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ダイジェスト

JB Review

Featured article of the month. Ultrastructural diversity between centrioles of eukaryotes

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Several decades of centriole research have revealed the beautiful symmetry present in these microtubule-based organelles, which are required to form centrosomes, cilia and flagella in many eukaryotes. Centriole architecture is largely conserved across most organisms; however, individual centriolar features such as the central cartwheel or microtubule walls exhibit considerable variability when examined with finer resolution. In this paper, we review the ultrastructural characteristics of centrioles in commonly studied organisms, highlighting the subtle and not-so-subtle differences between specific structural components of these centrioles. In addition, we survey some non-canonical centriole structures that have been discovered in various species, from the coaxial bicentrioles of protists and lower land plants to the giant irregular centrioles of the fungus gnat Sciara. Finally, we speculate on the functional significance of these differences between centrioles, and the contribution of individual structural elements such as the cartwheel or microtubules towards the stability of centrioles.

Keywords: centriole structure; cartwheel; SAS-6; triplet microtubules; centrosome.

Featured article of the month. RAB39A: a Rab small GTPase with a prominent role in cancer stemness

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RAB39A is a Rab small GTPase that localizes at distinct subcellular compartments and regulates intracellular membrane trafficking pathways in vertebrate cells. RAB39A interacts with various molecules and modulates vesicular trafficking that regulates multiple biological pathways such as neuronal differentiation and/or autophagy. Among these pathways are Hippo and Notch signallings, microtubular organization and mitophagy/autophagy. Although RAB39A has never been studied in cancer biology, it has been recently shown to promote cancer stemness and tumorigenesis. Molecular pathways regulated by RAB39A are transcriptionally maintained by the formation of molecular complex with RXRB, NCOR and HDAC that also contribute to cancer stemness. In this review, we provide current knowledge on the oncogenic function of RAB39A and summarize the effect of different microenvironments on RAB39A activity and subcellular localization in cancer cells.

Keywords: cancer stemness; membrane trafficking; oncogenic mutation; RAB39A; Rab small GTPase.

BIOCHEMISTRY

Protein Interaction and Recognition

Intramolecular H-bonds govern the recognition of a flexible peptide by an antibody

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Molecular recognition is a fundamental event at the core of essentially every biological process. In particular, intermolecular H-bonds have been recognized as key stabilizing forces in antibodyantigen interactions resulting in exquisite specificity and high affinity. Although equally abundant, the role of intramolecular H-bonds is far less clear and not universally acknowledged. Herein, we have carried out a molecular-level study to dissect the contribution of intramolecular H-bonds in a flexible peptide for the recognition by an antibody. We show that intramolecular H-bonds may have a profound, multifaceted and favorable effect on the binding affinity by up to 2 kcal mol1 of free energy.

Collectively, our results suggest that antibodies are fine tuned to recognize transiently stabilized structures of flexible peptides in solution, for which intramolecular H-bonds play a key role.
Keywords: antibodyantigen interaction; molecular dynamics simulations; molecular recognition; transient folding; unstructured peptide.

Glycobiology and Carbohydrate Biochemistry

Molecular characterization of second tomato a1,3/4-fucosidase (a-Fuc'ase Sl-2), a member of glycosyl hydrolase family 29 active toward the core a1,3-fucosyl residue in plant N-glycans

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In a previous study, we molecular-characterized a tomato (Solanum lycopersicum) a1, 3/4-fucosidase (a-Fuc'ase Sl-1) encoded in a tomato gene (Solyc03 g006980), indicating that a-Fuc'ase Sl-1 is involved in the turnover of Lea epitope-containing N-glycans. In this study, we have characterized another tomato gene (Solyc11 g069010) encoding a1, 3/4-fucosidase (a-Fuc'ase Sl-2), which is also active toward the complex type N-glycans containing Lea epitope(s). The baculovirus-insect cell expression system was used to express that a-Fuc'ase Sl-2 with antiFLAG tag, and the expression product (rFuc'ase Sl-2), was found as a 65 kDa protein using SDS-PAGE and has an optimum pH of around 5.0. Similarly to rFuc'ase Sl-1, rFuc'ase Sl-2 hydrolyzed the non-reducing terminal a1, 3-fucose residue on LNFP III and a1, 4-fucose residues of Lea epitopes on plant complex type N-glycans, but not the core a1, 3-fucose residue on Manb1-4GlcNAcb1–4(Fuca1–3)GlcNAc or Fuca1–3GlcNAc. However, we found that both a-Fuc'ases Sl-1 and Sl-2 were specifically active toward a1, 3-fucose residue on GlcNAcb1–4(Fuca1–3) GlcNAc, indicating that the nonsubstituted b-GlcNAc linked to the proximal GlcNAc residue of the core tri-saccharide moiety of plant specific N-glycans must be a pre-requisite for a-Fuc'ase activity. A 3 D modelled structure of the catalytic sites of a-Fuc' ase Sl-2 suggested that Asp192 and Glu236 may be important for binding to the a1, 3/4 fucose residue.
Keywords: a-fucosidase; a1–3 fucose; N-glycan metabolism; plant N-glycan; Solanum lycopersicum.

