extent of incorporation of the probe, the proportion of azido fatty acids in the total fatty acids was estimated by comparing the absorption band of the carbonyl group of carboxylic acids at 1700–1725 cm^{-1} and the sharp azido band at 2100 cm^{-1}. All experimental values given represent the mean of 5 separate experiments. The SE was ± 5%.

Results and discussion. The *cel* mutant[5] of *N. crasa* grew in the presence of supplemented fatty acids of appropriate size (C_{12} - C_{18}). The mutant when grown in the presence of 9-azido stearic acid, was found (from IR analysis and TLC) to incorporate the azido fatty acid into the membrane lipids of cells (110 h), the efficiency of incorporation being 17%. But when the wild type strain was grown in the presence of 9-azido stearic acid, it did not incorporate the probe into the membrane lipids. The growth rates of the mutant as well as of the wild type were studied in the presence and absence of the azido fatty acid (figure 1). A similar type of growth pattern was observed. However, the net amount of growth was decreased slightly in the presence of the azido fatty acids.

The *Saccharomyces* desaturase mutant was unable to desaturate the long chain fatty acid (18:0) tot the Δ^9-*cis* unsaturated fatty acid (18:1), and hence was auxotrophic for the latter. The mutant was grown in the presence of 12-azido oleic acid. Lipid analyses (IR and TLC) of cells collected after 30 h of incubation revealed incorporation of the intact probe into the membrane lipid. The efficiency of incorporation was found to be 25%. When the wild type *S. cerevisae* was grown in the presence of 12-azido oleic acid, a similar type of result was obtained to that with the wild type *N. crassa;* the azido fatty acid was not incorporated in the membrane lipids and the growth rate was almost unaffected (figure 2). But the mutant showed a reduced growth rate and an abbreviated exponential phase in the presence of the 12-azido oleic acid and the net amount of growth was also lower (figure 2).

Incorporation of photolabile azido fatty acids in appropriate mutants of *Neurospora* and *Saccharomyces* is a succesful application of the photo affinity labelling technique for probing the membrane structure developed by Chakrabarti and Khorana[9,15]. This is the first report applying this technique to eukaryotic microorganisms.

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Isoacceptor glycine tRNA species during bovine myocardium development

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Summary. The isoacceptor patterns of glycyl-tRNAs from fetal bovine myocardium during development, and of adult cardiac muscle, have been studied by reverse-phase chromatography. 4 isospecies were detected and quantitative changes in their relative abundance were noted. Moreover, upon testing their efficiency in transferring glycine into polypeptides a differential utilization of the cognate tRNAs was observed.

Both quantitative and qualitative changes in the distribution of isoaccepting tRNAs have been observed in different organisms and tissues under a variety of biological conditions[3–7]. The biological significance of this fact is not clearly understood. It is possible that the relative distribution of isoacceptor aminoacyl-tRNAs is adapted to the requirement of protein synthesis[8], but it could also be involved in the regulatory mechanism of the biosynthetic processes[9].

The bovine myocardium during development represents a specialized tissue where the molecular mechanisms of regulation involved with protein synthesis could exhibit particular characteristics, considering the nature and function of the synthesized proteins[10]. Hence, it was interesting to explore possible changes in the tRNA populations during myocardium development.

In the present study, the chromatographic behavior of isoacceptor $tRNA^{Gly}$ species from fetal bovine myocardium during development and adult cardiac muscle tissue were compared. Quantitative changes in the relative abundances were found. In order to know whether or not there was a differential utilization of glycyl-tRNAs species, experiments on the transfer of labeled glycine into polypeptides were done in a cell-free system for the synthesis of muscle proteins.

Table 1. Glycine acceptance of tRNA from bovine myocardium

Source of tRNA		pmoles/A_{260} unit*
Fetal	3-month-old	58.0 ± 8.0**
	5-month-old	57.6 ± 8.7
	7-month-old	85.5 ± 7.6
Adult	1-year-old	37.5 ± 4.3

The acceptance assays were performed as described in Materials and methods. * pmoles of ^{14}C- or ^{3}H-glycine bound per 1 A_{260} unit of tRNA. ** Average of data obtained in 3–5 experiments ± SD.

