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**Study of inhibition of Gq (G-protein) by a minigene encoding a C-terminal portion of the portein**

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Many signaling molecules such as hormones, neurotransmitters, chemokines and local mediators activate signal transduction pathways through transmembrane heptahelical receptors. Theses receptors are coupled with specific systems through appropriate heterotrimeric G proteins that function as transducer of the extracellular signals. Heterotrimeric G proteins (α, β, and γ subunits) transmit signals from cell-surface receptors to intracellular effectors by transiting a controlled cycle of GTP binding and hydrolysis. Several studies have pointed out specific regions on Gα subunits that participate in the interaction with the receptor. Among these, the C-terminus of the Gα subunits play a critical role in the selective activation of the G proteins by their cognate receptors. There are several reports showing that peptides corresponding to the last 11 amino acids of the Gα C-terminus act as inhibitors of signal transduction. As part of a work to study the link between Gαq and β-Catenin function, we have made a minigene plasmid construct, carrying an oligonucleotide sequence encoding the last 11 amino acids of the carboxyl-terminal of Gαq. Following transfection of the recombinant plasmids into HEK 293 cells, the expression levels of the peptide and its anti-Gαq activity will be measured. A similar construct encoding the last 11 amino acids of the carboxyl-terminal of Gαs will be used as a control.

***Keywords*:** G-protein; Gαq; C-terminal portion; β-catenin; Hek293cells

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**Observation of surface ultra-fine structure of magnetic nanoparticles and lipid vesicles with atomic force microscopy**

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As a new class of targeting agents, magnetic lipid vesicles are developed recently. The surface structure and the topographical changes of magnetic lipid vesicles in the liquid-crystalline state at scale of several hundred nanometers can be observed by tapping mode AFM. Meanwhile, we investigated the surface interaction and elastic behaviors of magnetic lipid vesicles in liquid crystalline state when the stylus is used to scan the surface of the magnetic lipid vesicles. 1g $Fe_3O_4$ was mixed with distilled water and emulsified by ultrasound. Then magnetic particles $Fe_3O_4$ were mixed with 5% SOD solution and the mixture were emulsified by ultrasound into magnetic SOD solution. A certain amount of DPPE and cholesterol in the weight proportion of 3:1 were dissolved and mixed in 3:1chloroform: methanol (v/v), then mixed with magnetic SOD solution. The final product magnetic lipid vesicle was acquired by supercritical $CO_2$ swelled, and vacuumed freeze drying. All the measurements were performed at room temperature and a relative humidity of 55%. Commercially available $Si_3N_4$ stylus with a spring constant of approximately 0.12 N/m and 0.06 N/m was used respectively. RESULTS: When the magnetic lipid vesicles were adsorbed onto the mica, the layers of vesicles can be formed naturally. In order to obtain the monolayer, the excess material was removed by washing with fresh solvent. After the repeated scanning, we observed some large and small magnetic lipid vesicles in the area of scanning. It was possible to observe differences in size and shape by using AFM for the magnetic lipid vesicles. The shape of the magnetic lipid vesicles is spherical for some spherical surfaces in these areas. The mean size of spherical lipid vesicle ranged from 200 to 350 nm can be observed from the AFM topography. On the other hand, we have observed the magnetic lipid vesicles' change into ellipsoid by the control of scanning force. The results have shown how surface structure of the magnetic lipid vesicles in liquid-crystalline state can be created by the interaction between an AFM tip and a lipid vesicle. Some large and small elliptic lipid vesicles in the area of scanning were shown. Some smaller vesicles were also obtained. The adsorbed magnetic lipid vesicles onto mica were scanned by contact mode AFM. While scanning over the surface of the adsorbed magnetic lipid vesicles, the cantilever tip exerts an elastic force on the vesicles surface. In this investigation, one can map out the surface topography through monitors the deflection of a cantilever. Calculations indicate that the operating force should not exceed 10-11N and that force of the order of 10-8 N can lead to large deformations of the surface. When the operating force is between 10-11N and 10-8N, the elastic force leads to the topographical surface of the magnetic lipid vesicles in liquid-crystalline state.

