Complementation of formyl peptide receptor-mediated signal transduction in *Xenopus laevis* oocytes

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Formyl-methionine-containing peptides (e.g. fMet-Leu-Phe) stimulate a variety of neutrophil functions by interacting with specific cell surface receptors which are coupled via G-proteins to stimulation of phospholipase C. Two markedly distinct cDNAs coding for formyl peptide receptors have recently been isolated from a rabbit and a human cDNA library respectively. To examine the hitherto unknown signal transduction properties of the formyl peptide receptor encoded by the human cDNA, we have used the PCR to clone this cDNA from poly(A)⁺ RNA of myeloid differentiated human leukaemia (HL-60) cells, and have injected the cDNA-derived receptor cRNA into *Xenopus laevis* oocytes. Receptor activity was determined electrophysiologically by measuring the agonist-dependent opening of intracellular Ca²⁺ concentration ([Ca²⁺]_i)-independent Cl[−] channels. Injection of pure formyl peptide receptor cRNA did not lead to peptide-dependent changes in membrane current. In contrast, marked alterations of membrane current were observed in response to formyl peptides when the receptor cRNA was supplemented with poly(A)⁺ RNA isolated from undifferentiated HL-60 cells. Injection of the latter RNA did not lead to formyl-peptide-dependent alterations of membrane current. Binding studies using a radioiodinated formyl peptide revealed that injection of formyl peptide receptor cRNA alone led to expression of the formyl peptide receptor on the oocyte surface, and that co-injection of poly(A)⁺ RNA from undifferentiated HL-60 cells did not alter the level of receptor expression. Size fractionation of poly(A)⁺ RNA from undifferentiated HL-60 cells showed that the mRNA required to complement formyl-peptide-dependent signal transduction in oocytes had a size of approx. 3–3.5 kb. These results strongly suggest that the human formyl peptide receptor requires a specific cofactor(s), which is lacking in *Xenopus* oocytes but is present in undifferentiated HL-60 cells, to activate the second messenger pathway in oocytes. Identification of this factor will provide important information about the molecular mechanisms by which G-protein-coupled granulocyte-activating receptors stimulate phospholipase C.

INTRODUCTION

Bacterially derived *N*-formyl-methionine-containing peptides are potent stimulators of a variety of neutrophil functions, including chemotaxis, release of lysosomal enzymes and production of superoxide anions [1–3]. Formyl peptides initiate these events by first binding to and then activating specific receptors, which are present on the cell surface of neutrophils and several other phagocytes, including differentiated human leukaemia (HL-60) cells [4]. The agonist-activated formyl peptide receptor is coupled via a pertussis-toxin-sensitive G-protein(s) to stimulation of phospholipase C, which catalyses the breakdown of phosphatidylinositol 4,5-bisphosphate, thus generating the second messengers InsP₃ and diacylglycerol (see [5,6] for reviews).

Similar to other Ca²⁺-mobilizing receptors, the formyl peptide receptor has been expressed for functional studies in *Xenopus* oocytes following the injection of poly(A)⁺ RNA from myeloid-differentiated HL-60 cells (HL-60_d) [7,8]. Two groups have recently reported the cloning of the formyl peptide receptor cDNA from a rabbit neutrophil [9] and a human HL-60 cell cDNA library [10]. Expression of the rabbit cDNA in *Xenopus* oocytes resulted in binding of radiolabelled formyl peptides to oocyte membranes, and led to formyl-peptide-mediated increases in the cytosolic Ca²⁺ concentration ([Ca²⁺]_i) [9]. The human cDNA was isolated on the basis of its ability to confer binding and endocytosis of radiolabelled formyl peptides to COS-7 cells

