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Genetic Mapping of the *tox-1000* Locus of *Vibrio cholerae* El Tor Strain RJ1

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The results of a genetic cross between a *Vibrio cholerae* RJ1 donor and a *V. cholerae* 3083-2 recipient suggest that the map position of *tox-1000* is between *his* and *trp*.

We previously reported that a gene affecting the antigenic structure of cholera toxin (CT) is likely to be located between the *met* and *trp* loci of the *Vibrio cholerae* linkage map (6). This gene was designated *vct*. As a result of mating experiments designed to map the position of *vct*, it was observed that a wild-type gene designated *tox-1000* cotransferred with *trp* from the El Tor biotype strain RJ1 to the El Tor biotype strain 3083-2 at a frequency of approximately 60%. Strain RJ1 and recombinants with *tox-1000* were negative in the Elek test for CT (9), whereas strain 3083-2 and recombinants that expressed the wild-type gene *tox-2000* were positive in this test. It was suggested that *tox-1000* and *tox-2000* are alleles of the *tox-1* locus of the classical biotype strain 569B. The *tox-1* locus was reported to cotransfer with the *his-1* and *trp-1* loci of 569B at frequencies of approximately 3% and less than 1%, respectively, in standard P-factor crosses (1). However, the data in our previous report were not adequate for positioning *tox-1000* relative to *trp* or *his* on the *V. cholerae* genetic map. Since *vct* is apparently located between *met* and *trp* on the linkage map (6), and the *vct-1* and *vct-2* alleles seem to correlate with specific CT structural gene arrangements (D. W. Saunders, G. J. Kubala, A. B. Vaidya, and M. G. Bramucci, manuscript in preparation), the map position of *tox-1000* was examined in more detail.

The transposon-facilitated recombination system of Johnson and Romig (4) was used in conjunction with an RJ1 donor strain and a 3083-2 recipient strain to map the position of *tox-1000*. The procedural details of the mating experiments have been previously described (6). Strain MB2020(pSJ13) (RJ1::Tn*1*-24, *vct-1*, *tox-1000*, and *rif-5001*) was used as the donor strain. The plasmid pSJ13 has a Tn*1* insertion in the (−) orientation (4). The recipient strain MB1823 (*vct-2*, *tox-2000*, *str-5000*, *met-5000*, *trp-5000*, and *his-5002*) was derived from the previously described strain MB1813 by mutagenesis with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (6). Recombinants were tested for expression of *tox-1000* or *tox-2000* with a variation (3) of the radial passive immune hemolysis assay for CT (2). Strain RJ1 and recombinants with the *tox-1000* allele were negative in this assay, whereas strain 3083-2 and recombinants with the *tox-2000* allele were positive.

The data in Table 1 indicate that when Met$^+$ was the selected phenotype, cotransfer of *trp* or *his* with *met* did not occur at a significant frequency. When either Trp$^+$ or His$^+$ was the selected phenotype, cotransfer of *met* with *trp* or *his* occurred infrequently or not at all. Therefore, recombination analysis could not be used to order *met*, *trp*, and *his* relative to each other. However, the frequency of marker transfer data in Table 1 suggests that the order of the loci relative to the origin of transfer was *met-trp-his*, which was consistent with the map order determined by other authors for phenotypically similar markers (5, 7). When Trp$^+$ was the selected phenotype, *his* and *tox-1000* cotransferred with *trp* at approximately equal frequencies. This observation was consistent with *trp* being either between *his* and *tox-1000* or *trp* having an external position approximately equidistant from *his* and *tox-1000*. When His$^+$ was the selected phenotype, the *tox-1000* locus cotransferred with *his* at a high frequency (approximately 83%), whereas *trp* cotransferred with *his* at a lower frequency (approximately 63%). Since *his* and *tox-1000* cotransferred at a high frequency and both loci appeared to be less closely linked to *trp*, *trp* seemed most likely to be an external locus in this group of genes. This conclusion was consistent with the linkage relationship of phenotypically similar markers previously reported by Baine et al. (1).

