news and views

Adenovirus amazes at Cold Spring Harbor

from J. Sambrook

The XLII Cold Spring Harbor Symposium on Quantitative Biology, entitled Chromatin, was held on 1–8 June, 1977. New insights into the ways in which gene expression is controlled in adenovirus were reported at the symposium and are described below.

IF success were exactly proportionate to effort invested, our understanding of the mechanism of gene expression in eukaryotes would be virtually complete. Unfortunately, despite intensive investigations for a number of years, our ignorance remains almost total. In part, this frustrating situation stems from the technical difficulty of analysing the expression of genes which may be represented only once in a huge and complex genome. However, the continuing dearth of useful mutations in control regions and the failure to reconstitute *in vitro* systems in which synthesis of mRNA or its precursors is initiated have also been strong contributing factors.

Despite these difficulties, there is circumstantial evidence of several sorts to indicate that extensive control of gene expression occurs at the level of mRNA synthesis. For example, mRNAs which code for specific differentiated products are detectable only in those cells which are committed to make those products. Thus, globin mRNAs are found only in reticulocytes, ovalbumin mRNAs only in cells of the oviduct and so on (see *Nature, News and Views* **267,** 203; 1977). Two competing theories have been used to explain how such gene regulation may occur. The first, based on experience with prokaryotic systems proposes that different regions of the cell's genome are transcribed into mRNA at different frequencies and that mRNA is either the direct product of the transcription event or is derived from precursors which need not be much larger than the mature mRNA itself. This scheme places control of gene expression firmly at the level of transcription, implies that there are control elements before each gene, and relegates post-transcriptional modification to a subsidiary role. The second hypothesis on the other hand suggests that mRNAs are derived from extremely long precursor molecules (identified with heterogeneous nuclear RNA) by a complex set of post-transcriptional steps involving specific endonucleolytic cleavage and modification of the 3′ and 5′ ends of the RNA by addition of poly(A) and methylated bases. This pathway, in which less emphasis is placed on transcriptional control, is attractive because it provides a plausible reason for the existence of mRNA.

To test these ideas, several groups of workers have studied gene regulation in animal viruses as a model system which might be expected to reflect the processes that occur in eukaryotic cells. Recent findings from one of these groups indicate that both mechanisms of control may be active.

Most of the data have been obtained with adenovirus 2, a human virus which grows well in cultured cells. Its genome consists of a linear complex of DNA about 35,000 base pairs in length, sufficient to code for 30–40 polypeptides of average size. The expression of these polypeptides is under temporal control: some of them (confusingly called early proteins) are synthesised at all times during lytic infection; others (late proteins) are sythesised only at late times. The early genes are collected into four widely-spaced regions and during the early stage of infection the only virus-specific mRNA present in the cell is complementary to one strand of the DNA sequences of these four regions (for a review see Flint *Cell* **10,** 153; 1977).

At the Cold Spring Harbor symposium, J. E. Darnell (Rockefeller University) presented evidence to show that each of these four groups of early genes has at least one promoter sequence. Nuclei were isolated from cells early during lytic infection and incubated in the presence of radioactive precursors of RNA in conditions which do not allow the initiation of new RNA chains, but merely the extension by a hundred or so nucleotides of those already begun in the intact cell. At the end of the brief incubation period the RNA was extracted and divided into different size classes by sedimentation through sucrose gradients. The shortest RNA molecules to be labelled *in vitro* should be those that had just begun to be synthesised *in vivo*. By hybridising the short RNA chains to different segments of the viral genome, obtained by cleavage of adenovirus 2 DNA with restriction endonucleases it was a straightforward task to identify the sites at which RNA synthesis had begun. Darnell and his coworkers (R. Evans, N. Frazer, S. Goldberg, T. Weber and N. Wilson) found four starting points for early RNA—two on the *r* strand at positions 1.8 and 80 and two on the *l* strand at positions 98 and 72. Because they lie at the beginning of the genes which code for early proteins it seems likely that these four regions serve as classical promoters—sequences which direct RNA polymerase to begin synthesis of an RNA chain. When infection is stringently blocked in the early phase, no viral RNA sequences are detectable in the nucleus other than those copied from the early regions. Thus expression of early viral genes seems quite clearly to be under transcriptional control.

