building process goes on to the next step. Steps 1 to 5 correspond to the enlargement of the top of the nest, including the first sessile comb of cells (step 3). Steps 6 and 7 represent the construction of the external envelope, from which parallel floors are built (steps 8 and 9). The final step 9 determines the location of the entrance and access holes at the different levels (in the periphery of the architecture). Highly structured architectures with a complex long-range order can be generated with our simple model (Fig. 4). We suggest that the pattern in Fig. 4 is reminiscent of helicoidal ramps that make wide communication structures between the successive floors observed in the nest of the termite *Apicotermes arquieri* (*10*).

In summary, we have shown, within the framework of a simple computer model, that distributed algorithms have to belong to a particular class of algorithms, which we named "coordinated algorithms," to generate coherent structures in a strictly stigmergic mode. In such algorithms, local patterns of matter that result from past construction provide the exclusive cues necessary to direct and coordinate the building activities of the swarm. Therefore, any coherent architecture naturally induces coordination, which may then be seen as a by-product of the architecture; moreover, coordination severely constrains in turn the space of possible coherent architectures. In view of these results, ethologists may be able to take advantage of our approach "despite" its formal nature.

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Molecular Identification of an IgE-Dependent Histamine-Releasing Factor

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An immunoglobulin E (IgE)–dependent histamine-releasing factor (HRF) produced by lymphocytes of atopic children and present in biological fluids of allergic patients has been identified and purified. Amino-terminal sequencing revealed extensive homology to a mouse protein, p21, and its human homolog, p23. Both recombinant proteins caused histamine release from the human basophils of a subpopulation of donors, and this release was dependent on IgE. Polyclonal antibodies recognized and removed the biological activity of recombinant and native HRF. HRF identifies a heterogeneity of IgE and is believed to play a prominent role in chronic allergic disease processes.

Several in vivo systems are used to study human allergic disease. The model currently favored is the late response in humans that occurs hours after the immediate reaction to allergen challenge (*1*). The pathophysiological events, such as decreased airway function, are characterized by infiltration of inflammatory cells and by the presence of mediators such as histamine, which are findings similar to those observed in people with chronic allergic diseases such as asthma. The histamine released in this late-phase reaction (LPR) results from activation of basophils that, along with eosinophils and lymphocytes, infiltrate tissues during this response (*1*, *2*). Because the allergen that initiated the response is no longer present, two questions require study: the nature of the agent that triggers the release of inflammatory mediators from the basophils in the LPR, and the basis for the observation that only about half of allergic individuals experience an LPR.

Many laboratories have been attempting to answer these questions by identifying cytokines with histamine-releasing activity on human basophils. The first such molecule was described 15 years ago in the supernatants from peripheral blood mononuclear cell (PBMC) preparations (*3*). A decade ago we described an HRF in fluids derived from the LPR in the skin (*4*). This HRF was dependent on IgE bound to basophils, and it defined an IgE heterogeneity: Only half of atopic individuals had basophils that responded to HRF. Responders had serum IgE—which we designated as IgE^+—which could passively sensitize normally unresponsive basophils to release histamine in response to the HRF (*5*). A variety of cytokines with histamine-releasing activity have recently been discovered, including several C-C chemokines—MCP-3, MCP-1, and RANTES—as well as interleukins such as interleukin-3 (IL-3) (*6*–*9*). None of these molecules cause basophils to release histamine by a mechanism that involves cell surface IgE. This report describes the subcloning, sequencing, and expression of a unique IgE-dependent HRF.

