# Incorporation of photolabile azido fatty acid probes in *Neurospora crassa* and *Saccharomyces cerevisiae*[1,2]

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*Summary.* Photolabile azido fatty acids supported the growth of fatty acid auxotrophs, the *cel* mutant of *Neurospora crassa* and the KD115 mutant of *Saccharomyces cerevisiae*. These fatty acids were incorporated into the membrane lipids of the *cel* and KD115 mutants, but were not used by the wild type strain.

Fatty acid auxotrophs of microorganisms are useful tools for the study of membrane organization. The present investigation deals with 2 such mutants, a fatty acid chain elongation (*cel*) mutant[5] of *Neurospora crassa* and a fatty acid desaturase (KD115) mutant[6] of *Saccharomyces cerevisiae* and their wild types used to study the incorporation of photolabile azido fatty acids as a tool for photo affinity labelling.

*Materials and methods.* The *cel* mutant of *N. crassa* was grown at 34 °C in Vogel's minimal medium[7] (VMM) supplemented with stearic acid or 9-azido stearic acid at a concentration of $10^{-3}$ M in presence of 2% Brij-58. The wild type *N. crassa* (I.T.C.C.F., 1701) was grown at 34 °C in VMM as well as in VMM supplemented with $10^{-3}$ M 9-azido stearic acid and 2% Brij-58. The growth rate was measured as described by Henry and Keith[5].

The KD115 desaturase mutant of *S. cerevisiae* was grown at 25 °C in a minimal medium[8] supplemented with $10^{-3}$ M 12-azido stearic acid and 1% Brij-58. The wild type *S. cerevisiae* (S288C) was also grown at 25 °C in the minimal medium or in the minimal medium supplemented with $10^{-3}$ M 12-azido oleic acid and 1% Brij-58. The growth rate was followed by measuring the turbidity of the growth culture in a Systronic double cell photoelectric colorimeter. The azido fatty acids were synthesized by the method of Chakrabarti and Khorana[9].

Lipids were isolated from the washed and freeze-dried cells according to the following procedure. The cells were stirred for 60 min with chloroform:methanol, 2:1 (v/v) (20 ml of extractant per g of dry weight of cells) containing 50 mg antioxidant (2,6-di-*tert*-butyl-4-methyl phenol) per l. The cells were then centrifuged and the residue was treated twice as before. The residue obtained was extracted 3 times with chloroform:methanol:acetic acid (glacial):water, 25:15:4:2 (v/v/v/v) (20 ml of extractant per g of wet weight of cells) containing 50 mg of the above antioxidant per l. Lipids extracted using the above 2 solvents were pooled, washed with 0.88% KCl solution according to Folch et al.[10] and evaporated to dryness under vacuum.

Mild alkaline hydrolysis of phospholipids was carried out by the method of Dittmer and Wells[11]. The organic phase containing the fatty acid and azido fatty acid was collected.

TLC was carried out to separate the azido fatty acids from the fatty acids on silica gel G coated glass plates. The TLC plates were developed with a solvent system consisting of petroleum ether (b.p. 60-80°C):diethyl ether: acetic acid (glacial), 80:20:1 (v/v/v) using the double development technique. The separated components were visualized with iodine vapour[12-14]. The respective $R_f$-values of azido fatty acids and fatty acids were 75 and 83.

IR-spectra of the fatty acids were recorded in a Perkin-Elmer spectrophotomer (model 567). To determine the

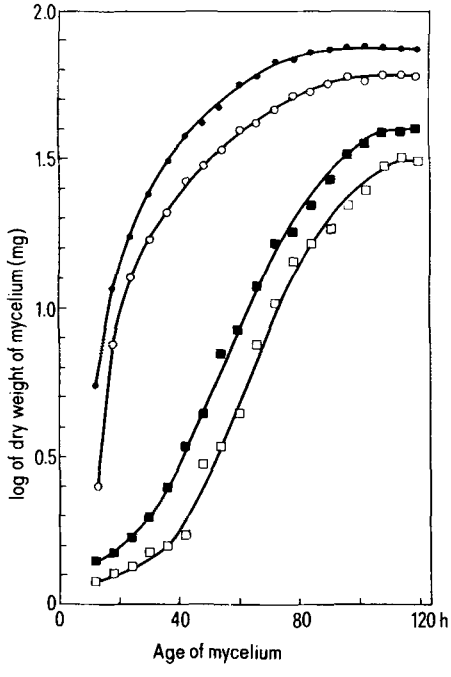


Fig. 1. Growth of wild type and *cel* mutant of *N. crassa*. ●——●, wild type grown on VMM + 2% Brij-58; ○——○, wild type grown on VMM + 2% Brij-58 + $10^{-3}$ M 9-azido stearic acid; ■——■ *cel* mutant grown on VMM + 2% Brij-58 + $10^{-3}$ M stearic acid; □——□ *cel* mutant grown on VMM + 2% Brij-58 + $10^{-3}$ M 9-azido stearic acid.

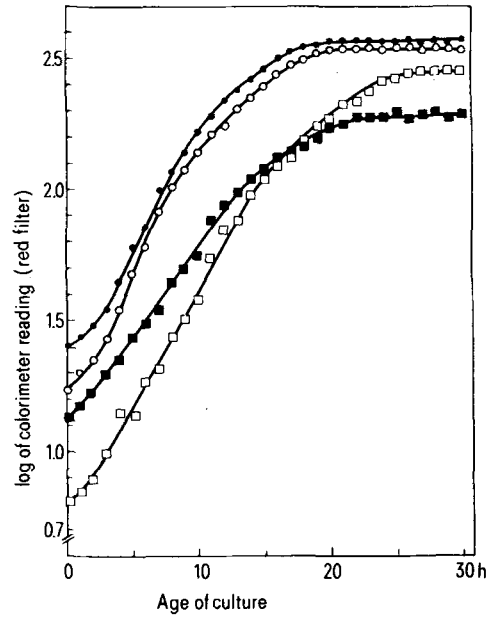


Fig. 2. Growth of wild type and KD115 mutant of *S. cerevisiae*. ●——●, wild type grown on minimal medium + 1% Brij-58; ○——○, wild type grown on minimal medium + 1% Brij-58 + $10^{-3}$ M 12-azido oleic acid; ■——■, KD115 grown on minimal medium + 1% Brij-58 + $10^{-3}$ M oleic acid; □——□, KD115 grown on minimal medium + 1% Brij-58 + $10^{-3}$ M 12-azido oleic acid.

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 $\Delta^9$-*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.