Since *trp* was most likely proximal to the origin of transfer, *trp* could not be used as a selected marker in a three-factor recombination-

TABLE 1. Linkage of *his* to *trp* and *met* by two-factor crosses

Selected phenotype	Expt	Frequency of transfer[a]	Total no.	No. with nonselected marker (% of total)						
				Met+	Trp+	His+	Met+ Trp+	Met+ His+	His+ Trp+	Tox−[b]
Met+	1	1.03×10^{-6}	158	—[c]	0	0	—	—	0	NT[d]
	2	2.0×10^{-6}	251	—	0	0	—	—	1 (0.4)	NT
Trp+	1	2.12×10^{-7}	147	0	—	84 (57.1)	—	1 (0.7)	—	77 (52.4)
	2	4.31×10^{-7}	318	2 (0.6)	—	151 (47.5)	—	3 (0.9)	—	154 (48.4)
His+	1	1.46×10^{-7}	114	0	73 (64.0)	—	0	—	—	94 (82.5)
	2	3.32×10^{-7}	237	0	150 (63.3)	—	3 (1.3)	—	—	201 (84.8)

[a] Recombination frequencies are the ratios of recombinants to the input number of donors.
[b] Phenotype of *tox-1000*.
[c] —, Selected marker.
[d] NT, Not tested.

al analysis, because linkage values would be distorted by accidental mating pair disruption before entry of the more distal markers. An alternative approach to mapping in this region of the chromosome would be to use MB2020(pSJ5) as the donor strain. The plasmid pSJ5 has a Tn*1* insertion in the orientation that is opposite to the Tn*1* insertion in pSJ13 (4) and should transfer *trp* as a distal marker and *his* as a proximal marker in MB2020. When MB2020(pSJ5) was mated with MB1823, *his* was transferred at a frequency approximately 20-fold lower than the frequency at which *met* was transferred (data not shown). Since it was expected that the transfer frequency of *his* would be higher than that of *met*, this observation suggested the possibility that an unidentified factor was interfering with the transfer of *his* as a proximal marker.

The data in Table 2 indicate the percentage of the His+ recombinants from Table 1 that could be assigned to each of the possible recombinant classes when *trp* and *tox* were scored as nonselected markers. Approximately 93% of the His+ Trp+ recombinants were Tox−. In contrast, only approximately 69% of the His+ Trp− recombinants were Tox−. Since *trp* seemed likely to have an external position and to influence acquisition of the Tox− phenotype by the recipient when His+ was the selected phenotype, the data in Table 2 are consistent with the His+ Trp+ Tox+ recombinant class resulting from a double crossover and from the order of the three genes being *his-tox-1000-trp*.

TABLE 2. Trp and Tox phenotypes of His+ recombinants[a]

Phenotype of recombinants	No.	% of total
His+ Trp+ Tox+	16	4.6
His+ Trp+ Tox−	207	59.0
His+ Trp− Tox+	40	11.4
His+ Trp− Tox−	88	25.1

[a] Combined data from experiments 1 and 2 in Table 1.

The mechanism by which the net level of CT production is affected by *tox-1000* and *tox-2000* is not known. However, the *tox-1000* allele is apparently an important determinant of the amount of CT produced by RJ1, since introduction of *tox-1000* into strain 3083-2 reduces the normally high level of CT produced by this strain to levels that are no longer detectable in the relatively sensitive radial passive immune hemolysis assay. Our data suggest that a possible location of CT structural gene information (i.e., the *vct* locus) is separated from a potentially important regulatory locus (i.e., *tox*) by at least one gene that is not related to CT synthesis (i.e., *trp*). This suggestion implies that *tox* may code for a diffusable product that regulates CT synthesis. The phenotype associated with *tox-1000* is similar to the Tox− mutants of the *tox-1* locus of strain 569B (1) and the low-level allele of *tox-1* located in classical strain 162 (8). It is not known whether *tox-1000* and *tox-1* affect the same aspect of CT production. However, if *tox-1000* and *tox-1* are alleles, then the gene order of the *his* region of the El Tor biotype genetic map is inverted relative to the classical biotype genetic map since Baine et al. positioned *his-1* between *tox-1* and *trp-1* (1). A similar inversion of the El Tor genetic map relative to the classical map has been reported for the *ilv-lys* region of the chromosome (4).

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