heliocentric revolution.

There is a comment about whether the 'Kerala astronomers maintain the distinction between the mean and the centre of the epicycle of an interior planet, even though both move together in the sky?'. What is perhaps meant is the possible distinction between the mean sun and the centre of the manda deferent circle of an interior planet. For the Kerala astronomers there is no distinction between these two, and in their texts there is no ambiguity on this issue.

The suggestion about the Kerala astronomers having 'studied infinite series some years later while the rest of the country was going through an academic dark age', is historically inaccurate. The discovery and study of infinite series is much prior to the work of Nilakantha and dates back to Madhava of Sangamagrama who lived in the middle of 14th century.

K. RAMASUBRAMANIAN
M. D. SRINIVAS
M. S. SRIRAM

Department of Theoretical Physics
University of Madras
Guindy Campus
Madras 600 025, India

Licence to replicate DNA

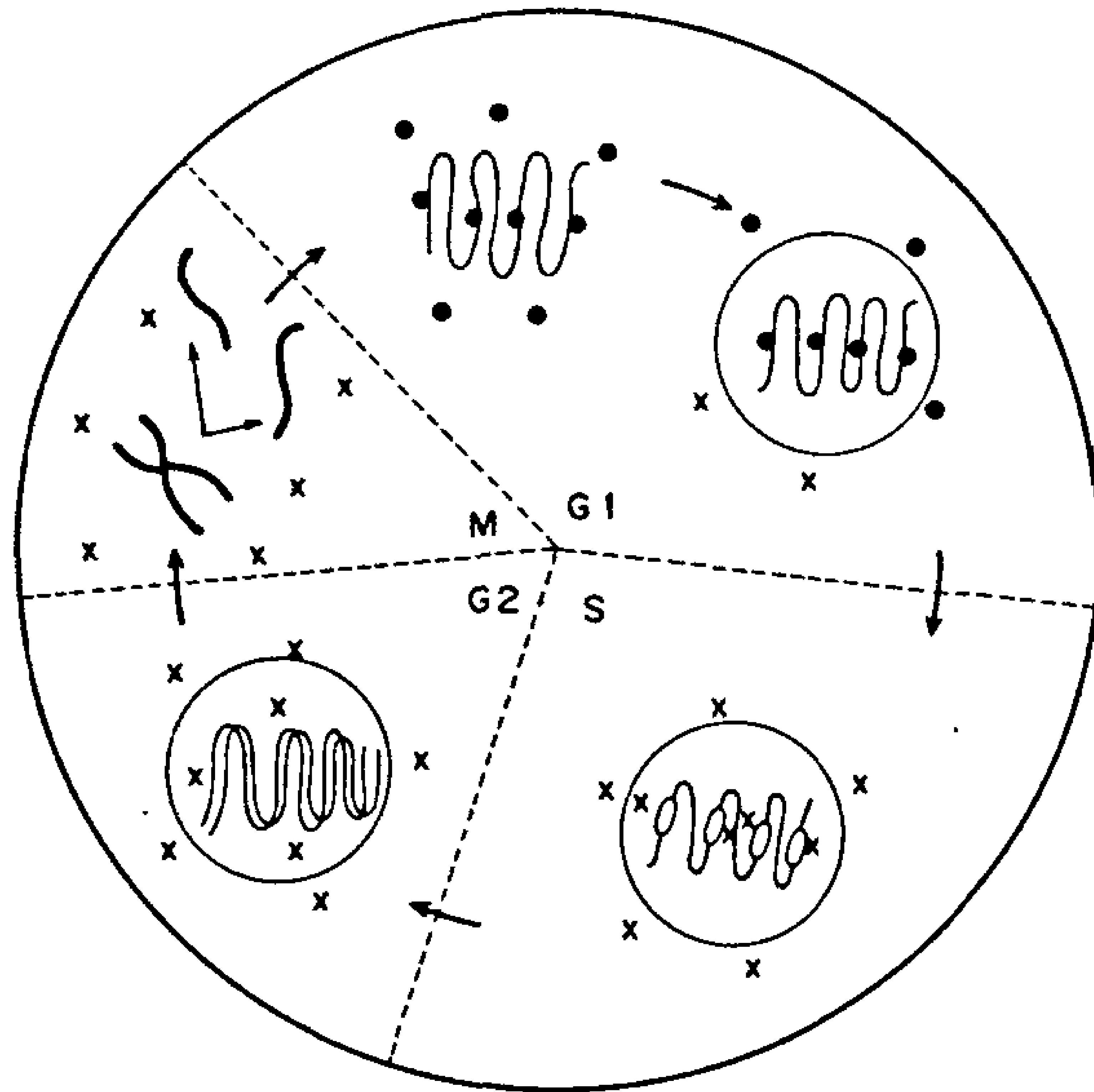
Veena K. Parnaik

The life cycle of a eukaryotic cell is divided into a phase of DNA synthesis or S phase, and a mitotic or M phase, that culminates in the division of the cell into two daughter cells. There are 'gaps' between these two events (G_1 before S phase and G_2 after S phase), during which time protein synthesis takes place. DNA replication in eukaryotes is initiated at multiple sites called replicons: a typical mammalian cell may have thousands of replicons, whereas a prokaryote such as *Escherichia coli* has only one replicon. Within a single S phase in a eukaryotic cell, all genomic DNA is replicated once and only once before mitosis ensues. The processes that coordinate initiation of replication at the multiple replicons must be precisely regulated to prevent re-replication or under-replication of DNA. What are these regulatory processes?

The classical cell fusion experiments of Rao and Johnson[1] first demonstrated that G_1 and G_2 nuclei differed in their capacity for DNA replication. G_1 nuclei were induced into premature S phase upon fusion of G_1 and S phase cells, whereas G_2 nuclei did not initiate DNA replication upon fusion of G_2 and S phase cells. In addition, cytoplasmic components of either G_1 or G_2 cells did not inhibit DNA synthesis of S phase cells. Thus G_1 and G_2 nuclei differed fundamentally in their replication capacity. Several years later, Blow and Laskey[2] proposed a simple though elegant model for the control of DNA replication in the cell cycle, whereby a factor essential for DNA replication was unable to cross the nuclear envelope but could gain access to DNA only when