A Genetic Method for Determining the Order of Events in a Biological Pathway

Jonathan Jarvik; David Botstein

Proceedings of the National Academy of Sciences of the United States of America, Vol. 70, No. 7. (Jul., 1973), pp. 2046-2050.

Stable URL:
http://links.jstor.org/sici?sici=0027-8424%28197307%2970%3A7%3C2046%3AAGMFDT%3E2.0.CO%3B2-U

Proceedings of the National Academy of Sciences of the United States of America is currently published by National Academy of Sciences.

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at http://www.jstor.org/about/terms.html. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at http://www.jstor.org/journals/nas.html.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is an independent not-for-profit organization dedicated to creating and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact support@jstor.org.

http://www.jstor.org/
Mon Mar 13 09:26:49 2006

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 7, pp. 2046–2050, July 1973

A Genetic Method for Determining the Order of Events in a Biological Pathway

(cold-sensitive mutants/temperature-sensitive mutants/phage P22/morphogenesis/temporal sequence)

JONATHAN JARVIK AND DAVID BOTSTEIN

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

Communicated by Boris Magasanik, April 16, 1973

ABSTRACT **We describe the application of a simple genetic test that indicates the order of events in a morphogenic or biochemical pathway. The test uses temperature-sensitive (*ts*) and cold-sensitive (*cs*) mutants, and it orders the times at which the *ts* and *cs* defects are expressed.**

During the development of any organism, many events occur as parts of pathways in which intermediates are processed in a defined sequence. If, in a temperature-sensitive (*ts*) or cold-sensitive (*cs*) mutant, a step in such a pathway fails to occur at nonpermissive temperature, an intermediate preceding the step may accumulate. If the block is relieved by shift to a permissive temperature, the intermediate may be converted to the product of the pathway. Let us consider a double mutant that contains both a *ts* and a *cs* mutation. If the *ts* and *cs* lesions affect the same pathway, the pathway will fail to function at either the high nonpermissive temperature or the low nonpermissive temperature. But if the *cs* and *ts* events must occur in a particular order, a shift from low nonpermissive temperature (e.g., 20°) to high nonpermissive temperature (e.g., 40°) should have a very different outcome than a shift from 40° to 20°. Specifically, (*A* in Table 1) if the *cs* step precedes the *ts* step, then a shift from 20° to 40° will not result in completion of the pathway since the *ts* block ($I_2 \xrightarrow{ts} P$) will be imposed as the *cs* block ($I_1 \xrightarrow{cs} I_2$) is relieved. A shift from 40° to 20°, on the other hand, ought to result in successful completion of the pathway, since imposition of the *cs* block as the *ts* block is relieved will not prevent accumulated intermediate (I_2) from being converted to product. Therefore, by performing temperature shifts with appropriate *cs-ts* double mutants, we can determine the order in which functions rendered *cs* or *ts* are required.

Temperature-sensitive mutations can occur in many, if not all, of the genes of a given organism (1–4). Many *ts* proteins lose activity when shifted from permissive to nonpermissive temperatures, and many acquire activity when shifted from nonpermissive to permissive temperatures (5–7). Cold-sensitive mutants have been isolated in several organisms (8–12). Like *ts* mutations, *cs* mutations can occur in many genes.

The experiments we report here use *cs* and *ts* mutations that block the pathway of bacteriophage P22 morphogenesis. They allow the construction of an *order-of-function map* that reflects the temporal sequence in which the *cs* and *ts* defects are expressed in the phage assembly pathway. The success of these experiments leads us to believe that similar experiments can be useful in analyzing development in any biological system in which temperature mutants are available and construction of recombinants is possible.

Abbreviations: *ts*, temperature sensitive; *cs*, cold sensitive; TSS and CSS, temperature- and cold-sensitive synthesis, respectively; TL and CL, temperature and cold labile, respectively.

