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22. DNA oligomers were synthesized and purified as reported previously (*9*). NMR samples contained 10 mM sodium phosphate buffer (pH 7) in 0.5 ml 99.96% D_2O or 90% H_2O and 10% D_2O. ImPImP·HCl was dissolved in 99.96% D_2O (with 10 mM sodium phosphate buffer), yielding a stock solution of approximately 26 mM as determined by ultraviolet (UV) absorbance ($\epsilon_{312} \approx 4.5 \times 10^4\ M^{-1}\ cm^{-1}$). Stock solutions were stored at −70°C. The concentration of d(CGTAG,CG,CTACG)$_2$ was 1.4 mM duplex as determined by UV absorbance at 80°C ($\epsilon_{260} = 1.13 \times 10^5\ M^{-1}\ cm^{-1}$).
23. NMR experiments were done on a Bruker AMX-600 (Bruker Instruments, Billerica, MA) at 600 MHz or on a GE Omega-500 (General Electric, Fremont, CA) at 500 MHz. ImPImP was titrated into the NMR sample containing duplex DNA in approximately 0.2 mole equivalents per addition. 1D spectra in D_2O (128 scans), 2D NOESY spectra (100 ms and 200 ms mixing time; 550 to 743 t_1 experiments with 32 or 48 scans) in D_2O and H_2O, and double-quantum filtered correlation spectroscopy spectra in D_2O (775 or 810 t_1 experiments with 16 or 32 scans) were acquired as described previously (*13*, *15*). The data were processed with FELIX 2.30 (Biosym, San Diego, CA) on Silicon Graphics workstations. DNA and ligand resonances were assigned by standard sequential methods and as previously described (*13*, *15*).
24. Intermolecular distance restraints for the modeling of the 2:1 ImPImP:DNA complex with d(CGTAGCGCTACG)$_2$ were generated from crosspeak volumes in the 100 ms H_2O NOESY spectrum as described previously (*13*, *15*). The crosspeak volumes were classified semiquantitatively into three categories: strong (1.8 to 2.5 Å), medium (2.5 to 3.7 Å), or weak (3.7 to 4.2 Å), relative to the cytosine C5H-C6H crosspeak volumes. In all, two identical sets of 39 intermolecular ligand-DNA restraints, 3 intermolecular ligand-ligand restraints, and 6 intramolecular restraints for each of the symmetry-related ligands were used. Hydrogen bonds for standard Watson-Crick base pairing were included as NOE restraints as described previously (*18*). The model of the d(CGTAGCGCTACG)$_2$ duplex was constructed with the use of the Biopolymer module of InsightII (Biosym) from standard B-form DNA. The ImPImP ligand model was built based on coordinates of the 2-ImN–Dst heterocomplex model (*18*). Two ImPImP molecules were roughly arranged in the head-to-tail orientation and manually docked into the minor groove. Restrained energy minimizations were done with Discover (Biosym) (with AMBER forcefield) as described previously (*18*). The model fulfills all restraints to within 0.1 Å.
25. The ligand amide NH-2 hydrogen bonds (as assigned by InsightII) with C6 cytosine C2 oxygen, NH-3 with G7 guanine N3 nitrogen, NH-4 with C8 cytosine C2 oxygen, and NH-5 with T9 thymine C2 oxygen.
26. Hydrogen bonds were assigned by the InsightII program. Nitrogen-nitrogen distances varied between 2.8 and 3.1 Å, with nitrogen-hydrogen distances between 1.8 and 2.2 Å.
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30. We are grateful to NIH (grants GM-27681 to P.B.D. and GM-43129 to D.E.W.) and the National Foundation for Cancer Research for research support, to the Ralph M. Parsons Foundation for a graduate fellowship to M.M., and to the U.S. Department of Energy (DE FG05-86ER75281) and NSF (DMB 86-09305 and BBS 87-20134) for instrumentation grants. The authors especially thank T. J. Dwyer for her contributions to the project. B.H.G. and D.E.W. also thank J. P. Jacobsen, H. P. Spielmann, and P. A. Fagan for helpful discussions.

