traditional boundaries and achieve an unprecedented level of mutual understanding, innovation, and cooperation. These efforts will have to go across disciplines, across sectors of societies, and across nations.

Let us hope that fundamental values of freedom, curiosity, opportunity, diversity, excellence, and human decency will guide our institutions as they evolve. These enduring values can make it possible for us to cope with the great problems of our time, to work steadily towards the humane uses of science and technology, and to take advantage of unprecedented opportunities that are emerging for the benefit of people everywhere.

Activation of Dormant Genes in Specialized Cells

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Summary. In several experimental systems the genomic capacity in specialized cells can be assessed by examining the activation of dormant genes. Since some of these specialized cells can be induced to change cell phenotype, all cell specializations do not necessarily involve irreversible genetic changes.

In the development of multicellular organisms, specialized functions of organ systems are achieved by their specialized cell types. When cell types once acquire the metabolic pathways for performing a special function, no further changes usually occur. We call this the stability of the differentiated state. It is now widely accepted that the process and stability of cell specialization are under genetic control. However, whether or not the establishment and maintenance of cell specialization generally involve irreversible genetic changes still has not been answered adequately. The support for and against genetic irreversibility has varied from time to time, opinion often being influenced by the organism studied and the techniques available. During the 1960's, the general view was that adult organisms retain the same set of genes as the nucleus of the fertilized egg, and that cell specialization is accomplished through the differential recruitment of genes and expression of their products. Gene losses in, for example, *Ascaris* and *Sciara* were considered special exceptions. Then with the explosion of highly sensitive molecular techniques capable of quantifying genes and determining nucleotide sequences in DNA, we became aware that in some cases genes can increase in number (gene amplification) and DNA can undergo rearrangements (*1*). However, it has not yet been shown that generally there are nucleotide losses affecting genomic totipotency.

In several experimental systems, the phenotype of specialized cells can be converted into a different phenotype. This conversion, when analyzed by molecular techniques, is shown to be accompanied by the synthesis of new and different gene products (RNA's, polypeptides, or proteins), an indication of the activation of dormant genes. Such systems provide a means for evaluating the degree to which cell specialization can be modulated and the degree to which gene function can be reversed. In this article we examine the current status of genomic capacity in specialized cells by analyzing the degree to which dormant genes can be activated in the experimental systems of cellular transdifferentiation, cell hybridization, nuclear transplantation in amphibians, and some cancers. In addition to discussing our data on nuclear transplantation, we point out that the fundamental phenomenon of induced gene activation is observed in these diverse experimental systems, as well as others. Collectively, these results demonstrate that a high degree of genomic information inherited from the zygote must be maintained during cell specialization because the stable phenotypes are reversible under appropriate experimental conditions.

Cellular Metaplasia or Transdifferentiation

Specialized cells in vivo do not ordinarily change into another cell type. There are, however, a few exceptions to the stability rule among vertebrates where alteration in the specific characteristics of differentiated cells occurs. This phenomenon has been termed cellular metaplasia or transdifferentiation because it results in cell-type conversion. A well-known example of transdifferentiation is the Wolffian regeneration of lens from the pigment cells of the iris epithelium in several adult urodelen species. Almost a century ago, it was discovered that in the adult newt a new lens can form from the dorsal iris after partial or complete lentectomy (*2*). In vitro studies stongly support the widely held belief that iris epithelium cells (IEC's) are converted into lens cells after complete lentectomy of newt eyes. Dorsal iris epithelium, isolated and cultured in the presence of frog retinal complex, differentiated newt lens tissue that contained lens-fiber specific γ-crystallins and total lens protein (*3*). Even in primary culture, lens cells formed from IEC's derived from both dorsal and ventral irises and clones of IEC's have expressed the lens phenotype (*2, 4*).

Another example of cell-type conversion occurs during regeneration of the urodelen amphibian limb. Soon after the limb is amputated, epidermal cells migrate distally and cover the wound surface. The internal limb tissues (skeletal muscle, bone, cartilage, and other connective tissues) proximal to the site of amputation then dedifferentiate into un-

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Table 1. Activation of genes in cell hybrids.

