

SPATIO-TEMPORAL ANALYSIS OF MOLECULAR INTERACTIONS IN LIVING CELLS

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Immunocytochemistry (IC) and fluorescence in situ hybridisation (FISH) are methods of choice to visualise cellular molecules. In living cells however, IC is of limited value, and specific DNA sequences are difficult to detect by FISH in live cells, since denaturation is not compatible with living cell status. For live cell studies, GFP labelling is an excellent alternative, whereas single stranded RNA staining by FISH is well feasible using new labelling strategies, involving 2'-O-methyl modified oligonucleotides, PNA (peptide nucleic acid) probes or molecular beacons.

The labelling technology for RNA can be combined with GFP constructs allowing the study of the dynamics of proteins (labelled by GFP) involved in transcription, and in processing and transport of RNA. To demonstrate functional association of labelled proteins with RNA, spatial (co)localization studies are insufficient. More reliable information is obtained when functional association is assessed by measurements of fluorescence resonance energy transfer (FRET), either on the basis of spectral analysis or by life-time measurements. Finally, quantitative analysis of moving intracellular structures is facilitated by 3 and 4 D image analysis, or by applying quantitative fluorescence recovery techniques such as FRAP and FLIP.

We applied all technologies described above in an integrated way to study the role of heterochromatin protein 1 (HP 1), considered as a key component of constitutive heterochromatin and a player in gene silencing. GFP-HP-1 protein appeared to accumulate in a number of nuclear subdomains, such as PML bodies. We demonstrated that HP-1 proteins form complexes with SP 100 in PML bodies using CFP and YFP as tags, by measuring their interaction by FRET-FLIM.

We also studied the association of telomeres (stained by PNA probes) with GFP labelled PML bodies. A subfraction of telomeres was shown to associate and dissociate suggesting that in vivo telomere clusters are not stable but dynamic structures. Dynamic association with PML bodies is thought to be essential for the occurrence of homologous recombination events associated with alternative lengthening of telomeres (ALT).

To further study the dynamics of PML bodies we constructed fusion proteins of Sp100, PML and CBP with autofluorescent proteins. Using time-lapse imaging, we showed that PML bodies exhibit little movement but that small foci contain Sp100 but not PML are dynamic and fuse with PML bodies. Finally, by FRAP analysis it was found that Sp100, PML and CBP are dynamic components of PML bodies, suggesting that these proteins function at other sites in the nucleoplasm.