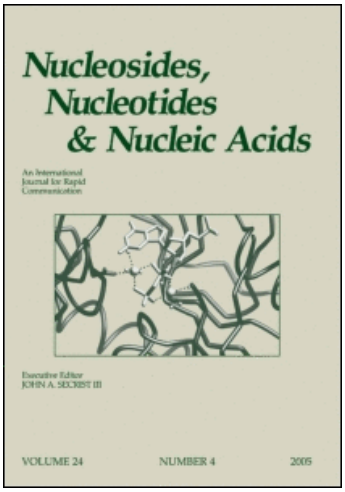

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5-ARYLETHYNYL-2′-DEOXYURIDINES: ENERGY TRANSFER AND SNP-DETECTION

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□ *Energy transfer between different fluorescent 5-alkynyl-2′-deoxyuridines in complementary and mismatched duplexes was studied.*

Keywords 5-Substituted-2′-deoxyuridines; pyrene; pyrlene; fluorescence energy transfer; mutations

At present, fluorescence measurements are one of the most popular methods of DNA studies.[1] The scope of classic types of fluorophores (fluoresceins, rhodamines, cyanines, etc.) is mostly realized. Therefore the important task now is to develop new fluorescent probes sensitive to microenvironment. Polyaromatic dyes conjugated with nucleobase through triple bond could serve as an example.[2–7] Recently, we studied oligonucleotides containing fluorescent nucleosides **A–D** (Figure 1).[2,3]

The fluorescence of nucleosides **C** and **D** in oligonucleotides is sensitive to changes in the environment. In the case of fluorophore **C** the intensity

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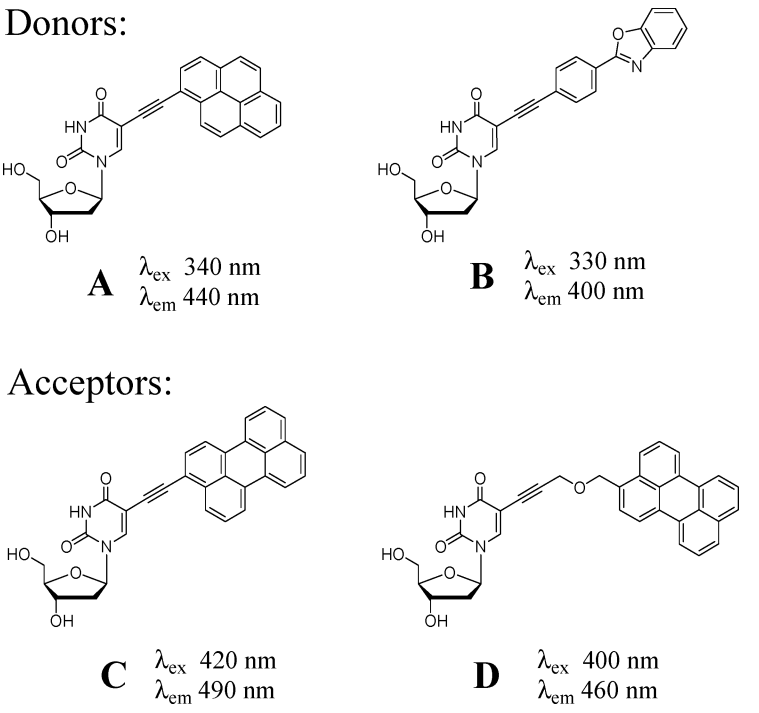

FIGURE 1 5-Alkynyl-2′-deoxyuridines bearing fluorescent polyaromatic dyes.

of fluorescence increases upon hybridization with a complementary strand by the factor of 2–4. The intensity of the nucleoside **D** fluorescence decreases upon hybridization by the same factor.[3] Spectral properties of oligonucleotides containing fluorophores **A** and **B** appeared to be less interesting. However, these nucleosides can be used as the donors of energy for fluorophores **C** and **D**. Such donor-acceptor pairs might be useful for DNA energy transfer studies and for the detection of DNA structure distortions—mismatches, deletions, etc. Here we describe the fluorescence energy transfer within different pairs of modified nucleosides (**A** → **C**, **A** → **D**, **B** → **C**, and **B** → **D**) that is possibly useful for SNP detection.

For the energy transfer studies, in the complementary oligonucleotides[8] thymidine residues were substituted by modified

(a) 5'-CCA-T**U**A-CAT-CCA-GAC-3'
3'-GGT-AA**U**-GTA-GGT-CTG-5'

(b) 5'-CCA-**U**TA-CAT-CCA-GAC-3'
3'-GGT-AA**U**-GTA-GGT-CTG-5'

(c) 5'-CCA-TTA-CA**U**-CCA-GAC-3'
3'-GGT-AA**U**-GTA-GGT-CTG-5'

FIGURE 2 Duplexes used for energy transfer studies (U—modified nucleoside).

