Hello Dolly!

K. P. Gopinathan

The recent creation of a true clone of a mammalian species in the laboratory, a sheep named Dolly, has evoked widespread interest and concern in all sections of people. The major question is how close are we now to generating human clones (see cover).

Hello Dolly, Well Hello Dolly,
It's so nice to have you back where you belong....
You are looking swell, Dolly, I can tell Dolly....

So goes the popular song from one of the greatest hits of Broadway, *Hello Dolly* of the 1960s. We say Hello now to another Dolly but this time near Edinburgh, Scotland. She has become the Darling of so many people already. Created by Ian Wilmut and his team of scientists at the Roslin Institute for Agricultural Sciences, Dolly is probably the first true clone of a mammal. The scientific team has developed a live sheep, now about 6 months of age, from a single cell derived from the udder tissue of a mother sheep by transplanting its nucleus into an egg cell from which its own original nucleus was eliminated. The egg cell containing the nucleus from the fully differentiated udder cell was then implanted into the reproductive tract of a foster mother, which developed to the normal baby sheep. The research paper published in *Nature* by Wilmut and coworkers[1] has raised many an eyebrow.

This indeed is a marvellous technical feat and an exciting piece of science but the hornet's nest has been opened. For, the major concern is – how far away are we from cloning of the human being? A Harvard University Professor is supposed to have written to *Nature* to stop the publication of this article, being genuinely concerned about its social and ethical repurcussions on our race. The US President Bill Clinton has already asked the 'Bioethics Advisory Commission' to review the implications on human beings and has banned federally-funded human cloning research, if any, till clearance is received. The French Government spokesman too has reacted in a similar way. The Pope and the Vatican also have expressed concern on the ethics of man interfering with God's work. Newspapers all over the world have carried stories on this 'development'. So, what is this excitement all about and do we really have to fear? Let us look at the scientific part first.

It is generally accepted that in multicellular organisms, every single cell contains the same set of chromosomes and therefore harbours the same information content. That it is so is established already in plants because from a single cell derived from any part of the fully grown plant a whole plant or tree can be regenerated. This capacity of a single cell to give rise to a whole organism, generally referred to as 'totipotency' was by and large considered to be restricted to the plants. How could a

