Transcription and Intragenic Recombination in Polar Mutants of Escherichia coli

Abstract. *Mitotic recombination within the β-galactosidase gene was followed in F′ merodiploids grown in the presence and absence of inducer. Inducer inhibited recombination when the two mutations in the merodiploid were nonpolar but had no effect when the mutations were strongly polar. These results are interpreted to mean that transcription inhibits intragenic mitotic recombination and that strongly polar mutations inhibit transcription.*

The presence of inducer for the lac operon reduces the frequency of recombination between two *Z* (structural gene for β-galactosidase) mutations, one nonpolar and one polar, carried by the chromosome and an F-merogenote of *Escherichia coli* K12 (*1*). It was proposed that the synthesis of *lac* messenger RNA (mRNA) in the induced cells inhibited recombination in the *Z* gene.

Polar mutations in the *Z* gene reduce or abolish the expression of the other genes of the *lac* operon. This polarity is frequently (if not exclusively) caused by the introduction of a nonsense codon within the *Z* gene as a consequence of the polar mutation (*2*). Nonsense codons signal the termination of message translation. Nonsense polar mutations in the *Z* gene also reduce the amount of *lac* mRNA in cell extracts, as detected by hybridization tests against *lac* DNA; and the extent of this reduction is correlated with the degree of polarity. Two proposals have been offered to account for this dual effect of nonsense polar mutations. (i) *Lac* mRNA is synthesized normally in polar mutants but is very rapidly degraded, either in vivo or during the extraction procedure, possibly because it is not protected by bound ribosomes (*2*, *3*). (ii) Transcription and translation are in some way coupled, so that the synthesis of *lac* mRNA is reduced when its translation is prematurely terminated by the nonsense codon. If the latter interpretation is correct, then inducer should have little effect on intragenic recombination in merodiploids carrying strongly polar mutations on both chromosome and F-merogenote. This prediction is confirmed by my results.

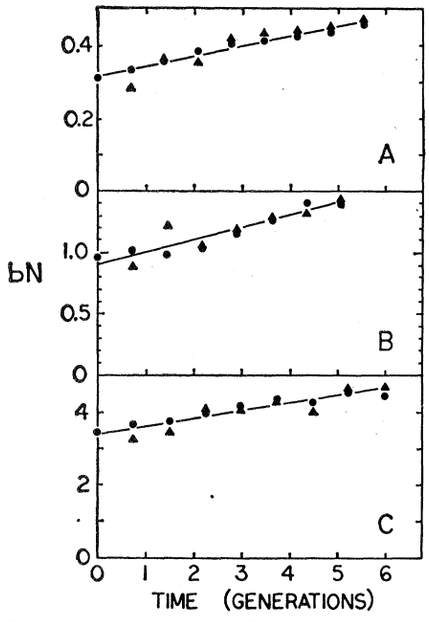

Fig. 1. Accumulation of Z^+ genes in F′ strains carrying strongly polar *Z* mutations on both chromosome and F-merogenote. The strains were grown in the presence (circles) and absence (triangles) of inducer. Units for *bN* are enzyme units per milliliter per optical density of culture at 540 nm; $bN = 1$ corresponds to a ratio of recombinants to nonrecombinants of about 2×10^{-8}. The *lac* genotypes of the three strains are as follows: (A) *Z2*/F *ZS*; (B) *Z2*/F *Z422*; (C) *Z545*/F *Z422*. Each of the four different *Z* mutations carried by these strains reduces the expression of adjacent *lac* genes by at least 90 percent (*8*, *9*). *Z2*, *Z422* and *Z545* have been identified as nonsense mutations (*8*, *9*).

