uptake activity between these two compounds. Another interesting relationship is the percentage of SHE cells that take up Ni_3S_2 relative to the incidence of morphological transformation. For 1.0, 5.0, and 10.0 μg/ml of Ni_3S_2 (SHE cells, Table 1) this ratio averages about 3. This suggests that one out of three cells that take up Ni_3S_2 particles undergoes morphological transformation. The uptake/carcinogenesis ratio may be governed by Ni_3S_2 toxicity or may be related to the occurrence of a critical mutation which initiates the carcinogenesis process. We have investigated the uptake and carcinogenic activity of other particulate nickel compounds and several other metals (*15*). There is a good correlation between the uptake of these metals and their respective carcinogenic activities; this result suggests that carcinogenic activity of other metal compounds is proportional to their uptake.

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References and Notes

1. R. Doll, J. D. Matthews, L. G. Morgan, *Br. J. Ind. Med.* **34**, 102 (1977); L. Kreyberg, *ibid.* **35**, 109 (1978); R. Lessard, D. Reed, B. Maheux, J. Lambert, *J. Occup. Med.* **20**, 815 (1978).
2. F. W. Sunderman, Jr., *Prev. Med.* **5**, 279 (1976).
3. _____, *Fed. Proc.* **37**, 40 (1978).
4. _____, *Biol. Trace Element Res.*, in press.
5. _____ and R. M. Maenza, *Res. Commun. Chem. Pathol. Pharmacol.* **14**, 319 (1976).
6. _____, P. R. Allpass, J. M. Mitchell, I. Damjanov, P. J. Goldblatt, in *Inorganic and Nutritional Aspects of Cancer*, G. N. Shcrauzer, Ed. (Plenum, New York, 1978), pp. 57–67.
7. A. D. Ottolenghi, J. K. Haseman, W. W. Payne, H. L. Salk, H. M. MacFarland, *J. Natl. Cancer Inst.* **54**, 1165 (1977).
8. M. Costa, J. S. Nye, F. W. Sunderman, Jr., P. R. Allpass, B. Gondos, *Cancer Res.* **39**, 3591 (1979).
9. M. Costa, *Adv. Chem. Ser.* **172**, 73 (1979).
10. J. A. DiPaolo and B. C. Casto, *Cancer Res.* **39**, 1008 (1979).
11. Nickel particles probably enter the nucleus but apparently not in a physical form large enough to be easily detected with the electron microscope. Numerous nickel particles (2 to 5 μm) were seen pushing against the nuclear membrane. On the basis of current dogma and in vivo biochemical studies of nickel carcinogenesis, the nucleus is a primary site of nickel deposition during the course of malignant transformation. Our results suggest that nickel may enter the nucleus only as very small particles or as nickel ions broken down from larger Ni_3S_2 particles contained within cytoplasmic vacuoles. We are currently conducting studies in which measurements of total nickel by x-ray fluorescence will be correlated with particulate nickel visible in the light and electron microscopes.
12. The SHE cells were isolated from hamster embryos after 13 to 14 days of gestation. These cultures have all the properties of a normal cell (that is, orderly growth, defined life span in culture, inability to form three-dimensional colonies in soft agar, and lack of tumorigenicity when cells are administered to appropriate host). After exposure to a carcinogen such as Ni_3S_2, some of the normal cells undergo morphological transformation characterized by disordered growth. Cultures that exhibit this disordered growth pattern also have acquired the other properties of a cancer cell (growth in soft agar and tumor formation in nude mice). Therefore, on the basis of the incidence of morphological transformation, the carcinogenic activity of the substance may be accurately determined [see (*13*)].
13. J. A. DiPaolo, P. Donovan, R. Nelson, *J. Natl. Cancer Inst.* **42**, 867 (1969); C. Heidelberger and P. F. Boshell, *Gann Monogr.* **17**, 39 (1975); E. Huberman, R. Mager, L. Sachs, *Nature (London)* **264**, 360 (1976).
14. The LC_{50} was determined by exposing log-phase cultures of CHO cells to various concentrations of the metal compounds for 4 days, with fresh metal and media added every day. At the end of the exposure period, 100 to 1000 cells were plated into 100-mm tissue culture dishes to form colonies. The total numbers of surviving colonies in each dish were counted and expressed as a function of the number of cells plated to determine the plating efficiency. The control plating efficiency was 80 to 90 percent, and the concentration that reduced this 50 percent was the LC_{50}. The LC_{50} could also be measured in SHE cells, but the plating efficiency of these cells is lower (1 to 10 percent) and more variable than that of CHO cells.
15. The uptake of crystalline Ni_3Se_2 and crystalline NiS in SHE cells was approximately 70 to 80 percent that of Ni_3S_2 for similar exposure conditions. Both these compounds are potent carcinogens in experimental animals and in tissue culture transformation assays (*9*). Nickel metal was taken up about 10 percent more than amorphous NiS and has been shown to be weakly carcinogenic in experimental animals (*4*). Out of the CHO cells exposed to iron dust for 24 hours at 10 μg/ml, only 2.7 percent of the cells contained particulate material. Of CHO cells exposed to chromium and cobalt dust under similar conditions, 8.2 percent and 5.2 percent, respectively, contained particulate material. Iron dust is not carcinogenic, but chromium dust has been shown to be carcinogenic in experimental animals and has been implicated as a human carcinogen on the basis of epidemiological studies (*2*). Cobalt has induced tumors in experimental animals (*2*).
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Developmental Potential of Somatic Nuclei Transplanted into Meiotic Oocytes of *Rana pipiens*

