EXPERIENTIA

Vol. 37 - Fasc. 1 Pag. 1-102 15. 1. 1981

GENERALIA

The structure and function of tRNA genes of higher eukaryotes*

by Eric Kubli

Zoologisches Institut der Universität Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich (Switzerland)

Summary. The most recent findings concerning the structure and function of tRNA genes of higher eukaryotes are discussed in an exemplary way. The tRNA genes of higher organisms are either dispersed or clustered at different sites of the genome. Clusters contain tRNA genes oriented in both directions and on both strands of the DNA with spacers of various length inbetween. Some genes contain intervening sequences close to the 3′ side of the anticodon. The primary transcription product possesses a 5′ leader and a 3′ trailer sequence which are removed by several maturation steps in a strict temporal and spacial order. Internal transcription control regions (promotors) are located at the 5′ and 3′ ends of the mature tRNA coding section of the tRNA gene. External sequences modulating the efficiency of the expression are present at the immediate 5′ ends of the genes. Transfer RNA genes are located nonrandomly in the nucleosomes.

I. Introduction

The application of gene cloning and rapid DNA sequencing techniques to the study of eukaryotic gene structure and function has led to the discovery of some unexpected features of the eukaryotic genome (Abelson, 1980). Two of the most extraordinary findings are the presence of intervening sequences which interrupt the continuity of the genetic information (Gilbert, 1979) and the novel type of transcriptional control of genes transcribed by polymerase III (Bogenhagen et al., 1980; Sakonju et al., 1980). Transfer RNA genes have played an important role in the elaboration of these results for several reasons. They represent a group of small genes with related functions and their gene products are easily isolated and characterized. Furthermore, tRNA genes carried on plasmids are efficiently transcribed when injected into *Xenopus laevis* germinal vesicles (Kressmann et al., 1978) or in *Xenopus* oocyte nuclear extracts (Schmidt, O., et al., 1978). This powerful technique, combined with genetic engineering methods, has brought the most interesting insights into the control of the expression of tRNA genes. It is the aim of this article to summarize some of the most recent developments in this field. Due to limitations of space the treatment will be exemplary rather than comprehensive. The reader interested in more details is referred to 2 authorative volumes published recently covering the various aspects of tRNA research (Schimmel et al., 1979; Söll et al., 1980).

II. The arrangement of tRNA genes

How many genes? In general it can be stated that the number of tRNA genes increases roughly with increasing complexity of organisms (Long and Dawid, 1980). The range in number is impressive extending from about 60 tRNA genes in *E. coli* (Birnstiel et al., 1972) to several thousands in *Xenopus laevis* (Clarkson et al., 1973a; table). What are the factors which determine the number of tRNA genes in an organism? One of the most obvious influences is the balanced synthesis and accumulation of the components involved in protein synthesis, e.g. tRNA, 5S RNA, rRNA, ribosomal proteins etc. Because of this one would expect constant proportions of these genes to exist in every organism; however, the available data demonstrate that only a loose correlation exists between the numbers of these genes (Long and Dawid, 1980). Therefore, the requirements of the protein synthesis machinery are probably not the only factors determining the number of tRNA genes in a genome.

Although the *total* number of tRNA genes increases with the complexity of the organisms; interestingly,

Repetition of genes coding for rRNA, 5S RNA, and tRNA in eukaryotes (compiled after Long and Dawid, 1980)

Species	Number of genes per haploid genome		
	rRNA	5S RNA	tRNA
Physarum polycephalum	280	690	1050
S. carlsbergiensis	140	200	360
S. cerevisiae	200–290	330–780	800–1450
Caenorhabditis elegans	55		300
D. melanogaster	100–240	100–200	590–900
X. laevis	500–600	9000–24,000	6500–7800
Rattus norvegicus	150	830	6500
Homo sapiens	50–200	2000	1310

*The present article is an extended, English version of the lecture held on 14.6.1980 at the University of Zürich, Switzerland.

the number of *different* tRNA genes is nearly constant for all organisms investigated over the entire evolutionary range, i.e. the tRNA genes of higher organisms are redundant (Birnstiel et al., 1972; Weber and Berger, 1976). The number of genes coding for an individual tRNA species can vary by more than one order of magnitude (Clarkson et al., 1973; Chevallier and Garel, 1979; Tener et al., 1980). This fact certainly reflects the special needs of an organism. For example the high demand for $tRNA^{Gly}$, $tRNA^{Ala}$ and $tRNA^{Ser}$ in the posterior silk gland of *Bombyx mori* (due to the high levels of these amino acids in fibroin which is produced by these glands in great amounts) is matched by an increased number of genes coding for these tRNAs (Chevallier and Garel, 1979). The same gene proportions are present in the anterior part of this organ. Thus, the composition of the tRNA genes of the anterior part of this organ reflects the needs of the highly specialized posterior part. A similar situation might be responsible for the extraordinarily high number of tRNA genes in *Xenopus laevis*. Clarkson et al. (1973a) have calculated that a *Xenopus* oocyte requires 1 year to synthesize the 40 ng tRNA it contains. Since no tRNA gene amplification has been observed yet, the high degree of redundancy of the tRNA genes might be necessary to produce this enormous amount of tRNA during oogenesis. The lack of cell specific amplification mechanisms for tRNA genes seems to impose the number and composition of tRNA genes on the entire genome which in fact would be required only in certain types of cells or during specific stages of the ontogeny of an organism. The high degree of redundancy of the tRNA genes in higher eukaryotes raises the question of how sequence constancy is maintained for hundreds of genes which code for the same tRNA. Indeed, a comparison of tRNA sequences from different higher eukaryotes demonstrates an astonishing conservatism (Sprinzl et al., 1980). The sequences of tRNAs from organisms as evolutionarily distant as *Drosophila* and mammals can differ in only a few nucleotides or not at all (e.g. $tRNA_2^{Lys}$). It is not clear whether strong selection pressure and/or a correction mechanism is responsible for these remarkable facts. The high degree of nucleotide modification observed in the tRNAs of higher eukaryotes might be a consequence of this conservation of the tRNA sequences over millions of years (Kubli, 1980). Indeed, many tRNA isoacceptors (tRNAs carrying the same amino acid but separable with physico-chemical means) differ only in their posttranscriptional modification, i.e. they are coded by the same genes. Modification produces a wide variety of tRNA structures without increasing the number of different tRNA genes.

Arrangement of the tRNA genes on the genome level: The following questions can be asked concerning the organization of the tRNA genes on the genome level. Are all tRNA genes localized at one site? If not, are the different tRNA species separately clustered or intermingled? Is there a functional logic behind the arrangement? Three methods have been used to answer these questions: a) distribution analysis of tRNA genes in DNA fractions after density gradient centrifugation, b) restriction enzyme analysis of genomic DNA and subsequent hybridization using radioactive tRNAs as probes to detect DNA pieces containing tRNA genes, and c) 'in situ' hybridization. The first 2 approaches do not give definitive answers, but almost all of the above questions can be answered by the powerful method of 'in situ' hybridization when it is applicable.

