LOCALIZATION OF tRNA GENES IN THE SALIVARY CHROMOSOMES OF DROSOPHILA BY RNA:DNA HYBRIDIZATION*

DALE M. STEFFENSEN AND D. E. WIMBER

Department of Botany, University of Illinois, Urbana, Illinois 61801 and Department of Biology, University of Oregon, Eugene, Oregon 97403

Received May 24, 1971

THE methods for *in situ* molecular hybridization to nuclei and chromosomes have been developed due primarily to the efforts of GALL and PARDUE (1969), PARDUE *et al.* (1970) and JOHN, BIRNSTIEL and JONES (1969). In theory the procedure permits the use of any fraction of labeled RNA or DNA to locate the site of specific binding in a genome, provided there is enough radioactivity for detection by autoradiography. During our recent successful hybridization study locating the genes coding for 5S RNA at region 56EF on chromosome 2 (R) in *Drosophila melanogaster* (WIMBER and STEFFENSEN 1970), it became apparent that numerous other sites on the salivary chromosomes were labeled due to the binding of [^{3}H]tRNA which was present in the 5S RNA fraction. The present experiments exploit these observations and provide evidence of cytological location of the genes which code for the transfer RNA molecules in *Drosophila melanogaster*. The analysis of the location of the [^{3}H]tRNA:DNA hybrids has been done for the *X* chromosome and most of chromosome 2, including the entire right arm (2R) and the distal half of the left arm (2L).

MATERIALS AND METHODS

Labeling Drosophila RNA: About 0.1 g of first instar larvae of *Drosophila melanogaster*, homozygous for sepia (*se*), was removed from unlabeled food by the sucrose method of MEAD (1964) and placed on 10 g of medium containing 1 mCi/ml of [5-^{3}H] uridine (New England Nuclear, 25 Ci/mM); the medium consisted of 0.8 g agar, 2.5 g brewer's yeast and 5.5 g of yellow corn meal made to 100 ml. After four days growth, third instar larvae were floated off and washed with sucrose solutions, homogenized in the sodium dodecyl sulfate-Tris buffer and the RNA extracted by the phenol method used by RITOSSA and SPIEGELMAN (1965). The RNA, precipitated from 2.5 volumes of ethanol, was washed in ethanol and ether, air dried and dissolved in 0.1 M NaCl. This RNA solution was loaded on a MAK column and eluted by a saline gradient according to the procedure of MANDELL and HERSHEY (1960). The specific activity was estimated using the nitrocellulose filter method (SUEOKA and YAMANE 1962), giving 4×10^6 dpm/μg, assuming 20 O.D./ml at 260 mμ equals 1 mg and the counting efficiency was 10%. The 4S and 5S RNA fractions were dialyzed against 0.9 M NaCl and 0.09 M sodium citrate, pH 7.4 and frozen until used for hybridization. Aliquots of the 4S and 5S RNA fractions were dialyzed extensively against

* This paper is dedicated to Dr. JACK SCHULTZ. He was one of the early few to realize the importance of RNA in the nucleus. A large part of the summary data on the location of Minute loci is the work of Dr. SCHULTZ, as is the cytology of additional puffing sites presented in the Figures. We are also indebted to him for his critical review of this paper a few weeks before his untimely death.

water, frozen, lyophilized and the RNA separated by electrophoresis on 9% polyacrylamide gels (LOENING 1967).

Hybridization to chromosomes: Salivary glands from late third instar larvae were squashed in 45% acetic acid, the cover glass removed by the dry ice method, the frozen slide immersed in 3:1 (100% ethanol: glacial acetic acid) for 10 min, transferred to 100% ethanol and air dried. The preparations were treated with RNase, 0.2 mg/ml (Sigma Ribonuclease A. type II-A protease-free) in 2 × SSC for about 1.5 hr at room temperature, and washed three times in 2 × SSC. SSC is 0.15 M NaCl, 0.015 M sodium citrate, adjusted to pH 7.4 with acetic acid. The denaturation of DNA in intact chromosomes was accomplished in 90% formamide in 0.1 × SSC at 65°C for 2.5 hr and then the slides were plunged into cold 2 × SSC. After several changes through 2 × SSC, two changes in 70% ethanol and one in 100% ethanol, the slides were air dried and stored. The remaining hybridization procedure with [^{3}H]RNA (0.5 μg/ml) was similar to that outlined by PARDUE *et al.* (1970). In autoradiography we employed the dipping method using Kodak NTB2 and exposure times of at least two months for these tRNA studies. After the autoradiographs were developed they were stained with 0.2% toluidine blue in 0.5% sodium bicarbonate buffer at pH 8.2, rinsed in water, air dried and mounted in immersion oil. A detailed discussion methods for *in situ* hybridization has been presented elsewhere (STEFFENSEN and WIMBER 1971).

Isolation of tRNA and 5S RNA for competition studies: About 132 g of third instar larvae, homozygous for sepia, were homogenized by mortar and pestle in buffer at pH 7.6 containing 0.25 M sucrose, 0.001 M $MgCl_2$, 0.025 M KCl, 0.05 M Tris and 0.001 M Cleland's reagent, and centrifuged at 10,000 × *g* for 10 min. The supernatant was layered over 1.25 M sucrose buffer after making the final mixture 0.5% for sodium desoxycholate, and centrifuged for 3 hr at 105,000 × *g*. The resulting upper two-thirds of the supernatant was spun again for five hr at 105,000 × *g* and this second supernatant was extracted with saline SDS-phenol (RITOSSA and and SPIEGELMAN 1965) to recover the tRNA. The ribosome pellet from the first 105,000 × *g* spin was resuspended in the starting buffer without sucrose but with higher Mg^{++} at 0.01 M $MgCl_2$, centrifuged for 20 min at 27,000 × *g* and the supernatant centrifuged again for 3 hr at 105,000 × *g*. The resulting ribosome pellets were resuspended and held overnight at 0°C in dissociation buffer at pH 7.6 containing 0.1 M Tris, 0.001 M Cleland's reagent, 0.002 M $MgCl_2$ and 50 μg/ml of puromycin. The dissociated ribosomes were layed over 20% sucrose in dissociation buffer without puromycin and centrifuged 5½ hr at 105,000 × *g* to sediment 60S particles. The clear pellets were extracted by the saline SDS-phenol to recover the RNA. A further purification of the tRNA from the post-ribosomal supernatant was done on a BD-cellulose column (GILLAM *et al.*, 1967). The 5S RNA from 60S particles was separated from 28S RNA by passage through a MAK column and the 5S RNA peak selected for use in competition studies.

Cytological analysis: The salivary chromosome map of BRIDGES, in BRIDGES and BREHME (1944), served as the standard for band identification. In puffed regions the photographs of ASHBURNER (1969a, b) were an aid in band identification. We also used our own photographic map for each arm, since there are differences in stainability between toluidine blue and the standard aceto-carmine or lacto-aceto-orcein preparations. Each chromosome was scored from glossy prints at a magnification of 3,300 ×, made from negatives at 400 × taken with a Zeiss photomicroscope in bright field using Kodak High Contrast Copy film.

