mal development, the dominant mode of action could arise from somatic segregation or mutation that eliminates or alters the normal allele at the *Min* locus or affects dosage of another locus (*6*). The question of whether loss of the normal allele at the *Min* locus is a necessary step in the process of tumorigenesis can be investigated when the *Min* locus has been mapped and linked probes are available. Then, analysis of tumor tissue for evidence of somatic segregation at this locus will be possible. By contrast, a gain-of-function mutation could generate a true dominant allele capable of inducing the transformation of intestinal cells.

In the human, a number of hereditary syndromes include intestinal tumor formation; examples are familial adenomatous polyposis, Gardner's syndrome, Lynch syndrome, and several other nonpolyposis cancer syndromes (*7*). The identification of the *Min* mutation demonstrates that mutant genes resulting in similar phenotypes can be recovered in mice. Mouse germline mutagenesis may also allow the identification of mutant genes that are not frequent in natural populations. The tumors in *Min*/+ mice arise as the result of an induced germline mutation and, unlike many mouse tumors, do not have a known viral etiology. Mouse models such as the *Min* mutant should provide a major resource for study of pathways of tumorigenesis and for the molecular identification and manipulation of genes that can control particular neoplastic processes.

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Cloning of an Interleukin-3 Receptor Gene: A Member of a Distinct Receptor Gene Family

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Interleukin-3 (IL-3) binds to its receptor with high and low affinities, induces tyrosine phosphorylation, and promotes the proliferation and differentiation of hematopoietic cells. A binding component of the IL-3 receptor was cloned. Fibroblasts transfected with the complementary DNA bound IL-3 with a low affinity [dissociation constant (K_d) of 17.9 ± 3.6 n*M*]. No consensus sequence for a tyrosine kinase was present in the cytoplasmic domain. Thus, additional components are required for a functional high affinity IL-3 receptor. A sequence comparison of the IL-3 receptor with other cytokine receptors (erythropoietin, IL-4, IL-6, and the β chain IL-2 receptor) revealed a common motif of a distinct receptor gene family.

INTERLEUKIN-3, PRIMARILY PRODUCED by activated T cells, stimulates colony formation of multiple lineages (multi-CSF), maintains spleen colony forming units (CFU-S), stimulates mast cell growth and histamine release, induces 20-α-steroid dehydrogenase and the Thy1 antigen, serves as a growth factor for megakaryocytes, and acts on pre–B cells and potentially on pre–T cells (*1*). Bone marrow stromal cells probably do not produce IL-3 (*2*); IL-3 may preferentially participate in the expansion of hematopoietic cells in response to inflammatory stimuli rather than in the constitutive hematopoiesis of the bone marrow (*3*).

Murine IL-3 binds to its receptor with both high and low affinities (*4*). Although the molecular size of the IL-3 receptor was initially reported as 60 to 70 kD (*5*), recent cross-linking results indicate the presence of additional binding proteins of 140 and 120 kD (*4*). A protein tyrosine kinase seems to participate in IL-3 signal transduction. Expression of oncogenes with tyrosine kinase activity abrogates IL-3 dependence (*6*). Particularly, a mutant v-*abl* with a temperature-sensitive tyrosine kinase abrogates IL-3 dependence in a temperature-dependent manner (*7*). Tyrosine phosphorylation of a set of cellular proteins is induced within a minute after addition of IL-3, which suggests the close association of a tyrosine kinase with the IL-3 receptor (*8*). IL-3 induced the tyrosine phosphorylation of a 140-kD IL-3 binding protein (*9*). Thus, either the IL-3 binding protein contains an intrinsic tyrosine kinase activity, or the IL-3 binding protein and the tyrosine kinase are distinct molecules.

Using IL-3–dependent murine cells as an immunogen, we obtained a monoclonal antibody (MAb), anti-Aic2, to a 105-kD cell surface protein present on various IL-3–dependent cell lines (*10*), whose expression correlates with IL-3 binding. The antibody partially inhibits the binding of IL-3 to its receptor and immunoprecipitates several ^{125}I-IL-3 cross-linked proteins. Thus, the Aic2 antigen is probably a component of the IL-3 receptor (*10*). We now have cloned and expressed the Aic2 cDNA, shown that the Aic2 antigen is a binding component of the IL-3 receptor, and identified a family of

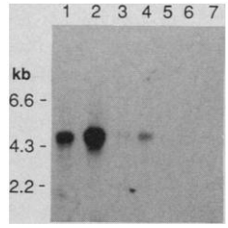

Fig. 1. An RNA blot analysis of IL-3 receptor mRNA. Polyadenylated RNA was isolated from MC/9 (lane 1), PT18 (lane 2), NFS60.8 (lane 3), DA3.15 (lane 4), HT-2 (lane 5), D9 (lane 6), and total liver cells (lane 7). The RNA (2 μg) was denatured and subjected to blotting analysis with the AIC2-2 cDNA. The numbers in the left lane indicate the position of Hind III fragments of λ phage DNA (in kilobases).