Two basic types of tRNA gene organization seem to be present in higher eukaryotes. In *Xenopus, Taricha, Bachytrops,* and *Drosophila* multiple copies of the same gene or a number of different genes are clustered (Clarkson et al., 1973b; Léon, 1976; Elder et al., 1980). In *Xenopus* a cluster containing different genes can be repeated many hundred times (Clarkson et al., 1973b; Müller and Clarkson, 1980). In *Tetrahymena, Physarum, Caenorhabditis,* and *Bombyx* the tRNA genes seem to be scattered throughout the entire genome (Tønnesen et al., 1976; Hall and Braun, 1977; Cortese et al., 1978; Garber and Gage, 1979; Hagenbüchle et al., 1979). However, unambiguous results are only available for *Drosophila melanogaster.* This is due to the fact that in this organism the tRNA genes can be very easily localized by 'in situ' hybridization with radioactively labeled tRNA to squashes of polytene salivary gland chomosomes (figure 1). The following discussion will therefore be restricted to results obtained by this elegant methodology in this organism.

'In situ' hybridization experiments have been carried out with crude, radioactively labeled tRNA preparations (Elder et al., 1980) and with purified, single isoacceptors labeled with [^{125}I] (Tener et al., 1980; Kubli et al., 1980). The results can be summarized as follows: 1. The tRNA genes are distributed more or less randomly over the whole *Drosophila melanogaster* genome with exception of the very small 4th chromosome where no tRNA genes could be found (the Y chromosome cannot be investigated, due to its heterochromatization in the centromere). 2. Genes coding for the same isoacceptor can be localized in different regions. 3. Different isoacceptors can hybridize to the same region. 4. No apparent pattern is indicated by the results obtained so far. Even the family of tRNAs characterized by the possession of the same hypermodified base Q in the 1st position of the anticodon ($tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{His}$ and $tRNA^{Tyr}$) do not hybridize to the same chromosome bands (Schmidt and Kubli, 1980). However, it has to be realized that presently only about a dozen purified

tRNAs have been hybridized. Hence, some pattern may emerge as more data become available.

Genes for almost every isoacceptor can be found at 2–4 sites in the *Drosophila* genome. It is tempting to speculate that this has something to do with the 4 developmentally distinct ontogenetic stages of this animal: embryo formation, larval stages, pupation, and imago. However, at the present time there are no hard data available to sustain this assumption.

The interpretation of the 'in situ' results are plagued by the limitations of all hybridization experiments. Furthermore, the grains obtained after autoradiography can only be assigned to regions, each of which contains several bands (an average band contains about 10–30 kilobases of DNA). Hence, this methodology is only able to give information on the locations of tRNA genes within a range of a few 100 kb.

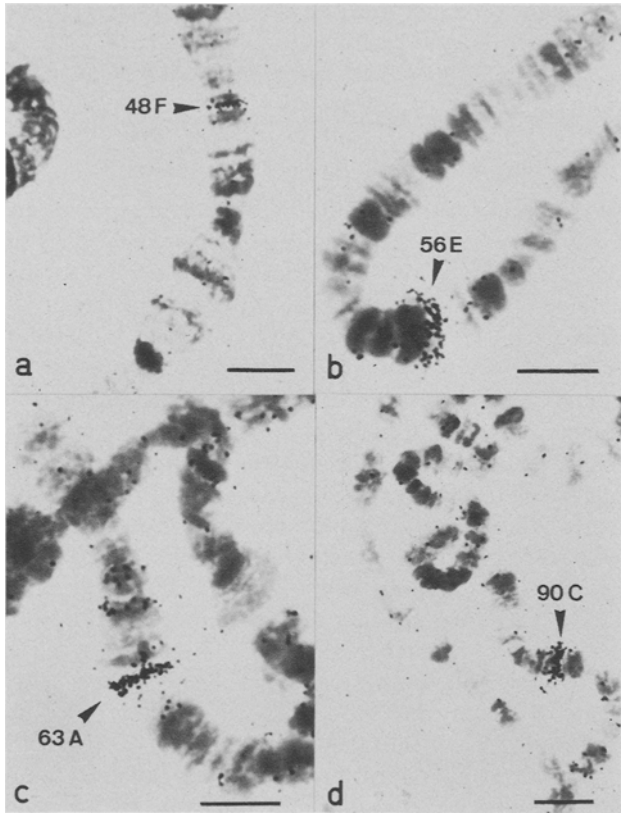

Fig. 1. *a* and *b* 'In situ' hybridization of [^{125}I]-tRNA^{His} to salivary gland chromosomes of the *Drosophila melanogaster* mutant *giant*. Labeling is found in the regions 48 F and 56 E. *c* and *d* 'In situ' hybridization of [^{125}I]-tRNA^{His} and [^{125}I]-tRNA^{Ala}. In addition to the regions 48 F and 56 E labeling is also found in the regions 63 A and 90 C. Scale 10 µm. (From: Schmidt and Kubli, 1980; with permission.)

More refined techniques are needed in order to determine the exact location and the order of individual genes in a cluster.

Clusters: Molecular cloning and rapid DNA sequencing techniques have also been applied to the analysis of tRNA genes in various organisms. The most comprehensive study available is of a tRNA gene cluster localized in the region 42 A (cytological map) of the *Drosophila* polytene chromosomes (Hovemann et al., 1980; Yen and Davidson, 1980). Since most of the findings of this study also apply to the tRNA gene organization studied in other organisms (*Xenopus laevis* (Müller and Clarkson, 1980), *Caenorhabditis elegans* (Cortese et al., 1980)) we shall concentrate on this particular gene cluster. The tRNA genes encoded in this region are found on both DNA strands and are oriented in both directions (figure 2). There is no regular spacing between the genes. In fact the distance between the individual genes can vary from only a few dozen nucleotides to up to hundreds of base pairs. Basically it has been found that the only constant sequences are the parts of the DNA coding for the mature tRNA. The sequences following the 3′ end are all AT-rich. In no case has the 3′ CCA-end (common to all tRNAs) been found to be coded by the tRNA genes (see below). The spacer regions do not usually show any sequence homologies with other regions of the genome, although the occurrence of repetitive elements has been described (Yen et al., 1979; Hosbach H., personal communication). It has been suggested that the diversity of the spacer sequences might help to suppress unequal crossing over between identical genes arranged in tandem fashion (Müller and Clarkson, 1980). The same result may be obtained by orienting identical genes in opposite directions.

