Human phagocytic cells express receptors for bacterial N-formyl peptides (formyl peptide receptor or FPR) which mediate chemotaxis, degranulation, and the respiratory burst. Although cDNA encoding a human phagocyte formyl peptide-binding protein has been reported recently (Boulay, F., Tardif, M., Bouchon, L., and Vignais, P. (1990) Biochem. Biophys. Res. Commun. 168, 1103–1109), functional coupling to signal transduction processes was not demonstrated. We describe corresponding full-length cDNA clones and prove that they encode the calcium-mobilizing human formyl peptide receptor by demonstrating functional reconstitution in the Xenopus oocyte. We further demonstrate that in contrast to all other cloned guanine nucleotide-binding regulatory protein (G-protein) coupled receptors expressed in this system, microinjection of FPR transcripts is not sufficient to confer ligand responsiveness to the oocyte: coinjection of phagocyte RNA encoding a complementary human factor that is not the α subunit of the heterotrimeric G-proteins G₃, G₂, or G₁ is also required. Whereas a 1.4-kilobase FPR transcript is expressed exclusively in differentiated phagocytic cells, the complementary factor activity localizes to a 3.5-kilobase RNA fraction and is expressed in both differentiated and undifferentiated myeloid cells as well as in liver. The deduced human FPR protein possesses seven hydrophobic putative membrane spanning segments, three sites for N-linked glycosylation, and a short 18-amino acid predicted third cytoplasmic loop. Surprisingly, the human FPR possesses only 28% amino acid identity with the rabbit FPR reported recently by Thomas and co-workers (Thomas, K. M., Pyun, H. Y., and Navarro, J. (1990) J. Biol. Chem. 265, 20061–20065). Moreover, the rabbit FPR does not require a complementary factor for calcium mobilization in the oocyte. Structural alignment reveals at most 20% amino acid identity of the human FPR with other G-protein coupled receptors, indicating a common ancestral gene. Functional reconstitution of the recombinant FPR will now permit precise delineation of its functional and regulatory domains. Moreover, discovery of a complementary factor for oocyte expression of the human FPR establishes a novel approach to the qualification by ligand screening of cDNA encoding other suspected G-protein coupled receptors.

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The migration of phagocytic cells from the blood to sites of infection and inflammation is an essential feature of the immune system and is mediated by chemoattractants such as bacterial N-formyl peptides, the activated fifth component of complement, and platelet-activating factor (1–3). A phagocyte activation program that results in directed motility, secretion of inflammatory mediators from intracellular granules, and the production of antimicrobial metabolites of molecular oxygen can be initiated by ligand occupancy of cell surface receptors for each of these stimuli. Specific features of the biochemical pathways resulting in these functional responses have been most extensively defined for the formyl peptide receptor (FPR) and include the activation of a pertussis toxin-sensitive G-protein, thought to be G₁₂ (4, 5), and a phospholipase C (6). Hydrolysis of membrane polyphosphoinositides results in the cytosolic accumulation of diacylglycerol and inositol 1,4,5-trisphosphate which in turn activate protein kinase C and mobilize intracellular calcium stores, respectively (7, 8). Other important activation pathways that are initiated by stimulation of phagocytes with formyl peptides include those subserved by phospholipases A₁, A₂, and D (10).

The structural basis of FPR-mediated signal transduction has been an area of great interest. Boulay et al. (11, 12) recently reported the sequences of two allelic HL60 neutrophil cDNA clones both of which conferred formyl peptide binding activity to transfected COS cells. However, the signaling capability of the putative receptor and tissue expression of corresponding transcripts were not described. Proof that a gene or cDNA encodes a receptor of interest and not just a ligand-binding protein or acceptor ultimately requires demonstration of functional reconstitution in a heterologous expression system.