MATERIALS AND METHODS

Media, Bacteria, and Phage. LB broth, nutrient agar, λ plates, and buffered saline have been described (13). Bacterial strains are derivatives of *Salmonella typhimurium* LT-2. DB53 and its amber suppressor derivative DB74 have been described (13). The following mutants of phage P22 have been described (15): c_1^7, 13⁻*am*H101 (in our notation, the gene assignment precedes the allele designation; 13⁻*am*H101 means that *am*H101 is an allele of gene 13), 1⁻*am*H21, 8⁻-*am*N15, 1⁻*ts*1.1, 2⁻*ts*2.1, 3⁻*ts*3.1, 12⁻*ts*12.1, 5⁻*ts*26.1, and 9⁻*ts*848. 8⁻*ts*RN15 was isolated as an am^+ revertant of *am*N15. 3⁻*ts*RH73 was isolated as a ug^+ revertant of *ug*H7 (16). 1⁻*cs*RH21 was isolated as an am^+ revertant of *am*H21.

Isolation of cs Mutants. 30 *cs* mutants, of which 2⁻*cs*H22, 2⁻*cs*H59, 2⁻*cs*H118, 8⁻*cs*H137, and 1⁻*cs*H139 were used in this study, were isolated as follows. Wild-type P22 was mutagenized for 26 hr with hydroxylamine (17). Phage survival was 0.01%, of which 0.5% formed clear plaques. Surviving phages were plated on DB53 at 40°, and after 2 hr the plates were transferred to 20°. All plaques that were small when the bacterial lawn appeared and that remained small as the lawn developed were picked with sterile toothpicks and tested on DB53 for growth at 37° and for failure to grow at 20°. All *ts* strains grow at 20° and 30° but fail to grow at 40°. All *cs* strains grow at 40° and 30° but fail to grow at 20°.

Genetic Mapping and Complementation Tests. Map positions for the *am* and *ts* markers have been reported (15). *cs* markers were mapped by the methods of efficiency of plating and permissive rescue on prophage deletion strains (13).

Complementation tests results for the *am*, *ts*, and *ug* alleles have been reported (15). *cs* alleles were tested at 20° against amber alleles in known genes by the complementation in liquid culture method (15).

Construction of cs-ts Recombinants. Exponential DB74 cells (2×10^8 cells per ml) were infected with 5 phage per cell of each parent. Progeny were plated permissively. Individual plaques were picked with sterile toothpicks and the phage were tested for ability to grow at 20°, 30°, and 40°. Phage that could grow at 30° but not at 20° or 40° were purified

and checked by complementation spot test (15) for failure to complement the *cs* parent at 20° and the *ts* parent at 40°.

Lysis Inhibition. All phages used in this study carried the marker $c_1{}^7$, to ensure entry into the lytic cycle, and the marker 13^{-}*am*H101 (lysis inhibition), to prevent cell lysis in the nonpermissive host DB53 and to allow linear phage or phage precursor accumulation throughout the temperature-shift intervals (15). During a 13^{-} infection, phage accumulation proceeds with linear kinetics by 200 min at 20° and by 50 min at 40°. The infected cells lyse rapidly and completely upon vigorous treatment with chloroform.

Temperature Shift-Down Experiments. Exponential DB53 cells (2.5×10^8/ml) were infected with phage at a multiplicity of infection (MOI) of 5. After 65 min of incubation at 40°, temperature shifts were performed by diluting 1:100 into LB broth at 20°, 30°, and 40°. At indicated times after the shifts, aliquots of each culture were lysed by dilution into buffered saline containing chloroform, and phage present were assayed by plating on the amber-suppressor strain DB74 at 30°.

Temperature Shift-Up Experiments. Exponential DB53 cells were infected at an MOI of 5. After 220 min of incubation at 20° (which is comparable physiologically to 65 min at 40°), temperature shifts were performed by diluting 1:100 into LB broth at 20°, 30°, and 40°. At indicated times after the shifts, aliquots of each culture were lysed by dilution into buffered saline containing chloroform, and phage present were assayed by plating on DB74 at 30°.