Interaction of receptor type of protein tyrosine phosphatase sigma (RPTPp) with a glycosaminoglycan library

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Receptor type of protein tyrosine phosphatase sigma (RPTPp) functions as a glycosaminoglycan (GAG) receptor of neuronal cells in both the central and peripheral nervous systems. Both chondroitin sulphate (CS) and heparan sulphate (HS) are important constituents of GAG ligands for RPTPp, although they have opposite effects on neuronal cells. CS inhibits neurite outgrowth and neural regeneration through RPTPp, whereas HS enhances them. We prepared recombinant RPTPp N-terminal fragment containing the GAG binding site and various types of biotin-conjugated GAG (CS and HS) with chemical modification and chemo-enzymatic synthesis. Then interaction of the RPTPp N-terminal fragment was analysed using GAG-biotin immobilized on streptavidin sensor chips by surface plasmon resonance. Interaction of RPTPp with the CS library was highly correlated to the degree of disulphated disaccharide E unit, which had two sulphate groups at C-4 and C-6 positions of the N-acetylgalactosamine residue (CSE). The optimum molecular mass of CSE was suggested to be approximately 10 kDa. Heparin showed higher affinity to RPTPp than the CS library. Our GAG library will not only contribute to the fields of carbohydrate science and cell biology, but also provide medical application to regulate neural regeneration.
Keywords: chondroitin; glycosaminoglycans; heparin; RPTPs; SPR.

Lipid Biochemistry

Phytosphingosine is a novel activator of GPR120

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GPR120 is a receptor for long chain fatty acids and is expressed in small intestinal endocrine cells, L cells and adipose tissue. Activation of GPR120 promotes the secretion of incretin GLP-1, which is known to have effects on anti-metabolic syndrome. As such, GPR120 is a potential target of pharmaceuticals for type II diabetes. In this study, we performed ligand-screening for

GPR120 on glycero- and sphingo-type lipids and their derivatives using a Transforming Growth Factor ashedding assay. We found that phytosphingosine (PHS) activates GPR120 in a manner comparable to the natural ligand a-linolenic acid (ALA) and superior to that of the synthetic ligand GW9508. The IC50 value of PHS was 33.4 kM, of ALA was 31.0 kM and of GW9508 was 41.7 kM. Additionally, PHS-induced activation of GPR120 was inhibited by the specific antagonist AH7614. Many of the natural or synthetic ligands found thus far are compounds with carboxyl groups. However, PHS does not possess a carboxyl group, suggesting that its manner of interaction with GPR120 may be significantly different from that of other ligands. Since PHS is rich in the plasma membrane of yeast, our results imply that PHS found in fermented food could have effects on anti-diabetes through activation of GPR120.

Keywords: GPCR; GPR120; phytosphingosine; sphingolipid; type II diabetes.

Enzymology

Purification and characterization of 1-acyl-sn-glycerol-3- phosphate acyltransferase with a substrate preference for polyunsaturated fatty acyl donors from the eicosapentaenoic acid-producing bacterium Shewanella livingstonensis Ac10

