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22. Mature *Xenopus* eggs were injected with heparin or ruthenium red in the presence of chlorobutanol (10 mM). The eggs were then washed in F1 medium of composition: 41.25 mM NaCl, 1.75 mM KCl, 0.063 mM $MgCl_2 \cdot 6H_2O$, 0.25 mM $CaCl_2 \cdot 2H_2O$, 0.5 mM NaH_2PO_4, 1.9 mM NaOH, and 2.5 mM Hepes (acid); pH 7.8 (*19*), and fertilization was attempted by addition of a sperm suspension in F1. We assessed fertilization by viewing the formation of a fertilization envelope and the contraction of the pigmented hemisphere toward the animal pole (*21*).
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28. *Xenopus* egg homogenates were prepared as described for sea urchin eggs (*7*) but with the following modifications: We removed egg jelly by swirling the eggs in F1 cysteine medium (*19*), and the eggs were washed in Ca^{2+}-free F1 containing 5 mM EGTA. The eggs were homogenized in a 70% intracellular medium (IM) solution (*7*). The resulting 20% homogenates were incubated with an ATP-regenerating system, mitochondrial inhibitors, and the fluorescent dye fura 2 (*7*). Portions (1 ml) were transferred to a cuvette, and fluorescence was monitored at 510 nm, with excitation at 340 and 380 nm, in a Hitachi fluorimeter at 22°C. The ratio of the signals at the two excitation wavelengths was used to determine the concentration of free Ca^{2+}, as described in (*32*). Calibrations were done in the presence and absence of heparin or ruthenium red. Each experiment in Figs. 1 to 4 is representative of at least three determinations that gave similar results.
29. The effect is not trivially explained as a consequence of the amount of Ca^{2+} in the store because this would affect the amount of Ca^{2+} available for release but not an increased sensitivity to maximal Ca^{2+} release by an agonist (*11*).
30. One of three experiments is shown in each case (each was performed at 20°C). Mature *Xenopus* eggs were obtained as described in (*19*). Eggs were stored in modified Barth's solution (MBS) medium containing the anesthetic chlorbutanol (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.8 mM $MgSO_4$, 0.5 mM NaH_2PO_4, 15 mM tris acid, and 10 mM chlorbutanol; pH 7.6) to reduce hydration of the jelly coat and to prevent egg activation during microinjection. Eggs were microinjected with Ca^{2+} indicator (calcium green: Molecular Probes, Junction City, OR). Just before insemination, eggs were transferred to the low-salt medium, F1. Images were obtained and processed with a Leica CLSM (Leica Laserteknik, Heidelberg, Germany) microscope with 480-nm excitation and emission filters and a ×5 objective. To obtain ratio images that eliminated dye concentration artifacts, we divided the experimental images pixel by pixel by a reference image obtained at the beginning of the experiment.
31. Eggs were obtained, microinjected, and fertilized, and the fura 2 signal was calibrated as described in (*32*). The confocal images are representative of at least five experiments for each condition. *Lytechinus pictus* eggs were maintained at 16°C.
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25 January 1993; accepted 10 May 1993

Calcium Mobilization by Dual Receptors During Fertilization of Sea Urchin Eggs

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Fertilization is accompanied by a transient increase in the concentration of intracellular Ca^{2+}, which serves as a signal for initiating development. Some of the Ca^{2+} appears to be released from intracellular stores by the binding of inositol trisphosphate (IP_3) to its receptor. However, in sea urchin eggs, other mechanisms appear to participate. Cyclic adenosine diphosphate–ribose (cADPR), a naturally occurring metabolite of nicotinamide adenine dinucleotide, is as potent as IP_3 in mobilizing Ca^{2+} in sea urchin eggs. Experiments with antagonists of the cADPR and IP_3 receptors revealed that both Ca^{2+} mobilizing systems were activated during fertilization. Blockage of either of the systems alone was not sufficient to prevent the sperm-induced Ca^{2+} transient. This study provides direct evidence for a physiological role of cADPR in the Ca^{2+} signaling process.

