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Non-Mendelian Inheritance of DNA-Induced Inositol Independence in *Neurospora*

(exosome/integration/meiosis/transformation)

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ABSTRACT **Inositol-independent ($inos^+$) revertants of *Neurospora* induced in inositol-requiring mutants by treatment with wild-type DNA in previous studies were found to be stable and to grow well in the absence of inositol. Genetic data presented in this paper show that a major proportion of these induced revertants rarely transmitted the inositol independence character ($inos^+$) to their sexual progeny. Non-Mendelian transmission of the transformed character ($inos^+$) was also found to occur in some of the sexual progeny in subsequent generations. These genetic data support the idea that the transforming DNA pieces carrying the genetic information (called exosomes) are not readily integrated into the host genome. It is suggested that elimination of most exosomes during meiosis causes a loss of the genetic information and leads to non-Mendelian transmission of the induced revertant character ($inos^+$).**

Since its elucidation by Avery and his coworkers (1), transformation has become a well-known process for the transfer of genetic information in bacteria. However, the study of such a genetic process in higher organisms has remained difficult due to several factors, including their diploid nature (2). Tatum (3) and Watson (4) have emphasized the significance of the use of eukaryotic microorganisms in similar studies. We have recently reinvestigated this problem in *Neurospora;* the results provide evidence for DNA-mediated transfer of genetic information in this eukaryotic haploid fungus (5). In the previous report we described the DNA-mediated transformation of three different genetic markers ($inos^-$, pdx^-, and rg^-), each of which determines a specific defective biochemical reaction. The transformed strains were found to be stable for their DNA-induced characters during the vegetative cycle of the organism. However, some of the inositol-independent ($inos^+$) revertants were found to be unable to transmit the DNA-induced character to their sexual progeny in ratios expected on the basis of Mendelian inheritance. These results can be explained on the basis of a rare genetic system involved in transformation of the $inos^-$ character in *Neurospora.* Such a genetic system has the following characteristics: (*a*) the transforming DNA pieces carrying the genetic information are able to replicate, transcribe, and express specific biochemical functions, and (*b*) in some cases these DNA pieces, called exosomes, (6), can remain unintegrated into the host genome and are subsequently liable to be eliminated during meiosis, thus leading to a non-Mendelian inheritance of the DNA-induced characters. In this paper we present genetic data which support an exosome model of transformation in *Neurospora.*

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MATERIALS AND METHODS

Strains. The inositol-independent ($inos^+$) revertant strains used in this study are those obtained during our earlier reported studies of transformation in *Neurospora* (5). These revertants, on the basis of their origin, belong to three groups: (*a*) those which originated in the control experiments without any DNA treatment, (*b*) those which also appeared in the control experiments where conidia or mycelial fragments from actively growing cultures of the mutant strain ($inos^-$) were treated with its own DNA (*i.e.*, iso-DNA), and (*c*) those which were obtained after similar treatment of the mutant strain ($inos^-$) with the wild-type DNA (*i.e.*, allo-DNA). The last group, from allo-DNA treatment, occurred with 25- to 30-fold higher frequency than those without any DNA treatment or with iso-DNA treatment (5) and are designated as induced revertants. The strains used carried other genetic markers such as rg^- (ragged) or os^- (osmotic) and the mating type A or a.

Other *Neurospora* strains used in our present study include the following: wild-type strains RL3-8A and RL21a; inositol-requiring ($inos^-$) strains 89601A and 89601a; osmotic mutant strains R2473a (os^-, a) or R2473-2-1A ($inos^-$; os^-, A); and inositol-requiring morphological mutants R2509-2-48A ($inos^-$; cr-3^-, A) and R2506-8-12a ($inos^-$; rg^-, a). These strains were obtained from the Rockefeller University collection. A few other strains used in genetic analysis were those obtained as inositol-independent progeny from crosses of revertants induced with allo-DNA ($inos^+$) with $inos^-$ strains, in studies of transmission of the induced revertant character ($inos^+$) in subsequent generaiions. Such strains were 26-2a, 26-3A, 26-4a, and 26-6A ($inos^+$; os^-) obtained from the cross of $inos^+$ revertant (26) × $inos^-$; os^-, A (R2473-2-1A). Strain 56-B-1 was obtained similarly from the cross of $inos^+$ revertant (26) × $inos^-$ (89601A). Other strains used were those obtained as inositol-independent progeny in subsequent generations. The $inos^+$ strains nos. 165, 166, 168, and 169 were obtained from the cross $inos^+$ (26-6A) × $inos^-$ (89601a).

