IN BRIEF

GENETICS

Recombination may cause genotoxicity

Bohin, N., Carlson, E. A. & Samuelson, L. C. *Stem Cell Rep*. https://doi.org/10.1016/j.stemcr.2018.10.014 (2018).

The Cre–*loxP* recombination system is popular in in vivo genetic studies because its expression can be induced temporally in a cell-specific manner. Researchers have applied inducible Cre drivers in intestinal stem cells (ISCs) to manipulate genetic recombination and study ISC function. Bohin et al. report that intestine-specific CreER^{T2} drivers, but not tamoxifen toxicity, can induce undesired DNA-cleavage events at cryptic *loxP* (*cloxP*) sites, which leads to reduced ISC function. Examining the Villin-CreER^{T2} mouse strain, they observed that tamoxifen (TX)-activated CreER^{T2} results in delayed crypt regeneration after epithelial cell damage induced by irradiation. They suggest that TX-activated CreER^{T2} introduces cleavage at *cloxP* sites and DNA double-stranded breaks. They also observed that genotoxicity reduces organoid-forming efficiency and impairs the function of crypt base columnar stem cells. Therefore, they advise that future CreER mouse studies should include control samples for Cre-introduced genotoxicity. *LT*

https://doi.org/10.1038/s41592-018-0278-6

SENSORS AND PROBES

RFPs made monomeric

Wannier, T. M. et al. *Proc. Natl. Acad. Sci. USA* **115**, E11294–E11301 (2018).

Commonly used 'monomeric' red fluorescent proteins (RFPs) are engineered from naturally oligomeric precursors. This monomerization is crucial for RFPs' performance as protein tags, as fusion to a dimeric or tetrameric fluorescent protein will lead to obligate oligomerization of the protein of interest, which can cause artifacts. However, monomeric variants of fluorescent proteins are often substantially dimmer than their precursors owing to extensive mutagenesis. Wannier et al. have developed a comprehensive approach for generating true monomers from oligomeric RFPs that treats separately the problems of protein stabilization, core optimization, and surface engineering. To demonstrate their approach, they developed monomeric variants of two far-red fluorescent proteins, mCardinal and HcRed. One of the developed variants, mKelly1, represents a promising far-red tag for biological applications. *RS*

https://doi.org/10.1038/s41592-018-0282-x

MICROSCOPY

Comprehensive correlation analysis

Scipioni, L. et al. *Nat. Commun.* **9**, 5120 (2018).

Fluorescence fluctuation techniques are established approaches for studying dynamic behaviors of labeled molecules within cells. However, various types of fluorescence fluctuation analyses have different acquisition parameters, which makes simultaneous acquisition challenging. Scipioni et al. have developed comprehensive correlation analysis, which allows many advanced fluorescence fluctuation techniques to be implemented in a single, simultaneous analysis. Their approach is based on the ultrafast Airyscan detector in the Zeiss LSM 880, which can operate at around 1 million frames per second. The researchers show that with this instrument one can study both fluorescently labeled probes and the environment in which they are diffusing, via the simultaneous acquisition of parameters such as oligomerization state, diffusion coefficient, diffusion modality, and barriers to diffusion. They demonstrated their approach by mapping GFP diffusion in cells. *RS*

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STRUCTURAL BIOLOGY

Fluctuation X-ray scattering

Pande, K. et al. *Proc. Natl. Acad. Sci. USA* **115**, 11772–11777 (2018).

In traditional solution-scattering experiments conducted at synchrotrons, the X-ray exposure time is longer than the time required for a particle to undergo a full rotation. In fluctuation X-ray scattering (FXS), solution scattering data are collected at time scales below particle rotational diffusion times, which means that much more information can be extracted from an experiment. Though proposed decades ago, this type of experiment has only now been made possible with the advent of X-ray free-electron laser (XFEL) technology, which provides high-intensity, femtosecond-length X-ray pulses, enabling the rapid capture of snapshots of thousands of individual particles. Pande et al. applied FXS to determine a nanometer-resolution structure of the giant *Paramecium bursaria* Chlorella virus. Their reconstruction indicates that the viral capsid has icosahedral symmetry, whereas the interior of the virus is disordered. FXS could potentially be applied in a time-resolved manner to obtain insights into structural dynamics. *AD*

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