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receptors distinct from previously described receptor families.

We constructed a cDNA library from an IL-3–dependent mast cell line, MC/9, expressed it in COS7 cells, and screened it with anti-Aic2 (*11*). Plasmid DNA recovered from the COS7 cells transiently transfected with the cDNA library was used to transform *Escherichia coli*. After three cycles of selection, we analyzed individual plasmids and obtained the AIC2-2 cDNA that expressed the Aic2 antigen in COS7 cells. Since cDNAs recovered from COS7 cells may have mutations or rearrangements that occurred during the propagation of plasmids in COS7 cells, we used the AIC2-2 cDNA as a hybridization probe to isolate additional cDNAs from the original cDNA library. Nucleotide sequencing of the resulting cDNA, AIC2-26, revealed a cell surface protein with a single transmembrane domain.

The Aic2 mRNA was examined by hybridization with a cDNA insert from AIC2-2 as a probe. The RNA blot analysis showed a 4.6-kb mRNA in IL-3–responsive cells such as mast cell lines (MC/9, PT18) and myeloid cell lines (NFS60.8 and DA3.15), but not in IL-3–nonresponsive cells such as T cell lines (HT2 and D9) and liver (Fig. 1). The mRNA expression was variable; the highest expression was in the mast cell lines, MC/9 and PT18, which is consistent with the expression of the Aic2 antigen. In contrast, NFS60.8, a GM-CSF–dependent subline of NFS60 that requires a higher concentration of IL-3 due to its low expression of the IL-3 receptor (*12*), expressed relatively little Aic2 mRNA (Fig. 1).

We examined whether the Aic2 antigen bound IL-3. The COS7 cells transiently transfected with AIC2-26 cDNA specifically bound ^{125}I–IL-3: IL-2, IL-4, and GM-CSF did not effectively compete at concentrations up to 10^{-5} *M* (Fig. 2A). To determine the affinity of the IL-3 binding, we assayed IL-3 displacement on L cell stable transfectants (cl26) (Fig. 2B). Iterative curve fitting indicated a single binding site with an apparent dissociation constant (K_d) of 17.9 ± 3.6 n*M* at 4°C (Fig. 2B, insert) and 5.7 ± 1.0 n*M* at 37°C, consistent with the low affinity binding site previously characterized on various IL-3–dependent cells (*4*). Similarly, a single equilibrium binding constant was obtained by radioligand saturation assays. Because the dissociation rate of IL-3 from the low affinity binding sites at 4°C is faster ($t_{1/2}$ = 4 min) than that from the high affinity site ($t_{1/2}$ = 4 hour) (*4*), we measured the rate of ^{125}I–IL-3 dissociation from COS7 cells and L cells transfected with AIC2-26 cDNAs (Fig. 2C) and found that the $t_{1/2}$ was about 3 min. At 37°C, the dissociation was faster ($t_{1/2}$ = 6 s), similar to the IL-2 receptor β chain (*13*). Thus the cloned cDNA encodes an IL-3 binding protein with a low affinity when expressed in fibroblasts.

As there was no ligand-dependent internalization of the AIC2-26 protein at 37°C and no stimulation of tyrosine phosphorylation in L cell stable transfectants (*14*), the protein expressed in fibroblasts was nonfunctional. In contrast, the Aic2 antigen expressed on IL-3–dependent cells was downregulated by IL-3 (*10*), indicating that a cell type–specific factor may modulate the properties of the Aic2 antigen.

