for rice cultivation was elaborated by R. P. Singh (Banaras Hindu University). A saving of 40 kg N ha^{-1} was observed by using *Eichhornia* leaf as green manure at 10 tons ha^{-1}. Three papers were devoted to use of microorganisms for the removal of pollutants. Arvind Kumar (Banaras Hindu University) described a strain of *Pseudomonas* capable of degrading phenol as well as acrylamide. V. Mishra (Delhi University) demonstrated degradation of phenol and BHC (an insecticide) by *Nocardia* sp. and *Sphingomonas paucimobilus*, respectively. Sarita Singh (Banaras Hindu University) discussed the use of microalgae for stripping toxic metals from wastewaters. Immobilized algae and encapsulated *Microcystis* were found to have exceptionally high metal-binding abilities.

The following recommendations were made: (i) Mass awareness campaigns be launched with a larger participation of school/college students, NGOs and mass media. (ii) Conventional and biotechnological methods be adopted to save the existing population of species listed as threatened or known to be sensitive. (iii) Management of national parks, wildlife sanctuaries and biosphere reserves be done in a more effective manner, keeping in view also the needs, aspirations and concerns of local people. (iv) Restoration of natural populations can be promoted only by acquiring information on floristics, populations, reproductive biology, genetic diversity, microclimatic condition and structure and functioning of ecosystems. Three or four multidisciplinary centers be created for training people in the above aspects. Such centers should be located at places, like the Botany Department of Banaras Hindu University, widely known for its infrastructural facilities and expertise. These centers should also be involved in mass awareness campaigns. (v) There should be greater cooperation between universities and institutes for conserving species and ecosystems. (vi) Multidisciplinary courses on conservation biology at the post-graduate level be started at select universities and institutes including the Banaras Hindu University. (vii) Static and predictive inventories of biodiversity for various regions be prepared. Environmental 'hot spots' be identified and their biodiversity assessed. (viii) Botanical gardens for *ex situ* conservation be set up in various phytogeographical regions. (ix) Appropriate technologies be evolved for managing species diversity of small protected systems (patches). (x) Taxa which are not only threatened but have economic potential as well be prioritized. (xi) Wild relatives of cereal, fruit, and vegetable plants be conserved. (xii) Site-specific technologies be developed for rehabilitation and restoration of degraded/derelict ecosystems.

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Chronobiology hits pay dirt: The identification and cloning of the first circadian clock gene in mammals

M. K. Chandrashekaran

The first recorded circadian rhythm was for the 'sleep' movements of the leaves of the tamarind tree by the Greek philosopher Androsthenes when he joined Alexander the Great in his march on India in the fourth century BC. Two hundred years ago Lamarck had constructed a 'floral clock' based on his knowledge of opening of flowers at different hours of day. The French astronomer de Mairan performed in 1729 a blemishless experiment in the modern experimental tradition and established that the closing and opening leaf movements of the touch-me-not plant *Mimosa pudica*, were endogenous. He removed *M. pudica* plants into a deep cave and demonstrated that the sleep movements *persisted* in the continuous darkness of the cave[1]. Buenning[2] demonstrated through means of crossing experiments that the daily rhythms in the sleep movements of the leaves of the bean plant *Phaseolus multiflorus* were heritable, i.e. had a genetic basis. Thus, there were early and convincing leads pointing to the genetic basis for the ubiquitous circadian rhythms (from the Latin *circa* and *dies* (day) and not *diem* as most authors writing in English write, e.g. ref. 3) which characterize the behaviour and physiology of organisms from fungi to humans. It will be of much interest to the students of the history of scientific ideas that until about 1960, even to proclaim the existence of an endogenous diurnal rhythm was regarded, even by some well-known biologists, as subscribing to a mystical or metaphysical notion. It was with the symposium on Biological Clocks (1960) held at Cold Spring Harbor, New York, with E. Buenning in the Chair, that an era of intensified experimental work was ushered in. Today several laboratories the world over are working on the molecular biology and behavioural expressions of circadian rhythms. With customary foresight The National Science Foundation in the USA, has established a full fledged Center for Biological Timing. This Center brings together geneticists, molecular biologists, endocrinologists and statisticians from Brandeis University, Rockefeller University, Northwestern University and the University of Virgina.