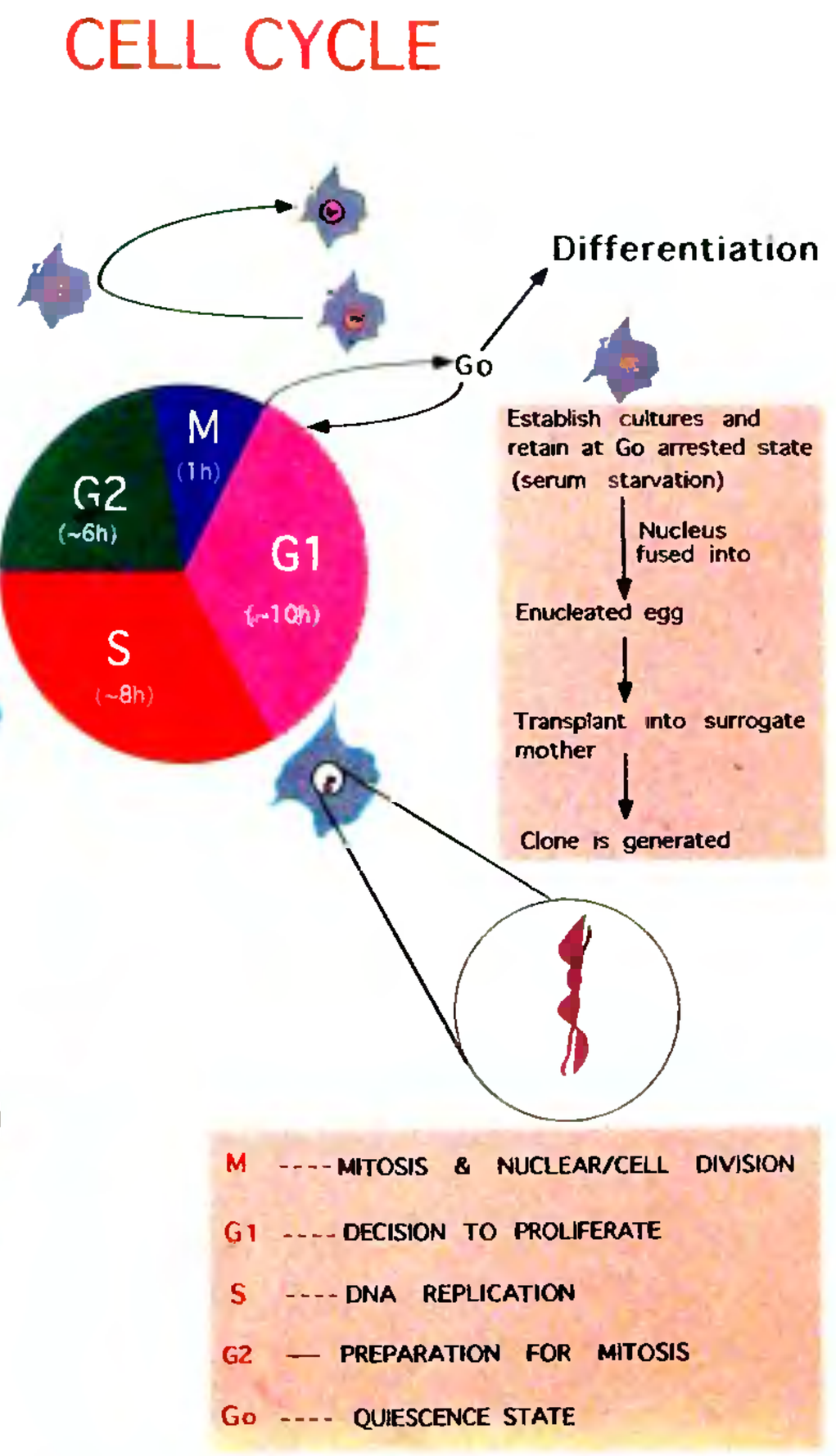

Figure 1. The cell cycle progression in a normal eukaryotic cell[2]. The shaded box on the top right outlines the approaches followed by Wilmut *et al.*[1], to generate the sheep clone, Dolly.

single cell from a fully differentiated organism derived from, say, the arms or legs of a human possibly give rise to a whole human being? It was widely assumed that such a feat will be impossible although theoretically conceivable. If one completely rips apart the whole human body, the entire set of cells numbering about 10,000 billion (10^{13} cells) should have the same set of chromosomes and genes each one harbouring the complete information to give rise to a whole human being. But how do we know that each cell contained the full complement of the genetic information? No one has sequenced so far the entire set of chromosomes/genes from different cells from a single individual or for that matter even from a single cell of an individual. The evidence is mostly circumstantial and deduced through indirect experimentations. As we know, the whole human body has been formed, after all, from a single egg cell derived from the mother following fertilization by a sperm cell originating from the father. The fertilized egg, the zygote, has gone on dividing to give rise to 2, 4, 8, 16, 32, ... and so on cells, each being the descendent of the immediate parent and consequently inheriting an identical daughter nucleus. In the development process, however, the axes in which the cell divides is an important consideration in the context of differentiation. Thus, the division of the embryonic cell on the X, Y or Z axes, decides the 'head or tail' (anterior/posterior), 'left or right' (proximal/distal) or 'front or back' (dorsal/ventral) development programming of the organism. It is clear therefore, that at precise points of time during the embryonic development, decisions are made about the fate of the cell and all its descendants then obey the order. At a molecular level, does this decision making involve a complete set of rearrangements of genetic information on each of the chromosomes within that cellular nucleus? For certain, we know that in the antibody producing white blood cells, the genome undergoes permanent rearrangements at the immunoglobin locus to give rise to only one particular type of antibody. If such rearrangements have taken place at other loci in other fully differentiated cells, it is possible that one can never retrieve the full genetic information to regenerate the whole animal from a single committed cell.

