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14. The 3T3-L1 adipoblasts were grown as previously described (*12*). Subconfluent monolayers (~5 × 10^5 cells, 100-mm-diameter dish) were transfected with 5 μg of pZip-NeoSV(X) and with either 50 μg of pMSV-C/EBP or pEMSV using the $CaPO_4$-precipitation method (*12*). After 5 hours, cells were shocked for 1 min with Dulbecco's modified Eagle's medium that contained 15% glycerol and then allowed to recover overnight in growth medium. Cells were subcultured in growth medium and subjected to selection in growth medium that contained G418 (400 μg/ml) for 2 to 3 weeks.
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19. After G418 selection, individual colonies were isolated with cloning cylinders and lysed in 10 mM tris-HCl (pH 7.5), 10 mM NaCl, 3 mM $MgCl_2$, and 0.5% NP-40 (50 μl) at room temperature for 1 min. The RT assay (20 μl) contained cell extract (4 μl), 50 mM tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 1 mM each of deoxyguanosine 5′-triphosphate, deoxyadenosine 5′-triphosphate, deoxythymidine 5′-triphosphate, deoxycytidine 5′-triphosphate, random hexamers (5 μM), 1 mM dithiothreitol, and 200 U of Moloney murine leukemia virus RT. The PCR assay (100 μl) was performed with the addition of the following to the RT mixture: 10 × PCR buffer [100 mM tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, and 0.1% (w/v) gelatin (10 μl)], 5′ and 3′ PCR primers (5 pmol, 1 μl), ^{32}P-labeled 5′ PCR primer (10^6 cpm, 1 μl), and *Taq* DNA polymerase (2.5 U, 0.5 μl). Samples were amplified in a DNA Thermal Cycler (Perkin-Elmer Cetus) for 30 cycles with denaturation at 95°C for 1 min, reannealing at 65°C for 2 min, and extension at 72°C for 1 min. Aliquots (10 μl) from the RT-PCR reactions were applied to 10% nondenaturing polyacrylamide gels. Gels were dried and analyzed by autoradiography. The PCR primers were: pMSV-C/EBP, 5′-GCGCCAGTCTTCCGATAGACTGCG-3′ and 5′-AAAGCCAAAGGCGGGCGTTGCTGG-3′, corresponding to nucleotides 3 to 26 of the MSV RNA (*25*) and nucleotides 209 to 232 of the rat C/EBPα sequence (*8*), respectively; mouse alpha 4 tubulin, 5′-GGCACCGGCTCTGGCTTCACCTCTC-3′ and 5′-GCCGTGGACACTTGGGGGGCTGG-3′, corresponding to nucleotides 459 to 483 and 546 to 568, respectively, of the mRNA sequence (*25*); and *aP2*, 5′-GTGGGAACCTGGAAGCTTGTCTCC-3′ and 5′-CACTTTCCTTGTGGCAAAGCCCAC-3′, corresponding to nucleotides 83 to 106 and 143 to 166, respectively, of the mRNA sequence (*20*).
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Cell Cycle–Regulated Binding of c-Abl Tyrosine Kinase to DNA

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The proto-oncogene c-*abl* encodes a protein tyrosine kinase that is localized in the cytoplasm and the nucleus. The large carboxyl-terminal segment of c-Abl was found to contain a DNA-binding domain that was necessary for the association of c-Abl with chromatin. The DNA-binding activity of c-Abl was lost during mitosis when the carboxyl-terminal segment became phosphorylated. In vitro phosphorylation of the DNA-binding domain by cdc2 kinase abolished DNA binding. Homozygous mutant mice expressing a c-Abl tyrosine kinase without the DNA-binding domain have been reported to die of multiple defects at birth. Thus, binding of the c-Abl tyrosine kinase to DNA may be essential to its biological function.

