Completion of a Programmable DNA-Binding Small Molecule Library

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For my family

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Abstract

Hairpin pyrrole-imidazole (Py-Im) polyamides are programmable oligomers that bind the DNA minor groove in a sequence-specific manner with affinities comparable to those of natural DNA-binding proteins. These cell-permeable small molecules have been shown to enter the nuclei of live cells and downregulate endogenous gene expression. We complete here a library of 27 hairpin Py-Im polyamides that bind 7-base-pair sequences of the general form 5'-WWGNNNW-3' (where W = A or T, N = W, G, or C). A table of binding affinities and sequence contexts for this completed 27-member library has been assembled for the benefit of the chemical biology community interested in molecular control of transcription. Quantitative fluorescence-based methods have been developed to determine the nuclear concentration of polyamide-fluorescein conjugates in cell culture. Confocal laser scanning microscopy and flow cytometry techniques are utilized to plot calibration curves, from which the nuclear concentration can be interpolated. Although confocal microscopy and flow cytometry generate disparate values, taken together these experiments suggest that the polyamide concentration inside the cell nucleus is lower than it is outside the cell. To further our understanding of C-terminal tail linkage effects on sequence specificity, the equilibrium association constants of hairpin polyamide conjugates were measured by quantitative DNase I footprint titration experiments. These results indicate that linkers and functional R groups on the tails of hairpin polyamide conjugates have recognition properties that should be considered in the design of these molecules to target DNA binding sites. Furthermore, these β-alanine-C_3-linked polyamide conjugates are shown to decrease hypoxia-inducible transcription of vascular endothelial growth factor (VEGF) in cultured HeLa cells. In addition, polyamide conjugates designed to target the Oct4 octamer DNA element modulate the expression levels of Oct4-driven genes in P19 mouse embryonal carcinoma cells and R1 mouse embryonic stem (ES) cells.

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