This is especially true for FPR. Binding sites for formyl peptides have been described on phagocytes as well as on endothelial cells (13). In contrast to phagocytes, however, endothelial cells do not exhibit formyl peptide-dependent functional responsiveness such as calcium mobilization. The molecular relationship of endothelial cell and phagocyte binding sites for formyl peptides is unknown. Nor is it known whether similar functionally silent formyl peptide binding sites are expressed by the phagocyte. The atrial natriuretic factor receptor provides an additional example of a receptor system for which abundant functionally silent ligand binding sites have been described (14).

To resolve this issue we have isolated corresponding full-
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length human monocyte and HL60 neutrophil cDNA clones and have tested the ability of RNA synthesized in vitro (cRNA) from candidate cDNA to confer a foray peptide-dependent calcium flux response to microinjected oocytes from Xenopus laevis. To this fraction we have discovered that a complementary factor expressed in both differentiated and undifferentiated phagocytic cells is absolutely required for expression of human FPR signal transduction in the oocyte. This unanticipated finding is of general relevance to the expression of human FPR signal transduction in the oocyte.

**EXPERIMENTAL PROCEDURES**

**cDNA Library Screening**—The materials and methods used to obtain RNA for library construction were as described previously in detail (18). The human promyelocytic leukemia cell line HL60 can be induced by treatment with dibutryl cyclic AMP to differentiate terminally into a cell type, designated the HL60 neutrophil, that expresses the FPR (19). A cDNA library was constructed in the unidirectional transcription competent vector UniZAP (Stratagene, La Jolla, CAL) from 1 kb fraction of the appropriate size-fractionated HL60 neutrophil poly(A)^+ RNA enriched in transcripts capable of endowing the microinjected Xenopus oocyte with a formyl peptide-dependent calcium mobilizing activity (20). The unamplified library contained 1.2 million independent recombinants. Direct examination of cDNA inserts from 80 randomly selected clones revealed an average insert size of 2 kb with a range from 1 to 2.5 kb. All clones examined had inserts. The library was divided into pools prior to amplification in the Escherichia coli host strain PLK^F (Stratagene). Library screening was performed by plaque hybridization analysis as described (21). A 33-mer oligodeoxyribonucleotide hybridization probe corresponding to nucleotides 214-246 of the published cDNA sequence (11) was synthesized (Pharmacia LKB Biotechnology Inc.). The probe was labeled with [α-^32P]dATP (Amersham Corp.) using a 5'-end labeling kit (Boehringer Mannheim). Samples of the library were amplified in the host cell XL-1-Stratagene on agarose plates, and DNA from duplicate plaque replicas was prepared as described previously (21) after transfer to nitrocellulose filters (Schleicher & Schuell). Filters were baked at 80 °C and then incubated at 40 °C for 12 h in a solution containing 5 × SSPE (1 × SSPE contains 150 mM NaCl, 10 mM NaH_2PO_4, and 1 mM Na_2EDTA, pH 7.4), 5 × Denhardt's (1 × Denhardt's = 0.02% each of Ficoll, bovine serum albumin, and polyvinylpyrrolidone), 50 μg/ml salmon sperm DNA, 0.5% SDS, and 10^5 cpm of radiolabeled probe/ml. The final wash was performed in a solution containing 5 × SSPE and 0.1% SDS at 55 °C for 5 min. Filters were then exposed to XAR-2 film (Kodak) at -80 °C for 12 h. Phages containing FPR cDNA were purified from plaques corresponding to duplicate hybridization signals, and Bluescript plasmids were rescued from recombinant UniZAP clones by R408 helper phage excision (Stratagene). The cDNA inserts were sequenced by walking with sequential 17-mer oligonucleotides using a T7 DNA polymerase based sequencing kit (Pharmacia) by the deoxyoligonucleotide chain termination method (22).

