In conclusion, it will be important to establish the mechanism of CdS nanoparticle synthesis in bacteria. The results of this work will undoubtedly prove useful to many fields, including bioremediation and nanotechnology. In addition, it will be interesting to see if the bacterial proteins responsible for CdS biomineralization bear any sequence resemblance to the CdS binding peptide sequences discovered via phage display techniques [11].

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Finding Their Groove: Bifunctional Molecules Arrest Growth of Cancer Cells

In this issue, Dickinson et al. describe an exciting advance in the search for inhibitors of transcription that function well in cells [1]. The authors screen for small molecules that selectively damage DNA and identify a histone gene as a potential new target for cancer therapeutic development.

Many human diseases exhibit altered patterns of gene transcription [2–4]. Overexpression of the human transcriptional inhibitor Mdm2, for example, has been correlated with a number of human cancers [5–7]. These altered patterns are a signature of a particular disease, they are useful for characterization and diagnosis, and they further offer an opportunity for targeting therapies specifically to diseased cells. One exciting approach is to home in on the affected genes themselves and interrupt or promote their transcription by using molecules that interact with specific DNA sequences [8–11]. So, for example, a triplex-forming oligonucleotide that prevents the transcription factor Sp1 from binding to DNA effectively inhibits the transcription of the *Src1* gene regulated by that protein in cell culture [12]. Among the historic difficulties with identifying small molecules that can accomplish this task is that such molecules must not only be cell and nuclear permeable but also must compete for DNA binding sites with a wide range of proteins in order to exert their function. An additional hurdle is that the molecules must interact with only a minimal number of binding sites within the genome in order to avoid affecting numerous biological processes. The most common approach taken to develop transcriptional inhibitors relies upon designing a molecule to target a specific DNA binding site associated with the gene of interest. The designed molecule is then tested first in vitro and subsequently in cell culture. However, it is often difficult to predict the behavior of the molecule in the complex environment of the cell based upon in vitro results due to issues of cell and nuclear permeability as well as the accessibility of the cognate DNA binding sites in the context of chromatin.

The approach taken by Gottesfeld and Dervan in this issue of *Chemistry & Biology* circumvents some of the difficulties outlined above and represents a departure from the typical mechanism of transcriptional inhibitor discovery [1]. Instead, the authors synthesized a small group of molecules and screened for activity in human colon cancer cells before investigating the origin of the observed effects. The molecules themselves are bifunctional, containing a sequence-specific DNA binding module and a functional group that damages DNA (Figure 1). The DNA binding module is a hairpin polyamide, a minor groove binding agent composed largely of heterocyclic amino acids that mediate sequence-specific interactions with the functional groups present in the minor groove. The mode of binding for hairpin polyamides is such that pairs of heterocycles bind side-by-side in the minor groove recognizing a specific base pair in a predictable manner—G•C versus C•G, for example—and it is thus possible to design a structure that recognizes a particular sequence. The authors prepared five polyamide-based structures for screening, each with a distinct

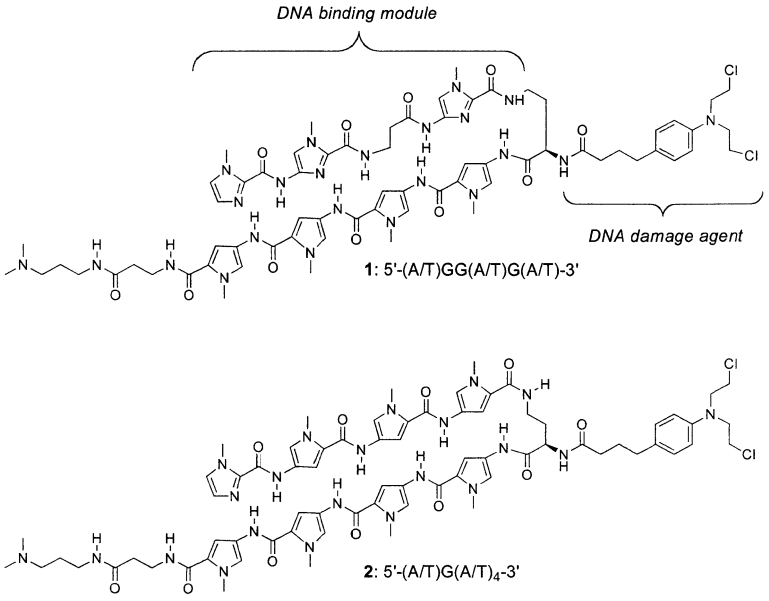

Figure 1. Two of the Five Polyamide-Chlorambucil Conjugates Prepared for the Study

The DNA sequence preference for each structure is indicated.

sequence preference. Interestingly, none of the five structures are designed to recognize a unique sequence within genomic DNA; each has a target binding site size of 6–7 base pairs, and within that sequence, 3–5 positions can be either an A•T or a T•A base pair. Thus, thousands of binding sites for the molecules exist in every cell.

