

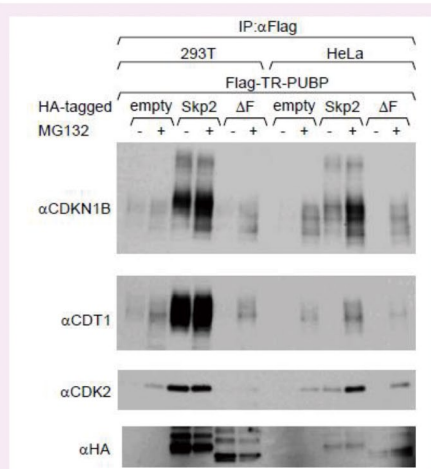
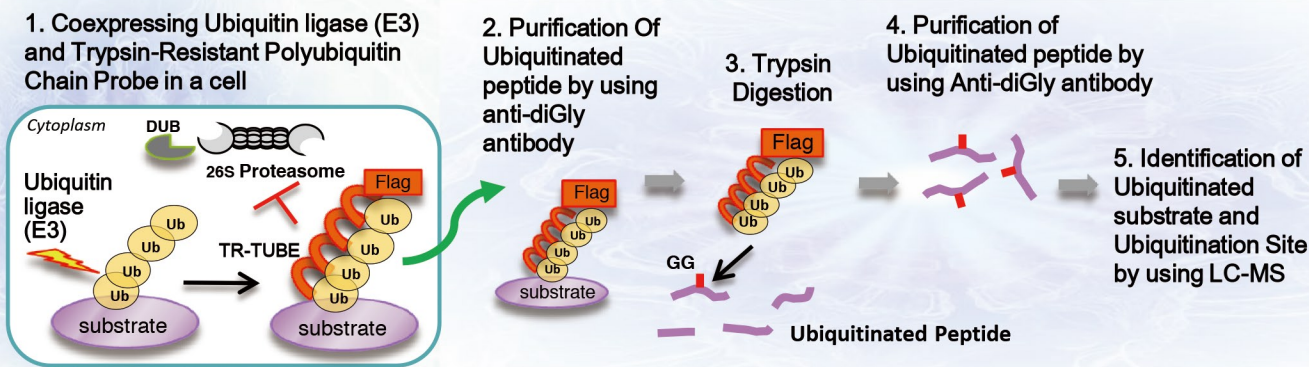
# TR-TUBE method

## — An Improved Method for Detecting Ubiquitinated Substrate —

### 【Background and Technical Problems for identifying ubiquitinated substrates】

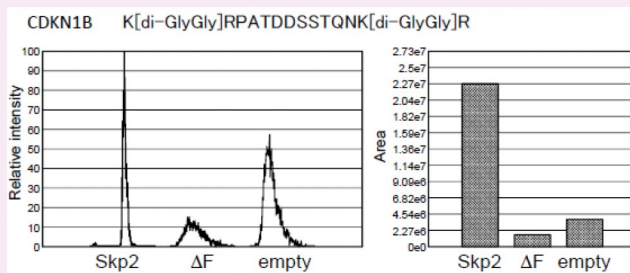
- ◆ Ubiquitination is very crucial cell system for keeping homeostasis, its abnormality leads to progression of many human diseases such as **cancer**, **neurodegenerative disease**, **inflammatory disease**, and **metabolic disease**.
- ◆ Most of polyubiquitinated proteins are rapidly degraded by **proteasome** *in vivo*. Moreover, poly-ubiquitin chain is rapidly removed by a deubiquitinating enzyme (**DUB**). Consequently, **it has been generally difficult to identify the polyubiquitinated proteins**.
- ◆ **Tandem ubiquitin-binding entities (TUBE)** is based on ubiquitin-associated domains and was developed for isolation of poly-ubiquitinated proteins in **extracted cell lysates**. Moreover, an antibody against the ubiquitinated remnant motif Lys-ε-Gly-Gly (**diGly**) that is exposed by tryptic digestion was developed for purifying ubiquitinated substrate peptides. **However, even with the conventional methods, it has been difficult to identify the ubiquitinated substrates due to which is done using *in vitro* reconstitution**.
- ◆ We have developed a **trypsin-resistant(TR)-TUBE** method for use *in culture cells* to capture of ubiquitinated proteins and to identify substrates of a specific ubiquitin ligase.

### 【Technical Strategies】



(Left) E3 ubiquitin ligase Skp2-specific ubiquitinated substrate proteins CDKN1B, CDT1, CDK2 were detected by TR-TUBE method, with co-transfection of Flag-TR-TUBE plasmid with an HA-tagged E3-ubiquitin ligase expression plasmid in 293T and HeLa cells.

(Below) Detection of ubiquitinated protein CDKN1B using MS after collecting from 293T cells.



**Paper** ■ Y. Yoshida et al., Proc Natl Acad Sci U S A. 2015 Apr 14;112(15):4630-5. A comprehensive method for detecting ubiquitinated substrates using TR-TUBE

**Patent Application**

■ US 61/901452, A Novel Versatile Method For Determining Ubiquitin Chain Length Reveals Functional Units Of Polyubiquitin Chains In Cells

■ PCT 2014/JP2014/008053, into the national stage [ US, EP, JP \* ] Method For Identifying Polyubiquitinated Substrate

\*JP; 2015-547782, EP; 14863005.6, US; 15/035357

**Our technology can make it even better! We are looking for companies that can take advantage of this technology in drug discovery and development research.**



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