At late times after infection a very different situation obtains. After viral DNA synthesis begins there is a vast increase in the rate of viral RNA synthesis as well as changes in the pattern of transcription. The cytoplasm contains a variety of new RNA species 1,000–4,000 bases long, that together account for virtually all the information of the viral genome. In addition the nucleus contains high molecular weight, virus-specific RNA molecules, some of which seem to be more than 20,000 bases long. More than 80% of both nuclear and cytoplasic transcripts are complementary to one strand of

Joe Sambrook is Senior Investigator at the Cold Spring Harbor Laboratory, New York.

viral DNA—the *r* strand. In two kinds of experiment described in the April issue of *Cell*, Darnell and his coworkers (Weber, Jelinek & Darnell *Cell* **10**, 611; 1977; Goldberg, Weber & Darnell *Cell* **10**, 617; 1977) have shown that the synthesis of the vast majority of the giant nuclear viral RNA molecules is initiated at a specific site on the adenovirus genome. Once again nuclei were isolated, this time from cells in the late stages of infection, and RNA chains were extended by the addition *in vitro* of a few hundred labelled nucleotides. The radioactive label incorporated into the longest RNA molecules was found to hybridise only to the right hand end of the viral genome: the shortest molecules, however, hybridised preferentially to a region mapping between positions 14.5 and 19.0 on the viral genome. Thus late viral RNA complementary to the *r* strand is synthesised from one long transcription unit, with a promoter near position 14.5 and a terminator near the right hand end of the viral DNA. This conclusion is confirmed by experiments in which infected cells were exposed to ultraviolet irradiation —a treatment which causes lesions in DNA through which RNA polymerase cannot pass.

The frequency at which a particular sequence of DNA will be transcribed will therefore depend on whether a lesion is introduced between the sequence in question and its promoter. If the lesions are randomly distributed throughout the population of viral DNA molecules, transcription should decrease exponentially with distance from the promoter. Late adenoviral RNA synthesised immediately after irradiation was found to hybridise most efficiently with sequences from the left hand 30% of the viral genome and progressively less well with sequences to the right. The results indicate that a late promoter is present near the left hand end of the *r* strand of adenoviral DNA.

Taken together, both types of experiment show, first, that late in adenoviral infection, transcriptional control still occurs, and second, that the site at which RNA synthesis begins is quite specific and efficient. By contrast to the early promoters however, the late promoter near 14.5 on the *r* strand is highly polar and controls the expression of all genes to its right. The primary product of transcription is a long RNA molecule complementary to DNA sequences between 14.5 and 100, which is cleaved endonucleolytically to yield mature mRNA. The shift from the early to the late phase of adenovirus transcription must therefore involve alterations not only in the genes that are expressed but also in the method by which their expression is controlled.

Mass of Saturn's rings

from David W. Hughes

MASS is one of the more awkward quantities to measure in an astronomical context. Usually it is derived from a careful analysis of the perturbing effect the gravitational field of one body has on the orbit of another or, less satisfactorily, by simply multiplying the volume of the object by an estimate of its density. Inevitably the problems and uncertainties increase as the body becomes smaller or more tenuous, so W. I. McLaughlin and T. D. Talbot from the Jet Propulsion Laboratory, California are to be congratulated on obtaining the reasonably precise value of $(3.5\pm1.4)\times10^{24}$ g for the mass of the rings of Saturn. Their calculations have been published in a recent edition of the *Monthly Notices of the Royal Astronomical Society* (**179**, 619; 1977).

The gravitational potential around Saturn is a function of the planet's mass, size and principal moments of inertia and also of the mass, size and orbits of the rings and the satellites. A knowledge of the rate of precession of the satellite orbits enables this potential to be estimated with considerable accuracy. Reliable observations of the six inner satellites, Mimas, Enceladus, Tethys, Dione, Rhea and Titan going back as far as 1888 have been analysed by Kozai (*Annls Tokyo astr. Obs. Ser.* 2. **5**, 72; 1957) and Garcia (*Astr. J.* **77**, 684; 1972) to give the precession rates of the line joining the ascending and descending nodes and also the line joining the apsides.

