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Styryl Molecules Light-Up RNAs

A combinatorial library of 1336 fluorescent styryl molecules was synthesized aiming to select dyes that are photostable, non-toxic, and specific for RNA molecules in living cells [1]. These dyes are potentially important to the study of gene expression in live cells.

In order to investigate the full complexity of the various processes that take place within the context of a living cell, there is a serious demand for compounds that selectively stain the individual components that play a role in these processes in such a way that they can be monitored at high resolution by live cell fluorescence microscopy. While approaches to analyzing pools of specific biomolecules in fixed cells are well established, live cell imaging is becoming an increasingly powerful tool in cell biology. This is a rapidly evolving field, and recent developments in labeling and microscopic fluorescence imaging approaches have already provided spectacular new insights in the functioning of biological processes [2].

In particular, the discovery and characterization of fluorescent proteins, such as green fluorescent protein (GFP), that are present in nature revolutionized the design of genetic tools, allowing us to literally look at the processes that take place within living cells. For this purpose, these fluorescent proteins are expressed either transiently or stably in cells as a fusion protein with any protein of interest and visualized by fluorescence microscopy. Furthermore, advanced microscopic techniques, like time-lapse imaging, fluorescence resonance energy transfer, and fluorescence recovery after photobleaching make it possible to extract spatial and dynamic information about the expressed fluorescent fusion proteins in live cells under physiological conditions. Hence, our current understanding of how proteins assemble in large protein complexes and function in cellular processes regulating the maintenance, growth, division, differentiation, and death of cells is developing rapidly.

Comparatively, the current status of technologies meant to detect nucleic acids in living cells is still relatively poor. There exist a few general fluorescent DNA stains which are compatible with live cell imaging. These include Hoechst 33258, DRAQ5, and the recently developed photoactivated green fluorescent DNA dye BENA435 [3]. Also, there exist a number of methods that allow detection of specific DNA sequences in living cells [4]. Each of these methods, however, has serious shortcomings or limitations hampering the detection of unique endogenous DNA sequences in living cells. Thus, the development of such a method is still a hurdle to be taken.

A similar situation exists for the visualization of RNA molecules. Selective RNA imaging reagents are arguably even more relevant for cell biologists because they offer the possibility of monitoring patterns and kinetics of gene expression and RNA transport. Dyes that stain specific RNA molecules do not exist yet, and approaches to detecting such molecules are currently based on in vivo hybridization principles using fluorescently labeled nucleic acid probes that are microinjected into cells [4]. Alternatively, a method has been developed based on the expression and detection of GFP-tagged MS2 proteins that can selectively bind to specific stem-loop structures that are introduced in transiently expressed mRNAs [5]. Each of these approaches has serious limitations that cannot be solved easily. For example, in vivo hybridization techniques lack sensitivity and, quite often, specificity, while GFP-MS2-based RNA detection methods do not allow detection of endogenous transcripts. Hence, there is still a strong demand for general RNA stains, which are compatible with live cell imaging analysis.

Currently, only a few compounds are available for this purpose. The classical dye is SYTO 14 [6], which has been applied in various studies. More recently, the SYTO-RNA Select dye was introduced [7]. Despite their preference for RNA, these dyes also bind to DNA, hampering the discrimination between the two. Furthermore, SYTO14 is not very photostable and, therefore, not very well suited for time-lapse imaging applications using living cells.

In this issue of Chemistry & Biology, Li and coworkers report the identification of RNA-selective fluorescent styryl dyes with properties that make them particularly suitable for live cell imaging applications [1]. Styryl dyes are fluorescent lipophilic cations that are synthesized by the condensation of aldehydes and pyridinium salts in the presence of pyrrolidine. Previously, this group reported the synthesis of a library of fluorescent styryl molecules, some of which revealed specificity for different subcellular compartments like mitochondria and endoplasmatic reticulum in living cells and covered a broad range of emission colors in the visible range from blue to red as well [8]. These compounds have therefore been propagated as organelle-specific probes that can be applied to living cells. Interestingly, some compounds from this library where shown to stain the nucleus or, more specifically, the nucleoli, but the specificity of this staining was not determined. The nucleolus is the most distinctive nuclear subcompartment and the site of the synthesis of ribosomal RNA and the assembly of the 40S and 60S ribosomal subunits. Since about one-half of the total amounts of cellular RNA are produced in this compartment, it contains huge amounts of RNA relative to other cell compartments.