Are any other genes located close to the tRNA genes? Hybridization of poly(A) containing RNA to plasmids covering 92 kb of the region 42 A has shown that genes coding for proteins are probably also present in this region (Yen and Davidson, 1980). However, the interspersion of tRNA genes with genes coding for ribosomal RNA as reported for *E. coli* has not yet been found in the nuclear genome of any higher organism.

Hosbach et al. (1980) have reported some interesting data on a tRNA^{Glu} gene cluster derived from the region 62 A. Their results may serve as a model for

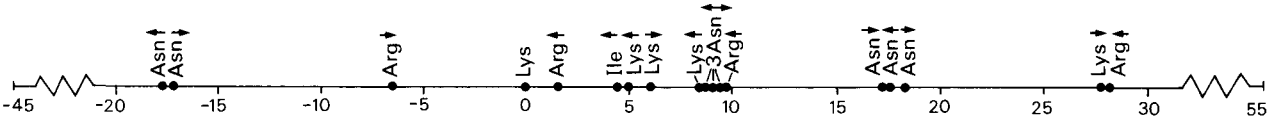

Fig. 2. The arrangement of the tRNA genes in the cytological region 42 A of the *Drosophila melanogaster* salivary gland chromosomes. The tRNA genes are oriented in both directions and coded by both DNA strands. No tRNA genes were found in the regions from 30 to 55 and from −20 to −45. The distance is given in kilobases (Adapted from Hovemann et al., 1980, and Yen and Davidson, 1980; with permission).

tRNA gene evolution (figure 3). Sequence analysis of the 5′ and 3′ nucleotides immediately flanking 3 $tRNA^{Glu}$ genes revealed striking homologies suggesting a possible evolutionary history of these genes. Two ancestral genes may have given rise to 2 gene pairs by duplication, and 1 of these gene doublets could then have given rise to a trio of genes as a result of unequal crossing over. The C-T transition found in gene 2 must have happened after the unequal crossing over event since this mutation is found only in this gene. This might be one of the mechanisms of evolution which increases the number of tRNA genes.

The special situation found in amphibia is also reflected in the arrangement of the tRNA genes in a 3.18 kb DNA fragment isolated from *Xenopus laevis* (Müller and Clarkson, 1980). This arrangement has been revealed by density gradient centrifugation, restriction enzyme and DNA sequence analysis. At least 8 tRNA genes have been found on this plasmid. Although the gene arrangement follows the general rules mentioned above, this fragment is repeated about 300 times in the *X. laevis* genome. Therefore, the main difference between the amphibian type of tRNA gene arrangement and the one occurring in other organisms seems to be the 100-fold repetition of tRNA gene clusters coding for different tRNA genes.

Introns: Many eukaryotic genes contain sequences (introns or intervening sequences) which do not code for the mature products of these genes, i.e. the genes and their products are not colinear. Such intervening sequences (IVS) have also been described for tRNA genes, mainly in yeast (Goodman et al., 1977; Valenzuela et al., 1978), but also in higher eukaryotes. One IVS has been found and sequenced in a $tRNA^{Tyr}$ gene of *X. laevis* (Müller and Clarkson, 1980) and IVS have also been reported for 2 chicken genes ($tRNA^{Lys}_{2}$ and $tRNA^{Lys}_{4}$, Wittig and Wittig, 1979). Interestingly they

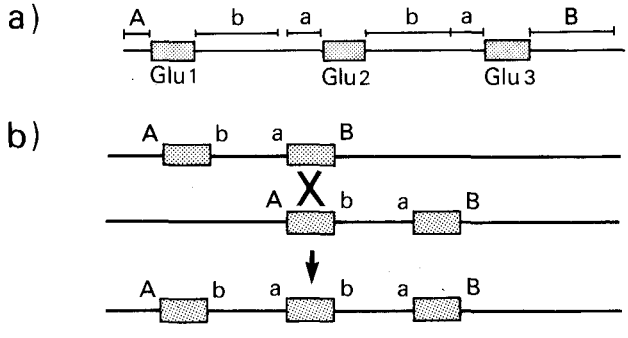

Fig. 3. Model for the evolution of a tRNA gene cluster in the cytological region 62 A of the *Drosophila melanogaster* salivary gland chromosomes.
a Sequence homologies around the 3 $tRNA^{Glu}$ genes. *b* Generation of a gene triplet from a hypothetical ancestral gene pair by unequal crossing over. The only difference in the tRNA coding region is a C-T transition in gene 2. This mutation must have happened after the unequal crossing over event since it is only found in this gene (Adapted from Hosbach et al., 1980; with permission).

have not yet been detected in the *D. melanogaster* tRNA genes, although this organism possesses genes with IVS. The size of the IVS in the tRNA genes is variable: 13 base pairs in $tDNA^{Tyr}$ from *X. laevis* (Müller and Clarkson, 1980), 29 and 31 base pairs in $tDNA^{Lys}_{2}$ and $tDNA^{Lys}_{4}$ from chicken, respectively (Wittig and Wittig, 1979). Some general rules (including the yeast tRNA genes) can be stated. All IVS have been found in the precursor tRNAs, i.e. they are fully transcribed (see below). Also, the acceptor stem, the dihydro-U and TΨC loops are conserved as in the standard cloverleaf model of tRNA. The anticodon stem is intact but is augmented by a 2nd helical region of variable length. This 2nd region is sometimes separated from the anticodon stem by a few unpaired bases. This helix includes the anticodon that pairs with 1–3 complementary nucleotides in the IVS. The IVS is always close to the 3′ end of the anticodon, although the exact position is not always clear since the sequences of the corresponding mature tRNAs are not known in all cases. Intervening sequences have been observed in several tRNA gene species, but tRNA genes which contain IVS in one organism might not contain them in another.

What is the function of the IVS? Recent experiments by Wallace et al. (1980) have shown that the IVS of the *S. cerevisiae* $tRNA^{Tyr}$ suppressor gene can be deleted without impairing the expression of this gene. This is in contrast to results obtained in similar experiments with genes from SV 40 and the mouse β-globin gene, where an IVS must be present in the transcriptional unit for the synthesis of stable mRNA, for its transport from the nucleus into the cytoplasm, or for both functions. It seems that the IVS of tRNA genes is not absolutely required for gene expression. Also, the splicing enzyme(s) used for the 1st reaction, excision, is probably not the same for tRNA and mRNA, whereas ligation might be performed by the same enzyme (Ogden et al., 1980). This does not mean that the IVS have no function in the tRNA genes. A comparison of the sequences which code for the mature part of the tRNA and those of the IVS provides relevant information on the possible function. Sequence comparison for the same tRNA gene in 2 different organisms shows that the portion which codes for the mature part is conserved whereas the IVS is not (Selker and Yanofsky, 1980). It has been suggested by Tiemeier et al. (1978) that IVS might help to suppress crossing over between identical genes arranged in tandem order. There are some sequence and structural similarities in the IVS studied so far, but they might be required for correct splicing rather than be related to the function of the IVS. Whether IVS have a role in speeding up the evolution of tRNA genes, as suggested for the IVS in protein coding genes (Gilbert, 1979), is very doubtful. At least a comparison of tRNA sequences does not indicate such

a hypothetical mechanism. If such a mechanism were operating one would most probably expect conservatism in the acceptor and anticodon stems, and more variability in the dihydro-U and the TΨU stems.

