GENETICALLY ENCODED LIGHT-INDUCIBLE SENSOR FOR NUCLEOLAR VISUALIZATION

Zhurlova PA, Besedovskaia ZV, Sokolinskaya EL, Putlyaeva LV ✉

Skolkovo institute of science and technology, Center for Molecular and Cellular Biology, Moscow, Russia

Nucleolus plays a vital role in enhancing rRNA production and maintaining ribosome biogenesis in tumor cells, making the nucleolus a desirable target for genetic and oncological research. The most convenient method for nucleolus monitoring is fluorescent microscopy, combining high efficiency and accessibility. Nevertheless, currently available fluorescent visualization methods are unsuitable for live-cell monitoring of nucleolus because they require continuous labeling. To address this issue, we have developed a genetically encoded Light-Activated Nucleolus Sensing (LANS) system for real-time nucleolar visualization. The combination of eMags domains and reader domain of DPF3 protein, responsible respectively for the light-induced dimerization and targeting the nucleolus, allowed LANS system to efficiently target nucleolus in several cancer cell lines without affecting cell morphology. This system makes it possible to increase the representation of the LANS2 sample in the nucleolus by 1.5 times relative to the fluorescence intensity values obtained before irradiation of the nucleolus. LANS holds the potential to accelerate the search for new drugs and enhance the primary screening of drug compounds in *in vivo* models.

Keywords: epigenetics, fluorescent proteins, eMags, optogenetics, nucleolus

Funding: this work was supported by the Russian Science Foundation grant № 22-24-01109.

✉ **Correspondence should be addressed:** Lidia V. Putlyaeva
Bolshoy Boulevard, 30, Moscow, Russia, 121205; lidia.mikhailova@mail.ru

Received: 27.11.2023 **Accepted:** 11.12.2023 **Published online:** 17.12.2023

DOI: 10.24075/brsmu.2023.048

СОЗДАНИЕ ГЕНЕТИЧЕСКИ КОДИРУЕМОГО СВЕТОИНДУЦИРУЕМОГО СЕНСОРА ДЛЯ ВИЗУАЛИЗАЦИИ ЯДРЫШКА

П. А. Журлова, З. В. Беседовская, Е. Л. Соколинская, Л. В. Путляева ✉

Сколковский институт науки и технологий, Центр молекулярной и клеточной биологии, Москва, Россия

Ядрышки участвуют в усилении продукции рРНК и поддержании биогенеза рибосом в опухолевых клетках, что делает их желанной мишенью в генетических и онкологических исследованиях. Наиболее удобным методом визуализации ядрышка благодаря своей эффективности и доступности является флуоресцентная микроскопия. В настоящее время методы визуализации ядрышек либо неточны, либо непригодны для живых клеток, либо требуют постоянного окрашивания для живого мониторинга. Целью исследования было создать генетически кодируемую систему фотоиндуцируемых сенсоров (light-activated nucleolus sensor, LANS) для визуализации ядрышка в реальном времени. Комбинация домена eMag для светоиндуцируемой димеризации и ридерного домена белка DPF3 позволяет системе LANS эффективно осуществлять направленное перемещение целевого белка в ядрышко посредством облучения клетки короткими импульсами синего света, не влияя на морфологию клеток. Данная система позволяет увеличить представленность пробы LANS2 в ядрышке в 1,5 раза относительно значений интенсивности флуоресценции, полученных до облучения ядрышка. Созданная система сенсоров LANS позволит ускорить поиск новых лекарств и улучшить первичный скрининг лекарственных соединений в моделях *in vivo*.

Ключевые слова: эпигенетика, флуоресцентные белки, eMags, оптогенетика, ядрышко

Финансирование: работа была поддержана грантом РНФ № 22-24-01109.

