sensitive cells to produce antibody without significant replication. Likewise, GAE must have stimulated some of the pathways, but not necessarily those involved in cellular replication. Further, Bennett and Bloom (*15*) have observed that transformation of lymphocytes was not a requirement for MIF production. In their studies MIF appeared in supernatant cultures within 6 hours after antigen stimulation, but morphologic alterations of lymphocytes were not seen prior to 24 hours. Godfrey *et al.* (*1*) demonstrated inhibition of macrophage migration as early as 2 hours. It is suggestive that mediators, such as MIF, which result from the interaction of antigens with sensitized lymphocytes are produced either as a continuum of sequential events along the same pathway that culminates in blast transformation and mitosis or by pathways separate from those that lead to mitosis. Although the pathway of mediation was not elucidated, our results showed that polysaccharide fraction GAE stimulated the sensitive committed lymphocyte to the point of MIF production which correlated with delayed skin reactivity but not to the point of DNA synthesis or cellular proliferation.

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Localization of 5S RNA Genes on Drosophila Chromosomes by RNA-DNA Hybridization

Abstract. *[^{3}H]RNA with a high specific activity was prepared from larvae of* Drosophila melanogaster *grown 4 days in contact with [^{3}H]uridine. Purified tritiated 5S RNA was annealed to the DNA of polytene chromosomes, which had been denatured in formamide. The 5S RNA genes are placed within the region 56E-F of the right arm of chromosome 2. This localization was determined from autoradiographs, where the radioactivity from hybrids of [^{3}H]RNA and DNA was confined to the 56E-F segment.*

The ribosome from prokaryotic and eukaryotic organisms contains three RNA species, one molecule each of 23 to 28*S*, 16 to 18*S*, and 5*S* RNA, as well as numerous proteins. The 5*S* RNA is associated with the 23 to 28*S* RNA in the larger ribosomal subunit. The haploid *Drosophila melanogaster* (*1*) genome has between 130 to 190 copies each of the genes coding for the 18*S* and 28*S* RNA. These are found at the nucleolus organizer sites on the X and Y chromosomes. Tartof and Perry (*1*) have recently concluded that in *D. melanogaster* the haploid genome contains about 200 5*S* genes, roughly a number equal to the number of either the 18*S* or 28*S* genes. Furthermore, they excluded the sex chromosomes as the site of the 5*S* genes. An earlier investigation by Brown and Weber (*2*) in the toad, *Xenopus laevis*, showed that the 5*S* genes are not interspersed with the 18*S* and 28*S* genes and that there are 20,000 to 27,000 copies per haploid genome. This redundancy is 45 to 60 times that of the 18*S* and 28*S* genes and is in marked contrast to the situation in *Drosophila*. However, there is agreement in the two studies in that the site of the 5*S* genes is placed outside of the nucleolus organizer. In this study we have located the 5*S* genes cytologically in the genome of *D. melanogaster* from autoradiographs produced after hybridizing tritiated 5*S* RNA to denatured DNA of polytene chromosome squashes in situ.

First instar larvae (about 0.1 g) were placed on 10 ml of low yeast medium (*3*) containing 10 mc of [5-^{3}H]uridine (25 c/mmole, New England Nuclear Corp.). After 4 days' growth the third instar larvae were harvested by being floated in 60 percent sucrose (*4*). The RNA was extracted from the larvae by means of a modification of the phenol method in which sodium dodecyl sulfate was used (*5*). Column chromatography on methylated albumin Keiselguhr (MAK) (*6*) was employed to separate the RNA species. The fractions containing the 4*S* and 5*S* RNA species from the MAK column were dialyzed against a solution of 0.9*M* NaCl and 0.09*M* sodium citrate, at *p*H 7.4, in preparation for hybridization (*7*) and frozen until used. The radioactivity of the RNA taken from the MAK column was measured in a liquid scintillation spectrometer (*8*). The RNA had a specific activity of about 4×10^5 count min^{-1} μg^{-1} as measured on membrane filters where the counting efficiency was approximately 10 percent (4×10^6 disintegration min^{-1} μg^{-1}).