III. The function of tRNA genes

Transcription and processing: In eukaryotes most tRNA genes are synthesized as precursors which are processed by nucleases in order to yield functional products. Transfer RNAs are not only processed but also modified at specific sites by a set of modification enzymes. Two basic types of questions arise concerning the maturation of tRNAs. 1. What is the size of the primary transcript - are any multimeric precursors formed as observed in *E. coli* (Mazzara and McClain, 1980) or in yeast (Mao et al., 1980; Schmidt et al., 1980)? 2. What are the processing and modification steps and where do they take place?

The discovery that the primary transcription products of tRNA genes are precursor molecules larger than the tRNAs which are engaged in protein synthesis was originally made in mammalian cells (Burdon et al., 1967). Due to the high content of some specific tRNAs in the posterior silk gland of *Bombyx mori* it was possible to characterize the $tRNA^{Gly}$ and $tRNA^{Ala}$ precursors of this organ (Garber et al., 1978). No multimeric precursors and no introns were found. Neither were the precursors fully modified. Unfortunately, only low specific activities of the RNA can be obtained by 'in vivo' labeling of cells from higher eukaryotes. Therefore, most investigators have turned to the analysis of transcription of tRNA genes by either injecting tRNA genes containing plasmids into *Xenopus laevis* germinal vesicles or using *Xenopus* germinal vesicle extracts (Kressmann et al., 1978; Hagenbüchle et al., 1979; Melton and Cortese, 1979; Silverman et al., 1979; Melton et al., 1980). The tRNA precursors of all higher eukaryotes studied so far possess additional nucleotides at the 5′ and 3′ end of the tRNA, a 5′ leader and a 3′ trailer sequence. No multimeric precursors have been found. Since no tRNA gene of any higher eukaryote encodes the CCA end of the mature tRNA, it has to be added posttranscriptionally. Illuminating experiments have been performed by Melton et al. (1980) with a yeast $tRNA^{Tyr}$ gene containing plasmid. Similar results were also obtained with a $tRNA^{Leu}$ gene isolated from *Caenorhabditis elegans* (Broach, J., Cortese, R., and Melton, D.A., cited in Melton et al., 1980). After injection of the $tDNA^{Tyr}$ containing plasmid into *Xenopus laevis* germinal vesicles, the nucleoplasm and cytoplasm were analyzed separately for processing products at different times (figure 4). The first steps which occur after transcription are the stepwise removal of the 5′ leader and 3′ trailer sequences. At the same time some bases are modified. The addition of the CCA end is also performed in the nucleus. The

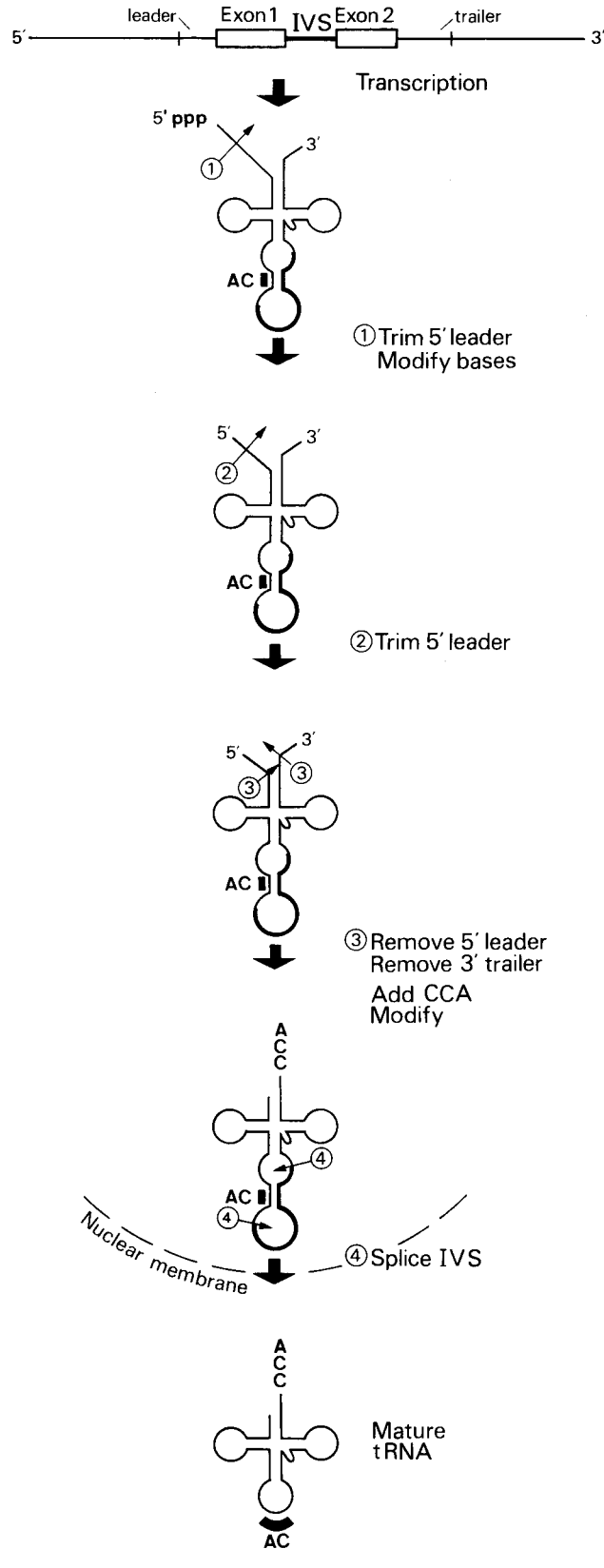

Fig. 4. Processing of yeast $tRNA^{Tyr}$ precursors into mature $tRNA^{Tyr}$ in injected *Xenopus laevis* oocytes. Size alterations and modifications occur in a strict temporal order. The anticodon (AC) is protected by base pairing with the IVS before the splicing takes place (Adapted from Melton et al., 1980; with permission).

last step before the mature tRNA appears in the cytoplasm is the excision of the IVS present in the $tRNA^{Tyr}$ gene, and consequently present also in the precursor. It is not clear whether this splicing event occurs in the nucleoplasm or in the nuclear membrane and then eventually coordinated with the transport into the cytoplasm. All the processing and modification steps occur in a strict temporal order. Since not all modifications have been monitored, some modification enzymes might be localized in the cytoplasm. Why is a tRNA synthesized by such a complicated processing scheme? One answer might be that the additional sequences of the precursors play a role in the ordering of the base modifications and the nuclear seggregation of the tRNA precursors (Melton et al., 1980). Consequently, only mature tRNAs would enter the cytoplasm for participation in protein synthesis.

