MECHANISMS OF SUPPRESSION

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I. Introduction

A. Suppressors in Genetics

Suppressor mutations are one class of secondary mutations ("modifiers") that modify the phenotype *in the presence of* the originally mutant gene. In contrast to "enhancers" that make the mutant phenotype more extreme, *suppressor mutations* yield organisms phenotypically more like the wild type: the mutant phenotype is "suppressed." In some cases the gross wild-type phenotype is completely restored; in other cases, restoration is only partial. Thus, suppressors are mutations that elicit a revertant or partially revertant phenotype. But suppressor mutations can be genetically separated, by recombination, from the mutation(s) that they suppress.

The first cases of genetic suppression were interpreted as gene duplications (Bridges, 1919; Morgan *et al.*, 1925) and, indeed, some were duplications (Morgan *et al.*, 1925; Schultz and Bridges, 1932). Later experiments showed that suppression also could result from interaction between nonallelic genes (Bonnier, 1927; Plough, 1928; Bridges, 1932; Schultz and Bridges, 1932). Biochemical analyses, coupled with microbial genetic methods, in the 1950s began to shed light into the mechanisms underlying particular nonallelic suppressor activities. Brief critical reviews of suppressor action appeared (Wagner and Mitchell, 1955, 1964; Yanofsky and St. Lawrence, 1960; Campbell, 1963; Gorini and Beckwith, 1966). The present review is intended as a supplement to these condensed reviews and more recent extensive summaries restricted to aspects of informational suppression (Garen, 1968; Davies, 1969; Gorini, 1970). Our intent here is to describe systems that seem to us particularly informative and/or illustrative of types of suppressor activity. Our summary of these few selected examples also calls attention to the vast potential offered by the study of suppressor activities.

Suppressor analysis can yield insight into arrays of problems not readily subject to more classic genetic experimentation. The examples described below indicate that suppressors can supply basic information on unsuspected interactions as well as providing probes so that direct selection for reversion through suppression may allow ready isolation of mutations of primary interest. For example, much of the critical experimentation in the *E. coli* lactose system depends in one fashion or another on mutants recognized by their abilities in suppression under defined sets of conditions (cf. Reznikoff, 1972). We believe that the analysis of suppressor mutations will find expanding and increasingly important use as the techniques of genetics and molecular biology are applied to more complicated biological situations.

Some ways in which suppressors act are summarized in Fig. 1. The figure and our outline show that we attempt to describe suppressor actions in terms of biochemical mechanisms. Contributions to genetics and the resolving power of suppressor studies also will be touched upon.

B. Nomenclature

In *Drosophila* the classical symbols for suppressors are *su* or *Su* for recessive or for dominant suppressors, respectively, followed by more specific designation (e.g., *Su-S* for a dominant suppressor of *S*, star) (Lindsley and Grell, 1968). In bacteria the standardized nomenclature of Demerec *et al.* (1966) has been supplemented to include compatible symbols such as *sup* for suppressor (Sanderson, 1970; Taylor, 1970),

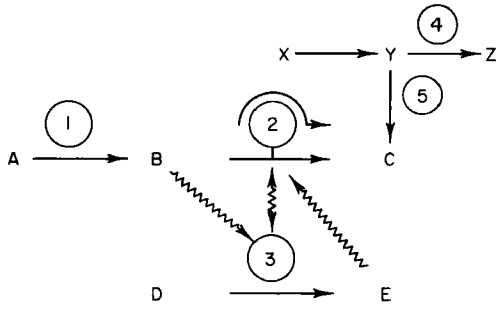

FIG. 1. General modes of suppression of enzyme defects. Metabolites are designated by capital letters, and genes and enzymes by numerals. A mutation in gene *2* the structural gene for enzyme 2, may be suppressed by a second mutation which:

1. Allows production of some wild-type or effective enzyme 2 from mutant gene *2* messenger RNA via an alteration in the protein-synthesizing system ("Informational" suppression).

2. Occurs in the same gene (intragenic suppression) but rectifies the effect of the first mutation by: (a) a change in a second letter in the same triplet codon affected by the first mutation, allowing insertion of an amino acid more compatible with functioning of enzyme 2; (b) a second, genetically separate mutation in gene *2* that leads to a "doubly mutant" enzyme 2: (1) enzyme 2 has regained functional activity, (2) reinitiation of messenger RNA or polypeptide synthesis relieves polar effects of nonsense codons or defective promotors, or (3) a toxic polypeptide is eliminated.

3. Increases the amount of partially defective enzyme 2 through increased gene dosage or through altered regulation of enzyme 2 production.

4. Releases an inhibition of mutationally altered enzyme 2 by ions, metabolites, or macromolecules (the wild-type enzyme 2 may be inhibited to some extent by the same agents or the mutationally altered enzyme 2 may be uniquely sensitive to these factors).

5. Increases substrate B by affecting the amount or the regulation of enzyme 1 activity.

6. Allows catalysis by an alternate protein that mimics enzyme 2 in its function ("duplicate gene"). To be effective in suppression the duplicate gene may be placed under new regulation or it may assume its new role through mutational alteration affecting substrate specificity.

7. Supplies metabolite C from a second pathway which may be parallel (X = B, Y = C) or which may be unique (X and Y distinct from B and C) by mutational alterations affecting accumulation of Y (defective enzyme 4) or conversion of Y to C (altered control or constitution of enzyme 5).

8. Relieves inhibition of other reactions by accumulated compound B through: (a) limit in synthesis of B via altered regulation or decreased efficiency of enzyme 1; (b) Lowered sensitivity of the inhibitory site, for example, enzyme 3, through an increase in the amount of enzyme 3, a decrease in its sensitivity to inhibition, or more ready availability of substrate D.

Numerous additions, rearrangements, and modifications of the above examples can be envisioned; they merely serve to point out some of the highlights of suppressor action.

suf for suppressor of frameshift mutations (Riddle and Roth, 1970), *sbc* for suppressors of *recB* and *recC* mutations (Barbour and Clark, 1970; Barbour *et al.*, 1970). In some cases the special phenotypic attributes imparted by the suppressor mutation (e.g., *crr* for catabolite repression resistant in bacteria) or knowledge of its biochemical mode of action (e.g., *pyr-3* for suppressors of *arg* mutants in *Neurospora*) have suggested special symbols for designation of loci that sometimes have other alleles which behave differently. In all cases the symbol + stands for the wild type which, most often, does not contain the suppressor activity in question. This usage has been distorted in the case of some papers on suppressors in bacterial systems where + conveys the idea that the suppressor strain *has* the particular suppressor and su^- is used for the strain lacking suppressor activity. While this unique usage is something to be wary of when reading the literature, our usage here will adhere to su^+ or an analogous symbol for wild type, in keeping with worldwide usage for a variety of genetic markers in a variety of organisms over a span of many years.

II. Intragenic Suppression ("Internal" Suppression)

We consider a gene as a polynucleotide stretch from which a functional segment of an RNA molecule is transcribed. Genes sometimes serve to dictate the base sequence, and thus the structure, of RNA molecules directly functional in the cell, for example, transfer RNA (tRNA) and ribosomal RNA (rRNA). Genes also serve as templates "transcribed" into messenger RNA (mRNA), which then guides synthesis of polypeptide chains. This "translation" into polypeptide product is achieved through initiation of the N-terminal amino acid at the proper site on the mRNA, reading of the subsequent base sequence in strict sets of triplets, each coding for an amino acid, and termination at a nontranslatable "nonsense" triplet at the C-terminal end of the polypeptide. Figure 2 shows the coding triplets (codons on mRNA) for the amino acids and the chain-terminating nonsense triplets.

Mutations occur through changes in the sequence of base pairs in the DNA. These may be (a) addition or deletion of one or more base pairs that disturb triplet reading at the site of mutation and, if not additions or deletions of sets of 3 base pairs, also affect subsequent reading ("frameshift" mutations), and (b) base substitutions leading to replacement in the polypeptide of one amino acid for another ("missense" mutations) or to premature chain termination ("nonsense" mutations: amber or UAG, ochre or UAA, and UGA).

First letter	Second letter U	C	A	G	Third letter
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Ochre nonsense	Nonsense	A
	Leu	Ser	Amber nonsense	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

FIG. 2. The genetic code. The abbreviated names of amino acids are given in the body of the table. For example, Met = methionine. Coding triplets (codons) on messenger RNA are read 5′ to 3′ using the first letter (left-hand column) and then the second letter (top heading) and thence the third letter (right-hand column). For example, the only codon for methionine is AUG. The three triplets that predominantly lead to polypeptide chain termination also are shown: these are UAA ("ochre"), UAG ("amber"), and UGA.

Below are examples of suppressors which have their effect as a consequence of a secondary change within the original mutant gene.

A. Different Letter of a Codon

a. Yanofsky and co-workers found two missense mutations that recombined at very low frequency to yield wild type and contained different amino acid substitutions at residue 210 of *Escherichia coli* tryptophan synthetase A protein (cf. Yanofsky *et al.*, 1967). These substitutions were Gly (GGA) to Arg (AGA) in mutant A23 (Helinski and Yanofsky, 1962) and the same Gly (GGA) to Glu (GAA) in mutant A46 (Henning and Yanofsky, 1962a). Various full and partial (weak enzyme activity) revertants were next described containing at position 210: Val (GUA),

Ala (GCA), and Gly (GGA) from mutant A46 and Gly (GGA), Ser ($AG\frac{U}{C}$) from mutant A23 (Henning and Yanofsky, 1962b; Allen and Yanofsky, 1963; Yanofsky, 1963; Carlton and Yanofsky, 1963). Later, additional substitutions at the same residue yielding ten different amino acids as well as studies of double frameshift mutants served to verify the *in vivo* codon assignments listed above (Yanofsky, 1965; Yanofsky *et al.*, 1966, 1969; Berger and Yanofsky, 1967; Berger *et al.*, 1968a). The substitutions are summarized on page 144 of Hartman and Suskind (1969).

The collective data conclusively show that any one of a variety of amino acids at residue 210 is compatible with enzyme catalysis while a few amino acids lead to inactive protein. Some revertants that are phenotypically wild type as judged by growth properties, accumulations, and even by some general properties of the enzyme in extract are in fact pseudo-revertants. They contain an amino acid coding triplet that is mutationally altered in *two different* base pairs, recombinationally separable at low frequency. Analogous observations have been made in yeast (Sherman *et al.*, 1970; Sherman and Stewart, 1971).

b. Studies similar to those described above have been performed in analyses of revertants of chain-terminating, nonsense mutations (Fig. 3). A particular nonsense triplet may arise by mutation of a number of different coding sequences in the DNA. Similarly, the nonsense coding sequence may revert either back to the original form (true reversion) or to any one of a number of other sequences that lead to the insertion of an amino acid compatible with functioning of the protein involved. These latter mutations often involve an alteration in a nucleotide pair not involved in the original mutational event. Figure 3 presents a compilation of the mutational changes found in several bacterial systems (Weigert *et al.*, 1966, 1967; Sarabhai and Brenner, 1967; Brenner *et al.*, 1967) and in yeast (Sherman *et al.*, 1970). These studies contributed to deduction of the *in vivo* genetic code and, today, lend caution in analyses of the action of chemical mutagens in cases where protein primary structure is not examined.

B. Active Conformation

Here we summarize cases where the deleterious effects of a mutation on the macromolecular gene product are partially or completely rectified by a balancing change at a second place in the same molecule. This applies both in cases involving polypeptide gene products and those involving RNA end products such as transfer RNA.

a. An amino acid substitution at one position in a polypeptide chain

sometimes can be compensated for by a second substitution at some distance away in the same polypeptide ("second-site reversion"). Thus, a Gly to Glu substitution at position 210 of the tryptophan synthetase A protein resulted in inactive enzyme but activity was present if a second substitution, Try to Cys, occurred at position 174 (Fig. 4). Both

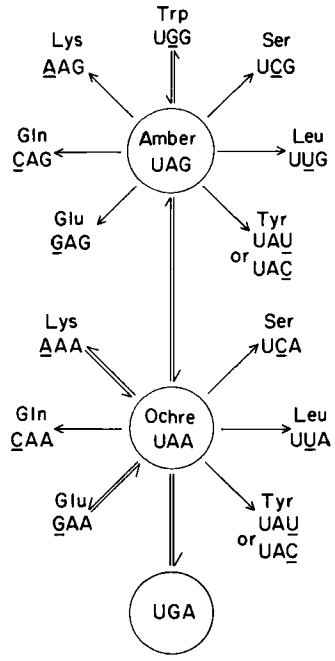

FIG. 3. Messenger RNA sequences due to base-substitution mutations leading to and from amber and ochre nonsense codons in *Escherichia coli*. Nonsense mutations (circled) were elicited. Where the amino acid originally present in the wild-type protein is known or can be strongly inferred, an arrow points from that amino acid with its appropriate codon to the nonsense codon. Revertants of the nonsense mutants have been obtained, and the particular amino acid replacement in each revertant has been determined or inferrred from genetic experiments. Reversions are indicated by arrows pointing from the nonsense codons to the respective amino acids. The compilation shows only those changes actually observed; in each case, changes in both directions are theoretically possible.

mutations were required for activity; the Tyr to Cys substitution alone led to inactive enzyme (Helinski and Yanofsky, 1963). The two mutations suppressed each other. A similar situation was found for a mutant, A187, which contained Val both at residue 120 and at residue 212 and lacked enzyme activity. Mutant A187 could revert by mutations leading to substitution of Ala at position 210, Ala at position 212, or by a

Leu to Arg substitution at position 176 (Yanofsky *et al.*, 1964; Carlton and Yanofsky, 1965). The arrangement of these compensating changes led Yanofsky *et al.* (1964) to suggest that residues 174–176 interact with residues 210–212 in the folded protein (Fig. 4). While the necessity for such direct interaction of different parts of the polypeptide chain is conjectural, there is no doubt that suppression of one genetic defect in a gene can be achieved by compensating amino acid substitution elsewhere in the same structural gene.

Brockman (1968) describes second-site reversion of an *adenine-3B* mutant in *Neurospora* that appears analogous to the cases just mentioned.

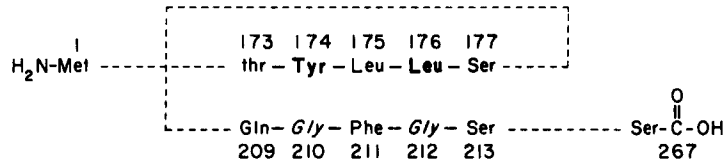

FIG. 4. Possible relationship between different regions of the folded wild-type tryptophan synthetase A protein molecule suggested by second-site reversion analysis. The amino acids changed in primary mutational events are shown in italics and those changes by second-site reversion are shown in bold letters. An amino acid replacement at residue 210 is compensated for by a second replacement in residue 174, and vice versa. Similarly amino acid substitutions at residues 176 and 212 compensate for each other. The spatial arrangement of compensating changes in the polypeptide chain had led to the speculation that the two segments of the chain interact when the protein achieves its final tertiary structure.

Mills and Ellingboe (1969) describe eight hydroxylamine-induced reversions of an arginine mutant (*arg-2*) in *Schizophyllum commune*. All eight grow on minimal medium but are recessive when combined in heterokaryons with *arg-2*. That is, *arg-2* possesses a dominant effect in complementation, as sometimes occurs in tests with mutant missense proteins when the enzyme is a multimer (e.g., Fincham, 1966; Foley *et al.*, 1965; Nashed *et al.*, 1967; Zimmermann *et al.*, 1969). Seven of the "suppressors" could not be separated from *arg-2*, but another (*su-1*) was separable. In crosses of *arg-2 su-1* to wild type, six progeny identical in properties to *arg-2* were recovered along with a new phenotype in 13 others (= *arg-2*$^+$ *su-1* ?) out of 87 progeny tested. These latter recombinants were arginine-requiring, like *arg-2*, but were effective in weak complementation with *arg-2*. Complementation is often found for widely spaced missense mutations in a gene which exhibits complementation. The easiest interpretation is that *su-1* is a new missense mutation in the *arg-2* gene. It also, however, could be defective in a gene coding

for a distinct protein species that aggregates with the product of the *arg-2* gene to form an active multimer. The other seven "suppressors" are either changes in the mutant codon of *arg-2* or are closely allied second-site intragenic missense mutations. Heterokaryons between *arg-2 su-1* and the other suppressed strains (e.g., *"arg-2 su-2"* = altered *arg-2* codon or nearby base-pair change) grow on minimal medium, indicating that the dominant blocking of complementation by *arg-2* has been alleviated by the intragenic "suppressor."

Mills and Ellingboe (1969) were perplexed by the high percentage of recombination between *su-1* and *arg-2*. However, an entirely analogous situation was more adequately investigated by Morgan (1966) in *Coprinus* where a spectrum of recombination values was obtained in a series of 41 suppressor mutations. And, in part, the observation of "allelic suppression" was previously described in *Aspergillus* for *paba-22* revertants by Luig (1962). So this seems something to be wary of in diploids and heterokaryons, namely, a second intragenic change that allows complementation under certain circumstances. Such situations mimic exactly the behavior expected of an extragenic suppressor. Material selected for in such studies, however, could supplement more conventional, randomly obtained mutants for studies of genetic map position and complementation behavior in relation to protein tertiary structure (cf. Fincham, 1966; Gillie, 1966, 1968).

b. An additional method potentially useful for studies of possible interactions between different parts of polypeptide chains *in vivo* may be regarded operationally as analogous to the cases of intragenic suppression just described. The method relies first upon reversion at a site in one protein that engenders a "semi-acceptable," but not optimal, amino acid sequence. One then screens for *new mutants* that again have lost the function in question. One can picture three types of mutants arising from the strain now "sensitized" by its possession of an altered polypeptide chain; the proportion of each type will be dictated by the remaining nucleotide sequence, the position of the initial change with regard to the active center of the enzyme, and on interactions of polypeptide chains (different segments of the same chain as well as interactions between independent chains): (a) mutants with new alterations at or near the "semi-acceptable" site, (b) mutations in parts of the gene dictating amino acid sequence in the portion of the polypeptide that interacts with the defective site (intrachain associations), and (c) similar interactions as in (b) but involving two different polypeptides (in cases where the polypeptide is part of an oligomeric complex).

In an apparent analysis of this type in *Salmonella*, Riyasaty and Dawson (1967) found a partial revertant of a tryptophan-requiring mu-

tant, *trpA47*, that gave rise to an unusually high frequency of mutants that again were tryptophan requiring. These comprised: (1) mutations that either were "silent" in wild-type genetic background or mapped at or very close to the original site of mutation, (2) mutations in at least five different nucleotide pairs more distantly located and presumed to lie within the *trpA* gene (but which actually could lie in the *trpB* gene and merely elicit a *"trpA"* phenotype), and (3) mutations in one (or more) nearby *trp* genes. Mutant *trpA47* is a frameshift mutation with defective anthranilate synthetase (ASase) specified by the *trpA* gene (Bauerle and Margolin, 1966). ASase function is activated by association of the *trpA* gene product with the polypeptide product of the *trpB* gene that, in addition, catalyzes the next step of tryptophan biosynthesis (Bauerle and Margolin, 1966; Ito and Yanofsky, 1966, 1969; Zalkin and Kling, 1968; Smith and Bauerle, 1969; Ito *et al.*, 1969; Tamir and Srinivasan, 1969; Henderson *et al.*, 1970; Nagano and Zalkin, 1970; Nagano *et al.*, 1970). Thorough analysis of this system might supplement other information (Hwang and Zalkin, 1971; Yanofsky *et al.*, 1971; Grieshaber and Bauerle, 1972) not only in revealing associations of different parts of one polypeptide chain but also how this polypeptide interacts with another to form an active complex (cf. Stuttard and Dawson, 1969). Chemical cross-linkage (Myers and Hardman, 1971) would seem a nongenetic method of potential usefulness in confirmatory study of protein tertiary and quaternary structure.

A second case illustrating the genetic approach is that studied by Koch and Drake (1970). They started with a leaky, "sensitizing" mutation in the bacteriophage T4 *rIIA* gene and isolated mutants with complete *rII* phenotype. Some of the newly isolated *rII* mutations were cryptic, exhibiting a pure *rII* phenotype only in the presence of the original sensitizing mutation. Cryptic mutations were found at certain regions of the *rIIA* gene as well as in the *rIIB* gene, indicating that the *rIIA* and *rIIB* proteins interact *in vivo*. In other systems, previously unsuspected interactions could well be revealed or useful mutants isolated by these and accessory genetic methods (see page 35).

c. Jinks (1961b) described interesting suppressors for mutants of the T4 bacteriophage *h* gene region. If the suppressors are internal suppressors, the mutations suppressed must be missense mutations or in-phase (triplet) deletions since the *h* gene product is involved with tail fibers, phage structures essential to phage maturation and adsorption. Of 129 revertants for the several host-range (*h*) mutants examined (Jinks, 1961a) all were found to be due to suppressors, and all but three mapped in the *h* region of the phage chromosome close to the original sites of mutation (Jinks, 1961b). This is the region where all but one of the

tail fiber protein and assembly genes are located (cf. Epstein *et al.*, 1963; Wood *et al.*, 1968). Some of the suppressors suppressed more than one *h* mutant, but none of the suppressors could be isolated from these original sites of mutation (i.e., they had a wild-type phenotype or were lethal when isolated). The suppressors primarily were selected on the basis of reversion of phage thermal sensitivity and secondarily for suppression of altered host-range phenotype. Therefore, they probably represent a unique class of revertants. Because they map close to the original sites of mutation, the suppressors could be second-site revertants of the type described above for tryptophan synthetase. On the other hand, their explanation may lie in the intricacies of phage tail fiber assembly (cf. King and Wood, 1969; Ward *et al.*, 1970; Takata and Tsugita, 1970). At least some suppressors may involve changes in one tail fiber component that compensate, through protein–protein interactions, for alterations in a separate constituent (see page 66).

d. Genes for tRNA molecules also can undergo second-site reversion. Studies of multiply mutant tRNAs are yielding information pertinent to the *in vivo* conformation of tRNA and its interaction with other macromolecules.