Both MC/9 and stable L cells transfected with the AIC2-26 cDNA had ^{125}I–IL-3 cross-linked complexes of about 160, 140, and 90 kD (Fig. 2D). The calculated size of

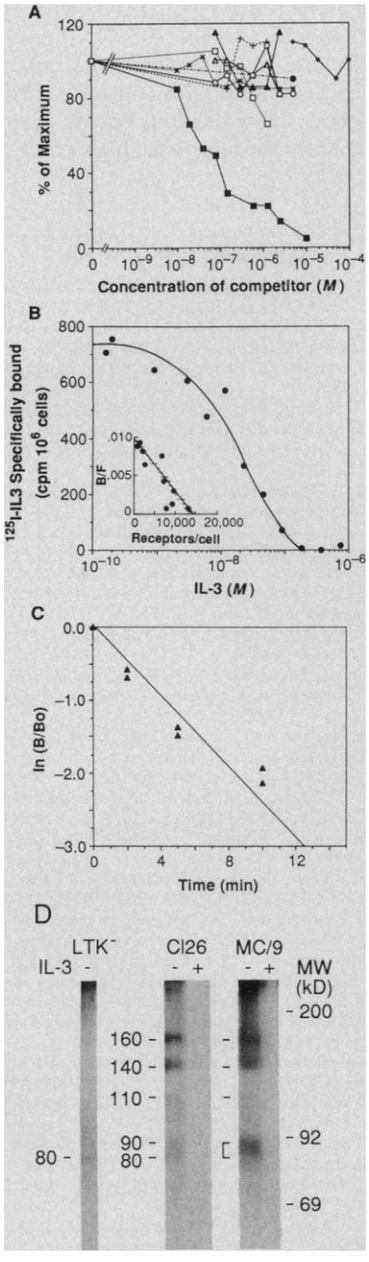

Fig. 2. (**A**) Specificity of IL-3 binding. COS7 cells transfected with AIC2-26 cDNA by the DEAE-dextran method (*27*) were harvested after 3 days, resuspended at 20,000 cells per 100 μl in Hanks balanced saline solution (HBSS), 20 m*M* Hepes, *p*H 7.0, bovine serum albumin (1 mg/ml), and incubated with ^{125}I–IL-3 (10 n*M*, 4°C, 3 hours) in the absence and presence of increasing concentrations of purified mouse IL-3 (–■–), IL-4 (--+--), GM-CSF (–□–), IL-2 (–△–), and EGF (–◇–) and human IL-3 (--×--), IL-4 (--●--), GM-CSF (–▲–), and IL-2 (–○–). Specifically bound ^{125}I–IL-3 was measured as described previously (*4*). Points are the mean of duplicate determinations. (**B**) ^{125}I–IL-3 displacement. The L cell stable transfectants were obtained by cotransfection of AIC2-26 cDNA with pcDneo (*28*) using the calcium phosphate precipitation method (*29*). A G418-resistant clone (cl26) expressing the Aic2 antigen was selected by fluorescence-activated cell sorting (FACS). Cells were incubated with ^{125}I–IL-3 (150 p*M*, 4°C, 2 hours) in the absence and presence of increasing concentrations of unlabeled IL-3. The K_d was calculated by the method of Cheng and Prusoff, as described (*4*). The inset shows a Scatchard plot for the data, with the best fit determined by iterative curve fitting: K_d = 17.9 ± 3.6 n*M*; B_{max} = 15,000 ± 1,500 receptors per cell. A similar K_d was obtained with COS7 cells transiently transfected with AIC2-26 cDNA. (**C**) Rate of dissociation. The COS7 cells transfected with AIC2-26 were incubated as in (A). Then, unlabeled IL-3 (1 μ*M*) was added for the indicated times, and specifically bound radioactivity (B) measured. B_0 represents ^{125}I–IL-3 bound at t = 0. (**D**) Cross-linking. Cells (cl26, LTK$^-$, and MC/9) were incubated with ^{125}I–IL-3 (3 n*M*) in the absence and presence of unlabeled IL-3 (0.5 μ*M*), pelleted, resuspended in 1 ml of 1 m*M* DSS, *p*H 8.2 for 5 min and analyzed by SDS–polyacrylamide gel electrophoresis as described (*4*).

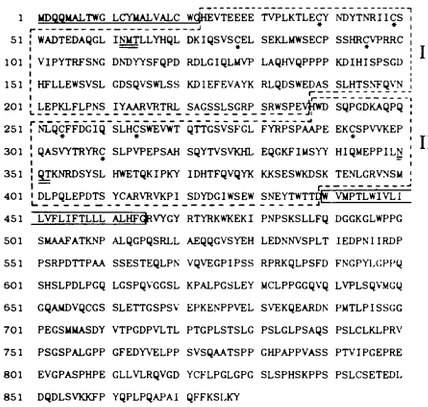

Fig. 3. The deduced amino acid sequence of the AIC2-26 cDNA (*31*). The dotted boxes indicate the two similar domains. The signal sequence is underlined and the transmembrane domain is marked by a box. The potential N-linked glycosylation sites are shown by double underlines. The conserved cysteines are marked by asterisks. The entire nucleotide sequence of the AIC2-26 cDNA has been deposited at GenBank (accession number M29855).