[10]. However, no data have so far been reported on the transmembrane signalling properties of the protein encoded by this human cDNA. This, together with the markedly dissimilar nucleotide sequences of the two cDNAs (only 56% identical nucleotides in the coding region, resulting in only 27% identical amino acids in the predicted proteins), prompted us to determine whether injection of mRNA transcribed *in vitro* from the human cDNA into *Xenopus* oocytes results in synthesis of a receptor protein capable of eliciting formyl-peptide-dependent cellular responses in these cells. Here we report that the human cDNA does in fact encode a signal-transducing formyl peptide receptor. More important, however, we show that expression of the receptor protein alone does not suffice to confer responsiveness to formyl peptides to *Xenopus* oocytes and that an additional exogenous factor encoded by an approx. 3.0–3.5 kb mRNA present in poly(A)⁺ RNA from undifferentiated HL-60 cells (HL-60_u) is required to complement formyl peptide receptor-mediated signal transduction in this system. A preliminary report of this study has been presented at a meeting and is published in abstract form [11].

MATERIALS AND METHODS

Materials

DNA-modifying enzymes and molecular biological reagents were from Boehringer (Mannheim, Germany). Moloney murine

Abbreviations used: HL-60_u, undifferentiated HL-60 cells; HL-60_d, myeloid differentiated HL-60 cells; Bt₂cAMP, dibutyryl cyclic AMP; fMet-Leu-Phe, *N*-formyl-methionyl-leucyl-phenylalanine; fNle-Leu-Phe-Nle-Tyr-Lys, *N*-formyl-norleucyl-leucyl-phenylalanine-norleucyl-tyrosyl-lysine; 5-HT, 5-hydroxytryptamine (serotonin); M-MLV, monkey murine leukaemia virus; [Ca²⁺]_i, cytosolic Ca²⁺ concentration.

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leukaemia virus (M-MLV) reverse transcriptase was from Gibco/BRL (Eggenstein, Germany). *Thermus aquaticus* DNA polymerase (*Taq* polymerase) was from Perkin-Elmer/Cetus (Überlingen, Germany). RNAasin ribonuclease inhibitor was from Promega (Heidelberg, Germany). Oligo(dT)-cellulose (type 3) was from Collaborative Research (Heidelberg, Germany). Formyl peptides were from Bachem (Heidelberg, Germany) or Bissendorf (Hannover, Germany). All other materials were from Sigma (Deisenhofen, Germany) or Merck (Darmstadt, Germany), or from sources previously described [12].

Cell culture

HL-60 cells were grown in suspension culture as described [12]. Cells were treated for 72 h with 500 μ M-dibutyl cyclic AMP (Bt₂cAMP) to induce myeloid differentiation [13].

Preparation and size fractionation of poly(A)⁺ RNA

Total RNA was prepared as described in [14]. Poly(A)⁺ RNA was selected from total RNA by chromatography (two passes) on oligo(dT)-cellulose as described [15], except that SDS was omitted from the elution buffer. Poly(A)⁺ RNA was size-fractionated by electrophoresis on methylmercuric hydroxide-containing agarose gels as detailed in [15]. The size of the resulting fractions was analysed by Northern blot analysis using ³²P-labelled poly(dT) as a probe [16].

Cloning of the formyl peptide receptor cDNA

Oligo(dT)-primed single-stranded cDNA was made from HL-60 poly(A)⁺ RNA using M-MLV reverse transcriptase as described [17]. A formyl peptide receptor cDNA (designated FPR-A) was amplified from single-stranded cDNA by the PCR [18] using the following oligonucleotides as primers: 5'-TATAGATCTATGGAGACAAATTCCTCTC-3' (upstream, sense) and 5'-GAGAGATCTCTTTCCTGTAAACGCCACCTC-3' (downstream, antisense). Reactions (in 10 mM-Tris/HCl, pH 8.4, 50 mM-KCl, 1.5 mM-MgCl₂, 0.8 mM each of dGTP, dATP, dCTP and dTTP, and 200 μ g of gelatin/ml) contained, in 50 μ l, cDNA from 2 μ g of poly(A)⁺ RNA, 50 pmol of each primer and 1 unit of *Taq* polymerase. Thirty cycles (94 °C, 1.5 min; 55 °C, 2 min; 72 °C, 2.5 min) followed by 72 °C for 5 min were done on a thermal cycler (Perkin-Elmer/Cetus). A second formyl peptide receptor cDNA (FPR-B) was amplified from random-hexamer-primed cDNA using PCR primers with the following sequences: 5'-CACAGATCTCCAGGAGCAGACAAGATGGA-3' (upstream, sense) and 5'-GAGAGATCTACTTTGCCTGTAAACGCCACCTC-3' (downstream, antisense). Reaction mixtures were electrophoresed on SeaPlaque (FMC) agarose gels (2 %), and products of the expected size were cut out and ligated into the single *Bg*/III site on the cloning vector pSP64T [19]. The cloned PCR products were sequenced with Sequenase (United States Biochemical). cRNA was made by *in vitro* transcription of the cloned cDNAs as described [15].