✉ **Для корреспонденции:** Лидия Викторовна Путляева
Большой бульвар, д. 30, стр. 1, г. Москва, Россия, 121205; lidia.mikhailova@mail.ru

Статья получена: 27.11.2023 **Статья принята к печати:** 11.12.2023 **Опубликована онлайн:** 17.12.2023

DOI: 10.24075/vrgmu.2023.048

The nucleolus is an intranuclear compartment that orchestrates cell cycle regulation, ribosome biogenesis, telomerase activity, p53 metabolism, and small RNA processing. Nevertheless, the primary role of nucleolus is the ribosome biogenesis, one of the most energy-intensive and tightly regulated processes in a cell [1]. Downregulation of rRNA gene transcription is related to the reduction in nucleolar size, while the amount of nucleolar rRNA has a positive correlation with the rate of protein synthesis and cell growth [2, 3]. Nucleolus undergoes changes during carcinogenesis, including increase in the size and abnormally stimulated functions, altering molecular pathways of such tumor suppressors as pRb, p53, c-Myc, cyclin D1, NF-kB, ErbB3, BCL-2, RAD51, and BCL-2 [4–8]. Numerous therapeutic strategies rely on targeting nucleolus, including using the selective inhibitors of RNA Pol I, mTOR, AKT, etc. [9] in therapy of haematological cancers [10, 11], non–small cell lung cancer [12], renal cell carcinoma, breast cancer, lymphoma [13], and others. Therefore, the development of new chemotherapeutic agents as well as studying their potential side effects may benefit from nucleolar visualization methods.

The most common method to visualize nucleolus is immunofluorescent staining using antibodies specific to nucleolar proteins [14]. Immunofluorescence is only compatible with fixed cells, therefore it cannot be used to study dynamic cell processes, while fluorescent microscopy appears to be a possible solution allowing for research of spatio-temporal changes in the nucleolus. [15]. Recently, a fluorescent probe for simultaneous mitochondria and nucleoli visualization was developed. It contained two emission metal complexes based on pyrazole linked to triphenylphosphine and copper (C1 and C2) [16]. Another fluorescent probe using a 9-(dicyanovinyl)julolidine (DCVJ) rotor was shown to efficiently target both mitochondria and nucleoli with minimal cytotoxicity [17]. These methods are useful for both imaging of live cells and monitoring their responses to stimuli, yet they are limited by the fact that fluorescent dyes degrade over time and are not inherited by daughter cells.

Genetically encoded fluorescent sensors overcome the limitations of the fluorescent dyes, providing the same flexibility, specificity, and compatibility with biological systems. Additionally, these sensors are non-toxic, suitable for real-time monitoring, and more time-efficient compared to fixed-cell analyses [18]. Recently, researchers have introduced a novel optogenetic system known as enhanced Magnets (eMags) [19]. This system is based on photodimerizing protein domains derived from the photoreceptor Vivid (VVD) found in Neurospora crassa. When exposed to blue light, oppositely charged VVD monomers, named eMagA and eMagB, undergo conformational changes forming dimers. The dimers dissociate without the presence of blue light, therefore the system can be used for the reversible light-dependent dimerization of the target proteins.

In this study, we developed the Light-Activated Nucleolus Sensing (LANS), a novel genetically encoded fluorescent sensor system for real-time nucleolar monitoring. LANS take advantage of eMags light-dependent dimerization, suggesting possible application of the sensor in recruiting proteins of interest to the nucleolus using light stimuli. The LANS system can be beneficial for biomedical research, specifically for testing drugs affecting nucleolus functioning.

METHODS

Molecular Cloning

All plasmids were constructed using the Golden Gate cloning system [20] and the MoClo Toolkit vector set (AddGene Kit #1000000044). The sequences of eMagA and eMagB were obtained from [19] and synthesized by "Cloning Facility" (Moscow, Russia) in the pAGM1301 vector. The DPF3 sequence was amplified using the following primers (DPF3_CCAT_FOR gttaGAAGACatCCATgggaacagtcattcccaataact actgtgacttctgcttgggggctccaacatgaacaagaagagtgggcggcc, DPF3_AATG_REV gttaGAAGACatCATTGTGGCGACCGG TCCGGATCCGCCCCCGCCGCTtttgagcagttcccag), which introduced a BpiI restriction site into the sequence, and then cloned into the pAGM1276 vector.