6 July 1994; accepted 2 September 1994

Transgenic *X. laevis* Embryos from Eggs Transplanted with Nuclei of Transfected Cultured Cells

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Transgenic *Xenopus laevis* embryos were produced by transplantation of transfected cultured cell nuclei into unfertilized eggs. A *Xenopus* cell line, X-C, was stably transfected with plasmids containing a hygromycin-resistance gene and genes for either β-galactosidase with a heat shock promoter or chloramphenicol acetyltransferase (CAT) with a muscle-specific actin promoter. Nuclei transplanted from these cells into unfertilized eggs directed development of embryos containing stably integrated copies of the plasmids in each cell. Transgenic embryos showed somite-specific expression of CAT and uniform expression of β-galactosidase. Transgenic embryos produced by nuclear transplantation should be useful for testing the function of cloned genes in amphibian development.

Xenopus laevis (the African clawed toad) is widely used for the study of vertebrate development because the embryos are easily manipulated and accessible at all stages. Gene products that mediate inductive and morphogenetic events in the early *Xenopus* embryo have been identified. However, it has only been possible to study the function of products of these cloned genes by analyzing transiently expressed RNA or DNA injected into fertilized eggs or early cleavage stage embryos. Injected RNAs are translated immediately and are often degraded before the inductive interactions and morphogenesis of the gastrula stage have begun. Thus, although RNA injection can be effectively used to study maternally expressed genes, the endogenous products of which are present at early stages in the embryo, it is unfavorable for the study of zygotic gene products expressed after the mid-blastula transition, which occurs when the embryo has about 4000 cells. DNA injection has been used for analyzing zygotic gene expression, but the usefulness of this approach has been limited by the extremely mosaic expression of such DNAs in the embryos after injection.

Transgenesis has been attempted in *Xenopus* by injecting plasmid DNA into fertilized eggs, raising embryos to adults, breeding the offspring, and screening them for transmission of injected sequences (*1*). However, the long generation time of *Xenopus* (8 months minimum) makes this approach cumbersome, and in the test case, the adults produced were mosaic for the introduced sequences, integrated the DNA into the germ line only at low frequency, and produced offspring that failed to express the introduced reporter gene.

We have developed an alternate approach for transgenesis in *Xenopus*. Cultured cells are transfected with reporter sequences, stable integrants are selected, and these cells are used as donors for nuclei that are then transplanted into unfertilized eggs. Nuclei from various *Xenopus* cells are able to support embryonic development after such transplantations, although the extent of development depends on the proliferative and differentiated state of the donor cells (*2*).

The *Xenopus* cell line X-C was generated from stage 34–38 whole tadpoles (*3*). Like other established lines, the line is slightly aneuploid (*4*). We used lipofection to stably

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transfect X-C cells (*3*) with the linearized constructs pRLCAR and pRLgal, which both contain the hygromycin B phosphotransferase gene (Fig. 1A) (*5*). The construct pRLCAR also encodes a CAT reporter under the control of a *Xenopus* muscle actin promoter; pRLgal encodes a β-galactosidase gene controlled by a *Xenopus* heat shock (HSP 70) promoter. Stably transfected cells were selected in the presence of hygromycin. Hygromycin B concentrations above 550 μg/ml were toxic for nontransfected X-C cells, whereas transfected cells were fully resistant (Fig. 1B). Stably transfected cell populations maintained resistance over several months in culture and after growth in nonselective media, indicating stable integration. Plasmid sequences were detected in long-term–resistant cells (*6*). The stable transfection efficiency was 2.8% for pRLgal (*7*).

To test whether hygromycin-selected cells stably transfected with pRLgal would express a cotransfected β-galactosidase reporter gene, cells were exposed to the vital substrate fluorescein di-(β-D-galactopyranoside) (FDG) (*8*). Intracellular β-galactosidase cleaves FDG to produce fluorescein, which is transiently retained within the cell. An average of 23% of pRLgal-transfected X-C cells were FDG positive (*9*), indicating effective cotransfection. Some heterogeneity with respect to the intensity of fluorescence was detected in positive cells, perhaps representing variation in the number of DNA molecules introduced into the cell (*10*), the number of copies maintained, or the amount of expression from each plasmid. It is possible that transformed cells could express too little β-galactosidase to be detected by FDG or that cells may have lost the transformed gene.

