Video Article

Purification of Mitochondria from Yeast Cells

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Abstract

Mitochondria are the main site of ATP production during aerobic metabolism in eukaryotic non-photosynthetic cells[1]. These complex organelles also play essential roles in apoptotic cell death[2], cell survival[3], mammalian development[4], neuronal development and function[4], intracellular signalling[5], and longevity regulation[6]. Our understanding of these complex biological processes controlled by mitochondria relies on robust methods for assessing their morphology, their protein and lipid composition, the integrity of their DNA, and their numerous vital functions. The budding yeast *Saccharomyces cerevisiae*, a genetically and biochemically manipulable unicellular eukaryote with annotated genome and well-defined proteome, is a valuable model for studying the molecular and cellular mechanisms underlying essential biological functions of mitochondria. For these types of studies, it is crucial to have highly pure mitochondria. Here we present a detailed description of a rapid and effective method for purification of yeast mitochondria. This method enables the isolation of highly pure mitochondria that are essentially free of contamination by other organelles and retain their structural and functional integrity after their purification. Mitochondria purified by this method are suitable for cell-free reconstitution of essential mitochondrial processes and can be used for the analysis of mitochondrial structure and functions, mitochondrial proteome and lipidome, and mitochondrial DNA.

Video Link

The video component of this article can be found at https://www.jove.com/video/1417/

Protocol

Materials and methods

Yeast strains and growth conditions

The wild-type strain BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) was grown in rich YEPD medium (1% yeast extract, 2% peptone, 2% glucose). Cells were cultured at 30°C with rotational shaking at 200 rpm in Erlenmeyer flasks at a "flask volume/medium volume" ratio of 5:1.

Isolation of crude mitochondrial fraction

1. Grow 1 L of the wild-type strain BY4742 culture for 48 h.
2. Pour culture in 500-ml Nalgene centrifuge tubes. Balance the load and harvest cells by centrifugation for 5 min at 3000 x g at room temperature.
3. Decant supernatant and resuspend pelleted cells in 250 ml of dH2O. Pellet cells by centrifugation for 5 min at 3000 x g at room temperature.
4. Decant supernatant and resuspend pelleted cells in 250 ml of dH2O. Pellet cells by centrifugation for 5 min at 3000 x g at room temperature.
5. Determine wet weight of cell pellet.
6. Resuspend pelleted cells in DTT buffer [2 ml of buffer/g (wet weight) cells].
7. Transfer the cell suspension to 50-ml Falcon plastic tubes.
8. Rotate the tubes at 70 rpm in a shaker for 20 min at 30°C.
9. Harvest cells by centrifugation for 5 min at 3000 x g at room temperature.
10. Resuspend pelleted cells in Zymolyase buffer without Zymolyase [7 ml of buffer/g (wet weight) cells].
11. Pellet cells by centrifugation for 5 min at 3000 x g at room temperature.
12. Resuspend pelleted cells in Zymolyase buffer without Zymolyase [7 ml of buffer/g (wet weight) cells].
13. Transfer cell suspension to a glass flask.
14. Add the powder of Zymolyase-100T [1 mg of Zymolyase-100T/ g (wet weight) cells] to the cell suspension.
15. Rotate the flask with cell suspension at 70 rpm in a shaker for 30 min at 30°C.
16. Transfer spheroplasts formed due to the digestion of the cell wall with Zymolyase-100T to 50-ml plastic centrifuge tubes.
17. Pellet spheroplasts by centrifugation for 8 min at 2200 x g at 4°C.

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All subsequent steps should be carried out at 4°C or on ice. Suspensions of spheroplasts should be handled gently using pipettes with cut tips to avoid breaking organelles.

18. Resuspend pelleted spheroplasts in ice-cold homogenization buffer [6.5 ml of buffer/g (wet weight) cells].
19. Pellet spheroplasts by centrifugation for 8 min at 2200 x g at 4°C.
20. Resuspend pelleted spheroplasts in ice-cold homogenization buffer [6.5 ml of buffer/g (wet weight) cells].
21. Transfer cells to a pre-chilled on ice glass homogenizer.
22. Using a tight pestle, homogenize the cells by making 15 strokes of the pestle.
23. Add 1 volume of ice-cold homogenization buffer.
24. Transfer homogenized spheroplasts to 50-ml plastic centrifuge tubes.
25. Pellet unbroken cells, nuclei, and large debris by centrifuging for 5 min at 1500 x g at 4°C.
26. Centrifuge the resulting supernatant for 5 min at 3000 x g at 4°C.
27. Centrifuge the resulting supernatant for 15 min at 12000 x g at 4°C.
28. Decant supernatant and resuspend pellet in ice-cold homogenization buffer [6.5 ml of buffer/g (wet weight) cells].
29. Centrifuge for 5 min at 3000 x g at 4°C.
30. Centrifuge the resulting supernatant for 15 min at 12000 x g at 4°C.
31. Decant supernatant and resuspend pellet in 3 ml of ice-cold SEM buffer.

