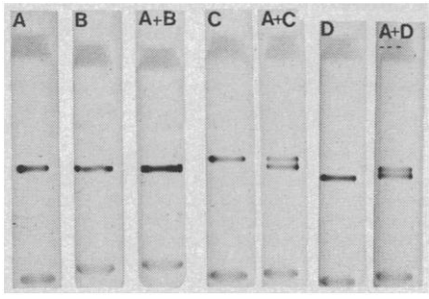

Fig. 2. Electrophoretic mobility of *2565*-suf2 histidinol dehydrogenase. Standard, *p*H 9.5, polyacrylamide gels were stained for histidinol dehydrogenase. For this experiment enzyme from *2565*suf2 cells, grown in 3 liters of E medium, was purified through the DEAE-Sephadex A-50 step by the standard procedure (*11*). Appropriate genetic tests showed the cells to be bona fide *2565*suf2 genotypes, containing no detectable intragenic revertants. All other samples used were crystalline preparations. The samples are: A, wild type, 6 μg; B, *2565*suf2, 300 μg; C, *497*R56, 10 μg. This enzyme lacks a glumatic acid residue (Ino, Hartman, and Yourno, unpublished results) and is, therefore, slower than wild type at *p*H 9.5; D, *3018*R95, 6 μg. This enzyme lacks an arginine residue (*14*) and is, therefore, faster than wild type at *p*H 9.5. Normal mobility appears in A, B, A + B, A + C, and A + D; C is above this line and D is below.

the suppressor is a tRNA with a quadruplet (+1) anticodon or a (+ or −) tRNA which is susceptible to slipping along a repeated mRNA sequence (*1*, *6*). This question can best be answered by direct isolation and characterization of the suppressor agent.

The accumulated data strongly suggest that the suppressor reads the quadruplet $CCC^{U}_{\bullet}$ as proline in each case (Fig. 1). If the overlapping quadruplets $^{U}_{\bullet}GA^{A}_{G}$ or •GU• were read instead by the suppressor as glutamic acid, the suppressed *2565* enzyme would carry a Val → Glu substitution and have greater than normal electrophoretic mobility at *p*H 9.5. The *3018* and *3749* mRNA's contain a $^{U}_{\bullet}GA$ codon in the (+) phase, overlapping the (+1) frameshift site. Possibly a nearby chain-terminating codon (UGA) plays some critical rephasing or rate-limiting role in suppression. However, the closest potential chain-terminating codon in *2565* is •(AG) (UAG) one codon removed from the frameshift site. In each case the quadruplet $CCC^{U}_{\bullet}$ at the frameshift site on mRNA is followed by a G residue. Again it is not clear whether this plays some role such as phasing in suppression. Whatever the detailed mechanism, the data indicate that the net effect of suppression is translation of the quadruplet $CCC^{U}_{\bullet}$ as proline.

About 1 percent of normal amounts of histidinol dehydrogenase are restored in each case by suppression. Yet about 5 percent more aminotransferase, product of the following gene, *hisC*, is produced in suppressed *3018* and *3749* strains (*7*). Suppressed *2565* strains do not produce measurably greater amounts of aminotransferase, which may therefore also be increased by 1 percent (Table 2). We do not understand the basis of this apparent hyperrelease from polarity in suppressed *3749* and *3018*. On the assumption that the enzyme assays are reliable, the hyperrelease may be due to reinitiation of mRNA reading after the frame shift site, dependent on the chain-terminating UGA codon generated at that point.

In summary, our results suggest that the quadruplet $CCC^{U}_{\bullet}$ is a sufficient if not necessary condition for external suppression of the *3018* class of frameshift. This is an exception to the triplet genetic code (*10*). However, the data do not rigorously define the specificity limits of the *3018* suppressors. A requirement for neighboring sequences, particularly chain-terminating codons, cannot be completely ruled out. Nor is it certain that the suppressors are absolutely specific for (+1) frameshifts in mRNA sequences of repeated C residues. The study of frameshift suppression is of considerable importance to further understanding of the genetic code and its translation into polypeptide structure.

