In Vivo Characterization of Protein-Protein Interactions in the AP1 System with Fluorescence Correlation Spectroscopy (FCS)

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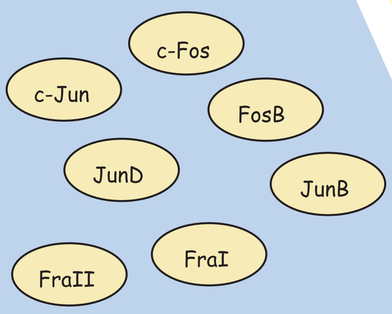
AP1 System



- group of transcription activator proteins
- subunits: c-Fos, FosB, c-Jun, JunB, JunD, FraI, FraII
-major components are c-Fos and c-Jun

Protein domains

Transactivation	DNA-Binding	Dimerization

Dimerization and DNA-binding

All proteins have a basic region leucine zipper (bZIP) for dimerization

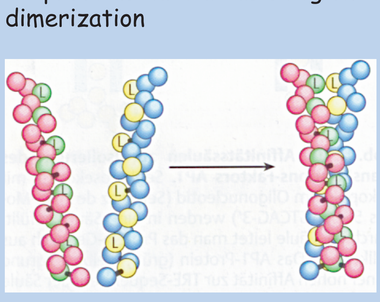


Fig.5 the leucine zipper is formed as a coiled coil

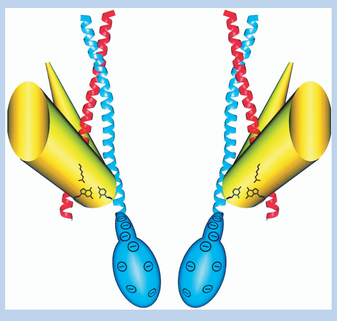


Fig.6 3D image of the AP1-DNA complex

Region around an AP1-regulated promoter

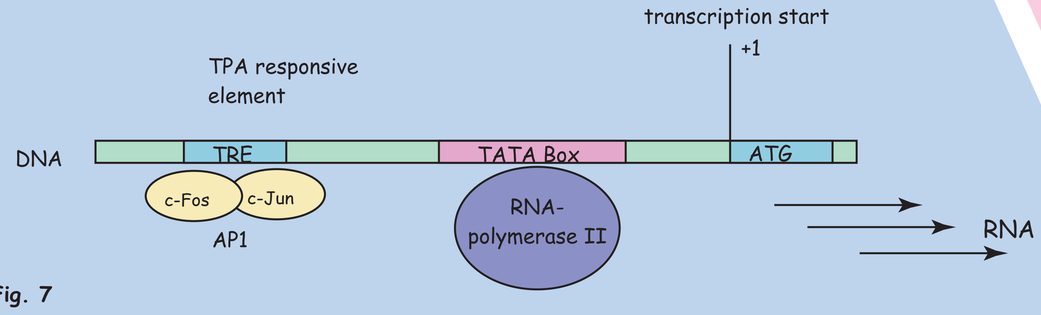
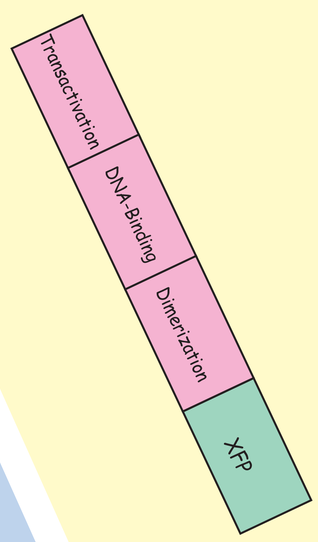


Fig. 7

- dimerization of AP1 is required for DNA binding before transcription
- AP1 binds to the TPA responsive element in the promoter region
- AP1 is controlled by phosphorylation and ubiquitination

