second book is right in its conclusions. The Ionic Centrifuge in the second type of discharge would seem to offer the possibility of successful nuclear fusion.

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*SUPPRESSOR GENE ALTERATION OF PROTEIN PRIMARY STRUCTURE**

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Some suppressor genes are known to act by restoring an enzymatic activity that is specifically lacking in a mutant strain. This could be accomplished in many ways, with or without the alteration of the enzyme in question.[1–3] Suppressor mutations have been detected which affect the A protein of the tryptophan synthetase of *Escherichia coli*. Previous studies have shown that alterations in the primary structure of this protein can result from forward mutation,[4, 5] reverse mutation,[6] and recombination[6] within the structural gene (the A gene) for this protein. The present paper indicates that a suppressor mutation in a region of the genome distant from the A gene also leads to a change in the primary structure of the A protein.

Pertinent Characteristics of the Tryptophan Synthetase System.—The *Escherichia coli* tryptophan synthetase consists of two separable protein subunits, designated A and B. Together these proteins catalyze the following three reactions:[7] (1) indole + L-serine → L-tryptophan; (2) indoleglycerol phosphate ⇌ indole + 3-phosphoglyceraldehyde; (3) indoleglycerol phosphate + L-serine → L-tryptophan + 3-phosphoglyceraldehyde.[8] Reaction (3) is believed to be the physiologically essential reaction in tryptophan biosynthesis.[7, 9] Many A mutant strains produce an altered A protein, designated A-CRM, which reacts with antibody to the normal A protein.[9] All of the A-CRM's detected to date can combine with the normal B protein component, and this complex can catalyze the In → Tryp reaction, but not the other two reactions, i.e., reactions (2) and (3).

Materials and Methods.—The A mutants and suppressed A mutants listed in this paper were produced by ultraviolet irradiation of the K-12 strain of *E. coli*.[9, 10] The methods employed for the preparation of transducing lysates of phage Plkc and for transduction with this phage have been described previously.[10] All cultures of suppressed A mutants used for the preparation of extracts were examined for possible changes in the cellular population, such as reversion in the A gene, by appropriate plating and transduction techniques.

Enzymatic assays,[7] and procedures for the heat-treatment and acid-treatment of crude extracts,[11] have been described previously. Procedures used for the isolation of the A protein,[12] as well as the methods for the digestion of the protein with

trypsin and chymotrypsin,[13] the isolation of peptides,[13] and the analyses of peptides[4] are described elsewhere.

Results.—Genetic characterization of suppressor mutations: A certain class of tryptophan-independent strains, designated as suppressed A mutants, were isolated in reversion studies performed with various tryptophan synthetase A mutants.[14, 15] These suppressed A mutants were clearly distinguished from revertants which arose by mutation in the A gene. Transduction experiments[15] indicated that the original mutation in the A gene was still present in the suppressed A mutant, and that the site of the suppressor mutation was not linked to the A gene.[16]

Allele specificity tests performed with the suppressor genes in strains A-11 su, A-3 su, and A-36 su indicated that each suppressor gene was allele-specific. Although 50 nonidentical A mutants were examined in tests with each of the suppressor genes, suppression was not detected, i.e., no tryptophan-independent colonies were observed.

Enzymatic characterization of crude extracts of suppressed mutants: Extracts of suppressed mutants differ from extracts of A mutants in that they exhibit low levels of InGP → Tryp activity (Table 1). The level of InGP → Tryp activity restored by a suppressor gene is characteristic of each suppressed mutant. In addition, the ratio of InGP → Tryp activity to In → Tryp activity is quite characteristic for each suppressed mutant, and shows little fluctuation when cultures are grown under similar conditions.

TABLE 1

THE ENZYMATIC ACTIVITIES OF THE A PROTEINS IN EXTRACTS OF VARIOUS MUTANTS AND SUPPRESSED MUTANTS

Strain	A protein-specific activity*		$\frac{\text{InGP} \rightarrow \text{Tryp}}{\text{In} \rightarrow \text{Tryp}}$ per cent
	In → Tryp	InGP → Tryp	
Wild-type	2.5	1	40
A-36	22	0	...
A-36 su	6	0.25	4.2
A-3	35	0	...
A-3 su	44	0.19	0.44
A-11	31	0	...
A-11 su	55	0.48	0.87

* Units/mg protein.