Using these and also values for the masses and semi-major axes of the satellites, McLaughlin and Talbot have solved the gravitational potential energy function to give values for Saturn's J_2 and J_4 (coefficients in the power expansion of the potential energy function, J_2 being directly related to the moments of inertia about the spin axis and an axis in the equatorial plane) and for the mass of the rings and of the satellite, Rhea. The first solution they presented however did not use a model for the mass distribution inside Saturn and had only a limited usefulness as far as calculating J_4 and the ring mass goes, given the latter imprecisely as $(5\pm33)\times10^{24}$ g.

A much more meaningful solution was obtained by incorporating the results of Hubbard, Slattery and De Vito's high precision modelling of the interior of Saturn (see *Astrophys. J.* **199**, 504; 1975) which led to a direct functional relationship between J_2 and J_4. The inclusion of this gives a ring mass of $(3.5\pm1.4)\times10^{24}$ g, a mass for Rhea of $(2.7\pm0.5)\times10^{24}$ g and values of $(1.664\pm0.001)\times10^{-2}$ and $-(8.7\pm0.1)\times10^{-4}$ for J_2 and J_4. The mass obtained for the rings is very close to the value obtained by Franklin, Colombo and Cook (*Icarus* **15**, 80; 1971) who used a completely different calculation based on the observations of the location of the Cassini division which occurs between the A and B ring. They found a 1,400 km discrepancy between the observed position and that predicted by assuming that the division was due to particles orbiting at that distance being in resonance with the orbit of Mimas, and thus being quickly removed by gravitational perturbation. They concluded that the shift was caused by the self gravitation of ring B thus leading to an augmentation of its outer boarder. To cause the observed shift they calculated that the rings had to have a mass not less than 3.4×10^{24} g a value which agrees surprisingly well with the McLaughlin and Talbot values of $(3.5\pm1.4)\times10^{24}$ g.

So in a mass league of planetary companions the rings of Saturn come in ninth, behind Ganymede, Triton, Titan, Callisto, Moon, Io, Europa and Titania. Also if all the mass in the rings were put together into one satellite with a density say like that of Dione and Rhea (~2.4 g cm^{-3}) this satellite would have a radius of around 700 km.

David W. Hughes is a lecturer in the Department of Physics at the University of Sheffield.

Following the leader

How mRNAs are cleaved from their nuclear precursors is totally unknown, but bizarre processes must be involved. The audience at the symposium was amazed, fascinated and not a little bewildered to learn that late adenovirus mRNAs are mosaic molecules consisting of sequences complementary to several non-contiguous segments of the viral genome. In every case so far examined, late adenovirus mRNAs contain at their 5′ ends about 150 nucleotides which are transcribed not from DNA sequences immediately adjacent to the structural gene, but from sequences which map at distant locations in the viral genome.

P. A. Sharp (Massachusetts Institute of Technology) and his colleagues, S. N. Berget, A. J. Berk and T. Harrison, described experiments in which purified mRNA coding for the major coat protein, hexon, was annealed to a restriction fragment (*Hind*III A) of adenoviral DNA which spans the hexon gene. When the resulting hybrids were examined by electron microscopy, only

15% were found to consist of perfect duplex structures. Protruding from the vast majority was a tail of RNA which was shown by its location and length to consist of about 150 nucleotides at the 5′ (or leading) end of the mRNA. All attempts to remove the leader sequence by incubating hexon mRNA in conditions which destroy RNA–RNA hybrids were unsuccessful, and Sharp and his colleagues were forced to conclude that the leader sequences are not complementary to DNA sequences which lie immediately upstream from the hexon structural gene. The full complexity of the situation was not apparent until hexon mRNA was hybridised to the isolated *r* strand of *Eco*RI fragment A DNA (see Fig. 1). Consistently observed were hybrids whose structure could be interpreted only as shown in Fig. 2*a*.