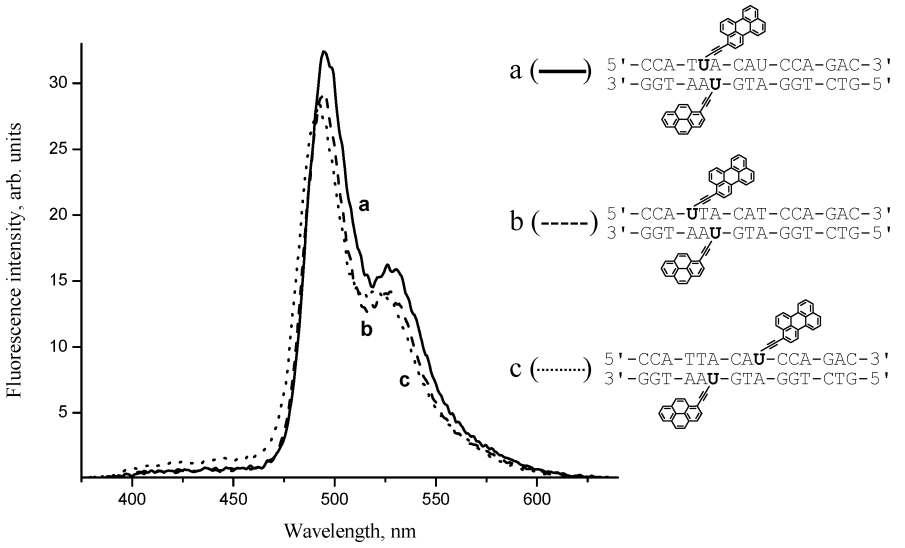

FIGURE 3 Energy transfer from 5-(pyren-1-ylethynyl)-2′-deoxyuridine (**A**) to 5-(perylen-3-ylethynyl)-2′-deoxyuridine (**C**) (λ_{ex} 340 nm).

nucleosides residues (Figure 2). In the case of duplex (**a**), the donor and acceptor are located in adjacent base pairs; in duplexes (**b**) and (**c**) they are separated by one and two base pairs, respectively.

Series A→C

In this series full energy transfer is observed (Figure 3). In addition, the intensity of the acceptor fluorescence does not depend on the distance between fluorophores. However, due to small changes in the distance, it is difficult to determine what type of energy transfer occurs—FRET, through-stack transfer, or both of them.

Series A→D

The data presented in Figure 4 are the strong evidence that the conjugation between the base and the fluorophore through the rigid ethynyl linker plays the important role in the effectiveness of the energy transfer process. Low intensity of the acceptor fluorescence is the consequence of non-effective energy transfer.

Series B→C

In the case of duplex (**a**), like in the **A** → **C** series, the donor fluorescence is not observed (Figure 5). The intensity of the acceptor fluorescence is somewhat higher than in the case of the **A** → **C** pair. However, in the

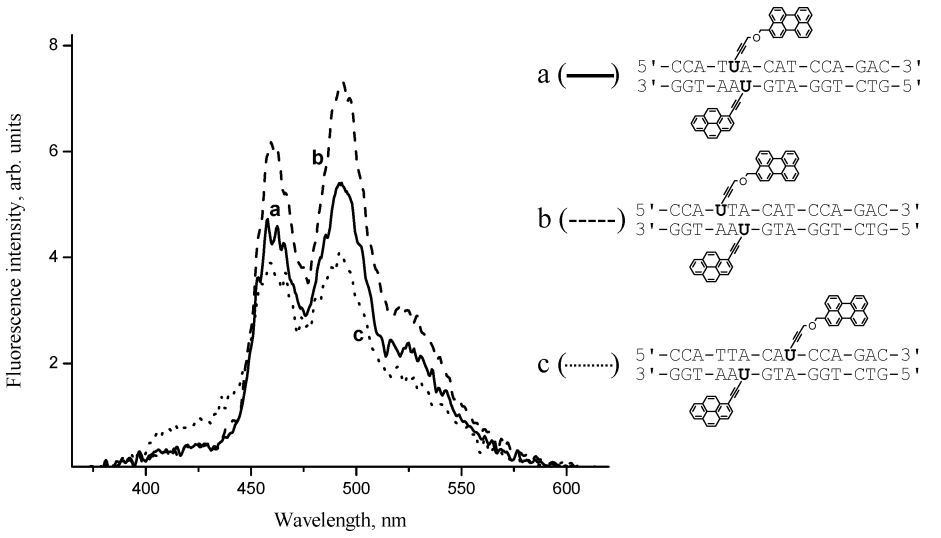

FIGURE 4 Energy transfer from 5-(pyren-1-ylethynyl)-2′-deoxyuridine (A) to 5-[(perylen-3-yl)methoxyprop-1-ynyl)-2′-deoxyuridine (D) (λ_{ex} 340 nm).

reversed duplex (a_R) spectra, the appearance of the donor fluorescence can be observed. This phenomenon can be explained in the following way: in the duplex (**a**) the residual donor fluorescence is quenched by the adjacent guanine; in the duplex (a_R) there is no quencher near the nucleoside **B**, and the band at 400 nm appears.

