Lambing by the Mother of All Techniques

Cloning by Nuclear Transplantation

Dolly is the world's first mammalian clone to have been created from a non-reproductive cell of an adult animal.

D Somashekar

Cloning was done in sheep by nuclear transplantation. A differential cell was made to develop into a complete organism by an unique gene cloning technique. The lamb born by this method, named Dolly, is the world's first mammalian clone to have been created from a fully differentiated adult cell.

'Dolly' – one of the most unique forms of life on our planet has created joy among a section of scientists and at the same time created terror among religious heads and politicians. Yes, I am referring to the innocent sheep Dolly, named after an American country singer, Dolly Parton. She is the world's first mammalian clone to have been created from a fully differentiated non-reproductive cell of an adult animal. She is an exact genetic 'xerox copy' of her mother, born without a biological father. Dr. Ian Wilmut and his team, from Roslin Laboratory of Scotland created her using what can literally be called the 'mother of all techniques' in mammalian cloning.

Let us see in some detail how this super-creature came into this world. Cloning is a technique by which one can propagate cloned cells/organisms. This technique is very popular in plant tissue culture, where one can generate a whole plant from a cell taken from any part of the plant and cultured on specially designed media. Somehow, this was not possible with animal cells, and it was thought that because animal cells are highly specialized, development in these cells is irreversible. For example, an udder cell in culture can give rise to only other udder cells, not to skin cells or an organ. Dolly is an exception to this limitation and with her creation one of the central dogmas of developmental biology

Box 1. What is a Clone?

A clone refers to a population of cells or organisms which were derived asexually from a single ancestor. Thus, they are genetically identical to each other, and to their common ancestor. A number of organisms naturally reproduce in this way, but adult mammals are not able to clone themselves. Identical twins, incidentally, can be considered, to be 'clones' produced as a result of a chance splitting of a fertilized egg in the course of normal, sexual reproduction.

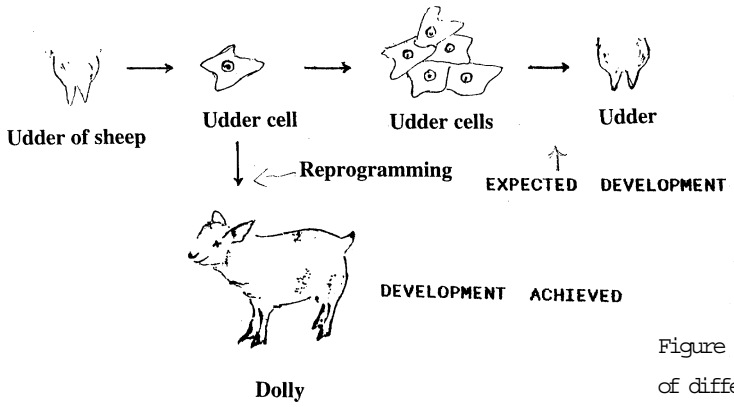

Figure 1. A schematic illustration of differentiation.

has been reversed. An adult mammalian cell can evidently be made to remember and reprogramme its development from the beginning, thus producing a complete viable organism (*Figure 1*).

In order to create Dolly, Wilmut took a cell from the udder of a six year old ewe and the cell was made to forget its existing role of developing into another udder cell by starving the cell with gradual reduction of nutrients (reprogramming). An unfertilized egg was then taken from another ewe and from it the nucleus containing the genetic material (DNA) was removed. Then the nucleus from the reprogrammed udder cell was taken and transplanted into the nucleus-free egg cell (*Figure 2*). The transplanted cell was allowed to grow and divide in a culture dish. After a week or so

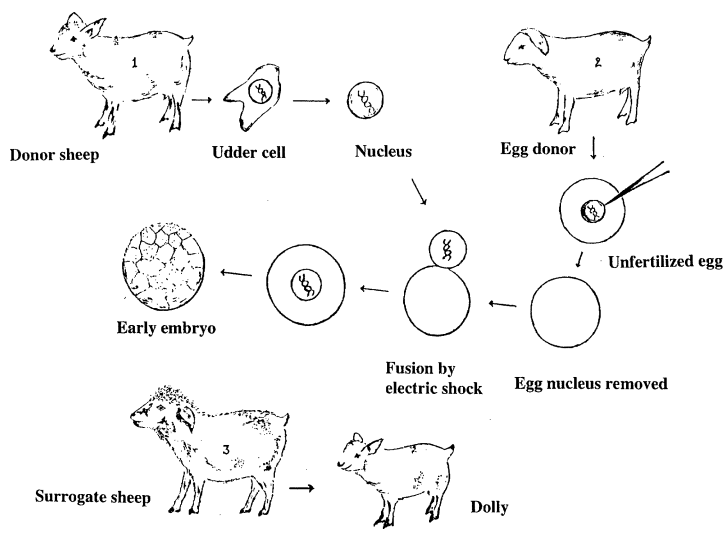

Figure 2. Cloning technique adopted for the creation of Dolly.