Mobilization of intracellular Ca^{2+} occurs in a wide variety of cellular processes and is mediated by at least two major mechanisms. One messenger molecule that links surface receptor activation to the release of Ca^{2+} from internal stores is IP_3 (*1*). Another major mechanism is Ca^{2+}-induced Ca^{2+} release (CICR), which is well characterized in muscle (*2, 3*) and may function in mechanisms of Ca^{2+} oscillation and Ca^{2+} wave propagation (*4, 5*). Cyclic ADP-ribose (cADPR) is a cyclic metabolite (*6*) synthesized by a ubiquitous enzyme, ADP-ribosyl cyclase (*7, 8*), that utilizes nicotinamide adenine dinucleotide (NAD^+) as substrate. The cADPR molecule occurs naturally in mammalian tissues (*9*) and is as potent as IP_3 in mobilizing Ca^{2+} in sea urchin eggs (*10*) and vertebrate cells (*11, 12*). Some evidence indicates that cADPR may be an endogenous regulator of the CICR process (*13–15*).

The similarity between ligand binding to surface receptors and the sperm-egg interaction during fertilization has led to the proposal that Ca^{2+} mobilization associated with fertilization is similarly mediated by IP_3. Indeed, phosphoinositide metabolism increased during fertilization coincident with the increase in the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and the cortical exocytotic reaction (*16, 17*). Microinjection of IP_3 into eggs activates a transient increase in $[Ca^{2+}]_i$ similar to that which occurs after fertilization (*18*). In hamster eggs, injection of antibody to the IP_3 receptor can block changes in $[Ca^{2+}]_i$ induced by IP_3 or fertilization (*19*). However, in sea urchin eggs, heparin (an antagonist for the IP_3 receptor) only delays the onset of the transient increase in $[Ca^{2+}]_i$ but does not prevent it, suggesting that other mechanisms for mobilizing Ca^{2+} may exist (*20, 21*).

We investigated the effects of heparin on the IP_3-induced Ca^{2+} mobilization in intact sea urchin eggs and homogenates. In egg homogenates, Ca^{2+} release induced by IP_3 (1 μM) was completely blocked by heparin (0.2 mg/ml) (Fig. 1) (*10*). However, heparin did not affect Ca^{2+} release induced by cADPR (Fig. 1). We used the cortical exocytotic reaction in intact eggs as an index for Ca^{2+} mobilization to determine the effective concentration of heparin needed to block IP_3-induced Ca^{2+} mobilization (*22*). Microinjection of IP_3 (0.15 μM, intracellular concentration) into *Lytechinus pictus* eggs induced the cortical reaction in 100% of the injected eggs (*10*). If eggs were first microinjected with heparin [H5765 (Sigma)] at a final intracellular concentration of 1.9 mg/ml and subsequently injected with 0.12 to 0.24 μM IP_3, seven out of seven of the injected eggs did not show a cortical reaction. Increasing the concentration of IP_3 to 0.72 μM partially overcame the block by heparin, with two eggs giving a full cortical reaction and two eggs out of a total of eight eggs injected showing a partial cortical reaction (50%). To block 0.72 μM IP_3 from inducing the cortical reaction, the heparin concentration had to be increased to 4.7 mg/ml (six out of six eggs were blocked). Thus, the blockage by heparin is competitive in nature (*20, 21*). The higher effective concentration of heparin in intact eggs as compared with homogenates is likely to result

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from the interaction of egg proteins with heparin, a polyanion with known affinity for various proteins.

Even at the highest concentration used (4.7 mg/ml), heparin was ineffective in blocking Ca^{2+} mobilization induced by fertilization. Of the 34 eggs injected with heparin (4.7 mg/ml) and then fertilized, 25 had full cortical reactions and 6 had partial reactions (91%). Changes in $[Ca^{2+}]_i$ in six of these eggs were also monitored with Indo 1, the Ca^{2+}-sensitive fluorescent dye (*22*), and the fluorescence ratio (405 nm/485 nm) increased from 0.23 ± 0.02 (± SE) before fertilization to 0.73 ± 0.08 afterward. The changes in the fluorescence ratio in these eggs were virtually the same as those in control eggs not injected with heparin, that is, 0.24 ± 0.03 (n = 5, ± SE) before fertilization and 0.73 ± 0.07 afterward. Ratios of 0.2 and 0.8 correspond to free Ca^{2+} concentrations of 0.2 and 1 μM, respectively, in Ca^{2+}-EGTA buffers (*22*). Inhibition of the IP_3 receptor with heparin, therefore, inhibited IP_3 from mobilizing Ca^{2+} but did not prevent the increase in $[Ca^{2+}]_i$ associated with fertilization.