Squashes of salivary gland chromosomes from late third instar larvae were prepared and treated with ribonuclease (*7*). The DNA of the chromosomes was denatured by a 2.5-hour treatment in 95 percent formamide in 0.015*M* NaCl and 0.0015*M* sodium citrate (*p*H 7.4)

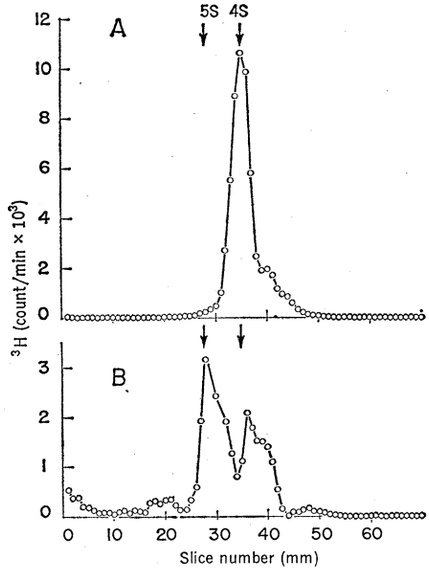

Fig. 1. Separation of tritiated 4*S* and 5*S* RNA on polyacrylamide gels (*9*). (A) The radioactivity profile of [^{3}H]RNA from the 4*S* peak from a MAK column. This peak of radioactivity coincides with the optical density maximum of unlabeled commercial transfer RNA (4*S*) from yeast (see arrow). (B) The radioactivity profile of the 5*S* RNA fraction taken from a MAK column. The main radioactivity peak agrees with the optical density maximum of 5*S* RNA taken from ribosomes from lily pollen (see arrow in A).

at 65°C. The [^{3}H]RNA was hybridized to the denatured DNA in the cytological preparations according to the methods outlined by Pardue *et al.* (*8*). Autoradiographs of the hybridized chromosomes were prepared with Kodak NTB2 liquid emulsion; slides were exposed for up to 2 months.

The 4*S* and 5*S* fractions taken from the MAK column were tested for purity by polyacrylamide-gel electrophoresis (*9*) (Fig. 1). The 4*S* fraction proved to be of high purity with no detectable 5*S* radioactivity. As expected (*1*), the 5*S* fraction from the MAK column contained a good deal of 4*S* RNA. Pure, unlabeled 5*S* RNA obtained from ribosomes of lily pollen was used as a marker in companion gels, and it banded very near to the drosophila 5*S* [^{3}H]RNA peak.

When the 5*S* RNA peak from the MAK column was used in hybridization, silver grains were found in great abundance over the nucleolus (*7*), indicating that ribosomal RNA was a contaminant in the 5*S* fraction, as found by others (*1*). In addition to the nucleolus, grains were encountered over many regions of the genome, the most heavily labeled of which was a segment of the 56E-F region of the right arm of chromosome 2. In nearly every chromosome spread a part of the 56E-F region was heavily labeled (Fig. 2). Indeed, it was possible to use the label as a cytological marker because of its very regular occurrence. The proximal, darkly staining bands of 56F (*10*) seem to be the site of localization, but the limits of resolution are such that the label could extend beyond the bands. Unfortunately there are very few genes mapped in this segment of chromosome 2R (*10*).

