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ΘΕΜΑ: **ENGINEERING PROTEIN-BASED MOLECULAR SWITCHES: *IN VIVO* REGULATION OF PROTEIN ACTIVITY AND THE CONSTRUCTION OF SIMPLE BIOSENSORS**

ΤΟΠΟΣ: Αίθουσα Σεμιναρίων ΙΤΕ/ΕΙΧΗΜΥΘ

ΗΜΕΡΟΜΗΝΙΑ: Τετάρτη, 11 Ιανουαρίου 2006

ΩΡΑ: 12:00

ΠΕΡΙΛΗΨΗ

The development of efficient tools for controlling protein function in living cells is one of the greatest challenges of the proteomic era. Traditionally, genetic approaches such as regulation of transcription and gene knockouts have been used extensively as they enable control of gene function in a highly specific and generalizable manner. Genetics, though, are hindered by transcriptional and translational delays before a functional product protein is formed. An alternate strategy is chemical genetics, where a small molecule is used to directly activate or inactivate a particular protein target. Although this method can provide dose-dependent control over gene products of interest at the post-translational level, a different small-molecule regulator must be discovered for each individual protein target. In this work, engineered protein-based molecular switches have been introduced as novel tools for regulating protein activity that combine the advantages of genetic and chemical genetic approaches. We have developed a small-molecule-controlled protein self-splicing element (intein), which can be used to inactivate arbitrary target proteins by genetic insertion and then reactivate them post-translationally when the splicing reaction is activated and intein self-excision takes place. Splicing and host protein activation in living cells can be achieved by simple addition of the appropriate small-molecule compound in the growth medium, with a fairly rapid response and in a dose-dependent manner that allows precise specification of the levels of active protein in the cell. In the second part of this work, it is demonstrated that protein-based molecular switches can be used to construct simple biosensors for

therapeutically relevant compounds. By creating chimeric fusion proteins that couple ligand binding to a nuclear hormone receptor with the catalytic activity of a highly sensitive reporter enzyme that is required for cell growth, engineered *Escherichia coli* strains have been generated whose survival relies on the presence of compounds with hormone-like properties. This allows the facile detection of novel small-molecule or peptide-based compounds with potential medical applications. Remarkably, these simple biosensors are also able to recognize important aspects of the pharmacological properties that a particular hormone analogue exhibits in higher animals. Finally, the catalytic activity of the chosen reporter enzyme was found to behave as a molecular sensor of the conformations of the hormone receptor, thus easily providing structural information that is very laborious to acquire with crystallographic methods.