Media. The revertant strains were maintained on minimal medium (7); the revertant strains of otherwise identical genetic background were found to grow similarly on medium with and without inositol. Other strains were maintained on inositol-supplemented minimal medium. The genetic crosses were made on synthetic crossing medium (8).

TABLE 1. *Genetic analysis of the wild-type revertants ($inos^+$) obtained without DNA treatment or with iso-DNA treatment*

Cross*	No. of progeny	
	$inos^-$	$inos^+$
1	271	302
2	817	789
3	101	111
4	1254	1238
5	83	68
6	44	38
7	42	45
8	67	73
9	493	511
10	83	95

* Revertant ($inos^+$) × $inos^-$ (89601A): other loci involved in these crosses were rg^-, a/rg^+, A.

Genetic Analysis. Random spores and ordered asci were analyzed by standard methods. A large sample of ascospores was analyzed with L-sorbose in the medium (9), as described by Giles (10). Equal aliquots of ascospore suspension were added to liquid agar sorbose medium with and without inositol supplement. After a heat treatment of 60° for 30 min, the medium containing the ascospores was distributed in sterile petri plates (25 ml per plate). The plates were incubated at 25° for about 70 hr before the growing colonies were counted. A small sample of ascospores were analyzed by transferring random spores individually onto inositol-supplemented agar slopes. For tetrad analysis, all eight ascospores from a particular ascus (one ascus per perithecium) were individually transferred onto inositol-supplemented agar slopes. Both random and ascus isolates, after sufficient growth, were examined for their inositol requirement. The forced heterokaryons, when required, were constructed by a described method (11).

Test for Different Phenotypes. The inositol requirement was examined by inoculating the individual culture on duplicate plates containing minimal sorbose agar medium with and without inositol. The ability of the culture to grow on the supplemented medium but not on the minimal medium indicated a requirement for inositol. Osmotic sensitivity of strains was determined by their inability to grow in the presence of 6% NaCl in the growth medium. The mating types of the isolates were determined by their responses to tester strains of known mating types (either A or a).

Test for Heterokaryosis at the Inositol Locus ($inos^+/inos^-$). The inositol-independent revertant strains were examined for heterokaryosis ($inos^+/inos^-$) by plating equal aliquots of the conidial (or mycelial fragment) suspension on sorbose agar medium with and without inositol supplement. For a heterokaryotic culture, the number of colonies growing on inositol-supplemented medium would significantly exceed the number growing on the minimal medium.

RESULTS

In order to ascertain the nature of the inheritance of the character induced with allo-DNA ($inos^+$), an extensive genetic analysis of the different revertants was undertaken. The revertants ($inos^+$) were crossed with the $inos^-$ strain and their progeny were analyzed for inheritance of the $inos^+$ character.

TABLE 2. *Genetic analysis of the wild-type revertants ($inos^+$) obtained with allo-DNA treatment*

Cross*	No. of progeny		Cross*	No. of progeny	
	$inos^-$	$inos^+$		$inos^-$	$inos^+$
1	92	89	17	1589	49
2	90	91	18	297	3
3	197	151	19	126	3
4	271	302	20	168	14
5	433	340	21	141	1
6	457	239	22	53	0
7	374	117	23	49	0
8	453	105	24	309	0
9	1502	521	25	126	3
10	1608	893	26	297	3
11	608	184	27	369	0
12	620	239	28	1781	3
13	1608	809	29	4878	10
14	374	117	30	3026	0
15	2571	302	31	700	0
16	2508	522	32	20,000	0
			33	100,000	0

* Revertant ($inos^+$) × $inos^-$ (89601). The cross numbers also represent the reassigned isolation numbers of the revertant strains. All revertants were rg^-, a (except for strain nos. 28 and 29, which were os^-, A); these markers segregated in the ratio of 1:1.

