tended the monitoring procedures by utilizing the infrared CO_2 gas analyzer (Beckman) in order to measure pCO_2 in the expired air. Two penetrations of blood vessels were performed percutaneously with a 20-cm hypodermic needle. In the last such experiment, 0.25 mg of thiopental per kilogram was injected directly into the abdominal *vena cava*. This small dose of barbiturate did not appear to affect the animal adversely in any perceivable way. Monitoring devices which measure arterial pO_2, *p*H, and pressure will probably be necessary, however, to test safely such agents as the barbiturates or halothane when given in effective anesthetic doses. Using nitrous oxide anesthesia, we have been able to insert electrode sleeve guides into the skull on four occasions in two animals, and to record successfully cortical electroencephalographic activity. These methods open the possibility of performing major surgery in this species for the first time. However, for some surgical procedures, supplementation of nitrous oxide anesthesia with other agents may be necessary (*11*).

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Protein Structure Relationships Revealed by Mutational Analysis

Abstract. *Studies of tryptophan synthetase A-protein mutants have shown a structural relationship between the positions of amino acid changes associated with forward mutations and second-site reverse mutations.*

In previous mutational studies of the tryptophan synthetase system (A-gene and A-protein) of *Escherichia coli* it was shown that seven different amino acids could occupy the same position in the A-protein (*1*, *2*). Two of the altered A-proteins were nonfunctional enzymatically (A-23 and A-46 A-proteins) while the others were active. One of the mutants, A-46, underwent reversion at a second site in the A-gene; this reversion event led to a change in the amino acid situated 36 residues from the position at which the original amino acid change occurred in mutant A-46 (*3*). Extensive reversion studies with mutant A-46 established that reversion only affects these two positions in the A-protein—that is, the position at which the original change occurred, and the position affected by the second-site reversion (*2*). In addition, analyses of the primary structure of six proteins from second-site revertants indicated that all the second-site changes involved the same *Tyr* → *Cys* (tyrosine, cysteine) replacement (*2*, *3*). These changes at the second site must therefore reflect some functionally significant relationship between the two specific regions of the folded polypeptide chain. Thus, just as studies of "forward" mutation reveal those mutational events which lead to a loss of function, reversion analyses reveal the spectrum of mutationally permissible changes in amino acids that can restore a functional protein.

Further reversion studies were undertaken in an effort to obtain additional information on the relationship between changes in primary structure and enzyme function. It was felt that the positions at which changes in amino acids occurred as a result of reversion events might reflect structural relationships between different regions of the protein molecule. Mutant A-187 was selected for this reversion study because the position of its amino acid change was very near the position at which the change occurred in the A-46 protein (*4*) (see Fig. 1). Mutant A-187 was isolated from strain A-46 PR9, a revertant of A-46 (*4*), as shown in Fig. 1. We have now performed genetic analyses with four revertants of A-187, and have also examined their A-proteins. The results of our studies are summarized in Fig. 1. Three different reversion changes were detected. There were two revertants of the A-187 SPR2 type, in which a valine in TP3 is replaced by alanine. In A-187 SPR3 the nearby valine in TP3 is replaced by alanine. Thus substitution of alanine for either valine in TP3 of the A-protein of mutant A-187 yields a functional protein (*4*). It should be pointed out that neither of these valine residues is present in the A-protein of wild-type *E. coli*. Of great-

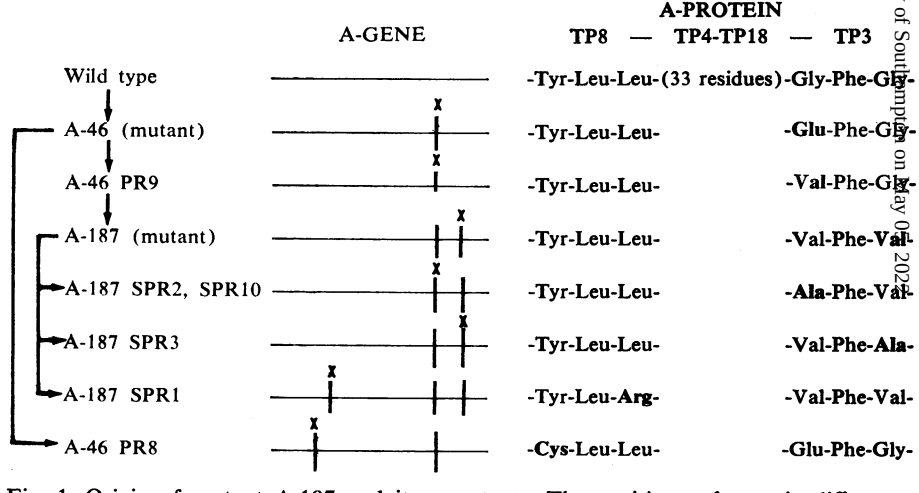

Fig. 1. Origin of mutant A-187 and its revertants. The positions of genetic differences from the wild-type A-gene and the site of each mutational change (marked X) are indicated on the left. Analyses of primary structure are given on the right; each amino acid that was changed as a result of mutation is shown in bold face type. TP8 and TP3 are two tryptic peptides of the A-protein that are joined by peptides TP4 (9 residues) and TP18 (11 residues). All the strains listed with the exception of A-46 and A-187 are prototrophic. (Abbreviations: Tyr, tyrosine; Leu, leucine; Gly, glycine; Phe, phenylalanine; Val, valine; Cys, cysteine; Ala, alanine.)