The proto-oncogene c-*abl* was first isolated from the mouse genome as a gene with similarity to the v-*abl* oncogene of Abelson murine leukemia virus (*1*). The c-*abl* gene encodes a protein tyrosine kinase that shares several common features with other cytoplasmic tyrosine kinases, for example, c-Abl has the Src-homology domains 2 (SH2) and 3 (SH3) (*2*). Unique to the c-Abl tyrosine kinase, however, is a large COOH-terminal segment. Mutant mice homozygous for a 3′-deletion of c-*abl* and expressing an active c-Abl tyrosine kinase truncated at its COOH-terminus have multiple defects at birth (*3*). This observation indicates that the COOH-terminal region of c-Abl is essential for its biological function. The c-Abl protein undergoes a cell cycle–regulated Ser-Thr phosphorylation and all the mitosis-specific phosphorylation sites are localized in the COOH-terminal segment (*4*). The mitotic phosphorylation does not appear to alter the tyrosine kinase activity of c-Abl (*4*). This result suggested that Ser-Thr phosphorylation might regulate other functions associated with the COOH-terminal segment.

While attempting to purify the c-Abl protein, we found that it was preferentially retained on a calf thymus DNA-cellulose column. When NIH 3T3 cell lysates were applied to DNA-cellulose columns (1.2 mg of protein per milliliter of bed volume in 100 mM KCl), less than 20% of the total cellular protein was bound, whereas 60 to 80% of the total c-Abl protein was retained

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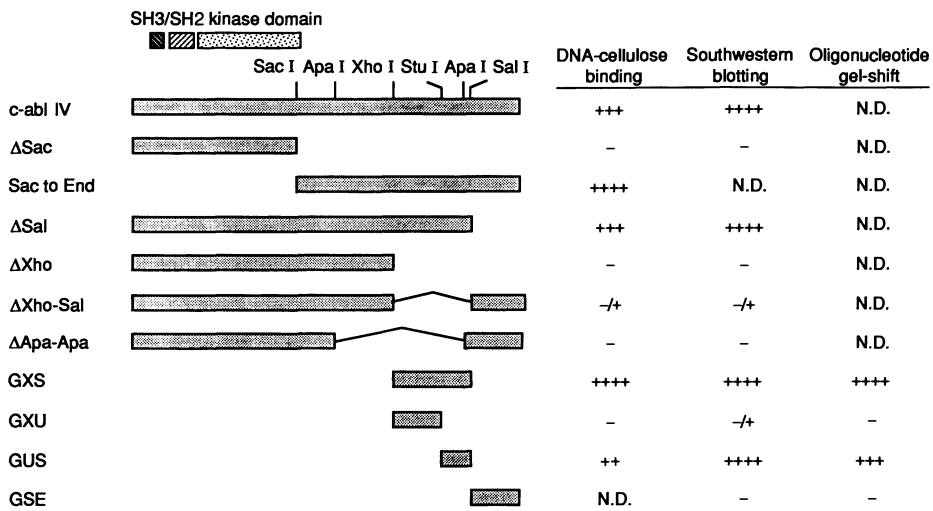

Fig. 1. Summary of constructs and results on DNA-binding assays. SH3, Src-homology domain 3; SH2, Src-homology domain 2; and N.D., not determined. The deletion and fusion proteins were prepared with the restriction enzymes indicated in the figure by standard recombinant DNA methods. Proteins were expressed in COS cells or in bacteria. DNA-cellulose binding (*5*): ++++ indicates 80 to 100% of the Abl protein in cell lysates was bound; +++ indicates 60 to 80% bound; ++ indicates 30 to 50% bound; −/+ indicates <5% bound; and − indicates no binding. Refer to Fig. 2 for Southwestern blotting and oligonucleotide gel shift results.

(5). Elution of the column with a linear KCl gradient showed that the bound c-Abl protein could be dissociated at 250 to 400 mM KCl.

