The Diabetes-associated 3243 Mutation in the Mitochondrial tRNALeu(UUR) Gene Causes Severe Mitochondrial Dysfunction without a Strong Decrease in Protein Synthesis Rate*

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**Cells harboring patient-derived mitochondria with an A-to-G transition at nucleotide position 3243 of their mitochondrial DNA display severe loss of respiration when compared with cells containing the wild-type adenine or otherwise identical mitochondrial DNA sequence. The amount and degree of leucylation of tRNALeu(UUR) were both found to be highly reduced in mutant cells. Despite the low level of leucyl-tRNALeu(UUR), the rate of mitochondrial translation was not seriously affected by this mutation. Therefore, decrease of mitochondrial protein synthesis as such does not appear to be a necessary prerequisite for loss of respiration. Rather, the mitochondrially encoded proteins seem subject to elevated degradation, leading to a severe reduction in their steady state levels. Our results favor a scheme in which the 3243 mutation causes loss of respiration through accelerated protein degradation, leading to a disequilibrium between the levels of mitochondrial and nuclear encoded respiratory chain subunits and thereby a reduction of functional respiratory chain complexes. The possible mechanisms underlying the pathogenesis of mitochondrial diabetes is discussed.**

Nucleotide substitutions in the mitochondrial tRNA genes are important factors in the pathogenesis of several multisystem disorders including mitochondrial encephalomyopathies and maternally inherited diabetes and deafness (MIDD)1 (1–6). A point mutation in the structural gene for a tRNA may be expected to influence protein synthesis either quantitatively or qualitatively. A strong reduction in mitochondrial protein synthesis, in particular in the proteins with a high lysine content, has been reported in the case of the 8344 mutation in the tRNA\(^{\text{tRNALys}}\) gene of myoclonus epilepsy with ragged red fibers (MERRF). This reduction is thought to be caused by the premature termination of mitochondrial translation on lysine codons and the subsequent formation of truncated polypeptides (7). Mitochondrial protein synthesis has also been found decreased in the case of the 3243 mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) (8, 9). Therefore, the general view has emerged that mutations in tRNA genes cause mitochondrial dysfunction by lowering mitochondrial protein synthesis and, as a consequence, the amount of mitochondrial encoded respiratory chain enzymes. Indeed, mitochondrial respiration is severely reduced in these cases (7–9). However, the large variation in clinical phenotypes that associate with tRNA mutations is difficult to explain solely by the lowered respiration rate.

MIDD accounts for 1–2% of the diabetic population in Europe and Japan and is clinically characterized by an early middle age onset of diabetes and sensorineural hearing loss, progressive insulin secretory defect, absence of islet cell antibodies, and absence of obesity (10, 11). As a first step in understanding the molecular mechanisms caused by the 3243 mutation, we have established mitochondrial transformants (cybrids) carrying either the wild-type adenine or the mutant-type guanine at position 3243 but having otherwise identical mitochondrial DNA sequences (12). Care was taken to ensure that all cell lines contained high and comparable mitochondrial DNA copy numbers. The mutant-type cells displayed a severe reduction of cellular respiration and respiratory chain activity, which has provided proof for the pathogenicity of the 3243 mutation in MIDD (12). In the present work, the analysis of these cybrids has been extended toward mitochondrial tRNA and protein metabolism in order to understand better the molecular mechanisms leading from the 3243 mutation to impaired respiration and MIDD.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The mitochondrial transformants (cybrids) used in this study had been generated earlier by the transfer of mitochondria from two genetically unrelated diabetes patients (V and A) into mitochondrial DNA-less B°-3 cells (12). Patient V displays the phenotype of Alport-like syndrome, which is characterized by progressive kidney disease, sensorineural hearing loss, and maternal inheritance (13). Diabetes also develops after transplantation and immunosuppressive therapy with steroids but is overshadowed by the kidney disease. Therefore, MIDD and Alport-like syndrome have in common maternal inheritance, sensorineural deafness, and diabetes, with renal failure as a prominent characteristic for Alport-like syndrome. From both donors we obtained fully wild-type cybrids (W6 and W7 from patient V; W20 from patient A) as well as mutant-type cybrids (M48 and M50 from patient V; M12, M26, and M30 from patient A) (12). Cybrids, 143B osteosarcoma cells and B°-3 cells were all cultured in Dulbecco’s modified Eagle’s medium containing 4.5 mg/ml glucose and 110 μg/ml uridine and 10% fetal bovine serum (complete DMEM). For long-term culturing (maximum ~3 months), the cybrids were cultured in DMEM supplemented with 10% dialysed fetal calf serum to preserve a high level of mitochondrial DNA. The mitochondrial DNA copy numbers of the cybrids used were all comparable with that of 143B cells. Cybrid cells were never cultured.
for longer than about 3 months. After this period a fresh vial of cells from the stock, stored in liquid nitrogen, was used.