***Keywords*:** magnetic lipid vesicles; magnetic nanoparticles; atomic force microscope; ultra-fine

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**ACAP4-ezrin is essential for acid secretion in gastric parietal cells**

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Stimulation of gastric acid secretion involves an activation of a cAMP-dependent protein kinase cascade that triggers the translocation and insertion of the proton pump enzyme, H, K-ATPase, into the apical plasma membrane of parietal cells. The stimulation-mediated relocation of the H,K-ATPase from the cytoplasmic membrane compartment to the apical plasma membrane is mediated by a small GTPases such as Rab11 and ARF6. To further understand the role of ARF6 in parietal cell secretion, we searched for proteins in parietal cell extracts that bind the GTPase-deficient ARF6Q67L mutant but not wild-type ARF6. Mass spectrometric analysis of a 97 kDa polypeptide specifically bound to ARF6Q67L led to the identification a 903 amino acid protein of unknown function. We named this protein as ACAP4 since ACAP4 contains two coiled coils, one PH domain, one GAP motif, and two ankyrin repeats. Our biochemical characterization indicates that ACAP4 displays a PIP2-dependent GAP specific for ARF6. Immunocytochemical study revealed that ACA4 is mainly located to the apical membrane of parietal cell within the gastric glands. Our deletion analysis revealed that ACAP4 binds to ezrin via its N-terminal 400 amino acids and is co-localized with ezrin to the apical canaliculi. The siRNA-mediated depletion of ACAP4 or a block of ARF6 GTP hydrolysis suppresses ARF6-dependent proton pump mobilization, suggesting that a continuous GTPase cycle is required for gastric acid secretion in parietal cells. Thus, ACAP4 provides a novel link between signal transduction, vesicular trafficking and membrane cytoskeletal dynamics underlying parietal cell activation.

***Keywords*:** exocytosis; PH domain; ARF6; ACAP6; ezrin

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**The damage repair effects of He-Ne laser on intanct chloroplasts (*in vitro*) exposed to enhanced UV-B radiation**

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The effects and mechanism of He-Ne laser (5 $mW \cdot mm^{-2}$) irradiation on the intanct chloroplasts (*in vitro*) of wheat cell induced by enhanced ultraviolet-B radiation were studied. Isolated intanct chloroplasts of wheat cell were divided into CK (control), L (He-Ne laser), B (UV-B) and BL (He-Ne laser+ UV-B) four different treatments. The chloroplasts of B and BL treated by different dose UV-B irradiation (0.42 $kJ \cdot m^{-2} \cdot d^{-1}$,0.84 $kJ \cdot m^{-2} \cdot d^{-1}$, 1.26 $kJ \cdot m^{-2} \cdot d^{-1}$), for BL, following He-Ne laser irradiation. The study results indicated distribution of excitation energy between PSI and PSII was changed, the rate of electron transport and ATPase activity was declined ,and the relative permeability of chloroplasts membrane was increased by UV-B irradiation, when the UV-B radiation dose increased to 1.26 $kJ \cdot m^{-2} \cdot d^{-1}$, the chloroplasts almost lost all photochemical activities, which all can be promted by He-Ne laser irradiation before losing it's activities completely. For BL,the damage extent induced by UV-B radiation was lower than B. The result suggests enhanced UV-B radiation change the photosynthetic characteristics of the chloroplasts and resulte in losing photochemical activities, which can be partly repaired by He-Ne laser irradiation.

