**F845** Transcriptional Inhibition of the *Drosophila raf* and *PCNA* gene by the Homeodomain Protein Engrailed

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Raf-1, a cytoplasm serine/threonine kinase located primarily in the cytosol, serves as central intermediate in many signaling pathway, ultimately regulating cell proliferation, differentiation, and development by connecting upstream tyrosine kinase with downstream serine/threonine kinase such as mitogen-activated protein kinase (MAPK) and MAPK kinase (MAPKK). A *Drosophila* homolog of the human *c-raf-1*, *D-raf* is also required for regulation of cell proliferation and differentiation. The *Drosophila* proliferating cell nuclear antigen (PCNA), an accessory protein of DNA polymerase $\delta$, is requred for cellular DNA synthesis and for cell cycle progression. The *Drosophila engrailed (en)* was originally identified as a segmentation gene affecting cells in the posterior compartments. The *en*, a homeobox-gene, encodes a nuclear protein that binds DNA specifically through its homeodomain and can function as a transcription factor. The promoter regions of *D-raf* and *DPCNA* contain the *Drosophila* DNA replication-related element (DRE) and the putative homeodomain-binding sites. In this study, we show that the expression of *DPCNA* and *D-raf* genes is transcriptionally inhibited by Engrailed.

**F846** Introduction of p53-independent p21 during ceramide-mediated G1 arrest in SK-Hep-1 cells

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Ceramide acts as a growth inhibitor in many cells, but its molecular mechanism linked to cell cycle is poorly understood. In this paper, we investigated expression of p21 and its relationship to G1 arrest induced by ceramide. SK-Hep-1 cells treated with 15M C6-ceramide resulted in a time-dependent G1 arrest and of pRB protein was dephosphorylated concomitantly Ceramide also inhibited cdk2 kinase activity during that process and the expression level of cyclin D1, E is decreased by ceramide. Furthermore, p21 protein and mRNA were significantly induced by ceramide, while the expression of p53 gene was not changed at the same condition. Immunoprecipitation of cdk2 from ceramide-treated cells revealed a 3-fold higher ratio of p21 bound to cdk2 compared with control cells. Interestingly ceramide did not induce G1 arrest in the hepatocarcinoma cell line, Hep3B lacking retinoblastoma protein. However, Hep3B/RB cells transfected with a pRB-expressing vector underwent G1 arrest in response to ceramide and this arrest was accompanied by the accumulation of dephosphorylated pRB protein. Taken together our results, we suggest that pRB is a critical component of ceramide-induced G1 arrest. Moreover, p21 is induced by ceramide through p53-independent pathway and participated in pRB dephosphorylation by acting inhibitory protein of cyclin E-cdk2 in human hepatocarcinoma cells.