Defining features of all circadian rhythms are (i) persistence in constant light (LL) or darkness (DD) with a *circa* 24 hour period, (ii) compensation of period in the face of changes in temperature and (iii) entrainability to LD cycles. The importance of circadian rhythms in human physiology and well-being is now widely recognized. The human circadian system is directly implicated in jetlag, shift work, space travel, sleep disorders, endogenous

depression and seasonal affective disorders. Knowledge of physiological properties of circadian rhythms has already paved the way to successful amelioration of the symptoms of these various disorders. Therefore, better understanding of the fine-structure of cellular and molecular mechanisms of circadian clocks holds great promise for the development of non-invasive, innovative treatment strategies for a wide range of clinical disorders ranging from insomnia through incidence and timing of heart attacks and asthma.

The three diagnostic characteristics of circadian rhythms are widely shared among organisms from fungi to mammals, suggesting that the circadian organization is evolutionarily an ancient and highly conserved process, and that different organisms may have similar clock mechanisms. In the meantime, the occurrence of circadian rhythms in mammals has been recognized to be so widespread that a publication on sleep arrhythmia in the eusocial naked mole rat claims 'This naked mole rat *Heterocephalus gaber* is the **only** (emphasis added) wild mammal that does not regularly exhibit circadian sleep–wake cycles'!

Circadian clock genes

The circadian rhythms of *Drosophila* and *Neurospora* have been intensively studied. These organisms offer the advantages of powerful genetic analysis and of circadian rhythms that are easily monitored (locomotor activity and timing of eclosion in *Drosophila* and conidiation process in *Neurospora*). Newcomers to this field are mouse[4], *Arabidopsis* and *Cyanobacteria*[3]. The *per* gene of *Drosophila* and *frq* gene of *Neurospora* are the best studied components of the circadian clock[3] (Figure 1). Furthermore the *per* and *frq* genes have a short region of sequence similarity, indicating that underlying mechanisms may be partly conserved from fungi to insects[5].

In *Drosophila*, two 'clock genes' *per* (period) and *tim* (timeless) have been cloned. Both are essential elements of the circadian timing system. The mRNA and protein products of both genes oscillate and the cycling of each is dependent on heterodimerization of their protein products, PER and TIM. It is still not clear, however, how PER and TIM turn off their own transcription after their temporally gated entry into the nucleus[3].

In comparison to *Drosophila* and *Neurospora*[6] little is known about the molecular biology of circadian rhythms and about the structure of clock genes in vertebrate animals. However, this lacuna in our knowledge about the molecular nature of vertebrate circadian clocks is about to be filled. We are at the very threshold of path-breaking new knowledge. King *et al.*[7] and Antoch *et al.*[8] report that the first candidate clock gene in mammals, aptly named Clock, has now been cloned in mice and the encoded protein has very interesting features[9].

Suprachiasmatic nuclei

This year marks the silver jubilee of the discovery of the circadian clock function of the mammalian suprachiasmatic nuclei (SCN). The SCN are site of a master circadian clock in brain that generates most circadian rhythms in mammals (reviewed in ref. 10). SCN are small-paired structures in the anterior hypothalamus just above the optic chiasmata, each of the paired neural tissue contains about 10,000 neurons. The nuclei receive visual inputs for light–dark entrainment, mostly directly through the retinal pathway.

The demonstration that the SCN contain a circadian clock became possible by the discovery of a spontaneous, semidominant mutation, *tau*, in the Syrian hamster. This autosomal mutation shortens circadian period from 24 to 20 hours in homozygotes. In elegant SCN transplant experiments using tau mutant hamsters, it was shown that the period of restored rhythmicity is determined by the genotype of the donor and not that of the host. This finding lends an unequivocal circadian clock function to the SCN. The tau (period) mutant hamsters could not be used for unravelling molecular mechanisms for want of adequately developed genetic markers in the hamster.

Clock genes in mice

Searching for clock genes is a chancy affair. Some succeeded and many did not. Several laboratories use molecular approaches that revolve around major pet assumptions about the nature of clock genes. Thus homology screening protocols (e.g. PCR with degenerate primers, yeast two hybrid screens for PER- or TIM-interacting proteins) assume that vertebrate clock genes will resemble those in *Drosophila*. Joseph Takahashi and colleagues recommend and employ a 'forward' genetic approach (from phenotype to gene) to identify clock genes in mice[11].

