devices, the mechanism of the maintenance and termination of pregnancy, and about functional myometrial disorders. The common basic regulatory mechanism of the myometrium must be thoroughly investigated before evaluating the importance of superimposed modifications. The recognition that it is fundamentally alike in the various mammalian species promises to provide order in an important field of reproductive biology where controversy and confusion have prevailed.

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*ROLE OF RIBOSOMES IN STREPTOMYCIN-ACTIVATED SUPPRESSION**

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It is known[1, 2] that when a streptomycin-resistant (Sm^R) mutation is imposed on a Sm-sensitive (Sm^S) strain bearing a Sm-suppressible (SSu) defect, i.e., an impairment phenotypically corrected by Sm, two types of mutants are obtained: one remains correctable by Sm (conditionally Sm-dependent phenotype = CSD), and the other does not (defective phenotype). Two Sm^R alleles, competent and incompetent, are thus recognizable on the basis of their ability to permit Sm-suppression of a given SSu defect; their ratio depends on the parent strain. Furthermore, some competent mutants were always found among the auxotrophs derived from every randomly chosen Sm^R parent so far tested. It therefore appears unlikely that Sm^R mutations exist which are absolutely incompetent, i.e., are unable to per-

mit Sm-suppression of any SSu defect, but rather each Sm^R allele permits correction of only certain defects.

It is also known[3] that *in vitro* Sm causes misreading by acting on the ribosome. If Sm-suppression occurs *in vivo* via the same mechanism, Sm^R alleles differing in their pattern of suppression in the cell should confer a different degree or pattern of Sm-misreading in extracts. However, no difference was detected earlier[3] in the behavior *in vitro* of ribosomes extracted from competent and from incompetent Sm^R cells: both appeared equally refractory to Sm-misreading. On the assumption that this failure was due to lack of sensitivity of the *in vitro* system, we have developed a more sensitive modification based on the use of a partially purified enzyme preparation, reconstituted ribosomes, and heavy labeling of specific amino acids. With this system incorrect incorporation of an amino acid can be detected at 0.05 per cent of the level of the correct amino acid.

Sm^R mutants, competent and incompetent for the suppression of a given SSu defect, have been isolated and tested for their ability to suppress a second SSu defect. Their ribosomes have been compared under identical conditions, and differences in sensitivity (or pattern) to the misreading action of Sm have been demonstrated.

Methods.—Bacterial strains, growth conditions, and selection of mutants: Escherichia coli strain B and its mutants have been used in all experiments. The cultures were grown at 37°C in minimal medium A[4] with glucose and the necessary requirements or in medium A enriched with 1% Difco Bactotryptone and 0.5% Difco yeast extract (medium L). Spontaneous mutants with low (R_L) and high (R) resistance to Sm were selected as survivors on agar plates of medium L with 10 or 500 μg/ml Sm, respectively. The auxotrophs were obtained by nitrosoguanidine (NG) mutagenic treatment and penicillin selection as previously described;[1] those able to form colonies on plates of minimal medium A with Sm after 1–3 days of incubation at 37°C are classified as CSD.

Preparation of supernatant enzymes: To prepare the enzymes involved in protein synthesis, the initial part of the method described by Allende, Monro, and Lipmann[5] was utilized with three major modifications. First, all traces of ribosome particles were removed by extensive centrifugation; second, no Sm (or protamine) precipitation was undertaken since we are studying a Sm-activated system; and third, a broader ammonium sulfate fractionation was performed in order to include the sRNA acylating enzymes as well as the transfer enzymes.

