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KINETICS OF THE ASSEMBLY OF PEROXISOMES AFTER FUSION OF COMPLEMENTARY CELL LINES FROM PATIENTS WITH THE CEREBRO-HEPATO-RENAL (ZELLWEGER) SYNDROME AND RELATED DISORDERS

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We have recently identified four complementation groups in fibroblasts from patients deficient in peroxisomes. Here we describe a kinetic analysis of the complementation process. The kinetics of peroxisome assembly was assessed in heterokaryons of complementary cell lines by measuring the rate of incoporation of catalase, initially present in the cytosol, into particles. In two combinations of cell lines assembly was rapid and insensitive to cycloheximide. Thus the components required for peroxisome assembly must have been present in the parental cell lines, at least one of which presumably contained peroxisomal ghosts. In three other combinations of cell lines assembly of peroxisomes was slow and sensitive to cycloheximide.

The role of peroxisomes in mammalian cells is now firmly established; the organelles are involved in many processes including the β-oxidation of very long chain fatty acids [1-3] and prostaglandins [4], the biosynthesis of ether phospholipids [5] and bile acids [6,7], the catabolism of pipecolic acid [8] and, in man, the catabolism of glyoxylate [9,10].

Morphologically distinguishable peroxisomes are deficient in the Zellweger syndrome [11] and related disorders [12-15]. In such

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diseases there is a deficiency of several peroxisomal enzymes [16] and enzyme proteins [17]. Some peroxisomal enzymes, however, e.g. catalase [18,19], are not deficient but are located in the cytosol. The detection in livers from Zellweger patients of integral peroxisomal membrane proteins [20,21] suggests that peroxisomal ghost-like structures occur.

Recently we showed by complementation analysis after somatic cell fusion that fibroblasts from patients deficient in peroxisomes can be divided into at least four complementation groups [22], indicating that at least four gene products are required for the biogenesis of peroxisomes. One method of assessing complementation is to measure the incorporation of catalase, initially present in the cytosol [18,19], into particles (presumably peroxisomes); this confers structural linked latency on the enzyme. We have now studied the kinetics of peroxisome assembly by measuring the rate of incorporation of catalase into particles in heterokaryons of complementary cell lines.

MATERIALS AND METHODS

Cell lines Cultured skin fibroblasts were obtained from patients with the cerebro-hepato-renal syndrome (cell lines W78/515 and GOM85AD), the neonatal form of Adrenoleukodystrophy (cell line AAL85AD), the infantile form of Refsum disease (cell line BOV84AD) and Hyperpipecolic Acidaemia (cell line GM3605) [see ref. 22].

Materials Ham F-10 medium and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Gibro (Glasgow, U.K.). Polyethylene glycol (mol•wt 1000) was purchased from Merck (Darmstad, FRG), enzymes from Boehringer (Mannheim, FRG) and radiochemicals from the Radiochemical Centre (Amersham, U.K.). All other reagents were of analytical grade.

Methods Cells were fused with polyethyleneglycol as described in [22]. Particle-bound catalase was assessed by digitonin titration as described in [18]. Acyl-CoA:dihydroxyacetonephosphate acyltransferase was measured according to [23].

RESULTS AND DISCUSSION

Figure 1 shows the results of fusions between cell lines derived from complementation groups 2 and 3 (see ref. 22). Complementation group 2 consists of cell lines from patients with the Zellweger syndrome (W78/515), the infantile form of Refsum disease (BOV84AD) and Hyperpipecolic Acidaemia (GM3605), whereas complementation group 3 comprises a cell line from another patient with the Zellweger syndrome (GOM85AD). In the fusion between the two complementary Zellweger cell lines rapid incorporation of catalase into particles took place and was maximal within 3 h after fusion. Comparable rapid complementation was obtained in a fusion between