The method used for following mitotic recombination in the *Z* gene has been described (*1*). Briefly, I assumed that the differential rate of β-galactosidase synthesis at any time is proportional to *N*, the number of Z^+ genes per unit mass in the bacterial population. In a culture grown for at least four generations in the presence of inducer, $bN = E/M + \mathrm{d}(E/M)/\mathrm{d}T$, where *E* is units of β-galactosidase per milliliter, *M* is the optical density of the culture at 540 nm, *T* is generations of growth, and *b* is a constant of proportionality. (The last term in the above equation is a small correction term.) In the absence of inducer, *bN* is determined by removing a sample from the uninduced culture and incubating it in the presence of inducer for 30 minutes. In this case $bN = (E_2 - E_1)/cM$, where *c* is a constant, E_1 is units of enzyme per milliliter immediately prior to the addition of inducer, E_2 is units of enzyme per milliliter at the end of the 30-minute induction, and *M* is the optical density at 540 nm of the subculture at the end of the induction.

Media and experimental procedures were as described previously (*1*). All of the experiments shown in the figures were of a single type. Cultures were grown up from about four to five cells in the presence of inducer. At $T = 0$, half of the culture was maintained in inducer, and the other half was quickly washed free of inducer by Millipore filtration. Thus, at $T = 0$, *bN* in the uninduced culture = *bN* in the induced culture. This relation determines the aforementioned constant *c* and allows us to compare the subsequent relative rates of accumulation of Z^+ genes, as given by the relative slopes of *bN* versus *T* in the induced and uninduced cultures. The sampling conditions for each culture were maintained nearly constant by regular dilutions into fresh medium (*1*).

Results obtained with three different F′ merodiploids carrying strongly polar mutations on both chromosome and F-merogenote are presented in Fig. 1. The presence of inducer has little effect on the rate of accumulation of Z^+ genes in these strains. By contrast, Fig. 2 shows that when the mutations in the merodiploid are both nonpolar, the rate of accumulation of Z^+ genes is inhibited by the presence of inducer. Except for the lower recombination frequencies, which are presumably attributable to the proximity of the *Z823* and *Z4* mutations, the latter strain behaves similarly in all respects to the strain previously studied (*1*). The lower rate of accumulation of Z^+ genes in the presence of inducer reflects a lower rate of formation of Z^+ recombinant genes.

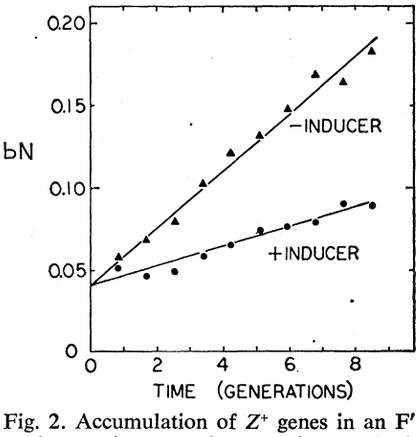

Fig. 2. Accumulation of Z^+ genes in an F′ strain carrying nonpolar mutations on both chromosome and F-merogenote. The *lac* genotype of this strain is *Z823*/F*Z4*.

The inducer is not directly responsible for the inhibitory effect of induction on intragenic recombination shown in Fig. 2, since the introduction of regulator constitutive mutations into the merodiploid has the same inhibitory effect (*1*). Moreover, the repressor molecule cannot be directly involved since the strongly polar mutants have wild-type regulator genes, and the removal of inducer does not increase recombination in these strains (Fig. 1). The control of the *lac* operon appears to operate most directly at the level of transcription (*4*); therefore, it seems very likely that the synthesis of lac mRNA is involved in the inhibition of intragenic recombination.

Translation of nascent mRNA molecules into polypeptides may begin before the synthesis of the message is completed (*5*). The nascent mRNA with its associated ribosomes is conceivably somehow responsible for the inhibitory effect of induction on recombination. If this were true, intragenic recombination in strongly polar mutants might be unaffected by induction, even if *lac* mRNA synthesis were induced normally, since the polar mutation could lead to release of ribosomes from the nascent message and rapid degradation of the message beyond the nonsense codon. But the *Z* gene is nearly as long as an *E. coli* cell (*6*).

It seems unlikely that the structure of the gene or its accessibility for participation in recombination with its homolog would be much affected by its nascent mRNA-ribosome complexes. Therefore, I favor the view that transcription alone inhibits intragenic recombination in merodiploids. It follows from this interpretation and the results illustrated by Fig. 1 that strongly polar mutations prevent normal transcription. This view is also favored by the work of Imamoto and Yanofsky on the kinetics of transcription of the tryptophan operon in polar mutants (*7*).