The data presented in Tables 1 and 2 show that the transmission of the $inos^+$ character to the progeny of the cross, revertant × $inos^-$, was dependent on how these revertants were originally obtained. Those revertants that were obtained in control experiments (without any DNA treatment or with iso-DNA) transmitted the $inos^+$ character to one-half of the progeny as a Mendelian gene (Table 1). This was, however, not the case with the revertants induced with allo-DNA; most of them showed aberrant transmission of the trait induced by allo-DNA (Table 2). In these crosses the transmission of the other loci (rg^- or os^- and the mating type loci A/a) was, however, in the Mendelian ratio of 1:1. The crosses involving revertants induced by allo-DNA can be classified into the following groups, based on the transmission pattern of the inositol-independence character: (*a*) normal Mendelian transmission in the expected ratio of 1:1 (crosses 1–4 in Table 2) and (*b*) non-Mendelian transmission (crosses 5–33 in Table 2). All crosses belonging to the latter group showed rare transmission of the $inos^+$ character, although the individual crosses varied in the proportion of the $inos^+$ progeny. Such non-

TABLE 3. *Analysis of the inositol-independent revertants for heterokaryosis of $inos^+/inos^-$ loci*

Revertant strains*	No. of colonies	
	Minimal	Inositol
1. *ragged*, a (26)	217	220
2. *osmotic*, A (28)	180	175
3. *osmotic*, A (29)	239	217
4. *ragged*, a (30)	193	205
5. *ragged*, a (33)	88	79

* *Numbers in parentheses* represent the crosses in which the strain was used for genetic analysis (see Table 2).

Mendelian transmission of the character induced by allo-DNA can be explained by either of the following suppositions: (*a*) that the revertant strains were heterokaryotic and carried a large number of *inos*⁻ nuclei in comparison to *inos*⁺ nuclei, or (*b*) that the revertant strains carried the *inos*⁺ genetic information as unintegrated pieces of DNA (exosomes) and that their transmission was prevented during meiosis by mechanisms to be discussed later. Results of genetic experiments presented in this paper favor the exosome model.

Examination for Heterokaryosis. Several inositol-independent revertants induced by allo-DNA were examined for heterokaryosis by plating equal aliquots of the conidial or mycelial suspension on minimal and inositol-supplemented media. The data presented in Table 3 show no significant difference in the number of colonies growing on the two kinds of media. These data suggest that the revertant strains were not heterokaryotic for the *inos*⁺ and *inos*⁻ alleles. Some of these *inos*⁺ colonies growing on minimal medium were isolated and then genetically analyzed. The conidial isolates in crosses with the *inos*⁻ strain showed non-Mendelian transmission of the *inos*⁺ character similar to the parental strain. These results provide additional evidence against the heterokaryotic state of the revertants induced by allo-DNA.

It is assumed that additional growth on minimal medium would select for *inos*⁺ nuclei if these strains were indeed heterokaryotic. Therefore, five revertants were grown on minimal medium in 35-cm-long growth tubes. Isolates were then obtained from the initial and terminal ends of each growth tube. These isolates (10 in number) were then crossed to *inos*⁻ strains. The results of such crosses, presented in Table 4, showed non-Mendelian transmission of the *inos*⁺ character. Thus, the results of all the foregoing genetic experiments clearly suggest that the revertants induced by allo-DNA were not heterokaryotic for the inositol locus (*inos*⁺/*inos*⁻).

TABLE 4. *Genetic analysis of the wild-type revertants induced by allo-DNA after growth* on minimal medium in growth tubes*

Cross†	No. of progeny *inos*⁻	No. of progeny *inos*⁺
1. (a) Initial end	189	0
(b) Terminal end	142	1
(c)‡ Parental strain	141	1
2. (a) Initial end	153	0
(b) Terminal end	149	0
(c) Parental strain	53	0
3. (a) Initial end	387	0
(b) Terminal end	369	0
(c)‡ Parental strain	309	0
4. (a) Initial end	168	2
(b) Terminal end	189	2
(c)‡ Parental strain	297	3
5. (a) Initial end	723	0
(b) Terminal end	803	0
(c)‡ Parental strain	3026	0

* These strains grow equally well on the medium with inositol.
† Revertant (*inos*⁺) × *inos*⁻; all revertants were *rg*⁻, a.
‡ Data from Table 2, corresponding to cross nos. 21, 22, 24, 26, and 30.

Inheritance of the Exosomes. On the basis of an exosome model, the transmission of the allo-DNA character would depend greatly on the fate of the exosomes during meiosis. Elimination of the exosome during meiosis by correction mechanisms will cause loss of the genetic information. However, occasional failure on the part of the correction mechanism will cause retention of the genetic information. Such handling of the exosome at meiosis adequately explains the

TABLE 5. *Transmission of the inositol independence induced by allo-DNA (inos*⁺*) in subsequent generations*