To localize the DNA-binding domain in c-Abl, several deletion mutants were expressed in COS-7 cells and tested for DNA binding (Fig. 1). The NH_2-terminal fragment (ΔSac) containing the SH3-SH2–tyrosine kinase domains showed no affinity for DNA-cellulose, whereas the COOH-terminal fragment (Sac-End, 80 kD) was retained on the column. Truncation of c-Abl at a 3′–Sal I site (ΔSal) had no effect, but truncation at the Xho I site (ΔXho) abolished DNA-cellulose binding. An internal deletion of an Apa I fragment (ΔApa-Apa) as well as a smaller deletion of the Xho I–Sal I fragment also eliminated DNA-cellulose binding (Fig. 1). To further demonstrate the DNA-binding activity, we probed immobilized c-Abl proteins with ^{32}P-labeled DNA fragments with the Southwestern blotting method (6). The full-length c-Abl and the ΔSal protein bound HeLa DNA fragments, but the ΔXho-Sal and ΔApa-Apa mutants did not (Fig. 2A). Hence, the amino acids encoded by the Xho-Sal fragment are required for binding to DNA.

To determine whether the peptide encoded by the Xho-Sal fragment is sufficient to bind DNA, we produced the fragment in bacteria as a glutathione-S-transferase (GST) fusion protein. The GST/Xho-Sal (GXS) fusion protein, but not the GST protein, bound to DNA-cellulose demonstrating the DNA-binding activity of the XS peptide (5). The Xho-Sal region was then subdivided and expressed as two other fusion proteins: GST-Xho-Stu (GXU) and GST-Stu-Sal (GUS) (Fig. 1). A fourth GST fusion protein containing the Sal-End fragment (GSE), which lacked DNA-binding activity, was used as a negative control. These GST fusion proteins were purified from bacterial lysates and tested in DNA-blotting assays (Fig. 2B). In keeping with the deletion analysis, GXS bound DNA, but GSE did not (Fig. 2B). Fusion protein GXU containing the NH_2-terminal half of the XS peptide bound DNA poorly, whereas the fusion GUS containing the other half of the XS peptide bound a level of DNA similar to that of GXS (Fig. 2B). The GXS protein also formed stable complexes with random oligonucleotides in gel mobility shift assays. A pool of 47-bp oligonucleotides with an internal random sequence of 16 nucleotides was used in these experiments. A stable GXS-oligonucleotide complex with a slow mobility was detected (Fig. 2C). The GUS protein containing only 99 amino acids of c-Abl also formed complexes with oligonucleotides in these experiments (Fig. 2C).

Taken together, the results summarized in Fig. 1 indicate that the DNA binding activity of c-Abl is localized primarily within the 99 amino acids encoded by the Stu-Sal fragment (7). These 99 amino acids do not share any recognizable similarity with the known DNA-binding proteins. This region is rich in proline (17%) and contains clusters of basic amino acids (7). The corresponding 99–amino acid fragments of the mouse and human c-Abl proteins are 72% identical (88% similar), but this region is not conserved in the *Drosophila* Abl protein (16% identity). It thus appears that the Abl protein has evolved in mammals to encompass a nuclear function, which includes binding to DNA.

The cdc2 kinase phosphorylates c-Abl on multiple Ser-Thr sites in the COOH-terminal region during mitosis (4). We therefore examined whether cdc2-catalyzed phosphorylation could affect the binding of c-Abl to DNA. By passing interphase or mitotic 3T3 cell lysates through DNA-cellulose columns, we found that the mitotic c-Abl did not bind to DNA (Fig. 3). The mitotic phosphorylation, however, did not affect binding to phosphocellulose (Fig. 3). Treatment of mitotic c-Abl with protein phosphatase 2A (PP2A) restored its DNA-binding activity (Fig. 3), but treatment of the interphase c-Abl had no effect on DNA binding (Fig. 3). PP2A did not remove all the phosphates in the mitotic c-Abl, as evident from the slower mobility, but some DNA-binding activity was restored (Fig. 3). At least seven mitotic phosphorylation sites are found in c-Abl, and the mobility shift can be induced by the phosphorylation of only one of those seven sites (4). The dephosphorylation result suggests that DNA binding may be regulated by a subset of the seven phosphorylation sites. The other phosphorylations may modulate other activities associated with the c-Abl COOH-segment, for example, the microfilament binding activity (8).