**Oxygen Consumption**—The rate of oxygen consumption by \(5 \times 10^6\) exponentially growing cells in 2 ml of complete DMEM but without glucose was measured at 37°C using a Clark-type electrode as described (14).

**Northern Blot Analysis**—Total RNA (20 \(\mu\)g), isolated from exponentially growing cells by the guanidinium thiocyanate method (15), was subjected to electrophoresis through a 1% agarose-formaldehyde gel, transferred onto Hybond N\(^{+}\) membranes and hybridized overnight at 42°C with \(^{32}P\)-labeled probes specific for the tRNA\(^{Leu(UUR)}\) gene (nucleotides 3245–3861) or the tRNA\(^{Val}\) gene (nucleotides 249–706), and washed at several changes of 1× SSC, 0.5× SDS at 42°C (16). tRNA bands were visualized by autoradiography and quantified using PhosphorImager detection and ImageQuant analysis.

The degree of *in vitro* aminoacylation of tRNAs was determined by high-resolution electrophoresis and subsequent Northern analysis as described (17). To prevent decay of tRNA during isolation, RNA was isolated under acidic conditions by the guanidinium thiocyanate method, immediately washed twice with 5 mM Tris-HCl, pH 7.5, and was then suspended in SDS-polyacrylamide gel electrophoresis followed by autoradiography.

**Mitochondrial Protein Synthesis and Degradation**—[\(^{35}\)S]Methionine incorporation into mitochondrially encoded proteins was analyzed on SDS-polyacrylamide gels essentially as described (18). In brief, \(5 \times 10^6\) exponentially growing cells in a 3.4-cm dish were washed twice with methionine-free DMEM and incubated for 30 min at 37°C with Promix (a 2:1 mixture of [\(^{35}\)S]methionine and [\(^{35}\)S]cysteine from Amersham Pharmacia Biotech; 10 \(\mu\)Ci/\(\mu\)l, 1,000 Ci/mmol) in 0.75 ml of methionine-free DMEM supplemented with 10% dialysed fetal bovine serum and emetine (100 \(\mu\)g/ml), which was added 15 min before the addition of label. The cells were washed three times with phosphate-buffered saline and finally dissolved in 100 \(\mu\)l of SDS sample buffer. Samples (40 \(\mu\)g) were analyzed on 15% SDS-polyacrylamide gels. Mitochondrial translation products were assigned according to Chomyn et al. (18). Protein concentration was determined by the bicinchoninic acid assay (Pierce).

**Western Blotting and Immunoprecipitation**—Western blotting and Immunoprecipitation—Western blotting was performed essentially as described (21). In brief, total cellular protein was separated on a 15% SDS gel and transferred to a polyvinylidene difluoride membrane (Immobilon P, Millipore). Subsequently, the proteins were specifically visualized using monoclonal antibodies against cytochrome c oxidase subunits I, II, and IV (Molecular Probes) and enhanced chemiluminescence (Amersham Pharmacia Biotech).