Cross*	Generation of progeny analyzed	Random No. of progeny *inos*⁻	Random No. of progeny *inos*⁺	Tetrad No. of asci in each ascus class (8 *inos*⁻ 0 *inos*⁺)	Tetrad No. of asci in each ascus class (4 *inos*⁻ 4 *inos*⁺)	Mode of transmission of allo-DNA character
1. 26 × *inos*⁻	F_1	297	3	20	0	Non-Mendelian
2. 26-B-1 × *inos*⁻	F_2	523	460	—	20	Mendelian
3. 26-B-1 × wild type	F_2	0	1284	0	—	Mendelian
4. 26-2 × *inos*⁻	F_2	1170	1124	0	10	Mendelian
5. 26-3 × *inos*⁻	F_2	1375	1319	0	10	Mendelian
6. 26-4 × *inos*⁻	F_2	714	0	—	—	Non-Mendelian
7. 26-6 × *inos*⁻	F_2	5582	4	15	0	Non-Mendelian
8. 26-6 × wild type†	F_2	60	622	—	5	Non-Mendelian
9. 26-6 × *inos*⁻	F_2	5700	21	—	—	Non-Mendelian
10. *inos*⁻ × 26-6	F_2	9700	50	—	—	Non-Mendelian
11. 26-6 × 26-4	F_2	515	45	5	8	Non-Mendelian
12. 26-4 × 26-6	F_2	627	63	9	1	Non-Mendelian
13. 169 × *inos*⁻	F_3	—	—	0	10	Mendelian
14. 165 × *inos*⁻	F_3	—	—	0	10	Mendelian
15. 168 × *inos*⁻	F_3	971	1054	—	—	Mendelian
16. 166 × *inos*⁻	F_3	460	42	6	2	Non-Mendelian

* All strains used in above crosses (except the *inos*⁻ and the wild-type strains) were the revertants induced by allo-DNA or their *inos*⁺ progeny (see *Methods* for a detailed description of these strains).
† Three asci each with 8 *inos*⁺ and 0 *inos*⁻ spores were also obtained from this cross.

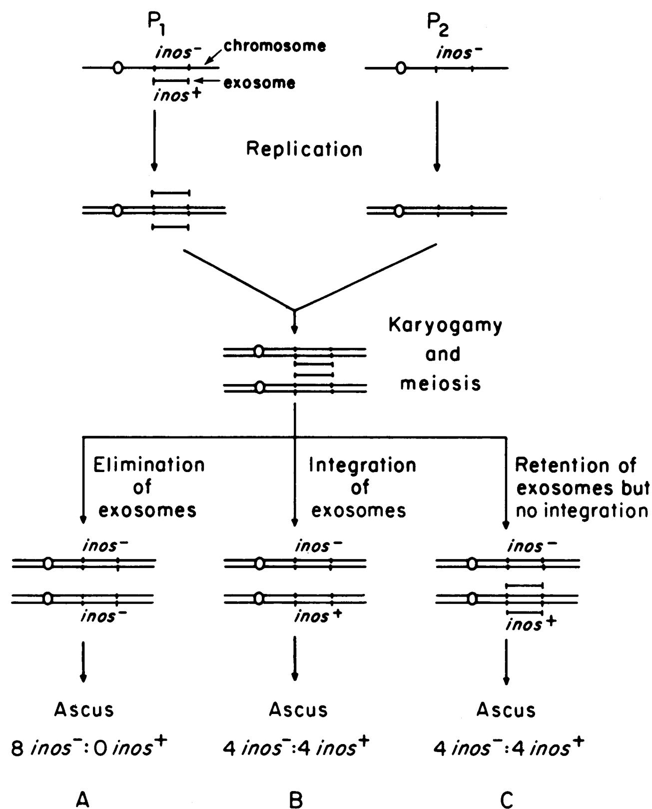

FIG. 1. The fate of exosomes during meiosis: (*A*) The loss of exosomes would cause a non-Mendelian inheritance of the parental *inos*$^+$ character. (*B*) As a result of exosome integration, the *inos*$^+$ progeny would be stable for the revertant *inos*$^+$ character and would transmit it as a Mendelian character in the subsequent generation. (*C*) Retention of the unintegrated exosomes would cause apparent Mendelian transmission of the *inos*$^+$ character. These exosomes, however, might be eliminated during subsequent meiosis and thus would then lead to a non-Mendelian inheritance; alternatively the exosomes might be integrated during the vegetative cycle and be inherited as a Mendelian character in subsequent generations.