Physical treatments of crude extracts of suppressed mutants: The low level of InGP → Tryp activity detected in extracts of suppressed mutants could be due to an alteration of the CRM molecules, making them all slightly active in this reaction, or it could be due to the formation of a small amount of a second A protein, which was active in the InGP → Tryp reaction. It would be possible to distinguish between these alternatives, if it could be shown that the physical properties of the A protein that is active in the InGP → Tryp reaction differed from the properties of the CRM protein. The three mutants listed in Table 1 were selected for this study because the A-CRM's in each one could be distinguished from the wild-type A protein by differences in heat- or acid-stability. In comparison to the wild-type A protein, the CRM of A-36 is heat-labile, the CRM of A-3 is heat-stable, while the CRM of A-11 is acid-precipitable.[11]

Figure 1 presents the heat inactivation curves for the two activities found in

the crude extracts of two suppressed mutants. The results obtained demonstrate two important facts about the A proteins in these extracts. First, the bulk of the In → Tryp activity in suppressed mutant extracts is associated with an A protein with the same heat sensitivity as the CRM in the original mutant (unsuppressed) extract.[11] Secondly, the A protein active in the InGP → Tryp reaction is clearly different from this CRM in its heat sensitivity, and furthermore resembles the wild-type A protein. As a control for one of the heat inactivation experiments, crude extracts of the wild-type strain and of strain A-36 were mixed to simulate an extract of A-36 su, and a similar heat inactivation experiment performed. The inactivation rates of the two activities in this mixture were the same as those observed with A-36 su extracts.

Heat treatment of an A-11 su extract did not differentially inactivate the InGP → Tryp activity or the In → Tryp activity in this extract. Since the CRM of strain A-11 is as heat-sensitive as the wild-type A protein,[11] no differences would be expected in the two inactivation rates, if the protein bearing the InGP → Tryp activity had the same heat stability as the wild-type A protein. However, acid treatment of an extract of A-11 su clearly showed (Table 2) that the InGP → Tryp activity was associated with a protein that was more acid-stable than the protein bearing the In → Tryp activity, and was similar in behavior to the A protein of the wild-type strain. Here again, the bulk of the A protein resembled the A-CRM found in strain A-11. Similar acid treatment of crude extracts of strains A-36 su and A-3 su led to a loss of activity in both reactions similar to that observed with extracts of the wild-type strain (Table 2). Since the A-CRM's from strains A-36 and A-3 have the same acid stability as the wild-type A protein,[11] it might not be possible to distinguish these A-CRM's from a second A protein, if that protein had the same stability as the wild-type A protein.

TABLE 2

ACID TREATMENT OF EXTRACTS

Extract	Per cent activity remaining*	
	In → Tryp	InGP → Tryp
Wild-type	58	55
A-11	11	...
A-11 su	17, 11	51, 47
A-36	60	...
A-36 su	59	57
A-3	58	...
A-3 su	61	53

* A protein activity in supernatant solutions following acidification of extracts to pH 4.0.

These findings are consistent with the view that the two types of A protein found in extracts of each suppressed mutant are: an A protein active in the InGP → Tryp reaction (hereafter designated as the su-A protein), which has certain physical properties in common with the wild-type A protein; and an A protein which closely resembles the A-CRM of the parental mutant.

Purification and separation of two A proteins by DEAE column chromatography: These findings, as well as previous observations,[2] suggested the presence of a second type of A protein in extracts of suppressed mutants. In further studies with extracts of strain A-36 su, it was possible to separate two A proteins by column

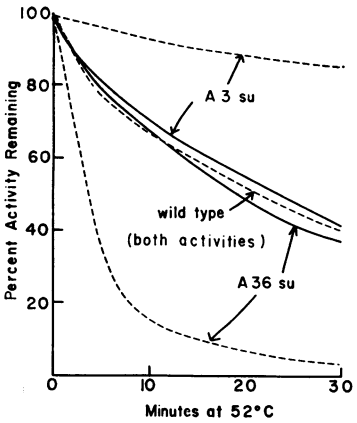

FIG. 1.—Heat inactivation of the A proteins in crude extracts of strains A-36 su, A-3 su, and the wild-type strain. InGP → Tryp activity———, In → Tryp activity - - - - -.