A gene, *su-3* (or su_{III}) in *E. coli,* can mutate to a form active in suppression of chain-terminating nonsense mutations of the amber (UAG) type leading to the insertion of tyrosine at the amber triplet (Weigert *et al.*, 1965; Kaplan *et al.*, 1965; Garen *et al.*, 1965). This informational suppression is achieved when the gene product, a tyrosine transfer RNA (Andoh and Ozeki, 1967; Landy *et al.*, 1967), contains an altered anticodon capable of "recognizing" UAG sequences of messenger RNA (Goodman *et al.*, 1968). Most recently, it has been demonstrated that intragenic secondary mutations that render the suppressor tRNA nonfunctional can be detected and the tRNA analyzed; further intragenic mutations that again result in active informational suppression also can be assessed. Such studies have provided evidence pertinent to *in vivo* conformation of this tRNA, its function and stability (Abelson *et al.*, 1970; Smith *et al.*, 1970), on the structure of the gene region involved in suppression (Russell *et al.*, 1970), and on some steps in the maturation of tRNA following transcription (Altman, 1971).

E. coli mutants with nonfunctional "suppressor" tRNA and revertants therefrom were isolated by setting up alternate conditions under which amber suppression is either lethal or is required for growth. Bacteria are poisoned by exogenous galactose if they possess functional galactokinase but no epimerase (see also page 58). Therefore, strains were constructed with an amber mutation in the galactokinase gene and a nonsuppressible mutation in the epimerase gene. Mutants whose suppres-

sor had been rendered nonfunctional lacked galactokinase and thus could not accumulate the toxic intermediate, galactose phosphate. In the absence of galactose, selection was made for suppressor function by virtue of an amber mutation located elsewhere in the chromosome.

Most mutations causing a loss of suppression cause a severe reduction in the amount of suppressor tRNA. This may be due to improper folding and premature degradation of the mutant tRNA (Abelson *et al.*, 1970). Possibly many base substitutions have little or no effect on activity

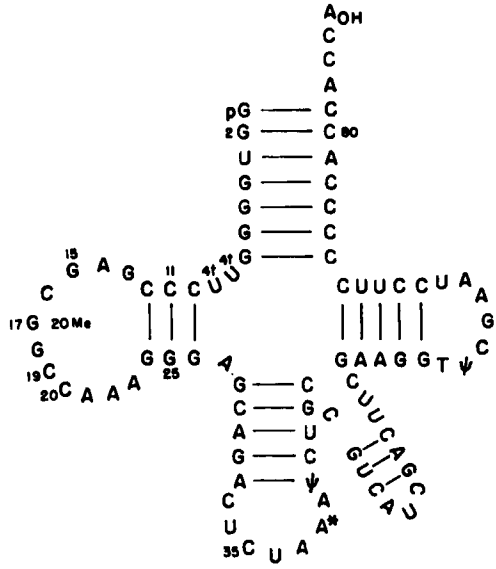

FIG. 5. Cloverleaf model of *su*$_{III}$ *tRNA*^{Tyr} *of Escherichia coli.* The nucleotide sequence is from Goodman *et al.* (1968). Residue numbers are referred to in the text and in Table 1.

but can alter the structure so as to cause gross instability. Mutants with temperature-sensitive tRNA, functional at 32°C but nonfunctional at 42°C, contain simple base substitutions in the amino acid acceptor arm of the standard "cloverleaf" arrangement (Fig. 5). One such temperature-sensitive mutation (A2) causes a G → A change at residue 2. This base substitution prevents the formation of the G:C base pair which normally occurs between residues 2 and 80, and leads to a temperature-sensitive tRNA. Among the revertants of A2 are second-site revertants which have a C → U change at residue 80. This change permits residues 2 and 80 to pair again, although the pair is now an A:U rather than the G:C in wild-type tRNA. With either pair in place, the tRNA

can function at both 42° and 32°C. The fact that this second-site revertant permits 2 and 80 to pair again, suggests strongly that bases 2 and 80 are in contact and that a cloverleaf arrangement (cf. Cramer, 1971; Arnott, 1971) has at least partial validity *in vivo*.

A second case of such restored pairing is provided by temperature-sensitive mutant A25, a G → A change at residue 25. In the "cloverleaf" arrangement, base 25 is paired with base 11 in the arm of the dihydrouracil loop (Fig. 5). Among the revertants of A25 is a second-site

TABLE 1
Summary of Second-Site Reversions Affecting Su_{III} Transfer RNA[a]

Genotype of tRNA[b]	Base change(s)	Function alteration
A2	G → A (residue 2)	Temperature sensitive
U80	C → U (residue 80)	Temperature sensitive
A2, U80	G → A (residue 2) C → U (residue 80)	Normal activity
A25	G → A (residue 25)	Loss of suppressor activity
A25, U11	G → A (residue 25) C → U (residue 11)	Fully restored suppressor activity
A25, U19	G → A (residue 25) C → U (residue 19)	Partially restored suppressor activity
A15	G → A (residue 15)	Loss of suppressor activity
A15, D19	G → A (residue 15) C → D[c] (residue 19)	Partially restored suppressor activity
A15, D20	G → A (residue 15) C → D[c] (residue 20)	Partially restored suppressor activity

[a] Data from Abelson *et al.* (1970) and Smith *et al.* (1970).

[b] All tRNAs carry the anticodon change G → C (residue 35) which permits reading of the amber codon.

[c] D = dihydrouracil; this is probably formed by modification of uracil.

revertant having a C → U replacement at residue 11. This change would restore a base pair between residues 11 and 25. This restored pairing apparently permits a functional tRNA at 42°C and 30°C even though a U:A pair replaces a C:G pair at that point in the structure.

Smith and co-workers (1970) have presented three other examples of second-site mutations restoring function to tRNA. These last three examples do not involve restoration of base pairing, but rather must cause other sorts of structural changes which compensate for the defect or the original mutant. These examples are presented in Table 1 (see Anderson and Smith (1972) for additional examples).

C. New Initiators and Elimination of Polarity

a. Sherman and co-workers (Stewart *et al.*, 1969; Sherman *et al.*, 1970) found second-site revertants of a yeast iso-1-cytochrome *c* mutant that lacked a normal polypeptide initiator codon (AUG = Met). The base substitutions in the second-site revertants created AUG codons effective in initiation of the cytochrome polypeptide chain at either of

Wild type	(Met)- Thr - Glu - Phe - Lys - Ala - Gly - -N N N-A U G-A C N-G A R-U U Y-A A G-G C N-G G N-
Revertant No. 1	Met - Ile - Thr - Glu - Phe - Lys - Ala - Gly - -A U G-A U A-A C N-G A R-U U Y-A A G-G C N-G G N-
Revertant No. 2	Met - Leu - Thr - Glu - - Lys -Phe Ala - Gly - -A U G-Y U G-A C N-G A R-U U Y-A A G-G C N-G G N-
Revertant No. 3	Met - Arg - Thr - Glu - Phe - Lys - Ala - Gly - -A U G-A G G-A C N-G A R-U U Y-A A G-G C N-G G N-
Revertant No. 4	(Met)- Val - Thr - Glu - Phe - Lys - Ala - Gly - -A U G-G U G-A C N-G A R-U U Y-A A G-G C N-G G N-
Revertant No. 5	(Met)- Ala - Gly - A U G-G C N-G G N-

Fig. 6. N-terminal amino acid and presumed messenger RNA sequences of yeast iso-1-cytochrome *c*. The sequences shown are for wild type and for 5 different second-site revertants of a mutant with a base-substitution in the normal AUG initiator (A U G in wild-type line). Methionine residues initiating polypeptide chains in the wild type and in revertants 4 and 5 were not detected owing to presumed elimination post-synthesis and are placed in parentheses. N = any of the four ribonucleotides, R = either of the two purine ribonucleotides, and Y = either of the two pyrimidine ribonucleotides. The complete amino acid sequence of the protein from yeast (Narita and Titani, 1969) also is given in Sherman *et al.* (1970).

two *new* sites, allowing synthesis of functional protein (Fig. 6). The studies elegantly demonstrate the initiator function of the AUG triplet in a eukaryote and the presence of an untranslated messenger sequence preceding the normal AUG initiation point. The studies also point out that in very special cases the corrective action of second-site suppressors operates on the translational level, not at the level of protein tertiary structure. Finally, the studies demonstrate an effect of the site of polypeptide initiation on protein level; Revertant 5 (Fig. 6) with an initia-

tion removed only 12 bases distally produces only about half of the normal iso-1-cytochrome *c* level.

b. Where several genes form a transcriptional unit, or operon, mutations in genes coding for proteins sometimes exert two effects: (1) an inactive gene product is made, and (2) the mutation exerts a "polar" effect. That is, the mutation limits the expression of distal genes in the same operon. This is a phenomenon apparent in a wide array of bacterial and phage operons (e.g., Franklin and Luria, 1961; Jacob and Monod, 1961; Ames *et al.*, 1963; Ito and Crawford, 1965; Henning *et al.*, 1965; Yanofsky and Ito, 1966; Bauerle and Margolin, 1966; Stahl *et al.*, 1966; Jordan and Saedler, 1967; Levinthal and Nikaido, 1969; Robertson *et al.*, 1970; Cordaro and Roseman, 1972). External suppressors can relieve polarity (e.g., Beckwith, 1963, 1964a; Jordan and Saedler, 1967; Morse and Primakoff, 1970; Carter and Newton, 1971). In addition, *intragenic* second-site mutations of two types also can suppress. The first type merely reverses the polar effects; the second type not only reverses polarity to some extent, but, in rare instances, also permits restoration of function to the product of the now doubly-mutant gene.

Polar mutations are nonsense mutations (Newton *et al.*, 1965; Henning *et al.*, 1965; Martin *et al.*, 1966; Yanofsky and Ito, 1966; Sambrook *et al.*, 1967; Jordan and Saedler, 1967; Zipser, 1967) or frameshift mutations (Whitfield *et al.*, 1966; Malamy, 1966) that place nonsense triplets in the phase of reading (Martin, 1967). In polarity a normal number of messenger RNA molecules is made, but many lack information distal to the general region of the nonsense mutation (Contesse *et al.*, 1966; Imamoto *et al.*, 1966; Imamoto and Yanofsky, 1967a,b). It is not clear whether polarity is due to a termination of transcription at or near intragenic nonsense triplets (Imamoto, 1970; Imamoto and Kano, 1971), to a low density of ribosomes beyond the nonsense triplet, allowing extremely rapid degradation of distal messenger RNA (Morse and Yanofsky, 1969b; Morse *et al.*, 1969; Morse and Primakoff, 1970; Morse, 1971; Morse and Guertin, 1971; Kuwano *et al.*, 1971), or to both factors. The extent of polarity often is a function of the distance of the intragenic nonsense triplet from the nucleotide sequence responsible for initiation of the next protein on the messenger RNA (Newton *et al.*, 1965; Yanofsky and Ito, 1966; Fink and Martin, 1967; Michels and Reznikoff, 1971). Revertants with restored levels of activity for distal genes may be selected without requiring return of function in the mutant gene. For example, mutations in the *E. coli* lactose operon that drastically decrease lactose permease levels by polar effects also are melibiose-negative at 40°C since melibiose enters only by lactose permease at this temperature. Revertants which are still lactose negative

but have lost polarity may be selected on melibiose (Beckwith, 1964a,b). In the case of the tryptophan system, regained ability to grow on a biosynthetic intermediate is selected (Balbinder *et al.*, 1968).

One mode of suppression brings the terminator triplet closer to the initiator for the subsequent protein by deletion of the intervening genetic material, thus reducing polarity and allowing gene function (Beckwith, 1964a,b; Newton, 1966; Zipser and Newton, 1967; Balbinder *et al.*, 1968). In some cases the site of the polar mutation is excised and polarity is eliminated by in-phase deletions (Beckwith, 1964a,b; Balbinder *et al.*, 1968; Elseviers *et al.*, 1969). Rechler and co-workers (Rechler and Bruni, 1971; Rechler *et al.*, 1972; Bruni *et al.*, 1972) report an interesting case where mutation affecting a polar restrictive site results in fusion of two genes to yield a bifunctional enzyme.

A second way polarity effects are relieved is through the introduction of a new polypeptide initiation site near and distal to the polar mutation. Such translational reinitiation mutations eliminate strong polarity and allow function of the distal portion of the mutant gene as well as distally located genes (Grodzicker and Zipser, 1968; Michels and Zipser, 1969; Newton, 1969). There are indications that structural genes sometimes already contain valid initiating sequences that are detected when chain termination occurs earlier in that gene (cf. Newton, 1966, 1969; Michels and Zipser, 1969; Zipser, 1970; Zipser *et al.*, 1970; Yanofsky *et al.*, 1971; Platt *et al.*, 1972).

Second-site reversions that allow polypeptide reinitiation also can restore function to the gene in which they occur. In these rare situations the original nonsense triplet must occur in a noncritical portion of the gene, and the resulting polypeptide fragment(s) also must be able to assume an active conformation. A situation of this type was described by Sarabhai and Brenner (1967) in the bacteriophage T4 *rIIB* gene.

Suppression eliminating polarity also can be achieved by mutations giving rise to new *transcriptional* start signals ("promoters"). These restore functional transcription of distally located bacterial genes after polar chain-terminating mutations and in mutants lacking the normal promotor (Margolin and Bauerle, 1966; St. Pierre, 1968; Fankhauser, 1971; Arditti *et al.*, 1968; Morse and Yanofsky, 1969a; Wuesthoff and Bauerle, 1970; Atkins and Loper, 1970; Callahan and Balbinder, 1970). Some of the mutations to new promoters lead to inactivation of the products of the genes in which the mutations lie (Morse and Yanofsky, 1969a; Atkins and Loper, 1970) and others are "silent," i.e., probably are acceptable missense mutations (St. Pierre, 1968; Wuesthoff and Bauerle, 1970). Some operons already contain "internal" low-level promoters that seemingly are not under specific regulatory control (*trp* operon: Margolin and Bauerle, 1966; Bauerle and Margolin, 1967;

Morse and Yanofsky, 1968; Jackson and Yanofsky, 1972; *gal* operon: Jordan *et al.*, 1968; *arg* operon: Cunin *et al.*, 1969; *his* operon: Atkins and Loper, 1970).

E. coli and *Salmonella* "promoter" mutants possessing low enzyme levels and low messenger levels also can be suppressed by very closely linked mutations (Scaife and Beckwith, 1966; Friedman and Margolin, 1968; Silverstone *et al.*, 1970; B. Ely, T. Kasai, D. B. Fankhauser, and P. E. Hartman, unpublished). These second-site mutations seem to alter the susceptibility of the nucleotide sequence involved in transcriptional control. They do not seem to give rise to entirely new "promoters" or to delete transcriptional "stop" signals and allow transcriptional "read-through" from another operon (cf. for *lac* system, Reznikoff, 1972). Deletions fusing operons and relieving polar effects also are known, however (Ames *et al.*, 1963; Beckwith, 1964a,b; Jacob *et al.*, 1964, 1965; Margolin and Bauerle, 1966).

D. Elimination of a Toxic Polypeptide

Among *rII* mutants of bacteriophage T4 a frameshift mutant (*r238*) produces a peptide fragment that is toxic to phage growth in a particular host. One manner in which the toxic effects may be suppressed is by intragenic mutations of the nonsense and frameshift types that lead to earlier chain termination and, thus, elimination of the toxic polypeptide (Barnett *et al.*, 1967).

A somewhat similar case of suppression occurs in *Salmonella*. Function of isopropylmalate isomerase, the product of the *leuC* gene, requires activation either by the product of the *leuD* gene or by the product of another gene, *supQ* (also see page 48). The *leuC* gene product can *not* be activated by the product of *supQ* if the *leuC* product is complexed with a defective, mutant product of gene *leuD* that has lost activating function. Kemper and Margolin (1969) demonstrated that elimination of the inhibitory *leuD* product through additional intragenic mutation or by deletion of the *leuD* gene restores the ability of the bacteria to grow in the presence of *supQ*.

Finally, some semidominant lactose-negative mutants in *E. coli* are mutant in the *i* gene and have repressor proteins with decreased affinity for inducer and, sometimes also an increased affinity for operator. This results in a drastic inhibition of enzyme synthesis dictated by the lactose operon. One mode of reversal is by an additional mutation in the *i* gene that eliminates the hyperactive regulatory protein (Jacob and Monod, 1961; Willson *et al.*, 1964; Bourgeois *et al.*, 1965; Bourgeois and Jobe, 1970).

Some dominant mutations in higher organisms might also lead to pro-

duction of toxic macromolecules. Two possible examples are *K-pn* (*Killer of prune*), discussed in Section III, D, c, and *Ns* (Nasobemia) in *Drosophila*. In both cases, reversion frequencies are high and include deletion mutations (Lifschytz and Falk, 1969a,b; Denell, 1972).

E. Double Frameshifts

Crick *et al.* (1961) proposed that deletion or addition of base pairs in other than multiples of three leads to alterations in the "reading frame" during translation of the "triplet" genetic code on messenger RNA (also see Barnett *et al.*, 1967). Alteration in the number of nucleotides leads to translation of the subsequent code letters in new multiples of three, as detected by alterations in the amino acid sequences of proteins. A second frameshift can compensate to restore the proper reading frame and lead to production of active protein so long as the amino acids inserted between the two frameshift sites are acceptable to catalytic activity and the nucleotide sequence does not lead to termination of the polypeptide chain. An example is given in Fig. 7.

Data on double frameshift mutations come predominantly from studies on bacteriophage T4 *rII* mutants (op cit), lysozyme (Terzaghi *et al.*, 1966; Okada *et al.*, 1966, 1968; Streisinger *et al.*, 1966; Inouye *et al.*, 1967), *E. coli* tryptophan synthetase A protein (Brammar *et al.*, 1967; Berger *et al.*, 1968a,b), and L-histidinol dehydrogenase of *S. typhimurium* (Yourno and Heath, 1969; Tanemura and Yourno, 1969; Yourno, 1970, 1971, 1972; Kohno *et al.*, 1970). The collective data indicate that frameshifts preferentially occur in sequences of repeating nucleotides or lead to repetitions (Streisinger *et al.*, 1966) although the mechanisms engendering frameshift mutations still remain unclear. Thus, "reversions" toward the wild-type phenotype may occur either by reversal of the original mutation or, frequently, by intragenic suppressor mutations (by deletions and insertions near the original mutation) leading to a protein that is catalytically active but contains one or more amino acid substitutions, additions, or deletions (Fig. 7). Double frameshifts also can be detected in eukaryotes (Brink *et al.*, 1969).

Malamy (1966, 1970) reported a case slightly different from those described above. Spontaneous mutants with absolute polar effects in the *E. coli* galactose and lactose operons are due to small insertions of genetic material (Jordan *et al.*, 1968; Shapiro, 1969; Michaelis *et al.*, 1969; Malamy, 1970). Reversion may be accomplished either by deletion of the inserted sequence (Shapiro, 1969; Malamy, 1970) or by a frameshift mutation (Malamy, 1966, 1970). The frameshift either disrupts a termination signal or creates a new promoter sequence. These two possibilities

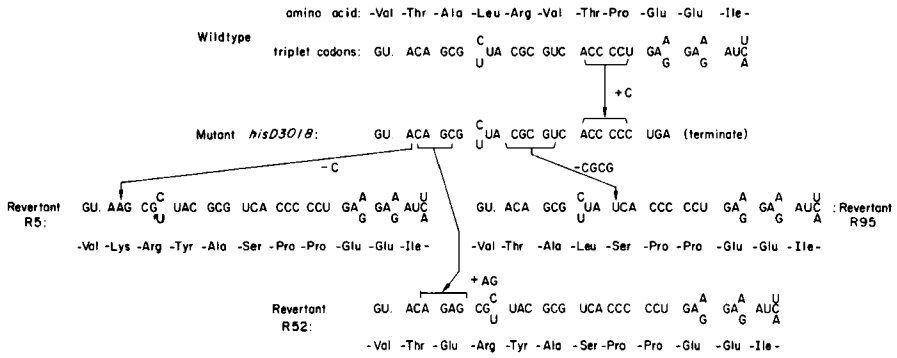

FIG. 7. Amino acid alterations and proposed genetic code in double frameshift mutants of *Salmonella typhimurium* L-histidinol dehydrogenase. The amino acid sequence and corresponding triplet codons for a portion of histidinol dehydrogenase from wild-type bacteria are shown at the top. Mutant *hisD3018* is assumed to have lost a G/C base pair in the DNA and a C in the messenger RNA (center) as based upon analysis of proteins produced by different double frameshift revertants (lower portion of figure). Frameshift mutant *3018* protein is not detected; presumably the protein is terminated at the UGA triplet whose "recognition" was created by the shift in reading frame. Revertant R5 has an altered string of 6 amino acids due to a −1 frameshift that compensates for the +1 frameshift originally present. The +1 frameshift also can be compensated by repetition of an AG sequence (revertant 52), again restoring in-phase translation and resulting in a protein one amino acid longer than the wild type, or by deletion of a CGCG sequence (revertant R95), resulting in a functional protein that is one amino acid shorter than the wild type. Additional classes of revertants, including true wild types, also have been described (Yourno and Heath, 1969; Yourno, 1970; Kohno *et al.*, 1970).

might be differentiated were it known whether the regained synthesis of enzyme (about 2–7% of wild-type activity) is constitutive or under normal repression control.