the envelope was disassembled at mitosis. This factor was named the 'licensing factor'. The model envisages the following sequence of events. The licensing factor binds to DNA during mitosis, with no unbound factor being available. After DNA is assembled into a mature nucleus, replication forks are initiated at the sites of bound licensing factor. At each site, the factor supports only one round of initiation and is then inactivated. This means that the entire genome is replicated precisely once. Nuclei in G_2 are unable to re-replicate because the licensing factor in the cytoplasm cannot gain access to the DNA until the nuclear envelope is broken down at mitosis. According to this model, there is a requirement for a 'positive' licensing factor. Alternative models involving the escape of a diffusible inhibitor ('negative' licensing factor) at mitosis were also suggested. In later experiments, it was shown that treatments that permeabilized the nuclear envelope caused reinitiation of replication in various *in vitro* systems, as predicted by the model.

Laskey and coworkers[3] and Blow[4] have recently provided direct experimental evidence for a 'positive' licensing factor. These investigators have employed cell-free extracts of *Xenopus* eggs to study DNA replication *in vitro*. Exogenous DNA added to the cell-free extract is organized into normal interphase nuclei by the assembly of a nuclear membrane from membrane precursors present in the extract. Isolated G_1 nuclei from synchronized mammalian cells can also be added directly to the cell-free extract. In both cases, the extracts support efficient initiation and completion of replication of DNA through a single round of replication. However, G_2 nuclei do not replicate their DNA in the egg extract unless their nuclear membranes are first permeabilized. In their recent studies, Laskey and coworkers have provided definitive evidence for a 'positive' licensing factor. They argued that if replication in G_2 nuclei was prevented by an inhibitor or 'negative' licensing factor which could escape when the nuclear membrane was permeabilized, then repair of the membrane before addition of the nuclei to fresh extract would still allow replication because the inhibitor would have escaped during the permeabilization. However, if DNA replication required the inward movement of a 'positive' licensing factor, then membrane repair before addition of nuclei to the extract would reimpose the block to replication. The authors then demonstrated that the repaired nuclear membrane blocked DNA replication in G_2 nuclei (from the mammalian cell line, HeLa), thus providing direct evidence for a 'positive' licensing factor.