Hybrids between		Test product	Source
Parent 1	Parent 2		
Rat hepatoma	Mouse fibroblast	Mouse albumin	(*11*)
Rat hepatoma	Mouse lymphoid cell	Mouse albumin	(*12*)
Rat hepatoma	Mouse lymphoid cell	Mouse tyrosine–aminotransferase and aldolase B	(*13*)
Mouse hepatoma	Human diploid peripheral leukocyte	Human albumin	(*14*)
Mouse hepatoma	Human amniocytes	Human albumin Human transferrin Human ceruloplasmin Human α-1 antitrypsin	(*15*)
Mouse Friend eythroleukemic cells	Human fibroblasts	Human α-, β-globin mRNA	(*16*)
Mouse Friend erythroleukemic cells	Mouse teratocarcinoma	α-Globin of mouse teratocarcinoma	(*17*)

specialized cells that proliferate and form a regeneration blastema (*5*). After a period of further proliferation, blastemal cells redifferentiate and reconstruct the missing part. The potential of the cells during limb regeneration has been studied by grafting labeled tissue into the host limb before regeneration begins. In addition, when the host limb is subjected to x-irradiation before grafting to prevent host tissues from participating in limb regeneration, triploid cartilage that had been grafted into diploid axolotl formed cartilage, perichondrium, connective tissue of the joints and dermis, and possibly muscle. On the other hand, epidermis is quite restrictive. It grows over the stump but fails to regenerate other cell types (*5, 6*). The two examples of cell-type conversion—lens and limb regeneration—both involve dedifferentiation, cell proliferation, and redifferentiation; but the transdifferentiation switch is confined to cell types from related primordial origin.

Heterokaryons and Cell Hybrids

Cell fusion experiments have provided evidence for activation of silent genes (*7*). In this system somatic cells with different phenotypes can be fused together. An outstanding example is the activation of the dormant genes in the hen erythrocyte. Fusion of hen erythrocytes to HeLa cells, resulted in the appearance of heterokaryons, which are cells containing separate nuclei from different types of parental cells. Each nucleus can be identified according to its cellular origin and then monitored. The erythrocyte nuclei enlarge, their highly condensed chromatin disperses, and they resume synthesis of RNA and DNA. The factors responsible for reactivation of hen erythrocyte nuclei are not species specific (*8*). They are due to components in the cytoplasm of active cells because reactivation occurred when erythrocyte nuclei were placed into cytoplasts prepared by cytochalasin-induced enucleation of mouse fibroblasts (*9*). Once nucleoli reappear in erythrocyte nuclei of heterokaryons, many newly synthesized gene products of the chick can be identified; these include surface antigens, surface receptors, nucleolus-specific antigens, α- and β-globin messenger RNA (mRNA) and polypeptides, at least three different enzymes involved in nucleotide synthesis, and approximately 40 constitutive polypeptides (*7, 8, 10*).

Another aspect of somatic cell genetics that has provided evidence for the activation of genes is the study of mononucleated cell hybrids. When heterokaryons are permitted to enter mitosis, the chromosomes of both parental nuclei form a single nucleus and a mononucleated cell is formed known as a cell hybrid. When cell hybrids are formed between parental cells exhibiting different facultative markers (that is, specialized functions), the markers can continue to be expressed or fail to be expressed (extinction); in some cases new functions that normally do not occur in that particular cell type can be activated. For the purposes of this article, the activations of new functions are considered (Table 1). Rat and mouse hepatoma cells normally synthesize certain liver-specific proteins. When mouse fibroblast (*11*) and mouse lymphoid (*12*) cells were each fused with rat hepatoma cells, the resulting hybrids produced mouse albumin, a liver protein that the mouse parental cells do not make. In another study, other mouse liver-specific proteins (tyrosine aminotransferase and aldolase B) were induced in mouse lymphoid cells after cell hybridization with rat hepatoma cells (*13*). Likewise, interspecific hybridization between mouse hepatoma cells and human leukocytes led to the activation of human albumin (*14*), and hybridization of mouse hepatoma cells and human amniocytes led to the activation of four human proteins (albumin, transferrin, ceruloplasmin, and α-1 antitrypsin) (*15*). Friend mouse erythroleukemic cells, after interspecific fusion with human fibroblasts (*16*) and intraspecific fusion with mouse teratocarcinoma cells (*17*), induced the formation of human α- and β-globin mRNA and mouse α-globin, respectively. In all the above cases the interpretation is that the regulatory molecules responsible for the transcription of those genes in one cell type activate the silent genes in the other cell type. Thus, the cited studies indicate that some genes that are stably repressed in eukaryotic cells can be activated.