The 8-amino derivative of cADPR is an effective antagonist (*23*). Addition of 8-amino-cADPR (8-NH_2, 100 nM) to egg homogenates did not produce any Ca^{2+} release but effectively inhibited Ca^{2+} release in response to cADPR (135 nM) added subsequently (Fig. 1). The inhibition appeared to be specific for cADPR-sensitive Ca^{2+} release because, in the presence of 8-NH_2, IP_3 (0.5 and 1 μM) still caused Ca^{2+} release (Fig. 1, trace e) as effectively as in homogenates not treated with 8-NH_2. On the other hand, heparin (0.2 mg/ml) completely prevented IP_3 (1 μM) from releasing Ca^{2+} but had no effect on cADPR-induced release. These results show that there are two independent Ca^{2+} release systems in the egg homogenates and that each can be blocked by its own specific antagonist.

The antagonist 8-NH_2 was also effective in intact eggs. We microinjected ten *L. pictus* eggs with 8-NH_2 (*22*); the injection volume was 0.05 to 0.15% of the egg volume, and the pipette concentration of 8-NH_2 was 240 μM. The final concentration of 8-NH_2 in the eggs was 0.12 to 0.36 μM. We then microinjected cADPR into the eggs (80 μM in pipette; 54 to 100 nM, final concentration in the eggs); nine of the ten eggs did not undergo the cortical reaction. Sperm were then added, and all nine eggs were fertilized and showed a full cortical reaction. As a control, ten eggs were injected with vehicle first and then with the same amounts of cADPR. Ten out of ten eggs underwent cortical reactions, an indication that 8-NH_2 was effective in blocking the cortical reaction induced by cADPR but not that induced by fertilization.

We verified that the blockage of the cADPR-induced cortical reaction by the antagonist was correlated with inhibition of Ca^{2+} mobilization by monitoring $[Ca^{2+}]_i$ with Indo 1 injected with 8-NH_2 (0.2 μM). Subsequent injection of two pulses of cADPR (64 nM each) did not produce any change in the $[Ca^{2+}]_i$ (Fig. 2). A third pulse of cADPR was observed under bright field to verify visually the success of the injection; in this case as well, neither a change in $[Ca^{2+}]_i$ nor a cortical reaction was observed. A single injection of the same amount of cADPR in a control egg

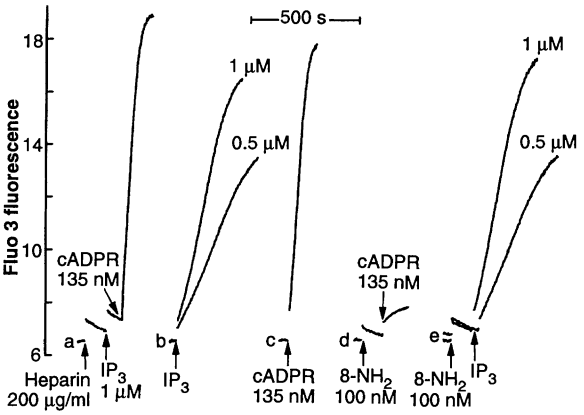

Fig. 1. Data showing the presence of two independent Ca^{2+} mobilizing systems in sea urchin egg homogenates. The release of Ca^{2+} in egg homogenates was monitored by Fluo 3 as described in (*15*). Homogenates (1.25% from *Strongylocentrotus purpuratus* eggs) were treated with the following: trace a, heparin (0.2 mg/ml) and subsequently IP_3 (1 μM) or cADPR (135 nM); trace b, two concentrations (0.5 and 1 μM) of IP_3; trace c, cADPR (135 nM); trace d, 8-NH_2 (100 nM) and then 135 nM cADPR; or trace e, 8-NH_2 (100 nM) and then two concentrations (0.5 and 1 μM) of IP_3. Cyclic ADP–ribose and 8-NH_2 were prepared as described in (*27*).