**Steady State Level of tRNA**—Northern blot analysis revealed that the level of tRNA\(^{Leu(UUR)}\) in the mutant-type cells was reduced to \(\approx 25\%\) of the level found in wild-type cybrids or the parental 143B cells, whereas the amount of tRNA\(^{Phe}\) remained constant within this set of cybrids (Fig. 2A). The relative ratio of tRNA\(^{Leu(UUR)}\) to tRNA\(^{Phe}\) was estimated to be 0.19 \pm 0.05 for the mutant-type and 0.80 \pm 0.12 for the wild-type cybrids. Furthermore, there was no change observed in the level of mature ND1, 12S, and 16S rRNA transcripts. tRNA\(^{19}\) however, which represents an unspliced transcript encompassing 16S rRNA, tRNA\(^{Leu(UUR)}\), and the ND1 mRNA, was increased in the mutant-type cells (not shown) comparable with the results previously described for the MELAS 3243 cybrids (9).

**Aminoacylation of tRNA**—To determine whether the 3243 mutation also affects the degree of aminoacylation of tRNA, we examined the ratio of mitochondrial leucyl-tRNA\(^{Leu(UUR)}\) available for mitochondrial protein synthesis in the mutant-type cybrids. RNA was isolated under acidic conditions, washed, and counted as described (19).

**Oxygen Consumption**—Stable mitochondrial transformants containing wild-type or mutant-type mitochondrial DNA have been generated and characterized earlier (12). Cybrids were cultured on selective media to obtain and preserve high and comparable mitochondrial DNA copy numbers. Mutant-type cybrids displayed an \(\approx 5\)-fold reduced rate of respiration (Fig. 1) and a 79–98% reduction of individual respiratory chain enzymes (12).

**RESULTS**

**Oxygen Consumption**—The rate of oxygen consumption by \(5 \times 10^6\) exponentially growing wild-type (VIW) or mutant (VM50) cells was monitored in a 2-ml reaction chamber using a Clark-type oxygen electrode. Oxygen consumption is expressed as the percentage of oxygen consumed from the amount originally present in the medium, i.e., \(\approx 0.25\,\text{ml O}_2\). Cells were added at \(t = 5\) min in both cases. A comparatively low respiratory rate was found in the other mutant-type cybrids (12).

**Fig. 1.** Highly reduced respiration rates of mutant-type cybrids. Oxygen consumption of about \(5 \times 10^6\) exponentially growing wild-type (VIW7) or mutant (VM50) cells was monitored in a 2-ml reaction chamber using a Clark-type oxygen electrode. Oxygen consumption is expressed as the percentage of oxygen consumed from the amount originally present in the medium, i.e., \(\approx 0.25\,\text{ml O}_2\). Cells were added at \(t = 5\) min in both cases. A comparatively low respiratory rate was found in the other mutant-type cybrids (12).

**RESULTS**

**Mitochondrial Protein Synthesis**—Because all mitochondrial mRNAs contain a number of UUR codons, it may be expected...
that a low level of leucyl-tRNA<sub>Leu(UUR)</sub> would reduce the rate of mitochondrial translation, at least when leucyl-tRNA<sub>Leu(UUR)</sub> is a limiting factor for mitochondrial protein synthesis. Therefore, we estimated the rate of mitochondrial protein synthesis by measuring the incorporation of [35S]methionine into protein in the presence of the cytoplasmic protein synthesis inhibitor, emetine. Unexpectedly, we did not observe a severe decrease in the rate of [35S]methionine incorporation into mitochondrially encoded proteins (Fig. 3, left panel). Rather, the overall rate of mitochondrial protein synthesis of most of the mutant-type cybrids (4 of 5) was very comparable with that of the wild-type cybrids. The MERRF cybrid, which was included as a control, did show a sharp decrease in mitochondrial translation (Fig. 3, right panel) (7, 18). We did not find in our cybrids a correlation between labeling intensity of a particular protein and the number of UUR codons in its respective mRNA (cf. Ref. 7). Remarkably, the synthesis of ATPase subunits, especially ATPase 8, was strongly enhanced in mutant-type cybrids (Fig. 3, left panel). Furthermore, mutant cells displayed four additional, but weakly [35S]labeled protein bands (indicated by arrowheads in Fig. 3, left panel), which may represent degradation products of the mutant mitochondrial proteins. The cybrids originating from the MIDD patient were indistinguishable from that of the patient with Alport-like syndrome, with the exception of M12, which showed a ~50% reduction of protein synthesis rate.