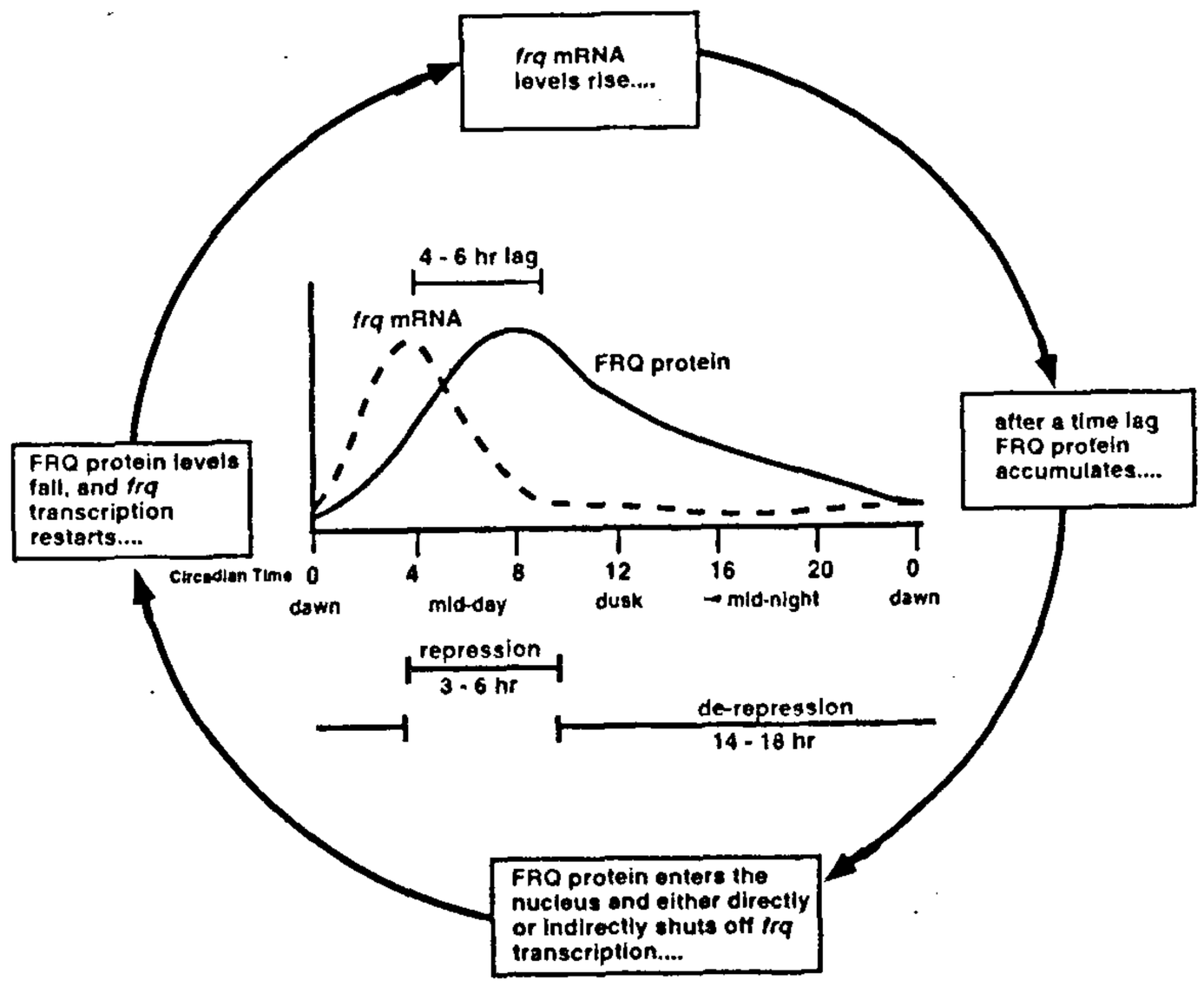