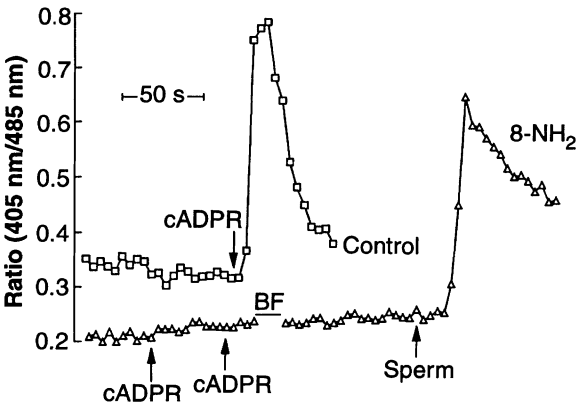

Fig. 2. Blockage of cADPR-induced Ca^{2+} changes in intact egg by 8-NH_2. Changes in $[Ca^{2+}]_i$ were measured with Indo 1 (*22*). Eggs were microinjected with 0.16% of the egg volume (two pulses of 0.08% of the egg volume each), and the injection buffer contained 0.5 M KCl, 0.1 mM EGTA, 10 mM Hepes (pH 6.7), 10 mM Indo 1, with (triangles) or without (squares) 8-NH_2 (120 μM). The final intracellular concentration of 8-NH_2 was 0.2 μM. Two pulses (0.08% of the egg volume each) of cADPR (80 μM in the injection buffer without Indo 1) were injected into the egg treated with 8-NH_2. Each pulse delivered a final intracellular concentration of 64 nM cADPR. Another injection of cADPR was observed in bright field (BF). The egg was subsequently fertilized (Sperm). The control egg received a single injection of cADPR and underwent a cortical reaction.

Table 1. Inhibition of the cortical reaction associated with fertilization by injection of antagonists of cADPR receptors and IP_3 receptors. Eggs were microinjected with 1 to 1.2% of the egg volume (four to five pulses, each consisting of 0.24% of the egg volume). The injection buffer contained 0.5 M KCl, 0.1 mM EGTA, 10 mM Hepes (pH 6.7), and 20 μM 8-NH_2 alone, heparin alone (0.4 g/ml) (Hep), the two together (Hep + 8-NH_2), or a combination of desulfated heparin (0.4 g/ml) and 20 μM 8-NH_2 (Desulf Hep + 8-NH_2). The intracellular concentrations of these substances were 0.2 to 0.24 μM 8-NH_2, heparin (3.8 to 4.7 mg/ml), and desulfated heparin (3.8 to 4.7 mg/ml). Three of the eggs were injected with ten pulses (0.24% of the egg volume each) of the combination of desulfated heparin (D4776, Sigma) and 8-NH_2, resulting in a doubling of the intracellular concentrations. The eggs were subsequently fertilized and the resulting cortical reaction was scored full (F) or partial (P), if the egg was surrounded fully or partially by the fertilization envelope, respectively.

Item	Treatment			
	Hep + 8-NH_2	Hep	8-NH_2	Desulf Hep + 8-NH_2
Eggs with cortical reaction (no.)	3P, 5F	6P, 25F	27F	14F
Eggs injected (no.)	56	34	27	15
Eggs with cortical reaction (%)	14	91	100	93