The MoClo technology and pICH47732 vector were used for the assembly of the final plasmids eMagB-mScarlet(LANS2) and DPF3-mNeonGreen-NLS-eMagA(LANS1), following the protocol described in [20]. BpiI (BbsI) and Eco31I (BsaI) restriction enzymes (Thermo Scientific, Waltham, MA; USA) and T4 phage ligase (Evrogen; Russia) were used for cloning.

Cell Culture and Transfection

HeLa Kyoto cells were cultured at 37 °C (5% CO_2) in DMEM medium (PanEco; Russia) supplemented with 10% fetal bovine serum (BioSera, France), 100 U/ml penicillin, and 100 μg/ml streptomycin (PanEco; Russia). For transfection, HeLa cells were cultured in DMEM-full medium in 35 mm Petri dishes with glass bottoms (SPL Life Sciences; Korea) and transfected using GenJect-39 reagent (Molecta; Russia) following the manufacturer's instructions.

Live Cell Fluorescence Microscopy

For live-cell visualization, cells were cultured in confocal dishes with glass bottoms (SPL Life Sciences). Just before microscopy, the DMEM medium was replaced with MEM visualization medium (PanEco) supplemented with 10% fetal bovine serum (BioSera) and 20 mM HEPES (Corning, New York, NY, USA). *In vivo* fluorescence microscopy was performed using the Keyence Biorevo BZ-9000 fluorescent microscope (Keyence; Japan). Cells were imaged at 60x magnification using the CFI Plan Apo λ60xH/NA1.40 objective. Images were acquired in two channels: green (GFP-B filter cube, excitation 480/30 nm) for blue light irradiation of cells for 200 ms and visualization of LANS1, red (Texas Red filter cube, excitation 560/40 nm, emission 630/75 nm) for detection of LANS2 fluorescence.

Image Analysis

To calculate relative nucleolus to cytoplasm distribution of the sensors over time, the “ROI manager” tool of the Fiji software was used. First ROI was manually set by tracing the nucleolus, second ROI was set as an area of the cytoplasm in the same cell. The values corresponding to nucleolus/cytoplasm ratio (Fluorescence intensity; Fig. 1) were obtained by first dividing intensity of the first ROI (normalized by the area) by the corresponding value of the second ROI, and second dividing the obtained value by the same value corresponding to the preinduction image. Graph was made with GraphPad Prism 8.

RESULTS

We engineered a genetically encoded fluorescent system LANS, which is based on a photodimerizing pair of probes, LANS1 and LANS2. LANS1 consists of the PHD domain sequence taken from the DPF3 protein, the green fluorescent protein mNeonGreen, a nuclear localization signal (NLS), and the eMagA (Fig. 1A, upper panel). eMagA is part of the light-dependent enhanced Magnets (eMags) system, based on the Vivid photoreceptor (VVD) from Neurospora crassa [19]. The complementary LANS2 contains the eMagB and the red fluorescent protein mScarlet (Fig. 1A, lower panel). Also, DPF3, is a domain with affinity for the histone modification H3K4me1 in its dimeric state, however, in our laboratory it has been experimentally demonstrated that the sensor based on the monomeric DPF3 accumulates in the nucleolus (Fig. 1C). Therefore, before blue light induction LANS1 was enriched in the nucleolar regions, while LANS2 was distributed uniformly across the cellular compartments. After the 200 ms induction LANS1 and LANS2 interacted by oppositely charged eMag domains, forming a heterodimer (Fig. 1B).

To demonstrate the reversibility of LANS2 binding to the nucleolus, we exposed cells expressing LANS to 200 ms of blue light, followed by incubation in darkness. We compared the relative changes in fluorescence levels before and after the exposure and revealed that the probe returned to its initial distribution within approximately 60 seconds (Fig. 1D).

Our findings demonstrate the potential of the developed LANS system for reversible light-dependent visualization of the nucleolus compartment in live cells. A combination of LANS1 enriching the nucleoli regions and LANS2 freely moving across the cell enabled recruitment of LANS2 to the nucleolar regions through brief exposure to blue light. Moreover, this sensor can be effectively used to investigate nucleolus dynamics and to directly transport proteins of interest to the nucleolus by fusing the proteins to LANS2.