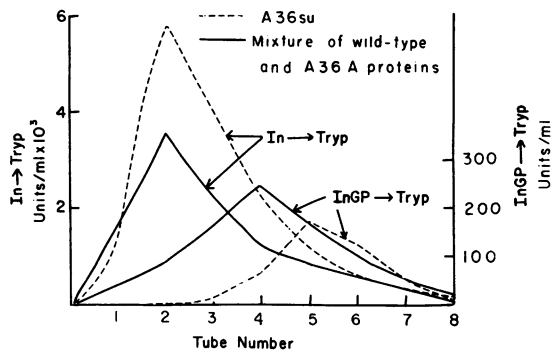

FIG. 2.—Elution pattern from DEAE-cellulose columns of the two A proteins in extracts of strain A-36 su, and of a mixture of the wild-type and A-36 A proteins. A linear phosphate gradient was employed (0.01 *M* → 0.30 *M*, pH 7.0), and fractions of 10 ml were collected. Tube #1 refers to the first tube that contains A protein activity.

chromatography (Fig. 2). As can be seen in this figure, the In → Tryp and InGP → Tryp activity peaks are partially separated.

To determine whether the chromatographic properties of the su-A protein were similar to those of the wild-type A protein, purified A proteins from mutant A-36 and from the wild-type strain were mixed to simulate a purified preparation from strain A-36 su. When this mixture was chromatographed employing the conditions used for the first column procedure (as described above), the In → Tryp and InGP → Tryp activity peaks were partially separated (Fig. 2). As shown also in Figure 2, the activity curves were very similar to those obtained by column chromatography of the preparation from strain A-36 su.

The su-A protein was purified further by combining the fractions containing InGP → Tryp activity from several DEAE columns, concentrating by $(NH_4)_2SO_4$ precipitation, and then rechromatographing on a 100 × 1.2 cm DEAE-cellulose column, using a linear gradient of 0.01 *M* → 0.15 *M* phosphate (pH 7.0). Since some of the early fractions from the previous column with InGP → Tryp activity contained the trailing portion of the In → Tryp activity peak, two activity peaks were also recovered from the second column. However, under the conditions of rechromatography, the two A proteins were completely separated. The purified su-A protein had an InGP → Tryp : In → Tryp activity ratio of 40 per cent, which is identical to that of the wild-type A protein. Heat inactivation studies showed that both of the enzymatic activities of the purified su-A protein were inactivated at the same rate, a rate identical to that for the heat inactivation of the wild-type A protein. These findings indicate that the su-A protein was completely free of the A-CRM protein. Furthermore, a comparison of the specific activities of the A protein peaks with the specific activity of pure wild-type A protein showed that the su-A protein was 50 per cent pure, while the A-CRM was greater than 90 per cent pure.

Analysis of peptides from the A-CRM and su-A proteins of A-36 su: The two A protein fractions isolated from strain A-36 su were examined in peptide pattern

studies. The peptide patterns of a trypsin plus chymotrypsin digest, as well as a chymotrypsin digest of the su-A protein, were found to correspond exactly to the peptide patterns of the wild-type A protein. The peptide pattern of chymotryptic digests of the CRM protein of strain A-36 su showed one difference from a similar wild-type A protein peptide pattern, the position of peptide CP-2, as shown schematically in Figure 3. Although the chymotryptic peptide pattern of the CRM from strain A-36 su differed from the wild-type peptide pattern, it was identical to the peptide pattern of the CRM protein from the original A-36 strain. It had previously been shown that the peptide difference in mutant A-36 was due to the replacement of a particular glycine residue in CP-2 by an arginine residue.[4, 17]

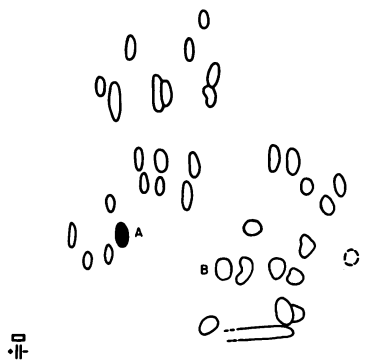

FIG. 3.—A schematic representation of the position of the major peptides in chymotryptic peptide patterns of both the A-CRM and the su-A protein of strain A-36 su. A = the position of peptide CP-2 from the su-A protein, B = the position of CP-2 from the A-CRM. Peptide CP-2 is in position A in wild-type peptide patterns and in position B in peptide patterns of the A-CRM from mutant A-36. The rectangle in the lower left-hand corner indicates the point of application of the sample.

Peptide CP-2 was isolated from the two A proteins from strain A-36 su and its amino acid composition determined. The results of these analyses are compared with the known sequence of amino acids in peptide CP-2 from the wild-type A protein,[17] and with the amino acid composition of CP-2 from A-36 CRM[17] (Table 3). The analyses indicate that peptide CP-2 from the su-A protein has the same composition as the corresponding peptide from the wild-type A protein, while CP-2 from the A-CRM of strain A-36 su has the same composition as CP-2 from the A-CRM of mutant A-36. It is clear from these data that an amino acid replacement occurs in the A protein as a result of the action of the suppressor gene.