Experiments

1. Construction of fusion proteins



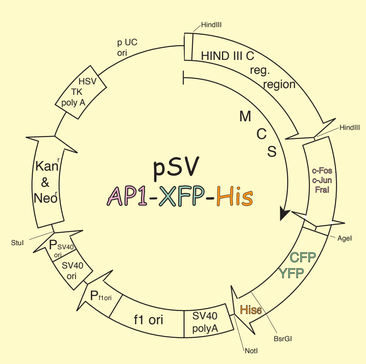


Fig. 1 plasmid map of pSV-AP1-XFP-His

2. Expression of fusion protein and FCS measurements

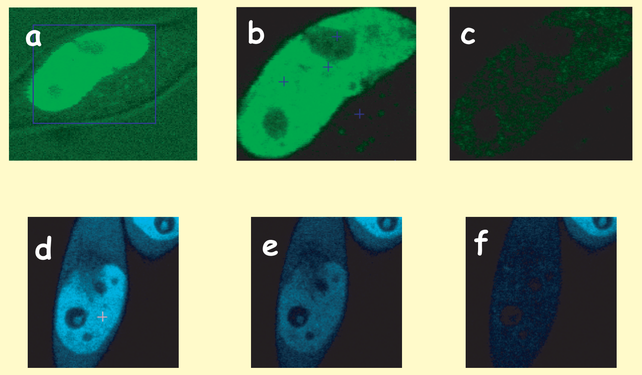


Fig. 2 Vektor **pSV-AP1-XFP** in HeLa cells stably transfected. The main fluorescence is in the nucleus (a+d). FCS measurement in the nucleus (b+e). The cells are bleached uniformly (c+f).

3. Results

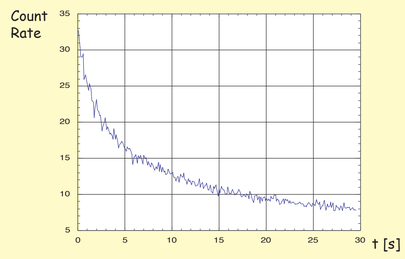


Fig. 3 Photobleaching in the nucleus of Fos-CFP cells: in the nucleus an equilibrium between the freely diffusing and the bound component of AP1 exists. By means of bleaching curves and an appropriate fit model we can calculate the dissociation rates k_{diss} in the nucleus, if the fraction of the immobilized component is known. We obtain retention periods between 10 and 20 seconds corresponding to the exchange between the free and the bound component.

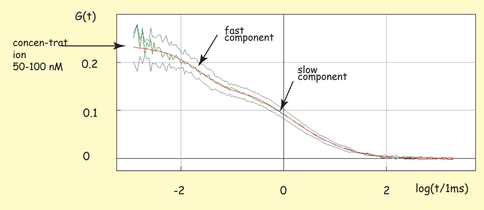


Fig. 4 FCS in the nucleus of Jun-YFP cells: a fast (μs) and a slow component (ms) of diffusion are obtained in the ACF corresponding to free and obstructed diffusion. A fast exchange in the nucleus is observed. Concentrations of the free component are 50-100 nM.

Conclusions

The aim of these studies is the quantitative investigation of protein-protein interactions in the AP1 system in vivo.

First results of FCS measurements show an exchange in the nucleus of the proteins Fos-CFP and Jun-YFP in the stably monotransfected HeLa-Cells. This is also shown by fitting the bleaching curves measured in the nucleus with an appropiate model. We obtained dissociation times between 10 and 20 seconds in the nucleus.

In the autocorrelation function a free and an obstructed component of diffusion are shown.

For further studies doubly transfected cells with both proteins, Fos-CFP and Jun-YFP, were prepared. These cells will now be characterized with FCCS to investigate the protein-protein interactions.

In order to obtain the dissociation rates of the complex in the cell nucleus bleaching curves will be recorded on these cell lines.

We also overexpressed and purified Jun-YFP and Fos-CFP for in vitro studies.

FCS and FCCS

One color FCS

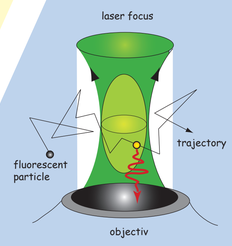


Fig. 8a

A laser is coupled into a microscope, illuminating a sub-femtoliter volume (Fig. 8a).