DISCUSSION

The nucleolus is a dynamic subnuclear compartment, which plays a crucial role in ribonucleoprotein assembly for ribosome biogenesis as well as in rRNA synthesis and processing. Therefore, nucleolar alterations are associated with aging and

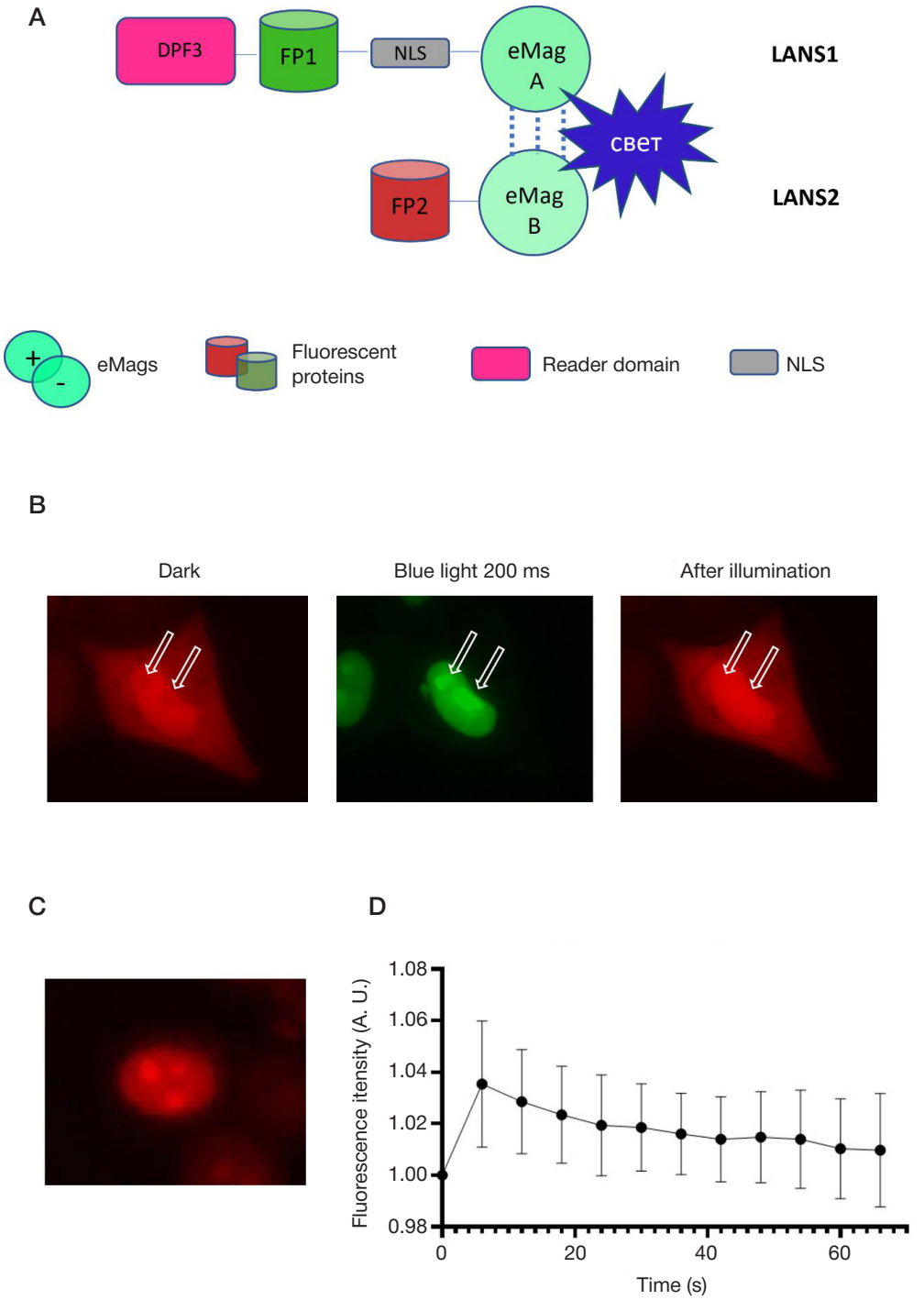

Fig. 1. Characterization of the genetically engineered LANS system enabling light-dependent recruitment of LANS2 to nucleoli. **A**. Schematic representation of the lightdependent dimerization probes LANS1 and LANS2. FP=fluorescent protein. **B**. Fluorescence microscopy images of HeLa cells transfected with LANS1 and LANS2 before (*left*), during (*middle*), and after (*right*) blue light irradiation. White arrows indicate the nucleoli. **C**. Fluorescence image of the nucleolus in HeLa cell expressing the DPF3-mScarlet sensor. **D**. Graph showing the nucleolus/cytoplasm relative fluorescence ratio for LANS2 during the experiment. Data represent mean ± range (n = 33 nucleoli)

are critical in development of different human pathologies, including cancer and neurodegeneration [21]. Numerous research laboratories are actively developing optogenetic delivery systems to target proteins to the nucleolus [22]. These systems rely on photosensitive proteins that undergo structural changes and form dimers when exposed to specific wavelengths of light. As a result, such systems bring target proteins together. Researchers have effectively utilized photodimerizing domains to control diverse cellular processes, including signaling pathways [23, 24], organelle transport [25, 26], nucleocytoplasmic transport [27, 28], cytoskeleton dynamics [29], and phase separation [30, 31].

The results obtained in this work indicating the possibility of light-induced targeting into the nucleus, are quite consistent with data obtained in other laboratories. For example, the photoinduced nuclear localization signal LINuS allowed researchers to translocate the mCherry protein from the cytoplasm into the nucleus, increasing its representation in the nucleus by approximately 1.8 times [32], while the LANS system allowed us to increase the representation of the LANS2 probe in the nucleolus by 1.5 times regarding to the fluorescence intensity values obtained before irradiation of the nucleolus. Thus, this sensor system may be useful for light-induced recruitment of target proteins into the nucleolus.

CONCLUSIONS

In this study we introduced LANS, a novel genetically encoded light-dependent sensor system, which exhibits minimal cellular toxicity. By triggering the light-induced heterodimerization of the sensor system by eMagA and eMagB photodomains, we observed the translocation of theLANS2 probe to the nucleolus, allowing us to visualize this compartment. It is important to mention that the nucleolus/cytoplasm fluorescence intensity ratio before and after irradiation is relatively modest (Fig. 1D), likely due to the high expression levels of the LANS1 and LANS2 in our experimental setup. This concern can be addressed in the future by optimizing the concentrations of sensor components or creating a stable cell line expressing LANS.