***Keywords*:** chloroplasts; wheat; He-Ne laser; UV-B

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**Effects of enhanced UV-B and He-Ne laser on chloroplasts (*in vivo*) of wheat**

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The conforamtion of photosystem components of plant cell was changed and resulted in photoinhibition which were induced by enhanced UV-B radiation, low dose He-Ne laser can stimulated cell activity and partly restore the damage induced by enchanced UV-B irradiation. The effects He-Ne laser (5 $mW \cdot mm^{-2}$) and ultraviolet-B (UV-B) (10.08 $kJ \cdot m^{-2} \cdot d^{-1}$) radiation on photosystem of wheat were measured, the wheat seedings were divided into CK (control), L (He-Ne laser), B (UV-B) and BL (He-Ne laser+ UV-B) four different treatments. The experimental results showed: In contrast to CK, The chlorophyll concentration, electron transport and net photosynthetic were decreased in wheat exposured to UV-B, however, which was increased in wheat treated by He-Ne laser irradiation, for BL, the change extent was lower than only exposured to UV-B. the excitation energy distribution between two photoystems was altered by He-Ne laser and UV-B irradiation. the excitation energy transnsfer from light harvesting

chlorophyll protein complexes to PSII was enhanced in the wheat irradiated by He-Ne laser, which was inhibited by UV-B that induced PSII core antenna degraded ,which play an important role in excitation energy transfer. There were not obvious differents on stomatal conductance, and transpiration rate between the four treatments.The results indicated:enhanced UV-B radiation induced photosystem biochemical activities inhibition, to some extent, which can be promoted by He-Ne laser irradiation , and thedamages to photosystem which induced by UV-B radiation can be partly repaired by He-Ne laser irradiation.

***Keywords*:** UV-B; He-Ne laser; chloroplasts; wheat

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**Characterization of HSP90 as a CB2 cannabinoid receptor interacting protein**

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CB2 cannabinoid receptor is expressed in the immune system and has been suggested to play an essential role in modulating immune responses. Using an immunoprecipitation and mass spectrometry based proteomic approach, we have identified several candidate proteins that interacting with human CB2 receptor. One of these candidate proteins is hsp90. Immunofluorescence microscopy studies showed that hsp90 and CB2 receptor co-localize with each other. Co-immunoprecipitation experiments demonstrated that Hsp90 is indeed interacting with CB2 receptor. It is known that 2-arachidonoylglycerol (2-AG), an endogenous cannabinoid agonist, causes cell migration. In the current study, knocking down hsp90 with specific short interfering RNAs (siRNAs) in HEK293 cells expressing recombinant human CB2 receptors, as well as in differentiated HL-60 cells expressing native CB2 receptors, markedly reduced 2-AG-induced cell migration. Treatment of cells with geldanamycin, a specific hsp90 inhibitor, also reduced 2-AG-induced cell migration. In conclusion, these data indicate that hsp90 is a CB2 receptor interacting protein that modulates CB2 receptor-mediated cell migration.

***Keywords*:** cannabinoid; receptor; HSP90

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**Regulation of neutral lipid storage by Rab GTPases and Mono-ADP-ribosylation of CtBP1/BARS**

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Aberration of lipid storage in lipid droplets/adiposomes has been linked with the development and progression of several common metabolic diseases including obesity, type II diabetes, cardiovascular diseases such as atherosclerosis, and neutral lipid storage disease like Chanarin-Dorfman Syndrome. Our recent proteomic and immunoblotting analyses of isolated lipid droplets (LD) revealed over 17 Rab GTPases appear to be highly enriched in LD. Here we present three lines of evidence that Rabs involved in regulating membrane traffic are functionally associated with LD. First, we assayed for the ability of Rab-GDI to remove Rabs from isolated LD. Using immunoblotting we detected the removal of Rabs by GDI in the presence of GTPβs but not GTPγs. Next we used the GDI treated LD to see if Rabs could be recruited from cytosol. Multiple Rabs including Rab5 were recruited and recruitment was dependent on both GTP and the concentration of cytosol. Recruitment appeared to be saturable and individual Rabs did not compete with each other, suggesting the presence of Rab-specific binding sites on LD. The Rab5 effector EEA1 was also recruited to LD in the presence of GTP but not other nucleotides. When isolated LD and endosomes were incubated together, both EEA1 and transferrin receptor were detected in the LD fraction in the presence of GTPγs, suggesting a Rab5-dependent interaction of these organelles. The inhibition of this interaction by using Rab-GDI that released Rabs from both organelles further confirmed the finding. We also found that 12 h in the presence of BFA caused a complete loss of LD. LD loss required new protein synthesis, was blocked by multiple ribosylation inhibitors, and did not involve disruption of the Golgi apparatus nor the ER unfolded protein response. Importantly, reducing CtBP1/BARS by RNA interference recapitulated the effect of BFA. We conclude that Rab GTPases regulate membrane trafficking that leads to lipid transport in and out of LD and mono-ADP-ribosylation of CtBP1/BARS inactivates its repressor function, thereby activating genes that regulate the retention of neutral lipid in LD.