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1-Acyl-sn-glycerol-3-phosphate acyltransferase (designated as PlsC in bacteria) catalyzes the acylation of lysophosphatidic acid and is responsible for the de novo production of phosphatidic acid, a precursor for the synthesis of various membrane glycerophospholipids. Because PlsC is an integral membrane protein, it is generally difficult to solubilize it without causing its inactivation, which has been hampering its biochemical characterization despite its ubiquitous presence and physiological importance. Most biochemical studies of PlsC have been carried out using crude membrane preparations or intact cells. In this study, we succeeded in solubilization and purification of a recombinant PlsC in its active form from the eicosapentaenoic acid-producing bacterium Shewanella livingstonensis Ac10 using 6-cyclohexyl-1-hexyl-b-D-maltoside as the detergent. We characterized the purified enzyme and found that it has a substrate preference for the acyl donors with a polyunsaturated fatty acyl group, such as eicosapentaenoyl group. These results provide a new method for purification of the PlsC family enzyme and demonstrate the occurrence of a new PlsC with unique substrate specificity.

Keywords: 1-acyl-sn-glycerol-3-phosphate acyltransferase; lipid metabolism; membrane protein; phospholipid; PlsC.

BIOTECHNOLOGY

Biotechnology General

Impact in stability during sequential CDR grafting to construct camelid VHH antibodies against zinc oxide and gold

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Biomolecules which recognize inorganic materials and metal surfaces gain much attention for creating new type of nanomaterials and sensors. 4F2, a camelid VHH antibody, recognizes ZnO surface and has been applied for sensor applications. 4F2 was constructed sequential complementarity determining region (CDR) replacement on the parental VHH antibody, termed the Construction of Antibody by Integrating Grafting and Evolution Technology; CAnIGET procedure. Here, we evaluate the influence of CDR replacements during 4F2 generation using calorimetric technique. We found that the initial peptide grafting at CDR1 results in the stability reduction and subsequent CDR3 randomize and selection restore the stability during the construction of 4F2. Further examination using anti-gold VHH, AuE32, revealed that the final CDR3 randomize and selection step has little effect in stability while the initial CDR1 grafting reduces the stability as same as the case for 4F2. Our results showing here provide the detailed view of the stability alteration during the CAnIGET procedure.

Keywords: CDR grafting; protein stability; thermal denaturation; VHH antibody.

Gene Delivery Systems

Efficient delivery of large DNA from Escherichia coli to Synechococcus elongatus PCC7942 by broad-host-range conjugal plasmid pUB307

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Synechococcus elongatus PCC7942, a cyanobacterium that uses light and carbon dioxide to grow, has a high ability to incorporate DNA by transformation. To assess the effective delivery of large DNA in plasmid form, we cloned the endogenous plasmid pANL (46.4 kbp) into a BAC vector of Escherichia coli. The plasmid p38ANL (54.3 kbp) replaced the native plasmid. To assess the delivery of larger DNA into PCC7942, p38ANL was fused to the broad-host-range conjugal transfer plasmid

pUB307IP (53.5 kbp). The resulting plasmid pUB307IP501 (107.9 kbp) was transmitted from E. coli to PCC7942 by simple mixing of donor and recipient cultures. PCC7942 transcipients possessed only pUB307IP501, replacing the preexisting pANL. In contrast, the pUB307IP501 plasmid was unable to transform PCC7942, indicating that natural transformation of DNA may be restricted by size limitations. The ability to deliver large DNA by conjugation may lead to genetic engineering in PCC7942.
Keywords: conjugal transfer; cyanobacteria; endogenous plasmid; natural genetic transformation; plasmid stability.

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ダイジェスト

JB Review

Featured article of the month. CD169 macrophages regulate immune responses toward particulate materials in the circulating fluid

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Tissue macrophages comprise heterogeneous subsets that differ in localization, phenotype and ontogeny. They acquire tissue-specific phenotype in order to maintain normal tissue physiology. This review summarizes the current knowledge about the functions of CD169− positive macrophage subset residing in the lymphoid organs and intestinal tract. Strategically positioned at the interface between tissue and circulating fluid, CD169+ macrophages in the lymphoid organs capture blood- and lymph-borne particulate materials. Antigen information relayed by CD169+ macrophages to neighbouring immune cells is important for enhancement of antimicrobial and antitumour immunity as well as induction of tolerance. In the intestinal tract, CD169+ macrophages localize distantly from epithelial border. Following mucosal injury, they exacerbate inflammation by producing CCL8 that recruits inflammatory monocytes. As such, a better understanding of CD169+ macrophage phenotypes may enable the design of tissue-specific therapies for both immunological and non-immunological diseases.
Keywords: CCL8; CD169; intestinal inflammation; macrophage; tolerance.