Nomenclature: TSS, TL, CSS, and CL. We use the terminology of Sadler and Novick (5). If a *ts* gene product is synthesized at a permissive temperature and is subsequently shifted to a nonpermissive temperature, it may or may not be inactivated at the nonpermissive temperature. If it becomes inactive, it is called TL for "temperature labile." If it remains active, it is called TSS for "temperature-sensitive synthesis." We define CL and CSS analogously.

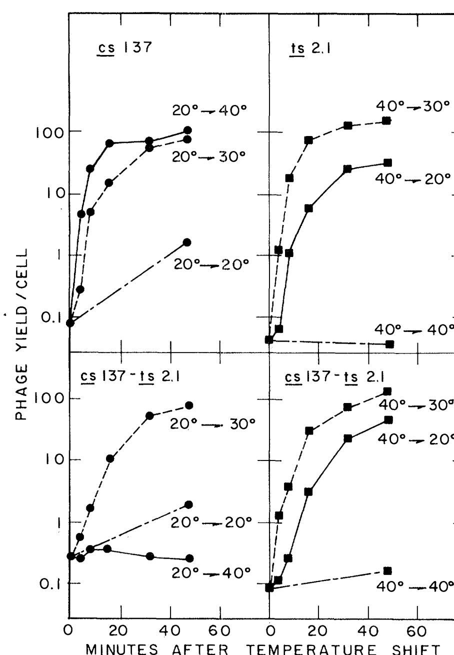


FIG. 1. Kinetics of phage accumulation after temperature shifts with *cs*137, *ts*2.1, and *cs*137-*ts*2.1. ●– — –●, 20° → 20°; ●- - -●, 20° → 30°; ●——●, 20° → 40°; ■– — –■, 40° → 40°; ■- - -■, 40° → 30°; ■——■, 40° → 20°.

We have also measured kinetics in *cs-ts* tests with *cs*137-*ts*1.1, *cs*137-*ts*3.1, *cs*59-*ts*2.1, *cs*59-*ts*12.1, and *cs*22-*ts*1.1. In each test, as in the figure above, the *cs-ts* double mutant behaved like its *cs* or *ts* parent for every shift except the 20° to 40° or 40° to 20° shift in which the *cs-ts* failed to yield phage.

RESULTS AND DISCUSSION

30 *cs-ts* double-mutant phage strains were constructed and tested in temperature-shift experiments. Each *cs-ts* infection was examined for phage yield after shifts from 20° to 30°, 20° to 40°, 40° to 30°, and 40° to 20°. Infections with all *cs* and *ts* single mutants were similarly examined. The results of a typical test are displayed in Table 2 and Fig. 1. In this example, *cs*137 produced substantial yields of phage after shifts from 20° to 30° and from 20° to 40°, and *ts*2.1 gave large yields after shifts from 40° to 30° and from 40° to 20°. Similar temperature-shift results were obtained with the double mutant *cs*137-*ts*2.1 with one significant exception: the shift from 20° to 40° was not productive. By the reasoning presented in the introduction, it appears that cs^{+} function is required before ts^{+} function.

The results of temperature-shift tests with all 30 *cs-ts* phage strains are shown in Table 3. 19 of the 30 gave results like those for *cs*137-*ts*2.1, in that both shifts to 30° were productive, but only one shift from one nonpermissive temperature to the other was productive. These 19 are grouped together in the table, and for each we have assigned a temporal order to the *cs* and *ts* defects. Eight of the 30 produced phage after both shifts to 30°, but they did not produce phage after either shift from one nonpermissive temperature to the other. One of the 30 gave phage after all shifts, and two failed to produce phage after one of the shifts to 30°. In all cases, infections with parental *cs* and *ts* single-mutant phages were productive after all shifts from nonpermissive to permissive temperatures.