However, in the amphibians, as early as 1960s the Oxford Zoologist Gurdon[2,3] had demonstrated that if the nucleus derived from the mature intestinal cells of the adult frog is implanted into the enucleated frog eggs, the embryos developed into tadpoles and matured into frogs. But then, a frog is a frog is a frog! The experiment was widely appreciated by the scientists and hardly led to any serious reactions from the general public. In essence, the present set of experiments conducted by Wilmut and coworkers are similar except that the system explored is mammalian. Obviously, we have moved several steps closer to the possibility of doing such experiments with humans and that indeed is the concern. In fact, the developmental potential of mammalian embryonic nuclei (but not those from adult or fully differentiated tissues) was reported previously in the 1980s by Illmensee and colleagues[4] by transplanting them into mouse eggs and obtaining live born nuclear transplant recipients; however this claim has been contested subsequently[5–7].

What are the special features of the Wilmut experiments reported presently? The research team had earlier established that viable lambs can be produced by transplanting nuclei from very early sheep embryos (blastocysts) to enucleated eggs. They now demonstrate that the same procedure can be used to obtain viable lamb from a cell line that was established from the udder (a fully differentiated tissue) of a six-year-old ewe. In the past, the attempts to clone mammals have had mixed outcomes. Nuclei taken from mouse embryos older than 8-cell stage from the totipotent embryonic stem cells or from inner cell mass failed to produce viable embryos, although adult sheep clones were produced using nuclei from early morula (8–16 cell stage embryos). The major problem in getting the transplanted oocytes to develop properly was the cell cycle incompatibility between the donor nucleus and recipient oocytes (see Figure 1, for cell cycle progression). In mammals, most of the embryonic nuclei used as donors were in either the S or G_2 (non-diploid) phases of the cycle, which is not compatible with the diploid metaphase II-arrested oocytes, the preferred recipient stage. Additional rounds of DNA replication and premature chromatin condensation in such cells result in abnormal development. In the present set of experiments, the nuclei from cells that have been arrested in G_0 phase of cell cycle by serum starvation were utilized for transplantation which perhaps provided the most favourable conditions for the proper development of the embryos.

By these experiments, Wilmut and coworkers[1] have clearly established that during the growth and differentiation of an embryo, irreversible modifications of the genome do not occur in somatic cells. Nuclear transplantation from somatic cells could be used to produce clones of sheep that have been selected for particular traits, although the number of successful transplants is still low. This approach may prove to be of great use to produce sheep of desired germ line, though still not easily practicable. Newspaper reports have already appeared stating that at the Oregon Primate Research Center in the US, Don Wolf and his colleagues have established the clones of simians, although by a different approach. Here, instead of nuclear transplantations into the egg cells, the reimplantation of portions of blastocysts derived from a single fertilized egg into surrogate mothers has been successfully attempted to generate the clones. However, the latest newspaper reports state that the technique employed here was also nuclear transplantation. Have we thus moved a step closer to the cloning of the human race or is it still a bug bear? Technically, the feat is not going to be very different but the issue is an ethical one, as to how far we want to go. If one were to clone a particular human being with the 'desired traits', the first task is to address the question of what the desired traits are. Is it fair skin or blue eyes or blond hair, or is it a higher level of intellect that makes a better human being? Are the presence of genes alone enough to confer the behavioural traits of the clone and what role does the environment play to dictate his/her gene functions? The desire to clone the idolized 'father' by an overaffectionate son or daughter, or for that matter, of any other kith or kin, if achievable, should also address the matters related to personal relationships and emotional attachments. In the above instances even if the clone is generated through the successful embryonic development, will the emotional linkage

between the individuals be sustained? In other words, can the soul be custom made? Duplications do occur naturally as in the case of identical twins, but the idea of a shadowy duplicate of oneself is undoubtedly a deeply disturbing thought. Are the clones of Frankensteins and Adolf Hitlers already in the production pipeline and if not can the days be far?