Nuclear Transplantation

For metazoan animal cells the only available technique that theoretically tests the entire genome within the confines of a living system is nuclear transplantation (*18*). This procedure, first developed in the anuran amphibian, *Rana pipiens*, by Briggs and King, demonstrated that many nuclei from undetermined regions of the embryo can direct eggs to develop into juvenile frogs (*19*), and if reared further, into sexually mature adults (*20*). These results indicated that all the genes required for normal development are present and functional; therefore, the donor nuclei are genetically equivalent to the zygote nucleus. The totipotency of early embryonic nuclei was confirmed in other anuran and some urodelen amphibian species (*21*) and extended to the fruit fly, *Drosophila* (*22*), and the fish (*23*). In the mouse, pronuclei (*24*) and nuclei of the inner cell mass (*25*) have now been shown to be totipotent.

In order to determine whether genes can be activated, it is necessary to test nuclei from specialized cells that are

Table 2. Pre- and posthatching tadpoles from nuclei of specialized cells. Stages 18 to 19 and 29 to 36 are postneurula embryos displaying muscular movements, heart beat, gill rudiments and, in some cases, initial hatching. Stages 20 and 37 to 40 are tadpoles which hatch from their jelly capsules, display blood circulation in the gills and initiate coordinated swimming. Stages 22 to 23^+ and 41 to 44 have well-formed external eyes, operculum folds, and intestinal coiling. Stage 25^+ in *Rana* have operculum folds completely covering the gills and normally would initiate feeding at stage 24^+.

Source of cells*	Total nuclei tested† (100%)	Number of injected hosts arresting at stage					Total (%)	Source
		18–19‡ 29–36§	20 37–40	22–23 41–44	25^+ 47^+	Feeding		
Male germ (*R*)	116	3				1	3.5	(*26*)
Cultured melanophores (*X*)	257	2					0.8	(*27*)
Cultured skin (*X*)	129	2	3‖	1			4.7	(*28*)
Spleen cells (*X*)	100		5‖	1			6.0	(*29*)
Erythroblasts (*X*)	442	8					1.8	(*30*)
Erythrocytes (*R*)	130	5	2	3	1		8.5	(*31*)

*Adult cells except larval melanophores. †Total number of nuclei tested includes results from serial transplantations. ‡Stages of Shumway (*68*) for *Rana pipiens* (*R*). §Stages of Nieuwkoop and Faber (*69*) for *Xenopus laevis* (*X*). ‖Probably greater than stage 36 but less than stage 41.

expressing a subset of genes required to maintain the specialized phenotype. Nuclear transplantation tests of specialized cells so far have been performed in the anuran amphibian species, *Rana pipiens* and *Xenopus laevis*. Although no adults have yet developed from the nuclei of specialized cells, pre- and posthatching tadpoles have resulted. In the case of prehatching tadpoles (postneurula embryos), the main embryonic organ systems are established in a rudimentary form and, in later stages, they display muscle and nerve function. Thereafter, they hatch from their jelly capsules, become free-swimming larvae (tadpoles), and display differentiated cell types in the various organ systems. Nuclei capable of providing genetic direction for this extent of development are multipotential, since a significant portion of the genome must have been recruited to specify the various RNA transcripts and proteins required for forming the cell types in postneurula embryos and tadpoles.

The evidence accumulated so far in support of genetic multipotentiality of nuclei from specialized cell types is summarized in Table 2. The most advanced nuclear transplant has been a feeding tadpole obtained from a spermatogonial germ cell of adult *Rana* (*26*). Somatic nuclei from five specialized cell types have been tested; these are melanophores of larvae, adult skin cells, spleen cells including lymphocytes, erythroblasts, and erythrocytes (*27–32*). In all cases, nuclei from these cell types promoted development to prehatching stages, where muscular movements and heart beat were recorded. Furthermore, swimming tadpoles resulted from nuclei of adult skin, spleen cells, and erythrocytes.

Ordinarily test nuclei have been injected into eggs. However, nuclei from advanced stages of development only occasionally support normal development, and so far no adult nucleus has supported development beyond the early tadpole stage (*18*). These results suggested that: either irreversible genetic changes accompany cell differentiation or the genetic potential of advanced cell types had not yet been adequately tested. The latter possibility was explored primarily for two reasons: (i) adult spermatogonial nuclei are destined to form mature sperm that participate in normal development after fertilization, but after transplantation into enucleated eggs, they support at best development of a feeding larva (*26*); and (ii) most nuclear transplants from advanced cell types exhibit chromosome changes, reflecting an inability of the chromosomes to proceed normally through the cell cycles of the egg and embryo (*33*). Since the oocyte cytoplasm normally prepares its own nucleus to participate in fertilization, it was proposed that somatic nuclei from specialized cells might be conditioned by the cytoplasm of oocytes and later express enhanced genetic potential in eggs (*34*).