Statistics: Following the advice of Dr. H. W. NORTON (University of Illinois), the Poisson distribution was used to provide a reasonable assignment of confidence limits. Fortunately, the salivary map of BRIDGES can be converted directly into a histogram to plot data for distinguishing between randomly distributed silver grains due to background and grains consistently over labeled segments. The presence of one or more silver grains was considered positive label. The sample size of chromosomes scored for the 4S RNA fraction was 16 for the *X*, 19 for 2R and 14 for 2L. The sample for the 5S fraction was 21 for the *X*, 29 for 2R and 18 for 2L. In most cases the average frequency for chromosome sections without silver grains was 0.4, the zero class. Using this value, the tables for Poisson distribution of MOLINA (1942) provided the expected frequencies and the basis for calculating the confidence limits presented in Figures 4–8. Probability levels of <0.001 are considered meaningful. The values at <0.05 or 0.01 are subject to

error, since in an arm with 120 segments, six segments at the 0.05 level and one or two at 0.01 could deviate from expectation due to chance alone. The most reliable estimates for labeling are when both the 4S and 5S fractions are at $P < 0.001$, giving a chance for error less than one in a million.

RESULTS

After the RNA in Drosophila larvae had been labeled with [^{3}H]uridine, purified and separated by MAK column chromatography, the 4S and 5S RNA fractions were taken for analysis and purification by gel electrophoresis and for hybridization to salivary chromosomes. The 4S and 5S RNA fractions from the MAK column are the large and small peaks, that elute between 0.42 and 0.52 M NaCl and 0.53 and 0.63 M NaCl, respectively. The 4S RNA fraction consists primarily of tRNA, while the 5S fraction is seen to include 5S RNA, tRNA and detectable amounts of RNA larger than 5S when separated by polyacrylamide gel electrophoresis.

In Figure 1B the 4S RNA fraction is shown to be essentially free of 5S RNA and is primarily tRNA. The 5S RNA fraction (Figure 1A) is a mixture of 5S RNA and tRNA with a detectable amount of larger molecules (7, 8S?). The rapidly migrating material, which is smaller than 4S in both fractions in Figures 1A and B, is presumed to be radiation damaged molecules caused by the high tritium concentrations.

Uniformly labeled [^{14}C] RNA from *Escherichia coli* served as a marker in 2A and B. It is germane to note in these experiments that the Drosophila tRNA peak in the 5S RNA fraction migrated a little less than the peak [^{14}C] tRNA, the former peak corresponding to the left shoulder of unfractionated *E. coli* tRNA. As expected, the 4S fraction from Drosophila (Figure 2B) and the *E. coli* peaks coincide and there is less material in the left shoulder of the Drosophila tRNA. The point of this distinction is that one would expect the following: (a) some of the tRNA species may be only in the 5S fraction and (b) conversely some tRNA species should be exclusively in the 4S fraction. (c) The majority of tRNA species will be in both the 4S and 5S fractions in varying proportions. In Figure 3, the situations are illustrated diagrammatically. When the distribution of aminoacyl-tRNA was examined for 16 amino acids by SUEOKA and YAMANE (1962) on a MAK column, the following was observed: (a) While the peak of most tRNA species corresponds to the peak of optical density, there is a long tailing of the right shoulders into the 5S RNA fraction; (b) only a few tRNA's (i.e., the tRNA's for glycine, aspartic, lysine in *E. coli*) are found exclusively in the 4S fraction. (c) The 5S fraction contains a number of "unique" tRNA species as well as many of the tRNA species in the 4S fraction due to the tailing effect.

Hybridization of [^{3}H]RNA to DNA of salivary chromosomes: The salivary chromosomes from late third instar larvae of *D. melanogaster* were used for molecular hybridization with both the 4S RNA and the 5S RNA fractions separated on a MAK column.

X chromosome: In Figures 4 and 5 the localization of [^{3}H]RNA:DNA binding sites is illustrated for both the 4S RNA and 5S RNA fractions as previously

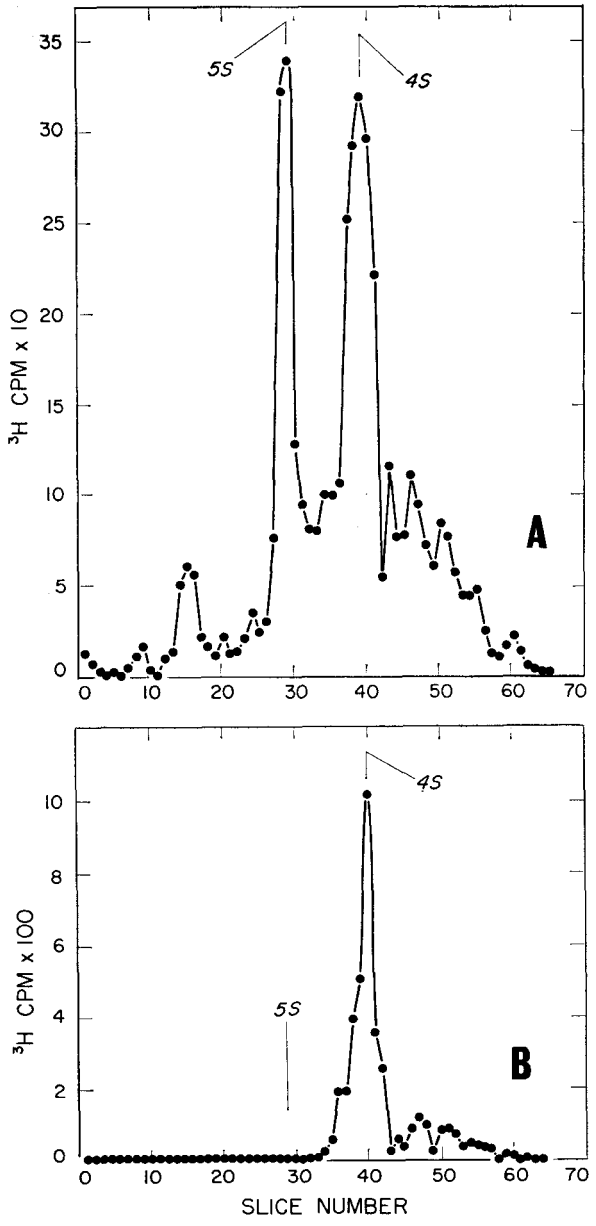


FIGURE 1.—Separation of 4S and 5S RNA fractions on 9% polyacrylamide gels. The Drosophila RNA, labeled with [^{3}H]Urd, was separated previously on a MAK column. The 4S and 5S notations were obtained from co-run markers, commercial yeast tRNA and 5S RNA from lily pollen ribosomes. (*A*): The 5S RNA fraction from the MAK column contains both 5S and tRNA. The small peak at slice 16 may be 7 or 8S RNA. (*B*): The 4S RNA fraction from the MAK column consists of tRNA and no detectable 5S RNA.

