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Direction of Translation in Bacteriophage S13

Abstract. *Suppressible* (amber-*type*) *mutants in gene IIIa of bacteriophage S13 show polarity in reducing the activity of only the neighboring gene IIIb. The polarity implies that the genome is translated in the direction of IIIa to IIIb. From the known homology of the messenger RNA with the single-stranded DNA, the orientation of the DNA with respect to the genetic map can also be inferred.*

Bacteriophage S13 is known to have at least seven genes, which have been estimated to account for at least 70 percent of the phage genome (*1*, *2*). The order of these seven genes and the circularity of the S13 genetic map have been established by three-factor crosses (*3*). Data have now been obtained from complementation tests indicating the direction of translation of two of the seven genes. It is inferred that this is the direction of translation of the entire genome.

In the original work (*1*) on complementation in phage S13, the complementation group designated III was considered to consist of two subgroups, IIIa and IIIb, because complementation between suppressible (*su*) mutants in IIIa and IIIb was much lower than complementation between other mutant pairs. However, the discovery of temperature-sensitive (*t*) mutants of group IIIa has led to the finding of strong complementation between *t* mutants of IIIa and *t* mutants of IIIb. A comparison of *su* × *su* tests with *t* × *t* tests is shown in the first part of Table 1. Consequently, IIIa and IIIb are considered to be separate complementation groups. Mutants in IIIb show a markedly altered heat stability of the mature virus, indicating that IIIb determines a viral coat protein (*4*).

Additional complementation tests with both *t* and *su* mutants of groups IIIa and IIIb show that *su* mutants of IIIa are defective in the IIIb function but that *su* mutants in IIIb leave the function of IIIa intact, and *t* mutants in either group have no effect on the function of the other group (Table 1). The table shows that very poor complementation occurs between IIIa and IIIb whenever an *su* mutant in IIIa is used. The difference between *su* and *t* mutants is made more striking by the fact that *t*′43 is a false revertant of *su*43 and therefore both are presumably mutant in the same DNA triplet. These complementation results are qualitatively similar to those observed in bacteriophage T4 (*5*).

The *su* mutants in IIIa are polar, but their polarity does not extend past IIIb into IV. This is inferred from complementation tests between *su* mutants of I, IIIa, IIIb, and IV (Table 2) which show poor complementation only for IIIa × IIIb tests. The gene order is I-IIIa-IIIb-IV (*1*, *3*). The tests in Table 2 were done at 37°C, so the general level of complementation is higher than the tests in Table 1.

All the *su* mutants of S13 are suppressible by one or more of the known *Escherichia coli amber*-suppressor strains such as CR63 and C600, so it is concluded that each *su* mutant contains a nonsense codon and that polypeptide synthesis terminates at the point of the mutation, as has been shown for *amber* nonsense mutants affecting the head protein of bacteriophage T4 (*6*). The chain-terminating property of nonsense mutations is associated with the polar effect observed in genes which

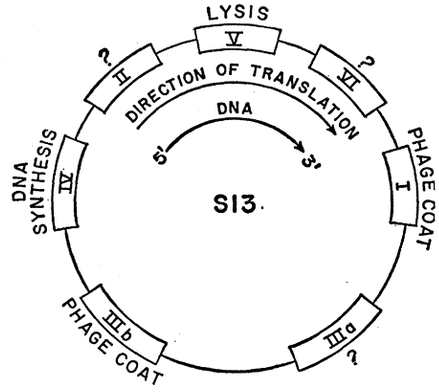

Fig. 1. Map of phage S13 (*1*, *3*) showing the gene functions where known. The direction of translation and the orientation of the single-stranded DNA was inferred from the polar properties of *amber*-type mutants in complementation tests between genes IIIa and IIIb.

normally are coordinately transcribed and translated; there is reduced activity of genes on the side of the nonsense mutation toward which translation would normally proceed (*7*). Accordingly, from the polar properties of the S13 *su* mutants we conclude that IIIa precedes IIIb in the coordinate translation of the two genes, and furthermore that the direction of translation of IIIa and of IIIb relative to the map order is IIIa → IIIb. Moreover, this direction of translation is presumably the same for the entire phage genome (Fig. 1) because of the observed homology (*8*) between the messenger RNA (mRNA) and the mature phage DNA.

It is significant that *su* mutants in other genes are not polar (*1*, *2*, *9*)

Table 1. Complementation tests between *su* and *t* mutants in complementation groups IIIa and IIIb. The tests were done at 42°C except for those marked with an asterisk, which were at 41°C. The method has been described (*1*). *Escherichia coli* C, which does not noticeably suppress any *su* mutant, was used as the host for the mixed infection. The burst sizes of *su* mutants were assayed on the permissive strain *E. coli* C600.1 (*2*), except for tests at 41°C in which cases *Shigella dysenteriae* Y6R (*1*) was used; *t* mutants were assayed on either C, C600.1, or Y6R. All burst sizes were corrected for the burst size of each mutant growing alone under the nonpermissive conditions; this correction produced negative results in two cases. The temperature-sensitive mutant *t*′43 is a false revertant of *su*43.

