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Early Detection of Gene Promoter Activation Using an Aggregating Fluorescent Protein

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Fluorescent microscopy requires bright genetically encoded tags. The brightest natural fluorescent proteins are typically selected for this, and then improved artificially. One of the best green fluorescent proteins thus obtained is called mNeonGreen.

In our work, we aimed to get a brighter genetically encoded fluorescent tag. We hypothesized that concentrating a fluorescent signal at a single point would significantly increase brightness compared to a signal from a tag which molecules are evenly distributed throughout the cell. To test this hypothesis, we created a highly aggregating tandem fluorescent protein TrioAG4 from three copies of the green fluorescent AG4 protein, which is tetrameric in solution. According to our assumption, the molecules of the triple tandem will effectively form large aggregates, due to the inability to form an intramolecular tetramer. Fluorescent microscopy of mammalian cells expressing TrioAG4 confirmed the assumption -individual bright particles in the cells were clearly visible.

We decided to check whether this method of signal amplification would help to detect fluorescence at a low protein concentration. For this, TrioAG4 protein was expressed in mammalian cells under the control of the histone H1 gene promoter. This gene is expressed only in the S phase of the cell cycle - thus, a small amount of protein is formed in the cell. As a result, we observed the appearance of green TrioAG4 particles in the S phase of the cell cycle, while the signal from mNeonGreen expressed under the same promoter was not detected.

We also tested TrioAG4 aggregates for cytotoxicity. As a result, TrioAG4 was a bit more toxic for the mammalian cells than mNeonGreen, but both toxicity levels were moderate. Simultaneously, larger aggregates can induce significant cytostatic and cytotoxic effects and thus such tags are not suitable for long-term and high-level expression.

Our results indicate that TrioAG4 is a suitable tool for early detection of gene promoter activation.