**Receptor Reconstitution in the Xenopus Oocyte**—In addition to Bluescript plasmids containing candidate FPR cDNA the following plasmids were obtained for use in receptor reconstitution experiments: pSR-1c (23), a Bluescript plasmid containing a rat cDNA encoding the serotonin 1c receptor (provided by D. Julius, University of California at San Francisco); BG41-2 (24), a PEG plasmid containing a human cDNA encoding G_a (provided by P. Bray (National Institute of Child Health and Human Development)); DJ18 and DJ33 (25), PGEM plasmids containing rat cDNA encoding G_a and G_b, respectively (provided by P. Reed, Johns Hopkins University School of Medicine). Capped sense RNA was synthesized by first transcription with T7 RNA polymerase type II and manual dissection. Oocytes were microinjected with RNA samples in a total volume of 50 nl/oocyte 1-2 days after harvesting and were then incubated at 18-20 °C for at least 3 days in ND96 medium. Oocytes were then loaded with 3Ca^2+ for 3 h as described (26). After 10 washes with ND96 individual oocytes were stimulated with 1 mM fMLP or 1 mM serotonin (Sigma) in 100 μl wells of a 96-well tissue culture plate containing 100 μl of ND96. Three 100-μl samples of bath solution were collected and analyzed by liquid scintillation counting (a) the final 100-μl wash of 20 min duration before application of ligand; (b) fluid containing the stimulus; and (c) the oocyte solubilized in 1% SDS in ND96 after the 20-min stimulation. Aquired ligand-dependent calcium efflux is reported as the percent of loaded 3Ca^2+ which is released in response to stimulation with ligand, defined as (net cpm released + cpm loaded) x 100, i.e., (b-a)/(b-a+c) x 100. Data are derived from experiments performed using oocytes from four frogs in each experimental group.

**RNA Survey**—Total cellular poly(A)^+ RNA was prepared as described previously (26) from: 1) fresh cadaveric human tissues; 2) cell pellets from human peripheral blood T cells and tonsilar B cells; and 3) the following transformed human blood cell lines: U937 promonocyte, THP-1 promonocyte, normal peripheral blood B cells transformed by Ebstein-Barr virus, HL60 promyelocytes, Jurkat T lymphocytes, and HL60 neutrophils (HL60 cells cultured for 48 h in 500 μM dibutryl cyclic AMP). Size-fractionated HL60 neutrophil poly(A)^+ RNA was prepared by sucrose gradient centrifugation as described previously (26). RNA samples were size-fractionated by electrophoresis on a 1.2% agarose gel containing 2.2 M formaldehyde and 20 mM MOPS and then transferred to Nytran membranes (Schleicher & Schuell) by electroblotting. Membranes were hybridized with cDNA from clone FPR10 labeled with [α-^32P]dCTP (Amersham) by the random primer method (27) to a specific activity of 5 x 10^6 cpm/μg DNA. Prehybridization conditions were buffer N (28), 37 °C for 60 min. Hybridization conditions were fresh buffer N containing 10^5 cpm/ml, 42 °C, for 12 h. Wash conditions were 0.1 x SSPE, 0.1% SDS, 68 °C, for 60 min. To estimate the size of FPR transcripts a separate blot was prepared with HL60 neutrophil poly(A)^+ RNA and DNA standards that established the positions of 1.35, 2.95, 4.4, 7.46, and 9.49 kb (Bethesda Research Laboratories).

**Sequence Analysis**—DNA and protein sequences were compiled and analyzed using the software package from the University of Wisconsin Genetics Computer Group (29) on a Cray supercomputer operated by the Frederick Cancer Research Facility, Frederick, MD.