III. Intergenic Suppression ("External" Suppression)

Many classes of suppressor mutations occur outside of the gene that carries the primary mutation. These are termed "extragenic" or "external" suppressors not because they occur outside of genes, but, rather, because they affect the structure of a second functional unit of the genetic material, i.e., are due to intergenic suppression. The external suppressors are sometimes subclassified. One subclass of intergenic suppressors "corrects" the amino acid sequence in the mutant protein. "Correction" is exerted through modifications in the protein-synthesizing system ("informational" or "direct" suppression). A second, more heterogeneous, sub-

class of intergenic suppressors allows continued production of the gene product in mutant form but compensates indirectly to allow expression of the wild-type phenotype by other means ("indirect" suppression).

A. Informational Suppression ("Direct" Suppression)

One commonly encountered class of suppressors in microorganisms is the group that alters the fidelity with which the genetic message is translated, a possibility first suggested by Yanofsky and St. Lawrence (1960) and by Benzer and Champe (1962). In these cases, the mutant gene still provides "mutant" messenger RNA but the mutant region of the message is occasionally "misread" in suppressor strains so that a functional protein is formed. These informational suppressors include external suppressors of missense, nonsense, and frameshift mutations. Informational suppressors contributed to our knowledge of the genetic code and still serve today to pinpoint particular kinds of mutational alterations. Furthermore, the allele specificity of informational suppressors allows differentiation of homoalleles that appear identical by other tests including recombination tests (cf. Barben, 1966).

Extensive prior reviews of this area exist (Gorini and Beckwith, 1966; Garen, 1968; Davies, 1969; Gorini, 1970), so we discuss here only some recent aspects of informational suppression not previously covered in detail. Our discussions focus on microorganisms, but we are convinced that higher organisms will shortly receive just as concerted attention. Nonsense suppressors may occur in *Drosophila,* for example, suppressor of *Hairy wing.* Suppressor of *Hairy wing* acts on some mutations at many loci (Lindsley and Grell, 1968) and, when homozygous, results in female sterility due to autonomous pathology in ovarian nurse cells (Klug *et al.,* 1968). In these cells protein synthesis is inhibited and RNA is labile (Klug *et al.,* 1970). Perhaps a minor tRNA species completely altered in the suppressor homozygote is used only once during development. Or, "oversuppression" might occur in genes uniquely used in nurse cells analogous to the host-dependent phage mutants uniquely sensitive to particular nonsense suppressors of bacteria (Horiuchi and Zinder, 1967). That is, other *Drosophila* genes may have double termination signals, but the suppressor-sensitive genes may have only one (cf. Lu and Rich, 1971). Also in *Drosophila,* "Minute" mutations have been associated with positions of deletions possibly involving redundant genes (Ritossa *et al.,* 1966a; Tartof and Perry, 1970) specifying tRNAs (Steffensen and Wimber, 1971). While no correlation of allele-specific suppression has been made with any of the "Minute" gene regions, Green

(1946) found that each of three *Minute* mutations enhanced the expressivity of various recessive *vg* (*vestigial*) mutations. The increased mutant expression was related to a prolongation of the third larval instar, characteristic of *Minutes*. Larval tumors also are enhanced by elongation of larval life (see Section IV, a). Perhaps these characteristic responses could be applied to selection for interesting kinds of suppressors for the *Minute* phenotype.

1. tRNA Suppressor Mutations External to Anticodons

Transfer RNAs can gain activity in suppression of nonsense (Goodman *et al.*, 1968, 1970; Gopinathan and Garen, 1970; Altman *et al.*, 1971) and probably of missense (Carbon and Curry, 1968; Carbon *et al.*, 1969; Squires and Carbon, 1971) codons through base substitutions occurring directly in the anticodon of the tRNA. However, in Section II, B, d tRNA mutants are described which lose and then regain ability of informational suppression through mutations scattered at other particular points in the tRNA gene. Several recent lines of evidence suggest that creation of nonsense suppressors from wild-type strains also may involve base substitutions in the tRNA outside of the anticodon or, alternatively, in some modification (Söll, 1971) of the tRNA molecule.

A strong UGA nonsense suppressor (Sambrook *et al.*, 1967) leads to production of an abnormal tryptophan tRNA (Hirsh, 1970, 1971; Hirsh and Gold, 1971; Chan *et al.*, 1971). Both wild-type and suppressor tRNAs have the same anticodon (CCA) which should, by the predictions of the "wobble" hypothesis (Crick, 1966), read only the tryptophan codon, UGG. The fact that the suppressor tRNA differs from wild-type by an A to G change elsewhere than in the anticodon suggests that a tRNA sequence alteration far from the anticodon region may affect codon–anticodon pairing, allowing the CCA anticodon of the tRNA to violate wobble rules and recognize the UGA codon as well as UGG.

A second sort of UGA suppressor seems to involve a defect in tRNA modification. Reeves and Roth (1971) found a UGA suppressor which is recessive in contrast to most bacterial nonsense suppressors, which are dominant mutations. The recessive UGA suppressor elicits undermethylated tRNA (Reeves and Roth, unpublished). Although it is uncertain which undermethylated tRNA is responsible for suppressor activity, it would seem likely that one undermethylated species occasionally miscodes, and thereby suppresses the mutant phenotype. An additional recessive suppressor (*sufF* on pages 26, 27, and 28) may represent another case of miscoding by unmodified tRNA. Both recessive suppressor genes control vital functions since temperature-sensitive lethal mutations of these genes are known.

Wild-type enteric bacteria exhibit a low-level reading of the UGA codon (Model *et al.*, 1969; Roth, 1970; Ferretti, 1971), but UGA triplets are predominantly chain terminating (Khorana *et al.*, 1966; Brenner *et al.*, 1967; Sambrook *et al.*, 1967; Zipser, 1967; Model *et al.*, 1969). The relationship of this low-level UGA suppression to the above UGA suppressors is not clear, but some of the activity may be due to miscoding by wild-type $tRNA^{Trp}$.

Recessive allele-specific suppressors have been described on several occasions in *Aspergillus* (for *ade-26* by Pritchard, 1955; and for *meth-2* by Luig, 1962). Other recessive nonsense suppressors have been found in yeast (Inge-Vectomov, 1965; Magni *et al.*, 1966). The mechanism of action has not been determined for either suppressor.

2. Lethal Suppressors

A recessive lethal amber suppressor (*su-7*) has been described in *E. coli* by Soll and Berg (1969a). Cells carrying this suppressor can survive only if they also carry a wild-type copy of the affected gene. This suppressor has been shown to insert *gln* in response to the amber codon (Soll and Berg, 1969b). Miller and Roth (1971) have described lethal amber and UGA suppressors in *Salmonella* which are allelic and map at a position analogous to that of *su-7* in *E. coli*. Tryptophan transfer RNA which reads the UGG codon should be capable of mutating so as to read UAG or, by a different mutation to read UGA. Thus the finding of an allelic UGA suppressor did not fit with the *gln* insertion of the *E. coli* lethal suppressor, *su-7*. Recently this discrepancy has been resolved by Yaniv, Soll, and Berg (personal communication; discussed in Berg, 1972), who found that the glutamine-inserting tRNA from *su-7* is actually an altered tryptophan tRNA. Apparently one mutation affecting the anticodon region can cause $tRNA^{Trp}$ to read UAG and be mischarged with the wrong amino acid, *gln*. The lethality of the *su-7* suppressor is probably due to the loss of the single *trp* transfer RNA species present in *E. coli*.

3. Substituted Proteins

The prevalence of nonsense mutations and the specificities of insertion of different amino acids by nonsense suppressors in *E. coli* has allowed the *in vivo* synthesis of proteins with particular amino acid substitutions at any one of a large number of locations. The effects of such substitutions on the properties of β-galactosidase have been described (Langridge, 1968a,b,c; Langridge and Campbell, 1968). Originally, suppressors with altered tRNAs and effective in inserting serine, glutamine, and tyrosine at amber codons and tyrosine at amber or at ochre codons

were described (reviewed by Garen, 1968). More recently, suppressors eliciting insertion of leucine at amber codons (Chan and Garen, 1969; Gopinathan and Garen, 1970), tryptophan at UGA codons (Chan and Garen, 1970; Hirsh, 1970, 1971; Hirsh and Gold, 1971; Chan *et al.*, 1971), and lysine at UAA and UAG codons (Kaplan, 1971) have been added to the substitution repertory. These suppressors should prove useful for construction of particular sorts of proteins in the study of enzyme action.

4. Nonsense Suppressors in Eukaryotes

Suppressors with properties of nonsense suppressors are present in yeast ("supersuppressors": Hawthorne and Mortimer, 1963; Mortimer and Gilmore, 1968) and *Neurospora* (Seale, 1968; Case and Giles, 1968; Chalmers and Seale, 1971). The following evidence supports the identity of supersuppressible mutations of yeast and the nonsense mutations, amber and ochre, found in bacteria:

a. The supersuppressors act on some, but not on all, alleles of many loci (Hawthorne and Mortimer, 1963; Mortimer and Gilmore, 1968; Gilmore *et al.*, 1971). For example, in the *tr-5* locus, approximately 40% of the known mutations are supersuppressible (Manney, 1964); in the *his-4* locus, 35% of the classified mutations are supersuppressible (G. Fink, personal communication). Additional frequency data are cited in Mortimer and Hawthorne (1969). This frequency is comparable to that of nonsense mutations in several *his* genes of *Salmonella typhimurium* (Whitfield *et al.*, 1966; Hartman *et al.*, 1971) and in the *lacZ* gene of *E. coli* (Langridge and Campbell, 1968).

b. As expected for organisms carrying chain-terminating mutations, supersuppressible mutants are not "leaky" or temperature-sensitive, nor does intragenic (intracistronic) complementation occur in most cases. The exceptional supersuppressible mutations exhibiting "intragenic" complementation show a polarized pattern explicable by participation of incomplete polypeptide chains in the complementation reaction (Manney, 1964; Fink, 1966, 1971).

c. Data on several systems indicate that supersuppressible mutations lead to gene products whose molecular size is decreased relative to native wild-type enzyme. This could be due either to premature chain termination, to polarity, or to effects on protein–protein interactions in aggregates of different polypeptide chains (for critical discussion, see Fink, 1971).

Native wild-type *Neurospora* and yeast tryptophan synthetase complexes have molecular weights close to 150,000 and catalyze two successive half-reactions: (I) indole-3-glycerol phosphate → indole + glyceraldehyde-3-phosphate, and (II) indole + serine → tryptophan.

The *Neurospora* enzyme (Bonner *et al.*, 1965; Lacy, 1965; also see

Lacy, cited in Hartman and Suskind, 1969, p. 66) and the comparable yeast enzyme (Duntze and Manney, 1968; Manney *et al.*, 1969) each are dictated by a contiguous genetic region predominantly concerned with elaboration of activity I from one subregion and activity II from a second subregion. The *Neurospora* enzyme may be composed of two different polypeptide chains (Carsiotis *et al.*, 1965), but neither it (Ensign *et al.*, 1964) nor the yeast enzyme (Duntze and Manney, 1968) readily dissociates into subunits under mild conditions. Supersuppressible yeast tryptophan synthetase mutants most often lose both half-reactions, but some contain an enzyme which has lost activity II but retained a substantial part of activity I. The molecular weight of the mutant enzyme (activity I) is reduced to approximately 35,000 as estimated by Sephadex gel chromatography (Manney, 1968).

Similarly, supersuppressible mutations in the yeast *his-4* (Fink, 1965, 1966, 1971; Shaffer *et al.*, 1969) and *Neurospora arom* (Case and Giles, 1968, 1971) genetic regions lead to enzyme aggregates considerably diminished in molecular weight from the wild-type enzyme complex.

d. Two supersuppressible mutations in the iso-1-cytochrome *c* gene of yeast (Sherman *et al.*, 1966) carry a UAA (ochre) codon at the mutant site (Gilmore *et al.*, 1968, 1971; Sherman *et al.*, 1970). Both mutant sites studied have a glutamic acid residue in the wild-type protein (Glu = GAA or GAG). Revertants specify mutant proteins carrying Gln (CAA), Lys (AAA), Leu (UUA), Tyr (UAU or UAC), or Ser (UCA), but none which carry Trp (UGG). Although it is not known whether cytochrome with a Trp substitution at either of these sites is functional, this region of the protein is tolerant of amino acid substitutions and thus the data favor UAA as the mutant codon (compare Fig. 3, page 7).

A second type of supersuppressible mutation has been shown to be of the amber UAG type. This type was first identified by the fact that it can be converted to ochre (UAA) by mutagenesis with hydroxylamine and ethylmethanesulfonate (Hawthorne, 1969a,b). This test, along with use of the informational suppressors mentioned below substantiate the UAA codon assignment made in the previous paragraph. This identification of the amber mutation was confirmed more recently by reversion studies analogous to those outlined above (Sherman and Stewart, 1971). In addition to the amber and ochre mutations, UGA mutations have also been identified by Hawthorne (personal communication).

Three general types of nonsense suppressors have been described in yeast: (a) amber-specific suppressors, (b) ochre-specific suppressors, and (c) suppressors which act on both amber and ochre mutations (Gilmore, 1967; Mortimer and Gilmore, 1968; Hawthorne, 1969a,b). Re-

cently Hawthorne (personal communication) has found a fourth class of suppressors which are UGA-specific. He has also found that the amber-ochre suppressors (type c above) are able to suppress UGA mutations as well.

The ochre-specific suppressors, not found in *E. coli,* may reflect the presence of inosine in tRNA of yeast whereas inosine is absent in tRNA of *E. coli.* The "wobble" rules, suggested by Crick (1966; consult Jukes and Gatlin, 1971, for recent discussion) to describe codon–anticodon base pairing, predict that inosine in the first position (5′ end) of the anticodon will pair with U, C, and A in the third position (3′ end) of the codon. Thus, a tRNA with the anticodon IUA would pair with UAC (Tyr), UAU (Tyr), and UAA (ochre). Several tyrosine-inserting nonsense suppressors have been described (Gilmore *et al.,* 1968, 1971). Other explanations of ochre-specific suppressors are possible if the general structure or modified bases of particular tRNAs can permit exceptions to the "wobble" pairing rules (discussed in Gilmore *et al.,* 1971). It is surprising to note that no yeast supersuppressor has been described corresponding to the ochre suppressors of *E. coli,* which suppress both ochre and amber mutations. The supersuppressors which act on UAG, UAA, and UGA may prove to be of the ribosomal type (cf. Gorini, 1970).

A second distinctive feature of yeast nonsense suppressors is the large number of suppressor loci. Gilmore (1967) described eight classes of nonsense suppressors in *Saccharomyces* based on their ability to suppress five different ochre mutations. Several of these suppressor classes were further divided into subsets based on the strength of the suppressive effect, leading to 21 phenotypic classes of ochre-specific and amber-ochre-UGA (see above) suppressors. Hawthorne (1969b) classified another group of suppressors into ten classes of which five were ochre-specific, three were amber-ochre-UGA, and two were amber-specific; only three of these classes overlap the suppressor classes described by Gilmore (1967). Sixteen map positions, scattered over eleven linkage groups, have been found for nonsense suppressors in *Saccharomyces;* three gave rise to amber-specific, three to ochre-amber-UGA, and eleven to ochre-specific suppressors (Hawthorne and Mortimer, 1968). A group of over 20 ochre-specific suppressors (Class I subunit 1; Gilmore, 1967) behave identically in suppression of a series of ochre mutations but are distributed among eight clearly genetic loci; each suppressor leads to insertion of Tyr at ochre codons but is ineffective in suppressing an amber mutation (Gilmore *et al.,* 1968, 1971). Of several hundred suppressors acting on five ochre mutations, all map in these same eight loci (Strömnaes and Mortimer, cited in Gilmore *et al.,* 1971).

Combination in a haploid yeast cell of two Class III (inefficient)

suppressors increases suppressor activity and has no overt effect on general growth properties (Mortimer and Gilmore, 1968), but combinations of two Class I (Tyr) suppressors is lethal or results in impaired and abnormal growth in nonrestrictive medium (Gilmore, 1967; Mortimer and Gilmore, 1968). Some strong ochre-specific suppressors are lethal in haploids containing the extrachromosomal factor, ψ (Cox, 1971), a factor that stimulates suppression by very weak ochre suppressors (Cox, 1965). In *E. coli,* a gene dosage effect on efficiency of nonsense suppression (Hoffman and Wilhelm, 1970) and a deleterious effect of ochre suppressors (Gallucci and Garen, 1966) also have been noted. It seems plausible that one cause of these deleterious effects is interference with normal chain termination at essential positions (cf. Lu and Rich, 1971; discussion in Gilmore *et al.,* 1971). In addition, withdrawal of tRNA genes from their normal function through suppressor mutations may cut down tRNA gene redundancy (Schweizer *et al.,* 1969) essential to synthesis of adequate tRNA (cf. Mortimer and Gilmore, 1968; Mortimer, 1969).

The mutagen ethylmethanesulfonate, which predominantly elicits G/C → A/T base pair substitutions, causes suppressor mutations of the ochre-specific and amber-specific suppressor type (Hawthorne, 1969b). It has been suggested that yeast (Magni and Puglisi, 1966; Magni *et al.,* 1966) and *Salmonella* (Whitfield *et al.,* 1966; Hartman *et al.,* 1971) nonsense suppressors also can arise through additions and deletions of base pairs since the ICR compounds (Ames and Whitfield, 1966) can induce suppressors. The nature of these suppressors and their relationship to other nonsense suppressors remains unexplored. This finding may not be surprising in the case of yeast, since there is some evidence that the mutagen ICR-170 causes largely base substitution mutations in that organism (cited in von Borstel, 1969; F. Sherman, personal communication).

Barben (1966) describes seven nonsense suppressor loci in *Schizosaccaromyces,* and five nonsense suppressor loci have been located in *Neurospora* (Seale, 1972).

5. Suppressors of Frameshifts

Additions or deletions of base pairs other than in multiples of three throws the organized triplet reading out of phase (see pp. 4, 18). The linear messenger RNA molecule is translated into a normal polypeptide chain up to the point of the mutation, but reading proceeds beyond the mutation in an improper phase. The protein-synthesizing apparatus merely translates consecutive triplet codons in the order they appear

on the mRNA (Crick *et al.*, 1961). Extended missense protein is made until a nonsense codon terminates translation (cf. Martin, 1967; Barnett *et al.*, 1967).

Extensive analyses of frameshift mutations were first carried out in bacteriophage T4 (Crick *et al.*, 1961; Streisinger *et al.*, 1966; Barnett *et al.*, 1967). The fact that most of the early work on frameshift mutants was done in phage may be one reason why intergenic, informational suppressors of frameshift mutations were not found earlier. A second explanation may stem from the apparent preponderance of base-pair deletions, as opposed to base-pair additions, in frameshift mutants unless they have been induced by intercalating agents or selected in back-mutation tests (discussed in Hartman *et al.*, 1971); we see below that all suppressible frameshifts are probably +1 frameshifts.

Indications of the presence of external suppressors for frameshift mutations (Riyasaty and Atkins, 1968; Oeschger and Hartman, 1970) were independently noted by Yourno *et al.* (1969), who next clearly demonstrated external suppression of the frameshift mutation *hisD3018* (Yourno and Tanemura, 1970). Comparison of the amino acid sequences of wild-type protein and those produced by various revertants showed that the suppressible mutation carries one extra G/C base pair (Yourno and Heath, 1969; Tanemura and Yourno, 1969; Yourno, 1970; Kohno *et al.*, 1970).

Additional suppression was found for thirteen of twenty-one tested frameshift mutations in the *Salmonella* histidine operon when the mutants reverted to prototrophy (Riddle and Roth, 1970). Forty-eight suppressor mutations were placed into six groups on the basis of map location (Fig. 8; Riddle and Roth, 1972a). Two general sorts of suppressors exist. One type (*sufA, B,* and *C*) never suppress mutations suppressible by the second type (*sufD, E, F*). The first suppressor type may correct the phase of +1 frameshift mutations located in runs of C in the messenger RNA since (1) three mutations suppressible by *sufA, B,* and *C* are known to lie in runs of C (Yourno and Heath, 1969; Yourno, 1971; Yourno and Kohno, 1972); (2) one suppressor of this type inserts proline at low frequency (Yourno and Tanemura, 1970); (3) mutations of the *sufA* and *sufB* genes affect different species of proline tRNA (Riddle and Roth, 1972b). Suppressors of the second general type (*sufD, E, F*) may restore proper reading phase to frameshift mutations in runs of G, since one suppressor of the *sufD* locus affects a glycine tRNA (Riddle and Roth, 1972b) which normally reads the codon GGG (Carbon *et al.*, 1970). This supposition agrees with the amino acid sequence data of Yourno (1972). Recently, a four-letter anticodon sequence has been found in a *sufD* $tRNA^{Gly}$ (Riddle and Carbon, 1973).

