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Radiolabeling of Drosophila embryonic RNA
and protein to high specific activity.

With the recent emphasis on the molecular approach
to the study of Drosophila embryogenesis, the availa-
bility of a method for the radiolabeling embryonic
macromolecules to high specific activity is highly
desirable. Although procedures for the labeling of
embryonic proteins have been published (Zalokar
1973; Raff et al. 1982), the incorporation of radioactive precursors is relatively low or unknown.

We have devised a protocol for radiolabeling Drosophila embryonic RNAs and proteins to high speci-
fic activity. It is based on our previous observations with other tissues (e.g., ovaries) that the net incorpor-
ation of radioactive precursors into macromolecules relates directly to the concentration and not to the
amount of labeled precursors in the surrounding medium. This paradigm was taken to its extreme in the
procedure illustrated in Figure 1. Dechorionated embryos were placed over dried radioactive precursor.
The embryos were then gently disrupted by crushing under light pressure. No buffer is used; the radioactive
precursor is dissolved in the embryo's own fluid. This provides for the maximum possible concentration
of labeled precursor. Incubation is at room temperature (about 22°C) and the contents are mixed
occasionally by applying gentle pressure over the cover slip. This procedure has been used for labeling
both RNA and protein.

The time course of incorporation of ^{3}H-uridine into RNA is illustrated in Figure 2. Incorporation
proceeds for at least 1 hr. The mean incorporation of ^{3}H-uridine into RNA in 4 independent experiments
was 5,100 (±1,300) CPM per embryo per 40 min for 20-hr old embryos. High incorporation was also obtained
when ^{35}S-methionine was used as a precursor. The mean incorporation of ^{35}S-methionine into protein
in 11 independent experiments was 68,900 (±26,400) CPM and 87,600 (±56,000) CPM per embryo per 40
min incubation at 22°C for 5-hr old and 19-hr old embryos, respectively.
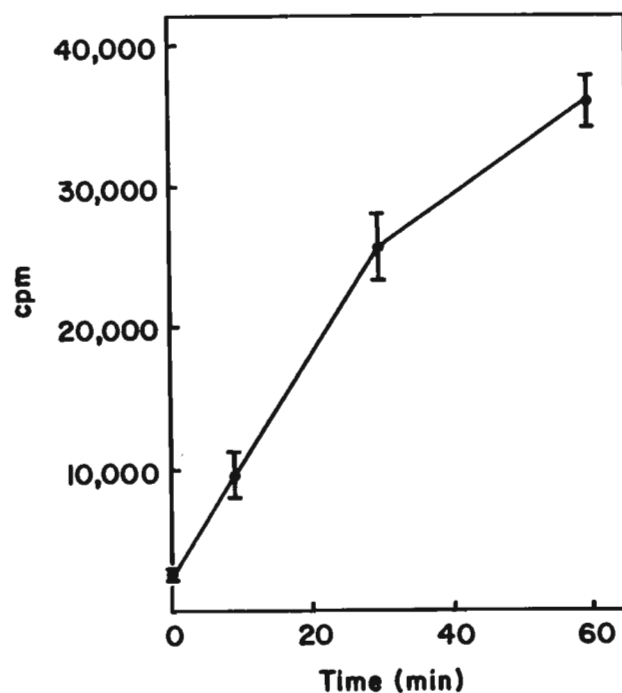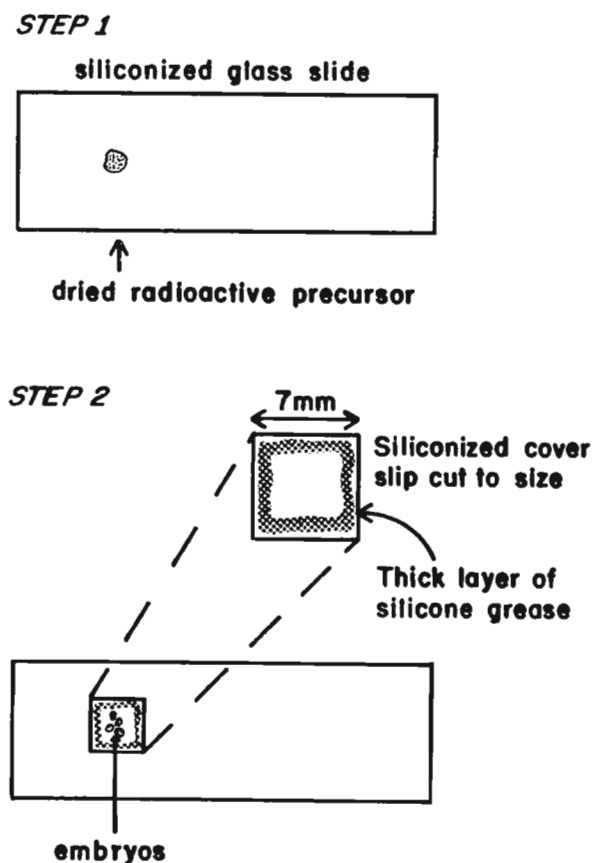
Figure 2. Time course of incorporation with ^{3}H-uridine.
Groups of 5 embryos (20-hr old) were labeled with 1 uCi
of ^{3}H-uridine for the specified length of time. At the
end of the incubation the cover slip was flipped over and
the contents were transferred with several 2 ul aliquots
of 1% SDS to a filter paper disc. The discs were washed
several times in the cold with 5% trichloroacetic acid and
the insoluble radioactivity was quantitated by scintilla-
tion counting. The background was not substracted.