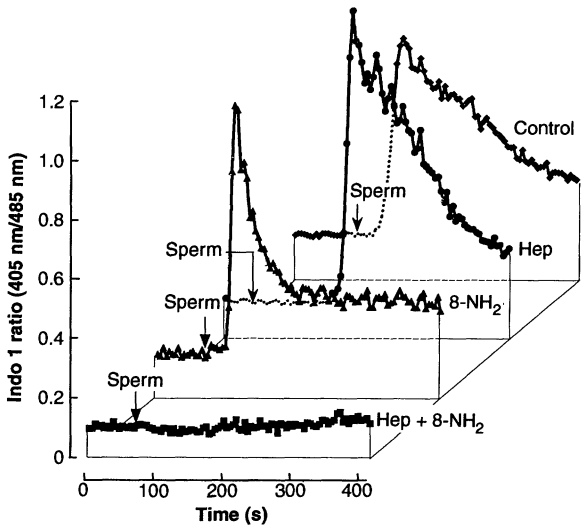

Fig. 3. Blockage of both the cADPR and IP_3 receptor by antagonists inhibits Ca^{2+} release associated with fertilization. Changes in $[Ca^{2+}]_i$ were measured with Indo 1 (*22*). Eggs were microinjected with the indicator together with either a combination of heparin and 8-NH_2 (Hep + 8-NH_2), or 8-NH_2 alone (8-NH_2), or heparin alone (Hep), or vehicle buffer alone (control). The injection volume was 1.2% of the egg volume (five pulses of 0.24% of the egg volume each). The buffer contained 0.5 M KCl, 0.1 mM EGTA, 10 mM Hepes (pH 6.7), and 10 mM Indo 1. When indicated, the vehicle buffer was supplemented with either 8-NH_2 (20 μM), heparin (0.4 g/ml), or both. The intracellular concentrations of 8-NH_2 and heparin were 0.24 μM and 4.7 mg/ml, respectively. At times indicated by arrows, sperm were added to fertilize the injected eggs and the resultant Ca^{2+} changes were recorded.

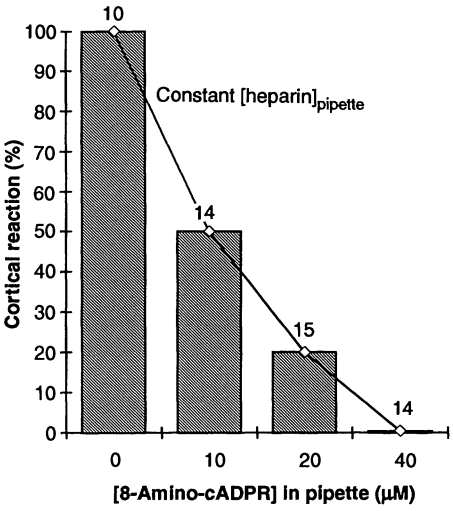

Fig. 4. Concentration dependence of the inhibition of the cortical reaction by 8-NH_2. Eggs were microinjected with heparin (4.7 mg/ml, intracellular concentration) and various amounts of 8-NH_2 and then fertilized (*22*). The injection volume was 1.2% of the egg volume (five pulses, each 0.24% of the egg volume). The injection medium contained 0.5 M KCl, 0.1 mM EGTA, 10 mM Hepes (pH 6.7), heparin (0.4 g/ml), and 8-NH_2 (10 to 40 μM) as indicated. The intracellular concentrations of 8-NH_2 were 0 to 0.48 μM. The number above each bar indicates the number of eggs injected. The bars indicate the percentage of these eggs that underwent cortical reaction after subsequent fertilization.

produced a large increase in $[Ca^{2+}]_i$ and a normal cortical reaction (Fig. 2).

These results indicate that neither cADPR nor IP_3 is solely responsible for the Ca^{2+} mobilization during fertilization. We investigated the possibility that both receptors are involved (Table 1). Eggs were injected with either 8-NH_2 (0.19 to 0.24 μM) alone, heparin (3.8 to 4.7 mg/ml) alone, or both antagonists together and subsequently fertilized. Only 14% of the eggs injected with both antagonists showed cortical reaction, whereas 100% of the eggs injected with the same concentration of 8-NH_2 alone underwent full cortical reactions when subsequently fertilized. Similarly, of the eggs injected with the same concentration of heparin alone, 91% showed cortical reactions. Fifteen eggs were injected with the same or a higher concentration of desulfated heparin (4.7 to 9.4 mg/ml) and 8-NH_2 (0.24 to 0.48 μM), and 14 of these eggs showed full cortical reaction when subsequently fertilized (93%).

These results indicate that the cortical exocytosis reaction associated with fertilization can be inhibited only when both receptors are blocked. To verify that the blockage did not prevent sperm incorporation, we stained 26 eggs with Hoechst dye (*24*) before injection with both antagonists; all of them had at least one sperm nucleus incorporated when subsequently fertilized. Of the 26 eggs, 17 contained more than one sperm nucleus and four of them had 11 to 14 sperm nuclei incorporated. Only 3 of the 26 eggs had a cortical reaction (12%).

Direct measurement of intracellular Ca^{2+} showed that the failure of the injected eggs to undergo a cortical reaction when fertilized was associated with suppression of Ca^{2+} mobilization. Injection of both heparin and 8-NH_2 blocked the fertilization-induced Ca^{2+} transient (Fig. 3). The Indo 1 ratio increased by no more than 0.05 ± 0.03 (± SE) from 0.25 ± 0.05 before to 0.30 ± 0.07 after fertilization (n = 6). In comparison, a ten times larger increase in the Indo 1 ratio of 0.5 to 0.56 was seen after fertilization in control eggs injected with either 0.24 to 0.48 μM 8-NH_2 alone (0.27 ± 0.03 to 0.83 ± 0.05, n = 6, ± SE), heparin (4.7 mg/ml) alone (0.23 ± 0.02 to 0.73 ± 0.08), or vehicle (0.24 ± 0.03 to 0.73 ± 0.07).