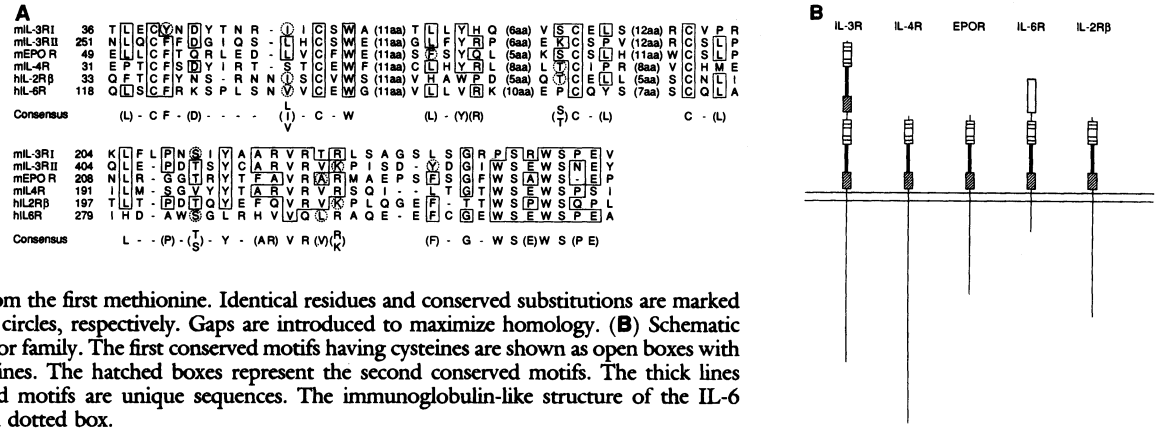

Fig. 4. (**A**) Alignment of the murine IL-3 receptor domains to cytokine receptors (*34*). Domains I and II of the murine IL-3, murine IL-4 (*20*), murine erythropoietin (*22*), human IL-2 β chain (*19*), and human IL-6 (*21*) receptors are aligned. The numbers at the left indicate the amino acid number starting from the first methionine. Identical residues and conserved substitutions are marked by solid boxes and dashed circles, respectively. Gaps are introduced to maximize homology. (**B**) Schematic representation of the receptor family. The first conserved motifs having cysteines are shown as open boxes with bars which represent cysteines. The hatched boxes represent the second conserved motifs. The thick lines between the two conserved motifs are unique sequences. The immunoglobulin-like structure of the IL-6 receptor is also shown by a dotted box.

the mature protein is 94,723 daltons with two potential N-linked glycosylation sites (see Fig. 3). The band at about 140 kD could be the IL-3 binding protein cross-linked to one IL-3, and the 160-kD band could be a cross-linking of two IL-3 molecules, as IL-3 may be a dimer in solution (*15*). The ^{125}I–IL-3 cross-linked 90-kD complex (Fig. 2D) was immunoprecipitated by the anti-Aic2 antibody (*10*) and may be a degradation product of the intact molecule. These results, however, do not preclude the existence of additional IL-3 binding proteins such as a 60- to 70-kD protein on IL-3–dependent cells.

The complete nucleotide sequence of the AIC2-26 cDNA revealed an open reading frame encoding a protein of 878 amino acids. The deduced primary structure of the protein (Fig. 3) predicts that the NH_2-terminal 22 amino acids are the signal sequence and that the mature protein is 856 amino acids. The protein has an external domain of 417 amino acids, a transmembrane domain of 26 amino acids, and a cytoplasmic domain of 413 amino acids. Although evidence (*6–9*) suggests the involvement of a tyrosine kinase in the IL-3 signal transduction pathway, no consensus sequence for a tyrosine kinase is present in the cytoplasmic domain, indicating that the IL-3 binding protein and the tyrosine kinase are distinct molecules.

