Doing it like DNA

The old stencil process and the modern Xerox, for images, may become possible for real things, says s ananthanarayanan

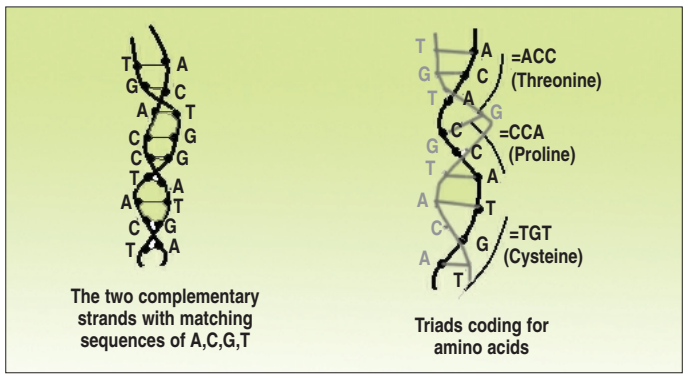
CREATING copies has many uses — one area is in images and documents, another is in the natural world, of biological reproduction. A group of scientists at New York University has made progress in mimicking the biological method for replicating molecules or artificial, nanometer-size structures. "Our findings raise the tantalising prospect that we may one day be able to realise self-replicating materials with various patterns or useful functions," say Nadrian C Seeman, Paul M Chaikin and others of the Centre for Soft Matter Research at NYU in a paper just published in the journal *Nature*.

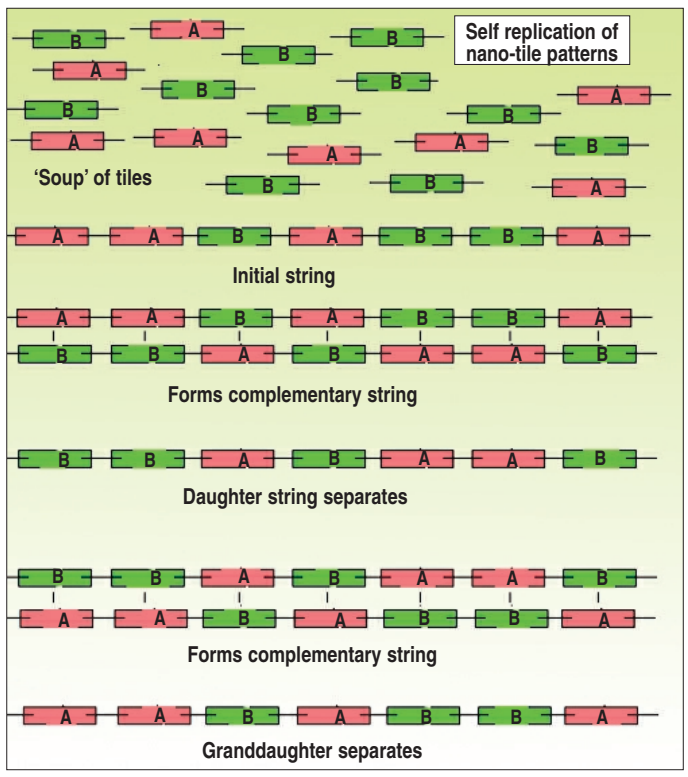
The work of Crick and Watson laid bare the elegance and ingenuity of the genetic code, both in function as well as the manner of its replication in the reproduction of living organisms. Each living cell contains, in its nucleus, a mega-molecule, the DNA, a string of triplets of just four basic molecular units. Each triplet forms the template for the synthesis of a specific amino acid, the building block of proteins.

As each unit in the triplet can take four forms, there can be 4x4x4=64 distinct triplets. But with provision for redundant forms and some housekeeping, the code describes only 20 amino acids, whose sequence in the DNA can define millions of proteins. The DNA thus contains the information for all processes and functions in an organism and makes for the complex yet exact and distinct construction of the enormous number of living species.

The definition of the four basic units to describe the amino acids also serves to enable the DNA to both remain stable and to replicate during reproduction of the cell. The act of reproduction, in fact, is nothing but the replication of DNA. The four basic units of the DNA, called A, T, G and C, have the property of attaching to each other, but in specific pairs — A with T and vice-versa or G with C and vice-versa. Thus, if there is a string, say, A, T, C, CT, G, as part of a strand of the DNA molecule, then each unit would attach, from the chemical soup within the cell, with units T, A, G, GA, C as complements of the original string. In this way, the millions of units in a full DNA string would have a complementary string alongside, and the two strings are joined at these pairs, right along their length, in the form of a helix.