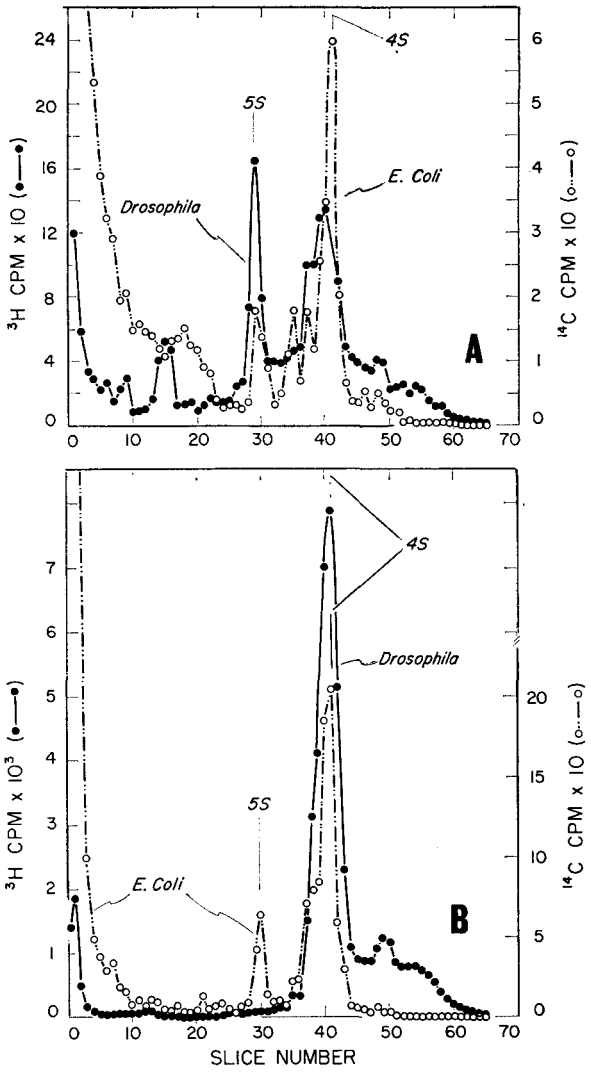


FIGURE 2.—Separation of 4S and 5S [^{3}H] RNA fractions from Drosophila with uniformly labeled [^{14}C] RNA from *E. coli* as a marker. (*A*): The 5S RNA fraction from Drosophila. The 5S RNA from Drosophila and from *E.* coli coincide. The peak of Drosophila [^{3}H] tRNA is in slice 40, on the left shoulder of the [^{14}C] tRNA from *E. coli*. (*B*): The 4S RNA fraction from Drosophila. The 4S or tRNA peaks—Drosophila and *E. coli*—occur in slice 41 with some of the left shoulder missing from the tRNA of Drosophila that is evident in the unfractionated tRNA of *E. coli*.

characterized. There are 16 sections which are labeled by the 4S fraction of $P<0.001$, using a Poisson distribution test; six of these are also labeled by the 5S fraction. Only three segments (4E, 8E, 13F) are "unique" to the 5S fraction with the absence of significant labeling ($P<0.05$) in the 4S fraction. There are four "unique" sites (5D, 7D, 17A and 18A) in the 4S fraction. In all there are 25

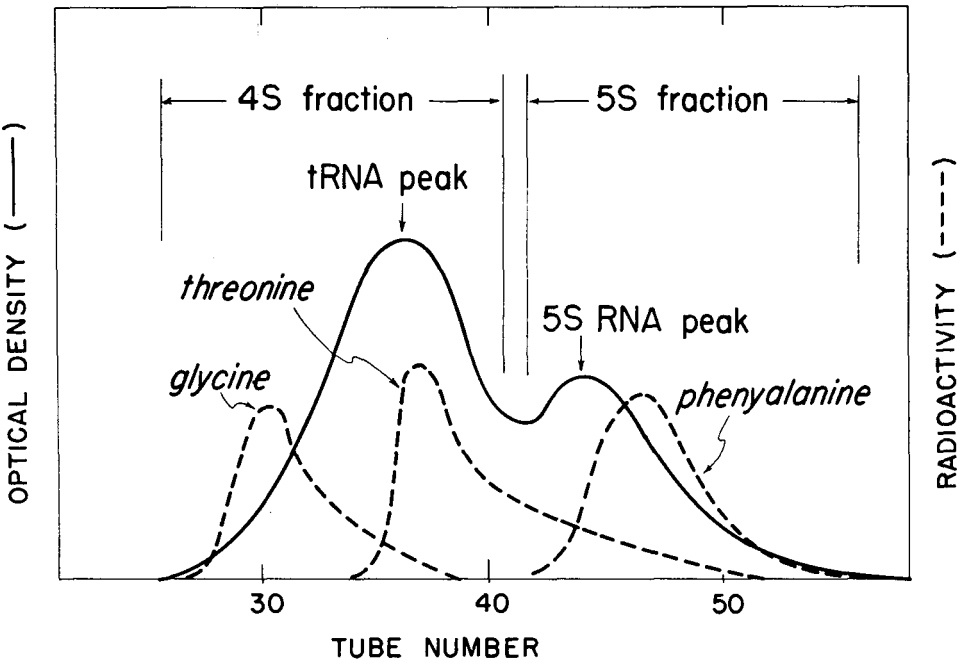


FIGURE 3.—A schematic diagram showing the separation on a MAK column of aminoacyl-tRNA charged with three different amino acids. The individual curves are redrawn from SUEOKA and YAMANE (1962). The inclosing lines indicate the way the [^{3}H] RNA was selected for hybridization studies with Drosophila.

independent locations on the *X* chromosome labeled by the 4S and 5S fractions at the <0.001 level which may represent tRNA genes.

The sites of Minute loci have been given in Figures 4–8, using the cytogenetic information from LINDSLEY and GRELL (1967). Some Minutes, such as *M(1)o^{Sp}* and *M(1)n*, correlate perfectly with putative tRNA genes at two of the major tRNA binding sites. *M(1)3E* does not correspond with a labeled site in Figure 4. The localization of grains to some bands could be in error in our cytological preparations, especially for a small puff like 3E. The precise localization of tRNA binding sites to specific bands is open to further refinement and analysis.

In Figures 4–8 the puffing patterns have been presented using the observations of ASHBURNER (1969a, b). Additional notations are made from LINDSLEY and GRELL (1967), citing both BECKER (B) and SCHULTZ (S), where the puffs were not indicated by ASHBURNER. In Figures 4 and 5 it is evident that eleven (44%) of the twenty-five labeled sites are in regions designated as puffs. Since the overall frequency of puffing is 34% (39 puffs/114 sections × 100), the percentage of puffs among the actual tRNA binding sites is higher than expected.