Although an IgE dependent HRF can be detected in nasal lavages (*5*), PBMC culture supernatants (*10*), and fluids from human LPR (*11*), we used supernatants from overnight cultures of U937 cells, a human macrophage cell line (*12*), for the isolation and sequencing of the HRF. Fifty liters of these supernatants were concentrated and the proteins contained therein purified by Sephadex G75 gel filtration, MONO Q anion exchange, and repetitive Superdex chromatography. The basophil-releasing activity was concentrated, subjected to SDS–polyacrylamide gel electrophoresis (PAGE), blotted onto a polyvinylidene difluoride membrane, and stained with Coomassie blue, revealing four major protein bands (at 60 kD and 29 kD and a doublet at 23 kD). The NH_2-terminal sequences of each of these four bands were determined by protein sequencing. The 18 NH_2-terminal amino acids of one of the 23-kD components are shown in Fig. 1. A GenBank search revealed 94% homology to p21, a predicted 21-kD murine peptide whose complementary DNA (cDNA) was isolated from mouse tumor cells (*13*), as well as identity to p23, the human homolog, described by Böhm *et al.* (*14*). The complete protein sequences of p21 and p23 are also shown in Fig. 1. Both were cloned on the basis of their abundant expression in tumor cells, and no function has been ascribed to either molecule. The p21 and p23 proteins do not have obvious hydrophobic residues at the NH_2-termini, which suggests that they are not secreted. However, because HRF is found in in vivo fluids, it may use a secretory pathway such as that described for IL-1β; or, again like IL-1, it may be released during apoptosis (*15*). Because there is a stop codon upstream from the initial methionine, it appears that p21 and p23 are not posttranslationally processed at their NH_2-termini. p21 cDNA was subcloned into the pGEX-2T plasmid (*16*), expressed as a fusion protein with glutathione-S-transferase (GST),

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purified, and isolated from GST by cleavage with thrombin. Due to the homology between p21 and p23, the same synthetic primers, based on the mouse p21 sequence, were used to isolate the human p23 cDNA from the U937 cell line. This protein also was expressed in *Escherichia coli* as a GST fusion protein and was subsequently cleaved from GST with thrombin.

The biological activities of the recombinant (r) p21-GST fusion protein, of cleaved rp21, of cleaved rp23, and of the HRF from nasal lavages, and from PBMC culture supernatants were assessed in basophil histamine-release assays. Because rp21-GST was cloned first, we have most experience with this molecule, but in each instance rp23 had exactly the same activity. The rp21-GST caused histamine release at protein concentrations of 0.2 to 200 μg/ml, or approximately 10^{-8} to 10^{-6} M (Fig. 2A). This recombinant product is less active than the HRF derived from the LPR fluids or from U937 supernatants, probably because it lacks posttranslational modifications. The rp21-GST caused histamine release from seven additional IgE$^+$ donor cell preparations and failed to release histamine from eight IgE$^-$ basophil preparations.

The kinetics of release by rp21-GST, by HRF obtained from biological fluids [nasal pool (NP) HRF], and by antibody to IgE were identical (Fig. 2B) and were much slower than the rate of mediator release induced by chemokines, in particular MCP-1, which causes complete release in 1 min (*7*). The kinetics were also significantly different from those of other cytokines, such as IL-3, which induce release much more slowly over a broad range of concentrations (*9*).

Figure 2C shows the results of a representative passive sensitization experiment. The cell surface IgE was removed from basophils with the use of lactic acid, and the cells were then passively sensitized with serum containing either IgE$^+$ or IgE$^-$. Release was initiated with rp21-GST, the biological fluid HRF (NP HRF), and antibody to IgE. Both the recombinant molecule and the native HRF released histamine only in the presence of IgE$^+$, whereas antibody to IgE was active under both conditions. From these data we conclude that the biological activities of p21 and p23 correspond to those of the human HRF.

Rabbit polyclonal antibodies to rp21 were generated by repetitive injections of the recombinant material in complete and incomplete Freund's adjuvant. The antibody raised recognized rp21, human rp23, and the native HRF from the U937 cell line (Fig. 3A). Rabbit polyclonal antibodies were also generated against human rp23. The rp23, native HRF (NP HRF), and PBMC culture supernatants were run over an affinity column prepared from an IgG fraction of these antibodies. Five milligrams of total rabbit IgG antibody to p23 were coupled to Sepharose, but only a small percentage of the total IgG was specific for rp23, because approximately 90% of rp23 (as measured by optical density) was recovered in the effluent. However, the rp23 protein eluted from this affinity column had the expected biologic activity. Also, the eluted NP and PBMC HRF caused basophil histamine release (39 and 42%, respectively) from basophils of an IgE$^+$ donor. On protein immunoblot, the PBMC starting material, the guanidine-eluted protein from the PBMC supernatant, and the authentic