References

1. Engbrecht M, Mangerich A. The Nucleolus and PARP1 in Cancer Biology. Cancers. 2020; 12.
2. Bersaglieri C, Santoro R. Genome Organization in and around the Nucleolus. Cells. 2019; 8.
3. Weeks SE, Metge BJ, Samant RS. The nucleolus: a central response hub for the stressors that drive cancer progression. Cell Mol Life Sci. 2019; 76: 4511–24.
4. Treré D, Ceccarelli C, Montanaro L, Tosti E, Derenzini M. Nucleolar size and activity are related to pRb and p53 status in human breast cancer. J Histochem Cytochem. 2004; 52: 1601–7.
5. Russo A, Russo G. Ribosomal Proteins Control or Bypass p53 during Nucleolar Stress. Int J Mol Sci. 2017; 18.
6. Peddibhotla S, Wei Z, Papineni R, Lam MH, Rosen JM, Zhang P. The DNA damage effector Chk1 kinase regulates Cdc14B nucleolar shuttling during cell cycle progression. Cell Cycle. 2011; 10: 671–9.
7. Andrique L, Fauvin D, El Maassarani M, Colasson H, Vannier B, Séité P. ErbB3(80 kDa), a nuclear variant of the ErbB3 receptor, binds to the Cyclin D1 promoter to activate cell proliferation but is negatively controlled by p14ARF. Cell Signal. 2012; 24: 1074–85.
8. Sasaki M, Kawahara K, Nishio M, Mimori K, Kogo R, Hamada K, et al. Regulation of the MDM2-P53 pathway and tumor growth by PICT1 via nucleolar RPL11. Nat Med. 2011; 17: 944–51.
9. Carotenuto P, Pecoraro A, Palma G, Russo G, Russo A. Therapeutic Approaches Targeting Nucleolus in Cancer. Cells. 2019; 8.
10. Bywater MJ, Poortinga G, Sanij E, Hein N, Peck A, Cullinane C, et al. Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. Cancer Cell. 2012; 22: 51–65.
11. Quin JE, Devlin JR, Cameron D, Hannan KM, Pearson RB, Hannan RD. Targeting the nucleolus for cancer intervention. Biochim Biophys Acta. 2014; 1842: 802–16.
12. Chan JC, Hannan KM, Riddell K, Ng PY, Peck A, Lee RS, et al. AKT promotes rRNA synthesis and cooperates with c-MYC to stimulate ribosome biogenesis in cancer. Sci Signal. 2011; 4: ra56.
13. Wall M, Poortinga G, Stanley KL, Lindemann RK, Bots M, Chan CJ, et al. The mTORC1 inhibitor everolimus prevents and treats Eμ-Myc lymphoma by restoring oncogene-induced senescence. Cancer Discov. 2013; 3: 82–95.
14. Stenström L, Mahdessian D, Gnann C, Cesnik AJ, Ouyang W, Leonetti MD, et al. Mapping the nucleolar proteome reveals a spatiotemporal organization related to intrinsic protein disorder. Mol Syst Biol. 2020; 16: e9469.
15. Farhy C, Hariharan S, Ylanko J, Orozco L, Zeng F-Y, Pass I, et al. Improving drug discovery using image-based multiparametric analysis of the epigenetic landscape. Elife. 2019; 8.
16. Mayank, Rani R, Singh A, Garg N, Kaur N, Singh N. Mitochondria- and nucleolus-targeted copper(i) complexes with pyrazole-linked triphenylphosphine moieties for live cell imaging. Analyst. 2019; 145: 83–90.