Box 2. Differentiation and Totipotency

The millions of cells in a multicellular individual, although originated from a single fertilized egg, are not the same. Some may be udder cells, others skin cells, still others nerve cells, *etc*. They look different and perform different functions. The processes by which these cells become specialized for their specific tasks are collectively known as *cellular differentiation.*

Earlier experiments on development and differentiation have shown that nuclei from early embryos can be used to produce adult clones or a complete viable organism. But this was not possible with the nuclei of cells of embryos beyond a certain stage. Hence, the nuclei from early embryos seemed to retain their potency to develop and differentiate into all types of cells (skin, udder, nerve cells, etc.) and so they were called *totipotent cells.* On the other hand adult differentiated cells in animals are typically not capable of giving rise to a complete viable organism or return to the state of totipotency. Today, however, Dolly is a living testimony to the fact that, given the right conditions, even a fully differentiated adult mammalian cell can be made to give rise to an entire organism.

this cell formed an early embryonic stage called *blastocyst*. This was implanted into the uterus of a third ewe that acted as a surrogate mother. After 5 months of gestation, at 4 pm on July 5, 1996, Dolly was born and almost immediately triggered off all kinds of controversies.

Although the technique seems simple in narration, it took Wilmut over 10 years and over Rs 2.5 crores in research funds to achieve the goal of cloning a whole mammal from a fully differentiated non-reproductive cell. In nature, during reproduction a sperm from the male parent and the egg of the female parent fuse. After development and differentiation, the fertilized egg eventually gives rise to an offspring. Thus, the sexually produced offspring inherits half of its genetic material from each parent. Dolly, on the other hand, is an identical clone of the ewe from which the udder cell was taken and does not have any genetic resemblance to either the ewe from which the egg was taken, or to the surrogate mother who bore and delivered her. Thus, Dolly truly has only a single parent.

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Another interesting fact is that Dolly was born by a process of asexual reproduction, even though there was fusion of a nucleus with an egg that lacked its own nucleus. The complete software (DNA) that controls

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the unfolding of development was provided by a somatic cell (in this case from the udder) rather than a reproductive cell. Asexual reproduction is commonly seen in lower forms of animal life like amoeba, hydra etc., but not in mammals.

Despite the furore created by this achievement, the success rate in this cloning endeavour was very low. Out of 277 nuclei that were transferred to enucleated eggs, only 29 eggs grew into embryos. Out of these, only 13 of the embryos could be successfully implanted into surrogate mothers, and, of these 13, just one ewe was successful in giving birth to an offspring – Dolly. The key to enhancing the success rate of this technique seems to lie in developing methods to make donor nuclei more acceptable to the cytoplasm of the recipient egg or oocyte.

Although the technique was a success in the case of a single Dolly, it remains to be seen whether it can be extended to other animals. As some researchers point out, Dolly could also have been a result of inadequate removal of genetic material (DNA) from the recipient egg. Even Wilmut admits that Dolly's genetic material might have come from a stem cell, which had retained in its memory the complete story of embryonic development. Well, the next thing haunting the mind is 'can we clone humans' by using this technique? If everything works well, as in the case of Dolly, it is certainly possible. Cloning has already been attempted in humans and monkeys using cells of the embryo, but not from an adult non-reproductive cell. Unlike in other mammals, in sheep there is enough time gap to remodel the transplanted adult nucleus to an embryonic state. It remains to be seen if a human adult cell/nucleus can be reprogrammed to an embryonic state and whether it can fuse harmoniously with an empty egg to begin the thread of life. If that becomes a reality, we may someday be able to manufacture clones of celebrities. Whether we would want to do so or not, is of course, another question altogether.

Suggested Reading

- **Collin Stewart. An udder way of making lambs. *Nature.* 385. pp. 769-771, 1997.**
- **I Wilmut, A E Schneike, J McWhir, A J Kind and K H S Campbell. Viable offspring derived from fetal and adult mammalian cells. *Nature.* 385. pp. 810-813, 1997.**

D Somashekar is a freelance writer. At present , he is working as Research Associate in the Food Microbiology Department at CFTRI, Mysore.

D Somashekar, Freelance writer, H.No.C/55, 'Vidhihasya', Petenarayan Temple Street, Sriranga-patna 571 438, India.