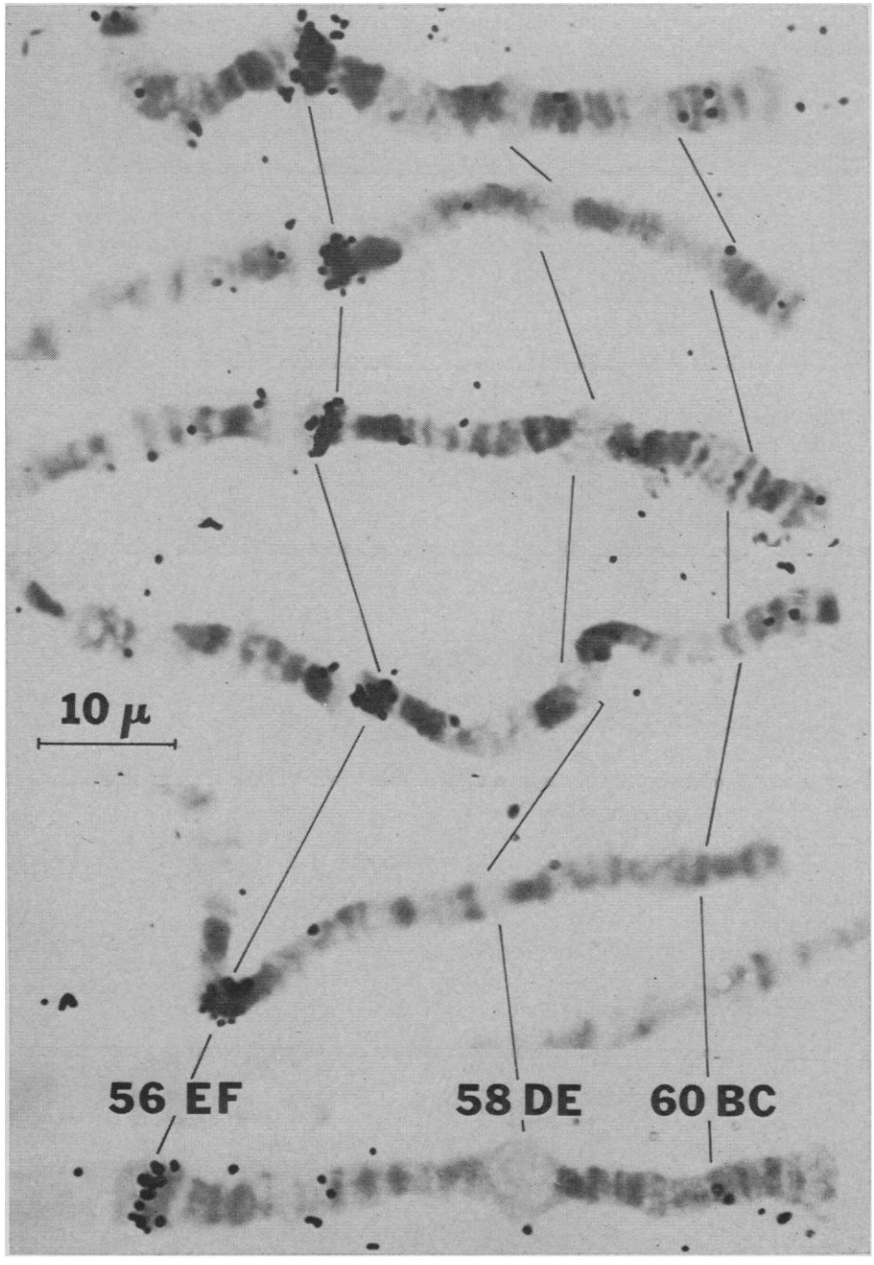

Fig. 2. Autoradiographs of the right arm of chromosome 2 of *D. melanogaster* after hybridization of the denatured DNA of the preparation with 5*S* [^{3}H]RNA. Silver grains are localized mainly over region 56E-F. Exposure time was 2 months.

In order to purify the 5*S* RNA more completely for hybridization, labeled RNA from the 5*S* peak was further separated electrophoretically on polyacrylamide gels (*9*). The gels were sliced, and the RNA was eluted from the slices and passed through a small chromatographic column containing benzoylated DEAE-cellulose (*11*). Again, the 56E-F region and nucleolus were labeled, with little, if any, radioactivity elsewhere. In these preparations, considerably enriched for 5*S* RNA, the ratio of silver grains found over the 56E-F section to those found over the nucleolus was about 1 : 2, whereas with the original MAK column 5*S* fraction the proportion was about 1 : 25. The 56E-F region was the only segment outside the nucleolus that was consistently highly labeled. We consider this to be indicative of specific binding of 5*S* RNA to the 56E-F region. We therefore conclude that the 56E-F segment of chromosome 2 contains a major portion, if not all, of the 5*S* genes. The fact that the nucleolus was slightly labeled demonstrates that even with purification on polyacrylamide-gel, there is a detectable amount of contamination from ribosomal RNA. This observation agrees with the conclusions reached by others (*1*) where contaminating fragments of 18*S* and 28*S* RNA were found in the low-molecular-weight RNA species.