17. Mukherjee T, Soppina V, Ludovic R, Mély Y, Klymchenko AS, Collot M, et al. Live-cell imaging of the nucleolus and mapping mitochondrial viscosity with a dual function fluorescent probe. Org Biomol Chem. 2021; 19: 3389–95.
18. Martin RM, Herce HD, Ludwig AK, Cardoso MC. Visualization of the Nucleolus in Living Cells with Cell-Penetrating Fluorescent Peptides. Methods Mol Biol. 2016; 1455: 71–82.
19. Benedetti L, Marvin JS, Falahati H, Guillén-Samander A, Looger LL, De Camilli P. Optimized Vivid-derived Magnets photodimerizers for subcellular optogenetics in mammalian cells. Elife. 2020; 9.
20. Werner S, Engler C, Weber E, Gruetzner R, Marillonnet S. Fast track assembly of multigene constructs using Golden Gate cloning and the MoClo system. Bioeng Bugs. 2012; 3: 38–43.
21. Corman A, Sirozh O, Lafarga V, Fernandez-Capetillo O. Targeting the nucleolus as a therapeutic strategy in human disease. Trends Biochem Sci. 2023; 48: 274–87.
22. Tan P, Hong T, Cai X, Li W, Huang Y, He L, et al. Optical control of protein delivery and partitioning in the nucleolus. Nucleic Acids Res. 2022; 50: e69.
23. Gray NW, Weimer RM, Bureau I, Svoboda K. Rapid redistribution of synaptic PSD-95 in the neocortex in vivo. PLoS Biol. 2006; 4: e370.
24. Grusch M, Schelch K, Riedler R, Reichhart E, Differ C, Berger W, et al. Spatio-temporally precise activation of engineered receptor tyrosine kinases by light. EMBO J. 2014; 33: 1713–26.
25. van Bergeijk P, Adrian M, Hoogenraad CC, Kapitein LC. Optogenetic control of organelle transport and positioning. Nature. 2015; 518: 111–4.
26. Duan L, Che D, Zhang K, Ong Q, Guo S, Cui B. Optogenetic control of molecular motors and organelle distributions in cells. Chem Biol. 2015; 22: 671–82.
27. Lerner AM, Yumerefendi H, Goudy OJ, Strahl BD, Kuhlman B. Engineering Improved Photoswitches for the Control of Nucleocytoplasmic Distribution. ACS Synth Biol. 2018; 7: 2898–907.
28. Niopek D, Wehler P, Roensch J, Eils R, Di Ventura B. Optogenetic control of nuclear protein export. Nat Commun. 2016; 7: 10624.
29. van Haren J, Charafeddine RA, Ettinger A, Wang H, Hahn KM, Wittmann T. Local control of intracellular microtubule dynamics by EB1 photodissociation. Nat Cell Biol. 2018; 20: 252–61.
30. Shin Y, Berry J, Pannucci N, Haataja MP, Toettcher JE, Brangwynne CP. Spatiotemporal Control of Intracellular Phase Transitions Using Light-Activated optoDroplets. Cell. 2017; 168: 159–71.e14.
31. Dine E, Gil AA, Uribe G, Brangwynne CP, Toettcher JE. Protein Phase Separation Provides Long-Term Memory of Transient Spatial Stimuli. Cell Syst. 2018; 6: 655–63.e5.
32. Niopek D, Benzinger D, Roensch J, Draebing T, Wehler P, Eils R, et al. Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells. Nat Commun. 2014; 5: 4404.