TABLE 1. *Predicted results of temperature shifts for pathways with reversible cs and ts steps*

		20° → 20°	20° → 30°	20° → 40°	40° → 40°	40° → 30°	40° → 20°
(A)	$I_1 \xrightarrow{cs} I_2 \xrightarrow{ts} P$	-	+	-	-	+	+
(B)	$I_1 \xrightarrow{ts} I_2 \xrightarrow{cs} P$	-	+	+	-	+	-

I_1 and I_2 represent intermediates on a pathway with product, P. A plus means that product is made after the indicated shift; a minus means that it is not made.

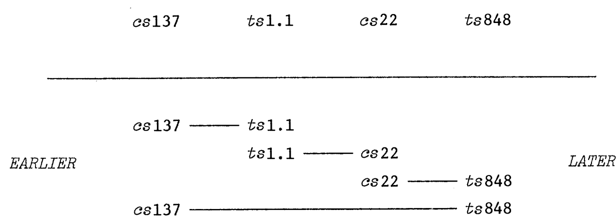


FIG. 2. Order-of-function map for *ts*1.1, *cs*22, *cs*137, and *ts*848. The order-of-function map is the *upper line* of the figure. The map is based on the four individual experiments whose outcomes are shown in the *lower part* of the figure.

The Execution Point. In order to interpret our experimental results, it is important to make the distinction between the *step* in a pathway that fails to occur in the presence of a *cs* or *ts* mutation and the *execution point* of the mutation. By a *cs* or *ts* step, we mean a point in the pathway at which the presence of wild-type function is required. The execution point is defined operationally; it is the latest time at which shift to nonpermissive temperature will produce the mutant phenotype. The execution point, therefore, indicates the end of the mutation's temperature-susceptible period (18). For a given mutation, the *cs* or *ts* step and the *cs* or *ts* execution point need not coincide. For example, if a *ts* enzyme is temperature sensitive at synthesis (TSS), its execution point is at the time of its synthesis, since shift to high temperature after that time will not produce the mutant phenotype. On the other hand, if the enzyme is temperature labile (TL), its execution point is at the time of its catalytic function. Thus for the TL enzyme the execution point and the *ts* step coincide, but for the TSS enzyme the execution point precedes the *ts* step. Similarly, if a *ts* protein is stabilized against heat inactivation by association with a structure, its execution point is at the time that the association is established.

Criteria for Inferring Temporal Order. Table 4 shows four basic patterns of temperature-shift behavior that *cs-ts* double mutants can generate in our experiments. For a given *cs-ts* double mutant, if the *cs* and *ts* steps are reversible upon temperature shifts and if the *cs* and *ts* steps must occur in a particular order, then results like those in *A* or *B* of Table 4 are expected. (See also *Introduction* and Table 1.) What should be expected if the mutant gene-products do not simply gain or lose activity as the temperature is shifted, or if the *cs* and *ts* events must not, in fact, occur in sequence? As the following analysis indicates, even where gene-products are not simply temperature-reversible, it is still possible to deduce the order of the *cs* and *ts execution points* in some cases. Even more important is the observation that, *whenever only one of the two shifts from one nonpermissive temperature to the other yields product, we can correctly infer the order of the mutant execution points.*

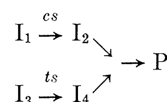
For this analysis, we shall assume that the mutants are *cs* or *ts* as a result of altered protein structure, although this need not be true for all temperature mutants and although our conclusions concerning order of function do not depend upon this assumption. We shall also assume that the *cs* and *ts* proteins are synthesized continuously, rather than sequentially, during the intervals in which temperature shifts are performed. This is, in fact, the case for the P22 "late" proteins with which our experiments are primarily concerned. We consider several cases:

(*i*) Suppose we have the pathway $I_1 \xrightarrow{cs(CL)} I_2 \xrightarrow{ts(TSS)} P$, in which *cs* protein is cold labile (CL) and the *ts* protein is temperature sensitive at synthesis (TSS). In such a situation, the *ts* execution point precedes the *cs* execution point. During incubation at 40° I_2 accumulates; shift to 20° results in formation of product (P), since I_2 is converted to P. If incubation is first done at 20°, I_1 accumulates; shift to 40° results in product formation, since the *ts* execution point has been passed. The pattern obtained is that of *D* in Table 4.