Fluorescent molecules cross this focus by Brownian motion.

Their fluorescence light is collected by the same optics and detected by confocally arranged detectors.

Single molecules can be detected due to the high sensivity of the detectors (avalanche photodiods).

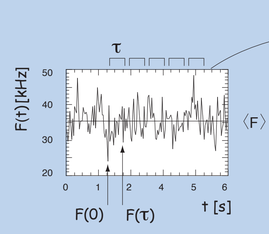


Fig. 8b

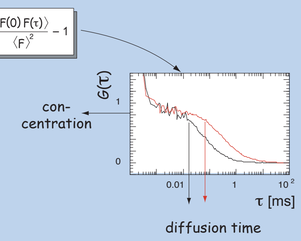


Fig. 8c

Signal fluctuations induced by molecules diffusing across the focus are recorded over time and the autocorrelation function (ACF) of the signal is calculated (Fig. 8b).

Fitting an appropriate model to the autocorrelation function results in: concentrations, diffusion coefficients and other physical parameters of the different fluorescent species in the system (Fig. 8c).

Two color FCCS

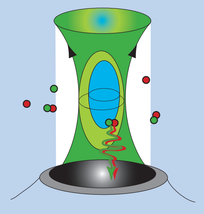


Fig. 9a

In Fluorescence Cross-Correlation Spec-troscopy (FCCS) two fluorophores with distinct spectra are detected simultaneously in two channels and their signals are cross-correlated (Fig. 9a+b).

Only particles which carry both fluorophores contribute to the cross correlation signal.

FCCS will enable us to measure for the first time protein-DNA and protein-protein interactions in living cells.

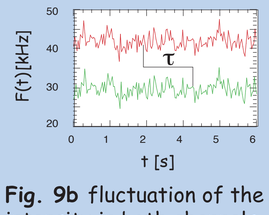


Fig. 9b fluctuation of the intensity in both channels

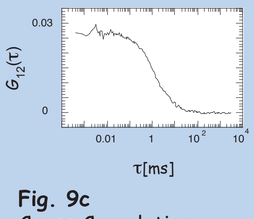


Fig. 9c Cross-Correlation

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5th Graduate Students Meeting of the German Cancer Research Center (DKFZ), Germany, May 2002.

Abstract

The aim of these studies is the quantitative investigation of protein-protein interactions in the AP1 system *in vivo*. First results of FCS measurements show an exchange in the nucleus of the proteins Fos-CFP and Jun-YFP in the stably mono-transfected HeLa-Cells. This is also shown by fitting the bleaching curves measured in the nucleus with an appropriate model. We obtained dissociation times between 10 and 20 seconds in the nucleus. In the autocorrelation function a free and an obstructed component of diffusion are shown. For further studies doubly transfected cells with both proteins, Fos-CFP and Jun-YFP, were prepared. These cells will now be characterized with FCCS to investigate the protein-protein interactions. In order to obtain the dissociation rates of the complex in the cell nucleus bleaching curves will be recorded on these cell lines. We also overexpressed and purified Jun-YFP and Fos-CFP for in vitro studies.

Keywords:

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome function, genetics, gene regulation, replication, transcription, repair, homologous recombination, simultaneous co-transfection, cell division, mitosis, metaphase, interphase, cell nucleus, nuclear structure, nuclear organization, chromatin density distribution, nuclear morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, chromatin loops, chromatin fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular transport, obstructed diffusion, anomalous diffusion, percolation, long-range correlations, fractal analysis, scaling analysis, exact yard-stick dimension, box-counting dimension, lacunarity dimension, local nuclear dimension, nuclear diffuseness, parallel super computing, grid computing, volunteer computing, Brownian Dynamics, Monte Carlo, fluorescence in situ hybridization, confocal laser scanning microscopy, fluorescence correlation spectroscopy, super resolution microscopy, spatial precision distance microscopy, auto-fluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, in vivo labelling.

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