One feature of the bonding is that the bond of the units along the length of the DNA is stronger than the bond of A to T and C to G, as complements. Thus, when the chemical signals of the time for a cell to divide are present, the lateral bonds between the strands of the DNA molecule separate but the backbone, which is bound by stronger ties, stays intact. And the two parts of the dividing cell carry away one strand each. As the units of each strand code for the corresponding matching unit, the single strands in the newly formed cells rapidly replace the missing, complementary units, which form bonds between themselves and the two parts of the DNA molecule are complete again, for the cell to function normally! These two cells can again split into two more, and the resulting four cells into another eight and so on, which can lead to very fast growth of tissue through cell division.





This elegant and simple method employed by living things holds out the prospect of using molecular templates for the generation of substances. Just as sequences of triads in the DNA code for specific proteins, we now have methods to build strands of molecules that could be capable of picking out components and assembling complex molecules. But equally attractive is the possibility of creating ways for existing molecules or materials to create copies of themselves, ideally, at the expanding rate of tissue growth. The work of Seeman, Chaikin and others is a first step in realising replication — through the use of components made of a combination of DNA segments.

The components used were a pair of only two *molecular tiles*, say A and B, to create a simple mosaic, like AABABBA. The objective was to get this string to attach to a complementary string, ÂÂBÂBBÂ, picking up the complementary units and assembling the string from the chemical environment.

The tiles were developed using 10 DNA strands each, in principle capable of millions of combinations, not just two forms. But two forms were used for the simple trial and each one was marked by chemical features so that they could be identified with the use of an *Atomic Force Microscope*. The first unit in the string was an "initiator", an A-like tile, attached to a magnetic bead — giving the string the form of: "I A B A B B A". The Atomic Force Microscope could then read off the sequence in the first, or "seed" string and then verify whether the "daughter" string that the seed was to clone was correctly formed.

In the experiment, the tiles were first separately formed from the constituent DNA segments, with ends fashioned so that the tiles would attach in a particular order, like AABABBA, in the example. When the tiles prepared like this were mixed, and they formed strings, a chemical marker was added to identify the "As" and the sequence order was verified with the Atomic Force Microscope. In the meantime, the complementary tiles, the "As" and the "Bs" were also prepared. When these were mixed with the "seed" strings, each complementary tile joined its counterpart and formed the "daughter" string. As in the case of natural DNA, the longitudinal adhesion was kept stronger than the lateral ties. Gentle heat could then separate the pairs of strings and a magnetic bead could be attached to one end of the "daughter" strings. The Atomic Force Microscope then verified if the replication was accurate.

The results showed a 70 per cent accurate creation of daughters. The next step was the creation of "granddaughters", or the replication of the daughters using the first set of tiles as complementaries. As the initial strings were not separated before the "second generation", separate markers were added to the second generation tiles for identification. The result was a similar level of creation of accurate "granddaughters", which then added to the number of the initial strings. Further generations would hence create larger numbers, as a growing progression.

The process is cumbersome and at 70 per cent replication is still not the runaway expansion of natural systems, But it does demonstrate that replication is possible, not just of DNA but of more complex forms, carrying information, shape and, conceivably, function. "We expect that... and other improvements will deliver a robust replication method that is applicable to molecular, nanometer-sized and colloidal systems displaying programmed recognition," say the authors.

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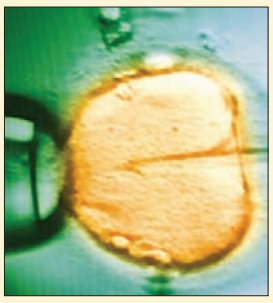
How old is too old?

jeremy laurance **reports on a clash over fertility advice**

A BRITISH fertility expert has clashed with a US specialist over when is the "right" time for single women who want children to resort to freezing their eggs. Gillian Lockwood, medical director of Midland Fertility Services in Aldridge, near Birmingham, defended the practice despite US research showing that the average age of women seeking egg freezing for social reasons is between 37 and 39 — by which time their eggs are rapidly deteriorating.

A review of 26 studies due to be presented at the annual meeting of the American Society for Reproductive Medicine in Orlando has found that women who froze their eggs when they were under 30 had higher rates of success than those who were older. Roger Lobo, president of the ASRM, warned women not to leave it too late. He said egg freezing was still "an experimental procedure" with "insufficient evidence" to show whether it was a "worthwhile undertaking". He said that "despite increasing numbers of clinics offering the procedure and the significant media attention paid to it in recent years, women are not pursuing elective egg freezing at an age when it would be most likely to help them accomplish their fertility goals".



US scientists claim that 38 is too old for eggs to be frozen effectively.

But Dr Lockwood said although it was true that egg freezing was not an option for many women in their late 30s, "we have to ask what the comparator is here. The comparator is how well will these women do with their own eggs in their early 40s? A 39-year-old frozen egg is going to do better in IVF than a 42-year-old fresh egg, because the drop-off becomes so sharp during these years".