Log phase cells from wild-type strain B grown on L medium were obtained from a culture at a density of 4×10^8 cells/ml in a medium able to support the growth of 10^{10} cells/ml. All procedures were done at 4°C. Eighty grams of frozen cells were ground with 240 gm of alumina. Of buffer A (0.06 *M* NH_4 Cl, 0.01 *M* Mg acetate, 0.01 *M* Tris-HCl, and 0.006 *M* β-mercaptoethanol; pH 7.4) 240 ml containing 5 μg/ml DNase (Nutritional Biochemicals Corp.) was slowly added. The suspension was centrifuged twice at 10,000 *g* for 10 min, then at 39,000 *g* for 1 hr, and finally at 105,000 *g* for $3\frac{1}{2}$ hr in a Spinco 40 rotor. The top $\frac{2}{3}$ of the supernatant was removed from each tube and centrifuged at 105,000 *g* for 15 hr. The upper $\frac{3}{4}$ of this supernatant, which was colorless and transparent, was carefully removed and pooled. Fourteen grams of solid ammonium sulfate per 100 ml supernatant was slowly added over a 15-min period with constant stirring, while maintaining the pH at 7.4 by addition of drops of 1 *N* NH_4OH. The suspension was then stirred for another 15 min and centrifuged at 10,000 *g* for 10 min. To the supernatant solid ammonium sulfate (18 gm/100 ml) was added as above. An additional hour of stirring was followed by centrifuging at 10,000 *g* for 10 min. The supernatant was discarded and the pellet dissolved in a minimal amount of buffer A and dialyzed for 24 hr against two changes of 100 vol of buffer A. The quantities of protein and sRNA in the preparation were estimated by analyzing the UV absorption spectrum from 235 to 300 mμ. The solution was then divided into small aliquots, shell-frozen in dry ice-acetone, and stored at −70°C until use. This single preparation was used for all experiments. The incubation system was saturated by an enzyme preparation

containing approximately 100 μg protein regardless of whether free amino acids or aminoacyl-sRNA was added, thereby indicating that both loading enzymes and Lipmann factors A and B are present.

Preparation of reconstituted ribosomes: A modification of the method described by Wood and Berg[6] was utilized. Cells of the desired mutant were grown to log phase (4×10^8 cells/ml) in L medium, a check being made for reverse mutation each time. The cells were then harvested by centrifugation, washed once in buffer A, and stored frozen until used. All procedures were done at 4°C. Frozen cells were broken by alumina grinding as described above. After centrifugation twice at 10,000 *g* for 10 min and once at 39,000 *g* for 20 min, 1.7 gm of solid ammonium sulfate per 10 ml supernatant were added slowly over a 10-min period with constant stirring while maintaining the pH at 7.4 by addition of drops of 1 *N* NH_4OH. The suspension was allowed to stir for another 20 min, then centrifuged at 10,000 *g* for 10 min, and at 150,000 *g* for 90 min in a Spinco 50 rotor. The supernatant was discarded, and the pellet, which was colorless and transparent, was composed of ribosomes. To dissociate the subunits, this pellet was gently homogenized over a 30-min period in 10 ml buffer B (0.5 *M* NH_4Cl, 0.001 *M* Mg acetate, and 0.01 *M* Tris-HCl; pH 7.4). Large aggregates were removed by centrifuging at 10,000 *g* for 10 min. The suspension was then run at 150,000 *g* in a Spinco 50 rotor for $4^1/_2$ hr in order to sediment the 50*S* and 30*S* subunits. The pellet, which again was colorless and transparent, was gently homogenized in a minimal quantity of buffer A which causes the subunits to recombine. The preparation was then dialized for at least 24 hr against two changes of 100 vol of this buffer, and the purity and concentration of the reconstituted ribosomal preparation were determined by measuring the 235/260 and 280/260 absorption ratios in a Gilford spectrophotometer. The preparation was then divided into small aliquots, shell-frozen in dry ice-acetone, and stored at −20°C until use. Although reconstituted ribosomes are less susceptible to inhibition by Sm of C^{14}-phenylalanine incorporation in the presence of poly U, they have a low background which allows the detection of lower levels of misreading as compared to nonreconstituted ribosomes.

Incubation conditions: Incubations were carried out with reagents at the following concentrations: 0.05 *M* Tris-HCl, pH 7.4; 0.015 *M* Mg acetate; 0.06 *M* ammonium acetate; 0.006 *M* β-mercaptoethanol; 10^{-4} *M* GTP (Na^+ salt); 10^{-3} *M* ATP (Na^+ salt), and 0.005 *M* phosphoenolpyruvate (K^+ salt). In addition, each 0.25 ml of reaction mixture contained 7.5 μg of phosphoenolpyruvate kinase; 200 μg of ribosomes (equal to 3.3 OD units/ml at 260 mμ), 108 μg enzyme preparation, 100 μg of added sRNA (from *E. coli* B stripped, General Biochemicals), C^{12}-amino acids at a concentration of 5×10^{-3} *M*, C^{14}-amino acids as specified, and 20 μg of poly U (always from the same lot) or poly C, poly A, or poly I (from Miles Chemical Co.). These values, which gave maximal C^{14}-phenylalanine incorporation in the presence of poly U at a ribosome concentration of 200 μg per tube, are used as the "standard" conditions. One μg of Sm was added when indicated. The order of addition of reagents is immaterial. The mixtures were incubated at 37°C for 30 min and the reaction was stopped by addition of 1 ml 10% trichloroacetic acid (TCA) per tube. After heating for 20 min in a boiling water bath, the precipitates were collected by Millipore filtration, washed with a solution of 5% TCA and 1% Casamino acids, dried, and counted in a Nuclear-Chicago gas flow low-background end-window counter.

