

MOLECULAR MECHANISMS OF GENE TRANSCRIPTION AND DNA REPAIR STUDIED BY QUANTITATIVE CONFOCAL MICROSCOPY

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The molecular mechanisms underlying DNA-interacting processes inside the living cell nucleus such as gene transcription and DNA repair are poorly understood. We use CFP, GFP and YFP-tagging of transcription and DNA-repair factors to study their function in living cells using various modes of confocal microscopy (Fig. 1), including spinning disk and high resolution 4Pi microscopy. In addition, we develop and apply quantitative fluorescence assays based on fluorescence recovery after photobleaching (FRAP) (Fig. 2, left), fluorescence correlation spectroscopy (FCS) (Fig. 2, right), and fluorescence resonance energy transfer (FRET). For optimal interpretation of the complex experimental results we use computer modeling to quantitatively analyze FRAP and combined FRET-FRAP assays (Fig. 3).

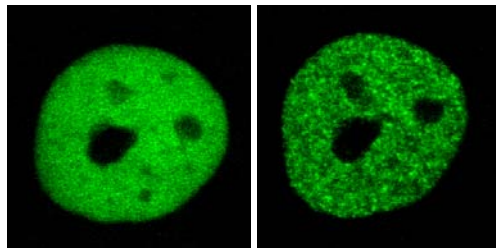


Figure 1: Living cell expressing the androgen receptor (AR) before (left) and after (right) activation with testosterone, showing that activity of this hormone induced transcription factor leads to a speckled distribution in the nucleus.

Figure 2: Complementary FRAP and FCS. FRAP (left) reveals DNA-binding of the AR for tens of seconds. FCS (right) suggests additional short term AR-DNA interactions.

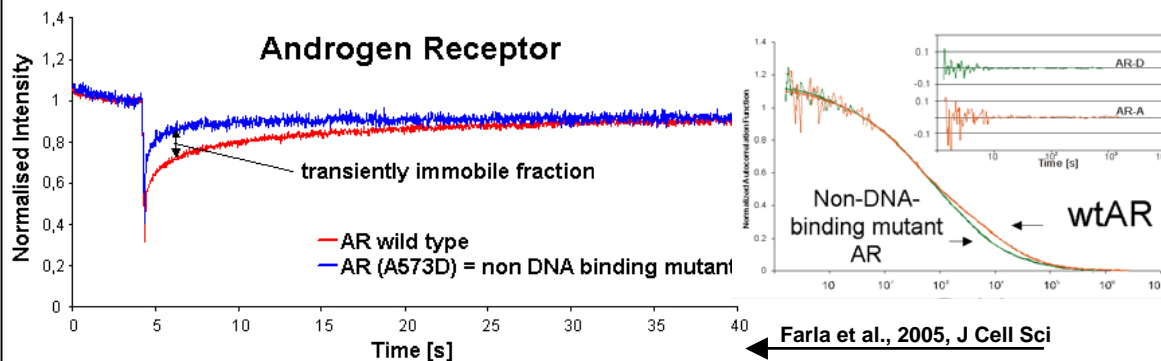


Figure 3: Combined FRET-FRAP assay. Donor fluorescence loss after acceptor bleaching (CFP signal = FRET-FRAP, lower left panel) specifically reveals the mobility of interacting proteins only.