p21	Met	Ile	Ile	Try	Arg	Asp	Leu	Ile	Ser	His	Asp	Glu	Leu	Phe	Ser	Asp	Ile	Try		
p23	-	-	-	-	-	-	-	-	-	-	-	-	Met	-	-	-	-	-		**18**
HRF	-	-	-	-	-	-	-	-	-	-	-	-	Met	-	-	-	-	-		
p21	Lys	Ile	Arg	Glu	Ile	Ala	Asp	Gly	Leu	Cys	Leu	Glu	Val	Glu	Gly	Lys	Met	Val	Ser	Arg **38**
p23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
p21	Thr	Glu	Gly	Ala	Ile	Asp	Asp	Ser	Leu	Ile	Gly	Gly	Asn	Ala	Ser	Ala	Glu	Gly	Pro	Glu **58**
p23	-	-	-	Asn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
p21	Gly	Glu	Gly	Thr	Glu	Ser	Thr	Val	Val	Thr	Gly	Val	Asp	Ile	Val	Met	Asn	His	His	Leu **78**
p23	-	-	-	-	-	-	-	-	Ile	-	-	-	-	-	-	-	-	-	-	-
p21	Gln	Glu	Thr	Ser	Phe	Thr	Lys	Glu	Ala	Tyr	Lys	Lys	Tyr	Ile	Lys	Asp	Tyr	Met	Lys	Ser **98**
p23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
p21	Leu	Lys	Gly	Lys	Leu	Glu	Glu	Gln	Lys	Pro	Glu	Arg	Val	Lys	Pro	Phe	Met	Thr	Gly	Ala **118**
p23	-	-	-	-	-	-	-	-	Arg	-	-	-	-	-	-	-	-	-	-	-
p21	Ala	Glu	Gln	Ile	Lys	His	Ile	Leu	Ala	Asn	Phe	Asn	Asn	Tyr	Gln	Phe	Phe	Ile	Gly	Glu **138**
p23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
p21	Asn	Met	Asn	Pro	Asp	Gly	Met	Val	Ala	Leu	Leu	Asp	Tyr	Arg	Glu	Asp	Gly	Val	Thr	Pro **158**
p23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
p21	Phe	Met	Ile	Phe	Phe	Lys	Asp	Gly	Leu	Glu	Met	Glu	Lys	Cys						**172**
p23	Tyr	-	-	-	-	-	-	-	-	-	-	-	-	-						

Fig. 1. The NH_2-terminal amino acid sequence of the 23-kD band from the native U937 HRF is compared with the NH_2-terminal sequences of the mouse p21 and human p23 proteins. The remaining amino acids of p21 and p23 are also compared (*21*).

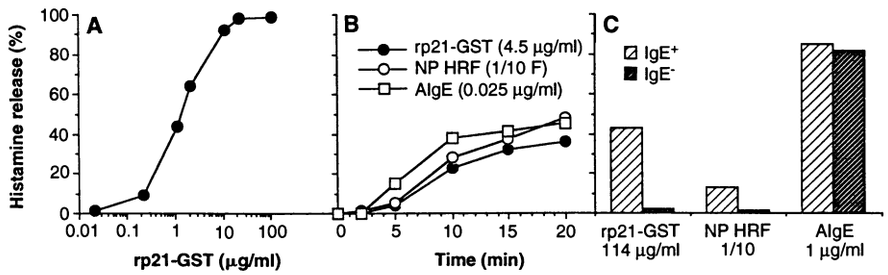

Fig. 2. (**A**) Dose-response curve of the histamine release from basophils of an IgE$^+$ subject by recombinant mouse HRF (rp21-GST). A representative example of seven IgE$^+$ individuals tested is shown (*22*). (**B**) Kinetics of histamine release by recombinant mouse HRF (rp21-GST), partially purified human nasal lavage HRF (NP HRF), and antibody to IgE (AIgE). With all stimuli, secretion is slow, with 50% of maximal release occurring at 5 to 10 min. A representative example of five experiments is shown (*22*). (**C**) Effect of passive sensitization of lactic acid–treated basophils with IgE$^+$ and IgE$^-$ molecules. Lactic acid–treated cells were incubated with sera containing IgE (2000 ng/ml). A representative example of five experiments with three basophil donors are shown. Two additional sera, one containing IgE$^+$ and one IgE$^-$, gave similar results (*23*).