Left arm of chromosome 2: The proximal part of 2L is cytologically difficult, so we chose to score the radioactivity in a segment including regions 21 through 32B (Figure 6) and postpone the analysis of the rest of 2L. There are twelve sites which are labeled by both the 4S and 5S fractions, where both are at the

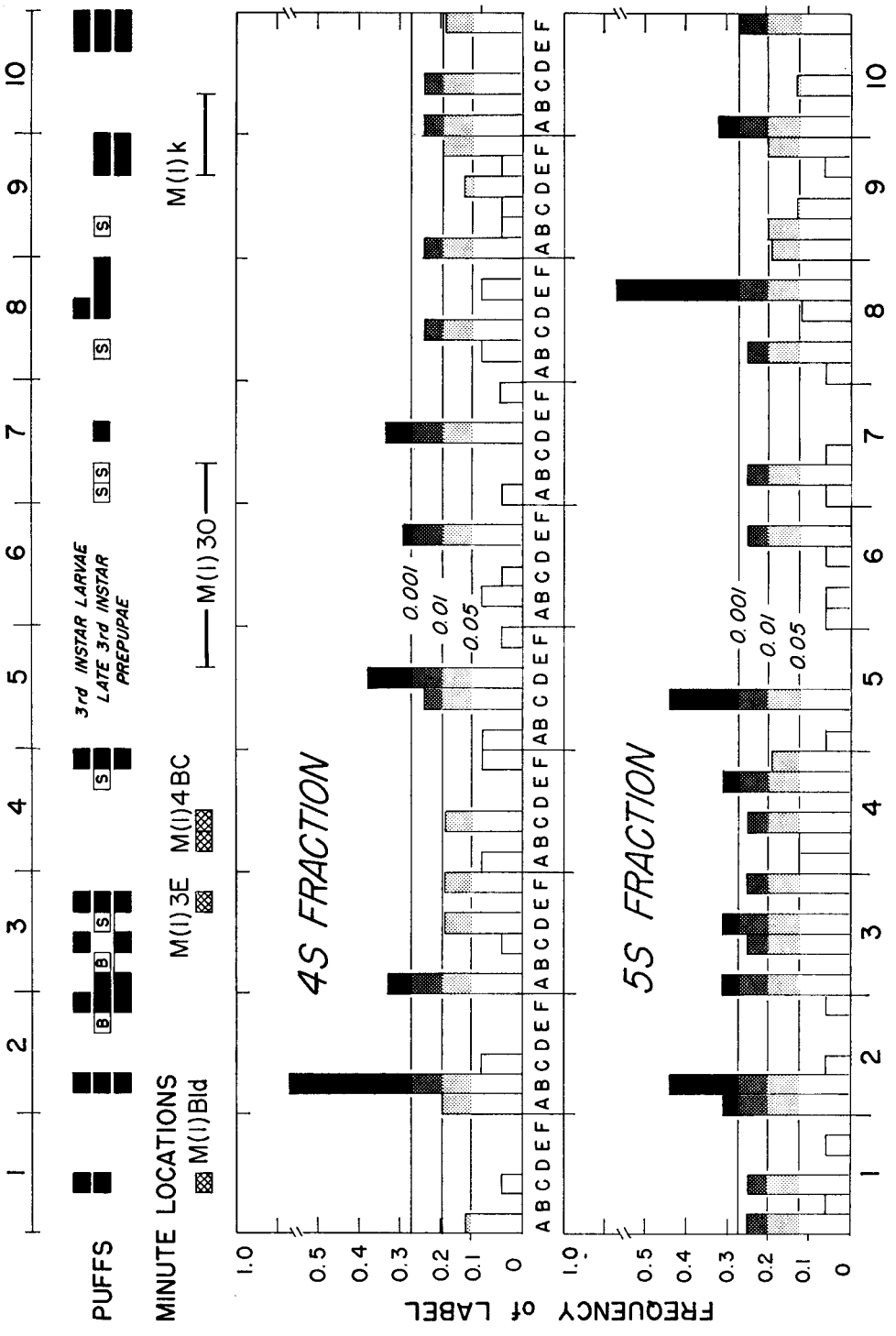
P <0.001 or with one at P <0.001 and the other at 0.01 or 0.05. Six unique binding sites are present in the 4S fraction (21D, 22A, 24F, 26B, 28C, and 28D), and three in the 5S fraction (21C, 27C and 32B). In all, there are twenty-one independent tRNA gene sites in the distal segments of 2L (regions 21 to 32B).

Three Minute loci, *M(2)21C1–2*, *M(2)Z* and *M(2)S1* in Figure 6, correspond very well to tRNA binding sites. The Minute, *M(2)e*, is not located accurately enough to be useful. Eleven of the twenty-one tRNA sites, or 52.5%, are located in segments that puff. The expected frequency is 32.4%; thus, potential tRNA genes occur in puffs in a percentage well over that expected.

Right arm of chromosome 2: There are eleven sites on 2R (Figures 7, 8) which are labeled by both the 4S and 5S fractions, where both are at significance levels of <0.001 or with one at <0.001 and the other at 0.01 or 0.05. These sections are: 57F, 57C, 55D, 53F, 50D, 50C, 50A, 47F, 45A, 42A and 41A–F. The last segment 41A–F, is heterochromatic and in our preparations the silver grains couldn't be located to sections, so the values represent the entire segment. Six sites (52F, 54E, 58D, 60A, 43B, and 49B) are exclusive to the 5S fraction and each is highly significant (<0.001). There are five unique sites that label with the 4S RNA fraction if the significance level of <0.01 is accepted. These segments are: 46C, 46D, 53A, 54F and 60C (60C is at <0.05 in the 5S fraction). In all, there are a total of seventeen sites on 2R and twenty-two possible if the five at the 0.01 level are included from the 4S fraction.

The majority of Minutes are within range of [^{3}H]tRNA sites. Minute *M(2)C* corresponds with section 60E in the 5S fraction with labeling at the 95% confidence limits. *M(2)S2* is in 41A but our localization of radioactivity within 41A–F is not good enough to draw a correlation. Six of seventeen sites (35.3%) on 2R are within segments which puff. The expected random frequency is 34.8% (39 puffs/112 segments × 100). The tRNA binding sites seem to be randomly placed with regard to puffing.