A significant correlation between the level of each glycyl-tRNA species and its transfer capacity for glycine into nascent polypeptide chains was found.

Materials and methods. ^{14}C-Glycine (56 mCi/mM) and ^{3}H-glycine (1500 mCi/mM) were purchased from New England Nuclear. The material for Plaskon chromatography (RPC-5) was obtained from Miles Lab. Cardiac muscles were excised from adult bovines and fetuses, immediately after death, and frozen at −60 °C. Transfer RNA freed of mitochondrial contaminants was prepared as described by Krauskopf et al.[11]. As a source of aminoacyl-tRNA synthetases and elongation factors, a S-150 was prepared from fetal myocardium essentially as described by Heywood et al.[12], except that G-100 column chromatography was performed to remove endogenous tRNAs[13]. The aminoacylation capacity assays and large-scale preparations of charged tRNA were performed as described previously[14,15]. The myocardium ^{3}H- or ^{14}C-glycyl-tRNA thus obtained were co-chromatographed on an RPC-5 column as described by Kelmers and Heatherly[16]. The 4 tRNAs^{Gly} isospecies detected were further purified by preparative RPC-5 chromatography. Myocardium tRNA free of tRNA^{Gly} was obtained by charging total tRNA with all the amino acids, except glycine. The mixture obtained was treated with $NaIO_4$[17] followed by a nonenzymatic hydrolysis of the aminoacylated-tRNAs in 0.2 M Tris-HCl buffer pH 8.7 at 37 °C. The periodate oxidation appeared to remove tRNA^{Gly} significantly, since the charging level for tRNA^{Gly} after this treatment was less than 5%. Myocardium polysomes from 3-, 5- and 7-month-old fetuses were prepared according to Palmiter[18] and purified by sedimentation in a linear sucrose gradient (15–40% w/w)[19]. The recovered polysomes were pooled and stored at −60 °C.

Results and discussion. Table 1 indicates that the extent of glycine acceptance of the fetal myocardium tRNAs was greater than in adult tissue. The increase in glycine incorporation was particularly important in myocardium tRNAs from 7-month-old fetuses, since it coincided with the exponential weight increase of heart muscle (data not shown). In order to explore the possible differences in the amount and quality of isoacceptor tRNA^{Gly} species, RPC-5 column co-chromatography was performed (figure 1). 4 isospecies designated I to IV according to their order of elution were detected in myocardium tRNAs from 3-, 5- and 7-month-old fetal calves and adult bovines. A quantitative evaluation showed clear differences in their relative abundances (table 2). In the second half of fetal development, the isospecies III and IV represent about 95% of the total myocardium tRNA^{Gly}, and this percentage is reduced to only 75% in adult myocardium. The most abundant species in 3-month-old fetal tissue, species III (47%), diminishes drastically in the myocardium tRNA from adult bovine (18%).

The quantitative modifications of the tRNAs^{Gly} populations would have a meaning, if the changes observed during development contributed to the optimal efficiency of the translational machinery[20]. 1 way to investigate this is to test the extent to which an individual aminoacyl-tRNA^{Gly} species participates in protein synthesis in vitro[13,21]. Therefore the level of transfer of glycine arising from each glycyl-tRNA species was measured (see details in the legend to figure 2). Panels 2A, 2B and 2C show that at the plateau level the glycine incorporation into polypeptides is approximately 3 times greater when the labeled amino acid arises from tRNA$^{Gly}_{III}$ and tRNA$^{Gly}_{IV}$ than when it comes from species I and II. A reproducible preference of the glycyl-tRNA$^{Gly}_{III}$ for decoding the bulk of mRNAs present in 3-month-old myocardium polysomes was found (figure 2, A). The polymerizing activity due to addition of a single tRNA^{Gly} species could suggest that the codons for species III and IV are preferentially utilized. But a basal level of contaminant tRNA^{Gly} isospecies might also be present in our uncharged tRNA preparation which escaped the in vitro aminoacylation assay. The results, nevertheless, clearly indicate that glycyl-tRNA$^{Gly}_{III}$ and glycyl-tRNA$^{Gly}_{IV}$ are preferentially decoded.

