Proc. Nat. Acad. Sci. USA
Vol. 72, No. 7, pp. 2738–2742, July 1975
Genetics

Conditional-lethal mutations that suppress genetic defects in morphogenesis by altering structural proteins

(protein interaction/temperature-sensitive mutants/cold-sensitive mutants/phage P22 morphogenesis/reversion)

JONATHAN JARVIK AND DAVID BOTSTEIN

Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. 02139

Communicated by Boris Magasanik, April 21, 1975

ABSTRACT **An analysis of revertants of missense mutants in phage P22 has shown: *(i)* New temperature-sensitive (TS) and cold-sensitive (CS) phenotypes are often acquired concomitant with reversion. *(ii)* In many cases, these new phenotypes are due to second-site mutations (suppressors) that correct the original defect. *(iii)* Sometimes the suppressor mutation is not in the same gene as the original mutation. *(iv)* Extragenic suppressors are almost always in genes whose products are known to interact physically with the original gene products. *(v)* The suppressor mutations typically retain their TS or CS phenotypes when crossed into wild-type genetic backgrounds. *(vi)* Some TS and CS mutants derived by reversion can themselves be reverted to produce additional mutations.**

We have shown that genetic reversion of missense mutants can be of value in producing new temperature-sensitive and cold-sensitive mutations affecting related functions. We suggest that our approach can be extended to organisms with large genomes.

Temperature-sensitive and cold-sensitive mutants allow an investigator to modulate protein activity *in vivo* and *in vitro* simply by varying the temperature. Such mutants thereby greatly aid in the analysis of biochemical and developmental events at the molecular, cellular, and organismic levels (1–7).

For conditional-lethal mutants, nonpermissive conditions provide an absolute selection for further mutation, since only revertant individuals can grow. Such revertants need not be true wild type; rather, they may have acquired suppressors—new mutations that act so as to correct, replace, or bypass the original defect.

The analysis of suppressors has proved to be of great value in diverse biological investigations (8). However, such analysis is operationally difficult if the suppressors under investigation lack characteristic phenotypes of their own—phenotypes that are expressed independent of the original mutations.

In this paper, we demonstrate, using bacteriophage P22, that new mutations with temperature-sensitive and cold-sensitive phenotypes frequently appear among the revertants of existing missense mutants. Our experiments were undertaken with two principles in mind. *(i)* Suppressors are often missense mutations (8, 9). *(ii)* Missense mutations often confer cold-sensitive or temperature-sensitive phenotypes (2). We therefore expected, and found, that some revertants of existing missense mutants would contain suppressor mutations that produce cold-sensitive or temperature-sensitive phenotypes in and of themselves.

Abbreviations: TS, temperature-sensitive phenotype; *ts*, genetic determinant of TS phenotype; CS, cold-sensitive phenotype; *cs*, genetic determinant of CS phenotype.

MATERIALS AND METHODS

Phage, Bacteria, and Media. The following mutations of bacteriophage P22 have been described (4,10): c_1-7, 13$^-$*am*H101 (in our notation, the gene designation precedes the allele designation; thus, 13$^-$*am*H101 means that *am*H101 is an allele of gene 13), 1$^-$*am*H21, 1$^-$*am*H58, 8$^-$*am*N26, 1$^-$*am*N23, 2$^-$*cs*H22, 2$^-$*cs*H59, 1$^-$*cs*H139, 5$^-$*cs*H126, 12$^-$*ts*12.1, 3$^-$*ts*RH203, 3$^-$*ts*3.1, 2$^-$*ts*2.1, 1$^-$*ts*1.1, 5$^-$*ts*N26, 5$^-$*ts*N105, 8$^-$*ts*N102, and 10$^-$*ts*10.1. 1$^-$*cs*RH21D was selected as an *am*$^+$ revertant of *am*H21. 8$^-$*cs*RN26D was selected as an *am*$^+$ revertant of *am*N26. 1$^-$*cs*H137 was erroneously reported to be in gene 8 in an earlier publication (4). Bacterial strains are derivatives of *Salmonella typhimurium* LT2. DB53 and its amber suppressor derivative DB74, and DB21 and its amber suppressor derivative DB28, have been described (10). DB7000 is *leu*$^-$*am*, a derivative of DB21. DB7004 is a derivative of DB7000 that contains the *su*$^+$ allele of DB74. DB7002 is a derivative of DB7000 that contains the *su*$^+$ allele of DB28. LB broth, nutrient agar, λ plates, and buffered saline have been described (11).