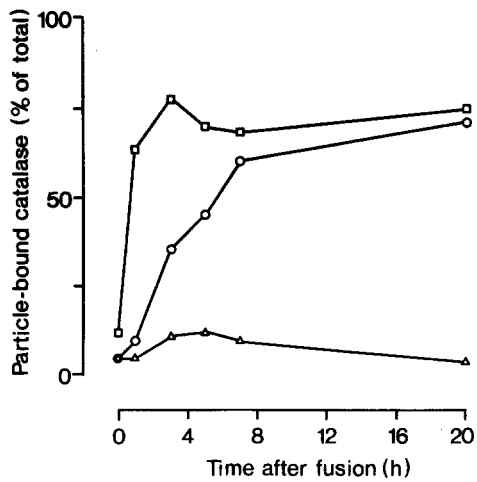

Fig. 1 Kinetics of complementation in heterokaryons obtained after fusion of complementary cell lines deficient in peroxisomes. Fibroblast were cultured in HAM F10 medium containing 10% (v/v) foetal calf serum. Cells were fused using polyethyleneglycol. Particle-bound catalase was assessed in the heterokaryons by the digitonin fractionation technique [18].
□——□, W78/515 (complementation group 2) x GOM85AD (group 3); ○—○, BOV84AD (group 2) x GOM85AD (group 3); △—△, self-fusion of GOM85AD cells.

cell line GOM85AD and a cell line from a patient in complementation group 4, which consists of the neonatal form of Adrenoleukodystrophy (AAL85AD; see ref. 22) (not shown).

In contrast, in a fusion between cell line GOM85AD and cell line BOV84AD, incorporation of catalase into particles was maximal only 8-10 h after fusion. There was a small but reproducible lag of about 1 h before incorporation of catalase was observed. Slow complementation was also found in fusions between cell lines GOM85AD and GM3605 and between cell lines W78/515 and AAL85AD (not shown). In a self-fusion experiment using cell line GOM85AD there was no increase in particle-bound catalase after fusion.

Complementation could also be monitored by measuring the activity of acyl-CoA:dihydroxyacetonephosphate acyltransferase (DHAP-AT) which is deficient in patients with the Zellweger syndrome and related disorders. It was observed that DHAP-AT activity increased slowly after fusion of all five combinations of cell lines (not shown).

Next we studied the influence of cycloheximide on the kinetics of complementation, measured both as the increase in particle-bound catalase and as the increase in DHAP-AT activity. The amount of cycloheximide used was sufficient to cause an almost

instantaneous 95-100% inhibition of incorporation of [^{35}S]methionine into trichloroacetic acid-insoluble material. Cell lines W78/515 and GOM85AD were fused in the absence and presence of cycloheximide and the heterokaryons were cultured in the absence and presence of the drug. Fig. 2a clearly demonstrate that no protein synthesis was necessary in this fusion combination for the appearance of particle-bound catalase. On the other hand, protein synthesis was necessary for the increase in DHAP-AT activity (Fig. 2b). Similar results were obtained in a fusion between GOM85AD and AAL85AD fibroblasts (not shown). After fusion of the complementary cell lines GOM85AD and BOV84AD, GOM85AD and GM3605 and cell lines W78/515 and AAL85AD protein synthesis was required for the assembly of particles (presumably peroxisomes) able to render catalase latent. Also in these fusions, restoration of DHAP-AT activity was dependent on protein synthesis in the heterokaryons (not shown).