To determine whether somatic nuclei would, in fact, function during embryogenesis after residing in oocyte cytoplasm, embryonic nuclei were tested first. These nuclei were injected in *Rana* oocytes at the time of first meiotic metaphase. At maturity, after the egg was activated parthenogenetically and the egg nucleus was removed surgically, the somatic nucleus was transformed into a pronucleus and supported development of the matured oocyte through embryogenesis (*34*). Thus, somatic nuclei can respond reversibly to both meiotic and mitotic cytoplasms.

Amphibian erythrocytes are noncycling, terminally differentiated cells, and are relatively dormant in RNA and protein synthesis (*35, 36*). In fact, the erythrocyte is genetically the least active cell type known. Adult erythrocyte nuclei of *Rana* after sequential exposure to the cytoplasm of oocytes and eggs directed the formation of swimming tadpoles (*31*) (Table 2 and Fig. 1). The most advanced tadpoles (stage 22 to 25^+) swam vigorously, the hearts beat regularly, and peripheral blood circulated through the capillaries of surface tissue of the body and tail. Internally, the brains and spinal cords differentiated into gray and white cellular components. The eyes formed lenses and also displayed neural and pigmented retinas. The guts fashioned pharynx, esophagus, stomach, liver, hindgut, and some degree of intestinal coiling. Well-formed notochords were present as well as hearts, pronephric tubules, and somites. In another series of experiments in which adult erythrocyte nuclei were injected directly into enucleated eggs, none proceeded beyond the early gastrula stage (*30, 31*). Comparison of the results from the two types of hosts demonstrates that (i) adult erythrocyte nuclei contain the genes to specify tadpole development and (ii) conditioning nuclei of terminally differentiated cells in the cytoplasm of oocytes leads to very widespread activation of dormant genes.

Although the molecular mechanism or mechanisms responsible for this gene activation in erythrocyte nuclei is unknown, it involves in some manner the ability of noncycling erythrocyte nuclei to undergo DNA replication again. Autoradiographic studies of the incorporation of ^{3}H-labeled thymidine triphosphate into *Rana* adult erythrocyte nuclei have shown that only a few of these nuclei when transplanted into eggs initiate a small degree of DNA synthesis. If, however, they are injected into diplotene oocytes which are later stimulated to mature in vitro with progesterone and incubated through meiotic maturation, more than 75 percent of the nuclei synthesize significant amounts of DNA in activated eggs (*36*). Perhaps molecular components in the maturing oocyte that prepare oocyte chromatin for fertilization and DNA synthesis might also act on the chromatin of specialized cells and contribute to the remodeling of chromatin proteins or organization with a subsequent change in genetic function. What-

ever precise mechanisms account for gene activation in *Rana* erythrocytes, it is clear that conditioning these nuclei in the cytoplasm of maturing oocytes leads to enhanced expression of their genetic potential.

The percentage of original erythrocyte nuclei that were tested in oocytes and that displayed genetic multipotentiality (8.5 percent) exceeds that obtained for nuclei from other somatic specialized cells and even adult germ cells that were tested in eggs (Table 2). The failure to demonstrate totipotency does not permit one to conclude that irreversible genetic changes have occurred. Various factors influence the success of the experiments. For example, many test nuclei synthesize significant amounts of DNA even though the eggs remain uncleaved or cleave abnormally. Also, some nuclear transplants form partially cleaved blastulas that cannot proceed through normal morphogenesis, yet nuclear retransfers from partially cleaved blastulas can promote tadpole development (*31*). The factor (or factors) responsible for these restrictions appear to reflect limitations in the cleavage mechanism and may not involve the genome. The fact that modifications in the procedure have led to increased success indicates that nuclei from advanced cell types have special requirements. Finally, since this is a complex system, there are known technical problems and presumably unknown ones that have yet to be solved. Amphibian nuclear transplantation has the potential to test the entire genome within the confines of a living system and, so far, has been technically a suitable animal model. Although nuclear transplantation in *Drosophila* and the mouse are technically more difficult, nuclei from specialized cells should be tested in these and other forms to determine whether or not their nuclei remain totipotent.