Boulay *et al.* [10] have reported two nucleotide sequences coding for formyl peptide receptors, designated fMLP-R26 and fMLP-R98, which differ from each other by two residue changes (Val¹⁰¹ and Glu³⁴⁶ in fMLP-R26; Leu¹⁰¹ and Ala³⁴⁶ in fMLP-R98). Interestingly, the nucleic acid sequences of both FPR-A and FPR-B predict a formyl peptide receptor with Val and Ala in positions 101 and 346 respectively. No other sequence differences were found between the constructs analysed here and the previously isolated human formyl peptide receptor cDNAs [10]. FPR-A differs from FPR-B in two respects. First, the FPR-A cDNA construct contains a *Bg*/III recognition site immediately upstream of the initiating ATG codon, whereas FRP-B contains an insertion of 16 nucleotides identical in sequence to the corresponding 5' non-coding region reported by Boulay *et al.*

[10] between the *Bg*/III site used for subcloning and the initiating ATG codon. Second, FPR-A is longer by five residues (Arg-Ser-Gly-Tyr-His) at its C-terminus relative to FRP-B, fMLP-R26 and fMLP-R96. Similar results were observed in this study using cRNA derived from both constructs. For simplicity, only the results obtained with FPR-A cRNA are reported here.

Preparation and microinjection of oocytes

Small pieces of ovary tissue were surgically removed from anaesthetized adult *Xenopus laevis* females (Kähler, Hamburg, Germany) and dissected into small groups of approx. 2–4 large oocytes as described [20]. Poly(A)⁺ RNA samples were injected in 50 nl of water (1 ng of RNA/nl) into fully grown oocytes. Following incubation for 72 h at 18 °C in modified Barth's saline (88 mM-NaCl, 1 mM-KCl, 2.4 mM-NaHCO₃, 0.82 mM-MgSO₄, 0.41 mM-CaCl₂, 0.3 mM-Ca(NO₃)₂, 100 units of penicillin G/ml, 100 μ g of streptomycin sulphate/ml, 2.5 mM-sodium pyruvate and 15 mM-Hepes/NaOH, pH 7.5 at 20 °C), the oocytes were defolliculated enzymically as described [21].

Electrophysiology

Single defolliculated oocytes were placed in a continuous-flow chamber perfused with clamp buffer (115 mM-NaCl, 1 mM-KCl, 1.8 mM-CaCl₂ and 10 mM-Hepes/NaOH, pH 7.5 at 20 °C) at a flow rate of 2.5 ml/min. Oocytes were impaled with two borosilicate glass microelectrodes filled with 2 M-KCl and maintained under a voltage clamp (–70 mV) using a Bio-Logic VF-102 dual microelectrode amplifier and a CA-100 clamp amplifier (Science Products, Frankfurt, Germany). Following equilibration of the membrane current to a constant level, fMet-Leu-Phe was included in clamp buffer for 1 min at 10 μ M.