Results.—Experiments in vivo: B40 is an arginine (or citrulline) auxotroph (derived from strain B by UV-mutagenesis) which bears an SSu defect in the structural gene of ornithine transcarbamylase (OTC).[7] In B40 itself this defect is manifested as a lack of OTC activity, but it becomes Sm-suppressible (CSD) in B40 derivatives which have undergone a second mutational step, unrelated to the OTC gene, which releases the non-endproduct repression of the arginine biosynthetic pathway peculiar to strain B.[8] This derepression is not essential for the mechanism of Sm-suppression itself, but it is required for its expression in B40.[1]

Since the CSD phenotype is not associated with any particular level of cell resistance to Sm,[9] CSD mutants have been derived from several strains of B40 differing in their sensitivity to Sm. From B40 itself (which is Sm^S) CSD mutants, able to dispense with the arginine requirement in the presence of sublethal amounts of Sm, have been selected on plates of minimal medium and 1 or 2 μg/ml of Sm.

CSD mutants from B40 Sm^{R_L} (indifferent to 10 but sensitive to 20 μg/ml Sm) and from B40 Sm^R (indifferent to >1000 μg/ml of Sm) have been selected in an analogous way, using 10 or 500 μg/ml Sm, respectively. The CSD derivatives from B40, B40 Sm^R, and B40 Sm^{R_L} arose with a frequency of about 10^{-7} and were found derepressed (to various extents) in the arginine pathway as expected, since in all three cases a mutation at the same repression site is assumed.

Figure 1 outlines the paths used for selection of competent and incompetent Sm^R mutants of strain B40. When (as in path 1) the Sm^R mutation is imposed prior to

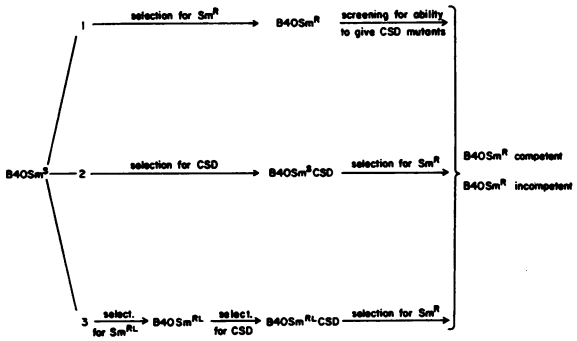

FIG. 1.—Derivation of B40 Sm^R mutants.

the derepression step, the competence of B40 Sm^R clones is tested by spreading them (10^8 to 10^9 cells/plate) on plates of minimal medium A with 500 μg/ml Sm. If the Sm^R clone was competent, 10–100 CSD mutants should appear while none should occur if it was incompetent. If (as in paths 2 and 3) the Sm^R mutation is imposed after the selection for the CSD phenotype, then the competence is seen directly, for with a competent Sm^R allele the arg CSD phenotype is maintained, whereas with an incompetent Sm^R allele the mutant appears as an arginine auxotroph. The per cent of competent and incompetent Sm^R mutants obtained from two B40 CSD parents, one Sm^S and the other Sm^{R_L}, are given in Table 1.

TABLE 1

COMPETENCE OF DIFFERENT Sm^R MUTANTS FROM B40 FOR CORRECTING THE OTC^{SSu} DEFECT

Parent (arg CSD)		Sm^R Clones		
Name	Sm genotype	Total	Incompetent (arg$^-$), %	Competent (arg CSD), %
B40-1	S	11	82	18
B40-2	R_L	23	61	39

The phenotype of the defective gene of ornithine transcarbamylase carried by B40 is given in parentheses.