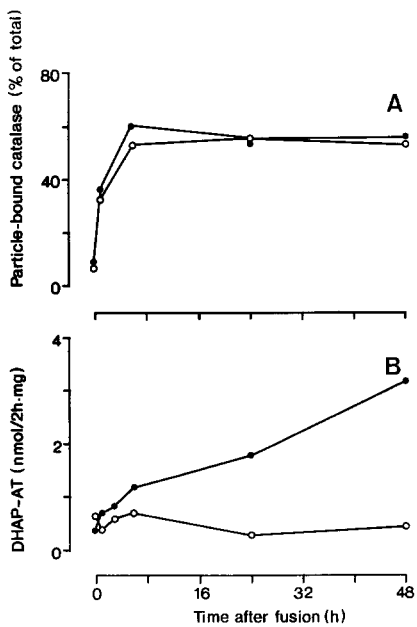

Fig. 2 Kinetics of assembly of peroxisomes in heterokaryons obtained after fusion of complementary cell lines deficient in peroxisomes: effect of cycloheximide. W78/515 fibroblasts and GOM85AD fibroblasts were fused in the presence or absence of 2.8 µg/ml cycloheximide. The heterokaryons were subsequently cultured for 48 h in the presence (open symbols) or absence (filled-in symbols) of the drug. Complementation was assessed at intervals after fusion either by measuring the increase in particle-bound (peroxisomal) catalase using the digitonin titration technique (A) or by measurement of DHAP-AT activity (B).

A summary of the results is shown in Table I. It is clear that assembly of peroxisomes after fusion of certain complementary cell lines deficient in peroxisomes occurs independently of protein synthesis. Thus components necessary for the assembly of peroxisomes can occur in a stable form in the cytosol of certain fibroblast cell lines but not in others. However, in all five combinations of cells studied, the increase in DHAP-AT activity is dependent on protein synthesis in the heterokaryons. In this respect DHAP-AT, a peroxisomal membrane protein, resembles the peroxisomal β-oxidation enzymes, which are synthesized in patients with the Zellweger syndrome and related disorders, but are rapidly broken down in the absence of a normal peroxisome [24].

Since the incorporation of catalase into particles is so fast in some of the fusions, and in view of current conceptions of peroxisomal biogenesis [25,26], it can be inferred that peroxisomal ghost-like structures which can act as a framework for the assembly of a peroxisome are present in at least some of the fusion partners. The demonstration [20,21] of the presence of several peroxisomal integral membrane proteins (i.e. sodium bicarbonate-insoluble proteins [27]) in homogenates of liver from Zellweger patients supports this possibility.

Finally, we observed that cell lines belonging to the same complementation group [W78/515, BOV84AD and GM3605], show a difference in the kinetics of appearance of particle-bound catalase after fusion with cell line GOM85AD. Thus the complementation component involved in peroxisome biogenesis, which is deficient in GOM85AD fibroblasts from complementation group 3, is stable in cell line W78/515 but unstable in cell lines BOV84AD and GM3605. This difference can be ascribed to different rates of breakdown of

Table 1. Kinetics and cycloheximide sensitivity of appearance of particle bound catalase after fusion of complementary cell lines deficient in peroxisomes

Cell lines fused	Incorporation of catalase	Effect of cycloheximide
ZS2 x ZS1	fast	none
ZS2 x NALD	fast	none
ZS2 x IRD	slow	inhibits
ZS2 x HPA	slow	inhibits
ZS1 x NALD	slow	inhibits

Cell lines: ZS1: W78/515, complementation group 2; ZS2: GOM85AD, complementation group 3; IRD: BOV84AD, complementation group 2; HPA: GM3605, complementation group 2; NALD: AAL85AD, complementation group 4 (see ref. 22).

the component. The stability of such components in certain cell lines should facilitate their identification and isolation; such studies are in progress.

