Isolation and characterization of cold sensitive pex6 mutant of the methylotrophic yeast Hansenula polymorpha

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Introduction. Studies on the interrelationship between the processes of peroxisome biogenesis and degradation to identify genetic elements mutual for both mechanisms have been initiated recently [1]. Although oppositely directed, previous studies demonstrated the existence of such overlapping factors, e. g. the H. polymorpha PEX14 gene product [2]. Besides Pex14p, the peroxisomal matrix protein import requires the action of two AAA ATPases, Pexlp and Pex6p. They form a complex of main importance for peroxisome biogenesis, and the mutations affecting this complex are the most common cause of the Zellweger syndrome in humans [3]. This work was carried out with a mutant of methylotrophic yeast H. polymorpha deficient in peroxisome biogenesis due to the mutation in PEX6 gene. To elucidate, whether this peroxin is involved in the opposite process of peroxisome degradation, the temperature or cold sensitive (ts or cs) pex6-derivative mutants can be utilized. The aim of this work was to isolate and characterize these mutant strains.

Materials and Methods. Strains, media and growth conditions. H. polymorpha NCYC495, auxotrophic derivatives thereof [4] and pex5-C79 (leu1.1) [5] were used in this study. Yeast were grown at 28 or 37 °C in the YPD medium (1 % yeast extract, 1 % peptone and 1 % glucose) or selective mineral media (MM) with 0.05 % yeast extract [6], supplemented with 1 % glucose, 0.5 % methanol (MM) or 0.5 % glycerol. Leucine (40 mg/l) was added to all MM. For solid media agar (2 %) was used. Sporulation/mating media and techniques were essentially as described in [4]. Cells were fixed and prepared for electron microscopy as in [7].

Conditional mutant isolation. To isolate ts and cs mutants the initial strain pex6 was grown in liquid YPD, washed twice with distilled water, spread on the plates with MMM (5·10^7 cells/plate) and UV-mutationized for 60 s. Afterwards the plates were incubated for 4—5 days at 28 or 37 °C, and replica plated on the plates with MMM for identification of the mutants with conditional phenotypes at 28 and 37 °C.

Biochemical methods. Cell-free extracts for enzyme assays were prepared as in [8]. The protein concentration was determined according to [9] using bovine serum albumin as the standard. Alcohol oxidase (AOX) (EC 1.3.3.13) was assayed as in [6].

Results and Discussion. Isolation of the cs pex6 mutant. To identify other than PEX14 genes possibly involved in both peroxisome biogenesis and degradation, we attempted to isolate UV-induced, condi-
tional (ts or cs) revertants from the *H. polymorpha* pex6 mutant as an initial strain. The pex6 cells were mutagenized, and the colonies with a restored ability to grow on MMM plates were selected. We were able to isolate only one cs clone with normal growth on methanol at 37 °C (permissive temperature) and very slow growth at 28 °C (restrictive temperature) among pex6 derivatives. The cs pex6 mutant exhibited the cs phenotype only in a methanol medium (Fig. 1, A, B), not in glucose, ethanol or glycerol media.

Characterization of the cs pex6 strain. To study the biogenesis of peroxisomes in the cs pex6 mutant cells, the electron-microscopic analysis was carried out (Fig. 2). It revealed the restoration of the peroxisome biogenesis at both temperatures after 12 h induction by 0.5 % methanol. The wild-type peroxisomes induced by methanol in the cs pex6 cells at the permissive temperature (Fig. 2, F), and the morphologically altered enlarged peroxisomes at the restrictive for methylotrophic growth temperature (Fig. 2, E) were observed. The intriguing question, why the cs pex6 mutant cells with restored peroxisome biogenesis are unable to grow in MMM at 28 °C, is now under investigation.

The genetic analysis revealed that the corresponding cs mutation is most probably tightly linked with, or resides in the *PEX6* locus. The biochemical analysis of pexophagy at permissive and restrictive temperatures did not reveal considerable differences between the cs pex6 cells and the wild-type strain (Fig. 1, C—F). Further biochemical and genetic analysis of the isolated conditional mutant is in
The nature of mutations in the utilized initial pex6 and derivative cs mutants will be established by isolation of both alleles and sequencing but it seems that the cs pex6 mutation has no effect on pexophagy in H. polymorpha. Together with the data on susceptibility of peroxisomal remnants in Δpex6 to the glucose-induced peroxisome degradation [1], it will be the second experimental evidence, that organelle development and turnover do not converge at Pex6p in H. polymorpha.

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Виділення та характеристика холодочутливого мутанта рехб метилотрофних дріжджів *Hansenula polymorpha*

Резюме
Виділено холодочутливий супрессорний мутант зі штаму рехб *H. polymorpha* з пошкодженим біогенезом пероксисом. Відновлення росту холодочутливого резб на метанолі при пермиссивній температурі корелювало з наявністю морфологічно нормальних пероксисом. Збільшені у розмірах пероксисоми при рестриктивній температурі не здатні підтримувати метилотрофний ріст холодочутливого штаму резб. Виділена му­тація не впливає на деградацію пероксисом *H. polymorpha*.

REFERENCES

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