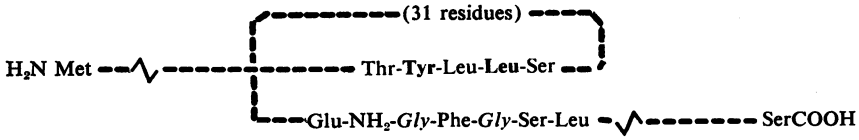

Fig. 2. Possible relationship between different regions of the folded wild-type A-protein molecule suggested by second-site reversion analysis. The amino acids changed in primary mutational events are shown in italics, while those changed by second-site reversion are shown in boldface type. (Abbreviations as in Fig. 1.)

er interest is the finding that, in mutant A-187, second-site reversion also occurs and results in a change in an amino acid situated 36 residues from the position of the original change (Fig. 1). The residue concerned, leucine, is two residues away from the tyrosine residue replaced in the second-site revertant of mutant A-46, revertant A-46 PR8. This leucine residue is replaced by arginine in the A-187 revertant, strain A-187 SPR1. Thus, mutational changes affecting amino acids situated two residues apart in the protein are reversed by second-site changes which are also two residues apart. This finding is diagrammatically represented in Fig. 2 in which a possible relationship between the respective regions of the folded wild-type A-protein is depicted. Whether or not the two regions are actually close together in the folded protein, it is clear that there is some functionally important relationship between the two regions of the A-protein. If structural relationships of this type continue to appear, the mutational approach may be of considerable help in the elucidation of those tertiary structure relationships which are of significance in determining enzyme activity *in vivo*.

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Reaction Time to Cortical Stimulation

Abstract. *A monkey was trained to release a lever when a stimulus was applied to the striate cortex. Reaction times to stimulation of the striate cortex were consistently 30 milliseconds shorter than reaction times to visual stimulation. The technique appears to be fruitful for analysis of neural mechanisms in a simple behavioral task.*

In a typical reaction-time experiment subjects are instructed to depress a key when a preliminary stimulus appears and to release the key at the onset of a second stimulus. The time between the preliminary stimulus and the second stimulus is termed the foreperiod. The interval between the appearance of the second stimulus and the response is termed the reaction time. Such experiments have yielded data which purportedly bear on problems extending from peripheral nerve conduction (*1*) to the underlying physiology and biophysics of perception (*2*). In most of these investigations, however, humans have been employed as subjects; thus neural processes could not be directly observed. Clearly, many of the conclusions from such studies might be established more firmly if they could be carried out with animal subjects.

In 1961 Stebbins and Lanson (*3*) described a technique for measuring reaction time in rats. We have used a modification of this technique for primates (*4*), to study reaction time to cortical stimulation.

In an adult monkey (*Macaca nemestrina*), bipolar platinium-iridium electrodes were implanted under direct observation in the occipital lobe at a point roughly 6 mm posterior to the lunate sulcus. This region lies near the central visual fields as determined by studies of photically evoked potentials (*5*, *6*). The electrode assembly was a modification of that described by Doty *et al.* (*7*). The uninsulated tips (about 1 mm² in surface area) of each pair of electrodes were separated by approximately 1.5 mm. Electrodes terminated in a miniature Winchester plug. The intracranial stimulus was generated by a well-isolated constant current stimulator, with a continuously variable 60-cy/sec output of from 1 to 800 μamp (root mean square, rms).

Although we were concerned about the possibility of dural rather than cortical excitation, several factors tended to minimize this problem. (i) The electrodes were inserted 1 mm below the pial surface, which made excitation of dural receptors unlikely. (ii) Threshold current was quite small, approximately 50 μamp (rms) for the right hemisphere and 85 μamp for the left. (iii) High-amplitude differential recordings of evoked potentials could be made by using peripheral flashes to evoke bilateral responses and unilateral electrical stimulation to evoke interhemispheric responses. (iv) With current intensities over 15 times threshold and durations of up to 5 seconds, no behavior suggesting discomfort was observed.

During training and testing the subject was restrained in a primate chair in a darkened, sound-insulated room. The subject was trained initially to press a telegraph key following the onset of a 1000-cy/sec tone (ready signal) and to release the key at the onset of a light of approximately 215 lumen/m² which appeared after a variable foreperiod (0 to 5 seconds). Later the light stimulus was replaced by cortical stimulation (700 μamp). Releases of the key in the presence of both the tone and light (or tone and cortical stimulation) were rewarded with a 1-g banana pellet (*8*). Inappropriate key responses—that is, releases prior to the onset of the visual or cortical stimulation or presses prior to the tone resulted in a 30-second "time out" period (the duration of the intertrial interval). The change from light to cortical stimulation was effected by introducing the cortical stimulation and then slowly fading out the peripheral stimulus. This procedure caused little disruption of performance. Latencies of key releases were recorded for each day's run in the form of a frequency distribution on a CAT computer (*9*) and individually on a Cramer chronoscope.

To obtain responses of minimal latency a "limited hold" was introduced (that is, only key releases occurring during a specified interval following on-

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