INTRACELLULAR TURNOVER OF MUTANT ENZYMES IN LYSOSOMAL STORAGE DISEASES

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Molecular analysis of genetic metabolic diseases has provided fruitful information about mutant genes and their products in somatic cells. In conventional biochemical analysis, the cells are disrupted mechanically or chemically, and a certain constituent or ingredient is extracted, purified and analyzed for structure and function in physiological or pathological state. However, this approach is valid only for the sum of the dynamic intracellular events at a given moment, and the fate of a specific molecule in the cell is not revealed.

A protein is subjected to various molecular modifications, and transported to exert its function at a predetermined organelle or site. We have been focusing our attention to lysosomes and lysosomal enzymes as a model system to analyze posttranslational changes of normal or mutant protein molecules. The sequence of events occurring intracellularly after the biosynthesis of lysosomal enzymes is listed in Table 1. This paper deals briefly with some recent results of molecular analysis in my laboratory, mainly for mutant proteins in the cells from lysosomal disease patients

ANALYSIS OF MUTANT PROTEINS IN FIBROBLASTS

For analysis of biosynthesis and processing of lysosomal enzymes, the protein in question was labeled with a radioactive isotope, precipitated with a specific antiserum, separated by electrophoresis, and visualized by fluorography (pulse-chase analysis) (1, 8). It has been particularly useful to analyze dynamic aspects of molecular events involving newly synthesized proteins.

Fibroblasts were cultured and pulse-labeled with ^3H -leucine in a leucine-free culture medium for a few hr, and the culture was further continued in the same

medium without radioactivity (chase). In our laboratory, a few lysosomal enzymes have been studied for posttranslational modification and maturation: β -galactosidase, α -glucosidase and protective protein/carboxypeptidase. Molecular mechanisms of α -glucosidase transport and targeting to lysosomes were also analyzed by this method,

β -Galactosidase activity is partially or completely lost under various molecular and environmental conditions. Two genetically different enzyme molecules have been separated and characterized: G_{M1} β -galactosidase encoded by a gene on chromosome 3, and galactosylceramide β -galactosidase encoded by another gene on chromosome 14. However, little information is now available for the latter. G_{M1} β -galactosidase has been the major target of our recent biochemical and genetic research. It is known to interact with another functional protein (protective protein/carboxypeptidase) to form a high-molecular-weight aggregate in lysosomes. We prepared an antiserum against the enzyme molecule and another against the complex, and started analysis of intracellular turnover of these two proteins (3-5). Then, we cloned cDNAs coding for human G_{M1} β -galactosidase (6) and protective protein (9), and subsequently started mutation analysis of human diseases with β -galactosidase deficiency.

In normal fibroblasts, an 84-kDa precursor was processed first to an 88-kDa intermediate form and then to a 64-kDa mature form (3). The intermediate form was secreted into the culture medium. All G_{M1} -

TABLE 1. *Intracellular turnover of lysosomal enzymes*

Transcription
Translation
Posttranslational modification
Translocation
Activation/Stabilization
Degradation

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gangliosidosis cell strains synthesized the precursor, but no further processing was observed (4). One of them showed a precursor of abnormal molecular mass (86 kDa), which corresponded to a partial duplication of the gene observed in a subsequent study (13). Enzyme deficiency in this disease occurred probably as a result of abnormal protein biosynthesis that was followed by rapid degradation of mutant molecules in infantile G_{M1} -gangliosidosis.

An inherited defect of protective protein is expressed clinically as a disease galactosialidosis that is deficient in two lysosomal enzymes, β -galactosidase and neuraminidase. This strange double enzyme deficiency is caused by defect of protective protein that activates these two enzymes intracellularly. We reported esterase/deamidase deficiency at neutral pH and carboxypeptidase deficiency at acidic pH. This protein has stabilization/activation function toward lysosomal enzyme proteins as well as catalytic activities toward other compounds as a multifunctional enzyme. The site or mechanism of interaction between protective protein and β -galactosidase/neuraminidase is not clear as yet.

INTRACELLULAR TRANSPORT OF NORMAL AND MUTANT PROTEINS

Some lysosomal enzymes are transported by way of the mannose 6-phosphate receptor system, but some others are not. This pathway is not effective for I-cell disease because of defect in phosphorylation in the carbohydrate moiety of the enzyme molecule. Activities of lysosomal enzymes are therefore reduced to various degrees (Table 2). This variation probably reflects the presence of not less than two different transport

mechanisms. We analyzed α -glucosidase and β -hexosaminidase in this disease (10, 11).