Competition with unlabeled tRNA and 5S RNA: Competition experiments were done as controls to see if the radioactive sites labeled by 5S RNA were really involved in specific binding of tRNA or if they resulted from contamination or non-specific binding. Unlabeled tRNA from third instar larvae was prepared from the post-ribosomal supernatant and used to compete against the ^{3}H-tRNA in the 5S RNA fraction. The concentration of cold tRNA was 100 times that of ^{3}H-RNA (50 μg/ml unlabeled tRNA and 0.5 μg/ml of ^{3}H-5S RNA fraction). The autoradiographs resulting from the latter hybridization had been exposed more than 8 months, four times longer than usual. The hundred-fold excess of unlabeled tRNA obliterated the labeling at the discrete bands. In precise scoring of 30 cells, silver grains were located on the chromosome ends of the *X*, 2L and 2R. With unlabeled tRNA competition, the radioactivity was either zero or at background levels at the major tRNA sites, such as 2B, 3A and 3D on the *X*, as well as 21A, 21B, 22C, and 25C on 2L and those on the end of 2R. None of these sites deviated from expectation using the Poisson distribution test. The only labeling observed in the entire genome was at 56EF, the 5S RNA genes, and over the nucleolus resulting from rRNA contamination.


PUFFS
MINUTE LOCATIONS
M(1)Bld
M(1) 3E
M(1)4BC
M(1) 30
M(1)k
3rd INSTAR LARVAE
LATE 3rd INSTAR
PREPUPAE
4S FRACTION
5S FRACTION
0.001
0.01
0.05
FREQUENCY of LABEL
1.0
0.5
0.4
0.3
0.2
0.1
0
A B C D E F
1
2
3
4
5
6
7
8
9
10

When unlabeled 5S RNA, isolated from 60S ribosomal particles was used to compete in concentrations 100 times higher than the ^{3}H- 5S RNA fraction, the radioactivity at 56EF was reduced to 3.5% of controls, averaging 0.71 silver grains per competed chromosome, against 20 grains per 56EF region in controls using the ^{3}H-5S RNA fraction without competition. This observation lends further support to our previous studies (WIMBER and STEFFENSEN 1970) where the 5S RNA genes were placed at 56EF on chromosome 2R. Competition with unlabeled 5S RNA did not affect the binding of the ^{3}H-tRNA in the 5S RNA fraction at the specified segments noted in Figures 4–8. With and without 5S RNA competition the frequency of label was about the same at the ^{3}H-tRNA sites. These experiments support the previous evidence that the major radioactive segments represent the cistrons which code for tRNA.

Refined band localization: In some of our best preparations it is possible to place the tRNA labeling at a specific band. For example, the radioactivity in the large 2B puff at the distal end of the *X* chromosome coincides best with the doublet, 2B3–4. Similarly, when another major site at 25C is puffed, the silver grains are most often over bands 25C8–9–10 but sometimes overlap to 25D1–2–3. Photographic illustrations of this region are presented by STEFFENSEN and WIMBER (1971). With intensive examination of excellent preparations, most of the putative tRNA gene sites can be placed more accurately than we have indicated in Figures 4–8. If one assumes the number of tRNA cistrons estimated by others (RITOSSA, ATWOOD and SPIEGELMAN 1966 and TARTOF and PERRY, 1970), then an average tRNA gene, serially repeated, would be smaller than a haploid DNA value of 10^6 Daltons, a size much smaller than the usual band. In theory then, one might expect to map a tRNA gene within part of one band.

DISCUSSION

The method of *in situ* hybridization has permitted us to indicate the probable locations of the genes coding for tRNA in about half of the genome of *D. melanogaster*. About 68 sites have been indicated; the expectation of an equal number from the remaining genome in the proximal half of 2L, 3R, 3L and chromosome 4 gives a total number of about 130 to 140. This is a value greater than the theoretical number of the sixty-four sites expected if each of the possible kinds of tRNA species corresponds to one specific site in the genome. This difficulty is resolved if some genes for tRNA species are in duplicate or in triplicate and are non-contiguous. We will be in a better position to discuss this question when the remaining half of the genome has been analyzed.

FIGURE 4.—Autoradiographic analysis of the [^{3}H] tRNA binding sites on the *X* chromosomes, sections 1 to 10. The data from the 4S and 5S fractions off the MAK column are presented separately. The location of Minute loci from LINDSLEY and GRELL (1967) are given. The puffs noted by ASHBURNER (1969a,b) are located as are some additional puffs described by BECKER (B) and SCHULTZ (S) cited by LINDSLEY and GRELL (1967). Frequency of label is defined as the number of segments with one or more silver grains divided by the number of segments scored. Confidence limits for significant labeling are derived from the data using observed and expected Poisson distributions.