JOSEPH YOURNO, TADAHIKO KOHNO
Biology Department,
Brookhaven National Laboratory,
Upton, New York 11973

References and Notes

1. J. Yourno, D. Barr, S. Tanemura, *J. Bacteriol.* **100**, 453 (1969).
2. The following abbreviations are used: tRNA, transfer RNA; mRNA, messenger RNA; ICR-191, 2-chloro-6-methoxy-9-[3-(2-chloroethyl)-aminopropylamino]acridine dihydrochloride; DES, diethyl sulfate; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; C, cytidylate; G, guanidylate; U, uridylate; A, adenylate; $^{A}_{G}$, either A or G; $^{U}_{C}$, either U or C; •, unspecified nucleotide residue; $^{U}_{\bullet}$, either U or •; (+1) frameshift, the addition of a single nucleotide pair in DNA and resultant addition of a single nucleotide residue in mRNA; (−1) frameshift, the deletion of a single DNA nucleotide pair and a single mRNA nucleotide residue; GC pair, a deoxyguanidylate-deoxycytidylate nucleotide pair in DNA; E medium, minimal salts medium of Vogel and Bonner [as described in (*1*)]; 2EM, E medium containing 2 percent by volume of Difco liquid nutrient broth; *his*n, a mutation involving histidine biosynthesis; *hisD*, the histidine *D* (histidinol dehydrogenase) gene; *hisO*, the histidine operator gene; Val, valine; Thr, threonine; Arg, arginine; Pro, proline; Glu, glutamic acid; Leu, leucine; Gln, glutamine; and Ser, serine.
3. N. Oeschger and P. E. Hartman, *J. Bacteriol.* **101**, 490 (1970).
4. J. Yourno and S. Heath, *ibid.* **100**, 460 (1969).
5. S. Tanemura and J. Yourno, *J. Mol. Biol.* **46**, 459 (1969); J. Yourno, *ibid.* **48**, 437 (1970).
6. J. Yourno and S. Tanemura, *Nature* **225**, 422 (1970).
7. D. Riddle and J. R. Roth, *J. Mol. Biol.* **54**, 131 (1970).
8. J. Yourno, *ibid.* **62**, 233 (1971).
9. G. R. Fink, T. Klopotowski, B. N. Ames, *ibid.* **30**, 81 (1967).
10. M. Nirenberg, R. Caskey, R. Marshall, R. Brinacombe, D. Kellog, B. Doctor, D. Hatfield, J. Levin, F. Rottman, S. Pestka, M. Wilcox, F. Anderson, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 11 (1966); H. G. Khorana, H. Buchi, H. Ghosh, N. Gupta, M. T. Jacob, H. Kossel, R. Morgan, S. Nurang, E. Ohtsuka, R. T. Wells, *ibid.*, p. 39.
11. J. Yourno and I. Ino, *J. Biol. Chem.* **242**, 3273 (1968).
12. B. N. Ames and H. J. Whitfield, Jr., *Cold Spring Harbor Symp. Quant. Biol.* **31**, 221 (1966).
13. R. G. Martin, M. A. Berberich, B. N. Ames, W. W. Davis, R. F. Goldberger, J. D. Yourno, *Methods Enzymol.* **17B**, 3 (1971).
14. We thank H. J. Creech for a supply of ICR-191. Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

6 August 1971; revised 7 November 1971 ■

Membrane Continuity between Plasmalemma and Nuclear Envelope in Spermatogenic Cells of Blasia

Abstract. *Ultrastructural study of liverwort antheridia showed that the spherical nuclei of some late-stage androgonial cells lie close to or appressed to the cell walls. In some cells the outer membrane of the nuclear envelope curves toward the wall and continues without interruption around the cell periphery as the plasmalemma. Subject to its confirmation as a natural occurrence, this evidence appears to support Robertson's concept of cellular organization.*

A modern, well-known concept of general cellular organization in part postulates continuity of the cytoplasmic membrane system (Robertson, *1*). According to this view, the continuous nature of nuclear envelope, endoplasmic reticulum, and plasmalemma membranes forms one or more open channels between the cell exterior and the so-called perinuclear space within the nuclear envelope. Such channels, even if intermittent in appearance, would play

important roles in both intra- and intercellular transport of various materials. Open passage to the cell exterior has special significance in virus transmission, since it has been shown that certain newly synthesized viruses are released into the perinuclear space in cells of both plant host and insect vector (*2*).