Thus, one suppressor tRNA contains a 4-base anticodon very likely capable of reading 4-base codons. In addition, tRNAs may have difficulty maintaining proper phase when monotonous base sequences undergo translation. Atkins *et al.* (1972) have demonstrated that ribosomal alterations can affect the frequency of mistakes in phase maintenance. The combined results suggest a critical role for tRNA in determining the distance traveled by the ribosome in the translocation step of protein synthesis and implicate ribosomal structure in the fidelity of this process.

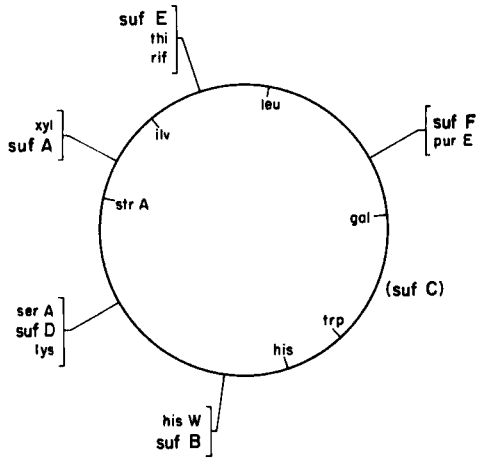

FIG. 8. Suppressors of frameshift mutations in *Salmonella typhimurium*. Genetic map of *S. typhimurium* chromosome showing locations of frameshift suppressor genes (*suf*) described in the text. Based on Riddle and Roth (1972a).

B. Reconstitution of Active Enzyme Conformation

Common among mutants are those that form polypeptide chains that are unusually sensitive to thermal denaturation. The thermal sensitivity often is expressed by the native protein but in some cases is restricted to the polypeptide at the time of synthesis, for example, while it exists as a monomer before joining with other molecules to form a "native" enzyme with coenzyme or other proteins attached. Temperature-sensitive tRNA mutants also are known. In addition, mutants sensitive to low temperature have been described; this last class is most often concerned with highly allosteric, multimeric proteins that undergo great conformational changes during union with various small or large ligands.

In view of the "plasticity" of protein molecules as exemplified by the examples just cited, we should expect to find it common that "inac-

tive" enzyme can be "reactivated" under appropriate environmental conditions. These conditions may vary, in separate cases, from what we might consider as quite general conditions of pH or ionic concentration to changes in the concentrations of more specialized molecules, effector molecules.

Effector molecules are those that influence catalytic activity of an enzyme through inhibition or activation and thus serve to regulate its activity. While, in this general sense, almost anything could be specified as an effector if high enough concentrations were examined, we restrict our definition here to effectors that are physiologically significant in intracellular metabolism.

Effector molecules are extremely important in bringing about the balanced regulation that dictates the flow of metabolites in normal microbial, plant, and animal systems (e.g., Atkinson, 1966; Umbarger, 1964, 1969; Kornberg, 1965; Koshland and Neet, 1968; Denburg and DeLuca, 1968; Tuli and Moyed, 1966; Whitehead, 1970). In fact, mutants contain defective proteins that are nominally active under the proper environmental conditions. Unfortunately for the cell, however, these conditions are special and do not mimic the intracellular environment; *in vivo* the protein is relatively inactive and leads to a mutant phenotype. Often, suppressor mutations lead to an adjustment of the intracellular milieu so that catalytic activity is restored to the defective protein. Here we discuss cases where the suppressor mutation serves to remove or modify normal molecules and thus releases a mutant protein from inhibition. On pp. 17–18, we mentioned cases where the original mutant protein itself has a deleterious effect on normal cell metabolism and where this effect is suppressed by removal of the toxic polypeptide by second-site mutation.

1. Inactivating Ion or Environment

Suskind and co-workers found that a temperature-sensitive *Neurospora* tryptophan synthetase mutant, *td24*, synthesized an enzyme protein abnormally sensitive to inhibition by zinc. (The wild-type enzyme is described on pp. 23–24.) Active enzyme was recovered once it had been purified away from other cellular constituents, including an inhibitor. Enzyme from a strain carrying an allele-specific suppressor mutation (Suskind and Yanofsky, 1961) retained the properties of the defective *td24* protein (Suskind, 1957a,b; Suskind and Kurek, 1957, 1959; Suskind and Jordan, 1959). This led to the suggestion that the suppressor gene acted to alter the intracellular environment to allow adequate function of defective enzyme. The suppressor mutation might lead to an alteration in the concentration or location of zinc ion.

A number of presumed missense mutants in fungi form enzymes that allow growth only on special media, for example, of high osmotic strength ("osmotic remedial" mutants: Hawthorne and Friis, 1964; Kuwana, 1961; Nakamura and Gowans, 1967; Lacy, 1968; Esposito, 1968; Metzenberg, 1968) or at high pH (Stokes *et al.*, 1943; B. S. Strauss, cited in Emerson, 1952). One surmises that the external environment has impact on the intracellular milieu sufficiently to allow the defective enzymes to assume active conformations, for example, by adequate binding of pyridoxal phosphate to apoenzyme in the latter instances cited above (cf. Guirard *et al.*, 1971). In these special cases it should be possible to obtain among "revertants" suppressor mutants of high interest, for example, organisms with alterations in intracellular osmolality or production of cofactor. The same line of reasoning applies to a case in which temperature or osmotic conditions circumvent a genetic block imposed by nonsense mutations and deletions in the *ade-3* locus of *Saccharomyces* (Jones, 1972).

2. Inactivating Metabolite

One would expect to find cases where a mutant enzyme for a limiting reaction was (hyper)sensitive to normal levels of a metabolite; mutations that cut down the rate of synthesis or increase the rate of utilization of the metabolite might then serve to suppress. At this time, however, we are unable to pinpoint cases of suppression that fall into this category.

3. Inactivating Macromolecule

a. tRNA. Mutations in one of the earliest suppressor genes analyzed (Schultz and Bridges, 1932) eliminate the presence of a species of transfer RNA that drastically inhibits particular mutant proteins (Jacobson, 1971; Jacobson and Grell, 1971; Twardzik *et al.*, 1971). *Drosophila* mutants at the vermilion (*v*) locus lack the brown constituent of eye pigment, accumulate nonprotein tryptophan (Green, 1949), and are deficient in the activity of an inducible enzyme (Kaufman, 1962), tryptophan pyrrolase (Tryptophan peroxidase) (Baglioni, 1959, 1960; Kaufman, 1962; Marzluf, 1965a,b; Tartof, 1969). Various *v* mutants fail to complement; i.e., the locus is a single cistron (Green, 1954; Barish and Fox, 1956). Tryptophan pyrrolase catalyzes the first step in the conversion of tryptophan to ommochrome pigment (Fig. 9). Kyurenine ("*v* substance") is not made by *v* mutants (Beadle and Ephrussi, 1936, 1937; Butenandt *et al.*, 1940; Tatum and Haagen-Smit, 1941; Kikkawa, 1941) although *v* mutants contain an excess of kynurenine formamidase (Glassman, 1956) and can use kynurenine to form eye pigment.

Kynurenine is hydroxylated to 3-hydroxykynurenine (Ghosh and Forrest, 1967a), and both compounds are able to react with products of tyrosinase activity to yield brown ommochrome pigments (Glassman, 1957).

Sex-linked mutations partially restore production of eye pigment (Schultz and Bridges, 1932), kynurenine (Beadle and Ephrussi, 1936, 1937), and tryptophan pyrrolase activity. The latter is detected in extracts of suppressor-sensitive (Green, 1952) *v* mutants (Baglioni, 1960; Kaufman, 1962; Marzluf, 1965a,b; Tartof, 1969). These suppressible mutants also produce some brown pigment and presumably possess slight enzyme activity under certain starvation conditions in the absence of the suppressor (Tatum and Beadle, 1939) whereas nonsuppressible *v* alleles fail to do so (Green, 1954; Shapard, 1960). Kynurenine production in the fat body (Beadle, 1937) is stimulated in the presence of tryptophan

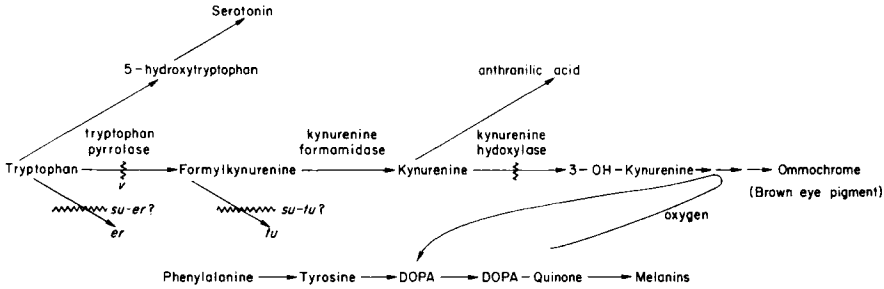

FIG. 9. Some aspects of tryptophan metabolism in *Drosophila melanogaster*.

or of the suppressor in suppressor-sensitive strains (Rizki, 1961b, 1963, 1964; Rizki and Rizki, 1968), and the suppressor alters the intracellular distribution of 420 Å halolike particles in fat body cells (Rizki *et al.*, 1970). The suppressors are recessive (Schultz and Bridges, 1932; Tartof, 1969), and even deletion of the suppressor locus results in suppression, suggesting that the su^+ locus is responsible for the synthesis of a product toxic to *v* mutants (Shapard, 1960). However, partial fractionation and mixtures of extracts failed to reveal the presence of inhibitors (Marzluf, 1965a,b; Tartof, 1969).

Recently, it has been found that treatment of extracts with ribonuclease leads to *activation* of latent tryptophan pyrrolase activity in suppressible mutants. Addition of a particular species of uncharged $tRNA^{Tyr}$ (tyrosine transfer RNA) inactivates mutant enzyme (Jacobson, 1971; Jacobson and Grell, 1971). Suppression of *v* is accompanied in *Drosophila* homozygous for suppressor by the *disappearance* of the inhibitory species of $tRNA^{Tyr}$ and an *increase* in another $tRNA^{Tyr}$ isoaccepting fraction. The

recessive nature of the suppressor and behavior of the $tRNA^{Tyr}$ species indicate that the suppressor locus does not code for the primary structure of tyrosine tRNA "but that it may control an enzyme that modifies the tyrosine tRNA" (Twardzik *et al.*, 1971). Mutants of four other genes involved in pigment formation (*sable, speck, purple,* and *black:* Bridges, 1932; Green, 1954; Lindsley and Grell, 1968; Rizki and Rizki, 1968) also are suppressed by at least one of the *v* suppressors.

One speculation is that $tRNA^{Tyr}$ plays a normal role in coordinating the two pathways that combine to elicit brown eye pigment (ommochromes) in wild-type *Drosophila* (Fig. 9). That is, the suppressible *v* mutant enzymes may be hypersensitive to *normal* effector molecule (Baille and Chovnick, 1971). Furthermore, there are many indications of coordination between the pathways of ommochrome synthesis and those involved in synthesis of red eye pigment. There are parallel decreases in red (pteridine) and brown (ommochrome) eye pigments in flies homozygous and heterozygous for various *w* (white) alleles (Morita and Tokuyama, 1959) and in flies grown under various conditions (cf. Ephrussi and Herold, 1945). There also seems to be some coordination between the synthesis of the pigments and the pigment "core granules" themselves (cf. Caspari, 1964) although the pigments are distributed differently in two distinct granule types (Shoup, 1966). These and other scattered data (Ziegler, 1961; Ziegler and Harmsen, 1969; Nolte, 1959; and others) as well as "intuition" lead to the assumption that coordination must exist between these "separate" reaction sequences; the question is: How? One possibility for coordination of eye pigment pathways has been indicated; tryptophan pyrrolase is noncompetitively inhibited by certain pteridines (Ghosh and Forrest, 1967b). Quite possibly many "morphological" mutants are affected in coordination rather than in catalytic activity per se. For example, mutations often result both in a reduction of, say, red pigment and simultaneously an increase of brown pigment (Nolte, 1955) or lead to accumulation of a new spectrum of pteridines (McIntire and Gregg, 1966). Close analyses of these situations and cases of suppression should assist to unravel the interrelationships of various genes and help define gene products and control mechanisms.

b. Mukai and Margolin (1963) present evidence indicating that a particular mutant of *Salmonella* is sensitive to an abnormal inhibition of enzyme synthesis. The mutant (*leu-500*) lacks activity for all three of the leucine biosynthetic enzymes that are dictated by the four adjacent *leu* genes in *Salmonella* (Fig. 10). Mutation *leu-500* is believed to be a single base-pair substitution in the promoter region of the leucine operon and essential for regulation of the cluster of genes (Margolin, 1963, 1971; Mukai and Margolin, 1963; Burns *et al.*, 1966). The *leu-500*

mutant may revert by back-mutation or it may grow on medium lacking leucine due to mutations in a particular suppressor gene (*su leu500* = *supX*) that maps at an entirely different region of the *Salmonella* chromosome. Among effective suppressor mutations are single-base transition mutations as well as a number of deletions of the entire *supX* gene region. Suppression is a recessive trait. Thus, elimination of an inhibitory and dispensable molecule restores gene function in mutant *leu-500* (Mukai and Margolin, 1963; Dubnau and Margolin, 1972). The *supX* mutations also affect functioning of other operons; the pleiotropic effects of *supX* mutations favor the hypothesis that *supX*$^+$ allows production of a molecule that directly or indirectly is toxic to transcription initiation of sensitive promoters (Dubnau and Margolin, 1972).

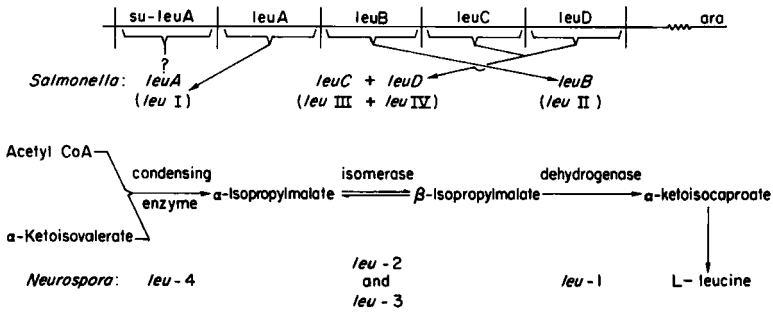

FIG. 10. Pathway of leucine biosynthesis in *Salmonella* and *Neurospora* and its genetic control.

In addition to this mode of suppression, the leucine operator region may undergo secondary suppressor mutation that, under certain growth conditions, prevents it from serving as a sensitive receptor to the "foreign" protein (Friedman and Margolin, 1968).

c. Dawson and Smith-Keary (1960, 1963; Smith-Keary, 1960) have described a curious system of suppression that could involve elimination of a "toxic protein" that exerts direct inhibition. Mutations in a short genetic region closely linked to the *Salmonella* leucine operon (Margolin, 1963) suppress only two *leuA* mutants out of 26 *leu* mutants tested (including an unknown number of *leuA* mutants). Although these suppressors have not been disproved as second-site reversions, one of the suppressor mutations may be a deletion, indicating the possibility of a distinct suppressor locus adjacent to the *leuA* gene (Dawson and Smith-Keary, 1960, 1963). Unfortunately, the map position of the suppressor region is not known in relation to the operator-promoter region of the leucine operon, also adjacent to *leuA* (Margolin, 1963; Burns

et al., 1966; Calvo *et al.*, 1969a,b). The product of the suppressor locus could be a feedback regulatory subunit that specifically inhibits altered proteins of the two suppressible mutants. The product coded by the wild-type *leuA* gene, isopropylmalate synthetase (Fig. 10), is highly labile in the absence of substrate and is feedback-sensitive to leucine (Jungwirth *et al.*, 1963; Burns *et al.*, 1966). Loss of feedback inhibition is deleterious (Calvo and Calvo, 1967), and its loss might be selected against, leading to mutational instabilities (cf. Dawson and Smith-Keary, 1963; Smith-Keary and Dawson, 1964). Reinvestigation of the suppressors from a biochemical point of view would seem worthwhile, and material for a more definitive genetical analysis also is currently available (e.g., Calvo *et al.*, 1969a,b, 1971).

d. Cell division is impaired on media containing high levels of carbon source in *Salmonella* containing mutations that engender excessive amounts of the histidine biosynthetic enzymes (Roth and Hartman, 1965; Murray and Hartman, 1972). Also, bacteria with high enzyme levels cannot grow at 42°C on minimal medium (Voll, 1967). The pleiotropic effects have been traced to overproduction of normal products of the *hisH* and *hisF* genes. These two proteins appear to act in concert, and neither presence of substrate nor ability to carry out the normal catalytic activity is requisite to their inhibitory effects (Murray and Hartman, 1972). Mutations in the histidine operon that lower levels of either *hisH* or *hisF* proteins suppress these pleitropic effects. Some of these suppressor mutations lead to a histidine requirement (Fink *et al.*, 1967) while others do not (Voll, 1967); all are in genes *H* or *F* or exert polar effects on one or both of these genes. Suppressors with similar effects on enzyme levels (and on cell division) also occur in the gene specifying histidyl-tRNA synthetase (Wyche, 1971).

A second type of suppression is exerted, namely, by mutations that do not alter the levels of the inhibitory proteins but, rather, affect some other cell process (*smo* mutations: Z. Ciésla and T. Klopotowski, cited in Sanderson, 1970). Perhaps suppressors at the *smo* loci alter the sensitive cellular site(s) where inhibition is exerted. One *smo* class is tightly linked to mutations in the histidyl-tRNA synthetase gene, indicating that this gene product may be part of the inhibitory complex (Wyche, 1971).

On two occasions mutants have been detected with abnormal cell division in the presence of *normal* histidine enzyme levels (Fankhauser, 1971; Savic, 1972). The retarded cell division can be alleviated by mutations that lower histidine enzyme levels below the normal (Fankhauser, 1971; B. Ely, personal communication). Analysis of these and other mutants of generally similar nature (*wrk* mutations; T. Klopotowski,

cited in Sanderson, 1970) may assist in analyses of the bacterial cell division mechanism.

e. Nash (1965, 1970) observed that the dominant mutation *Hairless* (*H*) in *Drosophila* is suppressed by deletion of a gene, *Su*(*H*), and enhanced by duplication, *E*(*H*), of the same gene. Both *H* and *Su*(*H*) are lethal when homozygous. Suppression is effected through deletion of only one of two homologs of the *Su*(*H*) gene and enhancement also has dominant effects. Interpretation is further restricted by the observation that homozygous *E*(*H*)/*E*(*H*); H^+/H^+ flies are phenotypically wild type. One possibility is that *H* specifies a protein that has lost normal regulation (Nash, 1965) or has widened specificity allowing production of an illicit metabolite. The $Su(H)^+$ gene, then, would be involved in a dosage-dependent role in control of *H* activity or in the metabolism of the illicit product to a compound active in causing the *Hairless* phenotype. A study of the interactions of *Su*(*H*) with various mutants already known to interact with *Hairless* (Lindsley and Grell, 1968) may furnish some clues as to the role of the toxic molecule produced through action of the $Su(H)^+$ gene.

Falk (1963) cites experiments indicating that *scute* (sc^7) suppresses *hairy* (*h*) in a manner indicating that *scute* is involved with synthesis of a structural component whereas *hairy* exerts a regulatory function. Other cases of dominant suppression of dominant mutations in *Drosophila* occur (Lindsley and Grell, 1968).

f. In the lactose system of *E. coli,* certain "promoter" mutants are thought to be unable to bind RNA polymerase and initiate transcription efficiently. The effects of these mutations can be partially suppressed and result in increased enzyme levels by mutations in the *i* gene, which eliminate repression of the *lac* operon (Scaife and Beckwith, 1966) or by mutations in a gene, *crp* (Plenge and Beckwith, cited in Reznikoff, 1972), that is concerned with dictating the structure of a protein thought to interact directly with a portion of the promoter nucleotide sequence (reviewed in Reznikoff, 1972).

4. Compensating Proteins

In Section II (pages 6–10), we mentioned in passing some methods potentially useful for studies of tertiary protein structure, that is, for analysis of interactions between different segments of one polypeptide chain. Since interactions between different macromolecules involve similar forces, the methodology would also seem to be applicable to studies of intracellular macromolecular organization, of cell regulation (examples in Calvo and Fink, 1971; Frieden, 1971) and in dissection of stages in the assembly of complex cell structures.