Mismatch detection. There is an example of energy transfer in complementary duplex (**a**) and mismatched one (**b**) (Figure 6).[9] As one can see,

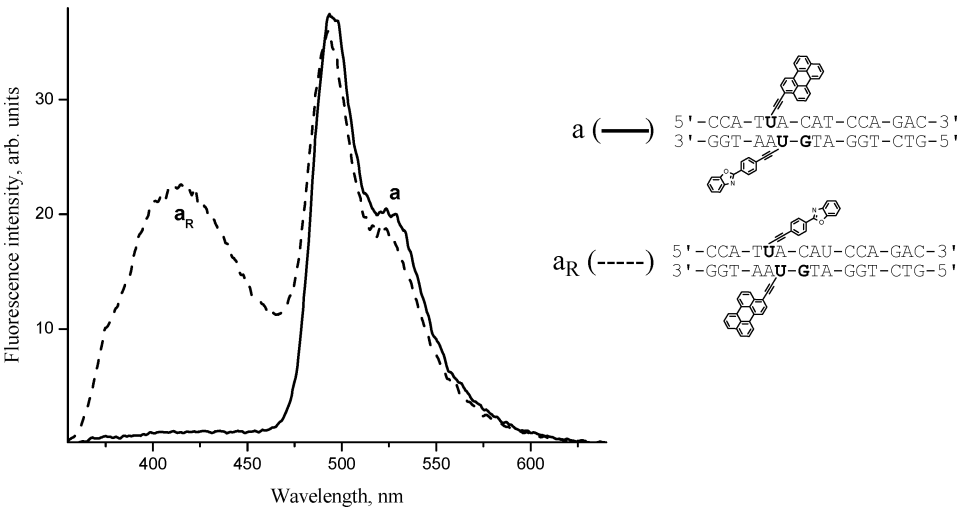

FIGURE 5 Energy transfer from 5-[4-(benzoxaloz-2-yl)phenylethynyl]-2′-deoxyuridine (B) to 5-(perylen-3-ylethynyl)-2′-deoxyuridine (C) (λ_{ex} 330 nm).

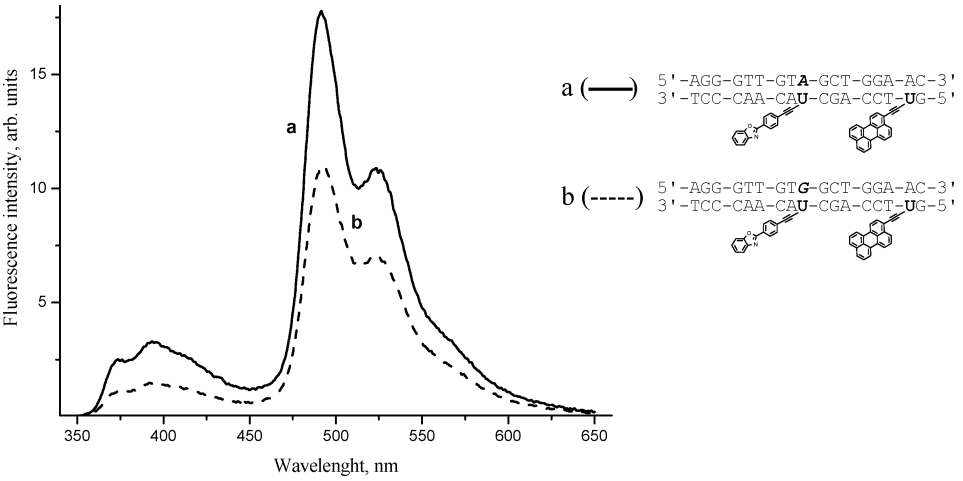

FIGURE 6 Mismatch detection using **B** → **C** donor-acceptor pair (λ_{ex} 330 nm).

the intensity of the **B** → **C** pair fluorescence decreases if the donor nucleoside is paired with a mismatched base.

To conclude, in all studied series full or partial energy transfer is observed. The mechanism of the transfer is not obvious; it possibly consists of two components—through space (FRET) as well as through-stack energy transfer. The most effective ET is in **A** → **C** and **B** → **C** donor-acceptor pairs. The fluorescence of donors **A** and **B** is quenched by adjacent guanosine residue. At the same time, guanine has a little influence on the fluorescence intensity of acceptor **C**. It is shown that fluorescence intensity of donor-acceptor pair **B** → **C** is two fold lower in the mismatched duplex than the matched one.

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9. Detailed studies on SNP detection will be published elsewhere.