Because the rate of translation does not seem to be seriously diminished in most cybrids, the 3243 mutation may possibly affect the accuracy of mitochondrial protein synthesis by inducing the incorporation of amino acids other than leucine at UUR codons. Therefore, we measured the rate of [3H]leucine incorporation into mitochondrial protein as a first estimate of the degree of misincorporation at UUR codons of the mitochondrial mRNAs. [3H]Leucine incorporation was measured at various cell densities because we have noticed that the rate of mitochondrial protein synthesis depends on cell density, probably reflecting a gradual shutdown of protein synthesis as soon as the cells become more confluent. Because the mitochondrial genome contains two leucyl-tRNA genes with UUR as the minor codon for leucine (89 UUR codons in Fig. 3, left panel), which may represent degradation products of the mutant mitochondrial proteins. The cybrids originating from the MIDD patient were indistinguishable from that of the patient with Alport-like syndrome, with the exception of M12, which showed a ~50% reduction of protein synthesis rate.

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FIG. 4. [3H]Leucine incorporation into wild-type versus mutant-type cybrids. [3H]Leucine incorporation into trichloroacetic acid-precipitable material appears to be highly dependent on cell density. Density is expressed as μg of whole-cell protein/cm² of tissue culture area available and incorporation as cpm/μg of whole-cell protein; 30 μg/cm² corresponds to ~50% confluency. The data obtained for wild-type cybrids (●) and mutant-type cybrids (○), respectively, were fit to a straight line by using a least-squares fitting procedure. Linear regression analysis of these data revealed that mutant-type cybrids incorporated significantly (p < 0.01) less [3H]Leu than wild-type cybrids.

FIG. 5. Enhanced degradation in mutant-type cybrids. The decay rate constant, λ, of individual mitochondrially encoded proteins was estimated from pulse-chase experiments as detailed under “Experimental Procedures.” Because measurements are based on equal protein load, the degradation rate is somewhat underestimated in the slower growing mutant cybrids. Well resolved and strongly labeled protein bands were selected, except for cytochrome c oxidase subunit II (COII), which represents rather the sum of COII and ATPase subunit 6 (A6) degradation. Note that the stability of the selected proteins is remarkably comparable, with half-lives estimated to be ~50 h. Open bars, wild-type; filled bars, mutant-type cybrids. Results are expressed as means ± S.D. When treated as one group, the mutant-type proteins were found to degrade significantly (p < 0.01) faster than wild-type proteins. When treated separately, only the differences of COII and A8 reached statistical significance (p < 0.05). A similar loss of stability of mutant-type cytochrome c oxidase subunit I was also observable after its immunoprecipitation from M50 versus W7 extracts, respectively. NDII, NADH dehydrogenase subunit II.

**DISCUSSION**

The total gene content of the mitochondrion, 2 rRNA, 22 tRNA, and 13 protein-coding genes, is completely involved in the synthesis of components for oxidative phosphorylation. Therefore, any mutation in mitochondrial DNA may be expected to affect mitochondrial respiration in the first instance. Indeed, in the case of most pathogenic mitochondrial DNA mutations, respiration is reduced in near-homoplasmic mutant cells, independent of the nature of mitochondrial DNA mutation. The clinical phenotype related to these mutations, however, varies strongly. For instance, MERRF, which is caused by a mutation in the tRNA^{Leu} gene, is a severe neuromuscular disease with a low life expectancy, whereas MIDD with the tRNA^{Leu} mutation presents as a milder disorder. Furthermore, the same mitochondrial DNA mutation can even produce distinct clinical phenotypes in different individuals (6). The latter is especially evident for the 3243 mutation in the tRNA^{Leu} gene, which may lead to MIDD, Alport-like syndrome, MELAS, or chronic progressive external ophthalmoplegia (12). This difference in pathogenesis may best be explained by assuming the involvement of additional nuclear or mitochondrial factors that determine the spatial and temporal distribution of the 3243 mutation over the different tissues and thereby determine the organ most affected. Because tRNA also seems to be involved in processes other than translation (23), it cannot be ruled out that the mutated tRNA gene product exerts its effect instead via other processes.