Binding of [¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Leu to intact oocytes

Single defolliculated oocytes were incubated for 30 min at 20 °C in a mixture (50 μ l) containing 15 mM-Hepes/NaOH, pH 7.5 at 20 °C, 88 mM-NaCl, 1 mM-KCl, 2.4 mM-NaHCO₃, 0.82 mM-MgSO₄, 0.41 mM-CaCl₂, 0.3 mM-Ca(NO₃)₂, 0.1 % (w/v) BSA and 2 nM (40 000 c.p.m.) *N*-formyl-norleucyl-leucyl-phenylalanyl-norleucyl-[¹²⁵I]tyrosyl-lysine ([¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Lys). The incubation was terminated by washing the oocyte rapidly with three changes (1 ml each) of ice-cold modified Barth's saline [supplemented with 0.1 % (w/v) BSA, but without antibiotics and sodium pyruvate]. Subsequently the oocyte-associated radioactivity was measured in a γ -radiation counter. Non-specific binding was defined as the binding not competed for by 100 μ M-fMet-Leu-Phe, and amounted to about 200 c.p.m./oocyte.

Miscellaneous

¹²⁵I was incorporated into fNle-Leu-Phe-Nle-Tyr-Lys as described in [22]. [¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Lys was purified by chromatography on a Sep-Pak C₁₈ cartridge (Waters) as described [23]. A single peak of radioiodinated material (400 Ci/mmol) was obtained. Oligonucleotides were synthesized on an Applied Biosystems model 391 DNA synthesizer.

RESULTS AND DISCUSSION

We have used the PCR procedure [18] to isolate the cDNA encoding the human formyl peptide receptor [10] and cloned this cDNA by insertion into the plasmid pSP64T [19]. pSP64T is an SP6 cloning vector designed to provide 5' and 3' flanking regions from an mRNA which is efficiently translated in *Xenopus* oocytes (*Xenopus* β -globin) to any cDNA which contains its own initiation codon. The cloned cDNA was used for *in vitro* synthesis of RNA, which was injected into *Xenopus laevis* oocytes to

Fig. 1. Electrophysiological responses of *Xenopus* oocytes injected with formyl peptide receptor cRNA or 5-HT₂ receptor cRNA to fMet-Leu-Phe and 5-HT

Oocytes which had been injected with formyl peptide receptor cRNA (50 ng) (A), 5-HT₂ receptor cRNA (50 ng) (B) or water (C) were exposed at the times indicated by the arrowheads to 10 μ M-fMet-Leu-Phe (F) or 10 nM-5-HT (S).

analyse the functional properties of the formyl peptide receptor. Receptor activity was determined electrophysiologically by measuring the opening of $[Ca^{2+}]_i$ -dependent Cl^- channels, which is triggered by receptor-mediated formation of $InsP_3$ [24,25].

The results shown in Fig. 1 clearly demonstrate that injection of even high quantities of pure formyl peptide receptor cRNA did not lead to changes in membrane current in response to fMet-Leu-Phe. The inability of the chemotactic peptide to elicit electrophysiological responses was not due to poor expression of foreign receptor proteins in the injected oocytes or to a failure of these receptors to elicit opening of Cl^- channels, since receptor-mediated changes in membrane current were easily detected in oocytes from the same batch which had been injected with cRNA encoding the rat 5-HT₂ receptor [26].

Next we examined the possibility that the formyl peptide receptor was expressed in the injected oocytes, but was unable to elicit electrical responses due to the absence from this system of a specific factor(s) that allows this receptor to stimulate generation of $InsP_3$. Formyl peptide receptor cRNA was supplemented with HL-60_{ud} poly(A)⁺ RNA, which does not contain mRNA coding for the formyl peptide receptor (see below), and receptor activity was determined in injected oocytes by electrophysiological analysis. Fig. 2 shows that neither the formyl peptide receptor cRNA nor HL-60_{ud} poly(A)⁺ RNA by themselves led to fMet-Leu-Phe-dependent changes in membrane current when injected into the oocytes. In contrast, marked electrophysiological responses were seen in oocytes that had been co-injected with the two RNA species.