About 70% of the α -glucosidase activity is preserved in fibroblasts, and mixed leukocytes preserved their activity. Although β -hexosaminidase activity was almost completely lost in heavy lysosomes as determined by Percoll density gradient centrifugation, α -glucosidase was transported there almost normally in I-cell disease fibroblasts. Furthermore, β -hexosaminidase activity was localized in heavy lysosomes in transformed lymphoblasts in spite of defective phosphorylation of mannose residue in the enzyme molecule. At present we do not know the molecular mechanism of the alternative transport system in I-cell disease.

MUTANT GENE AND ITS EXPRESSION PRODUCT

We recently identified numerous mutations in four types of primary β -galactosidase deficiency (β -galactosidosis); infantile G_{M1} -gangliosidosis, late infantile/juvenile G_{M1} -gangliosidosis, adult/chronic G_{M1} -gangliosidosis and Morquio B disease (7, 13). They express phenotypic manifestations of different severity and pathological distribution. Gene expression studies revealed almost complete loss of enzyme activity except for specific and common mutations in three of them; mutation A (I51T) in adult/chronic G_{M1} -gangliosidosis, mutation B (R201C) in late infantile/juvenile G_{M1} -gangliosidosis and mutation F (W273L) in Morquio B disease. Each of them was expressed in a fibroblast strain completely deficient in enzyme activity, and the processing and intracellular localization were studied. Mutant enzymes disappeared at the early stage of processing with defect in phosphorylation, degraded rapidly in lysosomes due to defect in aggregate formation with protective protein, or changed substrate specificity (Oshima, A. *et al.*, unpublished data; Table 3). These specific abnormalities in molecular modification and/or translocation may be clearly associated with phenotypic expressions. Histochemical or electron microscopic investigations have not been performed on these molecules as yet. Morphological approaches will reveal more details of pathological molecular mechanisms in the future.

We have been trying another approach for analysis of mutant proteins using various expression systems in culture. The baculovirus system was useful for producing a large amount of gene products. However, in an overexpression experiment of human β -galactosidase, the distribution of the enzyme molecule between cells and culture medium was found

TABLE 2. *Lysosomal enzyme activities in I-cell disease fibroblasts*

Relative activity	Enzyme
0-5 %	β -galactosidase α -fucosidase arylsulfatase A
10-30%	α -mannosidase α -galactosidase α -iduronidase β -hexosaminidase β -glucuronidase
50-80%	α -glucosidase
100%	β -glucosidase acid phosphatase

TABLE 3. Gene mutations and abnormalities in mutant proteins in human β -galactosidase deficiency

Phenotype ^{a)}	Genotype ^{b)}		Enzyme		Substrate Storage ^{e)}
			Turnover ^{c)}	Activity ^{d)}	
I. Neurosomatic					
Generalized	G _{M1} /inf	Hetero	Hetero	—	G _{M1} , OS>KS
II. Neuroskeletal					
Generalized CNS	G _{M1} /juv	R201C	Aggregation	+	G _{M1} >OS>KS
Localized CNS	G _{M1} /adult	I51T	Processing	+	G _{M1} >OS>KS
III. Skeletal					
Generalized	Morquio B	W273L	Normal	+	KS, OS>G _{M1}

a) CNS: central nervous system, G_{M1}: G_{M1}-gangliosidosis, inf: infantile form, juv: late infantile/juvenile form, adult: adult/chronic form.

b) Hetero: heterogeneous mutations. Each of the R201C and I51T mutations is common to a specific clinical form of β -galactosidase deficiency in Japanese patients.

c) Hetero: heterogeneous. Aggregation: defect of aggregation with protective protein, resulting in abnormal breakdown of the enzyme molecule. Processing: phosphorylation defect probably due to abnormal glycosylation. Normal: normal translocation and turnover, with abnormal substrate specificity.

d) —: not detectable, +: detectable.

e) Amount of tissue storage or urinary excretion. G_{M1}: ganglioside G_{M1}, OS: oligosaccharides, KS: keratan sulfate.

to be different from that for human fibroblasts (2). Glycosylation seems to be slightly different although it occurs in this insect cell culture system. We have to take into consideration the cell species for evaluation of normal and mutant molecules in the research of intracellular events using culture cells.

Molecular analysis is in itself a useful technique to evaluate the intracellular events but there is a limitation for information about transport or localization of gene products. Histochemical and electron microscopic approaches will overcome this disadvantage of analytical procedures.

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