It is evident from the above mentioned results that the *X. laevis* germinal vesicle is able to remove the IVS from the primary transcription product of the yeast $tRNA^{Tyr}$ gene. This is not astonishing since *Xenopus* $tRNA^{Tyr}$ genes also contain IVS (Müller and Clarkson, 1980). However, *X. laevis* germinal vesicle extracts also process dimeric yeast precursors (Mao et al., 1980; Schmidt, O., et al., 1980). Multimeric precursors have not yet been found in higher eukaryotes. It is therefore not clear whether this ability of the *Xenopus* germinal vesicle extract is due to a special enzyme(s) responsible for the processing of as yet undetected multimeric precursors, or whether the nucleases processing monomeric precursors can perform this function.

Regulation of gene expression: The control of transcription of one class of eukaryotic genes (those transcribed by polymerase III: 5S rRNA, tRNA, and some small RNAs) is quite different from that found in prokaryotes. In a series of elegant experiments it has been shown that the promotor of the 5S rRNA genes lies in the gene itself (Bogenhagen et al., 1980; Sakonju et al., 1980). The experiments described below indicate that the regulation of tRNA gene transcription might follow similar rules.

Short pieces of DNA containing a tRNA gene are capable of sustaining faithful transcription when injected into *X. laevis* germinal vesicles (Cortese et al., 1978). Telford et al. (1979) and Garber and Gage (1979) have shown in similar experiments that only 22

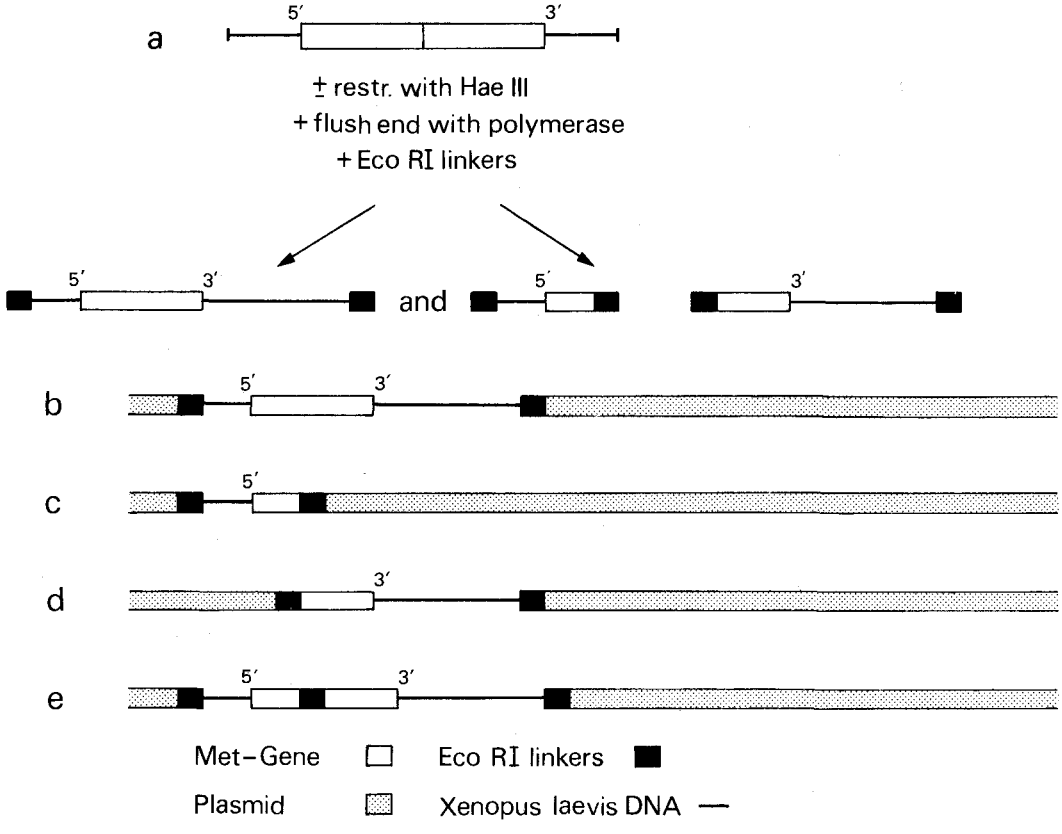

Fig. 5. Determination of the sites promoting transcription in a *Xenopus laevis* $tRNA^{Met}$ gene.
a Complete $tRNA^{Met}$ gene fragment with Hae III restriction site. *b* Recombinant containing the entire $tRNA^{Met}$ fragment. *c* Recombinant with anterior part of the fragment. *d* Recombinant with posterior part of the fragment. *e* Recombinant containing the tRNA gene with an Eco linker sequence as an intron.
Only the recombinants b and e are capable of promoting transcription. Hence, the presence of both the anterior and posterior portion of the tDNA unit are needed for transcription. The presence of an artificial Eco linker intron has no effect (Adapted from Kressmann et al., 1979; with permission).

