Epigenetics | Medicine an Pharmaceutics | Technology Offer

Fluorescence-based celluar assay for detection of gene regulatory activity of chromatin-associated proteins

Field of application

Gene expression in eukaryotic cells is regulated by a complex regulatory network of epigenetic signals including methylation of cytosine residues in the DNA and methylation, acetylaction or ubiquitination of histon proteins in nucleosomes. Epigenetic mechanisms are crucial for the development of different cell types and healthy cells, thus faulty epigenetic states can lead to oncogenetic transformation and tumor development. One challenge in research, as well as for the development of epigenetic therapies, is to identify and characterize these epigenetic (epi)-effector proteins and their (co-)regulators, as in fact many of them combine various functions and can be part of more than one complex at a time.

State of the art

So far, attempts have been made to uncover epigenetic effectors by co-immunoprecepitation followed by western blot or mass spectrometry analyses. Both approaches are complex, require specific antibodies and can identify only very abundant and extremely stable effectors. In view of the complexity of epigenetic mechanisms and their regulations, it is necessary to develop tools to significantly increase the level of knowledge about epigenetic proteins and complexes. This requires the development of reporter systems that allow the investigation of specific function of epigenetic proteins and direct analysis of the complex regulatory network within cells.

Innovation

Scientists at the University of Stuttgart have developed a novel epi-reporter assay to identify and characterize chromatin-associated proteins, their binding partners and factors that modulate the activity of said proteins inside cells. For this, the tetracycline-controlled transcriptional activation system (Tet system) is used. Analyses can be performed by standard methods including flow cytometry and fluorescence microscopy using a fluorescent reporter gene (like GFP) as readout. Moreover, not only effectors that are directly attached to the chromatin-associated protein can be identified, but also effectors with merely functional (but no direct) connection can be detected. This will not only allow to detect novel functional dependencies in epigenetic networks and the identification of essential components, but also enables the identification of new targets for drug development.

Your benefits at a glance

- ✓ Cellular *in vitro* assay
- ✓ No protein purification of chromatin-associated proteins is needed
- ✓ Hypothesis-free analysis of complex partners
- ✓ Quantification by standard methods like flow cytometry or fluorescence microscopy
- ✓ Identification of effectors which are only functionally linked to the chromatin-binding protein possible

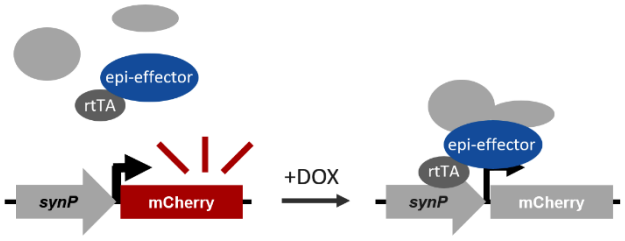

Figure: Schematic representation of the fluorescent reporter system to monitor epigenetic effector complexes. Recruitment of the reverse Tet-transactivator (rtTA) fused to an epigenetic effector protein (epi-effector) to the synthetic promoter (*synP*) is induced by the addition of Doxycycline (DOX). The induced change in fluorescence is a direct effect of the epi-effector recruitment, actively changing the chromatin environment at the synthetic promoter [Picture: University of Stuttgart].

Technology transfer

TLB GmbH manages inventions until they are marketable and offers companies opportunities for license and collaboration agreements.

Patent portfolio

A European patent application is pending.

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