Changes in the subunit pattern of myofibrillar proteins have been observed during muscle development[22–24] as the result of qualitative and quantitative variations of translatable mRNAs[25,26]. Since the transfer of the aminoacyl group into polypeptide nascent chains is directed by these mRNAs the differential incorporation of a particular amino acid could be due to changes in the frequency of occurrence of synonymous codons that may occur during development.

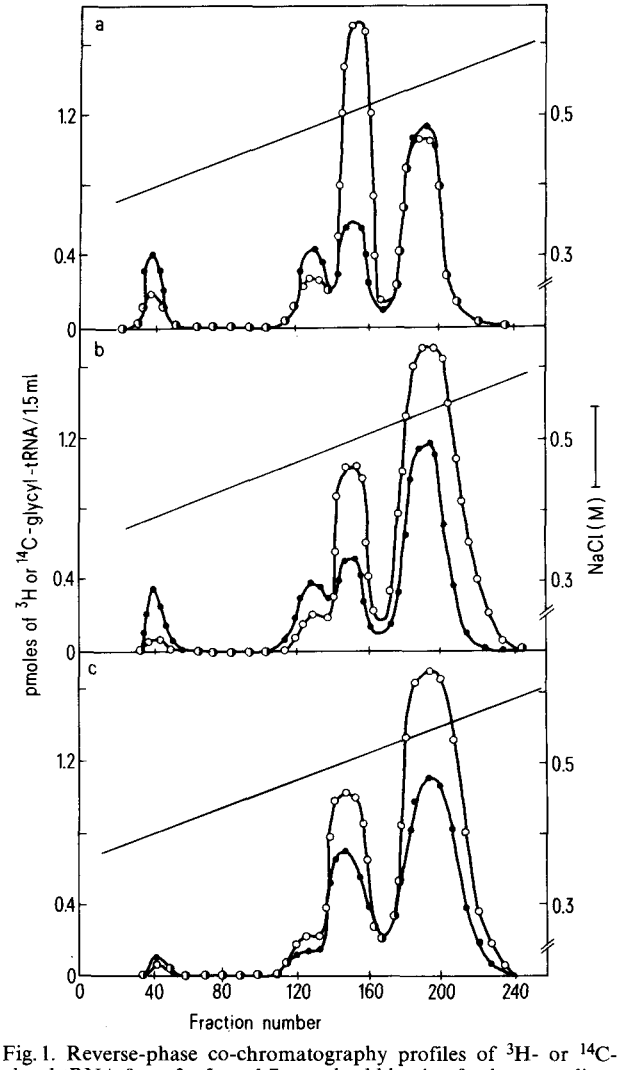

Fig. 1. Reverse-phase co-chromatography profiles of ^{3}H- or ^{14}C-glycyl-tRNA from 3-, 5- and 7-month-old bovine fetal myocardium and adult bovine cardiac muscle. A 0.9 × 90 cm glass column of RPC-5 was equilibrated with 10 mM sodium acetate buffer pH 4.7, 10 mM $MgCl_2$ and 0.35 M NaCl. Elution was performed at 4 °C with a linear NaCl-gradient from 0.35 to 0.75 M. Fractions of 2 ml were collected and the radioactivity was measured by counting 1.5 ml aliquots in a Triton-toluene scintillation mixture. *A* O——O, ^{14}C-glycyl-tRNA from a 3-month-old fetus; ●——●, ^{3}H-glycyl-tRNA from adult myocardium. *B* O——O, ^{14}C-glycyl-tRNA from a 7-month-old fetus; ●——●, ^{3}H-glycyl-tRNA from adult myocardium. *C* O——O, ^{3}H-glycyl-tRNA from a 7-month-old fetus; ●——●, ^{14}C-glycyl-tRNA from a 5-month-old fetus.