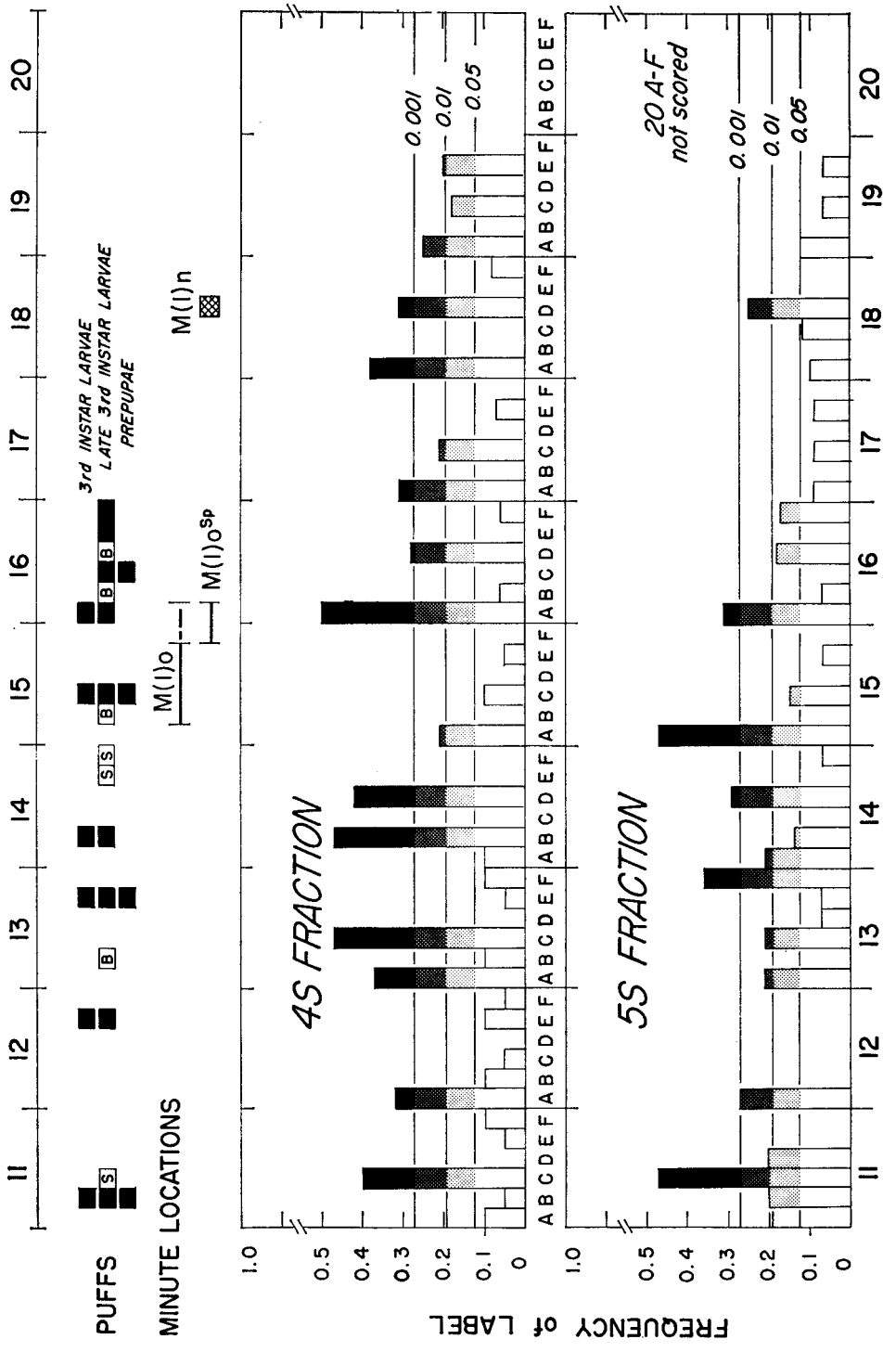


FIGURE 5.—Analysis of [^{3}H] tRNA radioactivity of the *X* chromosome, sections 11 to 20.

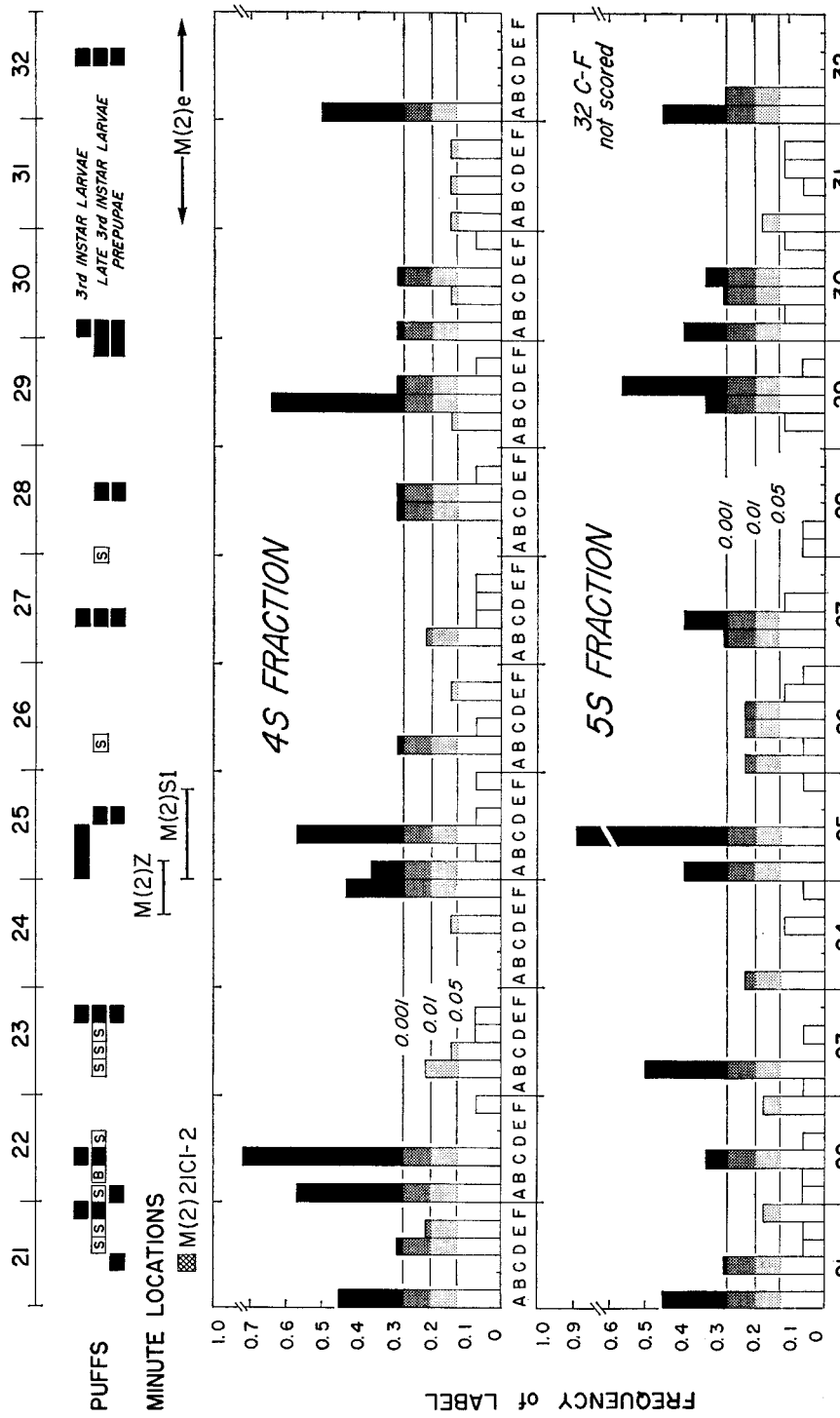


FIGURE 6.—Location of [^{3}H] tRNA radioactivity on 2L, sections 21 to 32B.