Nuclei from X-C cells stably transfected with pRLCAR or pRLgal were used for transplantation into unfertilized eggs to produce transgenic embryos. The classical transplantation technique (*11*) requires that each individual donor cell be broken manually. We chose instead to weaken the cell membrane with lysolecithin (L-α-lysophosphatidylcholine), allowing many cells to be drawn into the needle at one time and their intact nuclei injected singly and successively into unfertilized eggs (*12*). This modification allows several hundred nuclei per hour to be transplanted. When gastrula stage cells were so used as the source of nuclei, embryonic development of transplanted eggs was as frequent and as advanced as with the classical method.

Both enucleated and nonenucleated unfertilized eggs were used as hosts for transplantations. Nonenucleated eggs were often used because embryos from these transplantations developed to more advanced stages; this is presumably due to the ability of the haploid egg chromosomes to cover deficiencies of the aneuploid donor nucleus. The pseudotriploid embryos (*13*) generated by this type of transplantation are morphologically normal, as are triploids produced by other mechanisms, but do not develop to adulthood as do full triploids (*14*).

Both pRLCAR and pRLgal were expressed nonmosaically in transgenic embryos derived from nuclear transplantation of stably transfected cells. After transplantation into nonenucleated host eggs, development of embryos frequently reached neurula and tailbud stages and both reporter genes were expressed. CAT protein was detected by immunocytochemistry (*15*) in all cells of the somites of neurula stage transgenic embryos expressing pRLCAR (Fig. 2, D and E). In contrast, neurula stage embryos derived from pRLCAR plasmid injections into fertilized eggs showed highly mosaic although somite-specific CAT expression (Fig. 2, A and B). Only about 5% of the somite cells (an average of 12 cells per embryo) were CAT positive when 100 pg of DNA was injected per fertilized egg. Higher

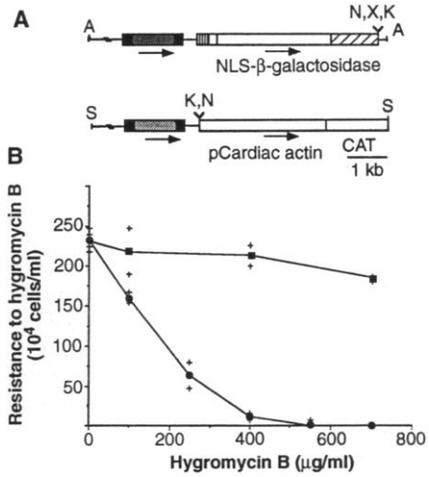

Fig. 1. X-C cell line stably transfected with pRLCAR and pRLgal vectors. (**A**) Structure of plasmids pRLgal (top) and pRLCAR (bottom). Both plasmids contain the hygromycin B phosphotransferase gene (gray) and thymidine kinase promoter and polyadenylation signals (solid). The plasmid pRLgal contains a *X. laevis* heat shock promoter (HSP 70, vertical stripe), a nuclear localization signal (NLS) and β-galactosidase reporter gene, and SV40 polyadenylation signals (diagonal stripe). We linearized pRLgal with Asp 700 (A) and Xba I (X) for transfections. The plasmid pRLCAR contains a cardiac actin promoter and CAT reporter and was linearized with Sph I (S). N, Not I; K, Kpn I. (**B**) Resistance of stably transfected and nontransfected X-C cells to hygromycin B. We seeded 60-mm plates with 4×10^5 nontransfected X-C cells (●) or X-C cells previously transfected with pRLgal and selected with hygromycin B (■) as described (*3*). Monolayers were treated with hygromycin B–containing media at the doses shown for 14 days. We counted 500 to 1000 cells to derive an average count (10^4 cells per milliliter) for each sample. Each data point represents the average of three to four samples from two separate experiments. Crosses show the range of data obtained.