Abstract. *Somatic nuclei, when transplanted into oocytes at the stage of first meiotic metaphase, were induced to undergo chromosome condensation and alignment on spindles. When the oocytes completed maturity in vitro and were activated, the somatic nuclei transformed into "fertilization" nuclei and promoted development through embryogenesis. Thus somatic nuclei can reversibly respond to cytoplasms directing either meiotic or mitotic events, and somatic nuclei from differentiated cells may be reversed by conditioning in oocytes.*

The genetic and developmental properties of somatic cell nuclei have been tested by transplantation of nuclei into mature eggs (*1*). Although somatic nuclei from young embryos promote development of amphibian eggs into normal larvae and adults, nuclei from well-defined cell types fail to promote normal development of the recipient eggs. These results suggest that genetic restrictions accompany cell specialization. However, nuclei from advanced cell types fail to integrate with the cytoplasmic division cycles of the egg and develop chromosomal abnormalities that lead to developmental restrictions of the nuclear transplants (*2*). Thus, conclusive proof of the theory of nuclear equivalence among specialized cell types is still lacking. One approach to distinguish between the above alternatives is to transplant somatic nuclei into oocytes where they may be first conditioned and then tested for developmental potential.

We report our initial studies on the developmental properties of somatic nuclei transplanted into maturing oocytes of *Rana pipiens*. The results demonstrate that blastula nuclei and endodermal nuclei of the tail-bud stage, when induced by meiotic cytoplasm to undergo chromosome condensation and alignment on spindles at the time of first meiotic metaphase, still retain the capacity to program for development. When the oocytes mature and are activated, the transplanted nuclei transform into "fertilization" nuclei and direct the formation of the various cell types required for the completion of embryogenesis. These results demonstrate that (i) somatic nuclei can reversibly respond to cytoplasms, directing either meiotic or mitotic events, and (ii) this system could be used for conditioning or reprogramming somatic nuclei from well-defined cell types that have so far exhibited genetic and developmental restrictions when transplanted into mature eggs (*1*).

Oocyte hosts utilized for nuclear transplantation were in the stage of first meiotic metaphase (first black dot). It is possible, by controlling the dose and time of pituitary and progesterone injections into frogs, to obtain maturing oocytes at first meiotic metaphase (*3*). These oocytes encased in jelly reach the uterus prematurely and can be stripped from the female, although they are not activatable or fertilizable at this time. About 24 hours later (18°C) when noninjected oocytes used for controls were

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Table 1. Nuclear transplantation into first meiotic metaphase oocytes: cleavage and development.