A second auxotrophic mutation has been imposed on several B40 Sm^R mutants, competent and incompetent, obtained through the different paths outlined in Figure 1. A large number of auxotrophs were then screened for CSD character of the new auxotrophic marker. Table 2 gives the results of such an experiment. It can be seen that the absence of competence for OTC^{SSu} suppression does not prevent a strain from being competent for Sm-suppression of a second SSu defect. Notice that competence for a second defect is seen in 3 out of 4 mutants incompetent for

TABLE 2

COMPETENCE OF DIFFERENT Sm^R MUTANTS FROM B40 FOR CORRECTING A SECOND DEFECT

Parents		Auxotrophs Obtained by NG Mutagenesis		
Name	Competence for OTC^{SSu}	Total tested	No. with new SSu marker	Nature of SSu markers
B40-P-4	Competent	230	5 (2.8%)	Arg and leu or met
" -19	"	87	7 (8.0%)	Arg and phe or met
" -2	Incompetent	67	2 (3.3%)	— leu or his
" -1	"	135	0	None
B40-N-16	Competent	94	9 (9.5%)	Arg and X
" -12	"	265	19 (7.2%)	Arg and X
" -1 to 9	Incompetent	300 each (2700 total)	0	None
B40-T-1	Incompetent	300	7 (2.3%)	— X
" -2	"	300	12 (4%)	— X

Strains B40-P = mutants Sm^R obtained from Sm^S B40 CSD (path 2, see Fig. 1); strains B40-N = mutants Sm^R obtained from Sm^{RL} B40 CSD (path 3, see Fig. 1); strains B40-T = mutants Sm^R obtained from B40 Sm^S (path 1, see Fig. 1). OTC^{SSu} = defective gene of ornithine transcarbamylase carried by B40. X = marker not determined.

OTC correction obtained through paths 1 or 2 but in none from the 9 obtained through path 3. With the reservations inherent in the interpretation of a negative result, this may reflect the genetic difference between Sm^R mutants obtained in one step (paths 1 and 2) and in 2 steps (path 3).

It is known[10] that when arginine prototrophs are selected from competent Sm^R mutants of B40, true revertants and suppressed mutants are obtained at the same time. They are distinguishable because in the latter the Sm^R character has become Sm-inhibited. Such a pleiotropic suppressor mutation occurs with a frequency of 10^{-7}. It was expected that a second SSu defect in the competent strain should be similarly suppressed. Five double CSD (OTC^{SSu} and X^{SSu} where X represents methionine, valine, or isoleucine) strains were spread on plates lacking either one or both of the two requirements. Out of more than 1400 "revertants" examined, only one class of Sm-inhibited suppressor mutants was found: it exhibited the OTC^+ and X^+ phenotype together and it also occurred with a frequency of 10^{-7}. This indicates that in the double CSD the SSu defect in OTC and X arose from the same type of code error.

Arginine prototrophs of the suppressed type from B40 Sm^R incompetent strains are indistinguishable from true revertants, since the Sm^R character remains unchanged. However, if the SSu defects in OTC and X were equivalent in strains which are OTC^{SSu} incompetent and X^{SSu} competent, one should obtain pleiotropic suppressed mutants with the phenotype $OTC^+X^+Sm^R$. However, no mutants of this type (nor of $OTC^- X^+$ Sm-inhibited type) were found among more than 1000 "revertants" obtained from five such strains ($OTC^- X$ CSD phenotype where X represents isoleucine or leucine) by spreading on media lacking either one or both of the two growth requirements.

Experiments in vitro: In preliminary experiments we tested 4 homopolymers (U,C,A, and I), using C^{14}-algal protein hydrolysate (New England Nuclear Corp.) and quenching the appropriate amino acids. The results indicated that poly U gave a higher level of misreading with B40 CSD Sm^R ribosomes than did the other 3 artificial messengers. We have therefore first studied those amino acids which are known to be incorporated through misreading of poly U in the presence of Sm.[11] Conditions were found for analyzing serine and isoleucine, but tyrosine could not be examined because of its high background. C^{14}-isoleucine or C^{14}-serine (240 and

120 mC/mmole, respectively, Schwarz BioResearch, Inc.) was used at a concentration of 2.1×10^{-4} *M* (no C^{12}-isoleucine or serine added). In order to ensure that the level of the labeled amino acid was not rate-limiting, an experiment was done using $^1/_{10}$ the quantity of C^{14}-isoleucine. It was found that the background was lowered but that the poly U-directed incorporation was unaltered. In the absence of enzyme, no incorporation occurred, thereby indicating that enzymatic catalysis is required for the finding of hot TCA-precipitable counts.