We have generated distinct clonal cell lines carrying the 3243 mutation from patients with MIDD and Alport-like syndrome in a near-homoplasmic form. The mitochondrial genome from the patient with MIDD has been extensively sequenced with the only heteroplasmic nucleotide found at position 3243 (3). Therefore, it may be assumed that upon mitochondria-mediated transformation the only difference between wild-type and mutant-type cybrids is the A-to-G transition at position 3243. Contrary to reports on cybrids from MERRF and MELAS patients (7, 8, 18, 24), our mutant-type cybrids show a serious deficiency in cellular respiration without a strong decrease in the rate of protein synthesis. The same applies for our mutant-type cybrids derived from Alport-like syndrome and also for some of the 3243 cybrids isolated in Dr. H. Jacobs’ laboratory (University of Tampere). In this context it should be mentioned that another panel of cybrids with low copy numbers of mitochondrial DNA often displayed a serious decrease of mitochondrial protein synthesis (25). Therefore, we took great care to ensure that all cell lines contained a high and comparable amount of mitochondrial DNA (12). Because we have generated cybrids with severe mitochondrial dysfunction without a strong decrease in protein synthesis rate, it is evident that a decrease in mitochondrial protein synthesis is not a necessary prerequisite for mitochondrial dysfunction. Therefore, our findings call into doubt the belief that a decrease in mitochondrial protein synthesis is a necessary prerequisite for loss of respiration and induction of pathogenesis. Our data suggest rather that the 3243 mutation reduces the stability of the mitochondrial

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2 H. Jacobs, personal communication.
encoded proteins, which may be due to misincorporated amino acids at UUR codons of the mitochondrial mRNAs (Fig. 4). Interestingly, a similar mechanism has been proposed to occur in SV40 transformed B-cells containing ~70% 3243 mutant mitochondrial DNA (26). Finally, it is important to mention that our cybrids did not contain a suppressor mutation in the anti-codon of the tRNA_{Leu(UUR)} gene as found recently by Jacobs and co-workers (27).

The low level of tRNA_{Leu(UUR)} itself may be explained by enhanced susceptibility for degradation, due to a result of decreased affinity toward its natural partners, leucyl-tRNA synthetase, EF-Tu, or the ribosome. In this context, it has been postulated that a tRNA is never free but is always bound to one of these three partners (28). On the other hand, because RNA19 is elevated, it cannot be ruled out that the synthesis of the mutated tRNA_{Leu(UUR)} is impaired at the processing level (29). It has been suggested in MERRF disease that a relative level of ~40% Lys-tRNALys is sufficiently low to reduce the synthesis of mitochondrial proteins in MERRF cybrids (7). However, with a level of ~15% Leu-tRNA_{Leu(UUR)}, we did not find such dramatic behavior, even though the absolute levels of tRNA_{Leu(UUR)} and tRNA_{Leu} are quite comparable in wild-type cells (30). How should the low level of leucyl-tRNA_{Leu(UUR)} be reconciled with the high rate of protein synthesis? With the assumption of an elongation rate of 20 amino acids/ribosome (31), which corresponds to an average transit time of 50 ms at each codon, the synthesis of a complete protein of 500 amino acids will require 25 s (500 × 50 ms). The ~6-fold decrease in leucyl-tRNA_{Leu(UUR)} may diminish the rate of elongation at UUR at most by a factor of 6 but probably by less (32). Thus, the transit time at UUR will rise to 300 ms at the most. Theoretically, the time to synthesize the complete protein, containing on average 10 UUR codons, would be elevated to (490 × 50 ms) + (10 × 300 ms) = 27.5 s, which corresponds to a 10% reduction of the rate of protein synthesis at the most.

The 3243 mutation induces enhanced degradation of mitochondrial DNA-encoded proteins (Figs. 5 and 6). Although we have not directly shown that the primary sequence of these proteins is different at the UUR-encoded leucine residues in mutant-type cells, two independently obtained results both demonstrate the decreased stability of these proteins, which at least strongly suggests an alteration of their primary structure.

