bases in naturally occurring nucleic acids and to the mode of action of clinical alkylating agents remains to be determined.

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Independent Action of Allele-Specific Suppressor Mutations

Abstract. *Two different allele-specific suppressor genes had an independent and nonadditive effect in restoring indoleglycerol phosphate → L-tryptophan activity in a double mutant strain lacking this activity.*

Suppressor mutations are those mutations in one gene which reverse the effects of mutations in another gene. Among the many different types of suppressor mutations are a class known as allele-specific suppressor mutations. This type of suppressor change appears to reverse the effects of only certain mutations in another gene. Examples of this type of specific suppressor effect have been reported in studies with the system described in this report, the tryptophan synthetase system of *Escherichia coli* (*1*, *2*).

The enzyme complex, tryptophan synthetase, is composed of two separable proteins subunits (*3*), designated A and B. Together, these proteins catalyze the following three reactions (*3*).

Indole + L-serine → L-tryptophan (In → Tryp) (1)

Indoleglycerol phosphate ⇄ indole + 3-phosphoglyceraldehyde (2)

Indoleglycerol phosphate + L-serine → L-tryptophan + 3-phosphoglyceraldehyde (InGP → Tryp) (3)

Many mutant strains of *E. coli* (A mutants) have been isolated which produce an altered A protein, designated A-CRM (cross-reacting material), which reacts with antibody to the normal A protein (*4*). All of the A-CRM studied to date can combine with the normal B subunit, and this complex can catalyze reaction 1, but not the other two reactions.

As reported previously, the effect of allele-specific suppressor mutations on CRM-producing A mutants and on A mutants lacking CRM was to restore small amounts of reaction 3 activity to mutant strains lacking this activity (*2*) (Table 1). The restored activity was shown to be associated with a small amount of *su*-A protein, which was similar in its properties to wild-type A protein (*2*). In the one mutant which was fully examined, the *su*-A protein was shown to differ from the corresponding CRM A protein by a single amino acid change (arginine → glycine) in its primary structure (*2*). From this observation and the extreme allele-specificity of different suppressor mutations, it appeared that each suppressor mutation had a specific and independent action in altering the primary structure of the A protein. In view of this conclusion, it was of interest to determine if two different suppressors in the same strain would have independent effects on the amount of active *su*-A protein that was formed.

A stock carrying two mutations in the A gene [CRM-producing double mutant A-11-36 (*5*)] plus the suppressors of each of these A mutations was prepared by transduction techniques for which phage Plkc was used (*6*). This stock was identified as the double mutant, double suppressor strain (designated A-11-36 su_{11}, su_{36}) on the basis of the following findings: (i) Lysates of Plkc prepared on this strain would only transduce the A-11 strain to a slow-growing *tryp*$^+$ type, identical to the A-11 su_{11} strain; (ii) the same lysates would only transduce the A-36 strain to a faster-growing *tryp*$^+$ type, identical to the A-36 su_{36} strain; (iii) the same lysates would not transduce the A-11-36 strain to any *tryp*$^+$ type.

These results confirmed that both the su_{11} and su_{36} loci were in this stock and indicated that these genes could not be co-transduced, since *tryp*$^+$ types were not found when A-11-36 was used as a recipient strain.

Two different isolates of the A-11-36 su_{11}, su_{36} strain were prepared and were grown on minimal medium and analyzed in duplicate for their A protein activities in reactions 1 and 3. The ratio of the activity of the A protein in these two reactions was considerably less in the double suppressor strain

Table 1. The enzymatic activities of the A proteins in extracts of various mutants and suppressed mutants. Abbreviations: In, indole; Tryp, tryptophan; InGP, indole-glycerol phosphate; su_{11}, su_{36}, the allele-specific suppressor genes that suppress the A-11 and A-36 mutations, respectively.

Strain	A protein activity of extracts *				InGP→Tryp/ In→Tryp activity ratio (%)
	In → Tryp		InGP → Tryp		
	Amount (units/ml)	Specific activity (units/mg)†	Amount (units/ml)	Specific activity (units/mg)†	
Wild type	75	2.5	30	1.0	40
A-36	725	22.0	<0.1	<0.003	
A-36 su_{36}	225	9.0	7.0	.28	3.2
A-11	1020	31.0	<0.1	<.003	
A-11 su_{11}	1380	55.0	12.0	.48	0.87
A-11-36	960	29.0	<0.1	<.003	
A-11-36 su_{11}, su_{36}	1640	41.0	1.60	.04	0.09

* Assayed in the presence of excess B protein. † Units per milligram of protein.

Table 2. Schematic representation of the types of A protein in the double mutant, double suppressor strain. X is the amino acid in the A-11 A protein that replaces a wild-type amino acid, Y is the amino acid in the A-36 A protein that replaces a wild-type amino acid, and *M* and *N* are the amino acids replacing X and Y, respectively, as a result of suppressor gene action.