Although widely popularized, this concept of cellular organization has lacked direct supporting evidence. Membrane continuity between nuclear envelope and endoplasmic reticulum is a familiar occurrence, but purported instances of continuity between plasmalemma and endoplasmic reticulum have tended to be morphologically ambiguous (*3*). In certain secretory cells internal extensions of plasmalemma are continuous with a system of membranous tubules (*4*); however, the tubules have not been shown in continuity with endoplasmic reticulum. The work I now report demonstrates continuity between the outer membrane of the nuclear envelope and the plasmalemma and offers qualified evidence supporting Robertson's concept.

Blasia pusilla L. was examined during an ultrastructural investigation of spermatogenesis in liverworts, a group of primitive, haploid land plants. Male specimens of healthy appearance were dissected, and their antheridia were fixed in buffered glutaraldehyde. After further treatment in osmium tetroxide, they were dehydrated in a graded ethanol series and embedded in Epon. Thin sections stained with uranyl acetate and basic lead citrate were examined with an electron microscope (Hitachi HU-11a).

In contrast to ordinary meristematic tissue, the androgonial cells within the antheridium become progressively smaller with each succeeding cell duplication. A late-stage androgonial cell characteristically has a prominent, spherical nucleus and a reduced complement of cytoplasmic organelles. Light microscopy has shown that the relatively large interphase nucleus usually occupies a central position in the cell, although occasionally it may lie very close to or actually appressed to one or more sides of the cell wall (*5*). An electron micrograph of a cell whose nucleus was situated in a parietal position revealed that the outer membrane of the nuclear envelope curved toward the nearby wall and continued without evident interruption as the plasmalemma (Fig. 1). The inner membrane of the nuclear envelope remained intact. Membrane-bound vesicles, often strongly resembling cytoplasmic ground substance, lie scattered between the nuclear envelope's inner membrane and the cell wall. Other, similar vesicles appear to lie in locally expanded regions in the perinuclear space. The vesicles are tentatively interpreted as cytoplasm that has been isolated during the establishment of membrane continuity (*6*).

Production of cytoplasmic vesicles and establishment of membrane continuity bear little resemblance to membrane responses manifest through cellular injury (*7*). Although the possibility exists that pathological or other factors may have induced this condition, the androgonial tissue appears to have been healthy and free of preparative artifacts. Perhaps a more likely causal influence is the progressive increase in ratio between nuclear and cytoplasmic volumes, a change which reflects the diminution in total cell volume and loss of cytoplasm that are correlated with spermatozoid development. If it can be shown that this continuity of membrane is a natural phenomenon of even sporadic occurrence in *Blasia* or other comparable tissue, it can be interpreted as supporting Robertson's concept,