(*ii*) Suppose we have the pathway $I_1 \xrightarrow{cs(CSS)} I_2 \xrightarrow{ts(TL)} P$. Here *cs* execution point precedes the *ts* execution point. During incubation at 40° I_2 accumulates; shift to 20° results in formation of product, since I_2 is converted to P. If incubation is first done at 20°, I_1 accumulates; shift to 40° does not result in product formation, since I_2 cannot be converted to product at 40°. The pattern obtained is that of *A* in Table 4.

(*iii*) Suppose we have the pathway $I_1 \xrightarrow[ts(TL)]{cs(CL)} P$, in which the same step fails to occur at both 20° and 40°. Here the *cs* and *ts* execution points are the same or are simultaneous. Neither the shift from 40° to 20° nor the shift from 20° to 40° is productive. The pattern is that of *C* in Table 4. If the *cs* is CSS, $I_1 \xrightarrow[ts(TL)]{cs(CSS)} P$, then the *cs* execution point precedes the *ts* execution point. Shift from 40° to 20° is productive, since the *ts* block is relieved and the *cs* execution point has been passed. The pattern obtained is that of *A* in Table 4.

(*iv*) Suppose the *cs* and *ts* steps are not in the same pathway.



Here there is not a necessary order for the *cs* and *ts* execution points. Shifts from 20° to 40° and from 40° to 20° are productive. The pattern obtained is that of *D* in Table 4.

(*v*) Suppose we have the pathway $I_1 \xrightarrow{cs(CL)} I_2 \xrightarrow{ts(TL)} P$ (with $I_2 \nearrow X$) in which, in the absence of the *ts* step, intermediate I_2 is irreversably converted to a nonviable form, X. Here the *cs* execution point precedes the *ts* execution point. Shift from 40° to 20° does not yield product, since X cannot be converted to

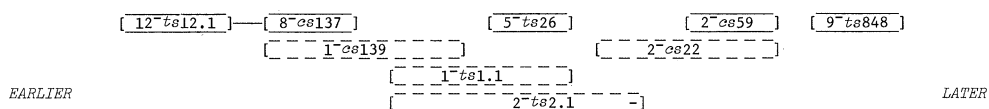


FIG. 3. Composite order-of-function map constructed from the data in Table 3. The map includes the gene assignment for each allele. Alleles that have not been tested in many *cs-ts* combinations (e.g., 3^{-}*ts*R73) are not included in this map.

P and since the *cs* block is imposed as the *ts* block is relieved. Shift from 20° to 40° also does not yield product, since the *ts* block is imposed as the *cs* block is relieved. The pattern obtained is that of *C* in Table 4. If the *ts* is *TSS*, $I_1 \xrightarrow{cs^{(CL)}} X \nearrow$, $I_2 \xrightarrow{ts^{(TSS)}} P$, then the *ts* execution point precedes the *cs* execution point. During incubation at 40°, X accumulates; shift to 20° does not yield product because the *cs* block is imposed and because *X* cannot be converted to P. Incubation at 20° followed by shift to 40° does result in product formation, since the *ts* execution point has been passed. The pattern obtained is that of *B* in Table 4.

From these examples a generalization emerges: Patterns like those in *A* or *B* of Table 4 indicate the order of the *cs* and *ts* execution points, whereas patterns like *C* and *D* do not, by themselves, indicate the order. Note that in the above examples we have made the simplifying assumption that a temperature-labile or cold-labile protein makes the active/inactive transition relatively rapidly upon shift to nonpermissive temperature. If, instead, the transition is relatively sluggish, then the protein takes on the character of a TSS or CSS.

In our experiments, then, we feel justified in inferring temporal order for a given *cs* and *ts* whenever the following conditions hold:

(*i*) All shifts of the *cs*, *ts* and *cs-ts* mutants from nonpermissive temperatures to 30° are productive. These controls tell us that incubation at nonpermissive temperature does not destroy the potential of the infected cell to produce phage after shift to permissive temperature.