In other studies of nuclear as well as gene injection, analyses by molecular techniques have demonstrated the cytoplasmic regulation of specific genes. Cell nuclei of adult liver from *Ambystoma texanum* were injected into diplotene oocytes and assayed for the synthesis of lactate dehydrogenase (LDH) and alcohol dehydrogenase (ADH) (*37*). The LDH is synthesized in the liver and oocytes, whereas ADH is synthesized in the liver but not in oocytes. Analysis of recipient oocytes revealed newly synthesized *A. texanum* LDH but not ADH. In another study, nuclei from cultured kidney cells of *Xenopus* were injected into oocytes and monitored for changes in their gene activity by two-dimensional gel electrophoresis of newly synthesized proteins (*38*). Genes normally expressed in cultured somatic cells became inactive, while genes normally expressed in oocytes, but formerly inactive in somatic cultured cells, were reactivated. Finally, reactivation of dormant oocyte-type *5S* ribosomal RNA genes was achieved in adult *Xenopus* erythrocyte nuclei following microinjection into the germinal vesicles of oocytes (*39*).

In contrast to the above studies, when genes are isolated from their neighboring DNA sequences and depleted of their normally associated chromosomal proteins, gene regulation is lost. Many cloned sequences of purified DNA have been microinjected into the nuclei of amphibian oocytes (*40*). Most of these genes are transcribed with varying efficiences, and the transcripts are translated into proteins even though oocytes do not normally make these products. On the other hand, tissue-specific gene expression has been obtained in *Drosophila* for injected genes that were integrated in the host genome of embryos (*41*). In both the mouse and the frog, tissue-specific expression has not yet been obtained for any integrated exogenous DNA sequences that were injected into fertilized eggs. However, the assessment of chromosome location on the expression of specific genes is now possible in the frog. Injected exogenous DNA sequences have been transmitted by nuclear transplantation and individuals of a clone have the DNA sequences integrated at the same chromosome location (*42*).

Cancer

Cancer may be considered as a disease of cell differentiation (*43*). Developmental biologists and geneticists have long held that cancer cells emerge when gene function in normal cells becomes misprogrammed. Whether the malignant phenotypes can be normalized has been tested. The results show that normal environmental signals can divert some types of cancer cells toward a normal phenotype.

In vivo cloning experiments of Kleinsmith and Pierce (*44*) showed that individual embryonal carcinoma cells are the stem cells of teratocarcinoma but can also form differentiated somatic cell types, and therefore they are multipotential. With the development of micromanipulative techniques for mouse embryos, these original observations have been extended (*45*). Multiple and single embryonal carcinoma cells carrying genetic markers were injected into mouse blastocysts and became associated with the host's inner cell mass, the progenitor of the embryo. In the resulting mice the

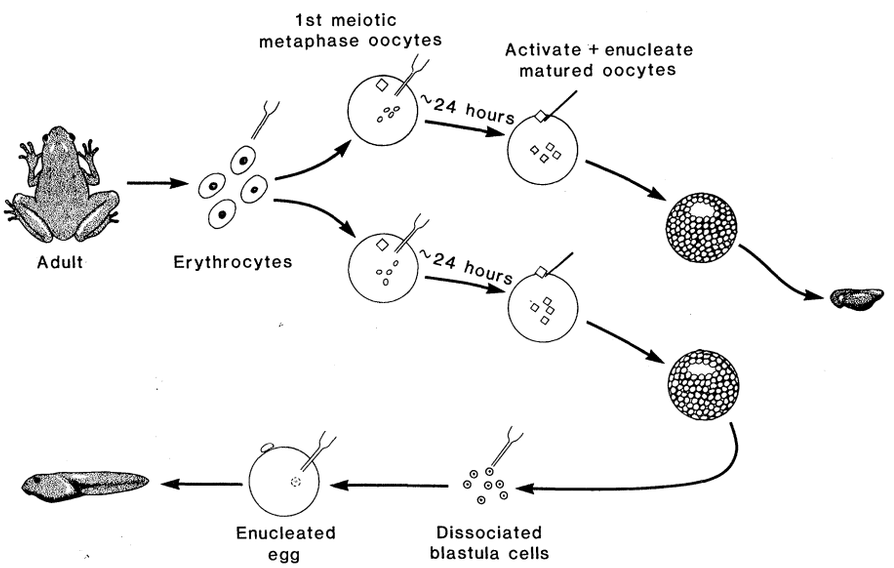

Fig. 1. Erythrocytes obtained by intracardiac puncture of adult *Rana pipiens* frogs were broken in distilled water by osmotic shock and microinjected into oocytes near the equator at first meiotic metaphase. Approximately 24 hours later (at 18°C) when the oocyte matured, the matured oocyte (egg) was activated by pricking with a glass needle, and the egg nucleus was removed microsurgically; (upper) original transplant generation, prehatching tadpoles resulted. In some cases, nuclear transplant blastulas were dissected and their animal hemisphere nuclei were transplanted singly into activated-enucleated eggs; (lower) first retransfer generation, swimming tadpoles resulted. Sixty-seven percent of the 12 clones produced in the retransplantation experiments formed prehatching tadpoles; 50 percent of the 12 clones formed swimming tadpoles (*31*). [Artwork by Bill Goren]