base pairs are needed upstream from the 5′ end of a $tRNA^{Met}$ gene of *Xenopus*, and that only 6 base pairs are necessary upstream from the transcription initiation site of a $tRNA_2^{Ala}$ gene of *Bombyx* in order to allow transcription. These results indicate that the control regions may be located quite close to the tRNA genes. The experiments which demonstrate that the 5′ and 3′ ends of the mature tRNA coding section of the gene are necessary are illustrated in figure 5 (Kressmann et al., 1979) and summarized here. Transcription of the $tDNA^{Met}$ injected into the germinal vesicle of a *X. laevis* oocyte is only obtained if both the 5′ and 3′ ends of the gene are present (figure 5,b and c). The insertion of a short artificial intron created by the Eco R I linkers does not hamper transcription (figure 5,e). Consequently the important parts of the tRNA genes are the 5′ and 3′ sequences, whereas the middle is not so decisive. This is understandable given the fact that many tRNA genes contain introns of variable length. Are the 5′ flanking sequences of any importance? DeFranco et al. (1980) have described 2 *D. melanogaster* $tRNA_2^{Lys}$ genes containing plasmids derived from different region of the genome, but which possess identical tRNA regions. These plasmids differ in their 5′ flanking sequences and the efficiency of transcription of the 2 genes in *Xenopus* germinal vesicle extracts differs by an order of magnitude. 'Switching' of the 5′ flanking sequences (figure 6) demonstrates that these sequences are responsible for the efficiency of transcription. Hence, besides the internal control regions responsible for initiation of transcription, external modulating sequences can be found at the immediate 5′ end of a tRNA gene. Replacement of the 5′ flanking sequence by plasmid DNA (figure 6,e and f) allows transcription at the same rate as for the unaffected (efficient) gene 4. Thus this finding supports the conclusion obtained by Kressmann et al. (1979) that the tRNA coding region contains the control region crucial for transcription initiation. Does a similar differential gene expression of the 2 $tRNA_2^{Lys}$ genes take place 'in vivo'? The answer is not known, since the isolation of the precursors from labeled animals or even cell cultures is difficult due to the low specific activity obtained. Furthermore, the presence of about 2 dozen $tRNA_2^{Lys}$ genes in the *Drosophila* genome (Tener et al., 1980) obscures the situation. However, the results can be interpreted in another way. Factors involved in the transcription of purified genes by polymerase III have been described by Roeder et al. (1979). The difference in the efficiency of transcription observed in the *Xenopus* germinal vesicle extract might therefore be due to the lack of a specific factor needed for the efficient transcription of the unefficient gene. Transcription experiments with polymerase III extracts which have been isolated from the homologous system may resolve this problem.

Despite this strong evidence in favor of a modulated

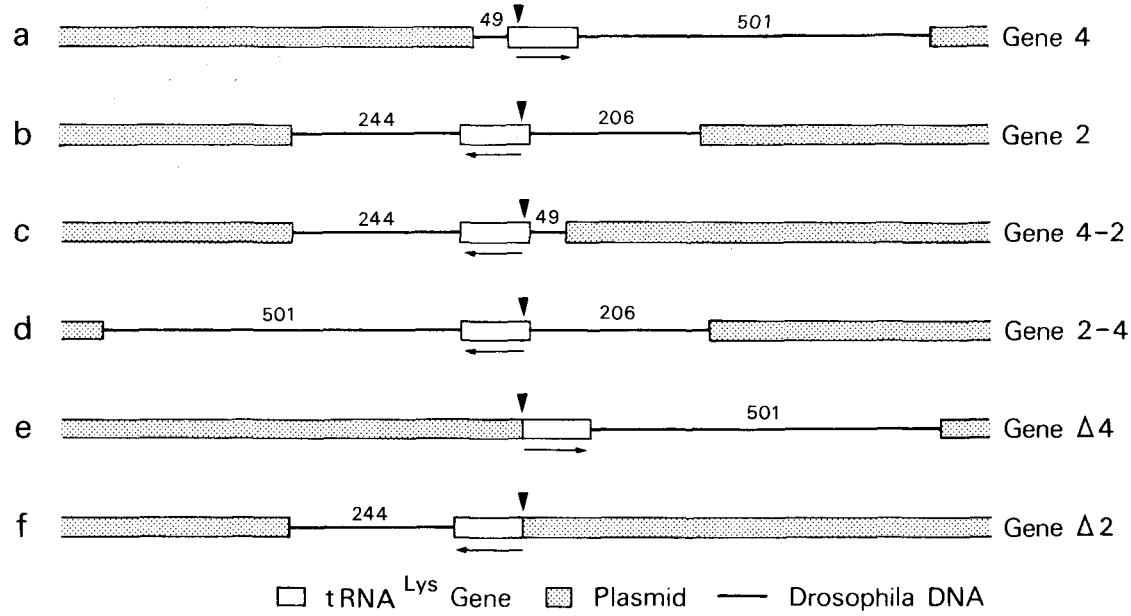

Fig. 6. Modulating effect of the 5′ flanking sequence on the transcription efficiency of a tRNA gene. The arrows indicate the orientation of the tRNA genes. The pointer indicates a Hae III restriction site. The numbers indicate the length of the fragments in base pairs. The 2 genes are transcribed with very different efficiencies in *Xenopus laevis* nuclear extracts.
a and *b* The 2 *Drosophila* $tRNA_2^{Lys}$ genes with identical tRNA coding sequences and different 5′ flanking regions. Gene 4 is the efficient gene; gene 2 the inefficient. *c* Combination of the 5′ flanking sequence of gene 4 with the structural gene sequence of gene 2. Transcription is efficient. *d* Combination of the 5′ flanking sequence of gene 2 with the structural gene sequence of gene 4. Transcription is inefficient. *e* and *f* The 5′ flanking sequences are replaced by plasmid DNA. Transcription is efficient in both cases (Adapted from DeFranco et al., 1980; with permission).

tRNA gene transcription it is not known whether this is a common phenomenon for tRNA genes. The occurrence of a tissue specific $tRNA^{Ala}$ in *Bombyx mori* (Sprague et al., 1977) demonstrates that tissue specific activation of tRNA genes can take place. However, measurements of the $tRNA^{Val}$ content of a *Drosophila* mutant containing a duplication for a $tRNA_{3b}^{Val}$ gene cluster (Tener et al., 1980) indicates a dosage dependent transcription of tRNA genes at least for *D. melanogaster.* Interestingly, the total $tRNA^{Val}$ content in the corresponding deletion mutant does not differ from the wild type value. Some correction toward a standard amount of $tRNA^{Val}$ seems therefore possible. It is not clear, whether this is due to increased transcription or decreased turnover of the involved $tRNA^{Val}$ species. In order to resolve this important questions tRNA mutants are needed; however, no tRNA dependent suppressors nor any other kind of tRNA mutants have been described in higher eukaryotes. The *Minute* mutants of *Drosophila* were originally thought to represent tRNA gene mutants (Ritossa et al., 1966) but do not show any correlation with the tRNA loci as determined by 'in situ' hybridization of radioactively labeled total *Drosophila* tRNA (Elder et al., 1980).

Phasing: Nucleosomes are the basic structural units of eukaryotic chromatin. They consist of about 140 base pairs of DNA coiled around a core of histones (2 each of the 4 histones H 3, H 4, H 2A and H 2B) and about 10–70 base pairs internucleosome linker DNA associated with histone H 1. The question arises whether the nucleosomes are distributed randomly along the DNA or whether there are specific relationships between the DNA sequence and the location of the nucleosomes. Wittig and Wittig (1979) have determined the position of chicken tRNA genes within cloned nucleosome DNA, and have found a nonrandom location. All tRNA structural gene sequences begin about 20 base pairs 'inside' the nucleosome core. A correct phase relationship is maintained over a distance of 4–6 subsequent nucleosomes. The presence of introns does not disturb the phasing of tRNA genes. However, phase readjustments have also been found between 2 tRNA genes. The functional significance of this phenomenon is not clear. But it seems, that this nonrandom location of tRNA genes is not due to any common sequences.