Genetic Mapping, Complementation Tests, and Genetic Crosses. All newly isolated *cs* and *ts* mutations were mapped by the methods of efficiency of plating or permissive rescue on prophage deletion strains (12). Any mutation that was not shown by deletion mapping to be between known markers in a known gene was assigned to a complementation group after tests against known amber alleles by the complementation in liquid culture method (10). Genetic crosses were performed as described (10).

Isolation of Independently Arising Revertants. Permissively grown plaques were touched with sterile needles and streaked on plates which were then incubated nonpermissively. Revertant plaques which grew on these plates were picked with a sterile capillary tube and suspended in buffered saline; the phage were tested for plaque-forming ability on the hosts and at the temperatures described in *Results*.

Nomenclature for Revertants. The phage to be reverted is called the parental mutant. A revertant is named by enclosing the name of the parental mutant in parentheses, preceding it with the letter "r," and succeeding it by a letter or number. All this is preceded by a designation of the revertant's phenotype (TS for temperature-sensitive and CS for cold-sensitive). This name stands for a phage of a particular phenotype, but it does not strictly define a genotype. The name of the new mutation should indicate its phenotype in a wild-type background. In naming the new mutation, the parentheses are dropped and lower case letters are used. For example, the first temperature-sensitive revertant of *cs*H137 isolated was given the name TSr(*cs*H137)A. The revertant

Table 1. Survey of revertants of missense mutants

Parental mutant	Conditions for selection of revertants	No. of revertants examined	Revertants with acquired CS or TS phenotypes	Gene in which new mutation is located	Is the new mutation a suppressor?
2⁻*cs*H22	18°	5	TSr(*cs*H22)A	Gene 2	ND
2⁻*cs*H59	18°	4	TSr(*cs*H59)A	Gene 2	ND
1⁻*cs*RH21D	18°	3	TSr(*cs*RH21D)3	Gene 1	No
1⁻*csrr*RH21D3A	18°	14	TSr(*csrr*RH21D3A)1	Gene 1	Yes
1⁻*cs*H137	18°	36	TSr(*cs*H137)A	Gene 5	Yes
			TSr(*cs*H137)B	Gene 5	Yes
			TSr(*cs*H137)C	Gene 5	Yes
			TSr(*cs*H137)D	Gene 5	Yes
1⁻*cs*H139	18°	30	TSr(*cs*H139)A	Gene 1	Yes
8⁻*cs*RN26D	18°	10	TSr(*cs*RN26D)1	Gene 8	ND
5⁻*cs*H126	18°	33	—	—	—
5⁻*csrr*H58G1	18°	48	TSr(*csrr*H58G1)A	Gene 5	ND
12⁻*ts*12.1	37°	40	—	—	—
3⁻*ts*3.1	41°	96	—	—	—
3⁻*ts*RH203	41°	32	—	—	—
2⁻*ts*2.1	37°	88	—	—	—
1⁻*ts*1.1	37°	58	—	—	—
1⁻*tsr*RH21D3	41°	14	CSr(*tsr*H21D3)A	Gene 1	No
8⁻*ts*N102	37°	32	CSr(*ts*N102)A	Gene 8	ND
5⁻*ts*N26	37°	94	—	—	—
5⁻*tsr*H58G	37°	114	CSr(*tsr*H58G)1	Gene 5	Yes
			CSr(*tsr*H58G)2	ND	ND
			CSr(*tsr*H58G)3	ND	ND
5⁻*tsr*H58E	37°	49	—	—	—
5⁻*ts*N105	37°	33	—	—	—
5⁻*tsr*H137B	41°	16	—	—	—
5⁻*tsr*H137C	34°	22	CSTSr(*tsr*H137C)19	Gene 12	Yes
1⁻*am*H58	Nonpermissive Su⁺ host at 30°	12	TSr(*am*H58)A	Gene 5	Yes
			TSr(*am*H58)D	Gene 5	Yes
			TSr(*am*H58)E	Gene 5	Yes
			TSr(*am*H58)G	Gene 5	Yes
			TSr(*am*H58)H	Gene 5	Yes
8⁻*am*N26	Nonpermissive Su⁺ host at 30°	21	CSTSr(*am*N26)K	Gene 5	Yes
1⁻*am*H21	Nonpermissive Su⁺ host at 30°	16	CSr(*am*H21)E	Gene 1	No