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REFERENCES
1. Goto, Y., Nonaka, I., and Horai, S. (1990) Nature 348, 651–653
2. Shoffner, J. M., Lott, M. T., Lezza, A. M., Seibel, P., Ballinger, S. W., and Wallace, D. C. (1996) Cell 86, 931–937
3. Van den Ouweland, J. M. W., Lemkes, H. H. P. J., Ruitenbeek, W., Sandkuijl, L. A., De Vilder, M. P., Straujsenberg, P. A. A., van de Kemp, J. J. P., and Maassen, J. A. (1992) Nat. Genet. 1, 368–371
4. Wallace, D. C. (1992) Annu. Rev. Biochem. 61, 1175–1212
5. Larsson, N. G., and Clayton, D. A. (1995) Annu. Rev. Genet. 29, 151–178
6. Grossman, L. I., and Shoulbridge, E. A. (1996) Bioessays 18, 983–991
7. Enriquez, J. A., Chomyn, A., and Attardi, G. (1995) Nat. Genet. 10, 47–55
8. Chomyn, A., Martinuzzi, A., Yoneda, M., Daga, A., Hurko, O., Johns, D., Lai, S. C., Nonaka, I., Angelini, C., and Attardi, G. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4221–4225
9. King, M. P., Kops, Y., Davidson, M., and Schon, E. A. (1992) Mol. Cell. Biol. 12, 490–490
10. Kadowaki, T., Kadowaki, H., Mori, Y., Tobe, K., Sakuta, R., Suzuki, Y., Tanabe, Y., Sakura, H., Awata, T., and Goto, Y. (1994) N. Engl. J. Med. 330, 962–968
11. Maassen, J. A., and Kadowaki, T. (1996) Diabetologia 39, 375–382
12. van den Ouweland, J. M. W., Maechler, P., Wolheim, C. B., Attardi, G., and Maassen, J. A. (1999) Diabetologia 42, 485–492
13. Jansen, J. J., Maassen, J. A., vanant, F. J. M., Lemmink, H. A., van den Ouweland, J. M., ‘t Hart, L. M., Screet, H. J., Bruijn, J. A., and Lemkes, H. H. (1997) J. Am. Soc. Nephrol. 8, 1118–1124
14. King, M. P., and Attardi, G. (1989) Science 246, 500–503
15. Chomynska, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
16. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Varshney, U., Lee, C.-P., and RajBhandary, U. L. (1991) J. Biol. Chem. 266, 24712–24718
18. Chomyn, A., Meola, G., Bredolin, N., Lai, S. T., Scarlato, G., and Attardi, G. (1991) Mol. Cell. Biol. 11, 2236–2244
19. Jansen, G. M. C., Maassen, J. A., and Moeller, W. (1990) Ribosomes and Protein Synthesis (Spedding, G., ed) pp. 51–68, Oxford University Press, New York
20. Chomyn, A. (1996) Methods Enzymol. 264, 197–211
21. Townsh, H., Starch, P., and Garden, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4550–4554
22. Kessler, S. W. (1981) Methods Enzymol. 73, 442–459
23. Lanker, S., Bishan, J. L., Hinnebush, A. G., Trachsel, H., and Mueller, P. P. (1990) Cell 70, 647–657
24. Enriquez, J. A., and Attardi, G. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8300–8305
25. van den Ouweland, J. M. W. (1994) A new Subtype of Non-insulin-dependent Diabetes Mellitus Is Associated with a Mitochondrial Gene Mutation. Ph. thesis, Leiden University
26. Fler, A., Reichmann, H., and Seibel, P. (1997) J. Biol. Chem. 272, 10228–10237
27. El Meziane, A., Lethinen, S. K., Hance, N., Nijtmans, L. G. J., Dunbar, D., Holt, I. J., and Jacobs, H. T. (1998) Nat. Genet. 18, 350–353
28. Negrutskii, B. S., and Deutscher, M. P. (1991) FEBS Lett. 278, 273–2736
29. Rossomnith, W., and Karwan, M. R. (1998) FEBS Lett. 443, 269–274
30. King, M. P., and Attardi, G. (1993) J. Biol. Chem. 268, 10228–10237
31. Kennel, D., and Riemman, H. (1977) J. Biol. Chem. 252, 1–11
32. Ogilvie, A., Hescha, U., and Kersten, W. (1979) Biochim. Biophys. Acta 565, 293–304