**RESULTS AND DISCUSSION**

While attempting to clone FPR cDNA by selection of functional clones in the Xenopus oocyte we became aware of the report by Boulay et al. (11) of a cDNA sequence derived from the coding block of a single HL60 neutrophil cDNA clone designated MLPR-R89 which conferred formyl peptide binding sites to transfected COS cells similar in affinity to those described for phagocytic cells. Subsequently this group reported the sequence and ligand binding properties of an allelic variant designated fMLP-R26 which contained two
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The open reading frame of all sequenced clones was 1,050 bp. The deduced polypeptide sequences of the seven clones were all 350 amino acids in length. All cDNA extended through the putative ATG initiator that was flanked by sequences satisfying the consensus criteria for translation initiation sites (31). The 3'-untranslated region (UTR) contained 189 bp; the 5'-UTR of the longest clone, designated FPR10, contained 93 bp. Thus, the 1,332-bp FPR10 cDNA contains only 282 bp of untranslated flanking sequence.

Although both deduced protein variants described by Boulay et al. (12) were represented in our collection of cDNA clones, we found only the shorter 3'-UTR described for fMLP-R26 associated with our variants. Clone fMLP-R98 cDNA is 1.9 kb in length and contains 0.85 kb of untranslated flanking sequence that includes an Alu repeat in the 3' UTR. A discrepancy of approximately 550 bp exists between the length of the 3'-UTR of clone fMLP-R98 and that of our largest of 50 HL60 neutrophil clones examined as well as that of the FPR transcript that we determined by Northern blot hybridization. Moreover, the largest of 10 FPR clones that we isolated from a Agt11 cDNA library constructed from endotoxin-stimulated human monocyte RNA (Clontech) also contained a 1.35-kb cDNA insert with a 3'-UTR identical in sequence to our HL60 neutrophil-derived clones. In this context the existence of an additional 0.55 kb of flanking sequence in clone fMLP-R98 must be regarded as anomalous. However, it is not artifactual since we have isolated human genomic clones that contain the extended 3'-UTR sequence of clone fMLP-R98.

Activation of the FPR is known to mobilize intracellular calcium stores in a pertussis toxin-sensitive manner both in phagocytic cells as well as in the frog oocyte injected with HL60 neutrophil RNA (20, 32). We therefore tested RNA synthesized in vitro (cRNA) from candidate cDNA for the capacity to confer formyl peptide-dependent "Ca2+" efflux activity to microinjected Xenopus oocytes. Surprisingly, none of the five clones tested represented both allelic variants was active on days 1, 2, 3, and 4 after injection of 5 pg, 50 pg, 500 pg, 5 ng, or 50 ng of RNA into oocytes using both FMLP and N-formyl Nle-Leu-Phe-Nle-Tyr-Lys as stimuli (not shown). In contrast, oocytes injected with cRNA encoding the rat HT1c receptor (23) acquired a serotonin-dependent calcium mobilizing response (Fig. 1). Moreover, for all cloned G-protein coupled receptors whose signaling properties in the oocyte have been reported, whether linked to adenylate cyclase (30) or phospholipase C (17, 23, 33, 34), microinjection of receptor RNA is both necessary and sufficient to establish an acquired ligand-specific signaling cascade.

Fig. 1. Functional expression of FPR in Xenopus oocytes requires co-injection of a complementary human factor that is not $G_{\alpha_i}$, $G_{\alpha_o}$, or $G_{\alpha_i}$. The RNA injected into, and the ligands applied to, each group of oocytes are coded as follows: FPR, cRNA synthesized from clone FPR15 cDNA; HT1c, cRNA synthesized from a rat serotonin 1c receptor cDNA (23); Gia, a mixture of cRNA encoding the subunits of human $G_{\alpha_i}$ (24), rat $G_{\alpha_o}$, and rat $G_{\alpha_i}$ (25); U, undifferentiated HL60 cell poly(A)+ RNA; N, HL60 neutrophil poly(A)+ RNA; SHT, serotonin. RNA samples were mixed with each other or with an equivalent volume of water to permit the microinjection of 50 nl/oocyte. The amount of RNA injected per oocyte was: U, 25 ng; N, 5 ng; FPR, 10 ng; HT1c, 10 ng; Gia, 2 ng, and Gia2, 2 ng each. Three days after microinjection, oocytes were stimulated with 1 $\mu$M FMLP or serotonin. The amount of "Ca2+" loaded per oocyte and the basal unstimulated "Ca2+" efflux differed by less than 10% between conditions. FPR cRNA injected alone was inactive. Data are from six oocytes/condition, except for H2O (four oocytes) and HT1c (nine oocytes) and are presented as the percent of loaded "Ca2+" which is released in 20 min in response to application of the stimulus. Data are representative of five independent experiments.