Experiment	Nuclear donor	Injected hosts (No.)	Blastulas (No.)		Gastrulas (No.)	Neurulae (No.)	Post-neurulae (No.)	Larvae (No.)	
			Partial	Complete				Abnormal	Normal
Enucleated eggs									
Control	Blastula	40	8 (78 %)	23	28 (70 %)	20 (50 %)	20 (50 %)	6 (15 %)	12 (30 %)
First meiotic metaphase oocytes									
Matured, then transplanted									
18°C	Blastula	10	1 (80 %)	7	7 (70 %)	7 (70 %)	7 (70 %)		7 (70 %)
10°C	Blastula	10	2 (20 %)						
4°C	Blastula	17	1 (18 %)	2	2 (12 %)	2 (12 %)	2 (12 %)	1 (6 %)	
Transplanted, then matured	Blastula	52	23 (64 %)	10	16 (31 %)	13 (25 %)	11 (21 %)	4 (8 %)	
	Stage 19 endodermal	80	33 (51 %)	8	17 (21 %)	4 (5 %)	1 (1 %)	1 (1 %)	

pricked with a glass microneedle and displayed evidence of activation (surface change from shiny to dull and elevation of the vitelline membrane and rotation), the experimental oocytes were transferred to 10 percent Ringer solution and activated; within 10 minutes the black dot indicative of the second meiotic metaphase of the host was removed surgically with a glass microneedle. Methods of handling oocytes and nuclear transplantation have been described (*4*, *5*). Nuclear transplantations were made into oocytes at two periods: (i) after completion of maturation in vitro and (ii) when they were in the stage of first meiotic metaphase.

Matured hosts served as a control for the in vitro system and also provided information on the essential requirement for proper time of oocyte activation. If the experimental conditions of the in vitro system are not harmful, then normal development should result when blastula nuclei are injected individually into matured hosts. Oocytes at first meiotic metaphase were allowed to complete maturation in vitro in Ringer solution; then they were activated, enucleated, and injected with single blastula nuclei. Seventy percent of the matured hosts that had been injected with a blastula nucleus developed into normal larvae (Table 1). When activated and enucleated eggs that had matured in vivo were injected with a blastula nucleus, 30 percent developed into normal larvae (Table 1). In an earlier experiment, Subtelny and Bradt (*6*) transplanted blastula nuclei singly into nonenucleated body-cavity oocytes that had previously matured in vitro from the first meiotic metaphase and found that 33 percent developed into normal larvae. Thus, the experimental conditions of the in vitro system permit normal larval development.

As was pointed out above, the time at which nuclear transplant oocytes acquire activatability is approximated by pricking noninjected control oocytes to determine when they are activatable. However, there is some temporal variability among the oocytes, and not all mature simultaneously. Oocytes obtained at first meiotic metaphase from the same female were matured at three different temperatures (18°, 10°, and 4°C) to determine whether activatability influences the success of nuclear transplants. Since amphibians are poikilothermic animals, the rate of maturation is controlled by environmental temperature. Only those oocytes incubated at 18°C promoted normal development or nuclear transplants (Table 1). Oocytes maturing at 10°C were used for hosts 2 hours later and those incubated at 4°C were used 4 to 5 hours later than the 18°C group. Thus, oocytes do not develop normally unless the hosts have attained complete maturity at the time of attempted activation.

In experiments with immature hosts, a single nucleus was transplanted into oocytes at first meiotic metaphase; approximately 24 hours later the nuclear transplants were activated, and the host nucleus was removed surgically. The objective of this study was to determine whether a somatic nucleus still retains developmental potential after exposure to cytoplasm that induces meiotic events.

A number of oocytes at first meiotic metaphase were injected with a blastula nucleus, fixed after transplantation (at 5 minutes, and 4, 8, 26, and 28 hours), and examined cytologically to determine whether the injected nucleus responds to the meiotic influence. Five minutes after nuclear transplantation the oocyte nuclei were in metaphase, and the injected nuclei were in condensed interphase (two cases). However, by 4 hours and up to 28 hours after nuclear transplantation, the injected nuclei had been converted to metaphase chromosomes on a spindle (seven cases) and exhibited the same cytological characteristics as the host nuclei (Fig. 1, A and B), confirming that the cytoplasm influences nuclear activity (*7*). These transplanted somatic nuclei still retained developmental potential, evidenced by the fact that of the oocytes that were allowed to develop and were activated and whose host nucleus was removed, 64 percent cleaved into blastulas, 31 percent gastrulated, 25 percent neurulated, 21 percent developed into postneurula embryos, and 8 percent formed larvae (Table 1). When endodermal nuclei from stage 19 tail-bud embryos were tested, 51 percent of the injected oocytes formed blastulas, 21 percent gastrulated, 5 percent neurulated, and one nuclear transplant proceeded through neurulation and postneurulation (Fig. 1C) and developed into a larva.