In the section below we cite studies indicating that a type of suppression occurs which also has particular potential in analysis of macromolecular architecture *in vivo*. In these cases, modification in one protein ("A") serves to suppress a modification in another protein ("B"). Suppression occurs because functional interaction of modified A and modified B can occur whereas wild-type A protein is unable to form a functional complex with modified B.

a. A system where protein–protein interactions are highly critical in assembly and function is the ribosome (for reviews, see Spirin, 1969; Nomura, 1970, 1972; Kaji, 1970). The effects of foreign molecules and of mutations on ribosome function and on the fidelity of protein synthesis have been extensively reviewed (Gorini and Beckwith, 1966; Davies, 1969; Schlessinger and Apirion, 1969; Nomura, 1970; Gorini, 1970; Pestka, 1971). Two examples can be briefly cited regarding genetic suppression. Two proteins, P13 and P5, exhibit cooperative binding to ribosomal RNA during ribosome assembly; a mutant P5 protein can suppress the defect in a mutant P13 protein by assisting in its binding and allow proper ribosome "maturation" (Nomura, 1970). In the second case, a mutant P4a protein can rectify restraints placed upon ribosome function by mutant P10 protein. P10 protein, dictated by the *strA* gene, is involved with the interaction of aminoacyl tRNA with the ribosome and facilitates inhibition by various antibiotics such as streptomycin. Mutations at various positions in the *strA* gene lead to functional resistance and even to dependence on streptomycin or other antibiotics (Momose and Gorini, 1971). Drug dependence is suppressed by mutation in the gene, *ram* (= ribosomal ambiguity), specifying a second ribosomal protein, P4a (Birge and Kurland, 1970; Deusser *et al.*, 1970; Kreider and Brownstein, 1971). Analysis leads to the conclusion that mutant P4a protein engenders a high level of misreading (mistakes in translation) and releases some restriction on translation imposed by the defective P10 (*strA*) protein (Bjare and Gorini, 1971; Zimmermann *et al.*, 1971a,b). Mutations in another gene, for ribosomal protein P4, also suppress streptomycin dependence (Kreider and Brownstein, 1972).

Genetic studies may be supplemented by other methodology, for example by chemical cross-linking of pairs of ribosomal proteins (Chang and Flaks, 1972) as well as reconstitution experiments (Nomura, 1970, 1972). [Also consult Davies and Stark (1970).]

b. Mutants of the gene for the lactose repressor counterbalance operator-constitutive mutations (J. Sadler, cited in Reznikoff, 1972). Presumably, the normal repressor protein has lowered affinity for the altered operator nucleotide sequence, but this affinity can be regained by modification of the attaching repressor protein. This is one method of deter-

mining which portions of the repressor polypeptide are involved in the DNA-protein interaction, complementing other genetic methods (cf. Platt *et al.*, 1972; Pfahl, 1972).

c. The suppression by interacting gene products may influence both ease of assembly of phage-specific proteins and the "specificity" of the resulting mature product, the intact phage particle. Mutations at many loci scattered over the phage genome were early shown by Baylor and co-workers (Baylor *et al.*, 1957; Baylor and Silver, 1961) to influence adsorption of T2 phage. They concluded that many "separate protein components . . . incorporated in different positions in the external phage coat influence host range of the phage although the main specificity resides in the product of the *h* gene." Further instances of suppression of *h* mutants are described on pages 10–11 and 66.

d. Genes *O* and *P* are suspected jointly to control an endonuclease activity in lambda phage (Freifelder and Kirschner, 1971). In a cleverly designed experiment specifically set up to see whether the two gene products cooperate directly, Tomizawa (1971) provided genetic evidence that there is indeed interaction in the formation of a functional molecule. Tomizawa found that the temperature-sensitivity of lambda replication due to a mutation in gene *O* was eliminated by a mutation in gene *P*. Presumably, the altered *P* product interacted with the thermolabile *O* product to yield a temperature-resistant complex.

e. Ito (1972) has provided analysis of interactions between compensating and noncompensating mutations in the genes for tryptophanyl tRNA synthetase (*trpS*) and the tryptophan repressor protein (*trpR*), indicating that these two proteins may interact directly *in vivo*.

Obviously, analyses of the types mentioned above form a powerful adjunct to other methodology in the detection and study of macromolecular interactions involved in the formation of aggregates, structures, and the performance of cellular functions.

C. Substitute Protein Activity

Alternate pathways and protein components are one commonly postulated mechanism of suppressor gene action. The suppressor mutation opens up an alternative biochemical pathway or alters a protein to widen its function so that it may serve in a capacity it is unable to serve in the wild-type organism. Or, regulation of enzyme production may be altered so that new functions may be served without actual alteration of catalytic activity. In such cases one would expect that ordinarily many mutant alleles of the suppressor locus would be effective in suppressing many or all alleles of the locus whose mutant phenotype is suppressed. Even deletions of the locus might be effectively suppressed.

The suppressors are thus expected to be "locus-specific" in their mode of action, and in fact the ability to be suppressed has constituted one criterion of allelism (Houlahan and Mitchell, 1947; Finck *et al.*, 1965). This characteristic may not be unique to bypass situations; for example, it is also possible to obtain "locus-specific" suppressors that decrease deleterious accumulations (see Section III, D).

The following examples illustrate some cases where metabolic lesions are overcome through the action of suppressor mutations that often are "locus-specific" and lead to a supply of substitute protein component(s). In terms of biochemical mechanisms, there are several quite different modes of action by which the phenotypic damage may be corrected.

1. Overflow in Channeled Pathways

The flow of metabolites often occurs in channeled pathways; that is, intermediates in the channeled pathway are not free to interact with other pathways. As a consequence of mutation the barrier between pathways may break down and thus lead to correction of the effects of genetic blocks. Analyses of these systems has proved valuable for the information they have given concerning channeling mechanisms and the evolution of distinctive control mechanisms. The discussion will focus on microorganisms, but it appears probable that similar phenomena exist in higher organisms, for example in *Drosophila* (see, e.g., Bahn *et al.*, 1971).

a. Arginine-Pyrimidine Pathways in Fungi. Studies on the mode of action of one series of suppressor mutations has afforded an especially well-documented example described below (also reviewed by Davis, 1967; Reissig *et al.*, 1967; O'Donovan and Neuhard, 1970). In these studies, analysis of the first suppressor mutation found in *Neurospora crassa* (Houlahan and Mitchell, 1947) has afforded interesting insights into metabolic flow *in vivo* (see Davis, 1972).

N. crassa contains two enzymes active in the synthesis of carbamyl phosphate (CAP). The first is an enzyme responsible for CAP used in arginine synthesis (Davis, 1963, 1965a,b), and the second is a CAP synthetase specific for pyrimidines (Davis, 1967; Reissig *et al.*, 1967; Williams and Davis, 1970). Figure 11 shows that mutations in the *arg-2* and *arg-3* loci block formation of CAP used in arginine biosynthesis (CAP^{ARG}) and certain mutations (*pyr-3* and *pyr-3a*) in the *pyr-3* gene block formation of CAP used in pyrimidine biosynthesis (CAP^{PYR}). Since the respective mutants require only arginine or only pyrimidines, respectively, the two pathways must be channeled; CAP made for one pathway is not used in the other pathway.

The mechanism of channeling is unclear but could involve as one com-

ponent the bifunctional nature of the *pyr-3* gene product. Gene *pyr-3* dictates the structure of an enzyme that carries out two consecutive steps in pyrimidine biosynthesis: the synthesis of CAP^{PYR} and the synthesis of ureidosuccinate from CAP^{PYR} and aspartate (aspartate transcarbamylase = ATCase in Fig. 11). *Pyr-3a* mutants lack CAP^{PYR} synthetase (Finck *et al.*, 1965; Davis, 1967; Reissig *et al.*, 1967; Williams and Davis, 1970) and have a kinetically altered ATCase (Hill and Woodward, 1968); *pyr-3d* mutants are partially or totally defective in ATCase (Davis, 1960; Davis and Woodward, 1962; Reissig, 1963a,b; Woodward and Davis, 1963); and mutants designated *pyr-3* lack both activities. Perhaps *in vivo*

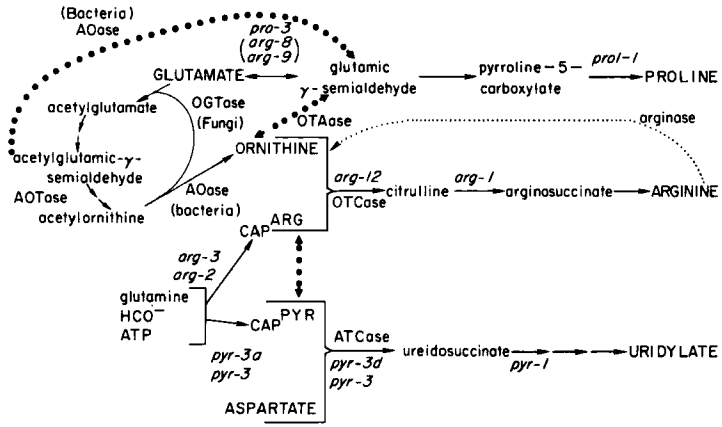

FIG. 11. Some aspects of proline, arginine, and pyrimidine metabolism in *Neurospora* and bacteria. Gene loci are italicized (e.g., *prol-1*), and enzyme names are abbreviated (e.g., AOase). Heavy dotted arrows indicate routes of overflow accounting for suppression in particular cases (consult text).

much of CAP^{PYR} is enzyme-bound and can thus be channeled specifically into pyrimidine biosynthesis by rapid interaction with aspartate (Davis, 1965b, 1967; Reissig *et al.*, 1967). Genetic studies on mutants of the *pyr-3* gene indicate that it dictates the structure of a single species of polypeptide chain with ATCase activity localized in the C-terminal portion (Radford, 1969a,b, 1970a,b, 1972). The two enzyme activities copurify (Williams *et al.*, 1970).

Mutations affecting CAP *utilization* in one pathway can serve to suppress mutations that decrease CAP *synthesis* in the second pathway. This genetically controlled suppression operates by creating a CAP "overflow," connecting the otherwise channeled pathways. Suppressors of *pyr-3a* mutants (Houlahan and Mitchell, 1947) occur in the *arg-12* gene and have reduced ornithine transcarbamylase (OTCase in Fig. 11)

activity (Davis, 1961, 1962a,b; Davis and Thwaites, 1963; Woodward and Schwarz, 1964). Davis (1961, 1962a,b) showed that one suppressor mutation in *arg-12* leads to a 98% reduction in OTCase activity with no change in growth rate in the absence of the end product, arginine. The residual OTCase activity is sufficient to maintain a supply of arginine adequate for growth but leads to an accumulation and overflow of CAP^{ARG} into the pathway of pyrimidine biosynthesis.

Similarly, mutations in the *pyr-3* gene of the *pyr-3d* type lead to accumulation and overflow of CAP^{PYR} and thus serve as a dechannelizing mechanism, suppressing *arg-2* and *arg-3* mutants (Reissig, 1960, 1963a,b; Davis and Woodward, 1962; Reissig *et al.*, 1965; McDougall *et al.*, 1969). Reissig *et al.* (1965) showed that *pyr-3d* mutants are effective in suppression if they contain 60% or less of the normal ATCase activity (and retain CAP^{PYR} synthetase activity), whereas a growth requirement for pyrimidine is not expressed until mutation has reduced the ATCase level to below about 25% of wild-type specific activity. A differential in minimum enzyme level leading to suppression and to pyrimidine requirement also can be inferred by the ability of an *arg-12/arg-12 pyr-3d* heterokaryon to grow on minimal medium (Reissig, 1958). The degree of suppression is a function both of the amount of reduction of ATCase level and the maintenance of CAP^{PYR} activity of the mutant bifunctional enzyme protein (Williams and Davis, 1970). Alterations in the K_m for aspartate of the altered ATCase proteins (Jobbágy, 1967) also probably are important.

A most important factor in channeling appears to be compartmentation of the duplicate enzymes (Bernhardt and Davis, 1972). Histochemical tests show that OTCase is located in the mitochondria while ATCase may reside in the nucleus. Since each enzyme is associated with its respective CAP synthetase, intracellular segregation of the two pathways serves to allow distinctive metabolic flow. This factor, plus the possible sequestering of CAP as an enzyme-bound intermediate also compartmentalized in the cell (Williams *et al.*, 1971) certainly must be a prominent factor in channeling.

A number of accessory observations is consonant with the above interpretations of suppressor action. Suppressed *pyr-3a* mutants are hypersensitive to arginine inhibition (Houlahan and Mitchell, 1947) since CAP^{ARG} synthetase activity is repressed by arginine (Davis, 1965b; Thwaites, 1967). One arginine-insensitive derivative has been indicated as having a reduced rate of arginine assimilation (Thwaites, 1967; Thwaites and Pendyala, 1968), presumably due to mutation in a transport system for basic amino acids (cf. Roess and De Busk, 1968).

Similarly, suppression of citrulline sensitivity (which gives rise to arginine, see Fig. 11) appears to be due to mutations affecting permeation of citrulline by a permease system primarily serving to transport neutral and aromatic amino acids (Thwaites *et al.*, 1970). These examples illustrate how new classes of mutations of primary interest may be obtained as suppressors for other systems.

There is a small amount of normal overflow in the direction CAP^{PYR} to CAP^{ARG}, as evidenced by slight residual growth ("leakiness") of *arg-3* mutants. This residual growth is eliminated by inclusion of uridine in the medium (Charles, 1962, 1964; Davis, 1963; Reissig *et al.*, 1967). Exogenous uridine also serves to decrease the strength of suppression of *arg-2* by *pyr-3d* mutations (Reissig *et al.*, 1967). These effects are due to repression and feedback inhibition of the *pyr-3* bifunctional enzyme protein by uridine triphosphate (Donachie, 1964a,b; Davis, 1965b; Caroline and Davis, 1969; Williams and Davis, 1970).

Overflow can be secured in the absence of suppressor mutations in high CO_2 atmospheres (Charles, 1962, 1964). The bifunctional ATCase of the *pyr-3* gene is induced by CO_2 (Nazario and Reissig, 1964), and suppression of *arg-2* by *pyr-3d* mutants is dependent upon the presence of CO_2 (Reissig *et al.*, 1967). Thus, this form of *phenotypic* suppression, like genotypic suppression, appears to rely upon mechanisms inducing overflow. Similarly, α-aminobutyrate supports growth of *pyr-3a* mutants by inhibition of OTCase activity (Fig. 11), again leading to dechanneling (Fairley, 1954; Fairley and Wampler, 1964; Charles, 1964).

There are further suppressor genes for *pyr-3d* mutants aside from *arg-12* (McDougall and Woodward, 1965); all may act by inducing CAP^{ARG} overflow. One class restricts ornithine production, limiting its coupling to CAP^{ARG} and thus releasing CAP^{ARG} to overflow. For example, mutants *arg-4* and *arg-7* suppress *pyr-3a* mutants (Mitchell and Mitchell, 1952a; McDougall and Woodward, 1965) and are deficient in ornithine–glutamate transacetylase activity (Vogel and Vogel, 1965) (OGTase in Fig. 11). In mutants of this type, addition of ornithine to the medium inhibits growth (Mitchell and Mitchell, 1952a; McDougall and Woodward, 1965). Still other *suppressor* + *pyr-3a* combinations are *stimulated* by exogenous ornithine (McDougall and Woodward, 1965); these strains could have readily inducible ornithine transaminase (OTAase in Fig. 11) so that the actual intracellular ornithine level is lower than normal. In addition, it is possible that some suppressors affect other control mechanisms. ATCase is repressible (Donachie, 1964a,b; Davis, 1965b; Caroline and Davis, 1969; Williams and Davis, 1970) and is influenced by CO_2 tension (Charles, 1962, 1964; Nazario

and Reissig, 1964; Reissig *et al.*, 1967) while the later enzymes in pyrimidine synthesis may be substrate inducible (Caroline and Davis, 1969); at least some enzymes involved in ornithine formation appear to be repressible (Vogel and Vogel, 1965).

Coprinus radiatus also contains a bifunctional ATCase, channeled CAP pools, and suppressor mutations similar in action to the *Neurospora arg-12* mutations (Cabet *et al.*, 1965; Hirsch, 1968; Gans and Masson, 1969). This could be the case also in *Drosophila* (Bahn *et al.*, 1971). *Saccharomyces cerevisiae* contains an arginine-specific CAP synthetase and a bifunctional pyrimidine CAP synthetase-ATCase with regulation similar to that of *Neurospora* (Lacroute, 1964, 1968; Lacroute *et al.*, 1965; Kaplan *et al.*, 1967, 1969); however, there is only weak channelling of CAP. The only *Saccharomyces* mutant described to be specifically deficient in CAP^{PYR} synthesis was originally isolated as a double mutant, grows on minimal medium when separated from the second mutation, and is only slightly inhibited by arginine (Lacroute *et al.*, 1965; Lacroute, 1968). Thus, CAP overflow appears to occur in the absence of suppressor mutations in *Saccharomyces* although some metabolic compartation occurs (Lue and Kaplan, 1970). CAP synthetases of other organisms are reviewed elsewhere (O'Donovan and Neuhard, 1970; Williams *et al.*, 1970; Gots, 1971; Jones, 1971). In Section III, C, 2, b, we discuss suppression of CAP synthetase by an entirely different mode of action in *E. coli*.

b. Arginine-Proline Pathways in Fungi. Channeling in the arginine and proline pathways (Fig. 11) also may be connected by overflow and consequent creation of a "new" pathway that circumvents a genetic block. In wild-type *Neurospora*, proline is synthesized from glutamate without appreciable flow of ornithine into proline biosynthesis (Vogel and Bonner, 1954; Abelson and Vogel, 1955; Yura and Vogel, 1955, 1959; Yura, 1959; Vogel and Kopac, 1959; Davis, 1968). Mutations in the *arg-8* and *arg-9* loci (now termed *pro* loci: Barratt and Radford, 1970) lead to inability in the conversion of glutamate to glutamic-γ-semialdehyde (Vogel and Bonner, 1954; Vogel and Kopac, 1959) and those at *pro-1* are blocked in the last step of proline biosynthesis (Yura and Vogel, 1955, 1959; Yura, 1959). Suppressor mutations at the *arg-12* locus that are effective in suppressing *pyr-3a* mutants also suppress *arg-8* and *arg-9* mutants (Mitchell and Mitchell, 1952a; Barry and Marsho, 1968). The accumulated ornithine in *arg-12* mutants induces an enzyme, ornithine transaminase (OTAase in Fig. 11), that converts ornithine to glutamic-γ-semialdehyde (Fincham, 1951, 1953; Fincham and Boulter, 1956; Vogel and Kopac, 1959; Castañeda *et al.*, 1967; Davis, 1968). The *arg-8* and *arg-9* mutants are slightly "leaky" and

this leakiness as well as suppression by mutations in the *arg-12* gene is eliminated in mutants lacking OTAase activity (Davis and Mora, 1968; Davis, 1968). Thus, accumulation and subsequent induction of a normal nonessential catabolic enzyme (Castañeda *et al.*, 1967; Davis and Mora, 1968; Davis, 1968) serve to join two pathways that are well-channeled in wild-type *Neurospora*. A suppressor mutation in the *arg-12* gene can simultaneously suppress a *pyr-3a* and an *arg-8* mutation (Mitchell and Mitchell, 1952a), the former by CAP overflow and the latter by ornithine overflow.

One important mechanism behind channeling is the intracellular compartmentation of enzymes and substrates. AOase, endogenous ornithine, and OTCase (Fig. 11) in wild type are predominantly located in the mitochondria whereas OTAase is a soluble enzyme located outside of the mitochondria (Weiss and Davis, 1972). This compartmentation probably plays a major role in the channeling of ornithine described above and explains some accessory observations reported below.

The *arg-8* and *arg-9* blocks (*pro-3*) can be phenotypically circumvented by addition of exogenous ornithine, citrulline, or arginine, accounting for their designation as *"arg"* loci (Srb *et al.*, 1950). Both exogenous ornithine and arginine induce OTAase activity, and arginine also induces an arginase that hydrolyzes arginine to ornithine and urea (Mora *et al.*, 1966; Castañeda *et al.*, 1967; Davis *et al.*, 1970; Morgan, 1970). Arginine also probably inhibits OTCase (Messenguy and Wiame, 1969) leading to further accumulation of ornithine. Exogenous ornithine is used for proline synthesis even in wild-type *Neurospora* (Abelson and Vogel, 1955; Vogel and Kopac, 1959) owing to induction of this very active OTAase activity that utilizes ornithine before it can be converted to citrulline (Davis and Mora, 1968). Ornithine may also inhibit the glutamate to glutamic-γ-semialdehyde reaction (Andersson-Kottö and Ehrensvärd, 1963) and thereby assist flow of ornithine into the proline pathway. *Arginase:pro-3* double mutants can be suppressed, but it is not known whether these suppressed mutants have constitutive or hyperactive OTA activity, are leaky *arg-12* mutants as discussed above, or are mutants having increased synthesis of ornithine (Morgan and Shaw, 1970). Other aspects of the channeling of ornithine in *Neurospora* are discussed by Davis (1967, 1968, 1972).

Aspergillus nidulans mutants analogous to the *N. crassa arg-8* and *arg-9* mutants carry mutations at the closely linked *pro-1* and *pro-3* loci (Forbes, 1956; see also Forbes, cited in Käfer, 1958; Weglenski, 1966). Mutations at these two proline loci are suppressed by mutations at a number of suppressor loci (Forbes, 1956; see also Forbes, cited in Käfer, 1958; Weglenski, 1966, 1967). One suppressor locus is involved

with the specific activity of OTCase and the mechanism of suppression is like that of the *Neurospora arg-12* suppressors mentioned above (Weglenski, 1967). Mutations at six other suppressor loci do not affect OTCase activity but produce altered regulation of arginase and of OTAase activity (Weglenski, 1967; Piotrowska *et al.*, 1969). The effect of these suppressor mutations appears to be exerted by the constitutive or increased presence of catabolic enzymes that divert ornithine (and arginine, via ornithine) into the proline pathway.

c. Arginine–Proline Pathways in Bacteria. The arginine and proline pathways of *E. coli* (cf. H. J. Vogel, 1953; R. H. Vogel *et al.*, 1971) and *S. typhimurium* have been joined by suppressor mutations to circumvent genetic blocks in proline biosynthesis, but the mechanism of overflow differs from that just described in Fungi. In the case of the bacteria, a deficiency in acetylornithine transaminase (AOTase in Fig. 11) leads to accumulation of acetylglutamic-γ-semialdehyde, and this is deacylated by acetylornithinase (AOase) to yield glutamic-γ-semialdehyde, a precursor of proline.