Литература

1. Engbrecht M, Mangerich A. The Nucleolus and PARP1 in Cancer Biology. Cancers. 2020; 12.
2. Bersaglieri C, Santoro R. Genome Organization in and around the Nucleolus. Cells. 2019; 8.
3. Weeks SE, Metge BJ, Samant RS. The nucleolus: a central response hub for the stressors that drive cancer progression. Cell Mol Life Sci. 2019; 76: 4511–24.
4. Treré D, Ceccarelli C, Montanaro L, Tosti E, Derenzini M. Nucleolar size and activity are related to pRb and p53 status in human breast cancer. J Histochem Cytochem. 2004; 52: 1601–7.

5. Russo A, Russo G. Ribosomal Proteins Control or Bypass p53 during Nucleolar Stress. Int J Mol Sci. 2017; 18.
6. Peddibhotla S, Wei Z, Papineni R, Lam MH, Rosen JM, Zhang P. The DNA damage effector Chk1 kinase regulates Cdc14B nucleolar shuttling during cell cycle progression. Cell Cycle. 2011; 10: 671–9.
7. Andrique L, Fauvin D, El Maassarani M, Colasson H, Vannier B, Séité P. ErbB3(80 kDa), a nuclear variant of the ErbB3 receptor, binds to the Cyclin D1 promoter to activate cell proliferation but is negatively controlled by p14ARF. Cell Signal. 2012; 24: 1074–85.
8. Sasaki M, Kawahara K, Nishio M, Mimori K, Kogo R, Hamada K, et al. Regulation of the MDM2-P53 pathway and tumor growth by PICT1 via nucleolar RPL11. Nat Med. 2011; 17: 944–51.
9. Carotenuto P, Pecoraro A, Palma G, Russo G, Russo A. Therapeutic Approaches Targeting Nucleolus in Cancer. Cells. 2019; 8.
10. Bywater MJ, Poortinga G, Sanij E, Hein N, Peck A, Cullinane C, et al. Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. Cancer Cell. 2012; 22: 51–65.
11. Quin JE, Devlin JR, Cameron D, Hannan KM, Pearson RB, Hannan RD. Targeting the nucleolus for cancer intervention. Biochim Biophys Acta. 2014; 1842: 802–16.
12. Chan JC, Hannan KM, Riddell K, Ng PY, Peck A, Lee RS, et al. AKT promotes rRNA synthesis and cooperates with c-MYC to stimulate ribosome biogenesis in cancer. Sci Signal. 2011; 4: ra56.
13. Wall M, Poortinga G, Stanley KL, Lindemann RK, Bots M, Chan CJ, et al. The mTORC1 inhibitor everolimus prevents and treats Eμ-Myc lymphoma by restoring oncogene-induced senescence. Cancer Discov. 2013; 3: 82–95.
14. Stenström L, Mahdessian D, Gnann C, Cesnik AJ, Ouyang W, Leonetti MD, et al. Mapping the nucleolar proteome reveals a spatiotemporal organization related to intrinsic protein disorder. Mol Syst Biol. 2020; 16: e9469.
15. Farhy C, Hariharan S, Ylanko J, Orozco L, Zeng F-Y, Pass I, et al. Improving drug discovery using image-based multiparametric analysis of the epigenetic landscape. Elife. 2019; 8.
16. Mayank, Rani R, Singh A, Garg N, Kaur N, Singh N. Mitochondria- and nucleolus-targeted copper(i) complexes with pyrazole-linked triphenylphosphine moieties for live cell imaging. Analyst. 2019; 145: 83–90.