***Keywords*:** lipid droplets/adiposomes; Rab; CtBP1/BARS; lipid storage; membrane trafficking

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**Dissection of protein quality control in the early secretory pathway**

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Newly synthesized (glyco)proteins are monitored by a quality control machinery which is important for the maintenance of the endoplasmic reticulum homeostasis. Three major processes can be distinguished during the life of a folding incompetent glycoprotein. The first involves recognition by quality control machinery proteins. This is followed by retrotranslocation, deglycosylation and ubiquitination. Finally, misfolded glycoproteins are retrotranslocated for proteosomal degradation. We have applied high resolution quantitative immunolabeling and serial section analysis to establish the *in situ* subcellular distribution of the quality control proteins glucosidase II and glucosyltransferase and the quality control receptor EDEM1. Immunolabeling for glucosidase II and glucosyltransferase was not only detected in the endoplasmic reticulum but also found in the p58-positive pre-Golgi intermediates (pGI). Furthermore, we studied the intracellular sites of accumulation of a misfolded insulin (Cys96Tyr) and found it accumulating mainly in tubules of enlarged pre-Golgi intermediates and to a lesser extent in dilated endoplasmic reticulum subdomains. This provides evidence for the importance of pre-Golgi intermediates in a protein folding disease. EDEM1 immunolabeling was sequestered into rough endoplasmic reticulum-derived, not COPII coated ~150 nm vesicles through a novel pathway that did not involve the canonical transitional endoplasmic reticulum exit sites. EDEM1 vesicles also contained the misfolded Hong Kong variant of alpha-1-antitrypsin. Collectively, these results suggest that (i) protein quality control seems not to be limited to the ER and that the pGI appear to be involved as well, (ii) different quality control checkpoints and protein accumulation sites exist in the ER and the pGIs, and (iii) a novel vesicle budding transport pathway out of the rough endoplasmic reticulum exists, which appears to represent a vesicular sorting intermediate between the recognition of misfolded glycoproteins and their subsequent degradation.

***Keywords*****:** protein quality control; endoplasmic reticulum; pre Golgi intermediates; immunolabeling; ERAD

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**The endocytic pathway: mechanisms of entry and exit**

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The microdomains on the cell surface capable of internalizing trafficking membrane proteins will be described and discussed and the molecular interactions responsible for holding these proteins in place whilst the plasma membrane invaginates and pinches off will be identified. The subsequent fate of the internalized proteins and the mechanisms responsible for their return to the cell surface will also be described, particular reference being given to the part played by these mechanisms in growth factor signalling and neurotransmission.

***Keywords*****:** endocytosis; membrane proteins; protein trafficking; recycling; exocytosis

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**The effect of iron and $H_2O_2$ on plasma membrane of wheat roots studied by fourier transform infrared spectroscop**

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In this work, we studied the iron-induced change plant PM membrane protein second structure and membrane lipid composition in the PM vesicles isolated from wheat roots. The FTIR-ATR methods and Computer Aided Analysis were used to study not only the change of protein and secondary structure of PM membrane protein at tacked by the oxidant: $H_2O_2$ and $FeSO_4$, $FeCl_3$, but also the effect of oxygen on the membrane lipids and membrane fluidly in this paper. The results indicate that the content of α-helix in the secondary structure of protein clearly decreased because of the attack of the oxidant respectively. The number of the P=O, C=O and C= C in the lipids of the membrane was decline correspondingly. Finally these changes were caused by decline of the $H^+$-ATPase activity and finally declined the PM fluidly.

***Keywords*****:** PM; lipid composition; FTIR; iron

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