The 99–amino acid DNA-binding domain (Stu-Sal region) contains two potential cdc2 phosphorylation sites, Ser^{852}-Pro^{853} and Ser^{883}-Pro^{884}. There are no Thr-Pro motifs in this region (7). We phosphorylated GUS and full-length c-Abl with mitotic cdc2 kinase and then analyzed the phosphotryptic maps. By mixing peptides derived from c-Abl with those from

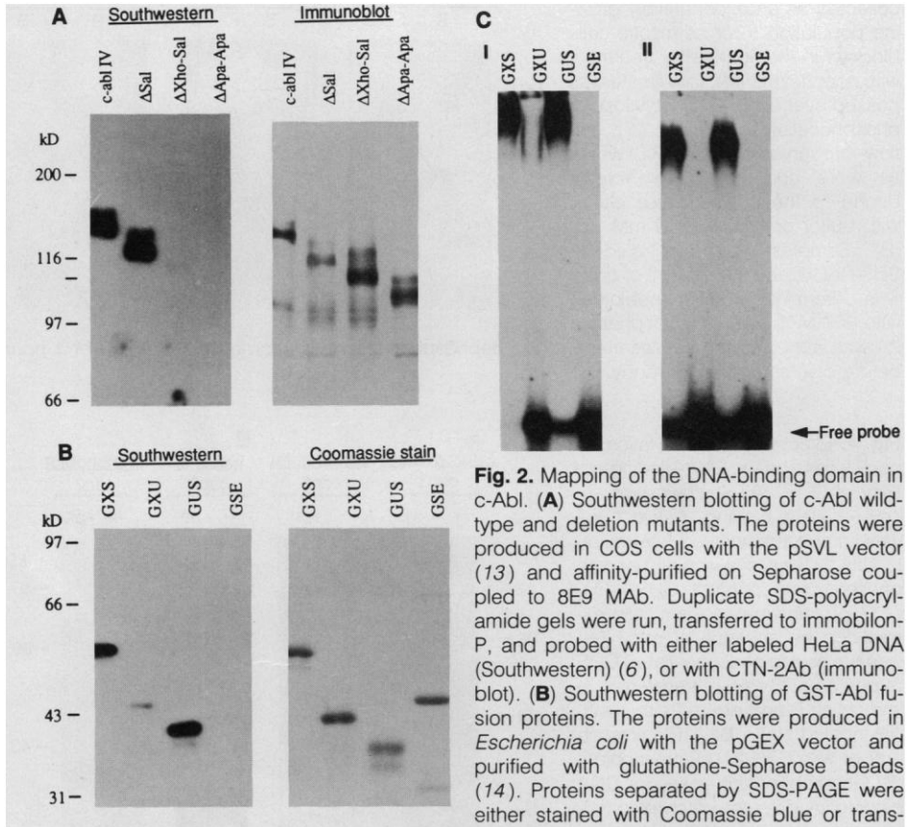

Fig. 2. Mapping of the DNA-binding domain in c-Abl. (**A**) Southwestern blotting of c-Abl wild-type and deletion mutants. The proteins were produced in COS cells with the pSVL vector (*13*) and affinity-purified on Sepharose coupled to 8E9 MAb. Duplicate SDS-polyacrylamide gels were run, transferred to immobilon-P, and probed with either labeled HeLa DNA (Southwestern) (*6*), or with CTN-2Ab (immunoblot). (**B**) Southwestern blotting of GST-Abl fusion proteins. The proteins were produced in *Escherichia coli* with the pGEX vector and purified with glutathione-Sepharose beads (*14*). Proteins separated by SDS-PAGE were either stained with Coomassie blue or transferred to nylon membrane and probed with labeled HeLa DNA, as indicated. (**C**) Binding of GST-Abl fusion proteins to random oligonucleotides determined by gel mobility shift. The proteins (500 ng each) were incubated with random oligonucleotides (0.5 ng) and poly(dI-dC)·poly(dI-dC) (25 ng in I and 200 ng in II). The reaction mixtures were separated by native PAGE (4% gel). The shifted bands correspond to nonspecific DNA-protein complexes.