Figure 1. Set up for radiolabeling of Drosophila embryos. Step 1: a droplet of the radioactive precursor is placed
over a siliconized glass microscope slide and dried under vacuum. Step 2: 5 to 15 hand-dechorionated embryos are
placed over the dried labeled precursor; these are immediately covered with the cover slip and gentle pressure is
applied over its center to disrupt the embryos and at the same time establish a hermetic seal by silicone grease.

For most purposes, analysis of the labeled macromolecules can be accomplished by dissolving the embryo in strongly denaturing detergents, such as sodium dodecyl sulfate (SDS). However, for certain applications this may not be desirable. For instance, we have recently used this labeling procedure to study the synthesis of ribosomal proteins at different stages of embryogenesis (Kay & Jacobs-Lorena, submitted). The two-dimensional gel system that was used for the analysis of ribosomal proteins is incompatible with the presence of SDS. Other denaturing agents such as urea resulted in incomplete extraction of the labeled proteins from the embryos. We therefore extracted the labeled embryos sequentially with a buffer containing Triton X-100 and then with 66% acetic acid, an excellent protein solvent. In this way the labeled proteins were completely extracted. The Triton was then removed by ether extraction. To introduce the labeled proteins into electrohoresis buffer, a Biogel P6 (BioRad) gel filtration column was prepared in a 1-ml disposable syringe and equilibrated in electrophoresis buffer. The column was placed in a test tube over a 1.5 ml conical tube. The radioactive sample (approx. 100 ul) was applied onto the column and centrifuged for 2 min at 1,800 RPM. All of the labeled ribosomal proteins were recovered in the first eluate, while non-incorporated label and undesired ions were retained in the column. The eluate could then be used directly for electrophoresis. Alternative procedures to change the ionic composition of the ribosomal proteins to that of electrophoresis buffer, such as precipitation with trichloroacetic acid or dialisys, were not satisfactory in that the recovery was low or unpredictable.

In summary, a simple procedure for very efficient radiolabeling and analysis of embryonic macromolecules is described. Because the embryos are disrupted, all tissues are equally exposed to the isotope allowing for uniform labeling of embryos at any developmental stage.

References: Limbourg, B. & M. Zalokar 1973, Dev. Biol. 35:382-387; Raff, E.C., M.T. Fuller, T.C. Kaufman, K.J. Kemphues, J.E. Rudolph & R.A. Raff 1982, Cell 28:33-40.

Jennings, N.J. and R.D. Seager. University of Northern Iowa, Cedar Falls, USNA. Larval substrates of wild Drosophila.

For the past several years we have been sampling Drosophila populations from different communities near Cedar Falls, Iowa (Jennings et al., submitted to Proc. Iowa Acad. Sci.; Seager & Jennings 1984). In order to add to our knowledge of natural breeding sites of Drosophila, in conjunction with our 1983 collections we attempted to rear adult flies from probable larval substrates found in a lowland forest community. These data were gathered in order to determine the possible success of a more thorough future study of this type. In addition we observed adult flies on some of these substrates and collected (aspirated) them for later identification. The data we report here are preliminary since our fungal identifications have not been verified by taxonomists. For future collections we have enlisted the aid of a fungal expert.

The substrates from which adults were either reared or aspirated and the species involved are:

Agaricus sp.: **D.tripunctata** and **D.falleni** were both reared and aspirated and **D.testacea** was reared from this species.

Tremella sp.: **D.putrida** and **D.falleni** were both reared and aspirated from this species.

Juglans nigra (black walnut): **D.tripunctata** was aspirated from walnuts.

Morus sp. (mulberries): **D.affinis, D.falleni, D.putrida,** and **D.tripunctata** were all aspirated from fallen mulberries.

In addition, four fungal growths which we were unable to key were studied. **D.quinaria, D.putrida, D.tripunctata,** and **D.falleni** were all reared from at least one of these, and **D.putrida, D.tripunctata, D.falleni,** and **D.testacea** were all aspirated from at least one of these.

The lack of suitable larval substrates will limit the distribution of a species. **D.tripunctata** was very common in our collections from a lowland forest community but was not found in samples taken at the same time from a nearby sand prairie community (Seager & Jennings 1984). At least two of the substrates that this species apparently uses, walnuts and mulberries, are present in the lowland community but are absent from the sand prairie community. Thus the absence of a suitable larval substrate may restrict the distribution of **D.tripunctata**. This is a possibility that we will explore in more detail in the future.

Reference: Seager & Jennings 1984, DIS 60:182-184.