BIOCHEMISTRY
Biochemistry General

A novel structure of exopolysaccharide produced by a plant-derived lactic acid bacterium Lactobacillus paracasei IJH-SONE68

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A lactic acid bacterium Lactobacillus paracasei IJHSONE68, which was isolated from a fig leaf by our group, was found to produce both acidic and neutral exopolysaccharides (EPSs). The nuclear magnetic resonance analysis demonstrates that the former EPS is composed primarily of mannose, and the latter one consists of the a-1, 6-linked glycan chains made of N-acetylglucosamine (GlcNAc). The presence of a-1, 6-linked GlcNAc polysaccharide is first reported in prokaryotes. Furthermore, to reveal the EPS-biosynthetic gene organization in the IJHSONE68 strain, in the present study, we determined the whole-genome sequence.
Keywords: exopolysaccharide; hyaluronidase inhibition; Lactobacillus paracasei; N-acetylglucosamine; whole-genome sequence.

Two J domains ensure high cochaperone activity of DnaJ, Escherichia coli heat shock protein 40

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Heat shock protein 70 (Hsp70) chaperone systems consist of Hsp70, Hsp40 and a nucleotide-exchange factor and function to help unfolded proteins achieve their native conformations. Typical Hsp40s assume a homodimeric structure and have both chaperone and cochaperone activity. The dimeric structure is critical for chaperone function, whereas the relationship between the dimeric structure and cochaperone function is hardly known. Here, we examined whether two intact protomers are required for cochaperone activity of Hsp40 using an Escherichia coli Hsp70 chaperone system consisting of DnaK, DnaJ and GrpE. The expression systems were generated and two heterodimeric DnaJs that included a mutated protomer lacking cochaperone activity were purified. Normal chaperone activity was demonstrated by assessing aggregation prevention activity using urea-denatured luciferase. The heterodimeric DnaJs were investigated for cochaperone activity by measuring DnaK ATPase activity

and the heat-denatured glucose-6-phosphate dehydrogenase refolding activity of the DnaK chaperone system, and they showed reduced cochaperone activity. These results indicate that two intact protomers are required for high cochaperone activity of DnaJ, suggesting that one homodimeric DnaJ molecule promotes the simultaneous binding of multiple DnaK molecules to one substrate molecule, and that this binding mode is required for the efficient folding of denatured proteins.

Keywords: chaperones; DnaJ; DnaK; Hsp40; Hsp70.

Protein Structure

Rim domain loops of staphylococcal b-pore forming bi-component toxin S-components recognize target human erythrocytes in a coordinated manner

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Staphylococcus aureus bi-component pore-forming toxins consist of S- and F-components, and form hetero-octameric beta-barrel pores on target blood cell membranes. Among them, c-haemolysin (Hlg2 and F-component of Luk (LukF)) and LukED (LukE and LukD) possess haemolytic activity, whereas the Panton-Valentine leukocidin (LukS-PV and LukFPV) does not lyse human erythrocytes. Here, we focussed on four loop structures in the rim domain of S-component, namely loops -1, -2, -3 and -4, and found that replacement of Loop-4 in both Hlg2 and LukE with that of LukS-PV abolished their haemolytic activity. Furthermore, LukS-PV gained haemolytic activity by Loop-4 exchange with Hlg2 or LukE, suggesting that Loop-4 of these S-components determined erythrocyte specificity. LOOP-1 and -2 enhanced the erythrocytes-binding ability of both components. Although Hlg2 and LukE recognize Duffy antigen receptor for chemokines on human erythrocytes, the ability of Loop4 was not complementary between Hlg2 and LukE. Exchange of Hlg2 with LukE Loop-4 showed weaker activity than intact Hlg2, and LukE mutant with Hlg2 Loop-4 lost its haemolytic activity in combination of LukD. Interestingly, the haemolytic activities of these Loop-4 exchange mutants were affected by F-component, namely LukF enhanced haemolytic activities of these Hlg2 and LukE Loop-4 mutants, and also haemolytic activity of LukS-PV mutant with LukE Loop-4.

Keywords: protein, bacterial; protein-protein interactions; toxins, drugs, xenobiotics, staphylococcal bicomponent toxin, S-components, CCR5.