non-Mendelian results of the genetic crosses presented in Table 2. Furthermore, the exosome model predicts that some of the F_1 *inos*$^+$ progeny (obtained from the cross: revertant × *inos*$^-$) should continue to manifest non-Mendelian transmission of the transformed character in subsequent generations. Results of genetic analysis presented in Table 5 meet these predictions of the exosome model. The data show that some of the F_1 progeny, when later crossed with the *inos*$^-$ or *inos*$^+$ strains, show similar non-Mendelian transmission to the F_2 generation (see crosses nos. 6–10 in Table 5). Also, the F_2 isolates (obtained as inositol-independent progeny from cross no. 7 in Table 5), in subsequent crosses with *inos*$^-$ strains showed both Mendelian and non-Mendelian transmission of the *inos*$^+$ character (see crosses nos. 13–16 in Table 5). These results suggest that in those strains showing non-Mendelian transmission of the *inos*$^+$ character, the allo-DNA is not integrated into the host genome. Data from tetrad analysis of these crosses, presented in Table 5, also support these conclusions. The *inos*$^+$ revertant strains (or their progeny in subsequent generations) in crosses with *inos*$^-$ strains produced two classes of asci, 8 *inos*$^-$:0 *inos*$^+$ and 4 *inos*$^-$:4 *inos*$^+$ spores (see Table 5). The occurrence of asci with 8 *inos*$^-$ and 0 *inos*$^+$ spores indicated non-Mendelian transmission of the *inos*$^+$ character. This ascus class was found to occur in greater number in all those crosses showing rare transmission of *inos*$^+$ on random analysis (see Table 5).

Location of Exosomes. The possibility that the exosomes may occur in the cytoplasm as extranuclear heritable factors was examined by (*a*) comparison of the results of reciprocal crosses and (*b*) heterokaryon test. Results of the reciprocal crosses between the *inos*$^+$ (revertant) × *inos*$^-$ strains did not show uniparental transmission of the *inos*$^+$ character (see crosses 9 and 10 in Table 5) and thus suggest that the exosomes are not cytoplasmic factors. This hypothesis is further substantiated by the rare transmission of the *inos*$^+$ character when both parents involved in the cross were revertants induced by allo-DNA (see crosses nos. 11 and 12 in Table 5).

The conclusion that exosomes were not transmitted as cytoplasmic factors was further supported by the results of a heterokaryon test. A forced heterokaryon was constructed between the revertant strain (*os*$^-$, *cr-3*$^+$, A; *inos*$^+$) and the tester strain (*os*$^+$, *cr-3*$^-$, A; *inos*$^-$). This heterokaryon grew like wild type on minimal medium containing 6% NaCl. Later, hemokaryotic isolates were obtained by plating conidia on medium containing inositol; isolates with the *cr-3*$^-$ phenotype of the tester strain used in the formation of the heterokaryon, were examined for their inositol requirement. Of the 100 conidial progeny examined, all were found to require inositol for growth (i.e., were *inos*$^-$). Thus, the fact that the inositol independence character could not be transmitted to the homokaryotic isolates from the forced heterokaryon would seem to exclude the possibility that the exosomes were extranuclear factors.

DISCUSSION

Genetic analysis of the revertants induced by allo-DNA shows both Mendelian and non-Mendelian inheritance of the transformed *inos*$^+$ character in *Neurospora*. The fact that a major proportion of these revertants and some of their progeny showed non-Mendelian transmission of the *inos*$^+$ character in subsequent generations strongly supports the hypothesis that alien DNA pieces carrying this genetic information were not readily integrated into the genome of these strains. Since all of these revertants were found to be stable for the induced character *inos*$^+$, it is suggested that the DNA pieces are capable of replication, transcription, and translation; such DNA pieces are designated as exosomes. In view of the fact that these exosomes do not seem to be carried in the cytoplasm, it is assumed that they enter the nucleus and replicate synchronously, perhaps attached to homologous sites on the chromosome.

The non-Mendelian inheritance of the allo-induced character in subsequent generations provides the most compelling evidence in support of the idea that exosomes are not readily integrated into the chromosome. Thus, the inheritance of genetic information carried by exosomes depends on their fate during meiosis.

Exosomes that are not integrated into chromosomes would be more liable to be recognized by the correction mechanism (4, 12–14) during meiosis and thus be eliminated as depicted in Fig. 1. Occasional failure of the correction mechanism may, however, permit retention and consequent transmission of the exosomes. The failure of the correction mechanism is suggested by our genetic data regarding the rare occurrence of *inos*$^+$

progeny from the cross revertant (*inos*$^+$) × *inos*$^-$. In circumstances where exosomes escape elimination, they may be integrated by breakage and reunion (15–17). Integration of an exosome by breakage and reunion in a particular meiotic cell may be assumed to be limited by the synaptinemal complex on spatial considerations (18, 19) and, therefore, integration could not possibly be achieved in all of the meiotic cells retaining exosomes. At least in some cases, the exosome would be transmitted as such largely or exclusively in the unintegrated form (Fig. 1). Those progeny carrying the integrated exosomes would show Mendelian transmission, whereas those receiving the unintegrated exosomes would show non-Mendelian transmission in subsequent generations provided the exosomes were not integrated during the vegetative cycle. These two classes of *inos*$^+$ progeny predicted on the basis of the exosome model were indeed obtained during our genetic analysis of the allo-DNA revertants (see Table 5).