GUS, we found that the previously described interphase site b and the mitotic site 7 were present in GUS. Both peptides b and 7 contain phosphoserine (*4*). Digestion with trypsin always generates a series of partial peptides around site 7 but only generates one tryptic peptide for site b (*4*). Ser^{852}, which is surrounded by multiple lysine and arginine residues, is likely to be the mitotic site 7 and Ser^{883} is probably the site b, which is phosphorylated throughout the cell cycle (*7*).

To determine directly the effect of cdc2-phosphorylation on the DNA-binding activity, we phosphorylated GUS with cdc2 kinase and then tested for DNA binding. Incorporation of 0.8 to 1.9 phosphates per GUS molecule was achieved in vitro, and the phosphorylated GUS migrated slightly slower on SDS–polyacrylamide gel electrophoresis (PAGE) than the unphosphorylated molecules (Fig. 4). DNA-binding activity was measured by Southwestern assay (Fig. 4A) or by gel mobility shift assay (Fig. 4B). In both cases, phosphorylation by cdc2 reduced or abolished the DNA-binding activity of GUS protein (Fig. 4). This is consistent with the observation that the mitotic c-Abl does not bind to DNA.

The c-Abl protein is localized to the cytoplasm and the nucleus (*9*). A correlation between DNA binding and nuclear localization could be shown in fractionation experiments. About 30% of the wild-type c-Abl was found in the nuclear pellet when it was expressed in COS cells (*10*). The nuclear c-Abl could not be extracted with 100 mM salt but was released with 500 mM salt. A similar result was obtained with the 80-kD Sac-End fragment, which was primarily localized in the nucleus (~70%). In contrast, in mutants lacking DNA-binding activity, only 15 to 20% of the ΔApa-Apa and ΔXho-Sal proteins were in the nuclear pellet and they were released by washing with 100 mM salt (*10*).

If c-Abl binds to cellular DNA, its concentration in the chromatin fraction should be higher than in the total cell lysate. We prepared chromatin fractions by the method of Hancock *et al.* (*11*) and determined the concentration of c-Abl in total lysates (L) and chromatin (C) by quantitative immunoblotting. The wild-type and the Sac-End proteins, when expressed in COS cells, were enriched fivefold in the chromatin fraction (C/L ratio of 4.7 to 5.6). The mutant proteins, ΔXho-Sal and ΔApa-Apa, were not enriched in the chromatin fraction (C/L ratio of 1 to 1.5) and the ΔXho protein was depleted in the chromatin fraction (C/L ratio of 0.5). These results suggest that the DNA-binding domain of c-Abl mediates its association with the cellular DNA. It remains to be determined if c-Abl interacts with specific DNA sequences.

The biological function of c-Abl tyrosine kinase is unknown. Mutant mice with homozygous deletions of c-*abl* can be born, but the neonatal animals have multiple defects and die of unknown causes soon after birth (*12*). The defects found with the c-*abl* null-mutant mice are also observed with mice that are homozygous for a c-*abl* ΔXho gene (*3*). The c-AblΔXho protein lacked DNA-binding activity and was depleted from the chromatin fraction. These findings suggest that binding of the c-Abl tyrosine kinase to DNA may be critical to its biological function.