(*ii*) The shift from 40° to 20° is productive for the *ts* mutant alone. This control tells us that any failure to get phage when the *cs-ts* double mutant is shifted from 40° to 20° is not due to the *ts* allele, and is therefore due to the additional presence of the *cs* allele.

(*iii*) The shift from 20° to 40° is productive for the *cs* mutant alone. This control tells us that any failure to get phage when the *cs-ts* double mutant is shifted from 20° to 40° is not due to the *cs* allele, and is therefore due to the additional presence of the *ts* allele.

(*iv*) With the *cs-ts* double mutant, either the shift from 40° to 20° is productive and the shift from 20° to 40° is not, in which case we conclude that *cs* precedes *ts*, or the shift from 20° to 40° is productive and the shift from 40° to 20° is not, in which case we conclude that *ts* precedes *cs*.

TABLE 2. *Phage yields after temperature shifts with cs137, ts2.1, and cs137-ts2.1*

	20°→ 20°	20°→ 30°	20° → 30°
cs137	1.5	72	98
ts2.1	-	-	-
cs137-ts2.1	1.7	74	0.2
	40°→ 40°	**40°→ 30°**	**40°→ 20°**
cs137	-	-	-
ts2.1	0.1	198	53
cs137-ts2.1	0.2	129	49

Table shows phage/cell 48 min after each temperature shift.

TABLE 3. *Phage yields after temperature shifts with all cs, ts, and cs-ts phages*

	20°→ 20°	20°→ 30°	20°→ 40°	40°→ 40°	40°→ 30°	40°→ 20°	Temporal order
cs22	0.5	181	208				
cs59	0.3	321	278				
cs118	0.3	129	305				
cs137	1.5	72	98				
cs139	0.5	156	150				
csRH21	4.0	166	35				
ts1.1				0.3	152	50	
ts2.1				<0.1	198	53	
ts3.1				<0.1	138	122	
ts5.1				<0.1	255	180	
ts10.1				<0.1	139	124	
ts12.1				<0.1	510	300	
ts26				1.0	500	112	
tsR73				0.8	268	40	
ts848				0.8	460	355	
wild-type	194	340	565	910	960	350	
cs22-ts1.1	0.3	168	125	1.1	48	1.7	ts-cs
cs22-ts26	0.3	392	165	0.6	310	0.6	ts-cs
cs22-ts848	0.1	85	2	0.6	154	112	cs-ts
cs59-ts2.1	0.9	225	34	0.1	84	0.1	ts-cs
cs59-ts12.1	0.7	194	141	0.1	260	0.1	ts-cs
cs59-tsR73	0.3	327	38	0.6	155	2.0	ts-cs
cs59-ts848	3.0	222	10	0.8	196	106	cs-ts
cs137-ts1.1	3.2	330	3.6	1.6	185	31	cs-ts
cs137-ts2.1	1.7	74	0.2	0.2	129	49	cs-ts
cs137-ts3.1	<0.1	65	0.1	<0.1	370	144	cs-ts
cs137-ts12.1	<0.1	87	44	<0.1	38	0.4	ts-cs
cs137-ts26	3.7	492	0.3	0.1	745	59	cs-ts
cs137-ts848	0.1	115	2.3	1.1	101	61	cs-ts
cs139-ts3.1	2.0	32	2.5	2.5	214	76	cs-ts
cs139-ts12.1	0.1	97	38	<0.1	22	0.1	ts-cs
cs139-ts26	0.3	226	4.6	3.5	129	44	cs-ts
cs139-ts848	7.5	234	9.2	<0.1	170	170	cs-ts
csRH21-ts848	3.4	99	2.2	0.7	67	46	cs-ts
cs59-ts26	0.1	570	25	0.1	378	0.1	ts-cs
cs22-ts2.1	0.8	265	2.4	0.1	135	2.4	
cs118-ts5.1	1.6	59	1.3	1.2	231	1.9	
csRH21-ts2.1	1.1	28	0.8	1.5	29	1.0	
csRH21-ts26	1.7	35	1.1	1.1	94	1.3	
csRH21-ts10.1	3.2	72	2.4	1.5	62	2.2	
cs139-ts2.1	1.7	73	1.4	2.1	38	1.3	
cs139-tsR73	0.3	82	3.1	0.4	65	0.5	
cs139-ts1.1	0.2	105	6.1	0.2	26	0.4	
cs137-ts10.1	1.6	121	6.9	0.1	5	2.3	
csRH21-ts3.1	0.3	8	0.4	0.3	27	3.8	
cs22-ts10.1	0.5	200	31	3.5	165	26	

