Application Note
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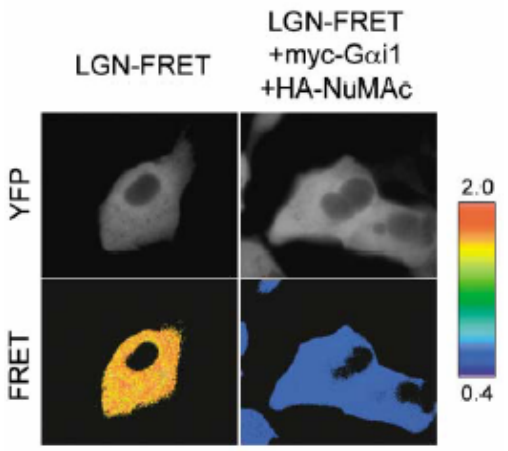
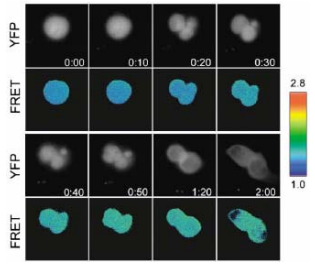
Using FRET to study mitotic spindle orientation





Time-lapse analysis of LGN-FRET probe during and after mitosis

Prof Ian Macara, from the Department of Microbiology at the University of Virginia School of Medicine, focuses his research activity on understanding cell asymmetry. One of the lab's ongoing programs is to study mitotic spindle orientation during asymmetric cell divisions. Spindle formation and orientation play a key role in accurate chromosome segregation and further details about this mechanism would enable researchers to better understand diseases caused by missegregation of chromosomes.

During asymmetric cell divisions, spindles align along the axis of polarization. In flies this requires the interaction of at least two proteins, Pins (Partner of Inscuteable) and a G-protein alpha subunit. LGN is a mamalian Pins that was used for the experiment. It binds to the alpha subunit of protein Gαi which is located at the cell cortex. The Gα protein is thought to play a role in a signalling pathway for spindle orientation control but little is known about its mechanism.

Once the nuclear membrane disassembles during mitosis, LGN also binds to a nuclear protein called NuMA. NuMA is known to have a Microtubule (MT) binding domain therefore allowing it to bind to both MTs and LGN, but this cannot be done simultaneously. Also, NuMA is necessary for LGN to bind the cell cortex. Prof. Macara and Dr. Quansheng Du used the FRET technique to understand how the trimeric NuMA/LGN/Gα complex regulates the interaction of MTs with the cell cortex.

An ***Openlab*** cell imaging system was used to carry out time-lapse experiments. After background substraction, FRET images were created using the ***Openlab FRET module***. The image on the left shows that FRET is taking place in cells transfected with LGN protein (High FRET: yellow) but stops when cells are also transfected with Gα and NuMA (Low FRET: blue). This result indicates that in the absence of Gα and NuMA, LGN is folded and its N and C labeled termini are physically close, whereas in presence of Gα and NUMA, LNG unfolds, the distance between its N and C termini rises and the FRET signal is decreased. Using ***Openlab*** for FRET analysis, Prof. Macara was able to demonstrate that LGN functions as a conformational switch, responding to Gα and NuMA binding.

Module configuration

Module Families	Critical Points
Camera ORCA CCD	High resolution, high dynamic range camera used to acquire high quality digital images
Hardware Focus Drive Filters & shutters	High quality excitation filters can be used with a triple band pass emission filter
File Filter TIFF file filter PICT file filter	Image sequences can be saved in other formats, individual PICTs or TIFFs or QuickTime movies Images can be annotated for publication
Application FRET module	Provides a choice of analysis expressions for the generation of FRET result images.
Automation Automator	Easy to use and powerful icon based programming language for automated imaging experiments