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Conjugal Transfer of a Chromosomal Gene Determining Production of Enterotoxin in Vibrio cholerae

Abstract. *Matings between strains of* Vibrio cholerae *differing in toxinogenicity, nutritional requirements, and antibiotic susceptibilities were performed in order to determine the location of the gene* tox *that controls production of cholera enterotoxin. Segregation analysis shows that* tox *is linked to a gene required for histidine biosynthesis. Our data indicate that the* tox *gene is located on the bacterial chromosome and not on a plasmid in the strains of* V. cholerae *studied.*

Although it is clear that a protein enterotoxin (known as choleragen) is responsible for the diarrhea of cholera, little is known regarding the regulation of toxinogenesis in *Vibrio cholerae* (*1*). In some strains of *Escherichia coli* the gene *ent* that controls production of enterotoxin has been shown to be located on a plasmid (*2*). Because the heat-labile protein enterotoxins of *V. cholerae* and of *E. coli* are immunologically cross-reactive and have similar modes of action (*3*), it is of interest to determine whether or not the production of enterotoxin by *V. cholerae* is also regulated by plasmids. Although a mating system resembling the F-mediated system of *E. coli* has been described in *V. cholerae* (*4*), conjugal mating promoted by the vibrio sex factor P has not previously been used to study the genetics of toxinogenesis in *V. cholerae*. In the present study, a gene controlling the synthesis of cholera enterotoxin has been transferred by conjugation from a toxinogenic (*tox*+) strain to a nontoxinogenic (*tox*−) strain of *V. cholerae*. In addition, segregation analysis indicates that the *tox* gene is linked to a chromosomal gene that controls the biosynthesis of histidine.

Several strains of *V. cholerae* were used in this study. *Vibrio cholerae* 569B Inaba, the widely used and highly toxinogenic strain (*1*), was found to be a genetic recipient (P−). It was converted to a genetic donor (P+) by methods previously described by Parker and Romig (*5*). *Vibrio cholerae* RV-31 (P−) and RV-102 (P+) are strains of Ogawa serotype and were provided by W. R. Romig (Department of Bacteriology, University of California, Los Angeles). Strain 569B is prototrophic as well as toxinogenic (*1*), while strains RV-31 and RV-102 have auxotrophic and antibiotic resistance markers characterized in earlier studies (*6*). All pertinent characteristics of strains 569B and RV-31 are given in Table 1, and strain RV-102 is described below. Strains RV-31 and RV-102 were tested for production of enterotoxin both in vivo and in vitro by methods described previously (*7*). Strains RV-31 and RV-102 produced no detectable toxin in vitro and did not elicit secretory responses in ligated ileal loops of adult rabbits in multiple tests.

The conditions for mating experiments found to be optimal by Parker *et al.* (*8*) were used. Briefly, cultures of a prototrophic, *tox*+ donor and a multiply auxotrophic, *tox*− recipient were mixed to give a 1 : 10 ratio of donor to recipient cells. The mating mixtures were incubated at 37°C for 2 hours with gentle agitation. Washed and concentrated samples of the mating mixtures were mixed with molten soft agar and plated on appropriately supplemented minimal media in order to select for individual nutritional markers from the donor. The donor was counterselected by its susceptibility to streptomycin. The frequencies of cotransfer of all unselected markers were then determined in each class of recombinants. In order to determine requirements for amino acids, recombinants were transferred to separate plates lack-

Table 1. Segregation of toxinogenicity during recombination in *Vibrio cholerae*. Donor: 569B (P+) *arg*+ *his*+ *ilv*+ *str*^{s} *tox*+; recipient: RV-31 (P−) *arg*− *his*− *ilv*− *str*^{r} *tox*−. ND = not done.

Selected marker from donor*	Experiment number	Recombination frequency†	Number of recombinants tested	Number of selected recombinants with unselected marker from donor			
				tox+	*his*+	*ilv*+	*arg*+
his+	1	2.5×10^{-7}	23	4		4	20
	2	1.1×10^{-6}	100	7		0	0
	3	1.0×10^{-6}	120	3		0	0
	4	1.2×10^{-6}	322	11		ND	ND
	5–10	3.6×10^{-6}	900	21		ND	ND
ilv+	1	5.0×10^{-6}	100	1	2		3
	2	8.5×10^{-7}	140	0	0		1
	3	5.2×10^{-7}	120	0	0		3
	4	1.1×10^{-6}	305	1	ND		ND
arg+	1	4.0×10^{-6}	100	0	0	3	
	2	8.5×10^{-7}	100	0	1	2	
	3	9.0×10^{-7}	120	0	0	0	
	4	7.1×10^{-7}	283	0	ND	ND	

* Selected marker from recipient was *str*^{r}. † Expressed as the number of recombinant colonies detected per input donor cell.

ing individual amino acids required by the recipient. The ability of the selected recombinants to produce enterotoxin was tested with the modified Elek technique previously described (*9*). Briefly, each recombinant was streaked on a brain-heart infusion agar plate perpendicular to a strip of filter paper soaked in specific equine antitoxin and embedded in the medium. If the recombinant was *tox*+, an antigen-antibody precipitin line formed in the agar at an angle to the filter paper and the growth from the streaks. This technique facilitated the testing of hundreds of recombinants for production of enterotoxin.