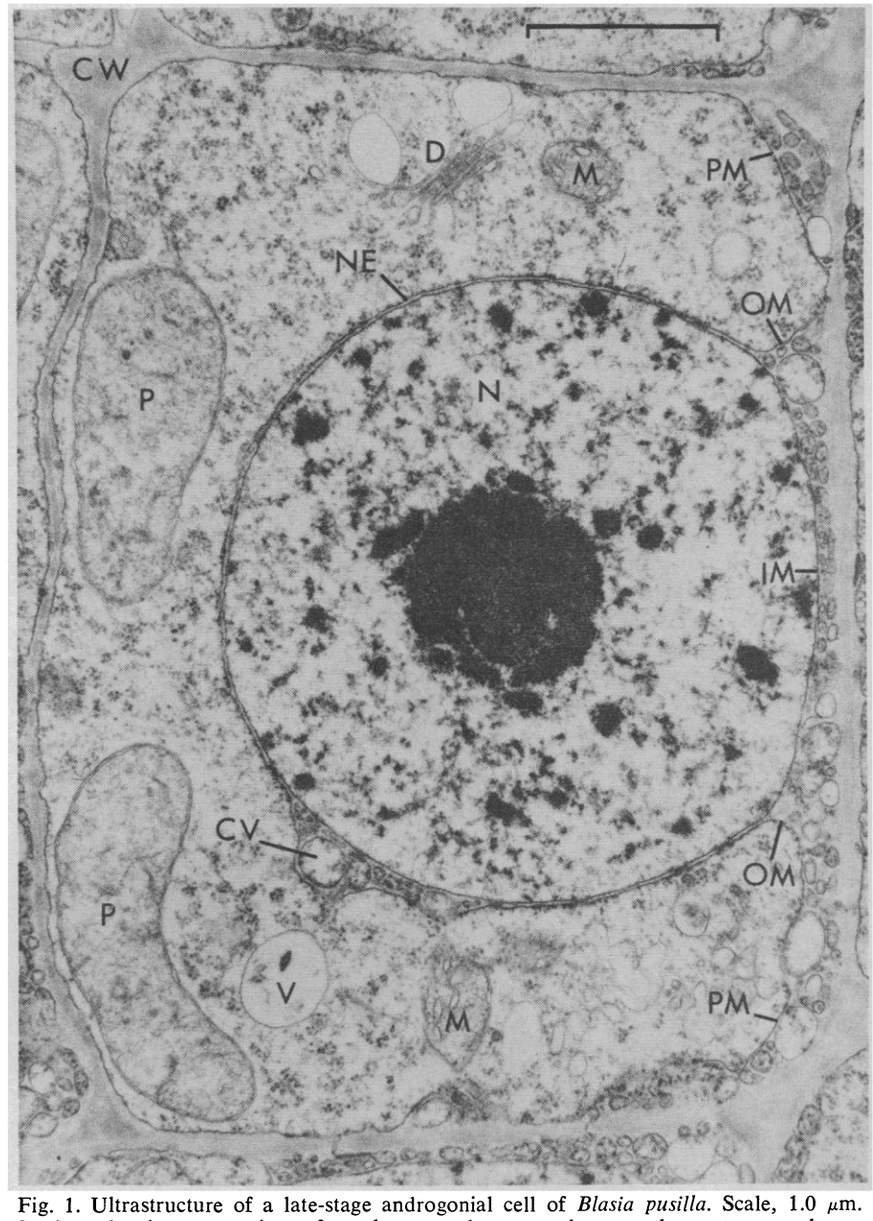

Fig. 1. Ultrastructure of a late-stage androgonial cell of *Blasia pusilla*. Scale, 1.0 μm. Section showing separation of nuclear envelope membranes, the outer membrane continuous with the plasmalemma. *CV*, cytoplasmic vesicle; *CW*, cell wall; *D*, dictyosome; *IM*, inner membrane; *M*, mitochondrion; *N*, nucleus; *NE*, nuclear envelope; *OM*, outer membrane; *P*, plastid; *PM*, plasmalemma; *V*, vacuole.

though in a somewhat extreme form. He presumed narrow channel openings to the cell exterior, while in *Blasia* the nucleus is broadly "exposed."

ZANE B. CAROTHERS
Department of Botany, University of Illinois, Urbana 61801

References and Notes

1. J. Robertson, in *The Structure and Function of Subcelilular Components*, E. Crook, Ed. (University Press, Cambridge, 1959), p. 3; ———, in *Cellular Membranes in Development*, M. Locke, Ed. (Academic Press, New York, 1964), p. 1.
2. R. MacLeod, L. Black, F. Moyer, *Virology* **29**, 540 (1966); R. Chiu, H. Liu, R. MacLeod, L. Black, *ibid.* **40**, 387 (1970).
3. M. Epstein, *J. Cell Biol.* **3**, 851 (1957); J. McAlear and G. Edwards, *Exp. Cell Res.* **16**, 689 (1959).
4. R. Ritch and C. Philpott, *Exp. Cell Res.* **55**, 17 (1969); A. Sedar, *J. Cell Biol.* **43**, 179 (1969).
5. J. Eymé, *Le Botaniste* **38**, 1 (1954).
6. A detailed report of these findings is in press (*J. Cell Biol.*).
7. W. Whaley, J. Kephart, H. Mollenhauer, in *Cellular Membranes in Development*, M. Locke, Ed. (Academic Press, New York, 1964), p. 135; W. Whaley, M. Dauwalder, J. Kephart, in *Origin and Continuity of Cell Organelles*, J. Reinert and H. Ursprung, Eds. (Springer, New York, 1971), p. 1.
8. I thank J. B. Hanson for suggestions.