Incomplete heterologous signal transduction pathways have been identified previously for the human $G_{\alpha_o}$ adrenergic receptor expressed in yeast, where mammalian $G_{\alpha_o}$ is a sufficient complementarly factor (35), and for the human interferon $\gamma$ receptor expressed in rodent cells, where an unknown complementary factor whose gene is located on chromosome 21 is required (36, 37). We hypothesized that an additional human factor might be required to link the FPR to calcium mobilization in the oocyte. Fig. 1 demonstrates that when clone FPR15 cRNA is injected as a mixture with RNA from either undifferentiated HL60 cells or HL60 neutrophils robust FMLP responsiveness is acquired by the oocyte. Identical results were obtained when cRNA from other FPR clones was tested and when N-formyl Nle-Leu-Phe-Nle-Tyr-Lys was used as the stimulus.

Complementary activity in HL60 neutrophil RNA is demonstrated by an augmentation of stimulated "Ca2+" efflux in samples that were mixed with RNA synthesized from clone FPR15 as compared with those that received an equal volume of water. As expected, admixture of an irrelevant RNA to each group of oocytes are coded as follows: FPR, cRNA synthesized from clone FPR15 cDNA; HT1c, cRNA synthesized from a rat serotonin 1c receptor cDNA (23); Gia, a mixture of cRNA encoding the subunits of human $G_{\alpha_i}$ (24), rat $G_{\alpha_o}$, and rat $G_{\alpha_i}$ (25); U, undifferentiated HL60 cell poly(A)+ RNA; N, HL60 neutrophil poly(A)+ RNA; SHT, serotonin. RNA samples were mixed with each other or with an equivalent volume of water to permit the microinjection of 50 nl/oocyte. The amount of RNA injected per oocyte was: U, 25 ng; N, 5 ng; FPR, 10 ng; HT1c, 10 ng; Gia, 2 ng, and Gia2, 2 ng each. Three days after microinjection, oocytes were stimulated with 1 $\mu$M FMLP or serotonin. The amount of "Ca2+" loaded per oocyte and the basal unstimulated "Ca2+" efflux differed by less than 10% between conditions. FPR cRNA injected alone was inactive. Data are from six oocytes/condition, except for H2O (four oocytes) and HT1c (nine oocytes) and are presented as the percent of loaded "Ca2+" which is released in 20 min in response to application of the stimulus. Data are representative of five independent experiments.

The identity of the complementary factor is not known. Co-injection of FPR cRNA with cRNA encoding the $\alpha$ subunit of the pertussis toxin target protein $G_{\alpha_o}$, which is thought to couple FPR to phospholipase C and calcium flux (1, 4, 38), does not confer formyl peptide sensitivity to the oocyte (Fig. 1 and Ref. 20).
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1). Similarly, the activity does not appear to reside in the pertussis toxin substrates $G_i$, or $G_o$, and $G_o$ is not expressed by differentiated or undifferentiated HL60 cells (5).

Other proximal signaling elements such as an additional receptor subunit, a low molecular weight G-protein, an as yet undiscovered preferred $\alpha$ subuinit of a heterotrimeric G-protein, or a preferred $\beta$ or $\gamma$ subunit of a heterotrimeric G-protein are also possibilities. It is distinctly possible that the complementary activity may be caused by the expression of more than one polypeptide chain or more than one factor. Additional elements situated distal to the G-protein in the signaling cascade and not expressed constitutively by the oocyte must also be considered, as must phagocyte proteins that may be specially required for post-translational processing and membrane targeting of the FPR. Under conditions in which we can demonstrate radioligand binding and affinity labeling of the FPR in neutrophil plasma membranes or whole cells (19), however, the FPR expressed in the oocyte is not detectable with or without a source of complementary factor activity. Postreceptor signal amplification by the endogenous oocyte signal transduction apparatus then is required to detect the presence of the receptor by functional assay.

