FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)-BASED DIRECT SENSOR OF RANGTP

Tech ID: 17267 / UC Case 2003-066-0

ABSTRACT

Scientists at the University of California Berkeley have designed FRET-based sensor of Ran-GTP that is a fluorescent protein construct consisting of Ran binding domain flanked by fluorescent proteins (donor and acceptor) capable of FRET.

The sensor functions as:

1) a direct sensor of Ran-GTP, and

2) an indirect sensor of Ran-GTP-binding proteins such as importin-b family proteins.

See:

P. Kalab, Weis, K., and Heald, R. (2002), Visualization of a Ran-GTP Gradient in Interphase and Mitotic Xenopus Egg Extracts. Science, March 29, 2002, Vol. 295, 2452-2456.

The small guanosine triphosphate Ran is loaded with guanosine tripohosphate (GTP) by the chromatin bound guanine nucleotide exchange factor RCC1 and releases import cargoes in the nucleus during interphase. In mitosis, Ran-GTP promotes spindle assembly around the chromosomes by locally discharging cargoes that regulate microtubule dynamics and organization. We used fluorescence energy transfer-based biosensors to visualize gradients of Ran-GTP and liberated cargoes around chromosomes in mitotic Xenopus egg extacts. Both gradients were required to assemble and maintain spindle structure. During interphase, Ran-GTP was highly enriched in the nucleoplasm, and a steep concentration difference between nuclear and cytoplasmic Ran-GTP was established, providing evidence for a Ran-GTP gradient surrounding chromosomes throughout the cell cycle.

APPLICATIONS

spatially resolved and quantitative analysis of Ran-GTP function in various systems, including living cells and organisms.

readout system in screens for the modulators and inhibitors of Ran, RCC1, Ran GAP and other components of Ran system.

ADVANTAGES

a direct sensor of Ran-GTP.

an indirect sensor of Ran-GTP-binding proteins such as importin-b family proteins.

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OTHER INFORMATION

KEYWORDS

research tool, vector

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2003-066-0

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