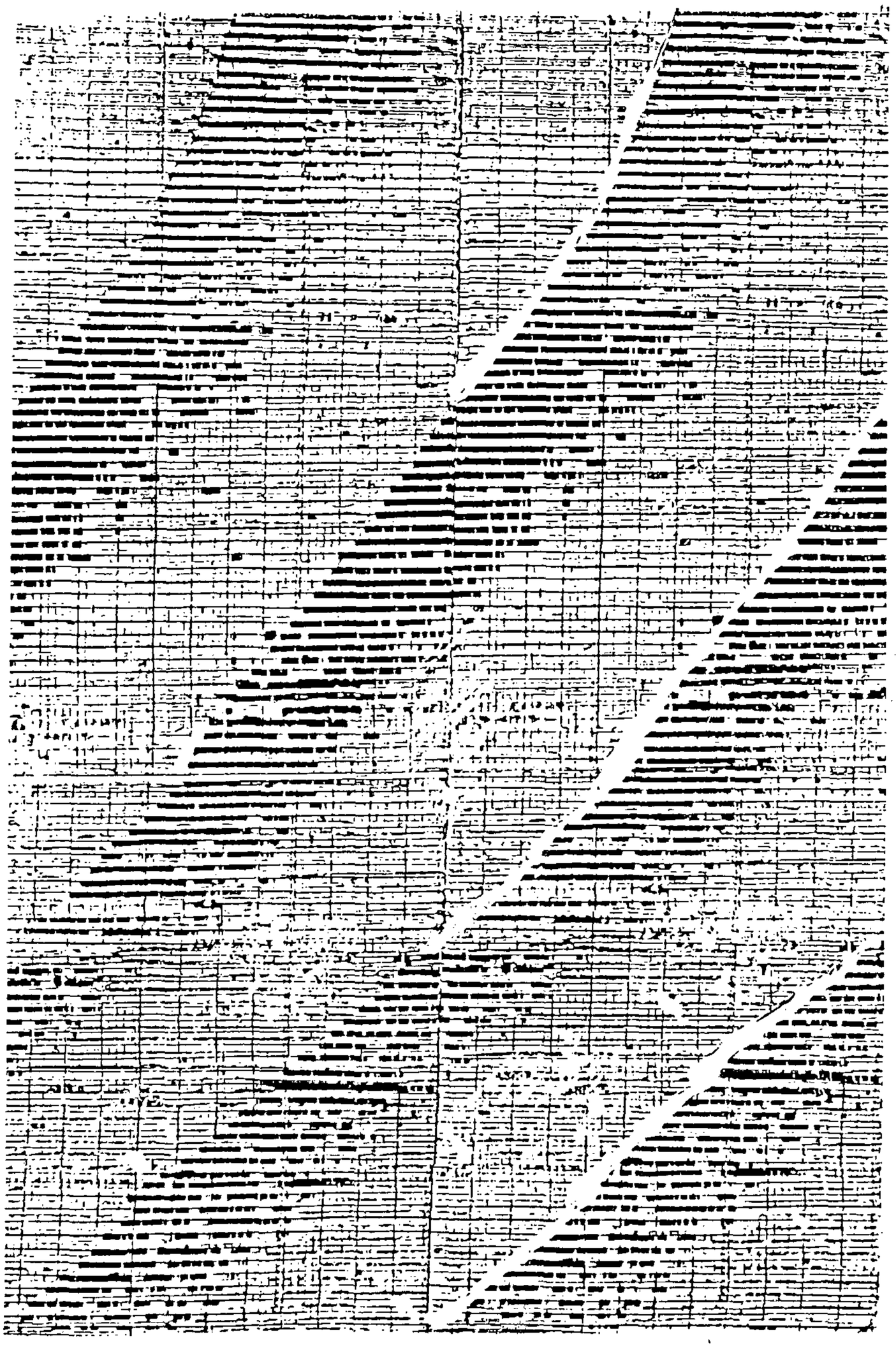