Blow[4] has designed experiments to explore the possibility that the licensing factor might be an enzyme which can phosphorylate specific sites on chromatin required for initiation of DNA replication. This was based on earlier studies which revealed that the initiation of DNA



Blow's recent model for action of licensing factor during the cell cycle (adapted from ref. 4). On exit from mitosis (M), licensing factor is rapidly activated and binds to future sites of initiation of DNA replication in the decondensed DNA. As the nuclear envelope reforms, some licensing factor remains in the cytoplasm and gradually gets inactivated. During S phase, a single initiation event is supported at each licensed site, after which the licensing factor is inactivated. Fully replicated DNA cannot rereplicate in G_2 due to absence of active licensing factor. ●, Active licensing factor; ×, inactive licensing factor. A single chromosome is depicted for clarity.

replication in *Xenopus* egg extracts was dependent on the activity of cdc2-like protein kinases[5] (cdc2-kinase is essential for entry of cells into mitosis), and also the fact that the *simian virus 40* protein, large T antigen, which is required to initiate viral DNA replication, is regulated by a cycle of phosphorylation and dephosphorylation[6]. Blow assayed various inhibitors of cdc2-like kinases for their ability to block the initiation of DNA replication in *Xenopus* egg extracts. He observed that metaphase-arrested extract treated with 6-dimethylaminopurine or staurosporine could not support initiation of DNA replication, whereas nuclear assembly and elongation of previously initiated forks could still occur in the extracts. Thus the licensing factor appears to be a cdc2-like kinase. An additional finding is that the levels of licencing factor activity vary considerably during the cell cycle. The factor is rapidly activated after entry of cells into interphase, in early G_1, and is inactivated in late S phase. In both the above papers, the authors discuss the possibility that the yeast licensing factor may belong to the CDC46 family of proteins. The CDC46 protein is essential for an early event in DNA replication and shows cell cycle-dependent changes in localization: it is nuclear in G_1 and S but is found in the cytoplasm throughout G_2 and mitosis.

Certain questions regarding the licensing factor model remain to be resolved. These have been discussed by Laskey and coworkers[3]. Blow and Laskey[2] originally proposed that the licensing factor enters the nucleus only when the nuclear envelope breaks down at mitosis. However, the nuclear membrane does not break down in yeast and other lower eukaryotes but they are still able to limit DNA replication to one round per cycle. In these cases, accumulation of proteins essential for replication, such as the licensing factor, should occur by regulated nuclear import in a specific phase of the cell cycle. This raises the possibility that in higher eukaryotes also, the licensing factor may enter the nucleus by active transport across the envelope prior to S phase, rather than at mitosis. Hence experimentally induced permeabilization of the envelope may only bypass this step rather than mimic the effects of envelope breakdown at mitosis, and repaired membranes may not exhibit all the features of authentic nuclear import. Clarification of some of these points must await future studies with *in vivo* systems, and availability of purified licensing factor.

1. Rao, P. N. and Johnson, R. T., *Nature*, 1970, **225**, 159–164.
2. Blow, J. J. and Laskey, R. A., *Nature*, 1988, **332**, 546–548
3. Coverley, D., Downes, C. S., Romanowski, P. and Laskey, R. A, *J Cell Biol.*, 1993, **122**, 985–992.
4. Blow, J J, *J. Cell Biol*, 1993, **122**, 993–1002.
5. Blow, J. J. and Nurse, P., *Cell*, 1990, **62**, 855–862.
6. McVey, D., Brizuela, L., Mohr, I., Marshak, D. R, Gluzman, Y. and Beach, D., *Nature*, 1989, **341**, 503–507.

Veena K. Parnaik is in the Centre for Cellular and Molecular Biology, Hyderabad 500 007, India