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Biogenicity of stromatolites and early life on Earth

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The earliest evidence for the origins of life on Earth go back to more than 3.5 billion years (b.y.) and this has been gathered from what is believed to be remnants of life on primitive Earth – certain odd dome-shaped structures called 'stromatolites'. These are actually made up of wavy and wrinkled layers, hardly 50–200 μm thick, and built out of fine marine sedimentary particles of silica, carbonates, barite or gypsum by microorganisms (bacteria, algae) layer upon layer (Figure 1). Earlier this century, these layered structures were mistaken as remains of other organisms like formaminifera, sponges and related marine species. However, their biological connections became established only after finding microbial fossils in some. Further, in modern times, cyanobacteria and algal communities are seen building up stromatolites off the west coast of Australia[1,2], and also in certain hot springs. Scientists were, therefore, convinced about the biogenicity of these primeval structures. Stromatolites have been reported from many parts of the world and some of the well-known occurrences are from Gunflint Formation in Canada (2.0 b.y.), Bulawayan Group in Rhodesia (2.5–2.8 b.y.), Pongola Group in South Africa (3.0 b.y.), Warawoona Group in Australia (3.3 b.y.) and in India they have been described from the Sandur region, Karnataka, dated around 2.9 b.y.

During the last few years, scientists have pushed back the beginnings of early life by reporting discoveries of microbial fossil-bearing stromatolites from the 3.4–3.5 b.y. cherts of Warawoona Group and Pilbara Block in western Australia[3,4], the 3.5 b.y. cherts in South Africa and 3.77 b.y. quartzites from Greenland[5]. Several of globally distributed Banded Iron Formations, some of them as old as 3.5 b.y. are themselves believed to be strong evidence for life, inasmuch as the conversion of iron to iron oxide in these formations requires free O_2 (absent 3.0 b.y. ago) which must have been supplied by O_2-producing microorganisms (cyanobacteria). These, no doubt point to the prolific activities of bacteria in those distant geological times and also imply that terrestrial life must have begun much earlier to have evolved sufficiently to build these organo-sedimentary stromatolites[6].

Though geologists were convinced about the role of microorganisms in building up the stromatolites and hence considered them as good evidence for life on primitive earth, doubts have now arisen whether some of the stromatolites can as well be non-biologic in origin, quite unrelated to life. This view has been particularly strengthened by the absence of fossil microorganisms in many stromatolites of Archaean age (4.5–2.5 b.y.), a view further supported by the latest observations by John P. Grotzinger and Daniel H. Rothman of MIT who found inorganic precipitates of aragonite and calcite on the ocean floor strongly simulating stromatolite structures[7]. The reliability of stromatolite as evidence for life has, therefore, come under a cloud and geologists have now felt a need for reexamination of the notable occurrences around the world as to their mode of genesis.

In their work, which has somewhat jolted the accepted notions, Grotzinger and Rothman have made critical morphological characterization of 1.9 b.y. old sub-tidal reef stromatolites from Wopmay Orogen, northwestern Canada; they examined the out-crops on the field and undertook laboratory measurement of thicknesses of calcite layers on polished samples and felt, on basis of microscopic textures and fractal dimensions, that these layered structures would very well be non-biologic or abiotic. According to the mathematical models they evolved using computer simulations, they could account for the development of these layered growth through the following four abiotic growth mechanisms: '(1) fall-out of suspended sediments; (2) diffusive smoothing of settled sediments (that is, sediments move downhill at a rate proportional to slope) and surface tension effects in chemical precipitation; (3) surface-normal precipitation; and (4) uncorrelated random noise representative of surface heterogeneity and environmental fluctuations'. The two, however, do not doubt the biogenicity of some of the world occurrences of stromatolites, but feel that in the absence of microfossils or other unambiguous clues in them, their biological origins cannot be taken for granted.

Grotzinger and Rothman's proofs about the abiogenicity of some of the stromatolites do not, however, rule out