IV. Conclusion

Rapid and fascinating progress has been made in the last few years in the understanding of the structure and function of the tRNA genes of higher eukaryotes. This is mainly due to the application of the techniques of 'in situ' hybridization, gene cloning, DNA sequencing, and transcription assays performed in *Xenopus laevis* germinal vesicles or oocyte nuclear extracts. However, it has to be emphasized that for none of these genes (with the exception of the yeast $tDNA^{Tyr}$) has an 'in vivo' function been demonstrated. Although it seems very likely for various reasons that an in vivo function would exist, it is imperative to isolate mutants affecting tRNA genes in order to confirm the in vitro results. Organisms with well known genetics *(Caenorhabditis, Drosophila)* are especially good candidates for such an approach. It is our belief that a combination of the powerful new methods of molecular biology together with sophisticated genetic techniques will reveal more interesting insights into this special class of eukaryotic genes in the near future.

Acknowledgments. Research carried out in the authors laboratory mentioned in this article was supported by grants from the Swiss National Science Foundation and the Hescheler-Stiftung. I would like to thank Dr B. Marcum for critically reading the manuscript.

Literature

Abelson, J., 1980. A revolution in biology. Science *209,* 1319–1321.
Birnstiel, M.L., Sells, B.H., and Purdom, I.F., 1971. Kinetic complexity of RNA molecules. J. molec. Biol. *63,* 21–39.
Bogenhagen, D.F., Sakonju, S., and Brown, D.D., 1980. A control region in the center of the 5S RNA gene directs the specific initiation of transcription: The 3′ border of the region. Cell *19,* 27–35.
Burdon, R.H., Martin, B.T., and Lal, B.M., 1967. Synthesis of low molecular weight ribonucleic acid in tumor cells. J. molec. Biol. *28,* 357–371.
Chevallier, A., and Garel, J.P., 1979. Studies on tRNA adaptation, tRNA turnover, precursor tRNA and tRNA gene distribution in *Bombyx mori* by using two-dimensional polyacrylamide gel electrophoresis. Biochimie *61,* 245–262.
Clarkson, S.G., Birnstiel, M.L., and Serra, V., 1973a. Reiterated transfer RNA genes of *Xenopus laevis.* J. molec. Biol. *79,* 391–410.
Clarkson, S.G., Birnstiel, M.L. and Purdom, I.F., 1973b. Clustering of transfer RNA genes of *Xenopus laevis.* J. molec. Biol. *79,* 411–429.
Cortese, R., Melton, D., Tranquilla, T., and Smith, J.D., 1978. Cloning of nematode tRNA genes and their expression in the frog oocyte. Nucl. Acids Res. *5,* 4593–4611.
Cortese, R., Melton, D., Tranquilla, T., and Smith, J.D., 1980. Transcription and processing of nematode tRNA genes microinjected into the frog oocyte; in: Transfer RNA: Biological aspects, pp. 287–293. Ed. D. Söll, J.N. Abelson and P.R. Schimmel. Cold Spring Harbor Laboratory.
DeFranco, D., Schmidt, O., and Söll, D., 1980. Two control regions for eukaryotic tRNA gene transcription. Proc. natl Acad. Sci. USA *77,* 3365–3368.
Elder, R.T., Uhlenbeck, O., and Szabo, P., 1980. 4S RNA gene organization in *Drosophila melanogaster;* in: Transfer RNA: Biological aspects. pp. 317–323. Ed. D. Söll, J.N. Abelson and P.R. Schimmel. Cold Spring Harbor Laboratory.
Garber, R.L., Siddiqui, M.A.Q., and Altman, S., 1978. Identification of precursor molecules to individual tRNA species from *Bombyx mori.* Proc. natl Acad. Sci. USA *75,* 635–639.
Garber, R.L., and Gage, L.P., 1979. Transcription of a cloned *Brombyx mori* $tRNA_2^{Ala}$ gene: nucleotide sequence of the tRNA precursor and its processing in vitro. Cell *18,* 817–828.
Gilbert, W., 1979. Introns and exons: playgrounds of evolution; in: Eukaryotic gene regulation. pp. 1–12. Ed. R. Axel, T. Maniatis and C.R. Fox. Academic Press, New York.
Goodman, H.M., Olson, M.V., and Hall, B.D., 1977. Nucleotide sequence of a mutant eukaryotic gene: the yeast tyrosine-inserting ochre suppressor SUP4-o. Proc. natl Acad. Sci. USA *74,* 5453–5457.
Hagenbüchle, O., Larson, D., Hall, G.I., and Sprague, K.U., 1979. The primary transcription product of a silkworm alanine tRNA gene: identification of in vitro sites of initiation, termination and processing. Cell *18,* 1217–1229.

Hall, L., and Braun, R., 1977. The organisation of genes for transfer RNA and ribosomal RNA in amoebae and plasmodia of *Physarum polycephalum*. Eur. J. Biochem. *76*, 165–174.

Hosbach, H.A., Silberklang, M., and McCarthy, B.J., 1980. Evolution of a *D. melanogaster* glutamate tRNA gene cluster. Cell *21*, 169–178.

Hovemann, B., Sharp, S., Yamada, H., and Söll, D., 1980. Analysis of a *Drosophila* tRNA gene cluster. Cell *19*, 889–895.

Kressmann, A., Clarkson, S.G., Pirrotta, V., and Birnstiel, M.L., 1978. Transcription of cloned tRNA gene fragments and subfragments injected into the oocyte nucleus of *Xenopus laevis*. Proc. natl Acad. Sci. USA *75*, 1176–1180.

Kressmann, A., Hofstetter, H., Di Capua, E., Grosschedl, R., Birnstiel, M.L., 1979. A tRNA gene of *Xenopus laevis* contains at least two sites promoting transcription. Nucl. Acids Res. *7*, 1749–1763.

Kubli, E., 1980. Modification of transfer RNA in eukaryotes: an evolutionary interpretation. Trends Biochem. Sci. *5*, 90–91.

Kubli, E., Schmidt, T., and Egg, A.H., 1980. The localization or the genes for $tRNA_4^{Glu}$ and $tRNA_2^{Asp}$ in *Drosophila melanogaster* by in situ hybridization; in: Transfer RNA: Biological aspects. pp.309–315. Ed. D. Söll, J.N. Abelson and P.R. Schimmel. Cold Spring Harbor Laboratory.

Léon, P.E., 1976. Molecular hybridization of iodinated 4S, 5S, and 18S+28S RNA to salamander chromosomes. J. Cell Biol. *69*, 287–300.

Long, E.O., and Dawid, I.B., 1980. Repeated genes in eukaryotes. A. Rev. Biochem. *49*, 727–764.