teratocarcinoma cells contributed to the coat and also to a wide range of internal tissues, including those in kidney, thymus, liver, and germ cells that had not been previously observed in teratocarcinomas. Five animals produced progeny, displaying genetic traits of the tumor genome, thus indicating genetic totipotency of the teratocarcinoma cells (*46*). The above studies indicate that "normalizing signals" emanate from normal embryonic cells and cause a change in gene expression in the cancer cells. Thus, the embryonic environment is decisive in causing reversion of this malignant phenotype.

The developmental potential of frog renal carcinoma nuclei has been tested by nuclear transplantation into enucleated eggs. Nuclei from spontaneous and induced mesonephric carcinoma and induced pronephric carcinoma directed development of the egg to pre- and posthatching tadpole stages free of tumors (*47, 48*). Development was indeed directed by the donor nucleus since either the egg nucleus was recovered in the exovate or a triploid nuclear marker was used for the donor nuclei, resulting in triploid nuclear transplants. Stromal and blood cells were virtually eliminated in the donor cell population because 98.5 percent of the dissociated cells that would serve as donors were epithelial cells, as determined by fluorescent staining. In another study, the donor cells were derived from epithelial cell cultures of induced pronephric carcinoma. Since normal pronephric cells die after the mesonephros' functions, the surviving cells of the induced pronephric carcinoma probably were transformed cancer cells. Perhaps enhanced expression of genetic potential can be revealed, if the cancer nuclei are conditioned first in oocytes like the erythrocyte nuclei (*31*). Nevertheless, the results indicate that molecular components in the cytoplasm of enucleated eggs can change the gene expression of renal carcinoma cells.

Conversion to a nonmalignant phenotype has also been observed in mouse and human myeloid leukemia in vitro, and in the mouse in vivo after the addition of a physiological inducer called MGI (macrophage and granulocyte inducer) (*49*). MGI is a family of proteins secreted by various types of normal cells and is required for differentiation of normal myeloblasts. When myeloid leukemic cells are exposed to MGI, they differentiate into macrophages and granulocytes. Thus, these malignant cells have not lost the genes that regulate normal differentiation.

Possible Mechanisms

The above studies demonstrate that certain normal specialized cells and cancer cells can be induced to change phenotype. In addition, the conversion of phenotype in those cases analyzed is accompanied by the production of new gene products, an indication of the activation of dormant genes.

The methylation of DNA sequences has been implicated in the regulation of gene expression (*50*). For example, one hypothesis suggests that DNA modification by methylation could be a mechanism of X chromosome inactivation. In females of eutherian mammals, one X chromosome undergoes inactivation during early embryogenesis and most of the genes are inactivated (*51*). Whereas X inactivation in somatic cells appears to be a permanent event, the inactivated X in murine and human germ cells is reactivated before or during entry into meiotic prophase (*52*).

Since 5-azacytidine (5-aza C) is a cytidine analog that can be incorporated into DNA and leads to hypomethylation of newly synthesized DNA (*53*), investigators have attempted to reactivate the X chromosome by treatment with 5-aza C. Mouse cells were transformed with the herpes simplex virus containing the viral thymidine kinase (TK) gene in an inactive state. When these cells were treated with 5-aza C, the viral TK gene was reexpressed. Analysis of the methylation patterns of the viral TK gene with restriction endonucleases showed that the gene induced by 5-aza C was ummethylated, whereas the inactive gene was methylated (*54*). Although there is not yet consistency among different systems in correlating gene activity with DNA hypomethylation, recent studies indicate that specific sites of DNA methylation are important. DNA methylation in the 5′ region of the human globin gene prevents transcription, whereas methylation in the structural gene does not (*55*). There is no evidence for the existence of a demethylase; however, demethylation of genomic DNA has been observed. During early embryogenesis in *Xenopus*, 18*S* and 28*S* ribosomal DNA (rDNA) becomes hypomethylated (*56*). Also, nucleoplasmic extracts from mouse erythroleukemic cells can remove methyl groups from bacterial DNA that was highly methylated (*57*). Therefore, it may be informative to examine the patterns of methylation at specific sites of DNA in nuclear transplant animals originating from nuclei of differentiated cell types such as erythrocytes, or of specific genes that have been reactivated in somatic cell hybrids.