Although this suspension is enriched in mitochondria, it also contains other organelles such as the endoplasmic reticulum (microsomes), Golgi, and vacuoles. To get pure mitochondria, this crude mitochondrial fraction can be subjected to further fractionation, as described below.

Purification of mitochondria devoid of contamination by other organelles

1. Place 1.5 ml of ice-cold 60% (w/v) sucrose in EM buffer into a Beckman Ultra-Clear centrifuge tube.
2. Overlay 60% (w/v) sucrose with 4 ml of 32%, 1.5 ml of 23%, 1.5 ml of 15% sucrose (all wt/v in EM buffer).
3. Place 3 ml of the crude mitochondrial fraction in SEM buffer on top of 15% (w/v) sucrose.
4. Centrifuge in a Beckman SW41 Ti swinging-bucket rotor for 1 h at 134000 x g (33000 rpm) at 4°C.
5. The intact mitochondria form a brown band at the 60%/32% sucrose interface.
6. Carefully remove sucrose until reaching the mitochondrial band.
7. Remove the mitochondrial band using a pipette with a cut tip and place it into a Beckman centrifuge tube for an MLS-5 rotor.
8. Fill the tube with SEM buffer.
9. Pellet the pure mitochondria by centrifugation in an MLS-5 rotor for 30 min at 10000 x g (31000 rpm) for 30 minutes at 4°C.
10. Decant supernatant using a pipette.
11. Use the pure mitochondria for the analysis of mitochondrial functions, mitochondrial proteome and lipidome, and mitochondrial DNA.

Reagents

1. DTT buffer [100 mM Tris/H2SO4 (pH 9.4), 10 mM dithiothreitol] (for 15 ml)
   - 1.5 ml of 1 M Tris/H2SO4 buffer (pH 9.4)
   - 150 µl of 1 M DTT
   - Volume to 15 ml

2. Zymolyase buffer [20 mM potassium phosphate (pH 7.4), 1.2 M sorbitol] (for 100 ml)
   - 2 ml of 1 M potassium phosphate buffer (pH 7.4)
   - 60 ml of 2 M sorbitol
   - Volume to 100 ml

3. Homogenization buffer [10 mM Tris/HCl (pH 7.4), 0.6 M sorbitol, 1 mM EDTA, 0.2% (w/v) BSA] (for 250 ml]
   - 2.5 ml of 1 M Tris/HCl buffer (pH 7.4)
   - 75 ml of 2 M sorbitol
   - 500 µl of 500 mM EDTA
   - 0.5 g of BSA
   - Volume to 250 ml

4. SEM buffer [10 mM MOPS/KOH (pH 7.2), 250 mM sucrose, 1 mM EDTA] (for 250 ml)
   - 2.5 ml of 1 M MOPS/KOH buffer (pH 7.2)
   - 31.25 ml of 2 M sucrose
   - 500 µl of 500 mM EDTA
   - Volume to 250 ml

5. EM buffer [10 mM MOPS/KOH (pH 7.2), 1 mM EDTA] (for 250 ml)
   - 2.5 ml of 1 M MOPS/KOH buffer (pH 7.2)
   - 500 µl of 500 mM EDTA
   - Volume to 250 ml

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Discussion

This method enables the high-yield isolation of pure mitochondria from yeast cells. Mitochondria purified by this method are essentially free of contamination by other organelles and retain their structural and functional integrity after their purification. The described method yields mitochondria that are suitable for cell-free reconstitution of essential mitochondrial processes. These highly pure mitochondria can also be used for the analysis of mitochondrial structure and functions, mitochondrial proteome and lipidome, and mitochondrial DNA.

Disclosures

The authors have nothing to disclose.

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