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# Isotope labeling of Neurospora DNA

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## Abstract

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Attempts to find the genetic relationships among microorganisms using the techniques of Bolton, Hoyer and McCarthy (1964 Science 144: 3621) are often frustrated by difficulties attendant on DNA labeling. This communication will describe observations on (1) the suitability of various isotopes for labeling Neurospora DNA and (2) the cultural techniques used to obtain maximum specific activity of labeled DNA.

The procedure used to isolate DNA from Neurospora is an adaptation of the method of Marmur (1961 J. Mol. Biol. 3: 208). Neurospora mycelia are grown in liquid culture, lyophilized (at room temperature) and powdered (through a 60-mesh screen) in a Wiley mill. Dispersion and solution, aided by sodium lauryl sulfate, was followed by ethanol precipitation and deproteinization with chloroform-isoamyl alcohol treatments. R Nase followed by isopropanol precipitation was used to purify the DNA.

Wild type 74A and ad-8 a strains were used in these experiments. The base medium used was according to Vogel's formula. Adenine-8-$C^{14}$ was obtained from Calbiochem Inc.; tritiated thymidine was obtained from Schwarz Bioresearch; and $P^{32}$ ($Na_2HP^{32}O_4$) was obtained from Oak Ridge National Laboratory via M. D. Anderson Hospital and Tumor Institute.

The quantitation of DNA was made with an ultraviolet spectrophotometer, correcting for protein contamination by the Lowry (1951 J. Biol. Chem. 193: 265) protein assay. The protein found with the purified DNA was always negligible (O. D. less than 0.003).

Maximum labeling is obtained by growing the ad-8 a mutant in adenine-8-$C^{14}$ supplemented medium (Table 1). DNA's with specific activities of several hundred c.p.m/ μg have been isolated using this approach. Supplements of more than 5 mg. % adenine decreased the specific activity of the DNA even though total uptake increased. The $P^{32}$ and $H^3$ compounds showed relatively low uptake and incorporation rates. The removal of $C^{14}$-adenine from the medium by 74A reached a maximum of 95% within 24 hours of growth, and the specific activity of the DNA decreased with time after 24 hours (Fig. 1).

The yield of DNA (DNA/weight of dry powder) was greatest in young cultures, and decreased with age in approximately linear fashion (Fig. 2). This decrease is associated with a reciprocal increase in protein.

When $H^3$ was used as label, there was low incorporation into DNA and high incorporation into the RNA-protein fractions. This observation was reported by St. Lawrence and Baer (1964 NN#6: 5). If this phenomenon obtains with other fungi, it may be necessary to isolate mutants of each species prior to incorporation of $H^3$. The use of orthophosphate as carrier of $P^{32}$ (McCarthy and Hoyer 1964 Proc. Natl. Acad. Sci. U.S.51: 915) has not been tried; it may yield better results than the $Na_2HP^{32}O_4$ used here.

Preliminary survey of other fungi and plant species indicated that the methods described here generally work well, but exceptions do occur. The degree of homology between related species will be published elsewhere.