Parker *et al.* have described three clusters of linked genes in *V. cholerae* which they have designated linkage groups I, II, and III (*8*). In order to determine whether or not *tox* belongs to any of these three linkage groups, we performed and analyzed matings between the prototrophic, *tox*+ donor strain 569B (P+) and the multiply auxotrophic, *tox*− recipient strain RV-31, which has mutant alleles (*his*−, *arg*−, *ilv*−) representing each of the three linkage groups. The results of several mating experiments are presented in Table 1.

Among 1465 *his*+ recombinants tested in ten experiments, 46 were *tox*+ (3 percent). In contrast cotransfer of *tox*+ with *ilv*+ occurred at a frequency of 2 in 665 (0.3 percent), and cotransfer of *tox*+ with *arg*+ was not detected (0 of 603). All *tox*+ recombinants were serotyped by slide agglutination tests, and all were Ogawa like the parental recipient strain. Therefore, the *tox*+ marker in strain 569B shows weak but significant linkage to the *his* gene of linkage group I, but little or no linkage to the *ilv* and *arg* genes of linkage groups II and III.

As an additional control to establish that the *tox*+ genotype of the recombinants arose by transfer of the *tox*+ allele from the donor to the recipient, a cross was performed using the *tox*−, prototrophic donor strain RV-102 and RV-31 as the recipient. No *tox*+ isolates were found among 100 *his*+, 100 *arg*+, and 100 *ilv*+ recombinants tested. The possibility of reversion from *tox*− to *tox*+ in strain RV-31 was also lessened by the observation that more than 500 single colonies of RV-31 tested were *tox*− by the Elek technique.

The results presented here demonstrate that a gene controlling toxinogenesis in *V. cholerae* can be transferred by conjugation from a *tox*+ donor to a *tox*− recipient strain. The linkage of the *tox* gene to the *his* gene, but not to genes *arg* and *ilv*, is inconsistent with control of toxinogenesis by a plasmid and provides strong evidence for the chromosomal location of the *tox* gene. Thus, in spite of the striking immunological and physiological similarities between the enterotoxins from *E. coli* and from *V. cholerae*, synthesis of enterotoxin in *V. cholerae* 569B is controlled by a chromosomal gene and differs from the plasmid-regulated system in *E. coli*.

We have recently isolated and characterized several different classes of mutants of *V. cholerae* strains 569B Inaba and 3083-2 Ogawa that are altered in toxinogenicity (*10*). Formal genetic analysis of such mutants should provide a feasible approach for the identification of the structural and regulatory genes that control toxinogensis in *V. cholerae*.

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Mapping of Functional Neural Pathways by Autoradiographic Survey of Local Metabolic Rate with [^{14}C]Deoxyglucose

Abstract. *If sufficient time has elapsed following an intravenous pulse of [^{14}C]deoxyglucose, the carbon-14 contents of the tissues of the central nervous system represent mainly the accumulated phosphorylated derivative of [^{14}C]deoxyglucose and reflect the rates of glucose consumption of the tissues. Altered functional activity alters metabolic activity and the uptake of [^{14}C]deoxyglucose in the tissues. By autoradiographic survey of sections of the central nervous system it is then possible to map all the regions with altered functional and metabolic activities in response to experimentally induced changes in functional state.*

A method has been developed for quantitative estimation of the rates of glucose consumption in the various structural components of the brain (*1*). The method is based on the uptake of ^{14}C in the various cerebral tissues following an intravenous tracer pulse of [^{14}C]deoxyglucose. If sufficient time is allowed after the pulse for the free [^{14}C]deoxyglucose to have been cleared from the tissue, then the ^{14}C concentration of the tissue represents the [^{14}C]deoxyglucose-6-phosphate content which, in turn, equals the integrated rate of [^{14}C]deoxyglucose phosphorylation with respect to time. With appropriate consideration of the time course of the relative concentrations of glucose and [^{14}C]deoxyglucose in the plasma, of the rate constants for the turnover of the free glucose and [^{14}C]deoxyglucose pools in the tissue, and of the ratios of the kinetic constants for the transport and phosphorylation of glucose and [^{14}C]deoxyglucose, the rate of glucose utilization in the tissue can be computed from its [^{14}C]deoxyglucose-6-phosphate concentration (*1*).

In order to achieve as fine a resolution and as broad a representation of the various cerebral structures as possible,