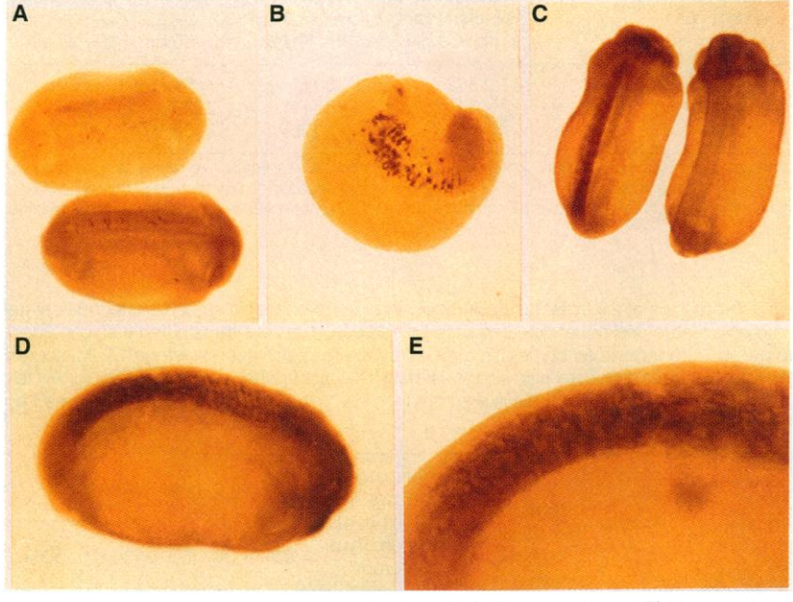

Fig. 2. Injected and transgenic neurulae expressing the pRLCAR vector. (**A** and **B**) Neurulae injected as fertilized eggs with 100 pg (A) and 300 pg (B) of pRLCAR–Sph I plasmid. (**C** to **E**) Transgenic neurulae generated after nuclear transplantations of pRLCAR-transfected X-C cells into nonenucleated eggs show somite-specific expression of CAT. In (C) one of two normal, neurula stage (stage 20–21) nuclear transplant recipient embryos shown is expressing CAT only in somites on one side of the body axis. (D) and (E) are side views of a transgenic embryo showing CAT expression in differentiated and undifferentiated somitic tissue.

doses of DNA (300 to 400 pg) increased the frequency of CAT positive cells slightly (to approximately 8% of somite cells), but embryonic development was frequently arrested (Fig. 2B). Thus, nuclear transplantation-based transgenesis produces embryos in which the spatial and temporal regulation of pRLCAR is preserved, expression is nonmosaic, and development proceeds through the stages when induction and morphogenesis occur. Transgenic embryos expressing pRLgal were also generated. Some of these embryos expressed β-galactosidase uniformly in each cell as expected (Table 2; β-galactosidase expression detected with X-gal) because the *HSP70* promoter is not tissue-specific.

Transgenic embryos expressing pRLCAR and pRLgal were produced at consistent frequencies: 20 to 50 neurula stage embryos resulted from each experiment in which 200 to 500 nuclei were transplanted and, of the surviving neurulae, 24% (6 to 12 embryos) expressed the pRLCAR transgene. Transgenic embryos expressing pRLCAR were identified at a somewhat higher frequency than those expressing pRLgal (Tables 1 and 2). This is probably due to differences in promoter strength or sensitivity of the assays used to detect transgene expression.

Some transgenic embryos expressed reporter constructs uniformly, whereas others exhibited chimeric expression (Tables 1 and 2). Most commonly, chimeric embryos expressed the transgene on only one side of the body axis (Fig. 2C). This chimerism (denoted as 50 to 95% expression in the Tables) is distinguishable from the mosaic expression resulting from plasmid injections. Right-left chimerism is probably due to segregation of chromosomes of the transplanted nucleus to only one blastomere at first cleavage, whereas those of the replicated haploid egg nucleus segregate to both blastomeres. Unilateral segregation occurs when transplanted nuclei from slowly dividing cells (X-C has a cell cycle of 35 to 40 hours) are unable to adjust to the more rapid 80-min cell cycle of the fertilized egg (*16*). In such right-left chimeras, the side of the body axis expressing the reporter gene is pseudotriploid whereas the nonexpressing side is haploid.

