Control of Intein-mediated Recombinant Protein Purification

In a Mammalian Cell Expression System

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Inteins are genetic elements that can self-excise from various host proteins in a post-translational protein splicing process. With a simple mutation of the intein, one can yield isolated, controllable intein cleaving activities at either the N- or C-terminus. By combining self-cleaving inteins with conventional affinity tags, self-cleaving affinity tags have been developed. The self-cleaving tags benefit the tag removal process by a simple, inducible cleaving reaction without any addition of protease. The phenomenal tag removal strategy works successfully in prokaryotic cell systems in our previous studies; however, the *in vivo* premature cleaving of the precursor protein remains the bottleneck of its application in eukaryotic cell systems. Here we present our most recent work in developing a next-generation intein for use in mammalian cells. In particular, we have engineered an innovative intein with enhanced control, where the control mechanism is compatible with our mammalian systems. This intein has been tested with several conventional affinity tags in mammalian systems. The target protein can be purified successfully with the new intein and shown activity after the downstream process. By controlling the *in vivo* premature cleaving in a mammalian system, we have generated a potential platform technology for the manufacture of non-antibody glycoprotein therapeutics at large scale. Additionally, this intein has been modified for high-throughput applications in drug and target discovery and development.