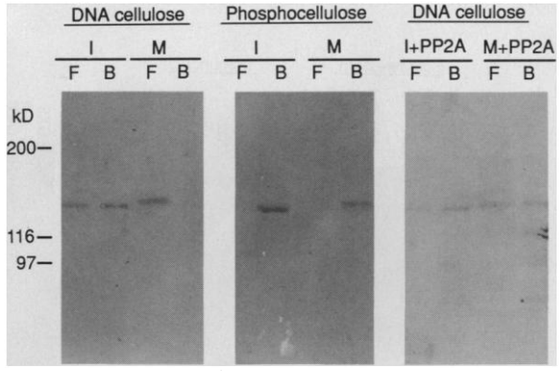

Fig. 3. Failure of mitotic c-Abl to bind DNA cellulose. NIH 3T3 cells were collected as an exponentially growing population (I) or as mitotic cells blocked in metaphase by treatment with nocodazole (M). Cell lysate was passed over either DNA-cellulose or phosphocellulose columns (*5*). The flow-through and 100 mM KCl washes were combined (F). Proteins bound to the column were eluted with buffer containing 800 mM KCl (B). Samples were subjected to SDS-PAGE, and the amount of c-Abl was determined by immunoblotting with 8E9 MAb. Protein phosphatase 2A was added to the cell lysates and the dephosphorylation was carried out at 37°C for 1 hour before column chromatography.

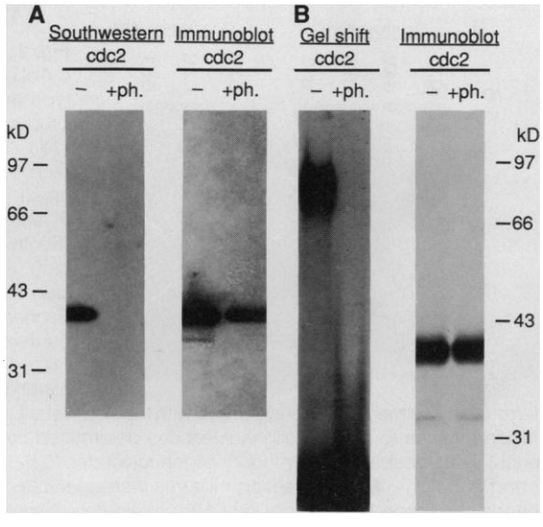

Fig. 4. Effect of phosphorylation by cdc2 kinase on the DNA-binding activity of GUS. (**A**) Southwestern assay for DNA binding. Active cdc2 was immunoprecipitated from mitotic NIH 3T3 cells and was used to phosphorylate purified GUS (*15*). GUS protein treated identically except for the absence of cdc2 (–) was run next to phosphorylated GUS (+ph.) on SDS-PAGE, transferred to immobilon-P and probed either with ^{32}P-labeled HeLa DNA (Southwestern) or with CTN-2Ab (immunoblot). (**B**) Gel mobility shift assay for DNA binding. A different batch of cdc2-phosphorylated (+ph.) or mock-phosphorylated GUS protein (–) were used in a gel mobility shift assay (gel shift) and were probed with CTN-2Ab (immunoblot).