Some embryos produced by transplantations into nonenucleated eggs developed to feeding tadpole stages and lived about 1 month. They had long, well-differentiated body axes and showed no signs of haploid syndrome, although there were subtle anatomical abnormalities. Of the 37 neurulae (Table 2) from nonenucleated hosts, 21 were stained at tailbud stage for β-galactosidase; three were positive. The remaining 16 embryos were raised to tadpole stages, at which time three began to feed; one of these expressed the transgene.

Enucleated unfertilized eggs were also used as hosts for transplantations. Enucleation was accomplished by ultraviolet irradiation of the animal hemisphere (*17*). Transplantation of nuclei into these hosts resulted in high frequencies of transgene expression and less chimeric expression (Table 2). However, fewer embryos survived, and those that did exhibited morphological abnormalities more frequently and did not develop beyond neurula stages. These embryos should make good candidates for serial transplantation (retransplantation of nuclei), which is known to increase the frequency of development to advanced stages (*18*). Therefore, even in an enucleated background, the transfected pseudodiploid X-C nucleus could support development to gastrula and neurula stages, by which time expression of many zygotic genes, such as that for muscle actin, has begun.

The nuclear transplantation-based transgenic approach described here allows *Xenopus* embryos to be produced that express introduced genes nonmosaically with the appropriate spatial and temporal regulation. This transgenic approach should be useful both for the ectopic expression of genes for dominant interference and blocking experiments and to analyze spatial and temporal regulation by promoters in the embryo. The use of euploid donor cell types (such as transfected embryonic cells or sperm nuclei) or the use of oocytes rather than eggs as hosts (*19*) may improve the extent of development after transplantation. Alternative integration strategies may also increase the frequency of transgenesis.

Table 1. Somite-specific expression of a CAT reporter gene in transgenic neurulae after nuclear transplantation of pRLCAR-transfected cells. Transgenic embryos were produced (*3, 12*), raised to stage 19-20, and immunostained to detect CAT (*15*). Three experiments (Exp) are shown.

Exp	Attempts	Neurulae produced	Number of CAT-expressing embryos (%)	Variation in somite-specific CAT expression*			
				95–100%	50–95%	10–50%	1–10%
E 1	468	53	13 (25)	2 (15)	7 (54)	4 (31)	0
E 2	360	37	9 (24)	1 (11)	2 (22)	6 (67)	0
E 3	216	17	4 (24)	1 (25)	2 (50)	1 (25)	0

*CAT-expressing embryos were classified into four categories; the CAT-expressing region of each embryo was scored as a percentage of the total somitic tissue in the embryo. The number of embryos in each catgory is shown; in parentheses, the number is expressed as a percentage of the total number of CAT-expressing embryos obtained in each experiment.

Table 2. Expression of a β-galactosidase reporter gene in transgenic embryos after nuclear transplantation into enucleated and nonenucleated unfertilized eggs. The cells transfected with pRLβgal (*3*) were used to produce transgenic embryos (*12*). Embryos were raised to neurula (stage 18-20, scored below) through tadpole stages and were fixed and stained with X-gal (*20*) when they appeared unlikely to develop further. Data for nonenucleated hosts is a summary of two experiments; data for enucleated hosts is a summary of four experiments.

Host	Attempts	Neurulae produced	Number of β-galactosidase–expressing neurulae (% of embryos scored)	Extent of β-galactosidase expression*			
				95–100%	50–95%	10–50%	1–10%
Nonenucleated unfertilized egg	612	94	7/57 (12)	2 (29)	2 (29)	3 (43)	0
Enucleated unfertilized egg	1008	65	33/65 (51)	20 (61)	9 (27)	1 (3)	3 (9)

*Embryos were scored for extent of expression and classified into categories, as described in Table 1, after X-gal staining before clearing. The number of embryos in each category is shown and is expressed in parentheses as a percentage of the total number of β-galactosidase–expressing neurulae obtained.