Revertants were selected and tested as described in *Materials and Methods*. "ND" in the table means "not determined." 1⁻*am*H58 fails to grow at any temperature on the Su⁺ host DB7004, but it does grow at 18°, 30°, and 41° on the Su⁺ host DB7002. 1⁻*am*H21 shows the same pattern of growth. 8⁻*am*N26 does not grow on DB7004 at any temperature, but it does grow on DB7002 at 30° and 41°. A mutation is called a suppressor only if it has been separated from its parental mutation by recombination. Thus, some revertants [e.g., TSr(*cs*H22)A] may contain suppressors but not yet have been identified as such. All four *tsr*H137 mutations are separable from one another by recombination, as are all five *tsr*H58 mutations. *ts*12.1, *ts*RH203, *ts*2.1, *ts*N102, and *cs*H137 phages carry c_1-7 (clear plaque) mutations. *cs*H22, *cs*H59, *csrr*H58G, and *ts*3.1 phages carry c_1-7 and *am*H101 (lysis defective) mutations. All other phages are wild type.

contains a new mutation at a different site from *cs*H137. If this mutation were shown to confer a temperature-sensitive phenotype when crossed away from *cs*H137, it would be named *tsr*H137A. (If the new mutation had been shown to be at the same genetic site as *cs*H137, it would likewise be called *tsr*H137A, since we would know that it confers a temperature-sensitive phenotype in a wild-type background). If *tsr*H137A were to produce a cold-sensitive revertant, that phage would be named CSr(*tsr*H137A)1; if the new mutation were shown to confer cold-sensitivity on a wild-type background, it would be named *csrr*H137A1, and so on.

RESULTS

Generation of New Mutants. We surveyed spontaneous independent revertants of three kinds of missense mutants for the presence of new temperature-sensitive and cold-sensitive phenotypes. CS⁺ revertants of cold-sensitive mutants were tested for temperature-sensitivity. TS⁺ revertants of temperature-sensitive mutants were tested for cold-sensitivity. Finally, revertants of a third class of mutant—amber mutants that do not grow in one of our standard amber-suppressing hosts—were selected for growth on the "nonpermissive" amber-suppressing host, and these revertants were tested for cold-sensitivity and temperature-sensitivity. (We presume that for this third class of mutant a defective protein with a missense amino acid at the amber position is made in the nonpermissive amber-suppressing host; the amber mutants are, therefore, phenotypically missense mutants in these hosts.) For each revertant with a newly acquired cold-sensitive or temperature-sensitive phenotype, we located the genetic determinant of the phenotype by deletion mapping, complementation tests, and, in some cases, by three-factor crosses.

Table 1 details the results of our survey. Of nine cold-sensitive mutants examined, eight yielded temperature-sensi-

Table 2. Phenotypes conferred by suppressors in parental and wild-type backgrounds

	18°	21°	26°	30°	34°	37°	41°
TSr(*cs*H137)B	+	+	+	+	+	(+)	–
*ts*rH137B	+	+	+	+	+	(+)	–
TSr(*cs*H137)C	+	+	+	+	–	–	–
*ts*rH137C	+	+	+	(+)	–	–	–
TSr(*cs*H137)D	+	+	–	–	–	–	–
*ts*rH137D	+	+	+	–	–	–	–
TSCSr(*am*N26)K	–	(+)	+	+	+	(+)	–
*ts*rN26K	(+)	+	+	+	+	(+)	–
TSr(*am*H58)A	+	+	+	+	+	+	–
*ts*rH58A	+	+	+	+	+	+	–
TSr(*am*H58)D	+	+	+	+	+	+	–
*ts*rH58D	+	+	+	+	+	+	–
TSr(*am*H58)E	+	+	+	+	+	–	–
*ts*rH58E	+	+	+	+	+	–	–
TSr(*am*H58)G	+	+	+	+	+	–	–
*ts*rH58G	+	+	+	+	+	–	–
TSr(*am*H58)H	+	+	+	+	+	+	–
*ts*rH58H	+	+	+	+	+	+	–
CSr(*ts*rH58G)1	–	–	+	+	+	+	–
*cs*rrH58G1	–	+	+	+	+	+	+

The upper member of each pair is the revertant itself; the lower member is a phage that carries the suppressor in a wild-type background.

