A simple and quick method to isolate nuclear extracts from pupae of *Drosophila melanogaster*.

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Abstract

Preparation of nuclear extracts is a critical step for biochemical identification of factors which function in the nuclei such as transcription factors. We have established a new method to prepare nuclear extracts from prepupae or pupae of *Drosophila melanogaster*. The method is simple and particularly useful for small-scale isolation from materials that are hard to get in large amounts such as specific tissues or animal bodies covered by pupal cases.

Introduction

Biochemical identification of factors is an important step to understand their function. In the case of transcriptional regulatory factors, it is important to know which factors bind to the regulatory regions of particular genes or which DNA elements are recognized by the factors for the understanding how organisms develop and maintain themselves. Gel mobility shift assay is a useful method to detect a DNA binding transcription factor which recognizes specific sequence. Although the assay itself is simple, preparation of extracts that is prerequisite for the assay is not easy and sometimes has limitations depending on their sources.

Drosophila melanogaster has been used as one of the best animals to study biology because it is amenable to both classical and modern genetics. In

contrast to genetics, not many researchers have employed biochemical approaches to study biology of the fly, because its body is so tiny that it is not easy to dissect it and use as biochemical materials. Nevertheless, some researchers have used the biochemical approach and have succeeded in getting results that are difficult to expect from other approaches. In most of these biochemical studies, embryonic nuclear extracts have been used because it is relatively easy to prepare large amounts of the extracts even from restricted developmental stages (Biggin and Tijan 1988; Jowett and White 1989; Ueda et al. 1990).

We have been using *Drosophila* nuclear extracts to identify factors which regulate gene expression (Murata et al. 1996; Kageyama et al. 1997). During analyses of gene regulation mechanisms at the onset of metamorphosis, staged nuclear extracts are essential to detect temporally induced factors

that are regulated by ecdysteroid hormone. However, in most cases large-scale collection of staged animals is not easy, because the staging depends on individual isolation based on their developmental makers. Furthermore, the hard pupal cases make homogenization of animals very hard. To overcome the problem, we developed a method to obtain nuclear extracts in small scale. In this new method, cortex was removed manually from each pupa before homogenization. Though it is difficult to treat many pupae, this method made it possible to obtain enough amount of nuclear extract from a small number of pupae for several trials of gel mobility shift assay.

Materials

Animals

Laboratory-bred *Drosophila* stock of strain *yw* (yellow white) are maintained at 25 °C.

Equipment

- (1) 1.5 ml centrifuge tube.¹
- (2) PELET PESTLE DISP W/TUBE.²
- (3) Centrifuge MRX-150.³
- (4) Optima TM TLX Ultracentrifuge.⁴
- (5) Rotor TLA1202.⁴
- (6) BASStation.⁵

Chemicals

- (1) Sucrose. Cat. No. 196-00015⁶
- (2) Hepes. Cat. No. 342-01375⁷
- (3) KCl. Cat. No. 163-03545⁶
- (4) MgCl₂. Cat. No. 136-03995⁶
- (5) PMSF. Cat. No. 504-45451⁶
- (6) Leupeptin. Cat. No. L2023⁸
- (7) Pepstatin A. Cat. No. P4265⁸

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⁵Fuji photo Film, Tokyo, Japan

⁶Wako Pure Chemical Industries, Ltd., Osaka, Japan

⁷Dojindo laboratories, Kumamoto, Japan

⁸Sigma-Aldrich Corporation, St. Louis, MO, USA

(8) Aporotinin. Cat. No. A6279⁸

(9) DTT. Cat. No. 045-08974⁶

(10) Glycerol. Cat. No. 072-04945⁶

(11) Phosphate buffered saline tablet. Cat. No. P4417⁸

(12) VECTASHIELD Mounting Medium with DAPI. Cat. No. H-1200⁹

Methods

Small-scale preparation of nuclear extract

The experimental procedure is shown schematically in Figure 1. Using forceps, remove front part of the pupal cases (cortices) from 20 animals in PBS and take out the interior bodies from their pupal cases. Transfer the bodies to 200 μ l of ice cold PBS in a 1.5 ml centrifuge tube. Perform following procedures under 4 °C. After removing PBS from the tube, homogenize the bodies by using a pestle in 300 μ l of homogenization solution (1 M sucrose, 10 mM Hepes [pH 7.9], 10 mM KCl, 3 mM MgCl₂, 0.5 mM PMSF, 5 μ g/ml Leupeptin, 2 μ g/ml Pepstatin, 0.75 μ g/ml Aporotinin). Centrifuge the homogenate in a micro centrifuge (MRX-150) at 2000 rpm for 10 min, save the supernatant into a new clean 1.5 ml centrifuge tube and centrifuge the obtained supernatant at 11,000 rpm for 10 min. Remove the supernatant by pipette carefully, and wipe off white lipid from the inside walls of the centrifuge tube with a cotton bud. Estimate the volume of the precipitated nuclei and suspend them in 3 volumes of Solution II (10 mM Hepes [pH 7.9], 0.4 M NaCl, 3mM MgCl₂, 0.5 mM DTT, 0.5 mM PMSF, 5% glycerol, 5 μ g/ml Leupeptin, 5 μ g/ml Pepstatin, 0.75 μ g/ml Aporotinin) and place on ice for 30 min with occasional shaking. To confirm the isolation of nuclei, mix small amounts of suspension with a mounting medium containing 1.5 μ g/ml of DAPI (4',6-diamidino-2-phenylindole) solution and observe with a microscope. Centrifuge (Optima TM TLX Ultracentrifuge) the suspension at 40,000 rpm for 60 min. Collect the supernatant as a nuclear extract and store it at -80 °C after quick freeze by liquid nitrogen.

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Figure 1. Scheme for the experimental procedure of newly designed small-scale nuclear isolation method. See materials and methods for the details.

Large-scale preparation of nuclear extract

Large-scale nuclear extracts from prepupae were prepared as described by Kageyama et al. (1997).

Gel mobility shift assay

The gel mobility shift assay was performed as described by Ueda and Hirose (1990). Signals were detected by using a Fuji BASstation.

Figure 2. Gel mobility shift assay reveals the same quality of an small-scale prepupal nuclear extract as that of a large scale one.

Complementary oligonucleotides carrying bp between -428 and -504 from the transcription start site of the *Edg78E* gene (Kawasaki et al. 2002) were annealed and were used as a D site probe after labeled with γ - ^{32}P -ATP and polynucleotide kinase.

Results and discussion

By the new small-scale method, roughly $10\ \mu\text{l}$ of nuclear extract was obtained from 20 prepupae. To compare the extracts prepared using the new method with those obtained by the previously used large-scale method (Kageyama et al. 1997), gel mobility shift assays were performed with D site as

a probe, which contains a binding site for the transcription factor FTZ-F1 (Kawasaki et al. 2002). Strong and clear signals were detected using $1\ \mu\text{l}$ of the nuclear extracts prepared by the new small-scale method as were the cases by the large-scale one (A typical example is shown in Figure 2). We confirmed that the signal is caused by FTZ-F1, because the shifted bands disappeared by the addition of anti-FTZ-F1 serum (data not shown). These results indicate that the new small-scale method can be used for gel shift assay as the usual large-scale method. The newly designed method is helpful for many other purposes: for example, preparation of nuclear extracts from specific tissues/organs or from a small number of individuals.

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