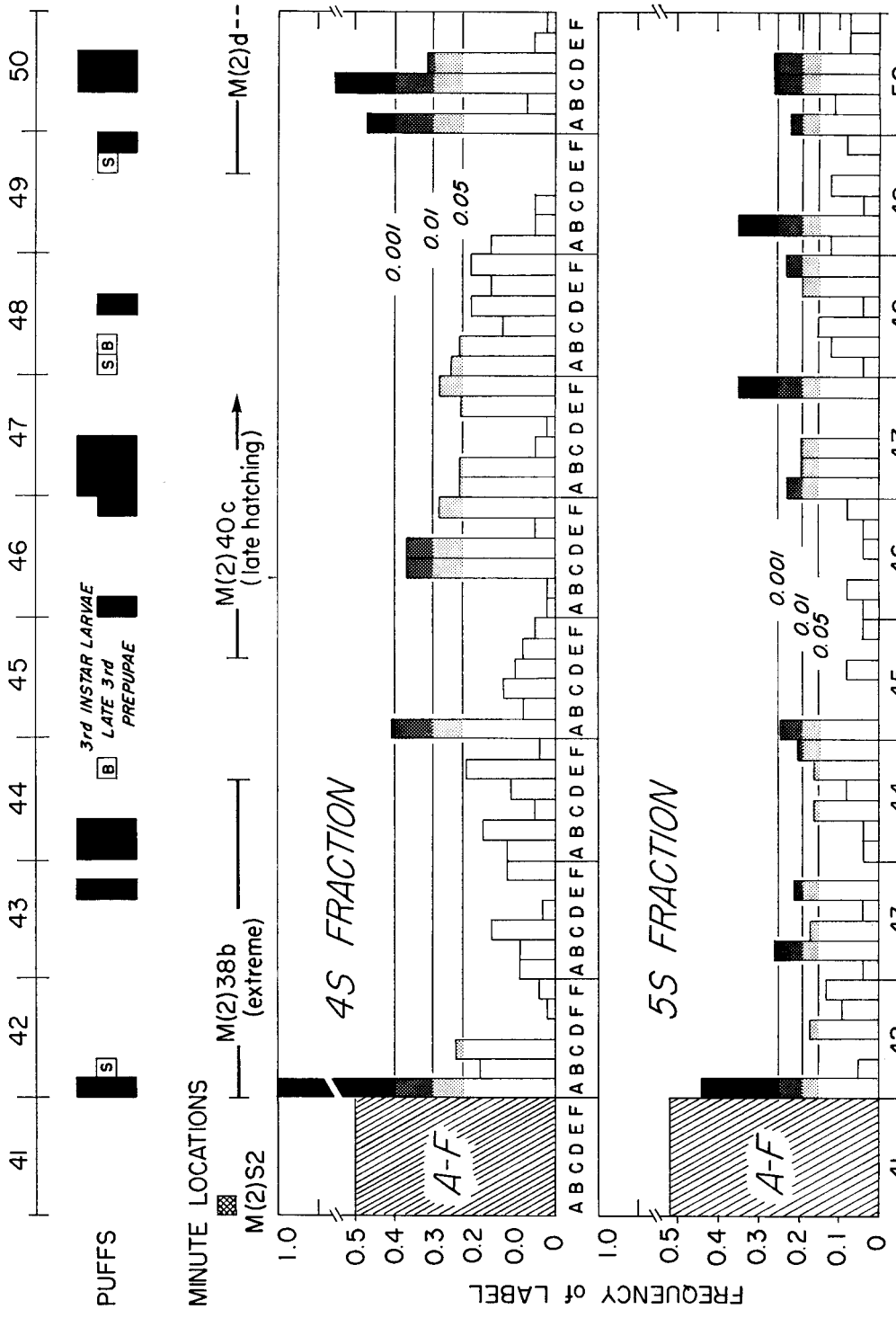


FIGURE 7.—Location of [^{3}H] tRNA radioactivity on 2R, sections 41 to 50.

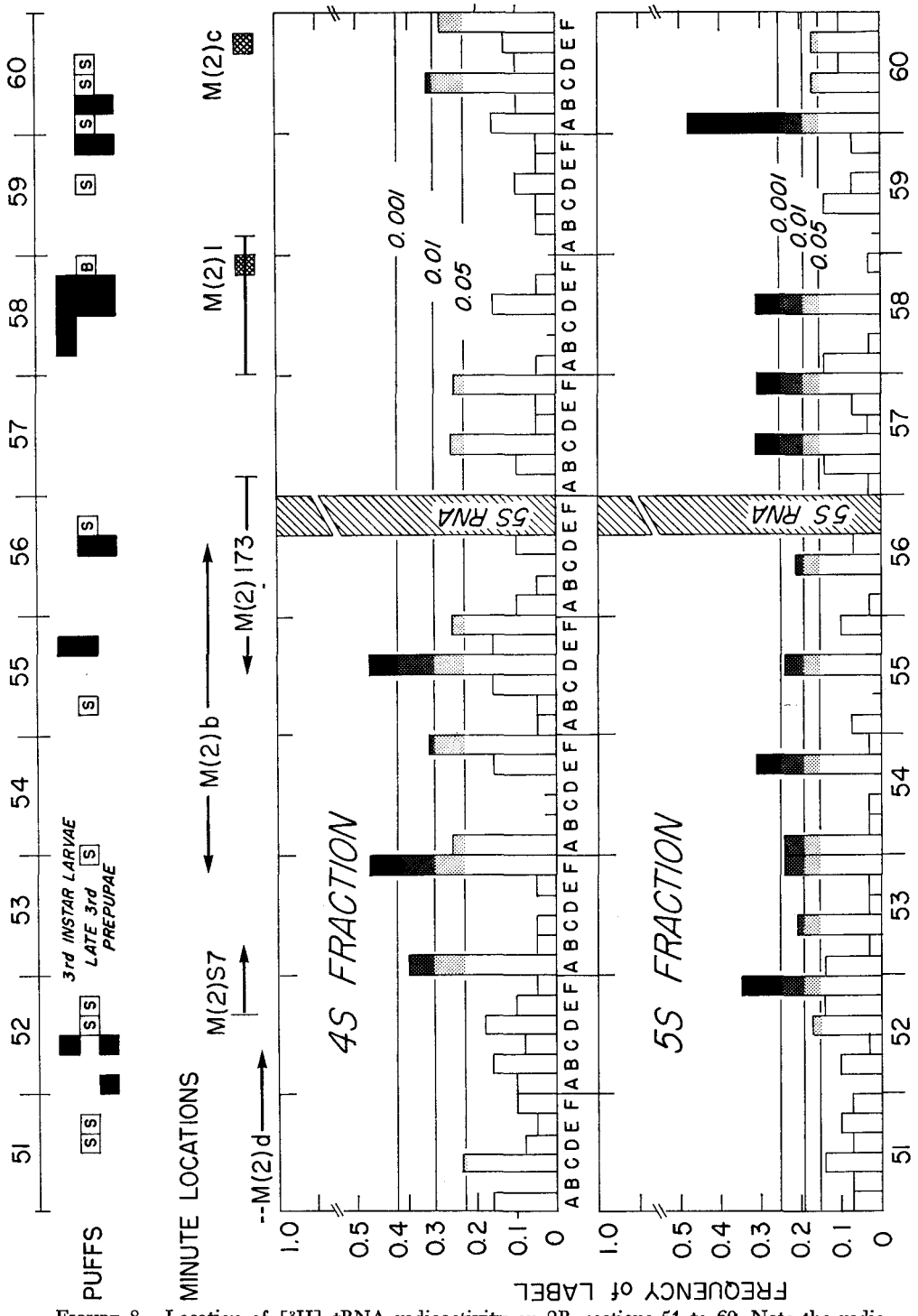


FIGURE 8.—Location of [^{3}H] tRNA radioactivity on 2R, sections 51 to 60. Note the radioactivity at 56EF is assigned to 5S RNA genes from a previous experiment (WIMBER and STEFFENSEN 1970).

It would seem that the Minute-tRNA hypothesis of K. C. ATWOOD, stating that the genes coding for tRNA are at Minute loci (RITOSSA *et al.*, 1966), has neither been proven nor disproven by the data presented. A decisive conclusion is not possible because: (1) most of the Minute loci have not been localized with sufficient accuracy on the salivary chromosome map and (2) some of our mapping of radioactivity may be in error, although we believe that the majority of binding sites have been correctly determined. In any case, the question can be resolved in the near future.

