Auxin Proteo Controller
(Auxin Inducible Degron System:AID System)

This Auxin Proteo Controller

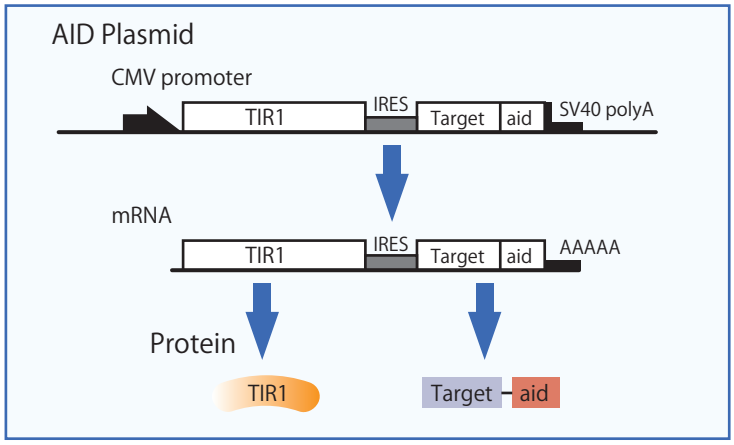
Can control expression of target protein in a reversible fashion in cultured cells.
Can regulate degradation of target protein in a different manner from promoter system so far.
Is an unique and a powerful new tool which has applied auxin-inducible degradation of target protein to animal cells and yeast.

Characterization of Auxin Proteo Controller

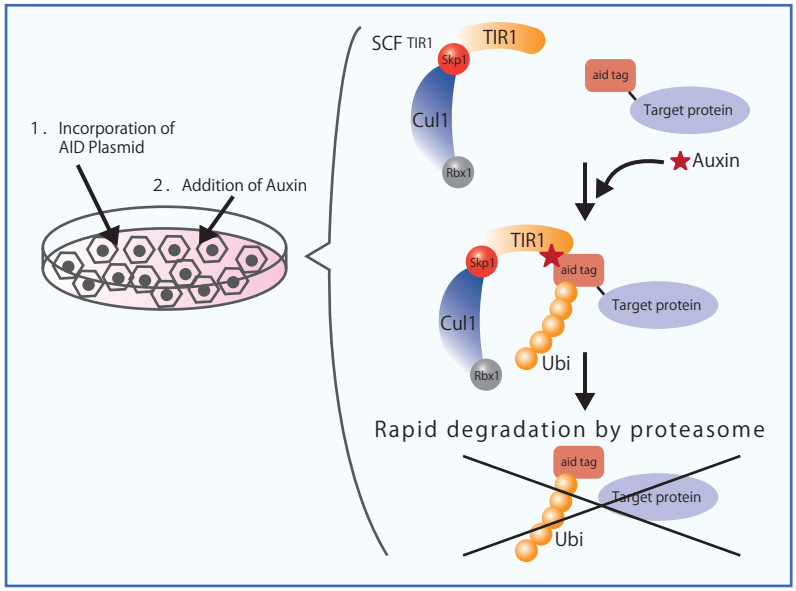
- Regulation of the amount of protein expression throughout induction of proteolysis system.
- Highly rapid expression control system in comparing with any other known systems.
- Rapid Proteolysis induced by the addition of Auxin to culture medium.
- Easy system construction by incorporation of one vector.

Principle of Auxin Proteo Controller (AID system)

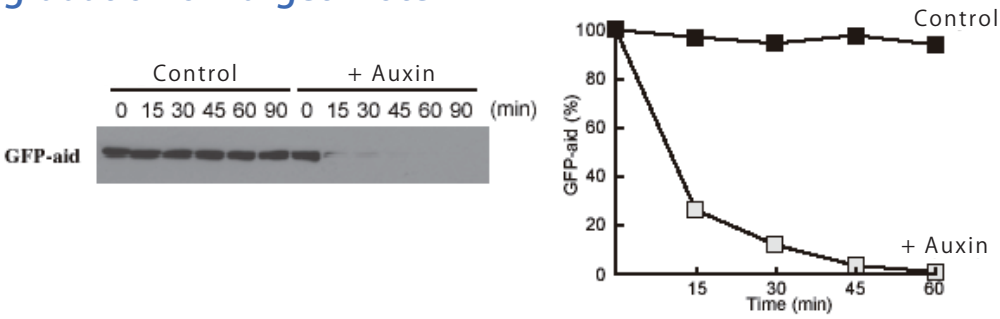
This system expresses target protein fused to IAA17(hereafter called the aid tag) And F-box TIR1 protein derived from plant at the same time. The target protein is desighed in the cells so that protein fused to aid tag is expressed by only the cloning at MCS in the vector. We have two vectors which is fused to the N-terminal or C-terminal of target protein. This vector expresses at the same time both proteins of TIR1 and target protein fused to aid by using the internal ribosome binding sequence (IRES) derived from the encephalomyocarditis virus.