These results mean that mature hexon mRNA consists of four separate blocks of sequences. Measurement of the lengths of single-stranded DNA and DNA–RNA hybrids show that closest to the 5′ end of hexon mRNA is a small number of nucleotides complementary to position 16.8 on the *r* strand of the viral DNA: next come approximately 80 nucleotides coded by position 19.8; then 110 nucleotides from position 26.9 and finally the coding sequences of the hexon gene itself which maps between positions 51.7 and 61.2. The structure of the hexon mRNA is shown in Fig. 2*b*.

An identical conclusion was reached independently and simultaneously by a number of groups working at Cold Spring Harbor itself, using several different late adenoviral messengers and a variety of different assay systems. Their results may be summarised as follows:

- many if not all adenoviral late mRNAs contain an identical capped oligonucleotide (7Me Gppp ACU [C_4U_3] Grp) at their 5′ end (R. E. Gelinas, R. J. Roberts)
- these capped structures are not complementary to viral DNA sequences near the major late structural genes but hybridise with DNA sequences which map between positions 14.7 and 21 (D. S. Klessig)
- many late adenoviral mRNAs which function in a cell-free system, hybridise not only to the appropriate structural gene, but also to DNA sequences which map between 16 and 32 (J. Lewis, A. Dunn, J. Hassell)
- mRNAs for fibre, 100 K, hexon and penton each contain sequences at their 5′ ends which can be seen by electron microscopy to hybridise to positions 16.7, 19.7 and 26.7 on the *r* strand of the adenoviral genome (L. T. Chow, Gelinas, T. R. Broker & Roberts).

These unexpected findings raise a number of interesting questions. How

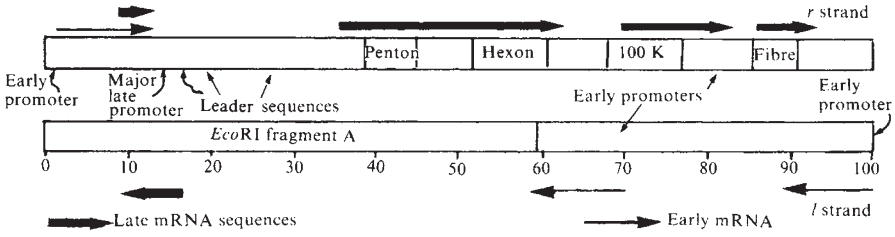

Fig. 1 Transcription map of adenovirus 2 (see Flint *Cell* **10**, 153; 1977).

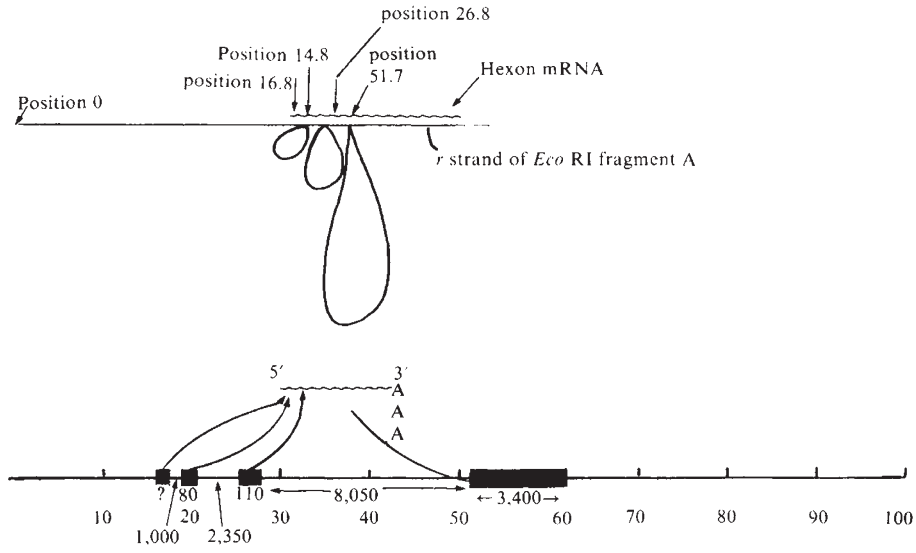

Fig. 2 *a*, Pattern of hybridisation between hexon mRNA and the *r* strand of *Eco*RI fragment A of adenovirus 2 DNA. *b*, Regions of adenovirus genome which contribute to hexon mRNA. Figures other than adenovirus DNA markers represent distances in nucleotide base pairs.