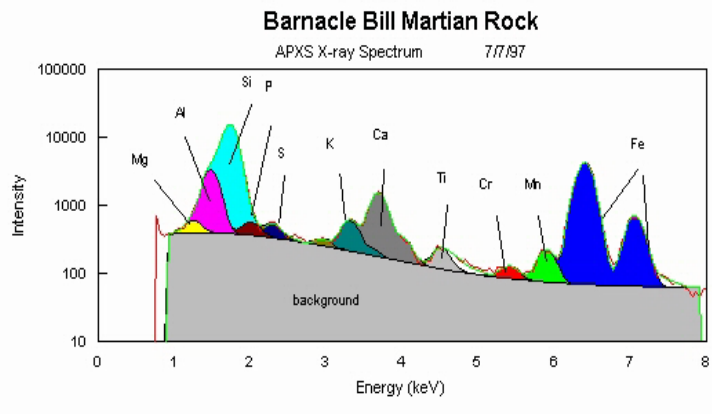
Pathfinder on Mars: A New Era of Planetary Exploration

Jitendranath Goswami

The landing of the *Pathfinder* spacecraft on the red planet Mars on July 4, 1997, marked a new chapter in the history of planetary exploration. For the first time we have a roving laboratory on another planet that can 'sniff ' the rocks and soils on its surface and can find out their chemical composition. The panoramic view of Martian landscapes, beamed back by the Pathfinder's camera, clearly shows the effect of devastating ancient floods that washed down rocks and boulders that are strewn all over the vast plain where the Pathfinder has landed. Although the timing of these ancient floods is not precisely known, they probably happened more than a billion years ago. The initial results obtained from analysis of Martian rocks by the alpha proton X-ray spectrometer (see *Box 1*) on board the rover, named *Sojourner*, yielded some unexpected results like their high silicon content. In terrestrial rocks we ascribe such high values to specific processes (e.g. plate tectonics, volcanism), that are not active on present day Mars. The one-time presence of water on the Martian surface is also reflected in the sodium and chlorine content of the soil. Detection of magnetized Martian dust particles by the magnets fixed on the Pathfinder in fact suggests the presence of iron-oxide that could have formed by long duration aqueous alteration of Martian rocks, a process akin to rusting on earth. We know that presence of iron oxide in the surface layers of Mars gives the planet its distinctive colour but the exact environment in which this oxidation process took

Box 1. Alpha Proton X-ray Spectrometer

This device attached to the rover Sojourner, 'sniffs' rocks and soils on the Martian surface and determines their chemical composition. It bombards the sample with energetic alpha particles that interact with the constituent atoms leading to the production of protons, X-rays and secondary alpha particles. The spectrometer then analyses the intensity of these emissions from the sample and provides information on its elemental composition.



Courtesy NASA

place is yet unclear. While all this exciting new pieces of information collected directly on the surface of another planet will keep planetary scientists busy for quite some time, we are going to have more news from Mars when other follow-up missions like the *Mars Global Surveyor* reach later this year and circle around the planet mapping its landforms and collect geochemical data. The results obtained by the Pathfinder, particularly spectral properties of rocks and soils analysed by the spectrometer, will serve as 'ground realities' while interpreting the remotely sensed data from the Mars Surveyor. This information will go a long way to improve our current understanding of the evolution of Mars.

If everything goes well (we should remember that a billion dollar Mars Observer mission failed in 1993), we can hope to get samples back from Mars by the middle of the next decade. However, many planetary scientists believe that they already have in their hands a dozen rock fragments from Mars in the form of meteorites. This group of meteorites is nicknamed SNC meteorites where S, N and C represent the first letters of the meteorites from the three sub-classes that belong to this group (see *Box 2*). Laboratory studies of these form the basis of our present ideas about the chemical evolution of Mars. The association of the SNC meteorites with Mars is based primarily on the fact that their trapped noble gas isotopic compositions (particularly for Ar and Xe) match those measured for the Martian atmosphere by the Viking spacecraft that landed on Mars 21 years back. These isotopic compositions are very different from those of the terrestrial atmosphere or those found in other meteorites.

The chemical analysis of the SNC meteorites suggests that just like earth, core and mantle formed in Mars very early in its evolutionary history. Magmatic