TABLE 3

AMINO ACID COMPOSITION OF PEPTIDE CP-2 FROM VARIOUS A PROTEINS

Wild-type	$AspNH_2$-Ala-Ala-Pro-Pro-Leu-$GluNH_2$-*Gly*-Phe
A-36	(Asp*-Ala-Ala-Pro-Pro)-Leu-$GluNH_2$-*Arg*-Phe
A-36 su	
CRM-A	(Asp,* Ala_2, Pro_2, Leu, *Arg*, Glu*)-Phe
su-A	(Asp,* Ala_2, Pro_2, Leu, *Gly*, Glu*)-Phe

* Not determined whether present as amide.

Discussion.—Physical and chemical treatments of extracts of three suppressed A mutants indicate that there are two types of A proteins present in each extract. The majority A protein component is indistinguishable from the original CRM protein, whereas the minority component (the su-A protein) has physical and enzymatic properties characteristic of the wild-type A protein. Peptide pattern studies of the two purified A proteins from strain A-36 su also show that the su-A protein resembles the wild-type A protein, while the CRM protein resembles the A-CRM of mutant A-36. Amino acid analysis of the relevant peptides (CP-2) confirmed the fact that there is a difference in amino acid composition of the two peptides. This fact, in itself, is critical since it indicates that the presence of the

suppressor gene in strain A-36 su has resulted in a change in the primary structure of the A protein.

The amino acid replacement in the A protein due to the suppressor gene of strain A-36 su is an arginine → glycine change in peptide CP-2. Only small amounts of purified su-A protein were available for study, so it was not possible to perform amino acid sequence analyses on peptide CP-2. However, if one makes the plausible assumption that the glycine residue in this peptide occupies the same position as the arginine residue that it replaces, then the effect of this suppressor gene is to restore an amino acid sequence identical to that of the wild-type A protein.[18] The presence of a glycine residue in this peptide is of particular importance in this case, since it has been shown that A proteins with any one of three other amino acids (alanine, serine, and valine) at this position are functional.[6] Although the amino acid found in the wild-type A protein (glycine) was restored in this case, it should be possible for amino acids other than the wild-type amino acid to be inserted at this position. In this regard, it is conceivable that a suppressor mutation could lead to an amino acid replacement in a protein which would not be possible by a single mutational event in the structural gene for this protein.

It has been found that the mutant phenotype of approximately one third of all the A mutants tested can be reversed by suppressor mutations.[15, 19] One mutant which is not suppressible, strain A-46, is particularly interesting in view of the studies reported in this paper. Although extensive reversion analyses and cross-suppression tests have been performed with this mutant, no suppressors have been found which restore InGP → Tryp activity. The amino acid change in the A-46 protein is a glycine → glutamic acid replacement, and this glycine residue is the same one that is replaced by arginine as a result of the A-36 mutation.[5] It is interesting that in the presence of the A-36 suppressor gene, glycine can replace arginine, but not glutamic acid, at this position in the A protein.

The results of these investigations can be most easily explained by postulating that the consequences of a mutation in a suppressor gene such as the suppressor of strain A-36 su is to produce an alteration in the specificity of incorporation of amino acids into proteins. The amino acid changes which result from this type of alteration could be called "mistakes" in protein synthesis. If the cell containing a mutated suppressor gene is incapable of always translating a certain specific DNA nucleotide sequence into the same amino acid without error, then it is important to determine the extent of this error in translation. The term "mistake level" will be used to indicate how often an amino acid at a particular position in a given protein is replaced at the same position by one or another amino acid. This definition does not include as "mistakes" the replacement of an amino acid by its analogues, or the translation of a nonsense sequence of nucleotides into a known amino acid. The "mistake level" might simply be expressed as follows: amino acid X is found to replace Y at a particular position in a protein in 8 per cent of the molecules. The "mistake level" might also be more complex if many amino acids could replace amino acid Y at this particular position and with different frequencies.[20] In any event, a given "mistake level" can be considered to denote a constant probability of misreading the genetic code independent of what proteins, or types of proteins, are being synthesized.

