IMAGING AND VISUALIZATION

Protein suicide highlights the cell cycle

By exploiting the process of regulated protein death, researchers create a new class of fluorescent sensors that highlights cell-cycle transitions in living cells.

Many signal transduction proteins can be compared to cellular samurai. Like samurai warriors of old, they show absolute obedience to their masters. When their existence interferes with the proper operation of the cell, they commit suicide on command. Their manner of death is not by slashing with a sword but by post-translational modification called ubiquitination that marks them for death in the proteasome.

Atsushi Miyawaki and his group at the RIKEN Brain Science Institute have been working with fluorescent proteins for years. They noticed that when a protein fused to a fluorescent protein is ubiquitinated and commits suicide, generally the fluorescent protein joins it in death. "We were very curious about how we could develop this observation as a sensor technology," says Miyawaki.

Of the many cellular functions regulated by signal transduction proteins, one of the most critical is cell-cycle progression. Some cell-cycle regulatory proteins must commit suicide after they have completed their role so that the cell can progress to the next phase of the cell cycle. Lead author Asako Sakaue-Sawano and Miyawaki thought that a sensor for highlighting the stages of the cell cycle in living cells would be a promising use of regulated protein suicide; they have now reported their first successes in *Cell*.

Existing cell-cycle sensors require cell fixation or function by tracking the location of fluorescently labeled proteins in the cell. The latter method works well for cells in monolayer culture where the protein location can be easily determined but is not applicable for the three-dimensional situation *in vivo*.

Hisao Masai at the Tokyo Metropolitan Institute, an expert in the field of cell-cycle regulation and one of the authors of this study, hypothesized that Cdt1 and Geminin would be good candidates for making fusion protein sensors that would undergo cell cycle–dependent suicide. Cdt1 accumulates specifically during the G1 phase but is degraded during the G1/S transition after ubiquitination. Geminin accumulates during S, G2 and M phases but is degraded in late M phase.

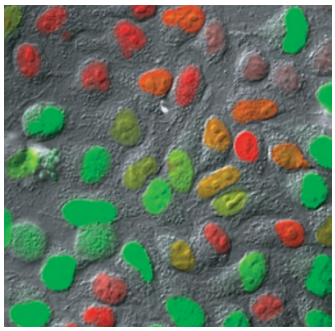

Figure 1 | Image of live HeLa cells stably expressing the Fucci cell-cycle sensors.

To begin developing their new class of cell-cycle sensors, Sakaue-Sawano and Miyawaki fused mKO2, a fast-folding variant of monomeric Kusabira orange, to full-length Cdt1. They stably transfected the gene fusion into mammalian cells, observed cell-cycle progression using time-lapse imaging, and compared this to untransfected cells. Unfortunately, the transfected cells did not divide normally. The authors reasoned that this was due to the activity of the full-length Cdt1. They proceeded to make deletion mutants of Cdt1 fused to mKO2 in an attempt to find a fusion that did not affect the cell cycle but retained the protein's nuclear localization and cell cycle–specific expression.

According to Miyawaki, "finding the appropriate fusion constructs was the most difficult task, and those shown in the paper are the tip of the iceberg. We had to very carefully design the construct to prevent it from acting as a dominant regulator." Eventually they found a fusion that worked well. This sensor displayed orange fluorescence in the nucleus only during G1 and had no effect on the cell cycle. They used the same methodology to generate an mKO2 fusion with Geminin.

Obviously, if both sensors are the same color it will be impossible to use them together. Unfortunately, when substituted for mKO2, other fluorescent proteins were not degraded along with Cdt1. Luckily, the Geminin-mKO2 fusion was immune to this effect and the authors replaced mKO2 with monomeric Azami green (mAG). This resulted in a pair of sensors that highlights cell nuclei orange during G1 and green during S/G2/M. They called this pair 'Fucci', which stands for fluorescent ubiquitination–based cell-cycle indicator (**Fig. 1**).

Fucci promises to be a valuable tool for analyzing cells in a variety of biological fields. For example, Fucci expression in tumorigenic and non-tumorigenic cell lines injected into nude mice showed that tumorigenic cells continued to proliferate while non-tumorigenic cells stopped doing so. Fucci also allowed the authors to observe tumorigenic processes occurring at specific points during the cell cycle.

Expression of Fucci in stem and progenitor cells in an intact animal would provide a powerful tool for developmental biologists by allowing them to track the activity and movement of these cells. As a first step on this path, the authors created a Fucci transgenic mouse line with each sensor under the control of a ubiquitous promoter, and examined the relationship of the cell cycle with the movement and development of neural progenitor cells.

Fucci will be a powerful tool for biologists in many fields, but Miyawaki is not satisfied with this progress and is already developing the next generation of suicidal sensors.

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RESEARCH PAPERS

Sakaue-Sawano, A. *et al.* Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* **132**, 487–498 (2008).