resides in the higher velocity with which the latter dissociates from the decoding site by reversal of the binding reaction or proofreading (*18*). We suggest that efficient suppressors are slow to dissociate so that modulation of their back-reactions has a negligible effect on the probability that they will donate their amino acids. Inefficient suppressors, like those created in these experiments by 5′ anticodon loop substitutions, may have fast reverse reactions that compete with guanosine triphosphate hydrolysis by translational elongation factor Tu and with formation of peptide bonds. The efficiency of this type of weak suppressor will be altered when these back-reactions are modulated.

The context effect does not depend on a fourth base pair. When we understand the biochemistry of the actions of context, then perhaps we will also be able to judge whether these effects have developed during the process of evolution to vary gene expression.

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A Balanced Translocation in Mice with a Neurological Defect

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A semisterile male translocation heterozygote [t(2; 14) 1Gso] that exhibited neurological symptoms and an inability to swim (diver) was found among the offspring of male mice treated with triethylenemelamine. All breeding and cytogenetic data showed a complete concordance between translocation heterozygosity and the neurological disorders. Homozygosity for the translocation seemed to be lethal at an early embryonic stage. Despite the distinctive neurologic symptoms, no anatomic or histological defects in either the ear or in the central nervous system were observed. Thus, a balanced chromosomal translocation can produce disease with an inheritance pattern that mimics a single dominant gene defect.

MOST TRANSMITTED BALANCED, reciprocal translocations cause no ill effects in heterozygous carriers, except for a reduced chance to reproduce (death of aneusomic offspring). In humans, however, certain apparently balanced translocations may have deleterious effects on carriers. Among mentally handicapped individuals, the incidence of carriers of balanced reciprocal translocations is higher than normal (*1*). Studies of several family pedigrees for syndromes with multiple skeletal abnormalities (*2*) and the Greig polysyndactyly-craniofacial anomaly syndrome (*3*) have shown that some of these morphological defects were associated with an inherited balanced translocation. The de novo occurrence of X-linked Duchenne muscular dystrophy in seven females is due to a balanced translocation involving one of the X chromosomes (*4*).

In the mouse, cases of association between balanced translocations and congenital defects have also been rare. For example, in heritable translocation induction studies with chemical mutagens and ionizing radiation, no abnormalities except semisterility or sterility were found in a large number of translocation carriers screened (*5–8*). On the other hand, among 37 independently induced (by ionizing radiation) dominant skeletal mutations, three were associated with balanced reciprocal translocations (*9*). Furthermore, several types of balanced reciprocal translocations can cause spermatogenic blockage that leads to complete sterility.

We have determined that one of the offspring of males treated with triethylenemelamine during a mutagenesis experiment was a semisterile male translocation heterozygote with obvious neurological symptoms and an inability to swim normally ("diver"). The translocation altered gene expression so as to mimic dominant Mendelian inheritance.

The original male translocation heterozygote was derived from a triethylenemelamine–treated male (SEC × C57BL) F_1 that had been mated to a (C3H × C57BL) F_1 female. The translocation heterozygote was mated to (SEC × C57BL) F_1 females to produce the second generation. Subsequent generations were produced from mating second generation males with (C3H × C57BL) F_1 females, and most of the pathology specimens came from this cross. Chromosomal analysis of the translocation was conducted on the meiocytes and on cultured kidney cells of neurologically abnormal males according to the methods of Evans *et al.* (*10*) and Cacheiro and Russell (*11*).