Although hairpin polyamides interact with their cognate DNA sites with high affinity and have been shown to compete with some DNA binding proteins for their cognate sites, polyamides provide no impediment to the polymerase machineries that transcribe or replicate DNA [11]. Thus, the authors included an additional functional group in their design: an alkylating agent that crosslinks the polyamide to DNA. They chose for this purpose the well-characterized DNA damage agent chlorambucil, a cancer therapeutic [13]. Conjugation of chlorambucil to a sequence-specific DNA binding molecule imposes the DNA binding specificity onto the DNA damage agent, and in the case of polyamides, this leads to alkylation of purine residues proximal to the DNA binding site [14]. In a recent pioneering study also appearing in *Chemistry & Biology*, Dervan and Gottesfeld demonstrated that polyamide-chlorambucil conjugates can localize in the nuclei of live cells and alkylate chromatin bound DNA at sites determined by the binding specificity of the polyamide moiety [15, 16].

Of the groups of human colon cancer cells treated with the five polyamide-chlorambucil conjugates examined in this study, only cells treated with **1** showed significant morphological changes and growth arrest largely unaccompanied by cell death; less specific conjugates such as **2** exhibited high cytotoxicity that is presumably related to extensive DNA alkylation. By examining the transcription levels of approximately 18,000 genes in cells either treated with **1** or with one of several controls, the authors found that 77 genes were upregulated and 35 were downregulated after treatment with **1**. Remarkably, only a single gene, H4c, was downregulated more than 2-fold. H4c is one of several genes encoding the histone protein H4, and due to the central role of histones in cell cycle progression, it is not surprising that downregulation of this gene would affect cell growth [17]. There were thus two key questions at this stage: (1) does downregulation of H4c contribute to the observed cellular changes?; and (2) is the downregulation a result of conjugate **1** binding and damaging DNA somewhere within that gene? The authors provide compelling evidence supporting affirmative answers to both of these questions. In the first case, downregulation of H4c gene transcription by an alternative mechanism, siRNA, produced similar changes in the appearance and growth of human colon cancer cells to those observed in the presence of compound **1**. Future experiments comparing the transcript levels of all genes from cells containing the H4c-specific siRNA with the levels found upon treatment of cells with **1** will provide additional insight into this question. Toward the second question, the authors identified four potential binding sites for **1** within the coding region of the H4c gene and used ligation-mediated PCR to demonstrate that one of those sites is specifically alkylated by **1** in cell culture. Extending the cell-based results to animal studies, the authors found that conjugate **1** inhibits tumor growth in mice, either upon dosing the animals with **1** or by pretreatment of the tumor cells with the molecule, a remarkable finding. As a result, the H4c gene is an exciting new target for the development of antiproliferative agents.

It is not surprising that a hybrid molecule such as **1** with both DNA binding and DNA damaging capabilities impacts cell growth, but the mechanism by which this occurs is an exciting and thought-provoking finding. The H4c gene is ubiquitous in human cells, yet it is overexpressed in only a subset of cell types, including the colon cancer cells examined in this study. In fact, the transcript analysis described in this work showed that the H4c gene accounted for fully 70% of the total H4 mRNA. Further, as discussed by the authors, it is likely that the transcriptionally active state of this gene is what allows conjugate **1** to specifically target H4c relative to the many other H4-coding genes that contain

the same cognate binding sites for **1** but are not affected by the compound. Taken together, these results illustrate the utility of a cell-based screening approach for discovery of transcriptional inhibitors, since a direct correlation between in vitro binding data and functional effects in the complex environment of the cell does not always exist. Certainly, there are difficulties inherent to the screening strategy, as evidenced by the additional 100 or so other genes affected by conjugate **1** whose roles have yet to be determined, but this is a small price to pay for success.

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