Type VI collagen a1 chain polypeptide in non-triple helical form is an alternative gene product of COL6A1

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Expression of type IV collagen a1 chain in non-triple helical form, NTH a1(IV), is observed in cultured human cells, human placenta and rabbit tissues. Biological functions of NTH a1(IV) are most likely to be distinct from type IV collagen, since their biochemical characteristics are quite different. To explore the biological functions of NTH a1(IV), we prepared some antiNTH a1(IV) antibodies. In the course of characterization of these antibodies, one antibody, #141, bound to a polypeptide of 140 kDa in size in addition to NTH a1(IV). In this study, we show evidence that the 140 kDa polypeptide is a novel non-triple helical polypeptide of type VI collagen a1 chain encoded by COL6A1, or NTH a1(VI). Expression of NTH a1(VI) is observed in supernatants of several human cancer cell lines, suggesting that the NTH a1(VI) might be involved in tumourigenesis. Reactivity with lectins indicates that sugar chains of NTH a1(VI) are different from those of the a1(VI) chain in triple helical form of type VI collagen, suggesting a synthetic mechanism and a mode of action of NTH a1(VI) is different from type VI collagen.

Keywords: cancer; non-triple helical a1(IV) chain; non-triple helical a1(VI) chain; type IV collagen; type VI collagen.

Lipid Biochemistry

Synthesis of omega-3 long-chain polyunsaturated fatty acid-rich triacylglycerols in an endemic goby, Gymnogobius isaza, from Lake Biwa, Japan

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It is commonly observed that freshwater fish contain lower amounts of omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), than marine fish species. In this study, we performed a detailed comparative analysis of phospholipids (PLs) and triacylglycerols (TAGs) from Gymnogobius isaza, a freshwater goby endemic to Lake Biwa inhabiting the lake bottom, and Gymnogobius urotaenia, a related goby that inhabits the shore of Lake Biwa. We found that tissues from G. isaza contain remarkably high amounts of omega-3 LC-PUFAs in both PLs and TAGs. Mass spectrometry analysis of TAGs demonstrated that the most abundant TAG molecular species were TAG (16:0/18:1/20:5), followed by TAG (14:0/18:1/20:5), in which EPA is incorporated into TAG at either the sn-1 or sn-3 positions. We isolated cDNAs encoding acyl-CoA: diacylglycerol acyltransferase designated as GiDGAT1 and GiDGAT2, from G. isaza. Expression studies using a neutral lipid-deficient Saccharomyces cerevisiae mutant strain demonstrated that both GiDGAT1 and GiDGAT2 possessed diacylglycerol acyltransferase activity, and preferential incorporation of LC-PUFA into TAG was observed in the presence of GiDGAT1. This study revealed the novel lipid profiles of G. isaza and identified the enzymes that were involved in the production of PUFA-containing TAGs.

Keywords: acyl-CoA:diacylglycerol acyltransferase; eicosapentaenoic acid; freshwater fish; phospholipid; triacylglycerol.

Enzymology

Cloning and characterization of the novel D-aspartyl endopeptidase, paenidase, from Paenibacillus sp. B38

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Paenidase is the first micro-organism-derived Daspartyl endopeptidase that specifically recognizes an internal D-Asp residue to cleave [D-Asp]-X peptide bonds. Using peptide sequences obtained from the protein, we performed PCR with degenerate primers to amplify the paenidase I-encoding gene. Nucleotide sequencing revealed that mature paenidase I consist of 322 amino acid residues and that the protein is encoded as a proprotein with a 197-amino-acid N-terminal extension compared to the mature protein. Paenidase I exhibits amino acid sequence similarity to several penicillin-binding proteins. In addition, paenidase I was classified into peptidase family S12 based on a MEROPS database search. Family S12 contains serine-type D-Ala-D-Ala carboxypeptidases that have three active site residues (Ser, Lys and Tyr) in the conserved motifs Ser-Xaa-Thr-Lys and Tyr-Xaa-Asn. These motifs were conserved in the primary structure of paenidase I, and the role of these residues was confirmed by site-directed mutagenesis.

Keywords: amyloid-beta (Ab); D-aspartic acid; endopeptidase; prokaryote; stereoselectivity.