17. Mukherjee T, Soppina V, Ludovic R, Mély Y, Klymchenko AS, Collot M, et al. Live-cell imaging of the nucleolus and mapping mitochondrial viscosity with a dual function fluorescent probe. Org Biomol Chem. 2021; 19: 3389–95.
18. Martin RM, Herce HD, Ludwig AK, Cardoso MC. Visualization of the Nucleolus in Living Cells with Cell-Penetrating Fluorescent Peptides. Methods Mol Biol. 2016; 1455: 71–82.
19. Benedetti L, Marvin JS, Falahati H, Guillén-Samander A, Looger LL, De Camilli P. Optimized Vivid-derived Magnets photodimerizers for subcellular optogenetics in mammalian cells. Elife. 2020; 9.
20. Werner S, Engler C, Weber E, Gruetzner R, Marillonnet S. Fast track assembly of multigene constructs using Golden Gate cloning and the MoClo system. Bioeng Bugs. 2012; 3: 38–43.
21. Corman A, Sirozh O, Lafarga V, Fernandez-Capetillo O. Targeting the nucleolus as a therapeutic strategy in human disease. Trends Biochem Sci. 2023; 48: 274–87.
22. Tan P, Hong T, Cai X, Li W, Huang Y, He L, et al. Optical control of protein delivery and partitioning in the nucleolus. Nucleic Acids Res. 2022; 50: e69.
23. Gray NW, Weimer RM, Bureau I, Svoboda K. Rapid redistribution of synaptic PSD-95 in the neocortex in vivo. PLoS Biol. 2006; 4: e370.
24. Grusch M, Schelch K, Riedler R, Reichhart E, Differ C, Berger W, et al. Spatio-temporally precise activation of engineered receptor tyrosine kinases by light. EMBO J. 2014; 33: 1713–26.
25. van Bergeijk P, Adrian M, Hoogenraad CC, Kapitein LC. Optogenetic control of organelle transport and positioning. Nature. 2015; 518: 111–4.
26. Duan L, Che D, Zhang K, Ong Q, Guo S, Cui B. Optogenetic control of molecular motors and organelle distributions in cells. Chem Biol. 2015; 22: 671–82.
27. Lerner AM, Yumerefendi H, Goudy OJ, Strahl BD, Kuhlman B. Engineering Improved Photoswitches for the Control of Nucleocytoplasmic Distribution. ACS Synth Biol. 2018; 7: 2898–907.
28. Niopek D, Wehler P, Roensch J, Eils R, Di Ventura B. Optogenetic control of nuclear protein export. Nat Commun. 2016; 7: 10624.
29. van Haren J, Charafeddine RA, Ettinger A, Wang H, Hahn KM, Wittmann T. Local control of intracellular microtubule dynamics by EB1 photodissociation. Nat Cell Biol. 2018; 20: 252–61.
30. Shin Y, Berry J, Pannucci N, Haataja MP, Toettcher JE, Brangwynne CP. Spatiotemporal Control of Intracellular Phase Transitions Using Light-Activated optoDroplets. Cell. 2017; 168: 159–71.e14.
31. Dine E, Gil AA, Uribe G, Brangwynne CP, Toettcher JE. Protein Phase Separation Provides Long-Term Memory of Transient Spatial Stimuli. Cell Syst. 2018; 6: 655–63.e5.
32. Niopek D, Benzinger D, Roensch J, Draebing T, Wehler P, Eils R, et al. Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells. Nat Commun. 2014; 5: 4404.