Backcrosses were performed as follows. For *ts*rH137 phages: A 1⁻*am*N23 mutation was crossed into the TSr strain to produce a phage with an AM,TS phenotype. The AM,TS phage was then crossed with wild type and an AM⁺,TS recombinant was recovered. The Am⁺,TS phage was checked to be sure that the parental *cs*H137 allele was absent by crossing it with a 1⁻*cs*H137 5⁻*ts*N26 double mutant phage and testing TS⁺ progeny for a CS⁺ phenotype (*am*N26 maps between the *ts*r mutation and *cs*H137). CS⁺,TS⁺ progeny indicate that the parental *cs* allele is absent from the AM⁺,TS phage. For *ts*rH58 and *ts*rN26 phages: The *am ts*r revertant was plated at permissive temperature on a prophage deletion lysogen (10) that contains the *am*⁺ allele but not the *ts*⁺ allele. *Am*⁺ recombinants were recovered. For *cs*rrH58G1: The phage, which is TS⁺ at 37° but still TS at 41°, was crossed with a 10⁻*ts*10.1 phage, and a fully TS⁺ recombinant that retains the CS phenotype was recovered.

tive mutants by reversion. Of 13 temperature-sensitive mutants examined, four yielded cold-sensitive mutants by reversion. Of three amber mutants examined, three yielded cold-sensitive or temperature-sensitive mutants by reversion. We conclude that *the probability is high that a missense mutant can yield new TS or CS mutants by reversion.*

Some of the New Mutations Are Suppressors. At least seven of the mutants in our survey yielded revertants with new mutations at genetic sites other than the parental sites. These revertants therefore carry suppressors. After separating some of these suppressors from their parental mutations by recombination, we examined the backcrossed phages for growth at various temperatures (Table 2). Clearly, the mutations confer their temperature-sensitive or cold-sensitive phenotypes in wild-type backgrounds, although some of them grow at greater extremes of temperature than with the parental mutations present. We conclude that in each case the mutant phenotype does not depend upon the presence of the parental mutation, and in this respect *the mutants behave like standard temperature-sensitive and cold-sensitive mutants.*

The Chain of Revertants. In several cases, a mutation that was generated by reversion was used as a parental mutation to generate further mutations. These mutations, therefore, comprise a *chain of revertants* in which each member is related by reversion to its predecessor. Three such chains are shown in Table 3. One of the chains contains only extragenic revertants, one contains only intragenic revertants, and one contains both. They illustrate clearly that several conditional-lethal mutations can be generated from a single missense mutant by sequential rounds of reversion.

Table 3. Three chains of revertants

1⁻*am*H58 → 5⁻*ts*rH58G → 5⁻*cs*rrH58G1 → 5⁻TSr(*cs*rrH58G1)A

1⁻*cs*RH21D → 1⁻*ts*rRH21D3 → 1⁻*cs*rrRH21D3A → 1⁻TSr(*cs*rrRH21D3A)1

1⁻*cs*H137 → 5⁻*ts*rH137C → 12⁻*cs*rrH137C19

DISCUSSION

The analysis of suppressors—mutations that can remedy the defects caused by other mutations—has been of value in the study of protein interaction, protein synthesis, and gene control (8, 14). However, a severe liability to the general use of suppressor analysis has derived from the fact that a suppressor typically does not have a phenotype in its own right. A suppressor whose only phenotype is an effect on another mutation's phenotype can only be observed in the presence of that other mutation, and so the examination of the suppressor itself is difficult. In the experiments reported in this paper, we have confined our attention to phage that have acquired, in the process of reversion, *new mutations that restore growth at one temperature and are lethal at another.* The conditional-lethal phenotypes of the new mutations, in turn, allowed us to show by standard genetic methods that in many instances the new mutation is in a gene other than the one in which the original mutation lies. Having confined our attention to conditional-lethal mutations, we are in a position to take advantage of their additional virtues: they can be crossed into diverse genetic backgrounds at will, they can be used in temperature-shift experiments *in vivo* and *in vitro* (15), and they can be used in schemes of enzyme or protein-complex purification (16, 17).