Cell surface receptors are classified into several groups based on their structure. The G protein–coupled hormonal receptors typically contain multiple transmembrane domains (*16*). Growth factor receptors for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), for example, share common structural features: a single transmembrane domain and a tyrosine kinase in the large cytoplasmic domain (*17*). A number of cell surface proteins, including T cell receptors and the IL-1 receptor, belong to the immunoglobulin superfamily (*18*). Although the receptors for IL-2, IL-3, IL-4, and erythropoietin do not belong to any of these families, comparative sequence analysis indicates that significant similarity is present in the external domains of the cytokine receptors for IL-2 (*19*), IL-3, IL-4 (*20*), IL-6 (*21*), and erythropoietin (*22*) (Fig. 4). There are two conserved motifs that are separated by a sequence of 90 to 100 unique amino acids. The first motif is composed of approximately 60 amino acids and contains four conserved cysteines. The second motif is located close to the transmembrane domain and is composed of approximately 30 amino acids (Fig. 4). A cDNA homologous to AIC2-2 isolated from a human cDNA library also agreed with the derived consensus sequence (*23*). Interestingly, the external domain of the IL-3 receptor can be divided into two units, both of which contain those common motifs (Fig. 4). All of these cytokine receptors may, therefore, belong to a new gene family and may have evolved from a common ancestor. The external domain of the IL-6 receptor interacts with a cell surface protein, gp130, that may function as a signal transducer (*24*). The common motifs found in the cytokine receptors may participate in these protein-protein interactions.

The cytoplasmic domain of the IL-3 receptor has 69 prolines and 50 serines (about 17 and 12% of the amino acids in the cytoplasmic domain, respectively). High percentages of proline and serine were also recognized in the cytoplasmic domain of the IL-2 receptor β chain (*19*), the IL-4 receptor (*20*), and the erythropoietin receptor (*22*). We also noticed significant homology among the cytoplasmic domains of the IL-3, erythropoietin, and IL-2 β chain receptors. The observations that IL-2–dependent cell lines were frequently obtained from IL-3–dependent myeloid cell lines (*25*) and that expression of the IL-2 receptor β chain in an IL-3–dependent cell line conferred IL-2 dependence (*26*) lead us to speculate that the IL-2 receptor may potentially interact with the signal transduction machinery of the IL-3 receptor. These conserved regions may have some role in the interaction with common proteins that are shared between cytokine receptors. Since the IL-3 receptor cDNA that we have cloned has only low affinity binding to IL-3 and has no kinase sequence, additional components must be required for the functional, high affinity IL-3 receptor.

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11. An expression cDNA library was constructed from polyadenylated RNA of the murine mast cell line MC/9 using a mammalian expression vector, pCEV4 (*31*). Double-stranded cDNA was attached with the Bst XI adaptors and cDNA larger than 1.5 kb was isolated by agarose gel electrophoresis. The size-selected cDNA was inserted in the Bst XI sites of pCEV4. We obtained 250,000 independent clones. The AIC2 cDNA was isolated as described (*32*) with the MAb to Aic2.
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31. The expression vector pCEV4 was constructed from pcDSRα (*33*). The polyoma virus origin (Bgl I–Bcl I fragment) was inserted into the Nde I site of pcDSRα and the Xba I fragment containing two Bst XI sites of CDM8 (*32*) was inserted in the Pst I site of the pcDSRα. A unique Not I site in the Xba I fragment was removed by filling in, and the Not I linker was inserted at the Cla I site of pcDSRα.
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34. During the revision of this manuscript, a new report showing homology among growth hormone, prolactin, erythropoietin, IL-6, and the β chain IL-2 receptors was published [F. Bazan, *Biochem. Biophys. Res. Commun.* **164**, 788 (1989)]. However, we found that the growth hormone and prolactin receptors are more distantly related to the family of cytokine receptors.
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Microinjection of a Conserved Peptide Sequence of p34^{cdc2} Induces a Ca^{2+} Transient in Oocytes

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The product of the yeast cell cycle control gene *cdc2*, and its homologs in higher eukaryotes (p34^{cdc2}), all contain a perfectly conserved sequence of 16 amino acids that has not been found in any other protein sequence. Microinjection of this peptide triggers a specific increase in the concentration of intracellular free Ca^{2+} that originates from intracellular stores in both starfish and *Xenopus* oocytes. Thus, p34^{cdc2} might interact through its conserved peptide domain with some component of the Ca^{2+}-regulatory system.