No. of phage per cell was measured 64 min after temperature shift for all experiments except the following: *cs*22, *ts*1.1, and *cs*22-*ts*1.1 infections were assayed 36 min after temperature shifts; *cs*59, *ts*2.1, *cs*137, *cs*59-*ts*2.1, and *cs*137-*ts*2.1 were assayed 48 min after temperature shifts; all shifts from 40° with *ts*12.1 were assayed 200 min after temperature shift, since no late gene expression can occur until after the shift (15). We consider a yield of more than 10 phage per cell productive and a yield of 10 or fewer nonproductive.

The Order-of-Function Map. For 19 of 30 *cs-ts* double mutants tested, we obtained results that meet our criteria for inferring temporal order. Accordingly, we have assigned an order to the *cs* and *ts* execution points for each pair, and these assignments are included in Table 3. We have combined some of these orders to produce the *order-of-function map* presented in Fig. 2. Here each *cs-ts* pair is drawn with the earlier of the two on the left. At the top of the figure is the map itself; at the bottom are representations of the outcome of each *cs-ts* test used to construct the map. The order-of-

TABLE 4. *Four patterns of results for temperature shifts with cs-ts double mutants*

	20°→ 20°	20°→ 30°	20°→ 40°	40°→ 40°	40°→ 30°	40°→ 20°
(A)	-	+	+	-	+	-
(B)	-	+	-	-	+	+
(C)	-	+	-	-	+	-
(D)	-	+	+	-	+	+

function map represents, in graphic form, the order of the mutant execution points during P22 assembly.

Events in sequence obey the transitive rule: if B is later than A and C is later than B, then C is later than A. For example, the transitive rule requires that *cs*137 precede *ts*848 (see Fig. 2). A *cs-ts* test with the double mutant *cs*137-*ts*848 gave the required order, as the figure indicates.

Using the information in Table 3, we have constructed the composite order-of-function map shown in Fig. 3. Here, as in Fig. 2, alleles with an earlier–later relation defined by the *cs-ts* test are placed with the earlier of the two on the left. If the earlier–later relation for a pair is not known, they are placed so that they overlap. (Note that mutant alleles with simultaneous execution points are expected to overlap on the order-of-function map.) When the figure is constructed by these rules, some alleles occupy discrete positions and are shown in solid boxes (e.g., *ts*26); others overlap two or more nonoverlapping alleles and are shown in dotted boxes (e.g., *ts*1.1). The map proves to be linear, thereby conforming to the transitive rule. The composite order-of-function map summarizes our conclusions about the order in which gene-products function during P22 assembly.

In a few of our tests, mutations that are in the same gene, and thus affect the same protein, do not show coincident execution points (see 2^{-}*ts*2.1 and 2^{-}*cs*22). This result is expected of two mutations that alter the same protein but are expressed at different times in the protein's life. For instance, the major capsid protein must not only form the shell of the virion, but it must also interact with the tail structure. There is every reason to believe that these two activities must occur in a fixed order. Therefore, two mutations in the gene for the capsid protein might show a sequence with respect to each other. We believe that such considerations ought to be applied to any protein unless they are excluded by specific information.