What is the probability that a revertant has a new phenotype?

Of nine CS mutants examined, eight yielded temperature-sensitive revertants, whereas only four of 13 TS mutants tested yielded cold-sensitive revertants (Table 1). In addition, we had to examine only a few CS⁺ revertants of each cold-sensitive mutant in order to obtain temperature-sensitives, but we had to examine many TS⁺ revertants of each temperature-sensitive mutant in order to find any cold-sensitives at all. If we pool the data for all nine CS mutants, we find that they yielded about one temperature-sensitive revertant per 20 total revertants (nine CS⁺TS revertants out of 183 CS⁺). A pooling of the data for the 13 TS mutants shows about one cold-sensitive revertant per 100 total revertants (six TS⁺CS out of 790 TS⁺). As shown by Tables 1 and 2, most of the revertants with new phenotypes carry suppressors. It must be stressed that these frequencies were obtained by examining independently arising revertants. Screening many revertants from a single mutant stock has not, in general, been so successful, probably because most of the revertants in each stock have a common ancestor (18).

We do not know why CS mutants revert to temperature-sensitivity more readily than TS mutants revert to cold-sen-

sitivity. It might simply reflect a relative rarity of genetic sites that can mutate to produce cold-sensitivity. Or it might indicate an intrinsic difference in the structural nature of cold-sensitive and temperature-sensitive mutant proteins. For example, temperature-sensitive proteins might typically be in disordered conformations at nonpermissive temperature, whereas cold-sensitive proteins might typically be in fixed, but inactive, conformations (19, 20). The structure at nonpermissive temperature, in turn, ought to influence the kinds of second-site mutations that could restore function (21).

How do our suppressors work?

We do not yet know how any of our suppressors restores growth. It is worth noting, however, that for all but one of our extragenic revertants, the suppressor is in a gene whose product is known to interact physically with the parental gene product (13). It is therefore an attractive possibility for the mechanism of suppression that the parental mutation and the suppressor mutation alter sites of mutual protein/protein interaction. By this hypothesis, the parental mutation destroys or distorts an interaction, and the suppressor produces a *compensating alteration* that restores the interaction. An intragenic suppressor produces a compensating alteration in the parental protein itself (9); an extragenic suppressor produces a compensating alteration in another protein that is in physical contact with the parental protein (22, 23). The hypothesis that the functional defect in a mutant protein can be corrected by physical interaction with another mutant protein finds support in the observation that antibodies can restore activity to some mutant enzymes *in vitro* (24), and in the many demonstrations that subunit interaction can restore activity lost by mutation in multimeric enzymes (25, 26).

Three of our parental mutants—1$^-$*cs*H137, 1$^-$*am*H58, and 8$^-$*am*N26—give rise to suppressors in gene 5. How might we interpret this in terms of the protein interaction hypothesis? The product of gene 5 (designated P5) is the major structural protein of the phage capsid, and the product of gene 1 (P1) is a structural protein that physically associates with the capsid (13). A suitable alteration in the structure of P5 might restore an interaction between P5 and P1 that was lost to the parental 1$^-$ mutant. Likewise, P5 and P8 interact, and so an alteration in P5 might restore a P5/P8 interaction that was lost to the parental 8$^-$ mutant. Thus, the parental/suppressor pairs that we obtained may indicate sites relevant to molecular interaction between P5 and P1 and between P5 and P8.

General advantages of experiments such as ours

By reversion of existing missense mutants, we have acquired many new *cs* and *ts* mutations in phage P22. These mutations should be of use in our ongoing study of P22 structure and function, and their further analysis may tell us much about the protein/protein interactions that occur during P22 morphogenesis. But here we wish to emphasize some ways in which experiments like ours might be useful in studying *any* organism.

1. Reversion Generates Mutations Suitable for Temporal Sequencing. We recently described a generally applicable method for determining the temporal order of events in a biological pathway (4). This method requires both *ts* and *cs* mutations in the genes of interest. The generation of both *ts* and *cs* mutations that affect related functions is intrinsic to the reversion system described in this paper, and so reversion is excellently suited for the acquisition of mutations for use in temporal sequencing.