Fig. 3 shows a summary of the electrophysiological analysis performed on more than 100 oocytes, obtained from several frogs, that had been injected with receptor cRNA alone, HL-60_{ud} poly(A)⁺ RNA alone, or a combination of these two RNAs. The response of oocytes injected with HL-60_{ud} poly(A)⁺ RNA is shown for comparison. It is obvious from these data that there were only minimal or no alterations in membrane currents in oocytes injected with receptor cRNA (1.4 ± 0.8 nA, mean $\pm$ S.E.M., $n = 40$), or HL-60_{ud} poly(A)⁺ RNA (0.9 ± 0.5 nA, $n = 30$) when

Fig. 2. Complementation of formyl peptide receptor-mediated signal transduction in *Xenopus* oocytes

Oocytes that had been injected with formyl peptide receptor cRNA (50 ng) (A), HL-60_{ud} poly(A)⁺ RNA (50 ng) (B), or a combination of these two RNAs (25 ng + 25 ng) (C) were exposed to 10 μ M-fMet-Leu-Phe at the times indicated by the arrowheads.

Fig. 3. Summary of the electrophysiological analysis showing complementation of formyl peptide receptor-mediated signal transduction in *Xenopus* oocytes

Oocytes that had been injected with formyl peptide receptor cRNA (50 ng) (A), HL-60_{ud} poly(A)⁺ RNA (50 ng) (B), a combination of these two RNAs (25 ng + 25 ng) (C) or HL-60_{ud} poly(A)⁺ RNA (50 ng) (D) were exposed to 10 μ M-fMet-Leu-Phe. Each value corresponds to the maximal current evoked in a single oocyte in response to agonist application.

injected alone. However, the combination of these two RNAs resulted in a marked response in almost all of the injected oocytes (350 ± 26 nA, $n = 49$). Note that an even greater electrical response was observed in the latter oocytes than in those which had received HL-60_{ud} poly(A)⁺ RNA (153 ± 24 nA, $n = 18$).

To examine whether the marked stimulatory effect of HL-60_{ud} poly(A)⁺ RNA or formyl peptide receptor function in oocytes was due to a stimulatory effect on the translation of the formyl peptide receptor cRNA, we examined the binding of the radioiodinated chemotactic formyl peptide [¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Lys to oocytes injected with receptor cRNA alone, HL-60_{ud} poly(A)⁺ RNA alone, or a combination of these two RNAs. Binding of [¹²⁵I]fNle-Leu-Phe-Tyr-Lys to oocytes injected with HL-60_{ud} poly(A)⁺ RNA was examined for comparison. As shown

Table 1. Binding of [¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Lys to *Xenopus* oocytes injected with formyl peptide receptor cRNA and/or HL-60_{ud} poly(A)⁺ RNA

Oocytes were injected with formyl peptide receptor cRNA (30 ng) and/or HL-60_{ud} poly(A)⁺ RNA (35 ng), or HL-60_a poly(A)⁺ RNA (60 ng). The amount of [¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Lys specifically bound to individual intact oocytes was determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. of the number of observations indicated in parentheses.

Injected RNA	[¹²⁵ I]fNle-Leu-Phe-Nle-Tyr-Lys bound (c.p.m./oocyte)
None	0 (11)
HL-60 _{ud} poly(A) ⁺ RNA	0 (11)
Receptor cRNA	785 $\pm$ 138 (10)
Receptor cRNA + HL-60 _{ud} poly(A) ⁺ RNA	978 $\pm$ 169 (11)
HL-60 _a poly(A) ⁺ RNA	152 $\pm$ 29 (11)

In Table 1, no specific binding of [¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Lys was found for non-injected oocytes or oocytes injected with HL-60_{ud} poly(A)⁺ RNA. In contrast, specific binding of the radiolabelled peptide was clearly detected with oocytes that had received either formyl peptide receptor cRNA alone or a combination of the receptor cRNA and HL-60_{ud} poly(A)⁺ RNA. The amount of [¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Lys was not significantly different for the latter two types of oocytes, but was clearly much higher than the amount of radiolabelled peptide bound to oocytes injected with HL-60_a poly(A)⁺ RNA. Taken together, these results clearly indicate that an enhancement of receptor expression is unlikely to be the mechanism by which HL-60_{ud} poly(A)⁺ amplifies the response of receptor cRNA-injected oocytes to fMet-Leu-Phe.