ROBERT K. HERMAN
Department of Genetics and Cell Biology, University of Minnesota, St. Paul 55101

References and Notes

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10. Supported by NIH grant AM11321-02. I thank Dr. D. Zipser for mutants and Mr. M. A. Larsen for technical assistance.

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Crystals from Cocoons of Malacosoma neustria testacea

Abstract. *The crystalline material covering the cocoon of* Malacosoma neustria testacea (*Lasiocampidae, Lepidoptera*) *was analyzed physically and chemically. The mean component was identified as calcium oxalate monohydrate, and the crystals were found to be whewellite in form.*

It has long been known that a crystalline powder covers the cocoons of some Lepidoptera (*1*), but the detailed nature of this material has not been worked out. We have analyzed the crystalline material of the tent caterpillar *Malacosoma neustria testacea* and found it to be calcium oxalate monohydrate.

About 400 cocoons were collected on the campus of Nagoya University and were repeatedly washed with 60 percent aqueous methanol. The suspension was centrifuged, and the yellow material packed in the bottom of the tube was dried in a desiccator under reduced pressure. The powder is composed mainly of crystalline material (Fig. 1).

Since preliminary analyses of the material revealed that the main component was calcium oxalate, calcium was estimated gravimetrically after the material was dissolved in dilute HCl. The calcium was precipitated with ammonium oxalate, and the oxalate was calcined in an electric furnace. Its content amounted to 33.7 percent by weight when calculated as CaO, or 87.7 percent when calculated as calcium oxalate monohydrate (*2*).

The content of oxalic acid was determined by titration with potassium permanganate. The material was dissolved in dilute HCl and passed through a Dowex-50 column. The fraction not adsorbed was neutralized, and the acid was precipitated with calcium chloride. The calcium salt, after being collected by centrifugation and washed repeatedly, was dissolved in dilute H_2SO_4 and titrated with permanganate. The material that could be titrated, with permanganate amounted to 80.6 percent when calculated as calcium oxalate monohydrate. The material also contained iron which was estimated colorimetrically with α,α′-dipyridyl, and the content was 0.35 percent by weight (*2*).

Identification of the material as calcium oxalate monohydrate was further confirmed by infrared spectrophotometry: the sample and the reagent of calcium oxalate monohydrate gave the identical pattern (*3*). The x-ray powder diffraction pattern for this material agrees well with that of whewellite (*4*), which is given in the ASTM card (Table 1) (*5*).

Table 1. X-ray powder diffraction data for cocoon crystals and $CaC_2O_4 \cdot H_2O$ (whewellite) (*5*); *d* (Å), spacing in angstroms; *I*, intensity.

Cocoon crystals		$CaC_2O_4 \cdot H_2O$ (whewellite)	
d (Å)	I/I_1	d (Å)	I/I_1
5.97	100	5.93	100
5.83	40	5.79	30
4.79	4	4.77	2
		4.64	1
4.55	10	4.52	4
3.79	20	3.78	6
3.66	100	3.65	70
3.35	10	3.41	2
		3.12	2
3.12	4	3.11	2
3.00	30	3.01	10
2.96	70	2.966	45
2.92	30	2.915	10
2.90	20	2.897	8
2.84	30	2.840	10
2.52	10	2.523	4
2.50	50	2.494	18
2.44	10	2.447	4
2.42	10	2.417	6
2.39	20	2.384	4
2.36	60	2.355	30
2.35	50	2.347	12
		2.320	1
2.30	4	2.301	2
2.26	40	2.263	8
		2.254	6
2.21	10	2.210	6
2.13	6	2.130	2
2.09	10	2.089	2
2.08	40	2.075	14
2.00	10	1.995	2
1.98	20	1.978	10
		1.957	2
1.95	30	1.950	10
1.93	20	1.933	8
		1.923	2
1.89	20	1.890	6
1.86	10	1.859	4