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5. DNA-cellulose binding was performed as follows: proteins were extracted with 800 mM KCl buffer A [10 mM tris (pH 7.2), 1 mM EDTA, Triton-X 100 (1%)] and protease inhibitors [1 mM phenylmethylsulfonylfluoride (PMSF), 10 μM benzamidine HCl, phenanthroline (1 μg/ml), aprotinin (10 μg/ml), leupeptin (10 μg/ml), and pepstatin A (10 μg/ml)]. Cell extracts (2×10^6 per milliliter) were centrifuged, diluted to 100 mM KCl, and passed over double-stranded DNA-cellulose (Pharmacia); (typically 0.5 ml of DNA-cellulose per 8 ml of diluted extract). The DNA-cellulose was washed with 100 mM KCl buffer A (20 column volumes). Bound proteins were eluted with 800 mM KCl buffer A (3 column volumes). Flow-through and bound proteins were concentrated by precipitation with trichloroacetic acid and subjected to SDS-PAGE. Immunoblotting was with a monoclonal antibody to an epitope in the c-Abl SH2 domain (8E-9 MAb) or with polyclonal antibody to the COOH-terminal region of c-Abl (CTN-2). Similar procedures were used for phosphocellulose (P-11, Whatman) binding.
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7. The minimal DNA-binding domain contains c-Abl common exon amino acids 837 to 935: SSEEMRVRRHKHSSESPGRDKGRLAKLKPAPPPPPAC TGKAGKPAQSPSQEAGEAGGPTKTKCTSLAMDAVNTDPTKAGPPGEGLRKPVPPSVPKPQST. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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collected by trypsinization, washed once with growth media, once with phosphate-buffered saline, and once with swelling buffer [10 mM Hepes (pH 7.5), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol (DTT), and protease inhibitors]. Cells were homogenized in swelling buffer (>95% cell lysis). One-quarter of the cell suspension was saved as total cell lysate. The remainder was divided into three portions and layered onto sucrose (25%) dissolved in swelling buffer. Nuclei were collected by centrifugation (2000*g* for 20 min). One portion of the nuclear preparation was saved. The other two portions were resuspended in 200 μl of swelling buffer with 0.5% NP-40, 1 mM PMSF, and either 100 mM KCl or 500 mM KCl and incubated at 4°C for 1 hour with gentle shaking. Nuclei were then centrifuged (13,000*g* for 5 min), and the supernatant was collected. An equal volume of each sample was analyzed by immunoblotting. Quantitation was by densitometric scan of autoradiographs.

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15. Immunoprecipitation of active cdc2 kinase from mitotic 3T3 cells was as previously described in (*4*). Seven-eighths of the cdc2 immunoprecipitate was resuspended in kinase buffer (35 μl) [20 mM tris (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, 100 μM adenosine triphosphate (ATP), and protease inhibitors] containing GUS (3.5 μg) and incubated at room temperature for 2 hours with gentle shaking. The reaction mixture was centrifuged, and the supernatant was used for immunoblotting and gel shift assays. The other one-eighth of the cdc2 immunoprecipitate was resuspended in kinase buffer (5 μl) containing GUS (0.5 μg) and 1.5 μM [γ-^{32}P]ATP (6000 Ci/mmol, ICN). The ^{32}P-labeled sample was run on SDS-polyacrylamide gel, dried, and autoradiographed. The labeled GUS band was cut out and counted in a scintillation counter to determine the stoichiometry of phosphorylation. Under identical conditions, GST protein was not phosphorylated by cdc2, thus phosphorylation of GUS was in the US peptide derived from c-Abl.

16. We thank E. T. Fouts who first observed the binding of the 80-kD Sac-End fragment to DNA-cellulose, J. McWhirter who prepared the GSE construct, C. Smythe who contributed protein phosphatase 2A, and W.-j. Li who prepared the Sac-End construct. This work was supported by grant CA 43054 to J.Y.J.W. from NIH.

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Appearance of Water Channels in *Xenopus* Oocytes Expressing Red Cell CHIP28 Protein

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Water rapidly crosses the plasma membrane of red blood cells (RBCs) and renal tubules through specialized channels. Although selective for water, the molecular structure of these channels is unknown. The CHIP28 protein is an abundant integral membrane protein in mammalian RBCs and renal proximal tubules and belongs to a family of membrane proteins with unknown functions. Oocytes from *Xenopus laevis* microinjected with in vitro–transcribed CHIP28 RNA exhibited increased osmotic water permeability; this was reversibly inhibited by mercuric chloride, a known inhibitor of water channels. Therefore it is likely that CHIP28 is a functional unit of membrane water channels.

Water slowly crosses cell membranes by diffusion through the lipid bilayer. However, certain cell types exhibit rapid transmembrane passage of water, probably through specialized water-selective channels. For example, water channels of mammalian RBCs permit cell swelling or shrinkage in response to small changes in extracellular osmolality (*1, 2*). Water is reabsorbed by channels in mammalian renal proximal convoluted tubules (*3*) and by vasopressin-regulated water channels in mammalian collecting ducts (*4*) and amphibian urinary bladder (*5*). Although water channel physiology has been studied extensively (*3, 5*), the molecular structure of the channel is unknown (*6, 7*). The glucose transporter (*8*) and band 3, an anion exchanger from RBCs (*2*), permit small amounts of transmembrane water movement, but neither is a water channel (*9, 10*).