Since variations in the *in vitro* incubation conditions can give rise to different levels of misreading, absolute values are not important. What is significant is the comparison under the same conditions of different ribosomes, identically prepared. Table 3 compares ribosomes isolated from one Sm^S and from 2 Sm^R strains for their ability, *without Sm*, to incorporate phenylalanine, isoleucine, and serine under the direction of poly U (each amino acid being checked in a separate experiment). Although comparably efficient for the incorporation of phenylalanine, the Sm^R ribosomes showed a 10-fold decrease in misreading of isoleucine and serine in comparison to the wild type.

TABLE 3

POLY U-DIRECTED AMINO ACID INCORPORATION WITH RIBOSOMES FROM STRAIN B WILD TYPE AND Sm^R MUTANTS

Strain		Amino Acid Incorporation into Polypeptide ($\mu\mu$moles)			% Misreading	
Name	Sm genotype	Phenylalanine	Isoleucine	Serine	Isoleucine	Serine
B wild type	S	605	14	6.5	2.3	1.1
B40-N-16	R	450	0.6	2.3	0.1	0.5
B40-P-1	R	980	1.7	1.8	0.2	0.2

B40-N-16 is a Sm^R competent for OTC^{SSu} correction (arg CSD phenotype). B40-P-1 is a Sm^R incompetent for OTC^{SSu} correction (arg^- phenotype). % Misreading = amount of isoleucine or serine incorporated in % of phenylalanine incorporation. Background readings were: phenylalanine: 34 ± 8 cpm; isoleucine: 160 ± 50 cpm; serine: 303 ± 20 cpm. 1 $\mu\mu$M of phenylalanine = 0.2 cpm; 1 $\mu\mu$M of isoleucine = 96 cpm; 1 $\mu\mu$M of serine = 48 cpm.

TABLE 4

SM-MISREADING IN STRAINS DIFFERING BY THEIR COMPETENCE FOR SM-SUPPRESSION OF THE OTC^{SSu} DEFECT IN B40

Ribosomes Supplied by Strains				$\mu\mu$moles Incorporated into Polypeptide When		
Name	Competence for Correction of OTC^{SSu} defect	2nd Defect	Amino acid misread	Sm is absent	Sm is present	+Sm/−Sm
Wild type (Sm^S)	—	—	Ileu	14	58	4.1
"	—	—	Ser	6.5	53	8.4
B40-N-16	Yes	Found	Ileu	0.6	7.3	12
"	"	"	Ser	2.3	5.8	2.6
B40-N-16-5	Yes	Found	Ileu	1.1	7.4	7.1
"	"	"	Ser	1.5	3.7	2.4
B40-N-2	No	Not found	Ileu	0.6	0.5	0.8
"	"	"	Ser	1.6	<0.5	(<1?)
B40-P-2	No	Found	Ileu	2.5	2.8	1.1
"	"	"	Ser	1.4	1.1	0.8
B40-P-2-2	No	Found	Ileu	1.6	1.9	1.2
"	"	"	Ser	<1.0	<1.0	(1)
B40-P-1	No	Not found	Ileu	1.7	2.1	1.2
"	"	"	Ser	1.8	1.0	0.6

Each value on the table is the average of 2–3 separate experiments run in duplicate. Moreover, B40-N-16-5 and B40-P-2-2 are mutants derived from B40-N-16 and B40-P-2, respectively, carrying a second defect correctable by Sm. The ribosomal structure should be unaltered between the parent and its mutant, since the only change should be on a structural gene of a biosynthetic pathway. Therefore, the values for parent and mutant should be and are similar. For background reading see Table 3.

Table 4 shows the effect of Sm on the poly U-directed incorporation of misread amino acids. Ribosomes from the wild-type Sm^S strains were compared with those

from B40 Sm^R competent and incompetent strains. It can be seen that there was a 2–2$^1/_2$-fold greater Sm effect on isoleucine incorporation with ribosomes from B40 Sm^R strains, which are competent for the OTC correction, than with those from the wild type. The Sm effect on serine incorporation was reversed, being ~3 times less in the mutants than in the wild type. In contrast, ribosomes from B40 Sm^R strains that are incompetent for the OTC correction showed no Sm effect for either isoleucine or serine, regardless of whether or not they were competent for a second defect.

Discussion.—The Sm^R mutants isolated from strain B40 have been classified as "competent" or "incompetent" on the basis of their ability to permit Sm-suppression of a given SSu defect, the OTC^{SSu} borne by this strain. By superimposing a second auxotrophic mutation on the competent and incompetent Sm^R derivatives of B40, it has been shown that CSD mutants may be found among the auxotrophs obtained from both.

