Analyses of the actin cytoskeleton using fluorescence resonance energy transfer (FRET)

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Actin is the principal component of microfilaments whose assembly/disassembly is essential for cell motility. It is present in the nucleus, where it may regulate gene expression. Cofilin is the principal regulator of actin assembly in cells. It can bind actin and translocate it into the nucleus during times of stress.

We used fluorescence resonance energy transfer (FRET) and confocal microscopy to analyse the interactions of cofilin and G-actin in the nucleus and cytoplasm. By measuring the rate of photobleaching of fluorescein-labeled actin ± Cy5-labeled cofilin, we show that most of the nuclear G-actin is bound to cofilin, but only half is bound in the cytoplasm. A significant proportion of cofilin in the nucleus and cytoplasm binds added TMR-labeled G-actin. These data suggest there is significantly more cofilin-G-actin complex and less free cofilin in the nucleus.

The actin cytoskeleton can also be probed in solution using FRET spectroscopy. This method can not only detect binding events but it can also detect structural changes in these proteins. We recently demonstrated that thymosin β_4 ($t\beta_4$) binding induces spatial rearrangements within subdomains 1 and 2 of G-actin. $T\beta_4$ binding increases the distance between Gln-41 and Cys-374 of actin by 2 Å and decreases the distance between bound ATP (βATP at the NUC site) and Lys-61 by 1.9 Å. The distance between Cys-374 and Lys-61 is minimally affected. Our results favour a model where $t\beta_4$ changes the orientation of actin subdomain 2. This conformational change presumably accounts for the reduced rate of nucleotide and amide hydrogen exchange from actin monomers.