The 28-kD integral membrane protein CHIP28 is abundant in mammalian RBCs and renal proximal tubules (*11*). The native CHIP28 protein is a noncovalent assembly of four subunits (*12*) and is physically similar to certain membrane channels (hence, CHIP28 = channel-forming integral protein of 28 kD). The cDNA encoding CHIP28 has been isolated (*13*), and the deduced amino acid sequence predicts an integral membrane protein with six bilayer-spanning domains (Fig. 1A). The CHIP28 protein is related to the major intrinsic protein from bovine lens, MIP26, a channel through which lens fiber cells may absorb interstitial fluid (*14*). CHIP28 and MIP26 are members of a family of bacterial and plant proteins with incompletely defined functions (*15*). Expression of one family member is induced in the roots and shoots of pea plants by water deprivation (*16*). Thus we tested whether CHIP28 is a membrane water channel by expressing CHIP28 in oocytes from *Xenopus laevis* and measuring osmotic water permeability.

We prepared an expression construct by inserting the CHIP28 coding sequence between the 5′ and 3′ untranslated sequences of the *Xenopus* β-globin cDNA (*17*). Defolliculated oocytes were microinjected with water (0.05 μl) or with up to 10 ng of in vitro–transcribed CHIP28 RNA (*18*). Expression of CHIP28 was monitored by immunoblot with an antibody to the COOH-terminal cytoplasmic domain of CHIP28 (anti-CHIP) (*11, 12*). A 28-kD protein was detected with anti-CHIP 24 hours after injection of oocytes with CHIP28 RNA, and it increased in abundance for up to 72 hours. A 35- to 45-kD glycosylated form of CHIP28 was also apparent (Fig. 1B).

We determined the osmotic water permeability after transfer of oocytes from a 200 to a 70 mosM solution by monitoring changes in cell volume with videomicroscopy (*19*). Control-injected oocytes swelled minimally and failed to rupture even after incubations of >1 hour, whereas oocytes injected with CHIP28 RNA consistently swelled to 1.3 to 1.5 times their initial volume and ruptured within 5 min (Fig. 2). The coefficients of osmotic water permeability (P_f) at 22°C were calculated from the rates of swelling of control-injected oocytes [$P_f = (26.3 \pm 16.5) \times 10^{-4}$ cm/s (± SD), $N = 18$)] and of CHIP28 RNA–injected oocytes [$P_f = (216 \pm 60) \times 10^{-4}$ cm/s, $N = 24$]. Osmotic water permeability was not increased by injection of oocytes with CHIP28 antisense RNA (10 ng) [$P_f = (18.7 \pm 2.2) \times 10^{-4}$ cm/s, $N = 2$]. When oocyte swelling assays were conducted at 10°C, the osmotic water permeability of the control-injected oocytes was greatly reduced [$P_f = (5.2 \pm 2.8) \times 10^{-4}$ cm/s, $N = 6$], whereas that of CHIP28 RNA–injected oocytes was only modestly reduced [$P_f = (177 \pm 23) \times 10^{-4}$ cm/s, $N = 3$]. The Arrhenius activation energy (E_a) (*20*) was lower in CHIP28 RNA–injected oocytes ($E_a < 3$ kcal/mol) than in control-injected oocytes ($E_a >> 10$ kcal/mol). These values are comparable to those reported for water channels in mammalian RBCs and renal proximal tubules ($P_f = 150$ to 200×10^{-4} cm/s; osmotic > diffusional water permeability; and $E_a < 4$ kcal/mol) (*1–3*).

Control experiments confirmed the specificity of the effect of CHIP28 expression on osmotic water permeability. Expres-

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