12 August 1971; revised 6 October 1971

Growth Retardation in Offspring of Female Rats Treated with Morphine Prior to Conception

Abstract. *Treatment of female rats with morphine sulfate for 5½ or 10 days, prior to drug withdrawal for 5 days and subsequent mating, results in retarded growth of offspring. The effect is not present at birth but appears at 3 to 4 weeks of age. It occurs even though offspring are not exposed to morphine in utero or postnatally. It is not eliminated by cross-fostering and is apparently of prenatal origin.*

Recent studies of tolerance to opiates (*1*), and in particular the persistence of tolerance to the analgesic effect 11 months after a single injection of morphine (*2*), suggest that some transferable factor induced by morphine treatment is involved in the development of tolerance to narcotic analgesics (*2*, *3*). Results of passive transfer of serum from tolerant to nontolerant animals, however, have been equivocal (*4*). To further characterize this elusive morphine factor, we sought to determine whether treatment of females with morphine prior to conception affects the responses of their offspring to opiates (*5*). Since, in both tolerant and nontolerant rats, total recovery of injected morphine occurs by 48 hours after the last injection (*6*), our animals were not mated until 5 days after morphine treatment was terminated.

In the course of this work we noted a transient but significant depression in body weight in the progeny of morphine-treated females in comparison with the body weight of offspring of saline-treated mothers, although the young had not been previously exposed to the drug either in utero or after birth. Growth retardation did not occur until after weaning at 25 days of age and was no longer apparent by 8 weeks.

Ten Holtzman female rats were injected subcutaneously for 5½ days with increasing doses of morphine sulfate: starting dose was a single 10 mg/kg injection; thereafter rats received 15 mg/kg twice daily on days 2 to 5 and 22 mg/kg once on the final day of treatment. Controls were similarly injected with physiologic saline. Five days after the last injection, the females were mated with drug-free males. Neonates were weighed at birth and weekly thereafter. Mean body weights were similar for the two groups of offspring at birth and for the period up to weaning. Figure 1 presents the results of a typical experiment illustrating the body weight changes in 4- to 7-week-old progeny. "Experimental" refers to the offspring of morphine-sulfate-injected females; "control" signifies offspring of saline-injected mothers. Each point represents mean body weight (in grams) of 13 to 27 rats. The decrease in growth rate among offspring of morphine-treated mothers appeared consistently in similar studies with 28 breeder females, although some variation occurred, in individual experiments, between sexes in both time of onset (4 to 5 weeks) and extent of growth depression.

In addition, differences in viability among female offspring were observed between control and experimental groups of offspring. Although such differences were not evident prior to weaning, the incidence of deaths among 4- to 7-week female offspring of drug-treated mothers (29 percent) was significantly higher than in the corresponding control group (3 percent; a chi-square test gave a $P < .01$).

To assess the effect of an increase in both the duration and extent of morphine treatment of mothers, female rats were injected with morphine sulfate or saline twice daily for 10 days before drug withdrawal and mating. Starting dose was 10 mg/kg per injection; the dose was increased daily, in 5 mg/kg increments, to a maximum total daily dose of 60 mg/kg on the fifth through tenth day of treatment. Although there were again no weight differences between groups at birth, growth retardation occurred prior to weaning in progeny from mothers treated with higher doses of the opiate (Fig. 2). There was also a marked increase in deaths among the pups of morphine-treated females (62 percent), so that statistical analysis of differences beyond 5 weeks of age was not feasible.

To assess the role of postnatal influences on the observed growth effect, cross-fostering experiments were performed. As in the first experiment, ten rats were treated for 5½ days prior to drug withdrawal and subsequent mating. At 1 to 3 days after birth, offspring of the two groups of females were exchanged; progeny of morphine-treated females were placed with saline-injected foster mothers, and offspring of the control females were placed with mothers which had previously received morphine. The neonates remained with their foster mothers until weaning. Although there is some modification of the growth effect among cross-fostered animals both in time of onset and in sex differences, the main effect of treatment is still evident and significant (Fig. 3). Weight differences between progeny of treated and control mothers remain and are not eliminated by the cross-fostering procedure. Since manipulation of infant rodents has residual effects both on behavior and on response to drugs (*7*), the additional handling required by the cross-fostering procedure itself may have contributed to the differences observed. An additional comparison, between nonfostered offspring of the saline-injected mothers and similar control offspring cross-fostered to morphine-treated foster mothers, revealed no detrimental effect of nursing by the morphine-treated mothers on growth of the young.

It thus appears that postnatal effects,