are the mosaic molecules synthesised? Why are the three leader sequences separated from each other and from the structural genes? Do the leader sequences serve any function? How general is the phenomenon?

At the symposium four possible ways were discussed by which mosaic late adenoviral mRNA might be synthesised: first, the polymerase responsible for late adenoviral transcription might jump from one segment of the viral genome to another in an ordered fashion. Second, there might be new DNA templates generated, perhaps by recombination, that would contain the appropriate arrangements of different segments of the viral genome. Third, each of the segments may be transcribed independently and the mature mRNAs may be synthesised by ligation of components. Fourth, all late mRNAs that contain an identical leader sequence may be derived by cleavage from the same nuclear precursor.

The first three hypotheses are inconsistent with the data of Darnell's group discussed above. The fourth hypothesis, however, is identical to the conclusions drawn by Darnell and his coworkers. All the mRNAs that have been shown so far to share the same leader sequence also belong to the same transcription unit and fall under the control of the promoter located on the *r* strand of viral DNA near position 14.5. By contrast a small late mRNA coded by sequences mapping between positions 9.7 and 11.1 on adenovirus 2 DNA and whose synthesis cannot be controlled by the promoter at position 14.5, contains a leader sequence which hybridises to DNA between positions 5.0 and 6.4 (Chow, Broker, Roberts & Westphal).

The best mechanism to explain these findings seems to be that espoused by Klessig. He believes that there may occur in the primary transcript, base-

pairing between the leader sequences and the coding sequences so that mature mRNA would be derived by recombination-like events. Each precursor would then generate one mature mRNA. Perhaps separation of the several leader sequences and those of the structural genes allows several different modes of base pairing whose relative frequencies may determine the rates at which different mRNAs are produced.

The major problem is to find out how widespread the phenomenon is. Already there is evidence presented at Cold Spring Harbor by S. Weissman, Y. Aloni, G. Khoury and Ming Ta-Hsu that a similar situation may occur during synthesis of late SV40 mRNAs. But the key question is whether analogous events happen in uninfected cells. Perhaps it is not too optimistic to hope that what has been so clearly shown for adenovirus 2 may also help to explain how host cells regulate their own genes. □

Genes, channels and membrane currents in *Paramecium*

from Roger Eckert

THE ciliated slipper animalcule first observed by Anton van Leeuwenhoek in 1674, and familiar to generations of introductory biology classes and students of cytoplasmic inheritance, has lately proved increasingly useful for novel interdisciplinary approaches to problems of broad neurobiological interest. This is not a completely new departure, however, for the very first intracellular electrical measurements from animal cells were made on *Paramecium* over 43 years ago by T. Kamada (*J. exp. Biol.* **11**, 94; 1934), a pioneer in the use of saline-filled capillary microelectrodes. More recently the surface membrane of this unicellular organism was found to exhibit properties of electrical excitability (see review in *Calcium in Biological Systems* ed. Duncan, C. J. page 233 Cambridge University Press, 1976), similar in essence to those shown by metazoan nerve and muscle preparations. Most prominent is a graded depolarisation produced by an inward regenerative current in response to depolarising stimuli. The entry of Ca ions carrying this current produces a transient rise in intracellular [Ca] that mediates a reorganisation of axonemal activity seen as reversed ciliary beating with backward swimming. The stimulus–response coupling sequence is summarised in Fig. 1.