Three types of controls were conducted to check the quality of the gametes, parthenogenetic activity, and enucleation efficiency. (i) Of the inseminated eggs whose siblings had provided donor cells for nuclear transplantations, 89 percent (230 cases) developed into normal larvae, attesting to the high quality of the eggs and sperm. (ii) Oocytes at first meiotic metaphase that had completed maturation in vitro were

activated (109 cases); these underwent abortive cleavages and none developed into blastulas, demonstrating that development of the experimental oocytes was not due to parthenogenesis. (iii) In another group of activated oocytes, the black dot (host nucleus) was removed surgically (45 cases); none of these displayed puckers or furrows of the egg surface within the first 5 hours after activation, and on the following day they remained uncleaved, indicating successful removal of the host nucleus.

The development and ploidy ensuing from nuclear transplantation into oocytes could be influenced by retention of the host nucleus and by meiotic reduction divisions of the transplanted nucleus. As a further check on the enucleation efficiency, we examined the time of first cleavage, estimated epidermal cell size of embryos during postneurula stages, and conducted cytological studies on nucleolar and chromosome number. Nuclear transplants that initiated cleavage at $2^1/_2$ to 3 hours after activation were diploids (13 cases), and those that were delayed one interval in cleavage were tetraploids (3 cases), indicating that transplanted diploid nuclei were responsible for the development obtained. If the host nucleus was responsible for development and the egg cleaved on time or was delayed one cleavage interval, the resulting embryos would be haploid and diploid, respectively; no such individuals were detected in our analyses. We do not yet know whether a transplanted diploid nucleus proceeds through anaphase and completes division; if it does, then the nuclei must fuse in order to retain the diploid state. In addition, only one black dot corresponding to the host nucleus has been observed. When the injections are made into the oocyte, the micropipette is inserted near the equator. Microscopic analysis of serial sections of nuclear transplant oocytes revealed that the injected nuclei were located just above the equator, approximately one-third to one-half the distance from the equator to the animal pole, but never close to the cortex. Thus even if division had occurred, an effective polar body could not be released from the oocyte.

Our studies show that many somatic nuclei residing in oocytes at first meiotic metaphase and responding to the meiotic events are capable of directing blastula formation. The oocytes that failed to cleave, or cleaved abortively, in most cases still underwent nuclear replication. Microscopic examination of these nuclear transplants and of partially cleaved blastulas revealed several to more than 100 nuclei in the uncleaved areas. Thus their developmental limitation appears mainly to be a restriction of cleavage but not of nuclear replication. Both blastula and tail-bud endodermal nuclei still retained the genetic potential to direct organogenesis. Thus, somatic cell nuclei transformed during meiosis can become "fertilization" nuclei and direct the formation of the various cell types required for the completion of embryogenesis. With improvement in the optimal time of oocyte activation, it may be possible to increase the developmental success of these nuclear transplants and to utilize this system for conditioning or reprogramming somatic nuclei from adult cell types that have so far exhibited developmental restrictions when transplanted into mature eggs (*1*). It is possible that nuclei of specialized cells still have the same complement of genes derived from the zygote nucleus, but when transplanted into eggs their chromosomes are unable to reverse fast enough to fit into the short cell cycles of the recipient egg (*2*). In fact, studies with erythrocyte nuclei from adult *R. pipiens* have shown that when they are transplanted into mature eggs, they show little or no response; but when they are conditioned in diplotene oocytes, which are then induced to mature with progesterone and are activated, the erythrocyte nuclei enlarge and incorporate [^{3}H]thymidine triphosphate, demonstrating that noncycling erythrocyte nuclei can be at least partially reversed (*8*).