The degree of inhibition of the cortical reaction depended on the concentration of 8-NH_2 (Fig. 4). Eggs were injected with a constant amount of heparin (4.7 mg/ml) and various concentrations of 8-NH_2 (from 0 to 0.48 μM) and then fertilized. Of the ten eggs injected with heparin alone (4.7 mg/ml), all displayed full cortical reactions after fertilization. Increasing the concentration of 8-NH_2 progressively decreased the percentage of eggs that underwent a cortical reaction. None of the 14 eggs injected with both 8-NH_2 (0.48 μM; 40 μM in pipette) and heparin (4.7 mg/ml) underwent a cortical reaction after fertilization. All eggs (five out of five) injected with 8-NH_2 (0.48 μM) alone showed a full cortical reaction when fertilized. Indeed, in the absence of heparin, even higher concentrations (4.7 μM) of 8-NH_2 did not block the fertilization-induced cortical reaction in 12 out of 13 eggs. These eggs were injected with two pulses of 1 mM 8-NH_2, and the injection volume of each pulse was 0.24% of the egg volume.

These results provide direct evidence that both cADPR and IP_3 are involved in mediating the increase in $[Ca^{2+}]_i$ during fertilization. This may represent redundancy to ensure that the Ca^{2+} transient does occur after fertilization, because this signal together with alkalization of internal pH constitute the two ionic signals responsible for activating the developmental program after fertilization (*25*, *26*).

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22. Eggs from three to five sea urchins (*L. pictus*) were mixed to minimize variations between batches of eggs. Egg jelly was removed and the eggs were placed onto small cover slips coated with protamine sulfate. The slips were stored in a petri dish at 17° to 20°C. For injections, a slip was transferred to a flow-through chamber and artificial seawater at 17° to 20°C was perfused through the chamber. The flow was stopped only during impalement and injection. Procedures for microinjection were described (*7, 10*). Varying numbers of injection pulses (one to five) were used to deliver substances to the eggs to the required intracellular concentration. The maximum injection volume was 1.2% of the egg (five pulses and each was 0.24% of the egg volume). Injection pulses were each separated by about 30 s, and the injected egg was allowed to recover for about 10 min after the last injection before fertilization. As soon as the surrounding eggs were fertilized, excess sperm were removed by flow. We used Indo 1 injected with various substances into the eggs to monitor intracellular Ca^{2+} changes (*7, 10*).
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27. Cyclic ADPR and 8-NH_2 were synthesized and purified on an anion-exchange column with the use of a gradient of trifluoroacetic acid (TFA) as described in (*8*) and (*23*), respectively. The residual TFA content in the purified cADPR samples after lyophilization was determined to be 0.9 to 1.7% of the weight of the sample (*15*). The TFA was removed from some preparations of 8-NH_2 by repurification on an anion-exchange column and elution with a gradient of HCl. Samples were neutralized immediately with KOH. In sea urchin egg homogenates, TFA (Na^+ salt) concentrations up to 4 mM had no effect on Ca^{2+} release. The Na^+-TFA did not release Ca^{2+} by itself nor did it inhibit cADPR from releasing Ca^{2+}. It had no effect on IP_3-induced Ca^{2+} release and did not potentiate Ca^{2+} release induced by either caffeine or divalent cation such as Sr^{2+} (*15*). In intact eggs, microinjection of 1.5 mM Na^+-TFA (intracellular concentration) alone (three out of three injected eggs) or in combination with heparin (4.7 mg/ml) (11 out of 11 injected eggs) did not cause cortical reaction or inhibit cortical reaction when the eggs were fertilized subsequently. The amounts of TFA injected in these controls were at least 80 times the residual amounts of TFA injected with the cADPR or 8-NH_2 samples.
28. This work was supported by grants from the National Institutes of Health (HD17484) to H.C.L. and from the Minnesota Medical Foundation to T.F.W.