In *Neurospora*, DNA replication occurs immediately before karyogamy and thus precedes meiosis (20). Therefore, each diploid nucleus entering meiosis would have at least two newly replicated exosomes (Fig. 1). As suggested earlier in this paper, two classes of asci with 8 *inos*$^-$ and 0 *inos*$^+$ and with 4 *inos*$^-$ and 4 *inos*$^+$ spores were obtained, depending on whether the correction mechanism was fully active or not. Theoretically, however, when the correction mechanism is only partly active and is able to remove only one exosome, there could be another class of ascus with 6 *inos*$^-$ and 2 *inos*$^+$ spores. No ascus of this kind has been obtained during our genetic analysis. This result may suggest that the correction mechanism in *Neurospora* is very efficient or that there are additional unknown parameters influencing the exosome elimination process at meiosis. The only information available in this respect is that *Neurospora* is abundantly rich in different kinds of nucleases (21).

These characteristics of transformation in *Neurospora* do not seem to have parallels in the typical phenomena of bacterial transformation. However, some of the features that we have described here are similar to those reported in *Drosophila* by Fox and coworkers (6).

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1. Avery, O. T., McLeod, C. M. & McCarty, M. (1944) *J. Exp. Med.* **79**, 137–158.
2. Ledoux, L. (1965) in *Progress Nucleic Acid Research and Molecular Biology*, eds. Davidson, J. N. & Cohn, W. (Academic Press, New York), Vol. 4, pp. 231–267.
3. Tatum, E. L. (1965) in *The Control of Human Heredity*, ed. Sonneborn, T. M. (Macmillan Co., New York), pp. 20–34.
4. Watson, J. D. (1970) *Molecular Biology of the Gene* (W. A. Benjamin, Inc., New York).
5. Mishra, N. C., Szabo, G. & Tatum, E. L. (1973) in *The Role of RNA in Reproduction and Development*, eds. Niu, M. C. & Segal, S. J. (North-Holland Publ. Co., Amsterdam), in press.
6. Fox, A. S., Duggleby, W. F., Gelbart, W. M. & Yoon, S. B. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1834–1838.
7. Vogel, H. J. (1964) *Amer. Natur.* **98**, 435–446.
8. Westergaard, M. & Mitchell, H. K. (1947) *Amer. J. Bot.* **34**, 573–577.
9. Tatum, E. L., Barratt, R. W. & Cutter, V. M. (1949) *Science* **109**, 509–511.
10. Giles, N. H., Jr. (1951) *Cold Spring Harbor Symp. Quant. Biol.* **16**, 283–313.
11. Beadle, G. W. & Coonradt, V. L. (1944) *Genetics* **29**, 291–308.
12. Boyce, R. P. & Howard-Flanders, R. (1964) *Proc. Nat. Acad. Sci. USA* **51**, 293–300.
13. Yasuda, S. & Sekiguchi, M. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1839–1845.
14. Whitehouse, H. L. K. (1969) *Towards an Understanding of the Mechanism of Heredity* (St. Martins, New York).
15. Bodmer, W. F. & Ganesan, A. T. (1964) *Genetics* **50**, 717–738.
16. Fox, M. C. & Allen, M. K. (1964) *Proc. Nat. Acad. Sci. USA* **52**, 412–419.
17. Hotchkiss, R. D. (1971) in *Advances in Genetics*, ed. Caspari, E. (Academic Press, New York), Vol. 16, pp. 325–348.
18. Lu, B. C. (1970) *J. Cell Sci.* **6**, 669–678.
19. Westergaard, M. & Von Wettstein, D. (1972) *Ann. Rev. Genet.* **6**, 71–110.
20. Rossen, J. M. & Westergaard, M. (1966) *C. R. Trav. Lab. Carlsberg* **35**, 233–286.
21. Linn, S. & Lehman, I. R. (1965) *J. Biol. Chem.* **240**, 1294–1304.