To further characterize the properties of the mRNA required for formyl peptide receptor-mediated signal transduction in *Xenopus* oocytes, HL-60_{ud} poly(A)⁺ RNA was fractionated by size and the fractions were supplemented with receptor cRNA prior to injection into oocytes. Table 2 shows that only two mRNA fractions obtained through the fractionation procedure were able to complement the receptor cRNA. The fraction encompassing mRNAs ranging in size from approx. 3.0 to 5.5 kb was more efficient than the fraction containing mRNAs ranging from approx. 1.7 to 3.0 kb. If one assumes that only a single species of mRNA is required for complementation of the signal transduction pathway, our results would indicate that this mRNA has an approximate size of 3.0 to 3.5 kb. However, our findings do not exclude the possibility that more than one mRNA species is involved in complementation.

The nature of the specific factor(s) required to complement the human formyl peptide receptor in *Xenopus* oocytes is unknown and requires further investigation. Our observations that the radiolabelled formyl peptide [¹²⁵I]fNle-Leu-Phe-Nle-Tyr-Lys binds to oocytes injected with receptor cRNA alone, and that this binding is not significantly enhanced by co-injection of HL60_{ud} poly(A)⁺ RNA, indicates that the complementary factor is unlikely to be involved in stabilization of the injected receptor cRNA or in enhancing its translational efficiency. It is also unlikely that the factor is important for post-translational processing or membrane integration of the receptor protein. On the other hand, our finding that the 5-HT₂ receptor can be functionally expressed in oocytes from the same batch without requiring complementation strongly indicates that the complementary factor acts at a step prior to the InsP₃-mediated release of Ca²⁺ from internal stores.

On the basis of these considerations, it appears likely that the

Table 2. Size approximation of the mRNA species required to complement formyl peptide receptor signal transduction in *Xenopus* oocytes

HL-60_{ud} poly(A)⁺ RNA was fractionated on a methylmercuric hydroxide-containing agarose gel. Each fraction was supplemented with a formyl peptide receptor cRNA (20 ng) prior to injection into the oocytes. Values are means $\pm$ S.E.M. of the maximal currents evoked in the numbers of oocytes given in parentheses.

Approximate mRNA size (kb)	Peak current (nA)
< 0.7	0 (4)
0.7–1.0	0 (5)
1.0–1.7	0 (7)
1.7–3.0	89 $\pm$ 39 (8)
3.0–5.5	151 $\pm$ 29 (9)
> 5.5	0 (5)

missing factor(s) corresponds to one or more subunits of a G-protein that is specifically involved in coupling the formyl peptide receptor to phospholipase C, or to a specific phospholipase C isoenzyme which is lacking in oocytes but present in granulocytes to mediate formyl peptide receptor action. In membranes from HL-60_a cells, the agonist-activated formyl peptide receptor interacts with G_{i2} and G_{i3} [27]. It is conceivable, therefore, that a specific subunit of these two G-proteins is either missing or present at too low a concentration in oocytes. However, we have so far been unsuccessful in attempts to reconstitute formyl peptide receptor-mediated signal transduction in oocytes by co-injection of cRNA encoding rat G_{i2} or G_{i3} [28] or human G β 1 [29]. Note, however, that we do not know whether the corresponding proteins were in fact synthesized in the injected oocytes. Of interest is the isolation of cDNAs encoding α -subunits closely related to mammalian G_{i1}, G_{i3}, G_{o2}, and G_{o3} from a *Xenopus* oocyte cDNA library [30]. One should keep in mind, however, that *Xenopus* oocytes accumulate large quantities of translationally blocked ('masked') maternal mRNAs [31]. Thus the cloning of a cDNA from a *Xenopus* oocyte library does not necessarily imply that the corresponding protein is actually produced by the oocyte.