Table 2. Relative amounts of bovine fetal and adult myocardium $tRNA^{Gly}$ isoacceptor species

Source of tRNA		Isospecies* I	II	III	IV
Fetal	3-month-old	3.9±0.7**	8.1±1.3	47.5±3.4	40.5±2.5
	5-month-old	2.2±1.0	5.3±1.5	27.5±3.0	65.0±2.5
	7-month-old	1.0±0.7	3.9±1.0	30.1±3.6	66.2±2.8
Adult	1-year-old	8.3±2.0	15.7±2.4	18.0±1.5	58.0±3.8

* The data correspond to figure 1. ** Average of data obtained in 3–4 experiments ± SD. Total glycyl-tRNA = 100%.

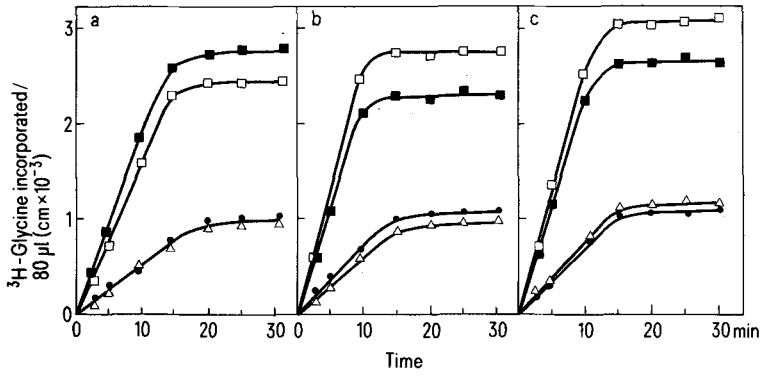

Fig. 2. Time course of transfer of glycine by isospecies: ●—●, I; △—△, II; ■—■, III and □—□, IV of ^{3}H-glycyl-tRNA into polypeptides. The cell-free amino acid incorporation assays were carried out in the following optimal conditions in a final volume of 1 ml: 50 mM Tes buffer pH 7.5, 6 mM $MgCl_2$, 100 mM KCl, 4 mM B-mercaptoethanol, 2 mM ATP, 0.5 mM GTP, 0.01 mM creatine phosphate, 0.3 mg of creatine kinase, 5 A_{260} units of homologous tRNA free of $tRNA^{Gly}$, 0.02 mM of each one of the cold amino acids less glycine and 50 µg of S-150 fraction. An equal amount (300 pmoles) of ^{3}H-glycyl-tRNA was tested individually in the incorporation system containing 3.5A_{260} units of polysomes from: *A* 3-month-old. *B* 5-month-old. *C* 7-month-old fetal bovine myocardium. The mixtures were incubated at 37 °C and duplicate aliquots of 80 µl, except for zero-time incubation, were assayed for hot-TCA insoluble radioactivity as described by Mans and Novelli[28]. Each point on the graphs is the average of 2 or 3 separate experiments.

The correlation of tRNA isoacceptor abundance in several kinds of animal cells with codon abundance has been presented as evidence that tRNA isoacceptor content and codon occurrence are adapted quantitatively[27]. Since a given mRNA in a specialized cell may contain a restricted set of codons, the translation of those mRNAs would be governed by the availability of one or several isoaccepting tRNAs[8]. Our results would indicate that a greater proportion of the translatable mRNA sequences contain more codons which are preferentially recognized by the more abundant glycyl-tRNA species in that stage of development. The existence of this quantitative correlation suggests that a specific-$tRNA^{Gly}$ codon could play a role in myocardium protein synthesis.

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