A-11-36 CRM protein	---X--------Y-----
Altered protein due to action of the su_{11} gene (A-36 CRM)	---*M*-------Y----
Altered protein due to action of the su_{36} gene (A-11 CRM)	----X-------*N*----
Altered protein due to action of the su_{11} and su_{36} genes (active protein)	--*M*-------*N*-----

than the ratio for either the A-11 su_{11} or A-36 su_{36} strains (Table 1). Since it had been shown that the A protein which was active in reaction 3 in the A-11 su_{11} and A-36 su_{36} strains was similar to the wild-type A protein (*2*), it was assumed that the A protein active in reaction 3 in the A-11-36 su_{11}, su_{36} strain was also similar to the wild-type A protein in its enzymatic properties (that is, an InGP → Tryp/In → Tryp activity ratio of 40 percent). If this assumption is correct, then the InGP → Tryp/In → Tryp activity ratio of the A proteins of suppressed mutants can be multiplied by 2.5 to give the ratio of *su*-A protein to A-CRM protein. That is, if a ratio of 40 percent were obtained it would indicate that all the A-protein molecules had wild-type activity. If an activity ratio of 10 percent were obtained it would indicate that only 25 percent of the A-protein molecules had wild-type activity. On this basis, 8 percent of the A-protein molecules in the A-36 su_{36} strain has wild-type activity, while 2.3 percent in the A-11 su_{11} strain and 0.22 percent in the A-11-36 su_{11}, su_{36} strain had wild-type activity. It was apparent that the value obtained from the experimental data (0.22 percent) was very similar to the value which would have been predicted on the basis of independent actions of the two suppressor genes—that is, 8 percent × 2.3 percent. Therefore, it was concluded that the two suppressor genes studied had independent and nonadditive actions in restoring InGP → Tryp activity.

Since it has been established that these particular suppressor genes produce changes in the primary structure of the A protein (*2*), the independent action of the two suppressor genes in the A-11-36 su_{11}, su_{36} strain could be interpreted in the following way: The su_{36} gene altered the primary structure of the A protein at one particular site of 8 percent of the A-protein molecules, while the su_{11} gene altered the primary structure at some other site in 2.3 percent of the A-protein molecules. On this basis then, at least four different A proteins could be formed in the A-11-36 su_{11}, su_{36} strain (Table 2)—that is, the A-11-36 CRM protein (the bulk of the A protein), the A-11 CRM protein (8 percent), the A-36 CRM protein (2.3 percent), and an *su*-A protein (0.22 percent). Although it was not determined if all four types of A protein were present, the finding of only low amounts of InGP → Tryp activity suggested that these two suppressor genes did affect different sites in the A protein.

At present, the mechanism of allele-specific suppression can be most reasonably explained as "mistakes" in the incorporation of amino acids into protein due to an alteration in the specificity of any one of the cell components which participate in protein synthesis (*2*, *7*, *8*). This cell component could be DNA, messenger RNA, ribosomes, transfer RNA, or amino acyl RNA synthetases (*8*, *9*), since it is conceivable that mutational alteration of any one of these components could lead to a mistake in amino acid incorporation. However, it remains to be determined which of these components are responsible for the independent, nonadditive effects observed in this study.

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Differences in Complement Titers of Serums from Spaced Bleedings in Guinea Pigs

Abstract. *Complement titers of serum samples collected 24 hours apart from an untreated guinea pig differed greatly. The mean of the differences between the titers at the beginning and 24 hours later for each of 11 animals was significantly different from zero (.01<p<.02).*

It has generally been accepted that the complement activity of a "normal" individual, animal or human, is relatively stable. We present evidence that this assumption may be without foundation.

In preliminary experiments we found that the antihypertensive drug 1-hydrazinophthalazine hydrochloride (hydralazine) (*1*) inactivated about 90 percent of the hemolytic activity of guinea pig complement in vitro. We then studied the complement activity of the serums of guinea pigs which had received an intraperitoneal injection of hydralazine. A serum sample was taken about 24 hours prior to the injection of the hydralazine. A second blood sample was obtained 15 minutes after the drug was injected. The titers of the two serums are designated T_1 and T_2, respectively, and are expressed as "percentage change," $100 \times (T_1 - T_2)/T_1$.

In several of the control animals which had received nonsterile 0.85 percent NaCl solution intraperitoneally we observed changes of 25 to 40 percent. In other experiments we found similar,

Table 1. Complement titers (C′), in 50 percent hemolytic units per milliliter of serum, of bloods drawn at 0 hours (T_1) and 24 hours (T_2); body weight at 0 hours; number of punctures (P); and number of bleedings (B).

C′ units		Wt. (g)	0 hr		24 hr	
T_1*	T_2*		P	B	P	B
122.25	173.16	380	1	1	1	1
139.68	210.06	270	3	1	1	1
179.10	231.12	370	2	1	8	2
196.21	258.84	350	1	1	6	3
220.91	275.48	450	4	2	1	1
223.05	237.15	410	1	1	1	1
247.52	309.63	430	5	2	2	1
253.59	281.42	415	1	1	2	1
283.55	313.41	440	3	2	1	1
287.91	243.90	405	1	1	7	2
291.54	271.00	420	1	1	1	1

* For computational purposes only. Not intended to denote five significant figures.