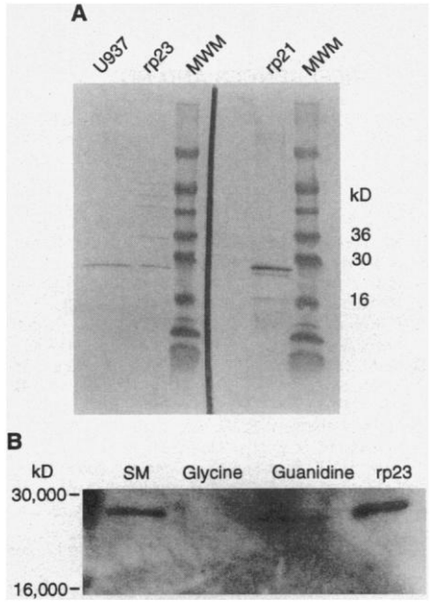

Fig. 3. (**A**) On a protein immunoblot, polyclonal rabbit antibody to rp21 recognizes the 23-kD band from the HRF isolated from the U937 cell line, the recombinant human HRF (rp23), and the recombinant mouse HRF (rp21) (*20*). MWM, molecular weight marker. (**B**) Polyclonal rabbit antibody to rp23 recognizes a protein in the column starting material (SM) from PBMC culture supernatants, nothing in the glycine eluate from a rabbit anti-p23 affinity column, and a protein in the guanidine eluate from the anti-p23 affinity column. Both proteins appeared in similar positions to that of authentic rp23, the recombinant human HRF (far right), (*24*) and had activity.

rp23 appeared as the expected 23-kD bands, whereas the glycine eluate did not have biologic activity or a protein band (Fig. 3B). With the use of p23-based primers, analyses by Northern (RNA) blot and by reverse transcription polymerase chain reaction (RT-PCR) indicated that mRNA for the IgE-dependent HRF was ubiquitous: It was found in B cells, T cells, mononuclear cells, and fibroblasts. The transcript could not be detected, however, in isolated human skin mast cells or in the rat basophilic leukemic cell line.

Efforts to identify an IgE-dependent HRF have occupied several laboratories for the past decade. Now that it is characterized, we expect that this molecule, which is linked to the intensity of the LPR (*17*) and to the severity of atopic dermatitis in children (*10*), will allow us to better understand the pathogenesis of human allergic disease and to appreciate the nature and significance of the heterogeneity of IgE, which may be a genetically determined polymorphism, may be due to differential glycosylation of the IgE molecule, or may be based on interactions with the alternatively spliced forms of human IgE reported to be present in human atopic serum by Zhang *et al.* (*18*). Efforts are ongoing to decipher whether this molecule interacts solely with IgE or, additionally, binds to its own receptor.