In *E. coli* the genes which code for tRNA are well known. The tRNA mutants often act as suppressors and usually involve a base substitution in the anticodon (GORINI 1970). There are a number of suppressors in Drosophila which, by the same analogy, could be tRNA gene mutants. Recently, TWARDZIK, GRELL and JACOBSON (1969) reported that *su(s)*^{2} homozygotes are missing one of the isoaccepting forms of tyrosyl-tRNA. It is not known yet if the *su(s)*^{2} gene codes for tyrosyl-tRNA. This gene is at the tip of the *X* chromosome. One could hybridize salivary chromosomes with ^{3}H-tyrosyl-tRNA to see if the tip of the *X* is labeled.

The localization of tRNA sites in the chromosomes of Drosophila is potentially of great importance for an understanding of the activity of tRNA in genetic regulation and differentiation. Most of the recent evidence and theories about the role of tRNA in development have been discussed by SUEOKA and KANO-SUEOKA (1970). In Drosophila many putative tRNA genes are located within major puffs which are actively synthesizing RNA, some of which is presumably a specific tRNA. Other tRNA gene sites in salivary chromosome bands are less active or shut off. Does the state of the tRNA bands have a regulatory role influencing the proteins being synthesized by the salivary gland? Drosophila may offer a unique system for the study of this problem.

The hybridization data with Drosophila using nitrocellulose filters indicate the genes for tRNA represent about 0.015% of the genome or about 750 cistrons according to RITOSSA *et al.* (1966) and TARTOF and PERRY (1970). If all of the 64 tRNA species are represented, then each gene on the average would be present twelve times with 3.0×10^5 Daltons each ($25{,}000 \times 12$) in haploids and a thousand times more in salivary chromosomes. As our data indicate, the highly sensitive procedure of RNA hybridization to salivary chromosomes allows one to localize genes with 3.0×10^5 Daltons or less of haploid DNA. Just how sensitive is the method? If the specific activity of the RNA were a little higher, say 1.0×10^7 dpm/μg, or if the autoradiographic exposure time was six months to a year, then almost any gene in the salivary chromosomes could be mapped, including unique sequences with just one haploid copy. This method may provide another approach to cytogenetics, as well as other applications for cell and developmental biology.

We wish to acknowledge the capable assistance of Mrs. LILLIE COOPER and Mrs. DORIS WIMBER in the study. This research was supported by NSF grant GB-18922 and NIH grants GM8465 and GM 11702.

SUMMARY

Two RNA fractions containing [^{3}H]tRNA were employed in molecular hy-

bridization studies using the DNA in cytological preparations of salivary chromosomes of *Drosophila melanogaster* to locate the genes coding for tRNA. The frequency of labeling at [^{3}H]tRNA:DNA binding sites was analyzed from autoradiographs and the data plotted on salivary chromosome maps. The *X* chromosome has 25 sites that are labeled at a frequency which is highly significant. The right arm of chromosome two (2R) possesses 22 independent tRNA gene sites in addition to the 5S genes at 56EF. The distal half of the left arm of chromosome 2 has 21 labeled segments. A number of the major [^{3}H]tRNA sites are located in chromosome segments which puff during larval or prepupal development. Competition with 100 times the concentration of unlabeled tRNA obliterates the radioactivity from ^{3}H-tRNA hybrid sites. Competition with unlabeled 5S RNA has no effect on the binding of ^{3}H-tRNA at the prescribed locations. The placement of presumptive tRNA genes was compared with the position of Minute loci but the overall data are not conclusive enough to establish a relationship.

LITERATURE CITED

ASHBURNER, M., 1969a Patterns of puffing activity in the salivary gland chromosomes of Drosophila. II. The *X*-chromosome puffing patterns of *D. melanogaster* and *D. simulans*. Chromosoma **27**: 47–63. ——, 1969b IV. Variability of puffing patterns. Chromosoma **27**: 156–177.

BRIDGES, C. B. and K. F. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. 552.

GALL, J. G. and M. L. PARDUE, 1969 The formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Natl. Acad. Sci. U.S. **63**: 378–383.

GILLAM, I., S. MILLWARD, D. BLEW, M. VON TIGERSTROM, E. WIMMER and G. M. TENER, 1967 The separation of soluble ribonucleic acids on benzoylated Diethylamino-ethylcellulose. Biochem. **6**: 3043–3056.

GORINI, L., 1970 Informational suppression. Ann. Rev. Genet. **4**: 107–134.

JOHN, H. A., M. L. BIRNSTIEL and K. W. JONES, 1969 RNA-DNA hybrids at the cytological level. Nature **223**: 582–587.

LINDSLEY, D. L. and E. H. GRELL, 1967 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. No. 627.

LOENING, U. E., 1967 The fractionation of high-molecular weight ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. **102**: 251–257.

MANDELL, J. D. and A. D. HERSHEY, 1960 A fractionating column for analysis of nucleic acids. Anal. Biochem. **1**: 66–77.

MEAD, C. G., 1964 A deoxyribonucleic acid-associated ribonucleic acid from *Drosophila melanogaster*. J. Biol. Chem. **239**: 550–554.

MOLINA, E. C., 1942 *Poisson's Exponential Binomial Limit*. Van Nostrand, New York.

PARDUE, M. L., S. A. GERBI, R. A. ECKARDT and J. G. GALL, 1970 Cytological localization of DNA complementary to ribosomal RNA in polytene chromosomes of Diptera. Chromosoma **29**: 268–290.

RITOSSA, F. M. and S. SPIEGELMAN, 1965 Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **53**: 737–745.

RITOSSA, F. M., K. C. ATWOOD and S. SPIEGELMAN, 1966 On the redundancy of DNA comple-

mentary to amino acid transfer RNA and its absence from the nucleolar organizer region of *Drosophila melanogaster*. Genetics **54**: 663–676.

STEFFENSEN, D. M. and D. E. WIMBER, 1971 Hybridization of nucleic acids to chromosomes. In: *Results and Problems in Cell Differentiation* 3, in press, Edited by H. URSPRUNG. Springer, New York, Berlin.

SUEOKA, N. and T. YAMANE, 1962 Fractionation of amino acyl-acceptor RNA on a methylated albumin column. Proc. Natl. Acad. Sci. U.S. **48**: 1454–1461.

SUEOKA, N. and R. KANO-SUEOKA, 1970 Transfer RNA and cell differentiation. Progr. Nucleic Acid Res. **10**: 23–55.

TARTOF, K. D. and R. P. PERRY, 1970 The 5S RNA genes of *Drosophila melaongaster*. J. Mol. Biol. **51**: 171–183.

TWARDZIK, D., E. H. GRELL and K. BRUCE JACOBSON, 1969 Genetic control of the iso-accepting forms of tyrosyl-tRNA in a suppressor of *Drosophila melanogaster*. Genetics **61**: s59.

WIMBER, D. E. and D. M. STEFFENSEN, 1970 Localization of 5S RNA genes in Drosophila chromosomes by RNA-DNA hybridization. Science **170**: 639–641.