requires more than one enzyme. Therefore, the step of the putative "Glycogen Initiatior" proposed by us before, requires at least two enzymes UDPGlc-transglucosylating activities.

S1.11

Identification and Developmentally Regulated Expression of Acid and Alkaline Peptide-N4 (*N*-acetyl-β-glucosaminyl)asparagine amidases (PNGase) in *Oryzias latipes* Embryos: The First Demonstration of the Occurrence of an Enzyme Responsible for De-*N*-Glycosylation in Animal Origin

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No report for PNGase from animal source had been found until we identified its presence in the early embryos of *Oryzias latipes* (1), though PNGase activity has been detected in the extracts of a variety of plant seeds and bacteria. Our previous findings of the accumulation of free *N*-glycan chains that retain di-*N*-acetylchitobiosyl structure at the reducing termini at the late stage of oogenesis (2) and blastulation stage of embryogenesis (3) have strongly supported the supposition that PNGase activity is expressed also in animal cells and its expression may be significant for the metabolism and function of certain glycoproteins. Two types of glycoproteins, phosvitin and hyosophorin, have been identified as the physiological substrates of the PNGases. Our current interest is to find if PNGase activity appears stage-specifically during oogenesis and/or embryogenesis, and to clarify the significance of de-*N*-glycosylation of phosvitin and hyosophorin in the fish embryos.

The carbohydrate units on glycoproteins may serve as recognition sites, regulate conformation of the molecule, and control proteolytic degradation. De-*N*-glycosylation of a glycopeptide or glycoprotein, which converts the carbohydrate-linked Asn residue to the Asp thereby introducing negative charge and alterating the peptide or protein conformation, may be a possible means to produce ***a functional conformation***. Our preliminary results showed that during *O. latipes* embryogenesis the PNGase activity appeared to rise rather progressively to a maximum at the late blastula stage, followed a decay. We detected two different PNGase activities in *O. latipes* early embryos, one is acid PNGase and the other alkaline PNGase.

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S1.12

Demonstration of the Presence of Peptide: *N*-Glycanase Activities in Mammalian-Derived Cultured Cells. A Possible Occurrence of *N*-Glycosylation/De-*N*-Glycosylation System in a Wide Variety of Living Organisms as the Universal Biologic Processes

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Recently, we found in fish embryos and oocytes the accumulation of free glycan chains with di-*N*-acetylchitobiose structure (GlcNAcβ1→4GlcNAc) at their reducing termini (1–3). Based on these findings, we predicted the presence of the enzyme (peptide:*N*-glycanase; PNGase) responsible for the detachment of free glycans from glycoproteins bearing *N*-linked glycan units and hypothesized "*N*-glycosylation/de-*N*-glycosylation system" as possible post-translational modification/remodification processes in eukaryotic cells. More recently, we found and partially purified PNGase from Medaka embryos (4) and this represents the first demonstration of the occurrence of PNGase in animal tissues.

We screened several mouse cultured cell lines and also some organs and tissues of BALB/c mouse to ascertain the universal occurrence of PNGase by using ^{14}C-labeled fetuin glycopeptide as a substrate. Current data indicate that the cultured cell lines including the C3H mouse fibroblast L-929 cells and BALB/c mouse myeloma P3Ul cells as well as BALB/c mouse organs and tissues examined (liver, kidney, brain, and spleen) were all found to express PNGase activity. In experiments designed to establish the nature of PNGase in the mammalian system, we have analyzed the reaction products, both the free glycan liberated and the peptide, formed on treatment of the fetuin glycopeptide with the soluble enzyme preparation obtained from L-929 cell lysate. The PNGase found in L-929 cells revealed its optimal pH at 6.5–6.8, suggesting that this enzyme is by no means a lysosomal enzyme. Although more experiments are necessary to elucidate the exact role of PNGase in cellular processes, the accumulated circumstantial evidence is irrefutable for a hypothesis of *N*-glycosylation/de-*N*-glycosylation system involving PNGase and one important conclusion that can be drawn from our experiments is that PNGase can no longer be the enzyme occurring only in plant kingdom and bacteria.

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S1.13

N-Acetylgalactosaminylation of Bovine Mammary Gland Epithelial Cell Glycoproteins

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N-Acetylgalactosamine is usually not a constitutive monosaccharide of asparagine-linked sugar chains. To date only a small number of glycoproteins has been shown to contain the sugar chains with the monosaccharide residue as the GalNAcβ1→4GlcNAc group. Our previous study showed that