Systemic autopsies with examinations of histopathology and the central nervous system were performed on ten diver and ten normal mice, (ages 3 months to 1 year), with multiple levels of the central nervous system examined by light microscopy after staining with hematoxylin and eosin and the Golgi stains. The sizes of the vestibular nuclei were compared in serial sections of the brain stem from one diver mouse and one control mouse. Electron microscopy of the cerebellar myelinated fibers was performed on two diver mice. The middle and inner ears of five diver mice were examined by microdissection, temporal bone histology, and scanning electron microscopy. Multiple histologic levels were examined from the brains of fetuses of diver male mice crossed with normal (C3H × C57BL) F_1 hybrid females at gestational ages 12, 14, 16, and 18 days as well as newborns and mice at 3, 12, 18, and 24 days of age (ten at each day). Twenty-eight fetuses from 13

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Table 1. Association between semisterility and neurological abnormality. Semisterility was determined by mating male offspring with at least three young virgin (C3H × C57BL) F_1 females that were killed for uterine analysis at midpregnancy. Neurologically abnormal males and females were also mated to identify the translocation homozygotes.

Cross (female × male)	Progeny (No.)	Diver					Normal				
		Males				Females	Males				Females
		Semisterile	Sterile	Fertile	Total		Semisterile	Sterile	Fertile	Total	
Diver × normal	188*	42	2	0	47	46	0	1	52	55	40
Normal × diver	441†	92	8	0	103	96	0	3	118	121	121
Diver × diver	129	11‡	0	0	37	38	0	0	15‖	24	30

*Five died before completion of the fertility test. (Three had diver phenotype and two were normal.) †Three died before completion of fertility test. (All had diver phenotype.) ‡Only 11 of 37 were tested for fertility. ‖Only 15 of 24 were tested for fertility.

matings between divers were examined as whole histologic sections. Seventeen offspring of diver males and (C3H × C57BL) F_1 (14 from normal mice) were weighed at intervals through 37 days of age and tested for the ability to swim and for neurological defects.

The affected mouse could be distinguished from its normal littermate as early as day 1 of age by its retarded ability to right itself as a result of poor lateral thrusting of the hind parts. The mutant mouse exhibited less spontaneous exploration during the first few days of life, and during the righting process would occasionally roll to the side and extend the hind limbs. The neonate tended to drag its extended hind limbs. By day 6, the most impressive and objective discriminator was the inability to swim, which had been evident on day 1. The limb and tail movements of the mutant were similar to those of normal animals, but the mutant swam in randomly tight circles, often on its side. Thus the mutant would eventually dive and continue the submarine circular swimming. This behavior persisted at all ages. In open spaces the mutant often moved backward from threats, in contrast to the "turn and run" behavior of the challenged normal mouse. No seizures, tremors, abnormal postures, compromised coordination, difficulty with fine movements, or visual or auditory defects were observed. Pain and position sensation seemed intact. Thus, the disease was present at birth and was neither debilitating nor progressive. Only minimal functional heterogeneity was observed. No changes were observed in four generations of backcrosses to the inbred SEC line.

Diver mice weighed the same as their normal siblings at birth, but from day 8 a disparity in weights became apparent. The mean weight of the diver group (6.7 g) was less than that of the control group (7.6 g) (t = 3.19, P = 0.002), and the difference persisted into adulthood. No dysmorphic features were seen on external examination, and the gross and histologic features of the internal organs were unremarkable. The brain, muscle, spinal cord, otoconia, and other inner ear structures of the diver mice were normal at all ages. The vestibular nuclei of the adult diver mouse and control mice contained comparable numbers of neurons. No ultrastructural myelin abnormalities were detected in the adult diver mice. The central nervous system histology of fetuses and neonates at different ages obtained from crosses between diver males and (C3H × C57BL) F_1 showed no abnormalities. In the 28 late fetuses obtained from crosses between diver translocation mice, 3 had abnormal limb positioning, 5 had broad shoulders and necks, and 1 had fusion of the lower limbs. Histologic examination of all but the latter was unremarkable; the brain of the later fetus had diffuse, nonspecific, degenerative changes with focal neuronal loss (a finding consistent with intrauterine death). No specific neuropathological defects were observed in the anatomic investigation of diver mice and fetuses from matings that would produce heterozygous and homozygous conceptuses.