Fig. 2 shows that cDNA encoding human FPR is not clonable by the conventional oocyte based Sib selection expression cloning strategy that has been used to isolate cDNA encoding other G-protein-linked receptors (23, 34) and ion channels (39). We have reported previously that the human formyl peptide receptor is encoded by a 2-kb transcript (20). This conclusion was based on the ability of 2-kb sucrose gradient size-fractionated HL60 neutrophil RNA to confer formyl peptide responsiveness to microinjected Xenopus oocytes. RNA fractions corresponding to 1, 3.5, and in particular 1.5 kb (the approximate size of our cDNA clones) were devoid of activity. We used this information to design an FPR cDNA cloning strategy based on functional expression of cRNA synthesized from pools of clones representing a cDNA library constructed from the 2-kb HL60 neutrophil RNA. However, cRNA representing 300,000 clones from this library conferred FMLP responsiveness to oocytes only when microinjected as a mixture with a source of complementary factor such as undifferentiated HL60 RNA (Fig. 2). This implies that the original FMLP-specific activity peak located at the 2-kb RNA size fraction was likely the sum of two overlapping transcript peaks: one that contained FPR transcripts, and at least one additional peak containing transcripts of a different length which encoded the complementary activity. Fig. 3a demonstrates this directly. When equal amounts of RNA from distinct sizes of sucrose gradient-fractionated HL60 neutrophil RNA are added to a constant amount of FPR cRNA, complementary activity peaks in the 3.5-kb fraction.

The 3.5-kb fraction by itself is unable to confer formyl peptide responsiveness to the oocyte when as much as 50 ng of RNA, the largest amount tested, is injected (not shown). That the absence of activity in this fraction is caused by a paucity of FPR transcripts is shown by RNA blot analysis in Fig. 3b and by functional reconstitution of this fraction with FPR cRNA in Fig. 3a. The 1.5-kb fraction of HL60 neutrophil RNA designated as fraction 2 in Fig. 3 contains peak amounts of FPR transcripts but is functionally inactive. The shoulder of the 1.5-kb receptor transcript peak overlaps with that of the 3.5-kb fraction containing peak complementary factor activity.

**Fig. 2.** Complementary factor activity is required for detection of functional formyl peptide receptor cDNA clones expressed in the Xenopus oocyte. The methods were exactly as for Fig. 1. L designates cRNA synthesized from cDNA representing a pool of 300,000 recombinant UniZAP clones from a 2-kb size-selected HL60 neutrophil library; $U$ designates undifferentiated HL60 cell poly(A)$^+$ RNA. Twenty-five ng of the indicated RNA was injected per oocyte. Three days after microinjection, oocytes were stimulated with 1 $\mu$M FMLP. The amount of $^{45}$Ca$^{2+}$ loaded per oocyte and the basal unstimulated $^{45}$Ca$^{2+}$ efflux varied by less than 10% between conditions. Ten oocytes were tested per condition.