She said she was frequently contacted by women in their mid- to late-30s who were still single and wanted to freeze their eggs. "They've discovered Mr Right has turned out to be Mr Wrong. They are deliberately and quite bravely trying to buy a bit of time." Dr Lockwood has seen around 100 women in the last few years who want to freeze their eggs for social reasons.

"With most of the women I see we agree that social egg freezing is not appropriate, either because they've left it too late or because their ovarian reserve is too low. I see it as unethical if you are only going to get three or four eggs because really you need 16, 18, 20 to have a realistic chance of achieving pregnancy. I would still say a woman up to her mid-30s who wishes to be a genetic mother one day and has not met Mr Right would be much better freezing her eggs than hoping Mr Right turns up before she's 40."

Scientists are developing a test that could dramatically boost IVF success rates from a single cycle of treatment. The technique, created at Oxford University, checks for chromosomal abnormalities in the developing embryo but also looks at two new markers that could potentially cause pregnancies to fail. Over time, researchers hope they can increase success rates towards the 100 per cent mark. At present, only around 30 per cent of IVF cycles worldwide result in a pregnancy, with many failing due to chromosomal abnormalities.

The Independent, London

Membrane function & the impact of molecular biology

Studies reveal that cells in the human body need more than 30 families of membrane proteins to facilitate transportation of the great variety of solutes that must be moved across membranes. tapan kumar maitra **elaborates**

BIOLOGICAL membranes play five related yet distinct roles: they define the boundaries of the cell and delineate its compartments; serve as loci of specific functions; possess transport proteins that facilitate and regulate the movement of substances into and out of the cell and its compartments; contain the receptors required for the detection of external signals; and provide mechanisms for cell-to-cell communication.

One of the most obvious functions of membranes is to define the boundaries of the cell and its compartments and serve as permeability barriers. The interior of the cell must be physically separated from the surrounding environment not only to keep desirable substances in but also to keep undesirable substances out. Membranes serve this purpose well because their hydrophobic interior is an effective permeability barrier for hydrophilic molecules and ions. The permeability barrier for the cell as a whole is the plasma (or cell) membrane — surrounding the cell and regulating the passage of materials both into and out of cells. In addition to the plasma membrane, various intracellular membranes serve to compartmentalise functions within *eukaryotic* cells.

Membranes have specific associated functions because the molecules and structures responsible for those functions — proteins, in most cases — are either embedded in or localised on them. One of the most useful ways to characterise a specific membrane, in fact, is to describe the particular enzymes, transport proteins, receptors and other molecules associated with it.

For example, many distinctive enzymes are present in or on the membranes of organelles such as the *mitochondrion*, *chloroplast*, *endoplasmic reticulum* (ER), *Golgi complex*, *lysosome* and *peroxisome*. Such enzymes are often useful as markers during the isolation of organelles from suspensions of disrupted cells. For example, *glucose phosphatase* is a membrane-bound enzyme found in the ER. When ER membranes are isolated and purified (as tiny vesicles called *microsontes*), glucose phosphatase can be used as a marker enzyme, enabling the investigator to determine the distribution of microsomes among the various fractions. Marker enzymes for other organelles can then be used to assess the degree to which it is free from contamination by these other markers.

Another function of membrane proteins is to carry out and regulate the transport of substances into and out of cells and their organelles. Nutrients, ions, gases, water and other substances are taken up into various compartments and various products and wastes must be removed.

The modes of transport differ. Many substances move in the direction dictated by their concentration gradients. A molecule that has no net charge moves in the direction dictated by its concentration gradient across the membrane. The movement of an ion, on the other hand, is determined by its electrochemical potential, which is the sum of its concentration gradient and the charge gradient across the membrane. This process, which does not require energy because movement is "down" the gradient, occurs via two different modes. Some molecules such as water, oxygen and ethanol can cross membranes by simple diffusion. Larger, more polar molecules such as sugars and amino acids move across membranes aided by specific transport proteins, a process called *facilitated diffusion*.

Alternatively, a substance can be transported against its concentration gradient if it is uncharged or against its electrochemical potential, in the case of an ion. This is an energy-requiring process called *active transport*. Solutes such as sugars and amino acid are often present in low concentrations outside the cell and are transported inward against their respective concentration or electrochemical gradients. The energy needed to drive such "uphill" transport is typically provided by the hydrolysis of ATP or a similar high-energy compound and the process is called *direct active transport*. Alternatively, the needed energy can be provided by coupling the "uphill" transport of the solute to the "downhill" transport of sodium ions or protons across the same membrane, a process that is called *indirect active transport*. The driving force for the "downhill" movement of the sodium ions or protons is their electrochemical potential, which depends on the prevailing charge gradient and the concentration gradient of the ion across the membrane.