2. Reversion Does Not Entail Mutagenesis. A hazard of mutagenic treatment is the possible occurrence of double mutants, and the more severe the mutagenesis the greater the risk. Since only revertant individuals will grow in nonpermissive conditions, reversion of conditional-lethal mutants is selective by nature, and so one can use reversion to isolate new mutations without the use of mutagenesis.

3. Reversion Is Function-Specific and Might Be Used to Advantage in Complex Organisms. Reversion by suppression is intrinsically function-specific, since the new mutation must remedy a very particular defect caused by the parental mutation. Even in a complex organism, the number of essential genes that can mutate to suppress any particular mutant phenotype is surely small—much smaller than the total number of essential genes. We therefore believe that the isolation of conditional-lethal suppressors could, in some cases, allow one to zero in on small subsets of essential genes (some of whose products are physically or functionally related) in organisms such as yeast, nematodes, and *Drosophila*. [We do not expect that *every* suppressor would affect a protein that is intimately related to the parental gene product (8), but we do suggest that a substantial number would.] Finally, suppressor analysis ought to be especially useful for investigating proteins that engage in strong noncovalent interactions with one another. These include not only the structural proteins (such as those in phage particles), but also the many enzymes that function as members of multi-protein complexes (27, 28).

We thank Larry Soll, Jonathan King, Van Jarvik, Ken Lew, Miriam Susskind, and George Weinstock for useful discussions. This work was supported in part by Grants VC18B and VC18C from the American Cancer Society and in part by Grant R01-GM21253-01 from the National Institutes of Health.

1. Horowitz, N. & Leupold, U. (1951) *Cold Spring Harbor Symp. Quant. Biol.* **16**, 65–72.
2. Campbell, A. (1961) *Virology* **14**, 22–32.
3. Edgar, R. & Lielausis, I. (1964) *Genetics* **49**, 649–662.
4. Jarvik, J. & Botstein, D. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2046–2050.
5. Hartwell, L., Culotti, J., Pringle, J. & Reid, B. (1974) *Science* **183**, 46–51.
6. Suzuki, D. (1970) *Science* **170**, 695–706.
7. Brenner, S. (1974) *Nature* **248**, 785–787.
8. Hartman, P. & Roth, J. (1973) *Adv. Genet.* **17**, 1–105.
9. Yanofsky, C., Ito, J. & Horn, V. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31**, 151–162.
10. Botstein, D., Chan, R. & Waddell, C. (1972) *Virology* **49**, 268–282.
11. Chan, R. & Botstein, D. (1972) *Virology* **49**, 257–267.
12. Chan, R., Botstein, D., Watanabe, T. & Ogata, Y. (1972) *Virology* **50**, 883–898.
13. King, J., Lenk, E. & Botstein, D. (1973) *J. Mol. Biol.* **80**, 697–731.
14. Floor, E. (1970) *J. Mol. Biol.* **47**, 293–306.
15. Luftig, R. & Lundh, N. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1636–1640.
16. Gefter, M., Hirota, Y., Kornberg, T., Wechsler, J. & Barnoux, C. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3150–3153.
17. Wright, M., Wickner, S. & Hurwitz, J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3120–3124.
18. Luria, S. & Delbruck, M. (1943) *Genetics* **28**, 491–511.
19. Tai, P., Kessler, D. & Ingraham, J. (1969) *J. Bacteriol.* **97**, 1198–1134.

20. Guthrie, C., Nashimoto, H. & Nomura, M. (1969) *Proc. Nat. Acad. Sci. USA* **63**, 384–391.
21. Cox, J. & Strack, H. (1971) *Genetics* **67**, 5–17.
22. Aperion, D. & Schlessinger, D. (1967) *J. Bacteriol.* **94**, 1275–1276.
23. Tomizowa, J. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.), pp. 549–552.
24. Rotman, B. & Celada, F. (1968) *Proc. Nat. Acad. Sci. USA* **60**, 660–667.
25. Fincham, J. (1966) *Genetic Complementation* (W. A. Benjamin, New York).
26. Crawford, I., Sikes, S., Belser, N. & Martinez, L. (1970) *Genetics* **65**, 201–211.
27. Case, M. & Giles, N. (1974) *Genetics* **77**, 613–626.
28. Jaenicke, R. & Helmreich, E. (eds.) (1972) *Protein–Protein Interactions* (Springer-Verlag, New York).