Evidence has accumulated linking the structural conformation of chromatin with the expression of specific genes. The precise physical conformation of active and inactive chromatin is not clear, but it is presumed that active chromatin contains fewer nucleosomal structures, is associated with at least two nonhistone chromosomal proteins (HMG 14 and 17) and in some cases is hypomethylated (*58*). In the globin gene region of the chicken red blood cell, the active chromatin is organized into a domain that is sensitive to digestion by deoxyribonuclease I. Within this domain are smaller stretches of DNA located at the 5′ end of the globin gene that are hypersensitive to deoxyribonuclease I. Groudine and Weintraub (*59*) have shown that the deoxyribonuclease hypersensitive sites once established in active globin genes are heritable through at least 20 cell generations, even after synthesis of globin mRNA has ceased. Whether the globin gene domain remains in the enzyme-sensitive configuration after nuclear transfer of erythrocyte nuclei into the oocyte or egg, or whether the chromatin is remodeled into a configuration more typical of a nonactive globin gene is not known. Similar uncertainties remain regarding the reversibility of X inactivation in the inactivation-reactivation cycle in mouse germ cells, activation of silent genes in somatic cell hybrids, transdifferentiation of iris epithelium into lens, and the alteration of phenotype observed in some neoplastic cells.

Alterations in chromatin conformation during cell phenotypic changes could involve the sequential replacement of chromosomal proteins. There is precedent for programmed changes in histone proteins during spermatogenesis when somatic histones are replaced by protamines or sperm-specific variants. After fertilization, certain male pronuclear histones in sea urchin are either replaced or modified (*60*); this is followed by the appearance of stage specific histone variants during development (*61*). When chick erythrocyte nuclei are reactivated in cells consisting of chick/HeLa nuclei, the erythrocyte-specific histone is lost and H1 histone and certain nonhistone proteins of HeLa origin migrate into the chick nuclei (*62*). There may also be changes in association of the DNA with specific regulatory molecules such as the 40,000-dalton protein involved in controlling the expression of the 5*S* ribosomal genes in *Xenopus* (*63*), and possibly nonhistone proteins such as the high

mobility group proteins 14 and 17 (*58*). In conjunction with these protein changes, a DNA replication cycle or a mitosis might be required for the expression of a gene that had not formerly functioned (*64*). It is likely that remodeling of the chromatin proteins would lead to changes in the pattern of gene expression that could account for alterations of cell phenotype.

Both plants and animals contain genetic elements capable of movement within the genome (*65*). Most of the transposable elements that have been found are probably not involved in the developmental regulation of gene expression since insofar as is known: (i) they are not part of a programmed sequence of events, (ii) the frequency of transposition of many of the elements is probably relatively low and therefore could not serve as a mechanism for controlling the expression of a large number of diverse genes during a rather short developmental program, and (iii) elements in *Drosophila* such as copia, do not differ in a developmental or tissue-specific manner. They do, however, probably play a significant role in producing variation and diversity in an evolutionary context. There are examples of genetic rearrangements and transposable elements that may serve as model systems by which cellular phenotypes may be established during differentiation, such as immunoglobulin gene rearrangement (*66*) and trypanosome antigen variation (*67*). Whether the genetic rearrangement of immunoglobulin genes is irreversible is not known. But, in cases where a copy of a gene is produced and inserted into a new locus, this copy might be replaced by a different gene copy produced later in the developmental program. For example, the parasitic protozoan, *Trypanosoma brucei*, avoids immunological destruction by the host by producing many different surface antigens at different times. Borst *et al.* (*67*) have proposed that the production of a new antigen occurs after a gene is copied and transposed to a new locus. Thus, it appears that a transpositional event results in the expression of a single cellular phenotype.