It is not known to what extent the bacterial cell could tolerate many types of

amino acid insertion mistakes. It is probable that very high "mistake levels," single or multiple, if they affected all proteins, would result in lethality. This may explain why all allele-specific suppressors of A mutants examined to date appear to have very low "mistake levels."[21] However, it could be possible to have a fairly high "mistake level" if there were more than one "coding unit" for a particular amino acid (so-called "degenerate coding units"), and if these amino acid coding units were not equally distributed in the DNA. If the suppressor mutation could lead to a translation "mistake" of a minority or infrequently found coding unit, then an amino acid switch found in one protein might not be found in other proteins. In cases where very high levels of an enzyme are restored by the action of a suppressor gene, different interpretations may be applicable.[3]

The finding that some suppressor mutations can reverse the effects of mutations in several genes lends weight to the "mistake in protein synthesis" hypothesis.[22, 23] The fact that a number of different point mutants in the T4r_{II} cistron[22] or in the alkaline phosphatase gene[3] are affected by a single suppressor gene is consistent with this idea and has been interpreted in a similar manner.

There are many ways that the presumed mistake in translation in strain A-36 su might occur. According to our present knowledge of protein synthesis, which

TABLE 4

POSSIBLE SUPPRESSOR "MISTAKES" THAT WOULD LEAD TO THE OCCASIONAL INCORPORATION OF GLYCINE INSTEAD OF ARGININE IN THE A PROTEIN OF STRAIN A-36 SU

Mutationally altered component	Effect of alteration	Result
(*a*) Arginine-activating enzyme	Arginine enzyme activates glycine in addition to arginine	Glycine or arginine coupled to same type of arginine-tRNA
(*b*) Glycine-activating enzyme	Glycine enzyme pairs with arginine-tRNA in addition to pairing with glycine-tRNA	Glycine coupled to both arginine-tRNA and glycine-tRNA
(*c*) Arginine-tRNA	Arginine-tRNA pairs with the glycine-activating enzyme in addition to pairing with the arginine-activating enzyme	Glycine or arginine coupled to same type of arginine-tRNA
(*d*) Glycine-tRNA	Glycine-tRNA pairs incorrectly with messenger RNA, in addition to pairing properly	Glycine-tRNA (charged with glycine), pairs with an arginine-coding unit, in addition to pairing with a glycine coding unit in messenger RNA

tRNA = amino acid transfer RNA.

may be incomplete, the possibilities listed in Table 4 can be considered. It is assumed that all of the alterations mentioned lead only to partial losses in specificity. At the present time, the specificities of these components in strain A-36 su are being investigated to determine whether any one of the components has been altered as a result of the suppressor mutation.

We wish to express our thanks to Deanna Thorpe, Patricia Schroeder, and John Horan for their assistance with various aspects of this work.

The following abbreviations were used: Tryp = Tryptophan; In = Indole; InGP = Indoleglycerol phosphate.

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† Predoctoral trainee of the U.S. Public Health Service.

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[18] It cannot be unequivocally stated that the entire amino acid sequence of the su-A protein is identical to that of the wild-type A protein, since amino acid sequence analyses of every peptide from both of these proteins would be required to establish this point.
[19] Yanofsky, C., D. Helinski, and B. Maling, in *Cellular Regulatory Mechanisms*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 26 (1961), p. 11.
[20] It was thought that perhaps a given suppressor gene, known to cause (for example) a histidine → tyrosine switch in the A protein, could be used as a genetic method for identifying tyrosine → histidine mutational changes in other proteins. However, this may not be possible until it is known whether single or multiple amino acid replacements are involved.
[21] "Mistake levels" in suppressed A mutants are estimated from the ratio of the A protein activity in the InGP → Tryp and In → Tryp reactions. If the A protein active in the InGP → Tryp reaction is similar to the wild-type A protein in its enzymatic properties, then this ratio of enzymatic activities can be taken as an indication of the amount of su-A protein relative to the A-CRM protein.
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*THE EFFECTS OF STREPTONIGRIN ON CULTURED HUMAN LEUKOCYTES**

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Streptonigrin (SN) is a derivative of *Streptomyces flocculus* which has been isolated and screened for its possible antitumor and antibiotic activity. It has not been released for clinical use execpt under experimental conditions. Studies with rodents indicated that it is an extremely cytotoxic compound.[1]

Recent work in this laboratory has shown that SN induces the production of bacteriophage in lysogenic bacteria while inhibiting the net synthesis of bacterial deoxyribonucleic acid (DNA).[2] Furthermore, it causes a marked increase in genetic recombination in mixed bacteriophage infections,[3] and initiates rapid *in vivo* degradation of the DNA of *Escherichia coli*.[4]

Because of these striking effects on macromolecular synthesis, tests of the