Orthologues of Streptococcus pyogenes nuclease A (SpnA) and Streptococcal 50 -nucleotidase A (S5nA) found in Streptococcus iniae

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Streptococcus pyogenes nuclease A (SpnA) and streptococcal 50 nucleosidase A (S5nA) are two recently described virulence factors from the human pathogen S. pyogenes. In vitro studies have shown that SpnA is a nuclease that cleaves ssDNA and dsDNA, including the DNA backbone of neutrophil extracellular traps. S5nA was shown to hydrolyse AMP and ADP, but not ATP, to generate the immunomodulatory molecule adenosine. S5nA also generates the macrophage-toxic deoxyadenosine from dAMP. However, detailed in vivo studies of the two enzymes have been hampered by difficulties with using current animal models for this exclusive human pathogen. Here we report the identification of two novel enzymes from the fish pathogen Streptococcus iniae that show similarities to SpnA and S5nA in amino acid sequence, protein domain structure and biochemical properties. We propose that SpnAi and S5nAi are orthologues of the S. pyogenes enzymes, providing a rationale to analyse the in vivo function of the two enzymes using a S. iniae-zebrafish infection model.

Keywords: cell surface-anchored enzyme; enzyme synergy; group A Streptococcus; LPXTG motif; Streptococcus iniae; Streptococcus pyogenes nuclease A (SpnA); Streptococcal 50-nucleotidase A (S5nA); zebrafish infection.

Biochemistry of Proteolysis

Apical-to-basolateral transepithelial transport of cow's milk caseins by intestinal Caco-2 cell monolayers: MS-based quantitation of cellularly degraded a- and b-casein fragments

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Casein (CN) is the major milk protein to nourish infants but, in certain population,it causes cow's milk allergy, indicating the uptake of antigenic CN and their peptides through the intestinal epithelium. Using human intestinal Caco-2 cell monolayers, the apical-to-basal transepithelial transport of CN was investigated. Confocal microscopy using component-specific antibodies showed that as1-CN antigens became detectable as punctate signals at the apical-side cytoplasm and reached to the cytoplasm at a tight-junction level within a few hours. Such intracellular CN signals were more remarkable than those of the other antigens, b-lactoglobulin and ovalbumin, colocalized in part with an early endosome marker protein (EEA1) and decreased in the presence of cytochalasin D or sodium azide and also at lowered temperature at 4C. Liquid chromatography coupled with mass spectroscopy analysis of the protein fraction in the basal-side medium identified the as1-CB fragment including the N-terminal region and the as2-CN fragment containing the central part of polypeptide at 1001,000 fmol per well levels. Moreover, b-CN C-terminal overlapping peptides were identified in the peptide fraction below 10 kDa of the basal medium. These results suggest that CNs are partially degraded by cellular proteases and/or peptidases and immunologically active CN fragments are transported to basal side of the cell monolayers.

Keywords: antigen processing; degradation; lysosomes; mass spectroscopy; polarity.

MOLECULAR BIOLOGY

Molecular Biology General

Consumption of N5 , N10-methylenetetrahydrofolate in Thermus thermophilus under nutrient-poor condition

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TrmFO catalyzes the formation of 5-methyluridine at position 54 in tRNA and uses N5 , N10-methylenetetrahydrofolate (CH-2THF) as the methyl group donor. We found that the trmFO gene-disruptant strain of Thermus thermophilus, an extremely thermophilic eubacterium, can grow faster than the wild-type strain in the synthetic medium at 70C (optimal growth temperature). Nucleoside analysis revealed that the majority of modifications were appropriately introduced into tRNA, showing that the limited nutrients are preferentially consumed in the tRNA modification systems. CH2THF is consumed not only for tRNA methylation by TrmFO but also for dTMP synthesis by ThyX and methionine synthesis by multiple steps including MetF reaction. In vivo experiment revealed that methylene group derived from serine was rapidly incorporated into DNA in the absence of TrmFO. Furthermore, the addition of thymidine to the medium accelerated growth speed of the wild-type strain. Moreover, in vitro experiments showed that TrmFO interfered with ThyX through consumption of CH2THF. Addition of methionine to the medium accelerated growth speed of wild-type strain and the activity of TrmFO was disturbed by MetF. Thus, the consumption of CH2THF by TrmFO has a negative effect on dTMP and methionine syntheses and results in the slow growth under a nutrientpoor condition.

Keywords: DNA synthesis; MetF; RNA modification; ThyX; TrmFO.