Mao, J., Schmidt, O., and Söll, D., 1980. Dimeric transfer RNA precursors in *S.pombe*. Cell *21*, 509–516.

Mazzara, G.P., and McClain, W.H., 1980. tRNA synthesis; in: Transfer RNA: Biological aspects. pp.3–27. Ed. D. Söll, J.N. Abelson and P.R. Schimmel. Cold Spring Harbor Laboratory.

Melton, D.A., and Cortese, R., 1979. Transcription of cloned tRNA genes and the nuclear partitioning of a tRNA precursor. Cell *18*, 1165–1172.

Melton, D.A., DeRobertis, E.M., and Cortese, R., 1980. Order and intracellular location of the events involved in the maturation of a spliced tRNA. Nature *284*, 143–148.

Müller, F., and Clarkson, S.G., 1980. Nucleotide sequence of genes coding for $tRNA^{Phe}$ and $tRNA^{Tyr}$ from a repeating unit of *X. laevis* DNA. Cell *19*, 345–353.

Ogden, R.C., Knapp, G., Peebles, C.L., Kang, H.S., Beckmann, J.S., Johnson, P.F., Fuhrman, S.H., and Abelson, J.N., 1980. Enzymatic removal of intervening sequences in the synthesis yeast tRNAs; in: Transfer RNA: Biological Aspects. pp.173–190. Ed. D. Söll, J.N. Abelson and P.R. Schimmel. Cold Spring Harbor Laboratory.

Ritossa, F.M., Atwood, K.C., and Spiegelman, S., 1966. On the redundancy of DNA complementary to amino acid transfer RNA and its absence from the nucleolar organizer region of *Drosophila melanogaster*. Genetics *54*, 663–676.

Roeder, R.G., Engelke, D.R., Ng, S., Segall, J., Shastry, B., and Weil, P.A., 1979. Factors involved in the transcription of purified genes by RNA polymerase III; in: Eucaryotic gene regulation. pp.521–540. Ed. R. Axel, T. Maniatis, and C.F. Fox. Academic Press, New York.

Sakonju, F., Bogenhagen, D.F., and Brown, D.D., 1980. A control region in the center of the 5S RNA gene directs specific initiation of transcription: I. The 5′ border of the region. Cell *19*, 13–25.

Schimmel, P.R., Söll, D., and Abelson, J.N., 1980. Transfer RNA: Structure, properties, and recognition. Cold Spring Harbor Laboratory.

Schmidt, O., Mao, J., Silverman, S., Hovemann, B., and Söll, D., 1978. Specific transcription of eukaryotic tRNA genes in *Xenopus* germinal vesicle extracts. Proc. natl Acad. Sci. USA *75*, 4819–4823.

Schmidt, O., Mao, J., Ogden, R., Beckman, J., Sakano, H., Abelson, J., and Söll, D., 1980. Dimeric tRNA precursors in yeast. Nature *287*, 750–752.

Schmidt, T., and Kubli, E., 1980. The localization of $tRNA_5^{Asn}$, $tRNA^{His}$, and $tRNA^{Ala}$ genes from *Drosophila melanogaster* by in situ hybridization to polytene salivary gland chromosomes. Chromosoma (Berl.) *80*, 277–287.

Selker, E., and Yanofsky, C., 1980. A phenylalanine tRNA gene from *Neurospora crassa:* conservation of secondary structure involving an intervening sequence. Nucl. Acids Res. *8*, 1033–1042.

Silverman, S., Schmidt, O., Söll, D., and Hovemann, B., 1979. The nucleotide sequence of a cloned *Drosophila* arginine tRNA gene and its in vitro transcription in *Xenopus* germinal vesicle extracts. J. biol. Chem. *20*, 10290–10294.

Söll, D., Abelson, J.N., and Schimmel, P.R., 1980. Transfer RNA: Biological aspects. Cold Spring Harbor Laboratory.

Sprague, K.U., Hagenbüchle, O., and Zuniga, M., 1977. The nucleotide sequence of two silk gland alanine tRNAs: implications for fibroin synthesis and for initiator tRNA structure. Cell *11*, 561–570.

Sprinzl, M., Grueter, F., Spelzhaus, A., and Gauss, D.H., 1980. Compilation of tRNA sequences. Nucl. Acids Res. *8*, r1–r22.

Telford, J.L., Kressmann, A., Koski, R.A., Grosschedl, R., Müller, F., Clarkson, S.G., and Birnstiel, M.L., 1979. Delimination of a promotor for RNA polymerase III by means of a functional test. Proc. natl Acad. Sci. USA *76*, 2590–2594.

Tener, G.M., Hayashi, S., Dunn, R., Delaney, A., Gillam, I.C., Grigliatti, T.A., Kaufman, T.C., and Suzuki, D.T., 1980. tRNA genes of *Drosophila melanogaster;* in: Transfer RNA: Biological aspects. pp.295–307. Ed. D. Söll, J.N. Abelson and P.R. Schimmel. Cold Spring Harbor Laboratory.

Tiemeier, D.C., Tilghman S.M., Polsky, F.I., Seidman, J.G., Leder, A., Edgell, M.H., and Leder, P., 1978. A comparison of two cloned mouse β-globin genes and their surrounding and intervening sequences. Cell *14*, 237–245.

Tønnesen, T., Engberg, J., and Leick, V., 1976. Studies on the amount and location of the tRNA and 5-S rRNA genes in *Tetrahymena pyriformis* GL. Eur. J. Biochem. *63*, 399–407.

Wallace, R.B., Johnson, P.F., Tanaka, S., Schöld, M., Itakura, K., and Abelson, J., 1980. Directed deletion of a yeast transfer RNA sequence. Science *209*, 1396–1400.

Weber, L., and Berger, E., 1976. Base sequence complexity of the stable RNA species of *Drosophila melanogaster*. Biochemistry *15*, 5511–5519.

Wittig, B., and Wittig, S., 1979. A phase relationship associates tRNA structural gene sequences with nucleosome cores. Cell *18*, 1173–1183.

Valenzuela, P., Venegas, A., Weinberg, F., Bishop, R., and Rutter, W.J., 1978. Structure of yeast phenylalanine-tRNA genes: An intervening DNA segment within the region coding for the tRNA. Proc. natl Acad. Sci. USA *75*, 190–194.

Yen, P., Hershey, N.D., Robinson, R.R., and Davidson, N., 1979. Sequence organization of *Drosophila* tRNA genes; in: Eucaryotic gene regulation. pp.133–142. Ed. R. Axel, T. Maniatis and C.F. Fox. Academic Press, New York.

Yen, P., and Davidson, N., 1980. The gross anatomy of a tRNA gene cluster at region 42A of the *Drosophila melanogaster* chromosome, Cell *22*, 137–148.