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20. Native material from the U937 macrophage cell line, human recombinant HRF (rp23), mouse recombinant HRF (rp21), and SEEBLUE prestained molecular weight markers (Novex, San Diego, CA), were run on a 4 to 20% tris glycine gradient gel (Novex) by an established method [U. K. Laemmli, *Nature* **227**, 680 (1970)]. The gel was blotted onto nitrocellulose. A rabbit polyclonal antibody generated against rp21 was used as a primary antibody, and the proteins were visualized with goat IgG to rabbit IgG, coupled to alkaline phosphatase. The additional bands that appeared in the lanes containing rp21 and rp23 were caused by nonspecific binding of the antiserum to *E. coli* proteins. The antibody recognizes a 23-kD band in each preparation, corresponding to the native HRF from the U937 line, to 0.04 μg of rp21, and to 0.14 μg of rp23.
21. The following primers were used to amplify the p21 cDNA from the Okayama-Berg vector: 5′ primer, 5′-AAAAGGATCCATGATCATCTACCGGGACC-3′; 3′ primer, 5′-AAAGAATTCTTAACATTTCTCCATCTCTAAGCC-3′. The resultant PCR product was restricted with Bam HI and Eco RI, ligated in frame with GST into the pGEX-2T plasmid, and transfected into JM109 competent cells (Promega). Production and purification of the recombinant GST fusion protein were done by means of affinity chromatography on immobilized glutathione (*16*). To separate p21 from GST, the GST p21 fusion protein was cleaved with thrombin with the use of the Pharmacia bulk GST purification module.
22. Partial purification of NP HRF was as previously described (*5*). After informed consent, peripheral blood was obtained from participants by venipuncture. Mixed leukocytes containing basophils were prepared by dextran sedimentation as previously described [R. P. Schleimer *et al.*, *J. Immunol.* **128**, 1632 (1982)]. Histamine release was done by a standard method (*5*), and histamine was measured by means of an automated fluorometric method [R. P. Siraganian, *J. Immunol. Meth.* **7**, 283 (1975)].
23. A lactic acid method [J. J. Pruzansky *et al.*, *J. Immunol.* **131**, 1949 (1983)] was used to dissociate IgE from basophils. Passive sensitization was accomplished as described [D. A. Levy and A. G. Osler, *ibid.* **97**, 203 (1966)]. After addition of the stimuli, the released histamine was measured as described above.
24. PBMC were isolated with the use of lymphocyte separation media (Ficoll-Paque, Pharmacia), and 5 × 10^6 cells per milliliter in 80 ml were stimulated with concanavalin A (5 μg/ml) (Sigma) for 24 hours at 37°C in 5% CO_2. Supernatants were harvested, centrifuged, dialyzed against a Pipes buffer, concentrated 26-fold, and placed on 5 ml of cyanogen-activated Sepharose CL-4B (*19*) beads covalently coupled to IgG anti-human p23 (1 mg/ml). The affinity column was washed with four column volumes of phosphate-buffered saline starting buffer, followed by two column volumes of 0.1 M glycine (pH 3) and then two column volumes of 5 M guanidine. The eluates were concentrated 100-fold and tested for basophil histamine-releasing activity as described above. Starting material from the concentrated PBMC supernatants, glycine eluate, guanidine eluate, human recombinant HRF (rp23), and SEEBLUE molecular weight markers were run on a 4 to 20% tris-glycine gradient gel as described above (*20*). The gel was blotted onto nitrocellulose (Schleicher and Schull, Keene, NH). A rabbit IgG polyclonal antibody generated against rp23 was the primary antibody, and a goat IgG to rabbit coupled to alkaline phosphatase was the secondary antibody. The protein immunoblot was developed with the use of enhanced chemiluminescence according to the manufacturer's instructions (Amersham).

25. We thank M. Koots and G. Brawerman (Tufts University, Boston, MA) for supplying p21 cDNA that was isolated from a mouse L cell library, ImmuLogic Corporation for NH_2-terminal sequencing of the four polyvinylidene difluoride transferred bands, and D. Redburn for scientific advice. Supported by NIH grants AI 32651 and AI 07290. For early studies, ImmuLogic Corporation supplied funds for obtaining 50 liters of U937 supernatants.

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Cloning and Characterization of a G Protein–Activated Human Phosphoinositide-3 Kinase

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Phosphoinositide-3 kinase activity is implicated in diverse cellular responses triggered by mammalian cell surface receptors and in the regulation of protein sorting in yeast. Receptors with intrinsic and associated tyrosine kinase activity recruit heterodimeric phosphoinositide-3 kinases that consist of p110 catalytic subunits and p85 adaptor molecules containing Src homology 2 (SH2) domains. A phosphoinositide-3 kinase isotype, p110γ, was cloned and characterized. The p110γ enzyme was activated in vitro by both the α and βγ subunits of heterotrimeric guanosine triphosphate (GTP)–binding proteins (G proteins) and did not interact with p85. A potential pleckstrin homology domain is located near its amino terminus. The p110γ isotype may link signaling through G protein–coupled receptors to the generation of phosphoinositide second messengers phosphorylated in the D-3 position.

Phosphoinositide-3 kinase (PI-3K) activity increases in response to numerous ligands, including those that signal through receptor tyrosine kinases, cytokine receptors, and G protein–coupled receptors (*1–3*). The activation of PI-3K by membrane-bound receptor tyrosine kinases results from recruitment of p110-p85 heterodimers to membrane-bound signaling complexes; this process is mediated by the SH2 domains of p85 that