As in the classical excitable membranes of nerve and muscle, depolarisation leads to an inward current (carried in *Paramecium* by Ca) followed by a more slowly développing outward K current. The electrically excitable membrane channels that carry the Ca current seem to reside almost exclusively in the portions of the surface membrane covering the cilia, for removal of the cilia eliminates the inward Ca current, which is restored gradually as this portion of the membrane regenerates with ciliary regrowth over a period of several hours (Dunlap & Eckert *J. Cell Biol.* **70**, 245a; 1977). Interest in Ca channels is heightened by reports of their occurrence and functioning in sensory, nervous, secretory, embryonic and motor tissues, and by growing evidence that Ca entering cells often acts as a second messenger in modulating certain metabolic activities as well as membrane conductances (Baker in *Calcium Movement in Excitable Cells,* Pergamon Press, Oxford, page 7; 1975; also various articles in *Calcium in Biological Systems op. cit.* Cambridge University Press, 1976).

Paramecium offers a unique combination of features useful for new approaches to the study of the Ca system and other components of excitable membranes. As a microorganism it can be mass cultured, cloned and manipulated and analysed genetically. It also possesses the fundamental electrical properties of an excitable cell. Because Ca channels are localised in the membrane covering the cilia a greatly enriched channel fraction can be produced by collecting the cilia which have a high surface area/volume ratio. Regrowth of the cilia provides a model system for studies of channel regeneration and insertion into the membrane. In addition, the coupling between membrane function and ciliary activity facilitates identification and isolation of membrane mutants by their sensory and locomotor behavioural phenotypes. The sensitivity of the ciliary apparatus to intraciliary [Ca] also provides an independent assay of Ca entry through the channels of the ciliary membrane (see below).

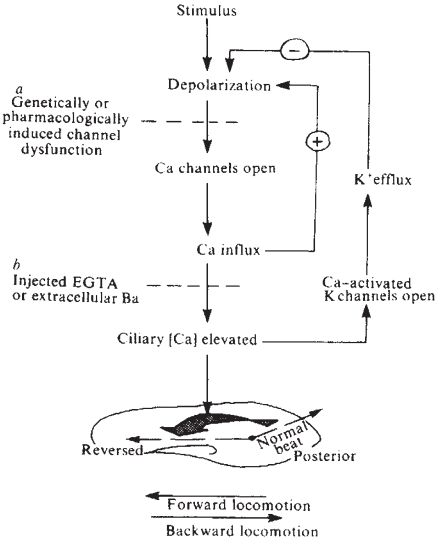

Fig. 1 Relations between channels, currents, and locomotor activity in *Paramecium*. The simulus-response coupling sequence, which proceeds from top to bottom, can be interrupted (dashed lines) in various ways; for example, by blocking the Ca channels (*a*) or by interference with intraciliary accumulation of free Ca^{2+} (*b*). Barium ions, like Ca^{2+}, promote ciliary reversal, so they do not prevent reversed swimming, although their substitution for intraciliary Ca does interfere with activation of the Ca-dependent K channels, causing Ba to promote long-lasting all-or-none action potentials with accompanying ciliary reversal in place of the brief, graded Ca response normally produced by the membrane. EGTA injection, by limiting Ca concentration buildup, interferes with both ciliary reversal and activation of the Ca-dependent K channels. This can produce prolonged all-or-none action potentials without ciliary reversal. A report in this issue (Oertel *et al. op. cit.*) demonstrates the utility of a membrane mutant in which a genetically determined Ca channel dysfunction (*a*) is used to analyse the normal behaviour of the membrane.

This issue of *Nature* contains an excellent example of the clever genetically-assisted analyses of membrane function that are being carried out on *Paramecium tetraurelia* by Ching Kung and his associates at the University of Wisconsin (Oertel *et al.* page 120). Membrane currents in response to step depolarisations of the wild type were compared with those of *pawn*, a class of mutant in which the Ca channels had been shown previously to be functionally defective. Predictably, the *pawn* membrane produced only the delayed outward K current, whereas the wild type produced, in addition, the previously reported early inward current carried through Ca channels. Averaged *pawn* current trajectories

Roger Eckert is a professor in the Department of Biology and in the Brain Research Institute at the University of California, Los Angeles.