Box 2. SNC Meteorites

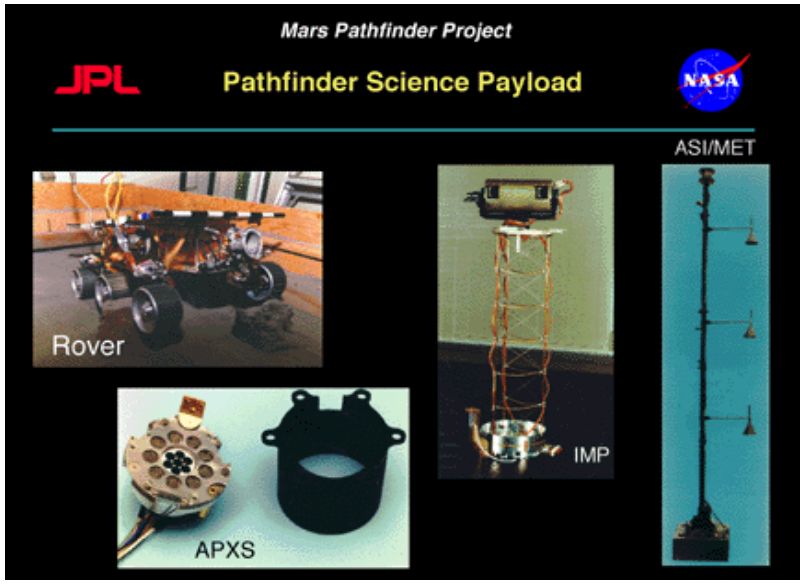
People who may have seen the meteorite that fell in Shergotty village in Bihar in 1865 could never have believed that they were looking at a rock fragment hurled down from the planet Mars. This meteorite, named Shergotty, was labelled as 'anomalous', because of its peculiar characteristics, for more than a hundred years. However, studies carried out in the seventies on this and several other anomalous meteorites like the Nakhla (that fell in Egypt in 1911) and Chassigny (that fell in France in 1815), showed distinct features that suggest that they could be from Mars. This was finally confirmed when their noble gas isotopic composition was found to be similar to that of Martian atmosphere. These and several other meteorites, called the SNC meteorites, are now considered to be of Martian origin.

The Rover positioning the APXS against Yogi.

Courtesy NASA

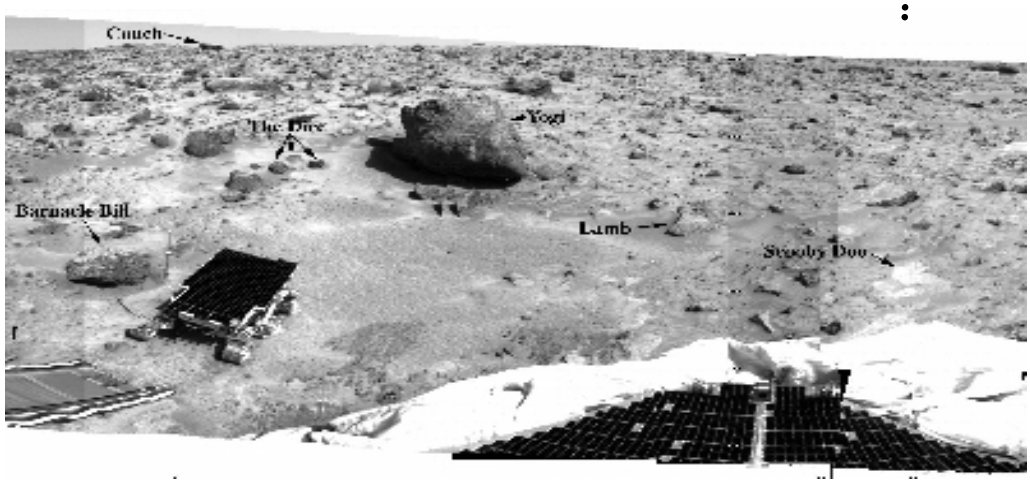
(volcanic) activity continued at least till 1.3 billion years ago as indicated by the formation ages of these meteorites. Microscopic analysis of samples of these meteorites clearly shows the presence of minerals that are not of terrestrial origin and are produced by aqueous activity, indicating the presence of water on Mars sometime during the last 1.3 billion years.

If water and an atmosphere are present in a planet and the temperature is not too harsh (the temperature on Mars varies from –20 to –80 degrees Celsius), one can always ask if some form of life could have developed in it. In fact, the main objective of the Viking mission was to look for possible signatures of life on Mars. Although Viking's results were negative, scientists claimed last year that they have found fossil records of life in Mars based on analysis of a Martian meteorite recovered from Antarctica. They argued that the presence of certain organic molecules (polycyclic aromatic hydrocarbons) and extremely small carbonate structures that resemble those produced in the terrestrial environment by nano-bacteria suggests biologenic activity within this Martian meteorite. Since terrestrial contamination can be ruled out for the observed effects, they suggest possible biogenic activity on Mars that was aided by Martian water. One can hope that Pathfinder and Sojourner and later the Mars Surveyor will be able to identify potential Martian rocks and soils



Instruments on the Rover.

Rocky Marscape with the names of the rocks.



Courtesy NASA

that may contain fossil records of biogenic activity which can then be targeted for collection by future sample return missions.

The pathbreaking success of the Pathfinder and Sojourner and the planned follow-up missions to Mars and other planets and satellites should provide us with a wealth of data to consolidate our understanding of the evolution of the Solar System. We can look forward to some exciting new discoveries in planetary sciences in the beginning of the 21st century.

Jitendranath Goswami, Physical Research Laboratory, Navrangpura, Ahmedabad 380 009, India.