Z506 Dephosphorylation of insulin-dependent Mr-20,000 phosphoprotein and expression of the heat shock response in *Drosophila melanogaster*.

Dong Hwan Won', Kyoung Sang Cho<sup>2</sup> and Chung Choo Lee<sup>1</sup>
Department of <sup>1</sup>Biology and <sup>2</sup>Molecular Biology, Seoul National University, Seoul 151-742, Korea

Protein synthesis activated by mitogens including insulin is severely inhibited by stress such as heat, osmotic pressure, and sodium arsenite. These regulations of protein synthesis are related to the level of phosphorylation of several proteins. In the present study, the state of insulin-dependent phosphorylation after heat shock was investigated to reveal the mechanism of repression of normal protein synthesis. Nevertheless insulin activated phosphorylation of Mr-20,000 protein (pp20) in ovaries of D. melanogaster more 10 times at normal state (24 $^{\circ}$ C), it was not effective at heat shock (37 $^{\circ}$ C). During recovery from a 30-min heat shock, rephosphorylation of pp20 was almost completed in 4 hrs and the recovery of normal protein synthesis was also restored in 2 to 4 hrs. Phosphatase activity did not show significant change after heat shock, and okadaic acid, phosphatase inhibitor, failed to recover phosphorylation state of pp20. These results implies that heat shock inhibit the normal protein synthesis via inactivation of phosphorylation of pp20 and phosphatase-independent pathway.

Z507 Mating stimulate protein phosphorylation and synthesis via rapamycin- and wortmannin-sensitive manner in male accessory glands of *D. melanogaster* 

Kyoung Sang Cho<sup>\*1</sup>, Dong Hwan Won<sup>2</sup>, and Chung Choo Lee<sup>2</sup>
Department of <sup>1</sup>Molecular Biology and <sup>2</sup>Biology, Seoul National University, Seoul 151-742, Korea

Mating increase protein synthesis 2-3 fold in male accessory glands of D. melanogaster. In the present study, mating-induced changes in synthesis and phosphorylation of accessory glands proteins were studied in vivo, to elucidate the components involved in the mechanism of stimulated protein synthesis after mating. At least 2 proteins (Mr-20,000 and -30,000 phosphoproteins; these are referred as pp20 and pp30 respectively), of which the level of phosphorylation was increased, were detected in one-dimensional SDS-PAGE and 5 proteins in high-performance liquid chromatography (HPLC). Their phosphorylation level was correlated with stimulated protein synthesis after mating. The stimulations in protein phosphorylation and synthesis were inhibited completely by rapamycin, inhibitor of mammalian target of rapamycin, but only partially by wortmannin, inhibitor of PI3 kinase. Insulin also activated the phosphorylation of the proteins and protein synthesis via rapamycin- and wortmannin-sensitive manner in the glands. The pp30 could be purified with pp20 from 7-methyl-GTP-Sepharose affinity chromatography, and another phosphoprotein (Mr-35,000) was also purified from the method. These results implies that pp30 and pp20 might be the Drosophila homologues of eIF-4E and eIF-4E binding protein, which are the central components in the eukaryotic translation initiation.