Bacon and Vogel (1963a) found that mutants blocked in conversion of glutamate to glutamic-γ-semialdehyde [comparable to the *pro-3* (*arg-8* and *arg-9*) mutants of *Neurospora*] require proline for growth whereas a second genetic lesion eliminating acetylornithine transaminase activity (AOTase) restores growth on minimal medium. The interpretation of the mechanism of suppression is that the double mutant accumulates acetylglutamic-γ-semialdehyde and that this overflows to supply glutamic-γ-semialdehyde sufficient for proline biosynthesis. Arginine is supplied at a slow rate by the low activity of some other transaminase with AOTase activity (Bacon and Vogel, 1963b; Albrecht and Vogel, 1964). This second transaminase is now suspected to be an enzyme that is present at low levels in wild-type strains but is induced by arginine to high levels in other strains (Vogel *et al.*, 1963, 1967; Bacon and Vogel, 1963b; Jones *et al.*, 1965, 1966).

The *Salmonella proA* and *proB* loci are concerned with the synthesis of glutamic-γ-semialdehyde (Kanazir, 1956; Miyake and Demerec, 1960; Itikawa and Demerec, 1968). Suppressor mutations have been detected that suppress mutations both in *proA* and in *proB* loci, including deletions that encompass both of these adjacent genes; the suppressed mutants are hypersensitive to inhibition by arginine (Itikawa *et al.*, 1968; Kuo and Stocker, 1969). The arginine-sensitivity can be attributed to four effects. First, in some strains arginine induces a protein with AOTase activity (Vogel *et al.*, 1963; Bacon and Vogel, 1963b; Jones *et al.*, 1965, 1966); this would tend to eliminate the pile-up of the precursor, acetylglutamic-γ-semialdehyde, that occurs when the normal, biosynthetic AOTase is absent. Second, arginine represses the arginine biosynthetic

enzymes, including those responsible for the synthesis of acetylglutamic-γ-semialdehyde (Baich and Vogel, 1962; Vyas and Maas, 1963; Vogel *et al.*, 1963). Third, arginine use is partially channeled (Sercarz and Gorini, 1964; Tabor and Tabor, 1969). Finally, it appears that one of the arginine biosynthetic enzymes, acetylornithinase (AOase in Fig. 11), is responsible for the conversion of the accumulated acetylglutamic-γ-semialdehyde to the proline precursor, glutamic-γ-semialdehyde. AOase acts on acetylglutamic-γ-semialdehyde at about 3% of the rate that it acts upon its normal substrate, acetylornithine (Itikawa *et al.*, 1968). Since AOase is repressed by arginine (Vogel, 1957, 1961; Maas, 1961; Vogel *et al.*, 1963; Itikawa *et al.*, 1968), suppressed strains that require extended activity of this enzyme would be sensitive to arginine repression. Mutations to constitutivity of the arginine enzymes relieve the arginine sensitivity (Itikawa *et al.*, 1968); suppression of this arginine sensitivity thus allows a direct selection for arginine regulatory mutations.

The experiments cited above, and additional ones (Bacon and Vogel, 1963a) support the view that suppressor mutations retarding arginine biosynthesis create a new flow of metabolites that circumvent a genetic block early in the pathway of proline biosynthesis.

2. Alternate Proteins

In the cases discussed above, "locus-specific" suppressor mutations lead to an altered flow of metabolites and essentially create new metabolic pathways carried out by enzymes common to wild-type organisms. Sometimes the pathways are quite novel as major pathways; sometimes they mimic the same sequence of biochemical steps normally functioning. In each instance, suppression results from substitute proteins, similar to cases described below. And again, in the cases we describe next, there is some locus specificity in suppression.

Morgan *et al.* (1925) and Schultz and Bridges (1932) were first to show that some cases of suppression in *Drosophila* result from shifts in gene arrangement; a wild-type locus translocated to a new position merely serves to "suppress" recessive alleles of that same locus in the proper genetic background. Muller and Oster (1957; Oster *et al.*, 1958) describe observations suggesting that a particular suppressor of forked (f^{+ih}) also is due to actual translocation of the *forked* genes, although other explanations could be suggested (see p. 68). As pointed out by Wagner and Mitchell (1955, 1964), cases of "duplicate genes," so often found in plant hybrids, could have origins stemming from translocations that occurred during the evolutionary separation and divergence of the strains involved.

In addition to transposed nucleotide sequences of reasonably recent vintage of separation, evolutionarily separate but functionally "duplicate" genes and "isozymes" are exceedingly common in microorganisms (reviewed by Datta, 1969; Umbarger, 1969; Mortimer, 1969; Mortimer and Hawthorne, 1966), plants (citations in Wagner and Mitchell, 1955, 1964) and animals (Markert, 1968; Harris, 1969; Wagoner, 1969; Ohno, 1970; Manwell and Baker, 1970).

Most often, these "duplicate genes" are not truly duplicate; during evolution their substrate specificity, enzyme kinetics, or regulation has diverged (examples in Vessell *et al.*, 1968). Sometimes the substrate specificity overlaps fairly precisely; in other instances, there is a slight or potential overlap that can be broadened by mutation. Sometimes one of the enzymes functions catabolically and the other anabolically, the two functions being separated by different control mechanisms and possibly also by channeling mechanisms. In such cases, gene mutations may alter the control patterns or the binding of metabolites so that the one enzyme may serve in place of the other enzyme under conditions where such replacement of function does not normally occur. Such divergence of nonallelic genes is superimposed upon allelic differences which contribute substantially to enzyme polymorphisms (cf. Fincham, 1972).

One wonders whether sudden substitution of a homologous protein, perhaps protected and allowed to evolve without the normal mode of selective pressure (cf. Boyer *et al.*, 1969, 1971), is not an important factor in evolution (cf. Ohno, 1970). Additionally, suppressor mutations that channel the flow of metabolites through a second pathway may become fixed in evolution and account for some metabolic differences among species (cf. Tanaka *et al.*, 1967).

a. Two types of suppression have been described for mutants of *E. coli* defective in the enzyme-mediated process of genetic recombination (Clark, 1971). One class of suppressors are defective in an enzyme that appears to interfere with a secondary mode of genetic recombination. A second class of suppressors involves an activation or derepression of one or a series of enzymes which either directly replace the missing function or participate in an alternate pathway of minor import to wild-type bacteria.

In *E. coli* several genes have been identified whose alteration leads to a deficiency in recombination (Willetts *et al.*, 1969; Willetts and Mount, 1969; Clark, 1971). Mutants of two of these genes, *recB* and *recC*, contain a low residual recombination ability and lack an ATP-dependent DNase activity (Wright and Buttin, 1969; Oishi, 1969; Barbour and Clark, 1970; Goldmark and Linn, 1970; Wright *et al.*, 1971; Gold-

mark and Linn, 1972; Nobrega *et al.*, 1972; Tanner *et al.*, 1972). One class of suppressor mutations (*sbcA*) restores high recombination ability and accessory phenotypic properties (e.g., resistance to ultraviolet light and to mitomycin C) to all strains tested carrying *recB* or *recC* mutations as well as to *recB-recC* double mutants (Barbour *et al.*, 1970). The *sbcA* revertants still lack the ATP-dependent DNase but now contain a high level of an ATP-*independent* DNase, presumably a substitute protein involved with their enhanced ability to perform genetic recombination.

The low residual recombination ability of *recB* and *recC* mutants is greatly enhanced by a second class of suppressor mutations (*sbcB*) that lead to defective DNA exonuclease I activity (Kushner *et al.*, 1971, 1972). The elimination of exonuclease I activity serves in some manner to release otherwise cryptic activity in genetic recombination and in resistance to irradiation.

Amplification of the secondary pathway(s) by *sbcB* suppressors has allowed Clark and co-workers to obtain exonuclease I mutants, which are difficult to identify by direct search. Isolation of further recombination-deficient mutants from *recB-recC-sbcB* suppressed strains have revealed mutations in genes hitherto unanalyzed from the point of view of the enzymology of the recombination process (Clark, 1971). Thus, the discovery of indirect suppression of *rec*$^-$ mutations greatly broadens the base for study of an involved physiological process critical in genetics.

Other instances of suppression and epistasis involving interactions of phage genes or of phage genes with the bacterial *rec* genes are reviewed by Clark (1971). One case is worth mentioning here, since it superficially resembles the bacterial system described above. Bacteriophage T4 DNA ligase-defective mutants are unable to grow on wild-type host bacteria; however, suppression (growth) occurs if the phage also carries a mutation in the *r*II genes (Berger and Kozinski, 1969; Karam, 1969). The suppressed mutants rely on host ligase for phage growth and exhibit an increase in genetic recombination that is due to alteration of some parameter of the recombination process rather than to alterations in DNA synthesis (Krisch *et al.*, 1971, 1972). It is possible that the *r*II genes specify a nonessential protein that is a nuclease or affects susceptibility to nuclease activity. T4 ligase-defective mutants also are suppressed by mutations eliminating phage-induced endonuclease II (Warner, 1971).

b. Action of an alternate protein may explain cases of suppression of *pyrA* mutants in *Salmonella*. The *pyrA* locus of *S. typhimurium* and the corresponding locus of *E. coli* dictate the structure of a single enzyme,

carbamyl phosphate (CAP) synthetase. CAP synthetase, in contrast to fungal systems, supplies CAP for biosynthesis *both* of arginine and of pyrimidine (CAP^{ARG} and CAP^{PYR} in Fig. 11). While a variety of phenotypes are engendered by mutations in the *pyrA* locus (reviewed by O'Donovan and Neuhard, 1970; Gots, 1971, Toshima and Ishidsu, 1971), the most common is an absolute requirement for the products of the two pathways requiring CAP, namely, arginine and pyrimidine. Yan and Demerec (1965) found suppressors for such mutants, allowing slow growth on arginine or citrulline in the absence of pyrimidine (Fig. 11). Such suppressors were found for 5 of the 15 mutants tested, and cross-suppression was observed between the two mutants tested (Yan and Demerec, 1965). It is possible that the suppressors allow growth when supplied with arginine through the synthesis of CAP adequate for pyrimidines by a second enzyme, carbamate kinase (acetokinase) (cf. Brzozowski and Kalman, 1966). Fungi lack carbamate kinase and possess duplicate CAP synthetases (see pp. 38–40) so this suppression mechanism would not be found in *Neurospora*. On the other hand, the structure and regulation of CAP synthetase activity is extremely complex; it is inhibited by UMP, especially in the presence of glutamine, and it is activated by ornithine and by ATP (Abd-El-Al and Ingraham, 1969; Trotta *et al.*, 1971; for reviews, see Anderson *et al.*, 1970; O'Donovan and Neuhard, 1970) so that alternate mechanisms of suppression can be visualized. Clearly, additional studies on these mutants would be of interest since the prediction of suppressor activity cited above should be locus specific rather than allele specific.

c. Kemper and Margolin (1969) have supplied evidence suggesting that suppressor mutations which arise in certain *Salmonella* leucine-requiring mutants supply a substitute component that replaces the function of the *leuD* gene product. In *Salmonella* and *Neurospora*, leucine biosynthesis proceeds by the pathway outlined in Fig. 10 (Calvo *et al.*, 1962; Gross *et al.*, 1962, 1963; Jungwirth *et al.*, 1963; Burns *et al.*, 1963). In *Salmonella* isomerase activity is coded for by two adjacent cistrons, *leuC* and *D* [termed *leuIII* and *IV* in Margolin (1963) and Gross *et al.* (1963)], while in *Neurospora* two unlinked genes code for the isomerase (Gross, 1962, 1965; Gross *et al.*, 1963; Gross and Webster, 1963). There is strong evidence that the isomerase of *Neurospora* is a multimeric enzyme composed of two different polypeptide subunits whose structures are dictated by the *leu-2* and *leu-3* genes, respectively (Gross, 1962, 1965; Gross and Webster, 1963). A similar situation is likely to exist in *Salmonella*. It can be proposed that the product of the *leuC* gene carries significant isomerase activity only when in combination with a polypeptide produced by the *leuD* gene, a common type of occurrence

in multimeric proteins (cf. Ginsburg and Stadtman, 1970; Trotta *et al.*, 1971).

Kemper and Margolin (1969) found that suppressor mutations affecting *Salmonella leuD* mutations map at a specific locus, *supQ* (cf. Sanderson, 1970). Different mutations in *supQ* suppress the leucine requirement to varying degrees; deletions of the entire *supQ* locus do not suppress. The suppressors are effective with mutations that lead to the most defective *leuD* gene products, namely, with deletions of parts or all of the *leuD* gene as well as with *leuD* nonsense mutations. Thus, *supQ* function replaces *leuD* function. The suppressors are not effective on mutants of other *leu* genes, nor are the suppressors active with *leuD* mutants that produce a protein that is only slightly altered. Failure of suppression in these cases is attributed to the formation of a tightly bound, enzymatically inactive complex of the *leuC* polypeptide with defective *leuD* polypeptide. [Subunit dissociation and reassociation do not appear to take place readily in mixed extracts of *leuC* and *leuD* mutants for they exhibit no isomerase activity (Gross *et al.*, 1963).] *SupQ*, then, could serve to elicit, through mutation, a protein product that replaces the function of normal *leuD* protein in activating previously uncomplexed *leuC* polypeptide to functional isomerase. This activation could occur either directly through protein–protein interactions (Kemper and Margolin, 1969) or, perhaps, indirectly by supplying an active metabolite. Due to the nature of *supQ* mutations, Kemper (1971) argues that the *supQ* gene product is normally tied up in a complex with a protein elicited by another gene, gene *W*, and is released for suppression when *W* is deleted or inactivated or by particular missense mutations in *supQ* that alter protein–protein interactions.

d. Suppressors of *cysA* mutants of *Salmonella typhimurium* appear to supply a novel component active in sulfate and thiosulfate transport across the bacterial cell membrane. Mutants of gene *cysA* can grow on cysteine, sulfide, or sulfite as sources of sulfur but cannot grow on sulfate or thiosulfate (Mizobuchi *et al.*, 1962; Dreyfuss and Monty, 1963). The *cysA* region is comprised of three adjacent cistrons (Mizobuchi *et al.*, 1962; Ohta *et al.*, 1971). Mutants with lesions in each of the three *cysA* cistrons contain all the enzymes necessary for sulfate assimilation into cysteine (Dreyfuss and Monty, 1963) but lack a component of a transport system necessary for accumulation of sulfate and thiosulfate from the medium (Dreyfuss, 1964). Mutants defective in sulfate transport may be isolated either as cysteine-requiring mutants (Mizobuchi *et al.*, 1962) or as chromate-resistant mutants (Pardee *et al.*,, 1966; Ohta *et al.*, 1971) since chromate is toxic and taken up by the sulfate permease.

Suppressor mutations isolated for any of a number of *cysA* mutants partially eliminate the mutant phenotype in all nine *cysA* mutants tested, including the deletion *cysA20* that has lost all three cistrons (Howarth, 1958). The suppressors are specific for *cysA* mutants. Mutations in the other *cys* loci tested [the *cysBa, Bb, Bc, C, D, Ea, Eb, G, H, I, J* loci of Mizobuchi *et al.* (1962) and Demerec *et al.* (1963)] are not suppressed (Howarth, 1958; Flatgaard and Hartman, 1962 unpublished); the suppressors are not linked by P22-mediated transduction with these other *cys* loci (Howarth, 1958; Flatgaard and Hartman, 1962 unpublished). The suppressors allow *cysA* mutants to grow either with sulfate or with thiosulfate as sulfur sources, and the suppressors can be selected for on either compound. The suppressor mutations have a deleterious effect in that they approximately double the generation time of wild-type bacteria growing in minimal medium both in the presence and in the absence of cysteine and of *cysA* mutants growing in the presence of cysteine (Howarth, 1958).

Sulfate is unable to passively diffuse into wild-type enteric bacteria at the concentrations normally used in culture media (Pardee, 1957), as also evidenced by the inability of the transport mutants to grow on sulfate. One might hypothesize that the *cysA* suppressor mutations nonspecifically allow more ready passage. This seems unlikely since some suppressor mutations allow better growth of *cysA* mutants on sulfate than on thiosulfate while the opposite is true of other suppressors (Flatgaard and Hartman, 1962 unpublished).

Some transport-negative *cysA* mutants, including the *cysA20* deletion, still bind sulfate to a specific protein that is located in the cell surface. The protein is repressed by cysteine, is regulated by the same genes active in regulating the cysteine biosynthetic pathway, and is thought to be involved in sulfate transport (Dreyfuss and Pardee, 1965, 1966; Pardee and Prestidge, 1966; Pardee *et al.*, 1966; Pardee and Watanabe, 1968; Ohta *et al.*, 1971). This binding component has been crystallized and some of its properties studied (Pardee, 1966, 1967, 1968). Other *cysA* mutants, including three nonsense mutants, each defective in one of the *cysA* complementation groups, exhibit little or no binding activity for sulfate (Ohta *et al.*, 1971).

S. V. Shestakov, Pardee, and Hartman (1967 unpublished) found that the four suppressors tested (Howarth's *su-2, -5, -7,* and *-8*) fail to suppress two transport-negative *cysA* mutants (SP-25 = *cysA1130* and SP-30 = *cysA1131*) that contain but low levels of binding protein (Pardee *et al.*, 1966; Ohta *et al.*, 1971), whereas these same suppressors are active on deletion *cysA20* and a number of other *cysA* mutants (Howarth, 1958; Flatgaard and Hartman, 1962 unpublished). The *cysA*

suppressors must restore sulfate transport (cf. Kaback, 1970) by supplying a component that can take the place of the products of the three *cysA* cistrons. It seems likely that the new component elicited by the suppressor gene(s) cooperates with the sulfate-binding protein in transport to form a novel transport system.

e. Mutations blocking transport of organic molecules also are readily reversed by suppressor mutations. One mechanism of suppression can be due to the revelation of alternate, cryptic permeases with incidental activity on a second substrate (cf. Schaefler, 1967; Schaefler and Maas, 1967; Arditti *et al.*, 1968; Lin, 1970; Hofnung and Schwartz, 1971; Saier *et al.*, 1972). In a second mechanism of suppression, the K_m and V_{max} of existing permeases may be altered to accommodate the excluded molecule. For example, *Neurospora* mutants with genetic blocks in the normal tryptophan transport system are suppressed by mutations that increase the affinity for tryptophan of a second, parallel transport system (Stadler, 1967; Brink *et al.*, 1969). In a third type of situation, a mutant of *Salmonella* defective in a phosphorylating system for "sugar transport" regained ability to utilize D-mannose as sole carbon source by a suppressor mutation. The secondary mutation resulted in a 25-fold elevation of a mannokinase that was ordinarily present at very low constitutive levels. It was suggested that phosphorylation of mannose activated a latent transport process by facilitating release of mannose from a membrane carrier into the cytoplasm (Saier *et al.*, 1971).

In a somewhat analogous case, *Aerobacter* mutants were discerned to lack components of the sugar transport system involved in the conversion of mannitol to mannitol 1-phosphate. Mannitol 1-phosphate is substrate for the only mannitol-specific dehydrogenase in this species of bacteria (Tanaka *et al.*, 1967; Tanaka and Lin, 1967). Growth on mannitol is restored by a mutation leading to *constitutive production* of D-arabitol dehydrogenase (DAD). DAD is an enzyme with loose specificity and able to convert mannitol to fructose. The *synthesis* of DAD is induced by D-arabitol, *not* by D-mannitol, so that constitutive synthesis is necessary for enzyme production in the presence of mannitol (Tanaka *et al.*, 1967). Penetration of mannitol appears adequate to permit its use as a carbon source once the "new" dehydrogenase is present whereas in the absence of the dehydrogenase, no detectable mannitol accumulation occurs (Tanaka and Lin, 1967).

Indirect effects of sugar permease mutants are overcome by still further types of mechanisms. Saier and Roseman (1972) found one type of suppressor mutation in *Salmonella* that released inhibition of sugar uptake, allowing operation of an alternate mode of transport. In the case of glycerol utilization, Lin's research group has found three classes

of suppressors that specifically allow growth on glycerol of an *E. coli* phosphotransferaseless mutant unable to grow because of *failure to induce* proteins necessary for glycerol uptake and catabolism: (1) mutations eliciting *constitutive* high production of glycerol kinase and glycerophosphate dehydrogenase (Berman *et al.*, 1970), (2) a suppressor that increases the levels of glycerol kinase and of a protein mediating facilitated diffusion of glycerol yet allows normal repression of these enzymes (Berman-Kurtz *et al.*, 1971), and (3) mutations in the structural gene for glycerol kinase that engender an enzyme no longer sensitive to feedback inhibition by fructose 1,6-diphosphate; this leads to increased production of glycerophosphate, which then induces even higher levels of the catabolic enzymes (Berman and Lin, 1971).

