distort the electric field in such a way that the growth of the wire can be controlled in a predetermined direction. The growth time and morphology of the wire is controlled by the field intensity which is typically 15- 45 V/cm – similar to current density controlling these factors in conventional electroplating. The process starts almost immediately and the wire contact is completed within 45 s. The wire branches thus formed are a few micrometers in diameter which is of the same order as in computer chips.

It is significant to note that the process involves no direct electrical contacts with the wire and the particle and is caused due to the phenomenon of field-induced polarization. Secondly, no metal ions are present initially in the medium unlike in electroplating.

The process works this way: the voltage applied to the platinum wires create an electric field that surrounds the particles. This field polarizes copper and forces positive charge to one side and negative on the other. This happens on both the particles which makes them face to face with opposite charges. When a strong field is applied, the, copper atoms are forced out of the particle as ions and these ions migrate to the opposite electrode to get deposited and the wire grows from then on. Ultimately the first branch to reach each copper particle would close the circuit and all growth would cease immediately. If the wire integrity is broken at any point during its growth by tapping the microscope stage-plate on which the process is conducted, quick regrowth was observed showing that the process is self-healing.

This process is well-known in fluidized bed electrodes, electrosynthesis and ultramicroelectrodes. This phenomenon called – bipolar electrochemistry – has been demonstrated on particle array to form microcircuits. This process is expected to help in the formation and construction of three-dimensional circuits, which should permit far denser information processing than that available from conventional 2-D lithographic process.

1. Bradley, J. C., Chen, H., Crawford, J., Eckert, J., Ernazarova, K., Kurzeja, T., Lin, M., McGee, M., Nadler, W. and Stephens, S. G., *Nature*, 1997, **389**, 268.
2. Wayt Gibbs, W., *Sci. Am.*, Nov. 1997, p. 24.

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Sequence-specific DNA-binding molecules: A futuristic approach in drug design

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Regulation of gene expression is the hallmark of subtleties and complexities of the biological world. Switching the pattern of gene expression changes the fate of a cell from one developing stage to another or, from a normal to a diseased one. Hence, it is needless to mention that understanding the components of regulatory switches and their interactions with other naturally occurring and synthetic molecules would be of unparalleled significance in controlling the dreadful diseases which are the consequences of misregulation of gene expression. Of the various approaches in this investigation, an important one is designing tailor-made small molecules that can recognize the specific sequences of base pairs on DNA. Although this seems to be a far cry, one group of investigators at California Institute of Technology has achieved remarkable success in controlling the 5S RNA expression using a synthetic sequence-specific DNA-binding molecule not only in the test tube but also inside a living cell[1].

The basic steps involved in synthesizing these molecules are: (i) identification of naturally occurring DNA-binding drugs; (ii) structural elucidation of DNA–drug complex; (iii) chemical modification of the drug to achieve an increase in length of binding sequences (a natural drug like netropsin binds to 4–5 AT pairs but it can be modified to bind to 13 bp by linking two molecules with a spacer like β-alanine[2]), binding specificity and affinity; (iv) *in vitro* and *in vivo* experiments to observe the effect of the drug on gene expression as well as its permeability across the cell membrane and stabilities in the cellular environment.

There are various naturally occurring DNA-binding drugs like netropsin and distamycinA, which have been a subject of great interest among investigators. In 1985, Richard Dickerson and coworkers published the X-ray structure of netropsin–DNA complex[3] which led some investigators to initiate the process of modification of netropsin to achieve its sequence-specific recognition. Since then, Dervan's group has taken a great lead in this study.

Netropsin (a polyamide with *N*-methylpyrrole backbone and guanidium tail, Figure 1) is a crescent-shaped molecule which binds to the minor groove of 4–5 successive AT base pairs of double helix DNA. The X-ray structure indicates that amide group of netropsin participates in bifurcated H-bond with N3 of adenine and O2 of thymidine. The pyrrole rings fit parallel to the bases through van der Waal interactions between its CH group and C2 of the bases. Its binding to GC region is probably sterically hindered by amino group of guanine present in the minor groove. Hence, incorporating another heterocyclic ring which could interact with the amino group of guanine as hydrogen bond acceptor (e.g. pyrimidine, *N*-methylimidazole, etc.) would facilitate the molecule to bind to GC region of the DNA. The molecule can be further improved by putting a linker (like γ-amino butyric acid) between two molecules. Thus, a variety of dimeric molecules (hetero, homo, parallel or antiparallel) can be designed to bind to the desired target sequences. The linker in an anti-parallel dimeric molecule would form a hairpin structure on binding to DNA and would facilitate the molecule

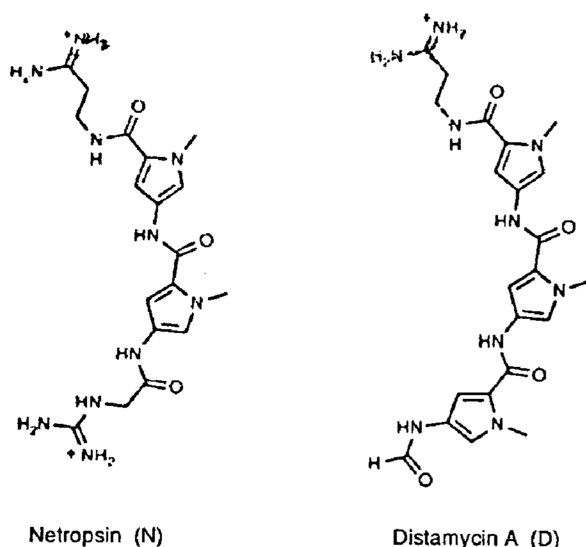

Figure 1. Natural products netropsin (N) and distamycin A (D) which bind to the minor groove of AT sequences of DNA.