Can other genes be localized by means of this technique of hybridizing RNA and DNA? After a 2-month autoradiographic exposure, 15 to 30 grains were consistently found over the 56E-F region. Approximately 200 5*S* genes are present in the haploid condition (*1*). With lateral redundancy of about 1000-fold, 2×10^5 5*S* genes should be present on the salivary gland polytene chromosome. We used [^{3}H]RNA with 4×10^6 disintegration min^{-1} μg^{-1}. The molecular weight of 5*S* RNA is 4×10^4

daltons (*1*), giving an estimate of 0.023 disintegrations per 5*S* RNA molecule per 2 months. The emulsion, NTB2, detects tritium disintegrations with nearly a 10 percent efficiency (*12*); therefore, 15 to 30 grains would accumulate over this region if the hybridization yield was between 3 to 6 percent. Such hybridization efficiency places the transfer RNA genes with their redundancy of 13 times or more (*13*) within the limits of resolution of the system. Indeed, certain sites along the chromosome arms are frequently lightly labeled with fractions containing transfer RNA (Fig. 2, region 60 BC) and need only to be cataloged to cytologically mark the location of the numerous transfer RNA genes.

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Cochlear Summating Potentials: Composition

Abstract. *The potential difference across the cochlear partition and the overall potential level of a given cochlear cross section were measured as functions of stimulus parameters and spatial location. It was confirmed that the potential difference is negative in the vicinity of greatest excitation, and it was discovered that in the same region the overall potential level is positive.*

Upon presentation of a brief tonal stimulus a d-c potential shift can be recorded from the cochlea (*1*). This potential, the summating potential (SP), is reported to be an elusive phenomenon and difficult to quantify (*2*), even though a large number of experiments have been devoted to it (*3*, *4*). The SP, just as the other inner-ear potential, the cochlear microphonic (CM), is presumed to originate in the hair cells; it is generally stated, or tacitly assumed, to appear in opposite polarity (referred to an indifferent reference electrode) in the two perilymphatic scalae at a given cochlear location. We show here that the latter contention is not generally correct, and that the recorded SP at any point in the cochlea can be thought of as the algebraic sum of the potential difference between scala vestibuli and tympani (heretofore designated as differential component or DIF) and the common potential of these scalae (to be identified as average component or AVE). These components vary independently; both are functions of stimulus parameters and cochlear recording site.

Data were obtained from 39 guinea pigs. The experimental animals were anesthetized with urethane; the auditory bulla was approached ventro-laterally and was opened widely; small holes were drilled through the bony cochlear wall at appropriate locations; and fine wire electrodes (Tu or Ag-AgCl) were introduced through these holes into the perilymphatic scalae. Electrodes were placed in pairs, one in the scala vestibuli and one in the scala tympani of a given turn. Either one or two pairs of electrodes were used. The indifferent electrode was an Ag-AgCl disk placed on the neck muscles. The signals from the two active electrodes were independently amplified (60-db gain, 3 second time-constant) and processed. The stimuli consisted of tone bursts 40 msec long, with 100 msec between bursts, and virtually instantaneous rise and fall times. The sound signal was delivered to the bony meatus of the animals in a closed acoustic system; it was continuously monitored near the eardrum with a calibrated probe-tube microphone. The amplified signals were processed with an averaging computer. The gated sinusoidal signal that provided the speaker voltage was not synchronized with the onset of the burst; consequently the cochlear microphonic phase was changing from burst to burst with the result that this a-c signal was simply averaged out. What remained was the onset and offset action potential, some onset CM in response to ringing in the acoustic system, and the SP. This method of eliminating the CM is much superior to filtering because it avoids wave-form distortion. The number of samples averaged ranged between 16 and 1024. This number depended on the magnitude of the signal; at high stimulus levels a few samples sufficed, but at low levels larger numbers were required. We would either average the scala vestibuli and scala tympani potentials (SV and ST) or utilize electronic means to subtract or add these signals before averaging. These latter two signals are defined as SV − ST = 2 DIF and SV + ST = 2 AVE. It is easy to demonstrate that either arithmetic or electronic generation of DIF and AVE components from SV and ST potentials yield virtually identical results. It should be noted that when obtained by electronic addition or subtraction, the DIF and AVE components are similar to the results of classic differential electrode recording when either action potential or CM is rejected (*5*).

The only difference between our method and the usual differential electrode recording is that we do not utilize an electronic balancing technique; instead, simple addition or subtraction is performed. Appropriate symmetry between the two electrodes is achieved by their careful placement and by the rejection of inadequate preparations (*6*). The DIF component should be in-