There is no information currently available on the nature of the phospholipase C isoenzyme(s) present in *Xenopus* oocytes. However, there is an increasing body of evidence suggesting that the main phospholipase C isoenzymes present in HL-60 cells differ structurally from the major forms identified in other tissues [32,33]. It is therefore tempting to speculate that one of these HL-60-cell-specific phospholipase C isoenzymes may be absent from *Xenopus* oocytes, but required in this system to couple the human formyl peptide receptor to trigger an increase in InsP₃.

An important question to be clarified by future experiments is whether the requirement of a complementary factor for successful transmembrane signalling in *Xenopus* oocytes is specific for the human formyl peptide receptor analysed here. Clearly there are a number of G-protein-coupled receptors fully capable of eliciting agonist-mediated responses in *Xenopus* oocytes without requiring exogenous cofactor(s) [34]. One of these receptors, the rabbit formyl peptide receptor, is a particularly interesting example, because it is activated by the same ligands as the human receptor examined in this report. An intriguing question is whether an analogue of the rabbit formyl peptide receptor exists in human granulocytes and, if so, whether it too has activity independent of any complementary factor when expressed in oocytes [9]. This would suggest that two isoforms of the formyl peptide receptor exist in human granulocytes and that they differ

not only in structure but also in the molecular mechanisms by which they activate phospholipase C.

A very important implication of our findings is that the *Xenopus* oocyte expression system may be suited to isolating the cDNAs of some, but not all, receptors coupled to activation of phospholipase C, since signals from receptors requiring complementation would almost certainly be lost during the screening procedure. Likewise, negative results obtained when putative G-protein-coupled receptors ('orphan receptors') (see refs. [35–37] for examples) are examined for activating ligands by injecting their cRNA into *Xenopus* oocytes have to be interpreted with great caution, because the receptor in question could also depend on complementation. It is clear that isolating the cDNA(s) encoding the complementary factor(s) described here is a very important and immediate goal, as co-injection of both receptor and cofactor cRNA would obviate these serious limitations of the *Xenopus* oocyte as an expression system for receptors coupled via G-proteins to stimulation of phospholipase C.

After completion of this work, Murphy & McDermott [38] reported that functional expression of formyl peptide receptors encoded by cDNAs corresponding in sequence to those published by Boulay *et al.* [10] in *Xenopus* oocytes requires a complementary human factor present in HL-60_{ud} poly(A)⁺ RNA. The ability of formyl peptides to elicit ⁴⁵Ca²⁺ efflux from injected oocytes was examined in that study. Our results are consistent with that report. One major difference, however, between the results reported here and the data obtained by Murphy & McDermott [38] is that no information on formyl peptide receptor expression in the injected oocytes was provided in the latter report. It was not clear, therefore, whether HL-60_{ud} poly(A)⁺ RNA acted by enhancing the stability or increasing the transcription of the receptor RNA, or by facilitating the co- or post-translational processing of the receptor polypeptide. This is an important issue, because there are several examples of regulated stability of receptor mRNAs [39–42], and because it is well known that a variety of mechanisms exist in *Xenopus* oocytes to suppress the translation of certain poly(A)⁺ RNAs [31,43–47]. Furthermore, there are several reports suggesting a requirement for cofactors for membrane insertion and post-translational 'activation' of G-protein-coupled receptors [48–50]. The results presented here clearly indicate: (1) that injection of formyl peptide receptor cRNA alone is sufficient to induce formyl peptide receptor expression in *Xenopus* oocytes, and (2) that co-injection of HL-60_{ud} poly(A)⁺ RNA does not influence the level of receptor expression in this system. These findings allow us to exclude a great number of potential mechanisms of action of the complementary factor described here.

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