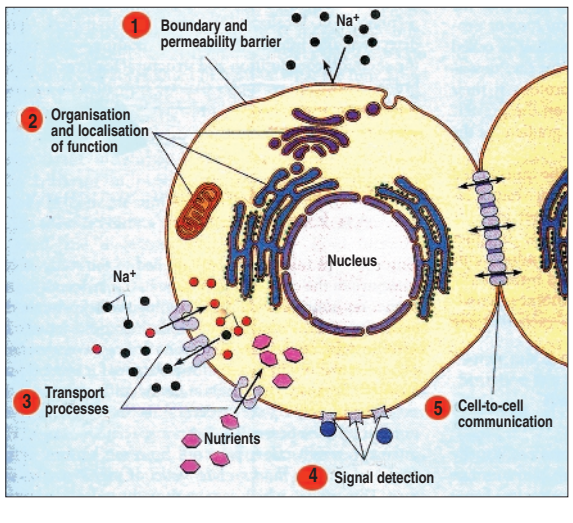
Even molecules as large as proteins can be transported across membranes. In some cases, intracellular vesicles facilitate the movement of such molecules either into the cell (endocytosis) or out of the cell (exocytosis). In other cases, proteins that are synthesised on the ER or in the cytosol can be imported into specific membrane-bound organelles such as *lysosomes*, *peroxi-somes* or *mitochondria*.

Cells receive information from their environment, usually in the form of electrical or chemical signals that impinge on their outer surface. The nerve impulses being sent from your eyes to your brain as you read these words are examples of such signals, as are the various hormones present in your circulatory system. *Signal transduction* is the term used to describe both the detection of specific signals at the outer surface of cells and the specific mechanisms used to transmit such signals to the cell interior.

In the case of chemical signal transduction, some signal molecules enter directly into cells and act internally. The hormone *estrogen* is an example. Because estrogen is a steroid, it is nonpolar and can therefore cross membranes readily. As a result, estrogen enters its target cells and interacts with regulatory proteins within. In most cases, however, the impinging signal molecules do not enter the cell but instead bind to specific proteins called receptors on the outer surface of the plasma membrane.

Binding of such substances, called *ligands*, is followed by specific chemical events on the inner surface of the membrane, thereby generating internal signals called *second messengers*. Membrane receptors therefore allow cells to recognise, transmit and respond to a variety of specific chemical signals. Membrane proteins also mediate adhesion and communication between adjacent cells. This intercellular communication is provided by gap junctions in animal cells and by *plasmodesmata* in plant cells.

Membrane proteins mediate a remarkable variety of cellular functions and are therefore of great interest to cell biologists. Only within recent years, however, has the study of these proteins begun to yield definitive insights and answers. Some of these answers have come from the application of biochemical techniques to membrane proteins.



Several such applications include SDS-polyacrylamide gel electrophoresis, hydropathy analysis and other procedures for labelling membrane proteins with radioactivity or fluorescent antibodies. Two other biochemical approaches that can be used to study membrane proteins are affinity labelling and membrane reconstitution.

Affinity labelling utilises radioactive molecules that bind to specific membrane proteins because of known functions of the proteins. For example, a compound called *cytochalasin B* is known to be a potent inhibitor of glucose transport. Membranes that have been exposed to radioactive cytochalasin B are, therefore, likely to contain radioactivity bound specifically to the protein(s) involved in glucose transport.

Membrane reconstitution involves the formation of artificial membranes from specific purified components. In this approach, proteins are extracted from membranes with detergent solutions and separated into their individual protein components. The purified proteins are then mixed together with *phospholipids* under conditions known to promote the formation of membrane vesicles called *liposomes*. These reconstituted vesicles can then be tested for their ability to carry out specific functions that are known, or thought to be mediated by membrane proteins.

In spite of some success with these and similar approaches, membrane biologists have often found themselves stymied in their attempts to isolate, purify and study membrane proteins. Biochemical techniques that work well with soluble proteins are not often useful with proteins that are hydrophobic. Within the past three decades, however, the study of membrane proteins has been revolutionised by the techniques of molecular biology, especially DNA sequencing and recombinant DNA technology.

Vital to these approaches is the isolation of a gene, or at least a fragment of a gene, that encodes a specific membrane protein. With a DNA molecule in hand, the first priority of the molecular biologist is almost always to determine its nucleotide sequence.

DNA sequencing is in fact one of the triumphs of molecular biology; it is now far easier to determine the nucleotide sequence of a DNA molecule than to determine the amino acid sequence of the protein for which it codes. Moreover, most of the sequencing procedure is carried out quickly and automatically by DNA sequencing machines. Once the DNA for a particular protein has been sequenced, the putative, or predicted, amino acid sequence of the protein can be deduced using the genetic code that equates every possible sequence of three nucleotides with a particular amino acid. The amino acid sequence can then be subjected to hydropathy analysis to identify likely transmembrane segments of the protein.

Knowing the amino acid sequence of the protein also allows the investigator to prepare synthetic peptides that correspond to specific segments of the protein.

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