Congruence of Our Conclusions with Those of Other Studies. In this paper we treat mutations as formal elements. However, let us briefly indicate the correlation between our conclusions and what is known about P22 assembly from other studies. The product of gene 12 is needed for P22 DNA synthesis, and DNA synthesis is required early in infection (20). Accordingly, we would expect gene 12 function to appear as an early event in our order-of-function map, and indeed it does. The product of gene 9 (designated P9) forms the phage tail, and purified tails can attach to purified tailless phage *in vitro;* hence addition of the tail is a terminal step in phage assembly (21). Accordingly, we expect at least some 9^{-} defects to appear as late events in the order-of-function map, and indeed the 9^{-}*ts* that was examined does so. The roles of the products of the other genes in which we have used temperature mutants are as follows: P5 is the major protein component of the phage head; P8 is required for production of a head precursor structure; P1, P2, and P3 are required for the encapsulation of DNA by the head precursor; and P10 is required soon after DNA encapsulation (22, 23). The order of function that we have derived from temperature shifts is evidently consistent with what is known from other investigations.

We have shown that *cs-ts* tests and complementation tests, considered together, indicate the order in which gene-products function in a morphogenic pathway. These tests do not require the experimenter to know the specific defects of the mutants used. Therefore they make a genetic analysis of development possible wherever temperature mutants are available and construction of recombinants is possible.

We thank Jon King, George Weinstock, Ira Herskowitz, and Harris Bernstein for useful discussion. This work was supported by grants from the American Cancer Society (VC-18A) and from the National Institute of General Medical Sciences (GM 18973). J.J. was supported by a National Defense Education Act title IV Graduate Fellowship; D.B. holds a Career Development Award (GM 70325) from the National Institute of General Medical Sciences.

1. Horowitz, N. H. & Leupold, U. (1951) *Cold Spring Harbor Symp. Quant. Biol.* **16**, 65–72.
2. Edgar, R. S. & Lielausis, I. (1964) *Genetics* **49**, 649–662.
3. Hartwell, L. H. (1967) *J. Bacteriol.* **93**, 1662–1670.
4. Suzuki, D. T. (1970) *Science* **170**, 695–706.
5. Sadler, J. R. & Novick, A. (1965) *J. Mol. Biol.* **12**, 305–327.
6. Karam, J. D. & Speyer, J. F. (1970) *Virology* **42**, 196–203.
7. Scotti, P. D. (1971) *Virology* **43**, 366–374.
8. O'Donovan, G. A. & Ingraham, J. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 451–457.
9. Tai, p., Kessler, D. P. & Ingraham, J. (1969) *J. Bacteriol.* **97**, 1298–1304.
10. Scotti, P. D. (1968) *Mutat. Res.* **6**, 1–14.
11. Cox, J. H. & Strack, H. B. (1970) *Genetics* **67**, 5–17.
12. Guthrie, C., Nashimoto, H. & Nomura, M. (1969) *Proc. Nat. Acad. Sci. USA* **63**, 384–391.
13. Chan, R. K. & Botstein D. (1972) *Virology* **49**, 257–267.
14. Levine, M. (1957) *Virology* **3**, 22–41.
15. Botstein, D., Chan, R. K. & Waddell C. H. (1972) *Virology* **49**, 268–282.
16. Lew, K. K. & Roth J. R. (1970) *Virology* **40**, 1059–1062.
17. Hall, D. H. & Tessman I. (1966) *Virology* **29**, 339–345.
18. Hartwell, L. H., Culotti, J. & Reid, B. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 352–359.
19. Benzer, S. (1955) *Proc. Nat. Acad. Sci. USA* **41**, 344–354.
20. Botstein, D. & Levine, M. (1968) *J. Mol. Biol.* **34**, 643–654.
21. Israel, J. V., Anderson, T. F. & Levine, M. (1967) *Proc. Nat. Acad. Sci. USA* **67**, 284–291.
22. Botstein, D., Waddell, C. H. & King, J. (1973) *J. Mol. Biol.*, in press.
23. King, J., Lenk, E. H. & Botstein, D. (1973) *J. Mol. Biol.*, in press.