Investigation of Fl\(^{1}\) - and TMR\(^{2}\)-dyes used for FRET

A stem-loop oligonucleotide complementary to the transactivation region of the HIV-1 genome (cTAR) was doubly labeled with a pair of dyes that are frequently used for FRET, Fl\(^{1}\) and TMR\(^{2}\). Labeling of the 5’ terminus with TMR was performed via an amino linker with a ten-carbon spacer arm. The 3’ terminus of the oligonucleotides was labeled with Fl using a special solid support with the dye already attached. Synthesis and labeling of the oligonucleotides was performed by IBA GmbH. Because both dyes are bound at the stem of the hairpin-loop, Bernacchi et al. could demonstrate that these dyes form a ground state heterodimer that shows a unique optical signature instead of the spectral properties of the individual dyes.

Figure: The oligonucleotide sequence of cTAR. (Kindly provided by Prof. Dr. Yves Mély)

REFERENCES

2-aminopurine substitution to analyze oligonucleotide – NC-peptide interaction

In this study hexanucleotides were substituted with 2-aminopurine (2Ap), a fluorescent adenine analogue, at positions 2 and 5 (custom manufactured by IBA GmbH). 2Ap is an environmentally sensitive fluorescent probe which can be used to detect oligonucleotide dynamics. Fluorescence of 2Ap within oligonucleotides is quenched due to interactions with its neighbour bases. During interaction of the oligonucleotide with NC-peptide (the HIV-1 nucleocapsid protein) quenching is reduced because of reduced oligonucleotide flexibility and 2Ap local mobility. This restriction in the oligonucleotide dynamics appears as an important mechanistic component of the nucleic acid chaperone properties of NC.

REFERENCES

A two-color fluorescence dye as alternative to FRET

Several oligonucleotides (e.g. SL2 RNA), custom produced by IBA GmbH, were used as interaction partners for the NC-peptide (the HIV-1 nucleocapsid protein) which is thought to be critically involved in the viral life cycle, mainly through interaction with nucleic acids. The NC-peptide was labeled with a fluorescent dye (3-hydroxycromone, 3HC) which has dual fluorescence emission. Upon interaction with nucleic acids, the ratio of the dye’s two emission bands changed detectably (Fig. 1). The described method is an alternative to FRET in interaction studies and has the advantage of requiring only single and not double labeling.

![Figure 1: Changes in the fluorescence emission spectra of the labeled peptide 3HC-NC on binding to SL2 RNA. The spectra of 0.4 µM 3HC-NC was recorded in the absence (black) and in the presence of 0.2 µM (red), 0.4 µM (blue) and 0.6 mM (green) SL2 RNA. Excitation wavelength was 340 nm. (Kindly provided by Prof. Dr. Yves Mély).](image)

REFERENCES

Time-resolved and FCS studies of the Hepatitis C virus core protein

The complementary oligonucleotides cTAR and dTAR form stem-loop structures and were synthesized and fluorescently labeled for time-resolved and FCS studies by IBA GmbH. In the case of the doubly labeled ODNs, the 5’ terminus was labeled with TMR\(^1\) or Rh6G\(^2\) via an amino-linker with a six carbon spacer arm, while the 3’ terminus was labeled with either Dabcyl\(^3\) or Fl\(^4\) using a special solid support with the dye already attached. The studies revealed that peptides from the Hepatitis C virus core protein chaperone the annealing of HIV-1 cTAR and dTAR, taken as models, and increase their annealing kinetics by at least three orders of magnitude. Two kinetic pathways were identified with a fast pre-equilibrium intermediate that then slowly converts into the final extended duplex. The nucleic acid chaperone properties of the core protein are mainly supported by its basic clusters.

\(^1\) carboxytetramethylrhodamine
\(^2\) Rhodamin 6G
\(^3\) 4-(40-dimethylaminophenylazo) benzoic acid
\(^4\) 5(and 6)-carboxyfluorescein

REFERENCES