Finally, defects in permease systems allow direct selection for bacterial suppressor mutants with defects in the cell envelope allowing a more ready penetration of substrate across this barrier (Lazdunski and Shapiro, 1972). Such "nonspecific" suppressor mutations should allow analysis of the constitution of the permeability barrier itself.

g. Guespin-Michel (1971b) describes two suppressor loci in *Bacillus* that restore wild-type polymyxin resistance to pleiotropic polymyxin-sensitive, sporulation-defective mutants. All mutants blocked early in spore-formation and representing five different loci are suppressed by each of the suppressors. While ability to sporulate is not returned, other secondary manifestations of the pleiotropic mutations (esterase, protease, and antibiotic production; regulation of nitrate reductase) also are suppressed to varying discrete degrees in a neatly ordered sequence. The various pleiotropic effects of the original mutations also are arranged in this same hierarchy. It is proposed that the suppressors restore lost membrane functions and affect catabolite repression, partially compensating for functions lacking in the primary mutants (Guespin-Michel, 1971b). Indeed, membrane transport systems are intimately tied to the commitment to sporulation (Freese *et al.*, 1970). There are at least two other loci effective in suppressing some of the pleiotropic effects in sporulation mutants (Guespin-Michel, 1971a).

h. Giles (1951) described a suppressor locus in *Neurospora* that mutates to partially relieve the methionine requirements of two nonallelic methionine mutants. The suppression is exerted on alleles of three genes, *me-2*, *me-3*, and *me-7* but not on *me-5* (Murray, 1960; Tokuno *et al.*, 1962). Mutants of each of the four genes are leaky (Tokuno *et al.*, 1962; Kerr and Flavin, 1970). It was originally thought that the suppressors, which alone lead to partial methionine requirement, eliminate secondary accumulations by creating a partial block at the early reaction specified by *me-5* (Tokuno *et al.*, 1962) (see Fig. 12). However, the

suppressor gene maps away from *me-5* (linkage group IVR: Murray, 1960) and on linkage group I near *albino-2* (Giles, 1951) as does *me-6* (Barratt *et al.*, 1954; Murray, 1960; Barratt and Radford, 1970). The *me-1* and *-6* mutants peculiarly appear defective in *two* steps required for methionine biosynthesis (Selhub *et al.*, 1969; Kerr and Flavin, 1970). Because all *me-2*, *-3*, and *-7* mutants are leaky and because of the pattern of suppression, it was suggested that the suppressor strain contained an activity (now known as sulfhydrylase utilizing, $S^=$ in Fig. 12) that opened up a minor "side pathway" of methionine biosynthesis circumventing the genetic blocks (Flavin, 1963). However, sulfhydrylase

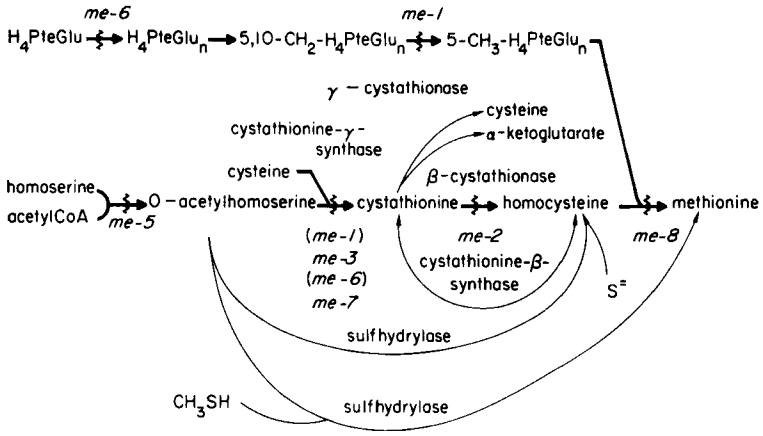

FIG. 12. Proposed main pathway (heavy arrows) of methionine biosynthesis and accessory reactions (light arrows) in *Neurospora crassa*.

activity of methionine-grown *Neurospora* is not significantly increased in *me-2* carrying the suppressor, so the suggestion was discarded (Kerr and Flavin, 1970). On the other hand, Wiebers and Garner (1967a,b) found an enzyme activity that could be the same as sulfhydrylase and which was repressed and feedback-inhibited by methionine. If native sulfhydrylase is indeed feedback-sensitive to methionine or to S-adenosylmethionine, as opposed to Kerr and Flavin's (1970) findings, then the suppressor could act to elicit feedback-resistant enzyme and open up the side pathway (Kerr, 1971). The sulfhydrylase pathway has been suggested as the normal major pathway in yeast (Cherest *et al.*, 1969), but this is questioned (Kerr and Flavin, 1970; Savin and Flavin, 1972).

Alternatively, the suppressor could release for biosynthesis cryptic γ-cystathionase and β-cystathionase (cystathionine-β-synthase in Fig.

12), activities that normally appear, for example, only on low sulfur medium. Such a γ-cystathionase activity was found by Flavin and Slaughter (1967); this and several other enzymes of sulfur metabolism are jointly under the control of two regulatory genes, *cys-3* and *scon* (Burton and Metzenberg, 1972). In addition, Fischer (1957) found both γ- and β-cystathionase activities "returned" in suppressed mutants. The observation of sulfur flow from *S*-methylcysteine into cysteine in the presence of high sulfate when the suppressor gene is present but not in the absence of the suppressor (Wiebers and Garner, 1964) and the failure of *cys-3* mutants to grow on *S*-methylcysteine (Burton and Metzenberg, 1972) also are consistent with this idea. One simple prediction is that *me-3*, *-7*, and *-2* mutants will be leakier on low than on high sulfate or cysteine media.

In any event, a "side shunt" seems to operate to circumvent and thus suppress genetic blocks in methionine biosynthesis. Analysis of methionine suppressors (as well as the *sfo* suppressors described in Section III, D, d) should allow better discernment of sulfur flow in *Neurospora*. The reduction of selenite (Zalokar, 1953) would seem a "marker" useful in the genetical portions of these analyses.

Five recessive methionine suppressor loci have been detected in *Coprinus* (Lewis, 1961) and at least six suppressor loci, only one of which yields dominant suppressors, in *Aspergillus* (Lilly, 1956; Gajewski and Litwińska, 1968).

The opening of side shunts could be a common phenomenon in eukaryotes. There are duplicate pathways ("conditional" pathways) that often are opened by mutation or unique cultural conditions (cf. Ulane and Ogur, 1972).

i. Gots and Gollub (1963) discovered a suppressor that relieves the purine requirement of all *purB* (adenylosuccinase) mutants in *Salmonella* (cf. Gots, 1971). The suppressor strains produce a substitute deacylase of unknown primary function that is immunologically distinct and fractionates differently from wild-type adenylosuccinase. The same research group (Benson *et al.*, 1972) also found evidence for two phosphoribosyltransferases in *Salmonella*. Deletion of the gene, *gxu*, coding for the transferase most specific for guanine and xanthine could be suppressed by mutation in the gene coding for the adenine-hypoxanthine specific transferase, allowing for its more efficient utilization of guanine.

j. Slonimski and co-workers (Sherman and Slonimski, 1964; Père *et al.*, 1965) describe a yeast mutant, *cy 1-1*, unable to grow on lactate even though containing lactate dehydrogenase, but completely devoid of the normally preponderant cytochrome *c*, iso-1-cytochrome *c* (iso-1). Mutation *cy 1-1* is an extensive deletion of the structural gene for iso-1

(Parker and Sherman, 1969; Clavilier *et al.*, 1969) yet *cy 1-1* readily mutates to growth on lactate. Among a small sample of revertants examined, mutations at five unlinked loci were detected. Each leads to growth on lactate and contains greatly increased amounts of a protein homologous to iso-1, namely iso-2-cytochrome *c* (Père *et al.*, 1965; Clavilier *et al.*, 1966, 1969). Each of these "compensator genes" is considered to be an independent locus, but at least one is probably due to a translocation of the *CY2* gene plus a mutation within the gene (Clavilier *et al.*, 1969). Perhaps all three loci, whose members are infrequently found in diploid strains and are dominant or semidominant, are translocations of this type. The other two suppressor genes, selected for in haploids where suppressor mutations are found with high frequency and which are recessive, may involve mutations in "regulator genes" (Clavilier *et al.*, 1969). In any event, it is clear that in all the above cases a normally minor protein species, iso-2, has come to substitute in activity for the normally predominant species, iso-1. This is not surprising, since Mattoon and Sherman (1966) showed that electron transport and oxidative phosphorylation in cytochrome *c*-deficient yeast mitochondria can be restored by either type of cytochrome *in vitro*. In addition, both cytochromes contain a methylated lysine residue (DeLange *et al.*, 1970) that may be critical to activity and respiratory control (see below).

Analysis of further *cy 1-1* revertants able to grow on lactate but containing no increase in cytochrome *c* (LAC mutants of Clavilier *et al.*, 1969) should reveal further modes by which the cytochrome *c* deficiency can be suppressed. Coupled oxidation of lactate in yeast proceeds exclusively by way of, and requires higher levels of, cytochrome *c* than does oxidation of substrates linked through cytochrome *b* (references in Sherman and Stewart, 1971). The LAC suppressors may circumvent this peculiar high-level requirement by opening a new channel or by adjusting electron flow through facilitated binding of other cytochromes *c* present in low concentration.

Suppression of cytoplasmic *mi-1* (*"poky"*) mutants in *Neurospora* might operate through action of substitute systems affecting protein methylation. "Poky"-type strains, of which there are several different isolates (Mitchell and Mitchell, 1952b; Garnjobst *et al.*, 1965; Diacumakos *et al.*, 1965; Griffiths *et al.*, 1968; Bertrand and Pittenger, 1969, 1972), are deficient in cytochromes *b* and $a + a_3$, cytochrome oxidase, and contain a great excess of nonparticulate cytochrome *c* (Haskins *et al.*, 1953; Tissieres *et al.*, 1953; Bertrand and Pittenger, 1969) and fatty acids (Hardesty and Mitchell, 1963). The fatty acid accumulation seems merely a secondary effect (Silagi, 1965). The content of other

mitochondrial enzymes has been reported as altered (Haskins *et al.*, 1953; Tissieres *et al.*, 1953; Tissieres and Mitchell, 1954; Woodward and Munkres, 1967; Woodward, 1968). Many of these observed differences could be due to secondary effects such as increased lability in *poky* strains (Edwards and Woodward, 1969; Eakin and Mitchell, 1970).

The slow-growing "poky" phenotype is reversed in some strains during continuous culture (Haskins *et al.*, 1953; Silagi, 1965) or through action of a chromosomal suppressor gene, *f* = *fast* (Mitchell and Mitchell, 1956; Griffiths *et al.*, 1968; Bertrand and Pittenger, 1972). Outgrowth also restores the wild-type cytochrome and respiratory patterns, but the suppressor has no marked effect on the cytochrome content of either *poky* or wild-type. The suppressor does *not* enhance growth in a second type of cytoplasmic mutants, *mi-3*, that has a different cytochrome spectrum (Mitchell *et al.*, 1953; Tissieres and Mitchell, 1954; Mitchell and Mitchell, 1956; Bertrand and Pittenger, 1972). Chromosomal suppressor *f* does not suppress two chromosomal mutations that influence cytochrome content, nor does a suppressor for one of those mutations suppress *poky;* in fact, *poky* blocks suppression of the nuclear mutation (Mitchell and Mitchell, 1956).

Strains *mi-3* and *poky* do not complement in common cytoplasm but certain strains of the "poky" phenotype do complement (Pittenger, 1956; Gowdridge, 1956; Bertrand and Pittenger, 1972). *Poky* also fails to complement a slow-growing cytoplasmic mutant, *SG*, that has normal cytochrome content (Srb, 1958, 1963).

An amino acid substitution was reported in a supposedly major protein species of mitochondria, a "membrane structural protein" (Woodward and Munkres, 1966, 1967; Woodward, 1968). The observation of a single major structural protein species in which such a change could be detected has been seriously challenged (Sebald *et al.*, 1968; Ashwell and Work, 1970), and the claim was retracted (Zollinger and Woodward, 1972). Alternative suggestions have been made that *poky* mitochondria lack several minor protein species in normal concentration (Sebald *et al.*, 1968), exhibit an imbalance in synthesis of mitochondrial ribosome subunits (Rifkin and Luck, 1971), and contain altered transfer RNAs (Brambl and Woodward, 1972). Let us simply postulate that *poky* mitochondria do not bind cytochromes with normal efficiency and contain altered cytochrome oxidase and other undermethylated molecules. This then triggers a multitude of secondary effects.

Wild-type *Neurospora* cytochrome *c* and cytochrome *c* in "old" (normal growth) "poky" cultures contain an unusual amino acid, ε-N-trimethyllysine, at amino acid residue number 72 that is present only as lysine in "young" (abnormal) "poky" cultures (Scott and Mitchell, 1969;

DeLange *et al.*, 1969). It is suggested that methylation of cytochrome *c* changes its binding affinity for proper sites on mitochondria; this binding could modify an otherwise normal respiratory chain and its regulation (Eakin and Mitchell, 1970). Methylation also might protect cytochrome oxidase from damage (see discussion in Edwards and Woodward, 1969) and could be critical to normal cytochrome oxidase function (discussion in Scott and Mitchell, 1969). Suppressor *f*, then, could merely serve to facilitate modification of cytochromes and other molecules to enhance proper binding and function. An altered route of methylation in suppressor *f* strains is a possibility.

More direct evidence of interaction of cytoplasmic and nuclear factors involved in one-carbon metabolism has been found in yeast (Lowenstein, 1971). The combined tools of genetics and biochemistry appear capable of presenting a detailed picture of factors involved in organelle enzyme content and function (for reviews, see Coen *et al.*, 1970; Linnane and Haslam, 1970; Sherman and Stewart, 1971; King, 1971).

k. A case of suppression in *Neurospora* appears to be due to induction of a catabolic enzyme that replaces the function of its constitutive biosynthetic counterpart. Case, Giles and Doy (1972) found that *arom-1* mutants, blocked in the conversion of 5-dehydroshikimic acid (DHS) to shikimic acid and lacking DHS reductase activity could revert by suppressor mutations in a second gene, *qa-4*. The *qa-4* mutations prevent metabolic destruction of accumulated DHS. Accumulation of high levels of DHS leads to induction of a normally catabolic enzyme, shikimic acid dehydrogenase, which supplies shikimic acid from DHS, fulfilling the function of the constitutive reductase.

l. Sometimes suppressors merely operate to create a more efficient flow of metabolites rather than truly circumventing the genetic block. One typical example is cited here.

E. coli and *Salmonella* mutants lacking thymidylate synthetase require 10-fold the expected level of thymine for growth (Cohen and Barner, 1954). Secondary mutants are readily selected with about one-tenth of this requirement (Harrison, 1965; Alikhanian *et al.*, 1966; Okada, 1966; Eisenstark *et al.*, 1968). This partial suppression is due to genetic blocks affecting either of two enzymes in the catabolism of deoxyribose 1-phosphate (dR1P) on the degradative pathway for deoxynucleosides (Breitman and Bradford, 1967; Beacham *et al.*, 1968). The second genetic block bestows an enzyme normally used in catabolism, thymidine phosphorylase, with a ready supply of substrate, dR1P, for use in the reverse reaction, that is, in the conversion of thymine to thymidine and prevents the destruction of thymidine. The suppressed strains are sensitive to deoxynucleosides if the genetic block in dR1P catabolism allows forma-

tion of deoxyribose 5-phosphate (dR5P) since dR5P is itself inhibitory (Lomax and Greenberg, 1968) or induces excess phosphorylase and leads to accumulation of another toxic compound, possibly dR1P (Becham *et al.*, 1968; Bonney and Weinfeld, 1971). Further mutants resistant to deoxynucleosides were isolated. These suppressors actually fit into the next section of this review, for they are mutations in earlier reactions that shut off the toxic accumulation (Robertson *et al.*, 1970).

D. Elimination of a Deleterious Accumulation

In Section II, D we discussed situations in which mutant *proteins* act as inhibitors of cellular processes. Second-site mutations eliminate the toxic protein. Below we cite examples of inhibitions by *metabolites* and the various means by which these inhibitions may be relieved.

The main phenotypic manifestations of mutations often arise simply from deficit of a metabolite beyond a genetic block; effects of accumulated metabolic intermediates, etc., often are secondary. However, cases are known where an end-product deficit does not cause the most readily observed or extreme phenotypic change. Rather, a slowed reaction results in accumulation behind a partial or complete genetic block of a metabolite in toxic concentration or of an excess of a metabolite that subsequently is converted to a toxic compound. Relief of the deleterious accumulation or of its effects through suppressor mutations can restore, or partially restore, the wild-type phenotype even though the initial genetic block persists.

a. Phosphate esters of sugars are toxic to bacteria when accumulated in large amounts such as behind a genetic block. If an alternate source of carbon is available, toxicity is overcome and growth can ensue due to suppressor mutations that prevent the accumulation of the toxic phosphate ester. The first such case analyzed in detail was the accumulation of galactose 1-phosphate by *E. coli* mutants blocked in the utilization of the phosphate ester (Yarmolinsky *et al.*, 1959; Nikaido, 1961; Fukasawa and Nikaido, 1961). Suppression is achieved by creation of a second block in galactose metabolism preventing accumulation of the phosphate ester, namely a block in galactokinase (Yarmolinsky *et al.*, 1959; Nikaido, 1961; Sundararajan *et al.*, 1962; Fukasawa *et al.*, 1963). Suppression of galactose sensitivity is widely used in selection of particular mutants of high interest (see pp. 11–12; also see Ippen *et al.*, 1971). L-Arabinose mutants accumulating L-ribulose 1-phosphate are arabinose sensitive, and this sensitivity is relieved by mutations leading to loss of L-ribulokinase (Englesberg *et al.*, 1962). Similarly, *E. coli* defective in 2-keto-3-deoxygluconate-6-phosphate (KDGP) aldolase cannot grow on gluconate although an intact alternate pathway for glu-

conate utilization is still present. Suppressor mutations allow growth on gluconate by channeling gluconate into the remaining pathway and eliminating its conversion to the toxic sugar phosphate ester, KDGP, which otherwise is accumulated behind the primary genetic block (Fradkin and Fraenkel, 1971; Fraenkel and Banerjee, 1972).

This type of situation is not merely a test tube creation. Just as in the case of mutant bacteria mentioned above, some wild-type bacteria are sensitive to the presence of particular sugars. For example, *Salmonella typhosa* strains are sensitive to L-rhamnose and cannot utilize it as a carbon source. Rhamnose apparently is metabolized to toxic rhamnulose 1-phosphate but no further in this species. Mutations blocking conversion to the phosphate ester lead to rhamnose resistance (Englesberg and Baron, 1959; Englesberg, 1960).

b. Studies with *Neurospora* were initially interpreted as indicating that a suppressor of acetate mutants opened up a secondary pathway of acetate production (Lein and Lein, 1952). The suppressor (*sp*) was active on all acetate mutants. Closer biochemical scrutiny, however, revealed that the suppressor acted by eliminating accumulation of an inhibitory substance (Strauss, 1953, 1955a,b; Strauss and Pierog, 1954). Strauss found that the block in the acetate mutants leads to the funneling of accumulated pyruvate into toxic acetaldehyde and thence into ethanol. Acetate inhibits the accumulation of pyruvate from glucose. The requirement for acetate is partially alleviated by mutations in either of two nonallelic genes, *sp* and *car,* that lower pyruvate carboxylase activity and thus limit the conversion of pyruvate to acetaldehyde.

c. Sturtevant (1956) described in *Drosophila* an autosomal dominant mutation, *K-pn* (*Killer of prune*), that is lethal when in combination with alleles of *pn* (*prune*) but has no phenotypic effect alone (i.e., pn^+ suppresses *K-pn*). The *pn* mutations result both in a reduction of red (pteridine) pigment and an increase in brown eye pigment (cf. Nolte, 1955). Lifschytz and Falk (1969a,b) speculate that *pn* flies accumulate a pteridine pigment precursor that is converted to a toxic substance in the presence of a single dose of *K-pn;* that is, the enzyme dictated by the *K-pn* locus carries out its normal or an analogous function but has either widened substrate specificity or altered regulation in flies carrying the *K-pn* mutation. An alternate possibility is mentioned on pp. 17–18.

There is speculation that lethals are very common in populations of *Drosophila* but that many are suppressed; recombination between the otherwise "silent" suppressors and the lethals reveal "synthetic lethals" such as in the instance just cited (Magalhães *et al.,* 1965).

d. Sometimes suppressor mutations occur that do not drastically affect

the overt phenotype but nevertheless lend a selective advantage under particular growth conditions. The suppressor mutations appear to shut off deleterious accumulations behind the primary genetic block and thus minimize secondary ramifications of this metabolic lesion. Therefore, in culture of a single mutant one sometimes ends up with a double mutant that retains the primary genetic block and, in addition, carries a new mutation affecting an earlier step in the same metabolic pathway. One example is the adenineless double mutant of *Neurospora* (Mitchell and Mitchell, 1950), and another example is the cysteineless double mutants of *Salmonella* (Gillespie *et al.*, 1968). We would guess that such occurrences are more common than reported and widely overlooked as potential adjuncts to other methods of genetic analysis.

e. Emerson (1948, 1952) described reversions of a sulfonamide-requiring mutant (*sfo*) of *Neurospora* sensitive to normal intracellular levels of *p*-aminobenzoic acid (Zalokar, 1948; Emerson 1949). The revertants proved to be heterokaryons carrying the *sfo* mutation in all nuclei and an additional "suppressor" mutation in some nuclei. These suppressors were mimicked in an artificial heterokaryon carrying in some nuclei *sfo* and a mutation (*pab*) blocking *p*-aminobenzoic acid synthesis (*sfo pab*) whereas other nuclei carried *sfo* pab^+ (Emerson, 1948). Growth on minimal medium occurs in mycelia containing the properly balanced gene dosage (nuclear ratio) that allows *p*-aminobenzoic acid synthesis sufficient for growth but inadequate for inhibition. Zalokar (1950) showed that the *sfo* mutation could be phenotypically reversed by adjustment of the ammonium concentration or by the addition of threonine, and that *sfo* was hypersensitive to inhibition by methionine. Besides the *pab* suppression, Zalokar (1950) detected restoration of growth by suppressors that limit methionine biosynthesis (also see Emerson, 1952). The *sfo* strain would appear an ideal tool for selection of new *arom* and *met* mutants but does not seem to have been used for this purpose.

f. The growth requirement of two "pantothenicless" (*pan*) mutants of *Neurospora* was shown by Wagner and Haddox (1951) to be alleviated by either of two mutations affecting aromatic amino acid biosynthesis. The data of these workers implicated the production of an inhibitor in the *pan* mutants whose production was reduced in the suppressed strains. They stressed "the concept that many biochemical mutants are due to internal upsets in the balance of metabolic systems."