**Fig. 3.** Size estimation of transcripts encoding complementary factor activity. a, peak complementary factor activity localizes to a 3.5-kb fraction of HL60 neutrophil RNA. Total HL60 neutrophil poly(A)$^+$ RNA was separated into seven fractions of increasing average length by sucrose gradient centrifugation. One ng of clone FPR15 cRNA and 5 ng of RNA from each fraction were injected into oocytes as a mixture in 50 nl of water. Oocytes were stimulated 3 days later with 1 $\mu$M FMLP. The amount of $^{45}$Ca$^{2+}$ loaded per oocyte and the basal unstimulated $^{45}$Ca$^{2+}$ efflux varied by less than 10%. Five oocytes were tested per condition. Only fraction 3 possessed activity in the absence of FPR15 cRNA admixture (not shown). The average size of transcripts in each fraction was estimated by blot hybridization with known internal standards as well as by comparison with chain length standards after agarose gel electrophoresis. b, distribution of FPR transcripts in the HL60 neutrophil RNA fractions tested in panel a. An RNA blot prepared with 250 ng from each fraction was hybridized with FPR10 cDNA. The blot was washed at 68 °C in 0.1 × SSPE and exposed to XAR-2 film for 48 h in a Quanta III cassette at −80 °C.
activity, each of which is functionally inactive by itself, to produce a size-fractionated HL60 neutrophil RNA functional activity profile with a peak at 2 kb. When clones containing full-length cDNA encoding FPR and the complementary factor from the 2-kb cDNA library are inevitably split into separate pools, no FMLP-dependent activity is possible.

We next examined the tissue distribution of transcripts encoding FPR and the complementary factor. Poly(A)+ RNA from a broad panel of human tissues (esophagus, kidney, brain, spleen, liver, adrenal, heart, thyroid, skeletal muscle, and lung) as well as from differentiated and undifferentiated blood cell types (THP-1, U937, Jurkat, HL60, EBV-transformed B cells, normal tonsillar B cells, PHA-activated normal peripheral blood T cells, and HL60 neutrophils) was analyzed by blot hybridization with radiolabeled cDNA from clone FPR10. A single 1.4-kb transcript was found only in RNA from HL60 neutrophils (not shown). As discussed above, it also must be expressed by human monocytes since we were able to clone cDNA with sequence identical to that of FPR10 from a human monocyte library.

In contrast to the highly regulated expression of FPR transcripts, complementary factor transcripts are less restricted as shown by microinjection of oocytes with mixtures of FPR cRNA with poly(A)+ RNA from human tissues and blood cell types, none of which possesses detectable FPR transcripts (Fig. 4). In particular, the complementary factor is not restricted to differentiated myeloid cells; in addition to the undifferentiated HL60 cell RNA, it is also detectable in RNA from liver but not from brain or spleen. The threshold for detection of complementary activity in undifferentiated poly(A)+ HL60 RNA is 1 ng (Fig. 5).

All other members of the G-protein coupled receptor family that is characterized by seven hydrophobic putative membrane spanning segments have been composed of a single polypeptide chain, Fig. 6 demonstrates directly that the FPR has primary structural features that are typical of members of this receptor family (30, 33, 40-46) and likely evolved from a common ancestral gene. Fifteen amino acid positions are identical in all sequences examined 10 of these reside in the third cytoplasmic loop, which is believed to participate in G-protein activation by these receptors, in which greater than 43% amino acid residues that could serve as phosphorylation sites involved in signaling cascade when expressed in the frog oocyte, without phosphorylation by protein kinase A. The divergent 45-amino acid carboxyl terminus contains 11 serine and threonine residues that could serve as phosphorylation sites involved in regulation of the receptor by other cellular kinases. Analogy with the adrenergic receptor and rhodopsin suggests that a specialized kinase involved in deactivation of the FPR may exist (48, 49). The third cytoplasmic loop contains 1 serine and 6 threonine residues.