Fortunately, Li and coworkers recognized the urgent need for RNA-selective dyes for live cell imaging applications. To this end, they sought to generate a new and more expanded fluorescent styryl library by using more aldehydes and a huge variety of functional groups in the condensation reaction [1]. The initial library contained 1336 members, but after a first selection for compounds that either bound RNA in vitro or selectively stained nucleoli in living 3T3 cells, 88 of them were selected and resynthesized for further characterization in a secondary screen using live cell imaging. The strong

point about this selection approach taken by Li and coworkers is that the final screen for putative RNA binding dyes is indeed performed on living cells. Finally, three library components were selected in the secondary screen (E36, E144 and F22) and notably E36, which gave a positive reaction in the nucleoli staining screen, was shown to be particularly highly selective for RNA.

Why are these fluorescent RNA binding dyes particularly suitable for live cell studies? First, they are small and cell membrane permeable. Therefore, they are rapidly and efficiently taken-up by cells when added to the cell culture medium. This feature eliminates the need for rather laborious microinjection procedures. Second, because of their small size, styryl dyes are expected to have easy access to the various compartments within a cell and to the RNA molecules that might be present in those compartments. This is an important issue because there has always been some debate concerning the accessibility of RNA molecules for dyes when they are assembled in ribonucleoprotein complexes in their native cellular environment. Third, the selected compounds have their excitation and emission wavelength in the visible range, are highly fluorescent, are photostable, and show hardly any cytotoxicity and phototoxicity. These are all essential characteristics for live cell imaging studies.

No doubt, RNA-specific fluorescent styryl dyes have the potential to find wide application in research fields related to cell biology. These innovative compounds are particularly relevant in analysis of the dynamic properties of RNA localization in living cells. Furthermore, because various color variants can be selected, these dyes can be easily applied in multicolor approaches by combining them with dyes selective for other cellular components like DNA.

Future studies may provide insight in the mechanism by which styryl dyes bind to RNA. If a structural feature or specific order of nucleotide sequence in the RNA turns out to be involved in binding, it might then be possible to refine the selection procedure to select for library components that bind specific types of RNAs; for example, messenger RNAs, ribosomal RNAs, transfer RNAs or small nuclear RNAs, or even specific gene transcripts.

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Myxing It Up to Study Chondramides

Considerable progress has been achieved in elucidating the biosynthesis of natural products with interesting cellular targets. In this issue of *Chemistry & Biology*, Rachid et al. [1] provide a new example, taming genetic manipulation of the producer of chondramide, an actin-interfering compound.

Natural products (NPs) have provided a large number of existing drugs and NPs, NP-derived, or NP-inspired compounds represented over half of the drugs approved during the 1981–2002 period [2]. A large number of NP-related compounds are also undergoing clinical trials, particularly in the anti-infective and anticancer fields [3]. However, NP-based drug discovery has been progressively abandoned by the pharmaceutical industry during the last two decades. Two reasons have probably contributed to the declining interest in NPs as potential drug leads: the labor-intensive nature of this type of endeavor, and the expectation that genomics, synthetic/combinatorial chemistry, and high throughput screening can provide a sufficient number of drug leads. This expectation has not been realized, and fewer drugs are being approved despite spiraling R&D costs [4].

While large pharma was becoming disinterested in NP research, the field attracted increasing attention from many academic laboratories and biotech companies, especially for the possibility of expanding NP diversity through biology-based approaches, such as mutasynthesis [5], combinatorial biosynthesis [6], or chemoenzymatic routes [7]. These approaches can complement chemistry in expanding NP diversity. In order to