E. Effective Dosage of a Limiting Gene Product

Several cases have been described where suppression results from an increase in a limiting gene product brought about through increased

effective gene dosage without apparent alteration in the product itself. Sometimes, the suppression occurs as the result of gene duplication (two chromosomal copies) or "gene magnification" (multiple chromosomal copies) and merely serves to make available more of the limiting gene product. No role of "gene amplification" (production of extrachromosomal gene copies: cf. Brown and Dawid, 1968, 1969; Gall, 1969) has been shown in suppression, but this mechanism remains a possibility. At other times suppression is achieved by elimination of a source of restriction of gene function, again making available more of the limiting gene product. Finally, mutation may alter the availability of another cellular component and suppress through restoration of a compatible balance between interacting molecules.

a. The first example of suppression by gene duplication stems from the studies of Stern (1929), who demonstrated quantitative effects of different doses of various *bobbed* (*bb*) alleles in *Drosophila* and interpreted his data in surprisingly modern fashion. We now know that the "*bobbed* locus" contains a string of repeated gene sequences for ribosomal RNA (Ritossa and Spiegelman, 1965; Ritossa *et al.*, 1966a,b; reviewed by Birnstiel *et al.*, 1971). Various of the *bobbed* mutant alleles are deficient to various extents in ribosomal RNA gene sequences. The mutants are unstable, due to what appears to be disproportionate gene replication (i.e., "gene magnification" rather than unequal crossing-over), yielding gene copies again adequate for wild-type levels of ribosomal RNA synthesis (Ritossa *et al.*, 1966c, 1971; Ritossa, 1968; Atwood, 1969; Ritossa and Scala, 1969; Henderson and Ritossa, 1970; Tartof, 1971). The study of various *bobbed* mutants and their suppression promises to yield interesting information regarding regulation of ribosomal RNA gene loci, possibly pertinent to extrapolation with regard to other sequences of redundant DNA.

b. Folk and Berg (1971) present evidence indicating that a glycine-requiring *E. coli* mutant (Folk and Berg, 1970a,b) containing a defective glycyl tRNA synthetase with increased K_m reverts frequently ($>10^{-5}$) to glycine independence through gene duplication. An increased gene dosage appears to allow increased production of synthetase subunits, albeit defective ones, to circumvent the metabolic block. Various genes surrounding the synthetase structural gene also were duplicated in some revertants, allowing indirect demonstration of the chromosomal location of the duplication. A further type of suppressor was also mentioned but not characterized by Folk and Berg (1971); its study might yield information pertinent to interaction of the synthetase with other cellular components.

Carbon *et al.* (1966a,b) have provided an example of biochemical

interactions leading to enhanced suppression. Suppression of a missense mutation resulting in a *Gly* for an *Arg* amino acid substitution (Brody and Yanofsky, 1963) was shown to be due to a genetically altered transfer RNA accepting glycine (Carbon *et al.*, 1966a,b). Suppression by the altered $tRNA^{Gly}$ was enhanced in a strain carrying a second genetic alteration leading to an approximately 7-fold enhanced level of Gly-$tRNA^{Gly}$ synthetase activity (Carbon *et al.*, 1966b). It appears that the suppressor $tRNA^{Gly}$ is only sluggishly charged by the synthetase so that enhancement of synthetase activity assists in overcoming this metabolic bottleneck (Carbon and Curry, 1968). The genetic mechanism underlying enhanced synthetase levels remains to be determined, i.e., if due to increased effective dosage of synthetase genes or to an alteration in the synthetase itself.

c. Unstable informational suppressors sometimes result from a duplication of a critical gene in a haploid organism and subsequent mutation of one of the two gene copies. Brody and Yanofsky (1963) found an unstable allele-specific suppressor in *E. coli* that contains a new tRNA species active in insertion of glycine instead of the usual arginine at the AGA codon (Carbon *et al.*, 1966a,b). The instability of suppressors of this type has been shown to be due to the involvement of gene duplication. Two copies of a glycine tRNA gene plus mutation in one of the twin genes leads to a su^+/su^- genotype effective in suppression. Due to genetic homology of the duplicated chromosome region, one of the two *su* genes is readily eliminated by crossing-over (Hill *et al.*, 1969, 1970; Carbon *et al.*, 1969). The gene duplication is necessary since the presence of at least one wild-type (su^+) gene is needed for normal growth (Carbon *et al.*, 1970).

2. Elimination of a Restrictive Site

In Section II, C we surveyed instances where second-site mutations result in a partial or complete suppression of the mutant phenotype by circumventing the effects of a restrictive site. This may be a chain-terminating nonsense codon, a promoter mutation, or a gene whose expression is blocked by repression of transcription. While these cases will not be reiterated here in detail, it must be realized that many of the papers cited in Section II, C afford examples where polarity is relieved by mutations in genes other than those originally affected. We will briefly survey below some representative examples.

a. Deletion or Modification of the Restrictive Site. Deletions of the normal promoter regions in the histidine and tryptophan operons of *Salmonella* essentially lead to failure of expression of the remaining,

intact genes of the operon. This failure may be due to lack of a promoter site for binding of RNA polymerase and initiation of messenger RNA transcription, or to fusion with repressed operons. Selection for function of the remaining genes of the operons reveals one class of revertants in which the original deletion is extended (Ames *et al.*, 1963; Margolin and Bauerle, 1966). These extended deletions may create new promoters, bring the remaining genes closer to existing promoters, or fuse the operons to entirely different operons which are not repressed. In the lactose operon of *E. coli*, expression is shut down by mutations in the *i* gene that lead to formation of "superrepressor." One class of suppressors which again permit enzyme synthesis are operator constitutive mutations that have alterations in the DNA sequence with which the repressor molecules interact (Jacob and Monod, 1961; Willson *et al.*, 1964; Jacob *et al.*, 1964, 1965; Bourgeois *et al.*, 1965; Bourgeois and Jobe, 1970).

We might point out here the opposite type of situation. Pseudo-revertants of constitutive operator mutants have been detected as mapping in gene *i* (J. Sadler, cited in Reznikoff, 1972). One imagines that the normal repressor protein has decreased affinity for the mutant operator nucleotide sequence whereas particular mutant *i* gene products have regained recognition and regulatory ability.

b. New Effective Promoters. Mutants which have lost an "activator" protein involved in enhancement of transcription of the arabinose or maltose operons of *E. coli* regain activity by suppressor mutations. These suppressors create new sites (promoters) at which transcription may occur in the absence of "activator" (Englesberg *et al.*, 1969; Englesberg, 1971; Gielow *et al.*, 1971; Hofnung and Schwartz, 1971). Other cases of suppressor mutations engendering new promoters are discussed in Section II, C.

c. Chromosomal Transposition. For a long time it has been appreciated that "suppression" can occur merely by the combination in a diploid of a translocated wild-type gene and a mutant recessive gene (cf. Morgan *et al.*, 1925; Schultz and Bridges, 1932). In such cases, the transposed gene and its control are not altered; the transposed gene carries out its normal function in a normal manner. Another possible instance of a similar effect, but where regulation is altered, is found in yeast with regard to dominant "suppressors" producing iso-2-cytochrome *c* (pages 54–55).

In *E. coli*, a gene involved in arginine biosynthesis and necessary for growth on ornithine is restricted in function in particular mutants. Function is restored by duplication and transposition to a new chromosomal location away from the restrictive site (Glansdorff and Sand, 1968; Elseviers *et al.*, 1969; Cunin *et al.*, 1970). Three arginine biosyn-

thetic enzymes are specified by three genes arranged in an operon in the sequence *argC, B, H*. The effects of a polar *argB* mutation in causing a drastic reduction in the expression of *argH* are eliminated in one revertant that carries an additional, transposed copy of *argH*. The functional *argH* gene maps close to the original *arg* gene cluster but is clearly located outside of it. The authors were quite careful in proving that the newly active gene was a newly arising duplication, not merely a previously present cryptic gene with overlapping function (Cunin *et al.*, 1970). Duplications of this sort may also explain some of the unstable revertants of promoter deletions in the tryptophan operon of *Salmonella* (Margolin and Bauerle, 1966; Margolin, 1971) and also of the histidine operon (Ames *et al.*, 1963; Levinthal and Yeh, 1972) as discussed below.

d. Translocation to an Episome. Ames *et al.* (1963) described reversions of histidine-requiring *Salmonella* mutants wherein function was restored to intact but nonfunctional genes through duplication and translocation to an extrachromosomal site. The accessory genetic structure (*pi*, for piece) containing the histidine genes was unstable and frequently lost, was not linked by transduction to the chromosomal histidine genes, and did not require recombinational events for its transfer by transduction to other bacteria. Further investigations showed that *pi* was sometimes transferred along with the chromosomally located histidine genes during bacterial conjugation. Levinthal and Yeh (1972) found unstable recombinants from such conjugal crosses that now contain two *chromosomal* copies of the histidine gene region, namely, the original set plus the additional set formerly contained on the extrachromosomal element. The old and new sets of genes are now linked by transduction, and their instability may arise from crossing-over between the duplicated regions with elimination of one block of genes.

3. Increased Substrate

Mutations in the *metG* gene of *Salmonella typhimurium* result in methionyl-tRNA synthetases with 100-fold or greater reduced affinity for methionine and a substantial growth requirement for this amino acid (Gross and Rowbury, 1969, 1971). Such synthetase K_m mutants are suppressed by mutations in two distantly located genes, *metJ* and *metK*, that are involved in the regulation of the levels of the methionine biosynthetic enzymes (Lawrence *et al.*, 1968; Chater *et al.*, 1970; Chater, 1970; Smith, 1971). Although suppression could be achieved by protein–protein interactions, the mode of suppression proposed by these workers is that the secondary mutations result in an elevation in the intracellular methionine pool sufficient to saturate the defective synthetase (Chater *et al.*, 1970; Smith, 1971). Feedback inhibition of the first

enzyme specific to methionine biosynthesis is incomplete *in vivo* except at excessive external methionine concentrations (cf. Lee *et al.*, 1966; Chater and Rowbury, 1970); mutations to high levels of the biosynthetic enzymes thus expand the methionine pool and lead to methionine excretion. Apparently, mutations to full feedback-resistance alone do not increase the methionine pool sufficiently for suppression (Chater *et al.*, 1970; Smith, 1971). The opposite situation applies with regard to histidine biosynthesis in the same organism. In this case, histidyl-tRNA synthetase mutants with altered K_m for histidine are suppressed by mutations eliminating sensitivity to feedback inhibition (Sheppard, 1964; Hartman *et al.*, 1971) and leading to a consequent increase in the internal pool of "free" histidine sufficient for suppression (Roth and Ames, 1966; Wyche, 1971).

4. Restoration of "Balance"

Many cellular structures and enzymes are composed of dissimilar protein subunits which aggregate in a specific ratio. It is possible that aberrant structures are formed through protein–protein interactions if a balanced supply of the different subunits is not maintained. In a theoretical example, formation of a functional structure of composition A_1B_4 will be limited if either a short supply of B or an excess of A subunits diverts complex formation into nonfunctional A_1B_1, A_1B_2, and A_1B_3 aggregates. This type of situation might be rectified, according to the case, by a decrease in formation of A or an increase in B, respectively. Here we are speaking of the *quantity* of a protein, not its *quality*. Particularly in cases where assembly involves partially defective subunits, an influence of "balance" would be expected to have a strong influence. Also, it is possible that imbalance at the level of protein subunits is one component of "genetic imbalance" associated with changes in chromosome number in higher organisms.

a. Mutations in the classic *w* (*white*) locus of *Drosophila* serve as "dominant" suppressors of *z* (*zeste*). Gans (1953) showed that two functional doses of the w^+ gene were necessary for expression of light eye-color in *zeste* flies. Through the mapping of point mutations and use of various types of duplication and deficiency stocks, the w^+ requirement was later narrowed down to a dosage of just the right-most portion of *w*. This is a gene region encompassing two mutationally and recombinationally separable sites (Green, 1959a,b,c, 1963, 1969; Judd, 1959, 1964; Rasmuson, 1965). Deletions of this portion of the *w* "locus" also suppress (Green, 1959c, 1969; Judd, 1959, 1964). Detailed knowledge of cytological fine structure in the *w* chromosome region support the genetic analysis (Gersh, 1962, 1967; Lefevre and Wilkins, 1966; Rayle

and Green, 1968). Abridged reports of immunological studies indicate that a protein ("w-1") is the product of some locus other than *w* and is modified differently in the suppressing *w* mutants than in the wild-type or in nonsuppressing *w* mutants (Fuscaldo and Fox, 1962; Fuscaldo and McCarron, 1965, 1967; McCarron and Fuscaldo, 1968a,b). Owing to the genetic homology known between the *zeste* and *white* chromosomal regions, it has been speculated that the "modified" protein is a joint multimeric protein combining products of the *z* and *w* genes (McCarron and Fuscaldo, 1968a,b). The suppressing *w* mutants lack 3-hydroxykynurenine (Fig. 9) and contain but one-half the kynurenine hydroxylase activity of wild-type flies whereas the nonsuppressing mutants contain 3-hydroxykynurenine (Ghosh and Forrest, 1967a).

b. Floor (1970) has shown that mutations affecting bacteriophage T4 tail fiber production (gene *37*) are suppressed by secondary mutations in genes affecting baseplate or head synthesis. Mutations in tail sheath gene *18* are suppressed by mutations affecting baseplate synthesis. Mutations affecting head structure (gene *23*) are suppressed by mutations in gene *20* (another head gene) or in gene *25* (baseplate). The suppressive effects can be attributed to "balance" of functional components in phage assembly. Further cases of suppressors in phage assembly also may involve such "balances" (cf. pp. 10–11, and 37).

IV. Other Interesting Cases

The compilation by Lindsley and Grell (1968) informs us of many situations in which analysis of suppression offers avenues for interesting exploration. The cases below are presented merely as examples of situations where suppressors offer a powerful probe into biochemical mechanisms.

a. The origin and nature of various hereditary tumors in *Drosophila* (Bridges, 1916; Stark, 1919) remain to be defined in spite of intensive investigations [see reviews, larval tumors (*tu*): Burdette, 1959; erupt (*er*): Glass, 1957; Tumorous head (*tu-h*): Gardner, 1970]. In each case tumor formation is dependent on a polygenic system; numerous cases of suppression and modification of tumor incidence and distribution have been found since the original observations of Stark and Bridges (1926).

Tumor incidences are differentially influenced by the presence of tryptophan and certain related compounds (see Fig. 9), such as phenylalanine, kynurenine, anthranilic acid, and 3-hydroxyanthranilic acid (Glass and Plaine, 1954; Plaine and Glass, 1955; Kanehisa, 1956; Brooks, 1967; Burnet and Sang, 1968). In some systems, effects of the feeding of tryptophan may have a component of "toxicity" (cf. Turner

and Gardner, 1960), but it seems unlikely nonspecific "toxicity" (for example, a mere lengthening of the normal larval period) rather than specific physiological effects could account for all the data. The response of tumor incidence to a given compound is influenced by particular modifier genes and metabolism by organisms in the larval food (cf. Burnet and Sang, 1968), probably accounting in part for the divergence of reports in the literature as to the relative efficiencies of these various compounds. Oxygen also stimulates tumor production (Glass and Plaine, 1952; Plaine and Glass, 1952, 1955; Plaine, 1955a; Brooks, 1967). Generally cysteine and methionine reverse the effects of tryptophan and related compounds (Plaine, 1955b; Burnet and Sang, 1968). The content of some free amino acids is altered in *tu* strains, but free tyrosine content remains constant (cf. Lewis, 1954; Yamazaki, 1968). These various responses led Glass (1957) and Brooks (1967) to suggest reactions affected by a suppressor of erupt (*su-er*) and by a suppressor of larval tumors (*su-tu*) as shown in Fig. 9. However, the proposed sites of action assume that effects are elicited directly by some metabolite rather than indirectly through regulation of another step in metabolism.

Melanotic larval tumors in *tu* strains are due to a particular type of blood cell, the "lamellocyte" (Russell, 1940; Rizki, 1957, 1960). The circulatory lamellocytes are precociously produced in *tu* strains (Rizki, 1957) or appear when larval life is extended beyond its normal period ("overaged larvae") such as in larval lethals unable to pupate (e.g., *ltl,* Kobel and van Breugel, 1967). Chemicals mimicking juvenile hormone (Bryant and Sang, 1969) and X-rays (Glass, 1944; Glass and Plaine, 1950; Hildreth, 1967) increase tumor incidence in particular suppressed *tu* stocks while ecdysone leads to repression (cf. Burdette, 1959). The spindle-shaped lamellocytes originate in the lymph gland, and some suppressors may influence their release from this source (Barigozzi, 1958; Barigozzi *et al.,* 1960). The lamellocytes next lodge between tissues, preferentially encapsulating caudal fat cells, a step inhibited by the feeding of glucosamine (Rizki, 1961a). Perhaps the effect of glucosamine is exerted through alterations on specific sites involving intercellular adhesion (cf. Oppenheimer *et al.,* 1969; Roth *et al.,* 1971). Later, melanization of the tumors takes place. This latter step (important in tumor recognition) is possibly separately inherited (Barigozzi *et al.,* 1960) and might accompany cell dissolution and release of stored tyrosinase substrates to enzyme action (cf. Henderson and Glassman, 1969), allow activation of DOPA oxidase (cf. Lewis and Lewis, 1963), or allow action of some other active phenoloxidase (cf. Parádi and Csukás-Szatlóczky, 1969). Preer (1971) reviews recent evidence that viruses form one component of certain melanotic tumors; similar claims from other labora-

tories in the past have not been reproduced or followed up. Reports of cytoplasmic effects on tumor incidence are common, however.

The above review, admittedly incomplete, suggests that tumor formation in *Drosophila* is a multistep process that can be stimulated by a wide variety of genes (Burdette, 1959) and which also can be blocked (suppressed) by mutations at many sites elsewhere in the genome. Probably different genes engender blocks at different points in the sequence of events culminating in tumor formation. We would surmise that intense cytological and biochemical analyses of these interactions would reveal phenomena of real importance to students of developmental biology.

b. A recessive temperature-sensitive suppressor, *su(f)*, shows interesting allele-specific effects on members of two loci that presumably are of diverse function. The suppressor acts to suppress a minority of mutations at the forked (*f*) "locus" and enhances one mutation (w^a) out of 14 tested at the *white* locus (Green, 1955, 1956, 1959c). Out of 19 *w* mutations tested, the w^a mutation also is the only allele suppressed by another recessive suppressor, *su-w*^{a}. Of two partial back mutants (presumably intra-locus) of w^a, the phenotype of one was unchanged by either suppressor while the second still behaved as w^a. That is, it was enhanced by *su-f* and suppressed by *su-w*^{a} (Green, 1959c).

c. A suppressor, *tol*, overcomes growth restriction imposed by the content of both *A* and *a* mating type loci in duplication strains of *Neurospora* (Newmeyer, 1968). Most often, restriction in growth is overcome by transitions to homozygosity or hemizygosity rather than to suppressor mutation (Newmeyer and Taylor, 1967). The occurrence of suppressors, however, may allow a more direct analysis of the biochemical basis of mating type incompatibility.

Acknowledgments

Ordinarily one feels embarrassed at dedicating a paper to anyone, much more so to the Editor. However, we feel Professor Ernst Caspari is such a staunch person and his Editorial Pen has covered such a broad spectrum in this series, in *Genetics*, and elsewhere that his modesty now might be prepared to withstand even a dedication to himself. So, we do fondly dedicate this review to Professor Caspari, a warm and inspiring person with a special interest in young scientists and an Editor who has contributed vastly to communication of scientific data and ideas. The origins of this paper go back to a conversation with Professor Caspari at the time Gorini and Beckwith must have been planning their own review of the same subject (1966), a subject not exclusively covered before that time (although citations in his papers suggest that such

an undertaking was contemplated by C. B. Bridges in his latter years). Dr. Sigmund R. Suskind deserves credit for keeping the idea of this review alive. We thank a number of our colleagues for responding graciously to our requests for updated information and for comments on certain sections during the preparation of this review. Our own work cited herein has been supported by U.S. Public Health Service Research Grants AI01650 (P. E. H.) and AM12115 (J. R. R.). Contribution No. 684 of the Department of Biology, The Johns Hopkins University.

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