The structure of the human FPR is exceptionally divergent from that of the rabbit FPR, which has been shown to bind N-formyl peptides and to couple to a calcium mobilizing signaling cascade when expressed in the frog oocyte, without the need for a homologous complementary factor (17). Despite the fact that both proteins are similar in length, have the same overall predicted transmembrane topography, bind the same ligands, and activate the same effector system, only 28% amino acid identity is found (Fig. 7). This is far less than for other transspecies homologues of other G-protein coupled receptors where the relatedness is usually greater than 85%. Moreover, it is much less than the relatedness exhibited by different subtypes within a given subfamily of G-protein coupled receptors, such as the adrenergic receptors or muscarinic receptors, in which greater than 45% amino acid.
FIG. 6. The formyl peptide receptor is a member of the G-protein coupled receptor superfamily. The following sequences were aligned (29, 51): FPR, M1 muscarinic acetylcholine receptor (M1) (40); mas oncogene (MAS) (41); rhodopsin (OPS) (42); substance K receptor (SKR) (33); serotonin 1A receptor (5HT1a) (43); and the β₁, α₁, and α₂ adrenergic receptors (β2AR) (30), (β1AR) (44), (α2AAR) (45), and (α2BAR) (46). All sequences are human with the exception of substance K receptor, which is bovine. Lines indicated by Roman numerals I–VII identify the locations of the putative transmembrane segments established by the Kyte-Doolittle algorithm (47). Arabic numbers above the sequence blocks enumerate the FPR sequence and are left justified. Numbers located on each line correspond to the number of amino acids in each loop which are not shown. The carboxy-terminal 19 amino acids of the α₁ adrenergic receptor are not shown. Asterisks denote the sites of potential N-linked glycosylation in FPR. **Shaded areas** contain amino acids that are identical in at least two other sequences compared with FPR (52). Dots indicate gaps assumed to optimize the alignment. Sequences were chosen with a human preference based on homology scores from a search (53) of the National Biomedical Research Foundation (Washington, D.C.) protein data base release 25.0 (6/90) using the FPR as the search sequence.
sequence identity is found for any pairwise comparison.

The extent of evolutionary drift of the human and rabbit FPR is highlighted further by comparison of their structures with those of receptors for the neuropeptides substance K and substance P (Fig. 7). The rat substance P receptor (50) is 48% identical in primary structure to the bovine substance K receptor. Both the human and rabbit FPR possess 23% amino acid identity (approximately 70–80 conserved residues) with both the substance K and the substance P receptors. However, only 31 residues are invariant among all four of these sequences, 27 of which are highly conserved among all G-protein coupled receptor subfamilies.

The divergence exhibited by the rabbit and human calcium mobilizing formyl peptide receptors is closer to that found when one compares members of different subfamilies of G-protein linked receptors (approximately 20%). A broad pharmacological comparison of the recombinant rabbit and human receptors, which includes an extensive panel of ligands and second messenger systems, may reveal important functional differences and ligand preferences inherent to this structural divergence. Furthermore, one would predict from this degree of divergence that a second human FPR isotype might exist whose structure more closely resembles that of the rabbit receptor and whose transcript would be approximately 3 kb, the size of the rabbit FPR transcript. If so, the data presented in Figs. 2 and 3 strongly suggest that it will almost certainly have evolved either to require the complementary factor for expression in the oocyte, to couple to an alternative effector system, or else to be a silent receptor analogous to certain forms of the atrial natriuretic factor receptor (14).

Taken together, the functional reconstitution and RNA expression data prove that the cDNA clones reported in this paper and, by extension, clones pMLP-R98 and -R26 reported by Boulay et al. (12), encode a calcium mobilizing formyl peptide receptor. This information lays the foundation for future experiments that will lead to a further understanding of the mechanisms of activation and deactivation of the receptor, as well as an understanding of the regulatory elements that limit its expression to mature myeloid cells. In addition, the absolute requirement for a complementary human factor for expression of FPR signal transduction in the frog oocyte was an unexpected finding that provides an opportunity to delineate more exactly the signaling pathways that are activated by the receptor. Precise identification of the molecular nature of the complementary factor may be possible by positive sib selection or hybrid deletion functional cloning strategies. Finally, the successful mixing experiments described in this paper identify a novel approach to identify functionally the increasing number of orphan cDNAs encoding suspected G-protein linked receptors whose ligands are unknown (15, 16).

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