ENTRY INTO AND EXIT FROM M phase of the cell cycle are controlled by changes in the activity of maturation promoting factor (MPF) (*1*), a two-subunit mitotic protein kinase (*2*). The catalytic and regulatory subunits of MPF are encoded by homologs of the yeast cell cycle control genes *cdc2*$^+$ (*3–7*) and *cdc13*$^+$ (*2*), respectively. The yeast *cdc2* product and its homologs in higher eukaryotes (p34^{cdc2}) all contain a perfectly conserved 16-residue sequence, EGVPSTAIREISLLKE (*8*) (called PSTAIR), which has not been found in any other protein sequence (*9*). Microinjection of the PSTAIR peptide is sufficient to induce meiotic maturation in starfish oocytes (*4*) and it accelerates the action of added MPF in the *Xenopus* system (*6*).

As well as inducing MPF activation and germinal vesicle breakdown (*4*), microinjection of the PSTAIR peptide into starfish oocytes results in cortical granule exocytosis (CGE) and elevation of a fertilization membrane (Fig. 1). The threshold intracellular concentration of PSTAIR peptide required to induce CGE is the same as for triggering MPF activation (~ 0.4 m*M*). A rise in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) induces CGE after fertilization or artificial activation (*10*). Co-injection of EGTA with the PSTAIR peptide suppressed CGE (Fig. 1), suggesting that Ca^{2+} is also required for PSTAIR peptide–induced CGE. In contrast, EGTA does not suppress MPF activation and germinal vesicle breakdown, confirming that a rise in $[Ca^{2+}]_i$ is not required for the prophase to metaphase transition during meiotic maturation of oocytes (*11*). To confirm that PSTAIR peptide–induced CGE was a Ca^{2+}-dependent event, we monitored $[Ca^{2+}]_i$ in oocytes loaded with the Ca^{2+} indicator indo-1. The $[Ca^{2+}]_i$ increased from ~0.1 μ*M* to 1 μ*M* or higher within 1 min after microinjection of the PSTAIR peptide, and then decreased slowly with a half-time of ~90 s toward its resting value (Fig. 2).

The product of the budding yeast gene *PHO85* is a 34-kD protein that is homologous to the *cdc2* product and contains a 16–amino acid sequence in which 14 residues are identical to those in the PSTAIR peptide (*12*). Thus, it was possible that the PSTAIR peptide was mimicking *PHO85* rather than

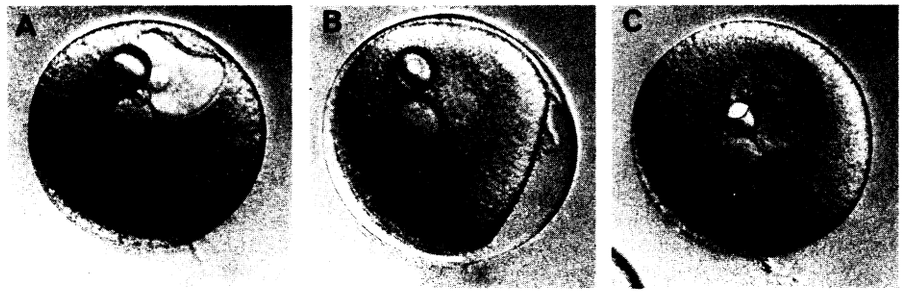

Fig. 1. Microinjection of the PSTAIR peptide induces germinal vesicle breakdown and elevation of the fertilization membrane in starfish oocytes. Oocytes of the starfish *Marthasterias glacialis* were prepared free of follicle cells (*4*) and then transferred to natural sea water. The PSTAIR peptide was solubilized in distilled water (20 mg/ml; ~12.5 m*M*) and microinjected (*30*), to give a final intracellular concentration of 1 m*M*, together with two oil droplets (preventing contact with sea water). About 50 oocytes were microinjected, giving identical results. (**A**) Micrograph taken 2 min after microinjection. The germinal vesicle (oocyte nucleus) is still limited by an intact envelope and CGE has not occurred. (**B**) The same oocyte as in (A), 25 min after peptide microinjection. The germinal vesicle has broken down and a fertilization membrane has elevated as a consequence of CGE. (**C**) Another oocyte injected first with 1 m*M* EGTA (*p*H 7.0), and then with 1 m*M* PSTAIR (both concentrations are intracellular). The micrograph was taken 17 min after peptide microinjection. The envelope limiting the germinal vesicle has disappeared, but the fertilization membrane did not elevate. It did not elevate and CGE did not occur even 1 hour later.

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