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3. The X-C cell line (E1C4, obtained from R. Reeder, Seattle, WA), generated in 1988, was maintained in 60% L-15, 2 mM glutamine, 10% fetal bovine serum, and gentamicin sulfate (50 μg/ml) at 21° to 22°C in closed flasks. For lipofections, 4×10^5 to 6×10^5 cells were plated in 4 ml of L-15 medium (in 60-mm plates) and grown for 48 to 72 hours (35 to 50% confluence). We used 7.5 μg of linearized plasmid and 25 μl of lipofectin (Gibco, BRL), diluted in Optimem I (1X; Gibco), per transfection after the standard protocol; after 18 hours transfection, cell monolayers were washed. After 48 hours recovery in L-15 medium, cells were selected with hygromycin B (700 μg/ml, Sigma; 1×10^6 U/g) for 14 days. A mock lipofection (without resistance plasmid) was always run in parallel to confirm hygromycin resistance.
4. For aneuploid X-C cells, $2n = 40$, whereas $2n = 36$ for euploid cells [J. Tymowska and H. Kobel, *Cytogenetics* **11**, 270 (1972)]; 30 metaphase spreads were analyzed.
5. To construct pRLgal and pRLCAR, we excised a BgI

II–Not I fragment containing TK and *hyg B* sequences from pRBK (Invitrogen); we then added Bgl II–Kpn I–Xba I–Not I sites to make pRBL. For pRLCAR, the Sph I–Kpn I insert of #254 {3-kb upstream sequences of *Xenopus* cardiac actin gene–CAT reporter CAT reporter [T. Mohun, N. Garrett, J. Gurdon, *EMBO J.* **5**, 3185 (1986)]} was cloned into pRBL cut with Sph I and Kpn I. For pRLgal, a 4.8-kb Xho I fragment (A. Hemmati-Brivanlou and R. Harland, unpublished material) that contains *X. laevis hsp 70* [M. Bienz, *EMBO J.* **3**, 2477 (1984)] and nuclear localized β-galactosidase [W. Smith and R. Harland, *Cell* **67**, 753 (1991)] sequences was inserted into pRBL.

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9. Transfected cells were trypsinized, pelleted in a clinical centrifuge (~1000 rpm for 3 to 4 min), and incubated in 0.4 mM FDG (Molecular Probes, stock in dH_2O) in L-15 medium for 20 to 40 min. After repelleting, cells were resuspended in saline or L-15 medium, and fluorescence assayed at 40 to 50 min.
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12. To make wells to hold eggs for injection, we laid a template made of Dow Corning Sylgard 184 silicone elastomer (with protruding egg-sized knobs) face down in a 35-mm plastic petri dish on a layer of melted 2.5% agarose (Sigma type V) in 25% modified Ringer solution (MR), pH 7.0. MR contains 100 mM NaCl, 1.8 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Hepes. Needles for transplantations were hand-made from 30-μl Drummond micropipettes and had a needle bore of ~300 μm and a tip diameter of 20 to 40 μm (2 to 3 times the cell diameter to prevent clogging and cell lysis). Tips were clipped to a beveled shape [J. Gurdon, *Methods Cell Biol.* **16**, 125 (1977)]. A dissecting microscope with ×20 eyepieces (~×60 magnification) was used for transplantation; cells were visible against a dark background throughout. For injection, an air-filled 20- to 40-ml glass Hamilton syringe (VWR Scientific) was mounted vertically (tip-upward) and connected to the needle by a length of tubing; a Syringe Microburet (Micro-metric Instrument, Cleveland, OH) was used to control the movement of the glass syringe plunger. The needle was held in a micromanipulator (Narishige, Japan). For preparation of the donor nuclei, we used cell monolayers at 50 to 75% confluence to assure that cells were actively dividing. Cells from one to two 25-cm^2 flasks were trypsinized, pelleted in a clinical centrifuge (3 to 4 min at ~500 rpm), and resuspended in 0.5 ml of transplantation buffer (TB) {0.25 M sucrose, .75 mM NaCl, 0.5 mM spermidine trihydrochloride, and 0.15 mM spermine tetrahydrochloride, pH 7.0 [J. Gurdon, *J. Emb. Exp. Morph.* **36**, 523 (1976)]}. We added 10 μl of lysolecithin (L-α-lysophosphatidylcholine, Sigma, Type I: a stock of 1 mg/ml in dH_2O), and cells were incubated 15 min in this solution. During this incubation, unfertilized eggs were dejellied in 2.5% cysteine hydrochloride, pH 8.0, and then maintained in 100% MR, pH 7.0. After incubation with lysolecithin, two volumes of TB containing 3% bovine serum albumin was added to the cell suspension, and cells were collected by centrifugation as before. Cells were resuspended in about 250 μl of transplantation buffer. Higher lysolecithin doses and longer times of exposure retard development after the gastrula stage promoted by the treated cells. Transplantations were done within 60 to 90 min after preparing X-C cells and unfertilized eggs. The cell suspension, pipetted onto a flat surface, settled into a one- to two-cell-deep carpet. The needle was loaded at the proper density by sweeping it slowly just above this carpet. For injection, a slow outward flow was generated such that single cells entered the needle tip every 1 to 2 s and could be injected in a minimal amount of buffer. The unfertilized egg membrane was pierced in the animal hemisphere with a single, sharp motion, then drawn back to deliver a cell below the membrane. After transplantation, eggs were left undisturbed in 25% MR (pH 7.0) until first cleavage. Cleaving embryos were incubated in 25% MR (pH 7.0) containing 5% Ficoll. After gastrulation, embryos were moved into individual wells of 24-well plates and raised in 25% MR (pH 7.0) containing gentamicin sulfate (50 μg/ml); the vitelline envelope was removed from embryos showing cell leakage after gastrulation, as this improved survival.
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17. A Mineralight lamp (model UVGL-25 from UVP, San Gabriel, CA) with the blue filter removed was used for egg enucleation. Eggs were dejellied and placed animal hemisphere-up in wells in agarose-covered dishes. The lamp was mounted 31 mm above the dish; eggs were submerged in 25% MR to 2-mm depth (from the well bottom) for irradiation. The ultraviolet dosage (short-wave setting) for our lamp at this distance was 3400 μW/cm^2. Using the method of J. Gurdon [*Q. J. Microsc. Sci.* **101**, 299 (1960)], we found an irradiation time of 45 to 60 s effectively destroyed egg chromosomes without affecting normal cleavage and development.
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1 June 1994; accepted 24 August 1994