Mutants crossed IIIa × IIIb	Burst size	Mutants crossed IIIa × IIIb	Burst size	Mutants crossed IIIa × IIIb	Burst size
su × *su*		*su* × *t*		*t* × *su*	
*su*10 × *su*44†	0.6	*su*10 × *t*330	0.8	*t*37 × *su*44†	27
*su*43 × *su*44	0.5	*su*10 × *t*11†	−0.6	*t*37 × *su*66†	40
t × *t*		*su*41 × *t*11†	−3.4	*t*′43 × *su*44†	24
*t*37 × *t*11†	10	*su*43 × *t*11†	4.5	*t*′43 × *su*66†	26
t′43 × *t*11†	10	*su*43 × *t*330	2.0	*t*37 × *su*63*	29
t′43 × *t*330	29	*su*10 × *t*330*	1.1	*t*163 × *su*63*	56
*t*37 × *t*330*	13	*su*43 × *t*330*	2.7	*t*163 × *su*66*	42
*t*163 × *t*330*	37				

* Performed at 41°C and assayed with Y6R when *su* mutants were involved. † Cyanide not used during adsorption.

Table 2. Complementation tests between *su* mutants at 37°C.

Mutants crossed	Burst size
IIIa × IIIb	
*su*41 × *su*66	6.7
*su*43 × *su*44	8.7
*su*43 × *su*66	8.0
I × IIIa	
*su*52 × *su*43	88
I × IIIb	
*su*52 × *su*44	163
IV × IIIa	
*su*100 × *su*43	79
IV × IIIb	
*su*100 × *su*66	97

and in particular, that they do not seem to reduce the activity of IIIb. Polarity might actually exist between genes other than the IIIa-IIIb pair and yet not be observed in complementation tests if the products of these other genes are adequate for phage growth, even if present only in small quantities. However, it is clear from our results that polar mutations can noticeably affect IIIb, perhaps indicating that the viral coat protein which IIIb makes (*4*) is a major phage component required in large quantities. Therefore, the fact that *su* mutants in genes other than IIIa do not reduce the activity of IIIb in complementation tests suggests that none of the known genes precedes IIIa in a coordinate translation with IIIa, for otherwise the polarity model would suggest that such mutants should be defective in IIIb. The other genes then must either follow IIIa and IIIb in coordinate translation or else be translated independently of IIIa and IIIb. By the same arguments, we would expect to find that IIIa and IIIb are coordinately transcribed into the same species of mRNA, but it remains to be determined whether any or all other phage genes are included.

Because the mature phage DNA is single-stranded (*10*), it too has a polarity, which can also be determined in reference to the genetic map. It is known for other systems (*11*) that the direction of translation of mRNA corresponds to the direction from the 5′- to the 3′-end of a nucleotide. Since the mature phage DNA is homologous (*8*) to the mRNA and has the same polarity (*12*), the direction of translation must also correspond to the 5′→3′ direction of the DNA (Fig. 1).

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Vitamin A Deficiency: Effect on Retinal Structure of the Moth Manduca sexta

Abstract. *Sphingid moths* (Manduca sexta) *were reared for several generations on an artificial diet deficient in vitamin A and its precursors. Retinal tissue from depleted moths was removed for histological examination. There was extensive histolysis in the retinal epithelium and underlying nervous and connective tissues. This pathology correlated with severe visual impairment, even though normal growth, metamorphosis, and reproduction occurred. In the adult this pathology could be reversed when the larvae were reared only on tobacco (its usual host) or on the artificial diet supplemented with β-carotene or vitamin A palmitate.*

Inadequate intake of the fat-soluble factor termed vitamin A produces a purulent eye condition in rats (*1*), which is usually accompanied by temporary night blindness. A similar type of nyctalopia in humans can often be relieved by dietary intake of large amounts of vitamin A (*2*), the biochemical rationale for this therapy being that the prosthetic portion of rod visual pigment is vitamin A aldehyde (*3*).

The existence of vitamin A aldehyde (retinal) in insects was first reported in the heads of honeybees (*4*) and later in the Lepidoptera (*5*). The role of retinal as the chromophore of the visual pigment of various insect species and the absorption spectrum of this substance have been reviewed (*6*). Others (cited in *7*) have demonstrated that vitamin A is a factor in insect reproduction and metabolism of integumentary pigment. As in other animals, there are no substantiated claims of vitamin A biosynthesis, and this factor must be supplied by the diet. We have found a relationship between vitamin A deficiency and a subsequent pathological condition (or its reversal upon carotenoid intake) of the photoreceptor elements of the nocturnal tobacco hornworm moth *Manduca sexta*. For the first time in an invertebrate, optic pathology has been correlated with vitamin A deficiency.

Earlier electrophysiological studies (*8*) on the compound eye of *M. sexta* reared in the wild revealed a spectral sensitivity peak at 5000 Å; from this observation a rhodopsin system was postulated. Further behavioral evidence indicated that a vitamin A deficiency might effect the development of the retinal structure of the adult moth.

In an attempt to establish the role of vitamin A in the development of the retina and its associated elements in *M. sexta*, we reared larvae for several generations on an artificial diet (*9*) deficient in vitamin A. Other larvae were reared on tobacco leaves (*10*) or on an artificial diet containing 15 mg of β-carotene or 13.75 mg of vitamin A palmitate per 100 g (wet weight) of artificial diet. After this induction period, and 24 hours after adult emergence, the retinulae, postretinal axons, and adhering lamina ganglionaris were excised from the remainder of the optic lobe. These tissues were fixed immediately in a 10 percent solution of acrolein (buffered to *p*H 6.9) and were left in this fixative at 4°C for 2 hours. After fixation, the tissues were transferred to distilled water at 4°C for a period of 12 hours, and were then dehydrated, cleared, and embedded in 2-hydroxyethyl methacrylate