These findings could conceivably open up new approaches to genetic analysis of somatic cells. For this research, the somatic nucleus was injected into the interior of the oocyte to prevent release of a polar body if reduction division occurred. However, if the somatic nucleus were injected under the cortex of the oocyte (*9*), a polar body might be released, resulting in meiotic reduction of the diploid somatic chromosomes. Such a result could be used to clone for homozygous individuals and selection of genetic complements.

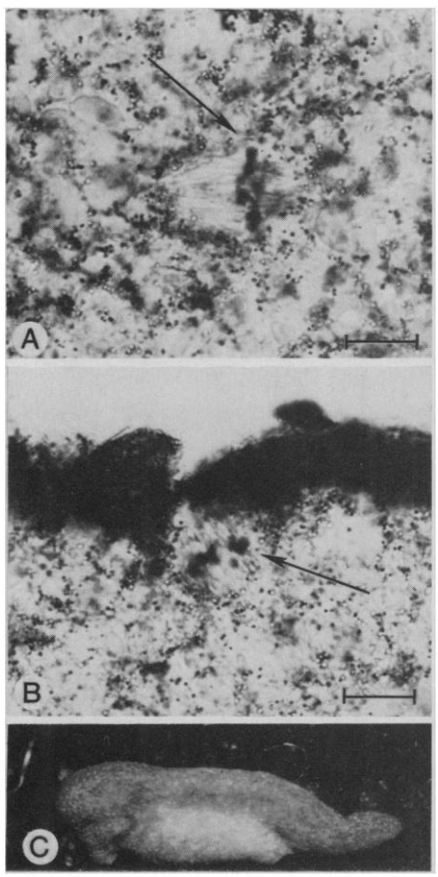

Fig. 1. Oocyte at first meiotic metaphase, injected with a single somatic nucleus. (A and B) Oocyte fixed 8 hours after nuclear transplantation. Scale bar, 10 μm. (A) Injected nucleus has transformed into metaphase chromosomes aligned on a spindle located approximately one-third down from the animal pole. (B) Oocyte metaphase chromosomes are aligned on meiotic spindle located just beneath surface coat of animal hemisphere. Note difference in size of spindles in A and B. The spindles of injected nuclei are 1.3 to 1.6 times longer (pole to pole) than the hosts' spindles. (C) Nuclear transplant derived from oocyte host (first meiotic metaphase) injected with a tail-bud endodermal nucleus (stage 19). The nuclear transplant developed normally through neurulation and then exhibited slight abnormalities during the postneurula and larval stages. Photographed at stage 19, the animals later developed into a swimming larva (stage 22$^+$). Length approximately 6 mm.

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References and Notes

1. R. Briggs and T. J. King, *Proc. Natl. Acad. Sci. U.S.A.* **38**, 455 (1952); R. G. McKinnell, *Cloning, Nuclear Transplantation in Amphibia* (Univ. of Minnesota Press, Minneapolis, 1978); R. Briggs, *Int. Rev. Cytol. Suppl.* **9**, 107 (1979); J. B. Gurdon, R. A. Laskey, E. M. DeRobertis, G. A. Partington, *ibid.*, p. 161; M. A. DiBerardino and N. J. Hoffner, in *Results and Problems in Cell Differentiation*, R. G. McKinnell, M. A. DiBerardino, M. Blumenfeld, R. Bergad, Eds. (Springer-Verlag, New York, in press).
2. M. A. DiBerardino, *Int. Rev. Cytol. Suppl.* **9**, 129 (1979).
3. R. P. Elinson, *J. Embryol. Exp. Morph.* **37**, 187 (1977).
4. M. A. DiBerardino, N. J. Hoffner, M. B. Matilsky, *Methods Cell Biol.* **14**, 141 (1977).
5. T. J. King, in *Methods in Developmental Biology*, F. H. Wilt and N. K. Wessells, Eds. (Crowell, New York, 1967), p. 737.
6. S. Subtelny and C. Bradt, *Dev. Biol.* **3**, 96 (1961).
7. Y. Masui and H. J. Clarke, *Int. Rev. Cytol.* **57**, 186 (1979).
8. R. A. Leonard, N. J. Hoffner, M. A. DiBerardino, *Am. Zool.* **19**, 951 (1979); *Anat. Rec.* **196**, 237A (1980).
9. S. Subtelny, *Am. Soc. Cell. Biol.* **39**, 131a (1968).
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