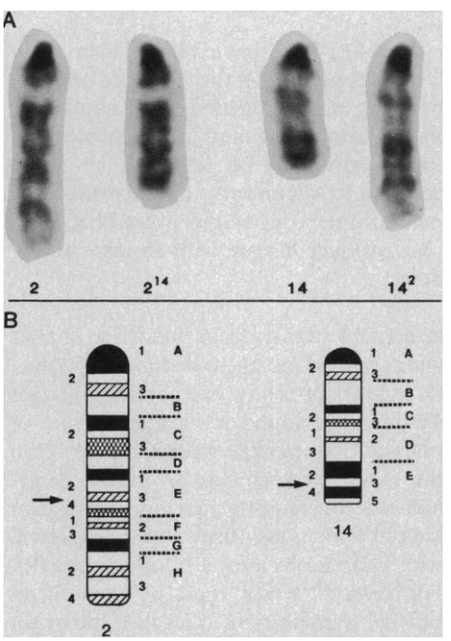

Fig. 1. Translocated chromosomes of the diver mutant. (A) Karyotype of Giemsa-banded metaphase chromosomes 2 and 14 and reciprocal rearrangements. (B) Idiogram of banding patterns of chromosomes 2 and 14 showing the approximate breakpoints (arrows) [adapted from (*16*)]. The breakpoint in chromosome 2 appeared to be located in band E4, and that in chromosome 14 was close to the distal end in band E3. One of the translocation products is formed from the centromere and proximal region of chromosome 2 and the distal region of chromosome 14. The other translocation product is formed from the centromere and major portion of chromosome 14 and the distal region of chromosome 2.

Results of the various crosses are shown in Table 1. All diver males producing offspring were semisterile, whereas all control males were fully fertile. On the average, semisterile diver males produced 57.6 percent (S.E., 5.8 percent) dead implants (from unbalanced segregants) when mated to at least three (C3H × C57BL) F_1 females, and normal males produced 3.75 percent dead implants (S.E., 3.3 percent). Thus, semisterility and the abnormal behavior are completely associated.

Cytological studies of meiotic chromosomes were conducted in three neurologically abnormal, semisterile grandsons of the original mutant, with 25 diakinesis–metaphase-I cells scored per testis. Most cells (77 percent overall; range, 68 to 88 percent) exhibited a large ring quadrivalent; 16 percent of cells (range, 12 to 20 percent) had chains of four chromosomes, and the remainder had 20 bivalents.

The original diver mutant was a clearly semisterile male. Since semisterility indicates the presence of balanced reciprocal translocations, we suspected that the neurological symptoms were attributable either directly to the exchange itself or indirectly to a gene in the vicinity of the breakpoint. All breeding and cytogenetic data showed a complete concordance between the translocation heterozygosity and the manifestation of the neurological symptoms. Homozygosity for the translocation seems to be lethal at early embryonic stages. If homozygotes were viable, we would have expected normal fertility associated with symptoms perhaps more severe than those of heterozygotes. On the contrary, all 11 affected males from matings between diver mice were semisterile (heterozygotes), whereas all 15 fully fertile mice were behaviorally normal.

The neurological symptoms of the diver translocation mouse resemble those observed in other mutant mouse strains. Reduced ability to swim is observed in the *pallid* (*pa*) mouse, whose defect in equilibrium is due to the absence of otoconia in the

vestibular labyrinth (*12*). This mutant gene is also located on chromosome 2. A chromosome 11 mutant (*teetering*) that also resembles the diver mutant is defective in swimming ability and does not have neuropathological defects visible by light microscopy (*13*). It differs, however, from diver translocation in that the homozygotes survive as long as a month after birth and have markedly reduced numbers of neurons throughout the central nervous system.

The neurological symptoms are clearly associated with the reciprocal exchange between chromosomes 2 and 14. As far as is known, this is the only neurological mutant in the mouse that resulted from a reciprocal nonhomologous chromosome exchange; all others are either recessive or dominant gene mutations. The translocation marks the genetic location of the defect and provides an avenue for future investigations. The findings also raise the question whether certain human genetic disorders that have been assumed to be the result of single gene mutations may instead be associated with chromosomal rearrangements.

