299b Ligand-Induced Protein Splicing: a General Way of Achieving Post-Translational Regulation of Protein Activity *in Vivo*

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Inteins are protein splicing elements that excise themselves post-translationally from a variety of protein hosts in a process known as protein splicing. In general, genetic insertion of an intein abolishes the activity of the host protein in the unspliced precursor, but host activity is subsequently restored upon intein excision. Thus, controllable inteins can be used as molecular switches for the control of arbitrary target proteins. Based on rational design, we have constructed a chimeric intein whose splicing activity is conditionally triggered *in vivo* by the presence of thyroid hormone. Initially this chimera was used to regulate the activity of a thymidylate synthase reporter enzyme in vivo. We then demonstrated that the activity of several different proteins in *Escherichia coli* can become thyroid hormone-dependent when expressed as gene fusions with this intein. In each case, genetic insertion of the intein leads to the expression of an inactive fusion precursor protein. This fusion can be induced to splice by the addition of thyroid hormone or synthetic analogues in a dose-dependent manner. Splicing restores the structure and function of the host protein, effectively allowing its activity to be induced by the addition of thyroid compounds. The generality of this method was further explored by the use of rational protein engineering and directed evolution to produce inteins whose splicing activity is inhibited by the presence of synthetic estrogen ligands. Finally, potential applications on controllable protein splicing will be discussed.