In the presence of Auxin,the expressed TIR1acts in harmony with many intracellular factors as additional enzyme of SCF ubiquitin. In the presence of Auxin,TIR1 is activated and combines to aid tag fused to target protein. Then, ubiqitin ligase SCF complex with aid tag fused to target protein is poly-ubiquitylated and is resulted in rapid degradation by the proteasome .TIR1 is inactive although Auxin is not present in the system.



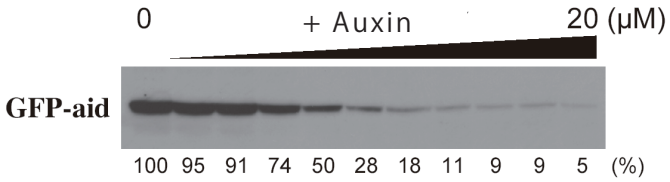
Rapid Degradation of Target Protein



Degradation of GFP protein fused to aid after addition of Auxin Clonning GFP fused with the aid plasmid was incorporated in 239 cells. Auxin of 500 μM IAA was added to 239 cells and then cells were recovered to determine the expressed amount of GFP-aid protein by westernblotting. As you can see in the figure, degradation of the most of GFP-aid was found at 15mitutes after addition of IAA.

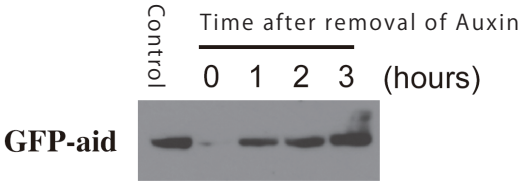
Free Expression Control

Expression Control depending on the concentration of Auxin 233 cells as described above was treated with different concentration of Auxin.
You can find that GFP-aid is expressed in the dose-dependent manner of Auxin.

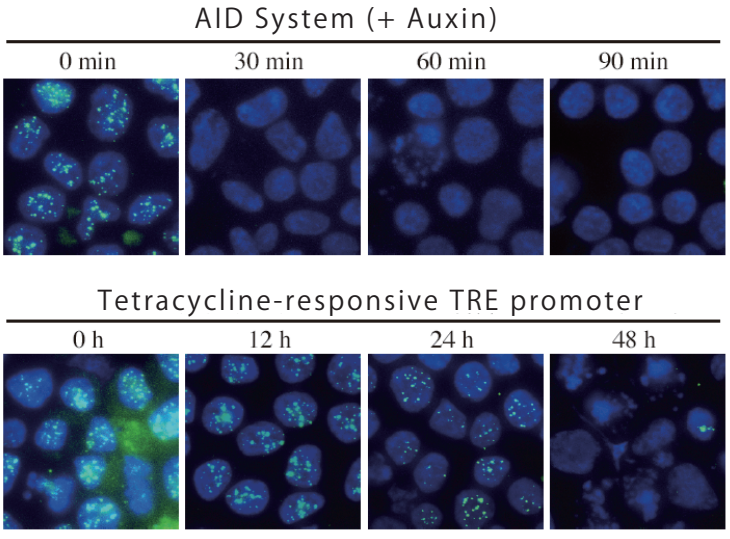


Reversibility of Switch On/OFF of Expression

After degradation of GFP-aid in 233 cells, cells were transferred to the culture medium not containing Auxin and they were recovered to determine the expression.
You can find that expression of GFP-aid is recovered after about 1 hour of withdraw.



Characterization of Auxin Proteo Controller (AID System)



Control system depending on tetracycline-responsive TRE promoter has been used as a expression control system of CENP-H mRNA. Therefore, degradation of protein depends on half time of various proteins in cells. This system made it possible to control a rapid expression inhibition of target gene throughout a direct proteolysis of expressed protein. Protein depletion of CENP-H by AID system was compared with mRNA depletion of CENP-H by the tetracyclin-repressive TRE promoter system in DT40 cells. Tetracycline treated or Auxin treated cells were collected at each time after the treatment Protein depletion of CENP-H in both cells was examined by immunofluoresence analysis (Green dots show protein of CENP-H and blue color shows nuclear DNA). CHNP-aid protein in SD40 treated with AID system disappeared within 30 minutes. On the other hand, CENP-H protein was still detected up to 24 hours when CENP-H mRNA was depleted in DT40 cells.

Contents of AID system

AID System consists of two types of plasmid and Auxin (IAA). One plasmid is pAID1.1-N Vector which is combined with N-terminal of target protein. The other one is pAID1.1-C Vector which is combined with C-terminal of target protein. IAA is Auxin which induces degradation of target aid protein.

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