Figure 1. The molecular basis of the *Neurospora* circadian clock. After ref. 6.



igure 2. Wheel running activity of an adult field mouse *Mus booduga* recorded under D conditions for a period of *ca.* 125 days. The aktogram is double plotted for easier sual inspection (Original).

lice are subjected to high efficiency iemical mutagenesis, and the second eneration progeny are then directly creened for dominant circadian clock iutations. The chief virtue of this pproach is that it makes no assumptions bout the nature of mammalian clock enes or their molecular biology. Since e circadian rhythm in wheel running ctivity in mice is very precise (Figure) and easily measured in individual ani- als, changes caused by mutations can be easily detected. Significant changes in period length (usually measured from onset of activity in one cycle until onset of activity in the next cycle and so on for several days of freerun) would imply changes in the basic clock mechanism. Once the mutants are identified, the task of positional cloning of these mutations is now easy[11].

Takahashi et al.[4] reported the discovery of the clock locus in 1994. They found one animal with a circadian period longer by 1 h than those of controls in DD. This long period phenotype was inherited as a semidominant autosomal mutation named Clock. Homozygous clock mice manifest very long periods (27–28 h) on placement in DD and gradually become arrhythmic after a few weeks in DD. Interestingly, if the arrhythmic mice are exposed to a 6 h light pulse, the long period rhythmicity is restored in DD. The Clock-mutation appears to relate to abnormalities of the circadian rhythm alone since no other obvious behavioural or anatomical changes were noticed. The clock phenotype apparently regulates two basic properties of circadian rhythms, the circadian period and persistence of the rhythmicity. A debate had in the meanwhile emerged as to whether Clock actually encodes a clock element or just a clock component effecting 'coupling'. The mouse circadian clock gene is now cloned and details reported in two papers in the 16 May 1997 issue of *Cell*. These reports describe two complementary approaches, positional cloning and functional rescue, that have led to the molecular identification of clock.

One clone produced 'full functional rescue' of the clock phenotype in both heterozygous and homozygous clock mutants, strongly suggesting that the 'clone' contained the entire clock gene, including coding region and regulatory elements. As the authors point out, the use of transgenes to rescue a mutant phenotype provides the most powerful means to prove that a cloned candidate gene is the one responsible for the mutant phenotype[7].

The identification and cloning of clock is a landmark discovery and has already been hailed in a minireview[9] as a 'nugget' of circadian gold. Chronobiology has hit pay dirt. These exciting researches have been carried out by Joseph S. Takahashi and colleagues working at the NSF Centre for Biological Timing, Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois. The mouse is the most complicated organism studied by the CBT researchers and also most closely related genetically to human beings. As Joe Takahashi points out, because of known relationships between the mouse and human genomes, it will take just a short time to identify the homologous gene in human beings now that a mouse clock gene has been discovered. In four decades we have indeed rapidly

progressed in chronobiology from (presumed) status of metaphysics to molecular insights into mammalian clock genes.

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The riddle of the avian ancestry

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The hot topic today in palaeontology is the postulated dinosaur–bird link. The participants in the controversy have pointed out a variety of physiological and anatomical traits to swing the views for a pro- or anti-dinosaur ancestry for the modern birds. How the essentially ground-based animals adapted their skeletal framework and gradually developed feathers and took to air has been a fascinating question engaging avian palaeontologists since the discovery of *Archaeopteryx*, the first bird-like fossil with a few reptilian features in Germany in 1861 (Figure 1). In the process of launching them into air, nature had apparently experimented and evolved many intermediate species with characters that can be interpreted in favour of one or the other theories going around then. For a long time, palaeontologists viewed the Jurassic period *Archaeopteryx* as unquestionably the first bird to flap its wings in the skies, but discoveries of bird fossils in still earlier geological times, hinted evolution of flight, perhaps even in the Triassic, and *Archaeopteryx*, the bird pioneer was dethroned. Several new finds of avian fossils have inundated literature during the past few years from diverse countries[1–4] and they have not only added a fund of data about evolutionary trends, but in their wake, fueled the ongoing controversy about the reptile–dinosaur–bird evolution. Today, what portends to be last straw for the anti-dinosaurian paternity camp has now surfaced in the reported find of a feathered dinosaur fossil in China[5] and this has further exacerbated their already ruffled feathers.