It has been generally assumed that balanced reciprocal translocations do not involve loss or gain in chromosomal components. For example, high-resolution karyotype analysis (at the level of light microscopy) of 13 human carriers of balanced translocations did not demonstrate any net loss or gain of chromosomal material (*14*). However, an increasing number of clear associations between balanced exchange and deleterious effects suggest that either the breakpoint may be located inside a structural gene or that it may affect the activity of genes in the immediate vicinity. In a female patient with Duchenne muscular dystrophy resulting from a translocation involving one X chromosome, the breakpoint is located close to the Duchenne muscular dystrophy gene (*15*). Little is known about the molecular mechanism involved in the formation of mutagen-induced nonhomologous chromosomal exchange. The diver translocation mutant may prove useful not only in neurobiology, but also in understanding the molecular nature of chemically induced chromosome exchange and the way in which deleterious effects of the exchange are expressed.

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A Blood Vessel Model Constructed from Collagen and Cultured Vascular Cells

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A model of a blood vessel was constructed in vitro. Its multilayered structure resembled that of an artery and it withstood physiological pressures. Electron microscopy showed that the endothelial cells lining the lumen and the smooth muscle cells in the wall were healthy and well differentiated. The lining of endothelial cells functioned physically, as a permeability barrier, and biosynthetically, producing von Willebrand's factor and prostacyclin. The strength of the model depended on its multiple layers of collagen integrated with a Dacron mesh.

A MODEL OF A BLOOD VESSEL THAT reproduces in vitro many of the physical and biological characteristics of a mammalian artery would be useful for the study of vascular cell biology, physiology, and pathology. Such a model might also be used as a living vascular prosthesis to replace or bypass small caliber arteries (<6 mm inside diameter) for which synthetic and processed biological grafts have not been entirely successful (*1*). Vascular cells have been extensively studied in tissue culture (*2*). Some aspects of the vascular wall have been replicated in vitro when endothelial cells were grown, not on plastic substrates, but in more physiological environments—on extracellular matrix materials, on layers of smooth muscle cells, under flow, or in a mock circulatory loop (*3*). Combining these approaches might result in the ideal blood vessel model, a multilayered tube capable of withstanding physiological pressures, allowing access to luminal and abluminal fluid compartments, and able to be incorporated into a mock circulatory loop. The layers corresponding to the intima, media, and adventitia would consist of a confluent monolayer of endothelial cells lining the lumen, a middle layer with a high density of smooth muscle cells and matrix materials, and an outer layer with adventitial fibroblasts and matrix materials.

We have developed a blood vessel model that meets these criteria. The construction of the model is based on the observations that fibroblasts can contract a hydrated collagen gel by a factor of 10 to 20 to produce a tough tissue-like lattice and that such a lattice is a suitable substrate for epithelial cells (*4*). In this report, we describe the construction of our model, demonstrate that the layer of endothelial cells functions much like the endothelium of a normal blood vessel, and examine the effects of various parameters on the strength of the model. A preliminary report of this model has been presented (*5*).

Bovine aortic endothelial cells, smooth muscle cells, and adventitial fibroblasts were isolated and cultured by standard methods (*2*). The middle layer of the blood vessel model, corresponding to the media of an artery, was prepared by casting culture medium, collagen, and smooth muscle cells together in an annular mold (*4, 6*). The mixture jelled after a few minutes at 37°C and contracted within a few days to produce a tubular lattice around the central mandrel. After 1 week, an open Dacron mesh sleeve was slipped over the lattice to provide additional mechanical support. The outer layer, corresponding to the adventitia, was cast around the first lattice with adventitial fibroblasts rather than smooth muscle cells. Two weeks later, when the outer layer was fully

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