Conclusions

The ability of some specialized cells to undergo gene activation and change phenotype indicates that all cell specializations do not necessarily involve irreversible genetic changes. The stability of the differentiated state could be due to various molecular mechanisms including DNA methylation, chromatin structure, DNA-protein interactions, and DNA rearrangements. Of the various methods for achieving stability of the differentiated state, only DNA loss would appear to be irreversible. All other putative mechanisms should be modifiable. Continued investigations in living experimental systems leading to the activation of dormant genes may uncover greater genetic potential than that already observed. In addition, the potential of genomes subjected to DNA rearrangements or nucleotide losses should be evaluated in these living systems. The search for additional cases of DNA rearrangements and nucleotide losses should continue and their effect on the cell phenotype evaluated. Only then can we conclusively answer the extent to which irreversible genetic changes govern cell specialization. Since a high degree of molecular and cellular phenotypic changes occurs in transdifferentiation, X reactivation, cell hybrids, nuclear transplantation, and some cancers, it is possible that the molecular mechanisms that confer genetic and cellular stability in these cases can be reversed by intra- and extracellular regulatory molecules. These regulatory molecules yet to be isolated and defined represent the most challenging aspect of explaining the stability and modulation of cell specialization. It is unlikely that these regulatory molecules will singly orchestrate negative and positive control of gene function, but most probably they will involve a series of steps acting in cascade or in concert.

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Uranium Power and Horizontal Proliferation of Nuclear Weapons

Chauncey Starr

The spread of nuclear weapons among nations has been of long-time concern to the industrial countries supplying nuclear equipment for civilian purposes. An increase in the number of states with nuclear weapons (horizontal proliferation) presents different issues than the growth of existing weapons stockpiles (vertical proliferation). In the early years of civilian applications, it generally was assumed that the arcane and costly nature of weapons technology and weapons material production would limit nuclear weapons to the major industrial powers. However, extensive efforts of these powers to introduce the world's technologists to the nuclear science and engineering useful for civilian applications also provided them the basic knowledge for a future entry into the military domain.

Early recognition by the major powers that the technical barriers to horizontal proliferation would decrease with time led to international political arrangements, such as the International Atomic Energy Agency (IAEA) in 1957 and the Treaty on the Non-Proliferation of Nuclear Weapons (NPT) in 1968, that were designed to inhibit the spread of military programs. A key assumption of these arrangements was that, in return for active support of civilian applications by supplier states, the states without nuclear weapons would abjure military programs. However, the growth of nuclear electric power and its technological infrastructure in some of the nonnuclear weapons states during the past decade has heightened concern about the adequacy of the barriers to nuclear weapons programs. In the United States, discussion of the risks of horizontal proliferation has tended to focus on technical issues—the adequacy of controls of weapons-usable material, the possible use of uranium power plants to produce such material, and export measures by supplier states to inhibit proliferation capability. It is time, in light of the experience of the past several decades, to examine the effectiveness of this primary technical focus and the role of nontechnical factors.

Because much of the relevant literature addresses only parts of this broad issue, the American Nuclear Society (ANS) appointed a special committee (*1*) to attempt an overview. The committee members had experience in both technical and international aspects of the subject. The committee did not attempt to propose solutions; rather, its objective was to assess present trends in the worldwide expansion of light-water reactors (LWR's) and their supporting facilities, the implication of these trends on the potential connection between civilian and military programs in the nonnuclear weapons states, and the influence of the policies of the major industrial suppliers, particularly the United States. This article is based on the report of the ANS special committee.

The principal question addressed is whether a nonnuclear weapons state that obtains commercial LWR's for electricity would be more likely to embark on a nuclear weapons program than if it had used only coal, gas, or oil. The states of particular concern have limited resources and military organizations, and could not produce or militarily benefit from a large arsenal of nuclear weapons in the foreseeable future. So the issue is their potential acquisition of a few nuclear weapons in the next 20 to 30 years through their use of commercial, uranium-fueled LWR power systems.

Nuclear weapons states have already developed indigenous means of producing weapons material. Except for India, which produced plutonium for its nuclear explosive device with a heavy-water research reactor, the major nuclear weapons states now use plutonium production systems dedicated to weapons material. Some of the earliest natural uranium reactors (moderated by graphite or heavy water) were designed to be dual-purpose, primarily producing plutonium for weapons, with electricity as a by-product. Subsequent improvements in reactor technology, the advent of relatively low cost slightly enriched uranium, and the large extension of fuel element operating lifetimes made single-purpose reactors for electricity production a commercial goal. As a result of these technological changes, the low-cost electricity fuel cycle differs from that best suited for production of weapons-grade plutonium. Although commercial nuclear power programs are now generally independent of military programs, the early history demonstrated that dual-purpose operation is technically possible, although with substantial increases in electricity costs.

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