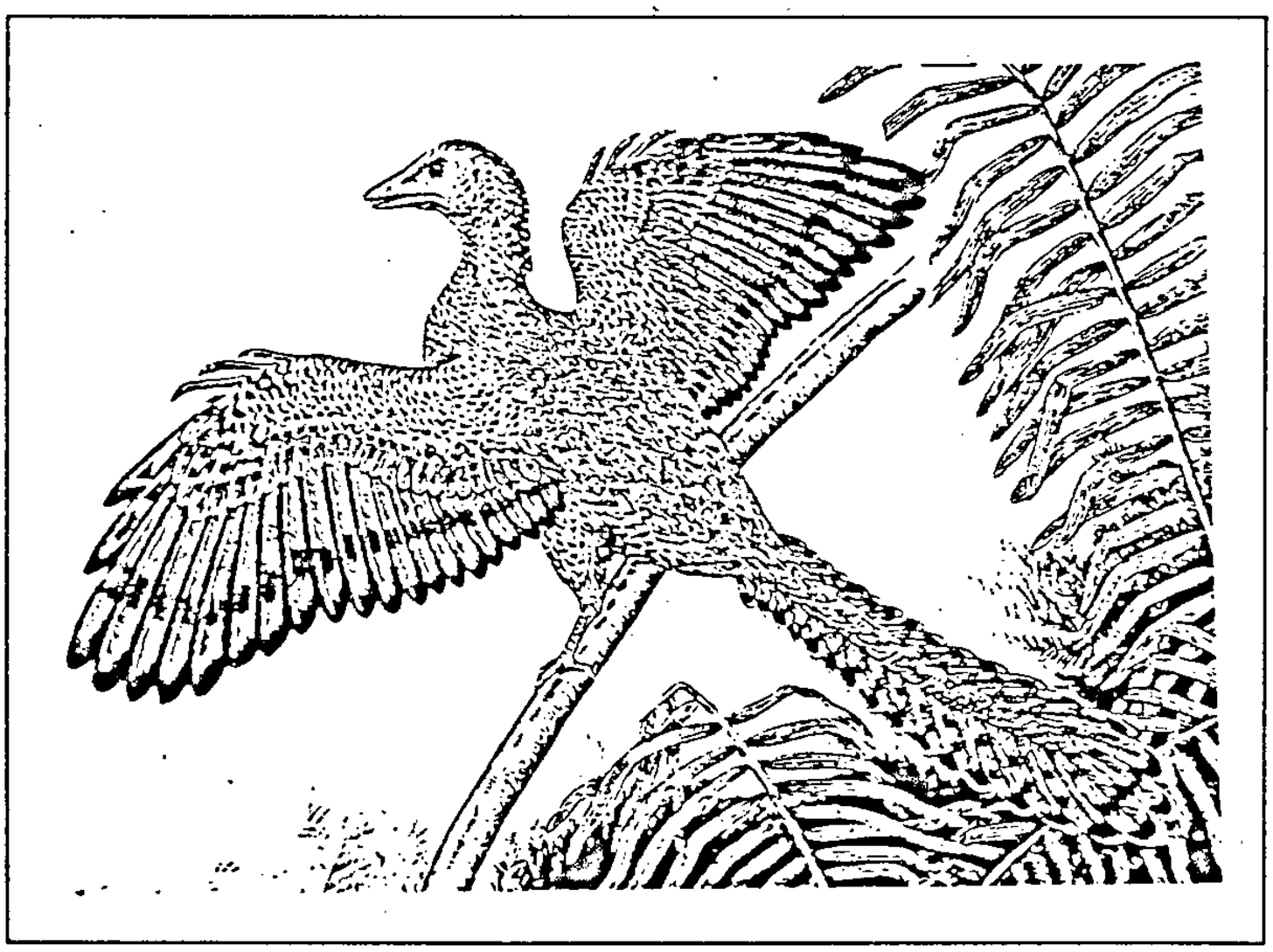

Figure 1. *Archaeopteryx*, the first bird fossil showing clawed wings, toothed beak and reptilian tail with feathers.

Compatible physiology and skeletal anatomy were two dominant aspects most palaeontologists were highlighting in their arguments for or against dinosaur pedigree for birds. Modern birds have skeletal framework specially suited for flight, like air-filled bones for buoyancy, a fused collar bone, breast bone with a deep keel for anchoring the flight muscles, lengthened forelimb (wing) with wrist and fused fingers and a composite pelvis and backbone with a remnant of a tail or pygostyle (Figure 2). Earlier scientists were, therefore, searching for evolutionary trends towards these skeletal modifications among some of the reptiles or dinosaurs they were suspecting as the ancestors. However, for