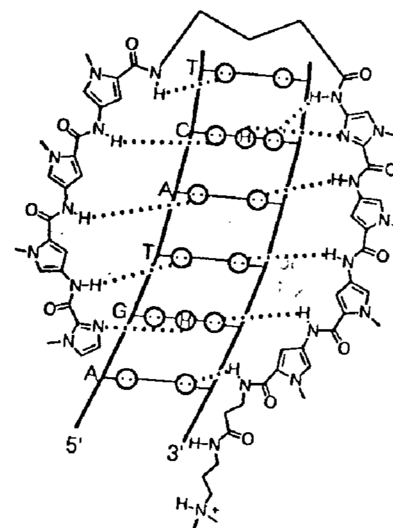

Figure 2. ImPyPyPy-γ-ImPyPyPy-β-Dp bind ing to GTAC region of TFIIIA-binding site (From ref. 1).

to sit on the anti-parallel strands of the DNA. This side-by-side pairing of molecules in the minor groove of DNA would increase the affinity of the dimer. To distinguish between the minor groove of the four base pairs, the position of H-bond donor and acceptor groups of the bases is targeted so as to participate in the drug–DNA interaction. As mentioned earlier, the N2 amine of guanine provides an overall asymmetry to minor groove of GC pair hence, interaction between N2 amine and a H-bond acceptor moiety in the drug (e.g. imidazole) would be energetically favoured providing specificity to the interaction. Thus, pairing of imidazole opposite pyrrole in an anti-parallel heterodimeric molecule would target a GC base pair and pyrrole opposite imidazole would recognize a CG base pair. The H-bond discrimination between AT and TA is more difficult because the H-bond acceptors at N3 of adenine and O2 of thyamine are almost identically placed in the minor groove. However, there is an asymmetrical cleft generated between thymine O2 and adenine N3 on the minor groove. This subtle asymmetry can be exploited to provide selectivity to drug–DNA interaction by substituting the C3-H of pyrrole with C3-OH. This strategy has been successfully applied to design a polyamide which contains a 3-hydroxypyrrole (Hp) in the place of pyrrole (Py) and has been shown that Hp/Py binds to TA pair 18-fold stronger than to AT pair and similarly Py/Hp binds to AT pair 77-fold stronger than to TA pair[4]. Thus, the pairing-rule (Hp/Py : TA, Py/Hp : At, Im/Py : GC and Py/Im : CG) can be generalized for targeting minor groove of any sequence.

Before achieving the AT–TA discrimination in the minor groove, Dervan's group had already achieved the regulation of gene expression *in vivo* by a synthetic molecule (ImPyPyPy-γ-ImPyPyPy-β-Dp, where Im, imidazole; Py, pyrrole; γ, γ-amino butyric acid; β, β-alanine; Dp, dimethyleaminopropylamide, Figure 2), which could specifically bind to GTAC region of TFIIIA-binding site[1]. TFIIIA is an eukaryotic transcription factor which positively regulates the transcription 5S RNA by RNA polymeraseIII. TFIIIA binds to the internal control region, a regulatory element present within the 5S RNA gene. TFIIIA has nine zinc finger motifs, of which zinc finger 4 binds to the minor groove of the AGTACT sequence. According to the pairing rule described earlier, this sequence would be bound in a hairpin motif by an eight-ring polyamide ImPyPyPy-γ-ImPyPyPy-β-Dp. The DNA footprinting results indicate that this molecule can compete with TFIIIA. The *in vitro* and *in vivo* transcription assays indicate a low level of 5S RNA in the presence of this molecule and thus prove this molecule as a drug.

The immediate challenge before the investigators is to look for the molecules which could bind in the major groove of the DNA where most of the DNA-binding proteins sit. Only then would the competitive inhibitors for the oncogenic proteins appear on the horizon. Although tailor-made DNA-binding drugs for desired regulatory sequences are still far from reality, the immense potential clinical applications of these futuristic drugs would provide a great inspiration for the future generation of drug designers to convert this idea into a reality.

1. Gottesfeld, J. M., Neely, L., Trauger, J. W., Baird, E. E. and Dervan, P. B., *Nature*, 1997, 387, 202–205.
2. Trauger, J. W., Baird, E. E. and Dervan, P. B., *J. Am. Chem. Soc.*, 1996, **118**, 6160–6166.
3. Kopka, M. L., Yoon, C., Goodsell, D., Pujra, P. and Dickerson, R. E., *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 1376–1380.
4. White, S., Szewczyk, J. W., Turner, J. M., Baird, E. E. and Dervan, P. B., *Nature*, 1998, **391**, 468–470.

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