It has been shown that for all Sm^R competent strains (phenotype = arg CSD) which have been tested, a second defect for which they are also competent can be found (phenotype = CSD for arg and for X at the same time). Moreover, from each one of these double CSD's it is possible to isolate, with a frequency of 10^{-7}, pleiotropic suppressor mutants with the following phenotype: arg^+ X^+ Sm-inhibited. It has been previously reported[10] that a suppressor mutation, termed "oversuppression," with the same characteristics (from CSD to positive and from Sm^R to Sm-inhibited) may be obtained from B40 CSD, which bears a competent Sm^R mutation and is the parent of the present double CSD's. It is highly likely that, as the suppressed product is the same, we are dealing with the same suppressor.

The majority of the Sm^R incompetent strains (phenotype = arg^-) were found competent for a second mutation (phenotype = arg^- X CSD). In their case no pleiotropic suppressor mutants (phenotype = arg^+ X^+ Sm^R or arg^- X^+ Sm-inhibited) were obtained. This indicates that a given Sm^R allele possesses a degree of selectivity for correctable codon errors similar to that possessed by a genetic suppressor. Finally, our results reveal another class of Sm^R mutants, incompetent (up to the level of detection) for any Sm-suppression.

The isoleucine and serine misincorporation in the presence of poly U has been studied *in vitro* by comparing ribosomes extracted from Sm^S and Sm^R, competent and incompetent, strains. In the absence of Sm there is a basal level of misincorporation of both amino acids. This level is about 1 per cent of the correct incorporation with Sm^S and 0.1 per cent with Sm^R ribosomes. It thus appears that, although Sm^S and Sm^R ribosomes are equally efficient for the correct amino acid incorporation, the structure of the Sm^R ribosome is somewhat less flexible in allowing misreadings. This finding indicates that ribosomes have different levels of ambiguity *per se* in the absence of Sm, and that these levels reflect differences in the ribosomal structure produced by mutations at the classical Sm locus as previously suggested.[12] Furthermore, it is significant that the responsiveness to Sm is quite different with different ribosomes. The index of misincorporation, i.e., the factor by which the basal level (in the absence of Sm) is changed, is different for isoleucine and serine and varies in opposite directions when Sm^S and Sm^R CSD ribosomes are compared: for isoleucine it is 2–3 times higher in the CSD's than in the Sm^S ribosomes; for serine it is 3–4 times lower. It is evident that a mutation to

Sm^R *per se* does not cancel the responsiveness to the misreading action of Sm. On the contrary, CSD ribosomes may be more responsive than the Sm^S ones, depending on the amino acid misread. The absolute figures obtained with CSD Sm^R ribosomes are less conspicuous, however, because the basal level of misreading is about 10 times less. This accounts, incidentally, for the failure to demonstrate misreadings using an *in vitro* system which is less sensitive.

It is also clear that different Sm^R alleles behave quite differently. In the particular case of competence and incompetence for OTC^{SSu} correction studied here, and considering only the misincorporation of isoleucine and serine in the presence of poly U, the Sm^R incompetent ribosomes are indifferent to Sm action, irrespective of whether competence for a second defect has been found. But presumably all strains which are competent, given the right nucleotide sequences, would misread in the presence of Sm. The same must hold for competence of all other phenotypes (Sm^S, Sm^D) of the Sm locus.

It would therefore seem that each Sm allele is able to alter the ribosome in a slightly different manner. It is postulated that some of these alterations provide the ability to misread specific codons. An SSu defect would be one in which the mutated codon of the structural gene of the deficient enzyme would be misread on the competent ribosome in such a way that the correct (or another usable) amino acid is incorporated. It is not so far possible, however, to discriminate between ribosomes permitting different levels or different patterns of ambiguity.

Summary.—Streptomycin (Sm)-resistant mutants have been isolated from a strain bearing a defective ornithine transcarbamylase phenotypically suppressible in the presence of Sm (OTC^{SSu}). Some of them remain competent and others become incompetent for the Sm-activated suppression of the OTC^{SSu} defect. Within each class the imposition of further auxotrophic mutations yields strains that are competent for the Sm-activated suppression of some of these additional mutations. *In vivo* these strains have different responses to one type of genetic suppressor. Ribosomes isolated from these different strains have been shown to differ in their response to the misreading action of Sm.

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