22 January 1993; accepted 19 April 1993

Colocalization of X-Linked Agammaglobulinemia and X-Linked Immunodeficiency Genes

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Mice that bear the X-linked immunodeficiency (*xid*) mutation have a B lymphocyte–specific defect resulting in an inability to make antibody responses to polysaccharide antigens. A backcross of 1114 progeny revealed the colocalization of *xid* with Bruton's agammaglobulinemia tyrosine kinase (*btk*) gene, which is implicated in the human immune deficiency, X-linked agammaglobulinemia. Mice that carry *xid* have a missense mutation that alters a highly conserved arginine near the amino-terminus of the *btk* protein, Btk. Because this region of Btk lies outside any obvious kinase domain, the *xid* mutation may define another aspect of tyrosine kinase function.

CBA/N mice, which bear the *xid* mutation, do not produce antibodies in response to immunization with polysaccharides or hapten-polysaccharide conjugates and have low serum immunoglobulin M (IgM) and IgG3 (*1*). These mice have moderately reduced numbers of B cells, and the B cells that are present have a high surface IgM to IgD ratio, an inability to form colonies in soft agar, and an absence of proliferation in response to surface Ig receptor cross-linkage. These animals do produce normal amounts of antibodies to protein antigens. Genetic characterization showed that *xid* is located between *Tabby* and *Hypophosphatemia* on the mouse X chromosome (*2*). Studies of F_1 female mice heterozygous at *xid* revealed a random pattern of X inactivation in pre-B cells, T cells, and other non-B cells. However, in the mature B cell population, a preponderance of cells have inactivated the *xid*-bearing X chromosome (*3*). Because the *xid* defect affects central aspects of B cell development and function, we set out to identify its molecular nature using a positional cloning approach (*4*).

CBA/N female (*xid/xid*) mice were mated to *Mus musculus* Skive (Skive) (+/Y), an inbred strain that has a high degree of genetic polymorphism relative to standard lab strains (*5*). Matings between the resulting F_1 female mice (+/*xid*) and CBA/N male (*xid*/Y) mice generated 1114 progeny. These "backcross" mice were immunized with trinitrophenyl (TNP)-Ficoll (*2*), and their phenotype (XID or normal) was determined by measurement of both serum IgM antibody to TNP (anti-TNP) and total serum IgG3 by enzyme-linked immunosorbant assay (ELISA) (*6*) (Fig. 1).

Because of the large number of backcross animals, we designed a highly focused strategy for mapping mouse X chromosome DNA markers relative to *xid*. We first analyzed a small, independently generated set of backcross mice using polymorphic markers that span the X chromosome. This analysis confirmed the previous mapping of *xid* (*2*). We therefore identified polymorphisms between CBA/N and Skive at three loci located in this region (DXCrc47, DXWas17, and DXSmh43) (*7*) and developed polymerase chain reaction (PCR)–based assays to facilitate rapid analysis (*8*). Alleles at each of these loci were determined with the use of genomic DNA that was isolated from backcross males (Fig. 2A). This analysis showed that *xid* lies between DXWas17 and DXSmh43. The 46 male backcross DNAs that were recombinant between these two loci were analyzed at several loci containing simple sequence repeats (SSRs): DXMit3, DXMit4, DXMit9, and DXNds2 (Fig. 2A) (*9, 10*).

We used a more restricted analysis to identify informative recombinants among the backcross females. All females were analyzed at three loci: *xid*, DXWas17, and DXMit4 (Fig. 2A). The 20 backcross females found to be recombinant between DXWas17 and DXMit4 were analyzed at DXMit3, DXMit9, and DXNds2 (Fig. 2A). The resultant map (Fig. 2C) is in good agreement with and extends the current map of the mouse X chromosome (*7*).

The X chromosome marker DXMit3 did not recombine with *xid* in any of the 30 backcross animals recombinant between DXWas17 and DXNds2, placing DXMit3 within 0.27 centimorgan (cM) of *xid* at the 95% confidence limits. Thus, DXMit3 defines an excellent entry point for the identification of candidate genes in the *xid* region, as had been our original goal. Our mapping results suggested that *xid* lies in the region of the mouse genome that is homologous to the region of the human genome containing the X-linked agammaglobulinemia (XLA) mutation (*7*). The defective gene in XLA, named *btk* (formerly *atk* or BPK), encodes a cyto-

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