Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602100419

Spatial and temporal assays to determine the dynamics of protein localisation and organelle movement in single living cells

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Visualisation and monitoring of protein localisation and function using Green Fluorescent Protein (GFP) tagged proteins is a well-established approach. We have sought to develop robust assays for quantifying the dynamic localisation of proteins at the single cell level. This approach allows us to determine properties of trafficking wild-type proteins and their mutated counterparts. It provides a functional genomics approach to screening drug effects and investigating the consequence of protein mutations. This opens the door to using combined GFP-tagging and laser scanning microscopy in a high-content medium-through-put arena.

A simple application of spatial localisation has been used to screen the targetting and delivery of chimeric proteins in sub-cellular compartments identified using vital cell markers to, for instance, endoplasmic reticulum, mitotracker and DNA.

A more advanced co-localisation approach has been developed for studying protein movement during gap junction assembly and collapse. Gap junctions are ubiquitous intercellular channels underpinning cell-cell communication. The sub-unit protein of gap junctions, connexins, are thought to be processed along the secretory pathway during which oligomerisation occurs into hemichannels. Many connexin proteins have been identified and also connexin mutations have been shown to be associated with key pathologies in which connexin (Cx) trafficking, gap junction assembly and also function are compromised. We have examined, using single and dual channel confocal microscopy, the delivery of tagged connexins to gap junctions in control conditions and after disruption of Golgi or microtubule function. To determine the kinetics of intracellular trafficking of different connexins we have acquired image sequences consisting of single optical sections collected over time(x,y,t). To quantify Cx-GFP movement multiple time points were compared and the temporal colocalisation coefficients or mobility coefficients were extracted. This works on a pixel-by-pixel basis that can be best represented in a scatter plot. However if we assign a colour red, green and blue to three chosen time points the combined or merged image provides a single view depicting the local movement of vesicles. The coefficient describes the amount of fluorescent pixels (at 30 seconds) overlapping with fluorescent pixels of a subsequent time point (at 15 minutes). If the mobility coefficient tended towards 1 then the extent of overlap was 100%, indicating no movement. If the value tended towards 0 then the extent of overlap was minimal and maximal vesicular movement had occurred. The approach is analogous to that previously described to measure spatial localisation [1]. A marked disruption of connexin movement was demonstrated in all connexin types with a typical shift in mobility coefficient from 0.7 to approximately 0.96 using nocodazole, a microtubule disrupting agent. However Brefeldin A (a drug which disrupts Golgi) had differential effects on connexin types, high-lighting alternative (or multiple) mechanisms for connexin trafficking [2]. Gap junctions are responsible for intercellular communication this is promarily via the transfer of calcium ions, however ironically intracellular calcium levels act to modulate connexin trafficking such that

sustained levels of calcium prevents gap junction assembly. We have been able to track and characterise connexin mobility and ER movement [3], using the colocalisation approach, at the same time as monitoring calcium levels in single cells. The potential implications on the regulation and modulation of gap junction communication during apoptosis where cells experience high levels of intracellular calcium will be discussed.

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https://doi.org/10.1017/S1431927602100419 Published online by Cambridge University Press