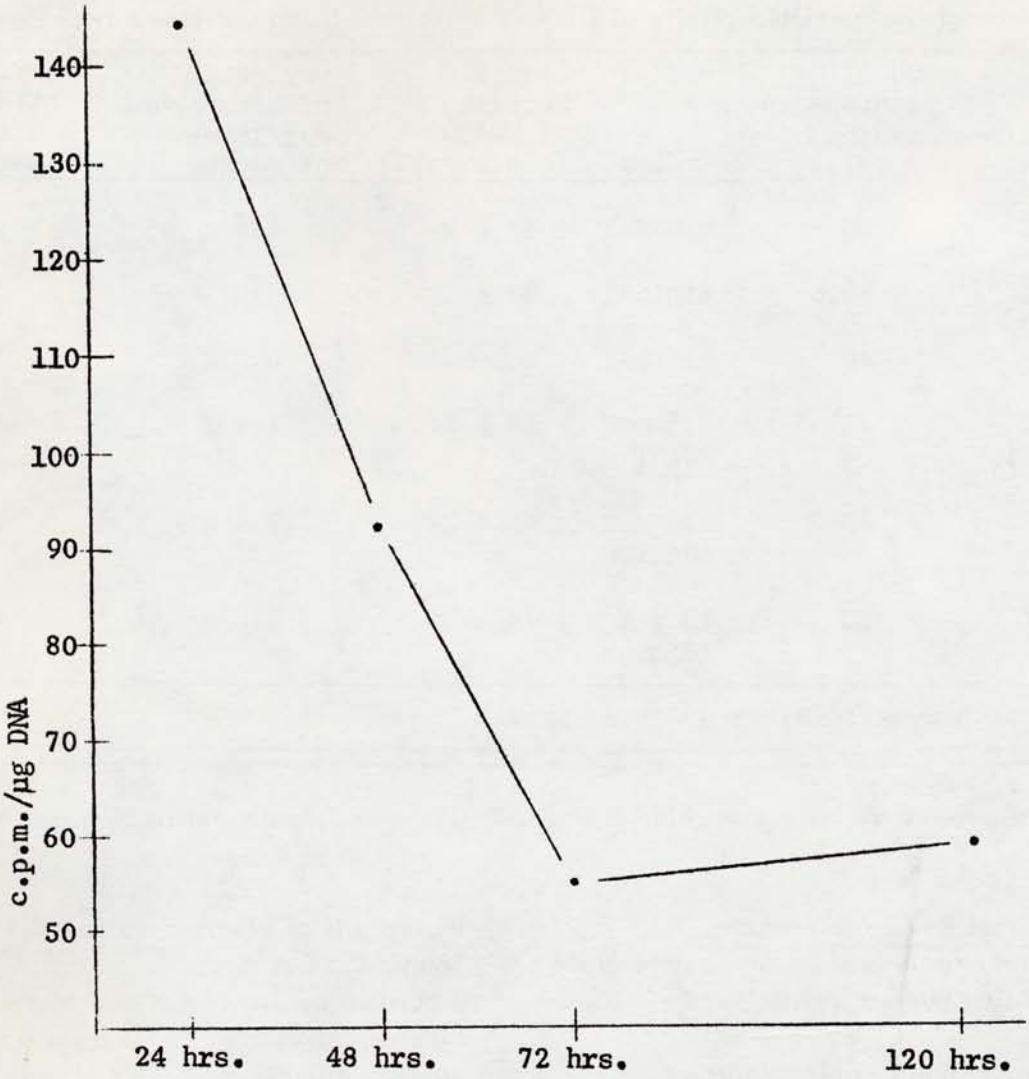


Fig. I. Specific activity of DNA of N. crassa as a function of age. (Strain 74A, 80 µc/l adenine-8-$C^{14}$.)

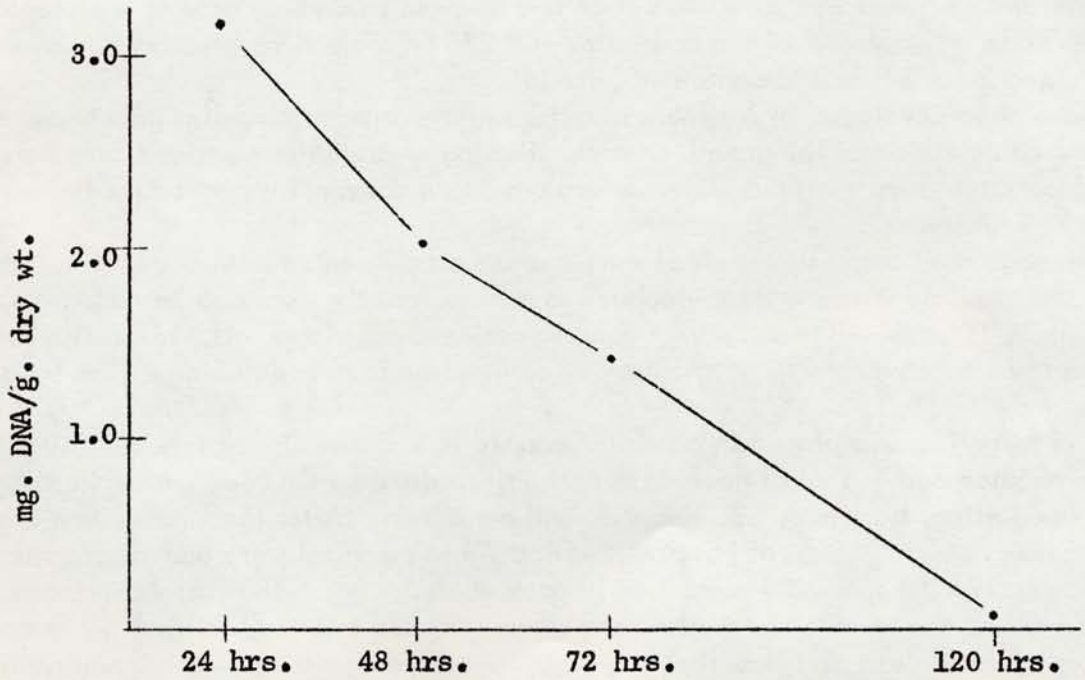


Fig. 2. Yield of DNA of N. crassa per gram mycelial powder as a function of age. (Strain 74A)

Table I. A comparison of the specific activity of DNA of N. crassa using various isotope compounds.*

| Strain | Isotope used | Quantity isotope in medium (μc/l.) | Medium | Specific activity of DNA (c.p.m./μg.) | Dry weight of Neurospora per liter of medium | Percentage uptake of label from medium |
|---|---|---|---|---|---|---|
| 74A | $C^{14}$ | 20 | minimal | 23 ± 2 | 4.8 g | 95 ± 2 |
| 74A | $P^{32}$ | 50.6 | minimal | 28 ± 2 | 5.0 g | 10 ± 2 |
| 74A | $H^{3}$ | 40 | minimal | 0.8 ± 0.2 | 5.0 g | 12 ± 2 |
| ad-8a | $C^{14}$ | 20 | 2.5 mg% adenine | 58 ± 2 | 1.4 g | 49 ± 2 |
| ad-8a | $C^{14}$ | 20 | 5.0 mg% adenine | 50 ± 2 | 2.4 g | 64 ± 2 |
| ad-8a | $C^{14}$ | 20 | 10 mg% adenine | 14 ± 2 | 4.5 g | 69 ± 2 |

* Mycelia were harvested after 48 hours growth.

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