gel and ligated in M13mp18 linearized with Sma I. The sequence was determined by the dideoxy chain termination method.
19. We transiently transfected COS cells by the DEAE-dextran method. Briefly, 7×10^6 cells in a 10-cm plate were incubated for 3 hours with 5 ml of Dulbecco's modified essential medium (DMEM), 2.5% fetal calf serum, 0.4 mg DEAE dextran per milliliter, 0.1 mM chloroquine phosphate, and 1 μg of expression plasmid per milliliter. This transfection medium was then replaced by DMEM containing fetal calf serum (10%). Binding experiments were performed after 72 hours.
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25. Fully grown oocytes (Dumont stage VI) were obtained from *Xenopus laevis*. The oocytes were defolliculated with collagenase (Sigma type IA, 1 mg/ml) and maintained at 19°C in modified Barth's medium (MBS) with Hepes (15 mM, pH 7.6), bovine serum albumin (1 mg/ml), penicillin G (100 U/ml), and streptomycin (100 μg/ml). The oocytes were injected with 50 nl of RNA solution or pure water (*23, 24*).
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29. The human recombinant 165–amino acid species of VPF-VEGF was expressed in Chinese hamster ovary cells (*9*) and purified to homogeneity as described [N. Ferrara, D. W. Leung, G. Cachienes, W. J. Winer, W. J. Henzel, *Methods Enzymol.* **198**, 391 (1991)]. VPF-VEGF was iodinated by an indirect iodogen method (*27*). The radioligand was tested for biological activity in an endothelial cell proliferation assay [D. Gospodarowicz, S. Massoglia, J. Cheng, D. K. Fuji, *J. Cell Physiol.* **127**, 121 (1986)].
30. Lysis buffer: 20 mM tris (pH 8.0) 137 mM NaCl, Triton X-100 (1%, v/v), glycerol (10%, v/v), 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride (PMSF) aprotinin (0.15 U/ml), 0.02 mM Leupeptin.
31. The $^{45}Ca^{2+}$ efflux from injected *Xenopus* oocytes was quantitated (*23, 24*). Two days after injection, the oocytes were incubated with $^{45}Ca^{2+}$ (100 μCi/ml) in 0.5 ml of Ca^{2+}-free MBS for 3 hours at 19°C. The oocytes were then washed with MBS and groups of eight oocytes were placed in 24-well plastic tissue culture plates. The medium was collected and replaced every 10 min, and the radioactivity in the medium was determined by liquid scintillation counting. After 30 min VEGF-VPF (20 ng/ml) was added to the medium for the first 10 min only. These experiments were performed at room temperature.

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Vegetal Messenger RNA Localization Directed by a 340-nt RNA Sequence Element in *Xenopus* Oocytes

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Contained within a single cell, the fertilized egg, is information that will ultimately specify the entire organism. During early embryonic cleavages, cells acquire distinct fates and their differences in developmental potential might be explained by localization of informational molecules in the egg. The mechanisms by which Vg1 RNA, a maternal mRNA, is translocated to the vegetal pole of *Xenopus* oocytes may indicate how developmental signals are localized. Data presented here show that a 340-nucleotide localization signal present in the 3′ untranslated region of Vg1 RNA is sufficient to direct RNA localization to the vegetal pole.

THE BASIS FOR PATTERN FORMATION in early development can be traced to the localization of maternal factors in eggs. Although there is evidence for the existence of localized cytoplasmic determinants in a number of systems (*1*), in no case is the localization process understood. This localized information can in principle be stored as RNA or protein. In vertebrate eggs one example of this phenomenon is Vg1 RNA, a maternal RNA found exclusively in the vegetal hemisphere of *Xenopus laevis* oocytes and eggs (*2*). Vg1 RNA codes for a protein that is homologous to the transforming growth factor β (TGF-β) (*3*), but its role in embryogenesis is not currently understood. Vg1 RNA is evenly distributed in young oocytes but is translocated later, during the vitellogenic phase of oogenesis, to the vegetal hemisphere in mature oocytes and unfertilized eggs (*4*). After fertilization, Vg1 RNA, and ultimately Vg1 protein, are inherited preferentially by the vegetal blas-

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