PHAS-I as a Link Between Mitogen-Activated Protein Kinase and Translation Initiation

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PHAS-I is a heat-stable protein (relative molecular mass ≈12,400) found in many tissues. It is rapidly phosphorylated in rat adipocytes incubated with insulin or growth factors. Nonphosphorylated PHAS-I bound to initiation factor 4E (eIF-4E) and inhibited protein synthesis. Serine-64 in PHAS-I was rapidly phosphorylated by mitogen-activated (MAP) kinase, the major insulin-stimulated PHAS-I kinase in adipocyte extracts. Results obtained with antibodies, immobilized PHAS-I, and a messenger RNA cap affinity resin indicated that PHAS-I did not bind eIF-4E when serine-64 was phosphorylated. Thus, PHAS-I may be a key mediator of the stimulation of protein synthesis by the diverse group of agents and stimuli that activate MAP kinase.

PHAS-I (*1*) was identified in rat adipocytes as one of several proteins that were phosphorylated in response to insulin (*2*). Rat PHAS-I is 93% identical to 4E-BP1, an eIF-4E binding protein cloned from a human placenta complementary DNA library (*3*). The mRNA cap-binding protein eIF-4E, which forms part of the larger eIF-4F complex, is limiting for the initiation step of translation, which is in turn usually rate-limiting for translation (*4*). PHAS-I (4E-BP1) inhibits eIF-4E function as it decreases translation of capped mRNA, both in vitro and when expressed in cultured osteosarcoma cells (*3*). Moreover, increased phosphorylation of PHAS-I in adipocytes with insulin is associated with decreased binding of PHAS-I to eIF-4E, indicating that the stimulation of translation by insulin may result from the release of eIF-4E from inhibition by PHAS-I (*3*). This mechanism would explain earlier findings that were suggestive of a stimulatory effect of insulin on eIF-4F activity (*5*). We found that PHAS-I was regulated by phosphorylation by MAP kinase. Thus, PHAS-I appears to mediate the regulation of protein synthesis by the large and diverse group of hormones, growth factors, and other stimuli that signal through the MAP kinase pathway (*6*).

The association of PHAS-I with eIF-4E was regulated by insulin in murine 3T3-L1 adipocytes. Insulin had no effect on the amount of eIF-4E in extracts of these cells,

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