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REFERENCES

1. Lazarow, P.B. and De Duve, C. Proc. Natl. Acad. Sci. 73: 2043-2046 (1976).
2. Bremer, J. Trends Biochem. Sci. 2: 207-209 (1977).
3. Singh, I., Moser, A.E., Goldfischer, S. and Moser, H.W. Proc. Natl. Acad. Sci. USA 81: 4203-4207 (1984).
4. Diczfalusy, U., Alexson, S.E.H. and Pedersen, J.I. Biochem. Biophys. Res. Commun. 144: 1206-1213 (1987).
5. Hajria, A.K. and Bishop, J.E. Ann. N.Y. Acad. Sci. 386: 170-182 (1982).
6. Pedersen, J.I. and Gustafsson, J.J. FEBS Lett. 121: 345-348 (1980).
7. Hagey, L.R. and Krisans, S.K. Biochem. Biophys. Res. Commun. 107: 834-841 (1982).
8. Trijbels, J.M.F., Monnens, L.A.H., Melis, G., Van den Broek van Essen, M., and Bruckwilder, M. J. Inh. Metab. Dis. 10: 128-134 (1987)
9. Noguchi, T. and Takada, Y. Arch. Biochem. Biophys. 196: 645-647 (1979).
10. Danpure, C.J. and Jennings, P.R. FEBS Lett. 201: 20-24 (1986).
11. Goldfischer, S., Moore, C.L., Johnson, A.B., Spiro, A.J., Valsamis, M.P., Wisniewski, H.K., Ritch, R.H., Nortan, W.T., Rapin, I. and Gartner, L.M. Science 182: 62-64 (1973).
12. Goldfischer, S. and Reddy, J.K. Int. Rev. Exp. Pathol. 26: 45-84 (1984).
13. Goldfisher, S., Collins, J., Rappin, I., Coltoff-Schiller, B., Chang, C.H., Nigro, M., Black, V.H., Javitt, N.B., Moser, H.W. and Lazarow, P.B. Science 227: 67-70 (1985).
14. Scotto, J.M., Hadchouel, M., Odievre, N., Laudat, M-H., Saudubray, J.M., Dulac, C., Beucler, I. and Beaune, P. J. Inh. Metab. Dis. 5: 83-90 (1982).
15. Ogier, H. Roels, F., Cornelius, A., Poll-Thé, B.T., Scotto, J.M., Odievre, M. and Saudubray, J.M. Scand. J. Clin. Lab. Invest. 45: 767-768 (1985).
16. Schutgens, R.B.H., Heymans, H.S.A., Wanders, R.J.A., Van Den Bosch, H., and Tager J.M. Eur. J. Pediatr. 144: 430-440 (1986).
17. Tager, J.M., Ten Harmsen Van Der Beek, W.A., Wanders, R.J.A., Hashimoto, T., Heymans, H.S.A., Van Den Bosch, H., Schutgens, R.B.H. and Schram, A.W. Biochem-Biophys. Res. Commun. 126, 3: 1269-1275 (1985).
18. Wanders, R.J.A., Kos, M., Roest, B;, Meyer, A.J., Schrakamp, G., Heymans, H.S.A., Tegelaers, W.H.H., Van Den Bosch, H., Schutgens, R.B.H. and Tager, J.M. Biochem. Biophys. Res. Commun. 123: 1054-1061 (1984).
19. Santos, M.J., Ojeda, J.M., Garrido, J. and Leighton, F. Proc. Natl. Acad. Sci. USA 82: 6556-6560 (1985).

20. Lazarow, P.B., Fujiki, Y., Small, G.M., Watkins, P. and Moser, H.W. Proc. Natl. Acad. Sci. USA 83: 9193-9196 (1986).
21. Just, A.W.W. and Schram, A.W. (personal communication).
22. Brul, S., Westerveld, A., Strijland, A., Wanders, R.J.A., Schram, A.W., Heymans, H.S.A., Schutgens, R.B.H., Van den Bosch, H. and Tager, J.M. J. Clin. Invest. (in press) (1988).
23. Schutgens, R.B.H., Romeyn, G.J., Wanders, R.J.A., Van den Bosch, H., Schrakamp, G. and Heymans, H.S.A. Biochem. Biophys. Res. Commun. 120: 179-184 (1984).
24. Schram, A.W., Strijland, A., Hashimoto, T., Wanders, R.J.A., Schutgens, R.B.H., Van den Bosch, H. and Tager, J.M. Proc. Natl. Acad. Sci. USA 83: 6156-6158 (1986).
25. Lazarow, P.B. and Fujiki, Y. Ann. Rev. Cell Biol. 1: 489-530 (1985).
26. Borst, P. Biochim. Biophys. Acta 66: 179-203 (1986).
27. Fujiki, Y., Hubbard, A.L., Fowler, S. and Lazarow, P.B. J. Cell. Biol. 93: 97-102(1982).