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Oncogenes: Growth Regulation and the Papovaviruses Polyoma and SV40

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Cellular oncogenes and their activated and retrovirus-coded counterparts play an important role in cellular regulation. Here the relationship between such oncogenes and the genes coding for the transforming proteins of the papovaviruses, polyoma viruses, and simian virus 40 (SV40) is discussed. It is concluded that polyoma virus may transform established cells by a mechanism involving activation of a cellular oncogene product, whereas SV40 may transform by a mechanism involving a previously little studied cytoplasmic form of the transforming protein.

Key words: DNA binding protein, polyoma virus, middle-T, retroviruses, oncogenes, transforming proteins, SV40 large-T

The oncogenes found in certain retroviruses are responsible for the ability of the viruses to cause tumours. Related genes are present in normal cells. Recent results indicate that both the viral oncogenes and their cellular homologues are involved in the regulation of cellular growth. Furthermore, malfunction of the oncogene or its product, brought about by a variety of recently identified mechanisms, may lead to uncontrolled proliferation of cells and this in turn may lead to the formation of tumours in animals including humans. Thus, a clearer, more unified view of the relationship between viral oncogenes and their normal and activated cellular counterparts and of their role in normal and abnormal cellular growth control has emerged. Certain DNA tumour viruses also code for proteins that are able to transform the growth of cells in culture and to give rise to tumours in animals, but in this case the relationship of the viral oncogenes to cellular genes is less clear. Here, some of the new data on retrovirus oncogenes and their cellular homologues are reviewed and ways in which the early genes of the DNA tumour viruses, polyoma virus, and simian virus 40 (SV40) may fit into the overall pattern of regulation by oncogenes are discussed.

RETROVIRUS ONCOGENES AND THEIR CELLULAR COUNTERPARTS

Much contemporary research in molecular and cellular biology seeks to understand growth regulation in molecular terms. It is usually assumed that many cellular

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proteins are involved in the process, that such proteins are likely to be present in cells in very small amounts, and that their expression may be transient, occurring at perhaps only limited periods of the cell cycle.

Although studies on the regulation of cell proliferation in normal mammalian cells are widely recognised as important, relatively little progress has been made by studying the problem directly. This reflects in part the considerations mentioned above but also, the lack of suitable laboratory systems that can be readily analysed. Some progress has been made in lower eukaryotes, especially yeast, where genetic analysis is possible and has allowed the isolation of temperature-sensitive cell division cycle (cdc) mutants [1]. The study of growth factors from mammalian cells has also proved fruitful partly because the factors have such potent activity and partly because their high stability makes possible the purification of the factors, even though they are present in very small amounts [2].

Most of the progress in growth control, however, has come from the study of tumour viruses [3,4]. Some such viruses can be isolated from animals bearing tumours, whilst others appear nontumourigenic in their natural hosts. When injected into susceptible animals all tumour viruses by definition are able to form tumours. A more important property in the present context is that they are able to alter or transform the growth regulation of established cell lines grown in tissue culture. Such virus-transformed cells provide a useful alternative laboratory system to study cellular growth control. Some of the tumour viruses, particularly the retroviruses and the papovaviruses, have the added advantage that they possess a small genome and their molecular biology is readily studied in great detail.

Retroviruses contain nucleic acid sequences that are dispensable for replication but are required for the ability to cause tumours [4]. These sequences are usually referred to as the viral oncogene (or v-onc). Such genes and their products have recently been characterised in some detail, at least in a few cases. A very striking result that has emerged from these studies is that normal cells contain nucleic acid sequences related to many of the viral oncogenes and that such sequences are highly conserved in vertebrates [4,5]. For convenience, the cellular sequences related to a viral oncogene are often referred to as a cellular oncogene (or c-onc). However, this term can be misleading, for in most cases it is not yet established that the normal cellular gene is capable of causing tumours. As will become clear, it seems more likely that in many cases such genes will only cause tumours following some kind of alteration or activation. For this reason, the cellular genes should more accurately be referred to as cellular proto-oncogenes. In spite of this, the term "cellular oncogene" is widely used.

The finding that normal cells contain highly conserved genes related to retrovirus oncogenes has several important implications. It provides an explanation of the likely origin of such sequences in the viruses; that is, they were fortuitously captured from the cell as a consequence of viral DNA integration into the host cell chromosome during virus replication. Perhaps more importantly, the finding also explains how a virus-coded and potentially foreign gene product is able rapidly to take over control of cellular proliferation. The gene product is not entirely foreign, it is closely related to a normal cellular protein. This same line of reasoning also leads to the conclusion that the conserved cellular gene product is likely to be involved in normal cellular growth control. A consequence of this is that as had always been hoped, many studies on viral oncogenes are directly relevant to normal cellular regulation.

The results mentioned above raise further questions: (a) How are cellular oncogenes activated? (b) what are the products of cellular oncogenes? and (c) what is the molecular basis of their activity?

ACTIVATION OF CELLULAR ONCOGENES

Several mechanisms for the activation of cellular oncogenes have recently been proposed, some of which are surprising and relate directly to human cancer.

The most obvious explanation for the transforming ability of retrovirus oncogenes is that the viral promoters are highly efficient so as to ensure high levels of transcription of the viral genes required for replication, but in consequence they also act to produce large amounts of the oncogene mRNA and hence its protein product. According to this model, the gene product would be identical to the normal cellular gene product, and overproduction of the latter would overwhelm some step in normal cellular regulation. Such a model would imply that cellular oncogenes are capable of transforming cellular growth in their own right, providing a sufficiently high dose of gene product is provided. This has been shown to be the case for example for the c-mos gene and the C-Ha-ras gene by elevating their expression by addition of strong transcriptional promoters [6,6a].

The model just described predicts that the coding sequences of viral and cellular oncogenes are identical. In practice, sequence studies to compare viral and cellular oncogenes have shown, at least in the cases studied, that the viral oncogene differs from its cellular counterpart. Thus, although the viral oncogene of Rous sarcoma virus (RSV), v-src, is very closely related to the cellular gene, c-src, there are differences that would result, for example, in an altered amino acid sequence at the carboxy terminus of the proteins [7]. This raises a different model for the activation of the cellular oncogene to make it transforming, that is, the viral oncogene product is different from its cellular counterpart and this, rather than a dosage effect, results in altered growth properties. Of course, it is possible that in some cases a high dose of the altered oncogene product is necessary for transformation and both models are correct. Studies to modulate the levels of expression of c-onc and v-onc sequences in cells to test these models have not yet resolved the issue unambiguously.

Another mechanism for the activation of a cellular oncogene has been proposed to explain the transforming ability of those retroviruses that lack an oncogene but which, nevertheless, are able to give rise to tumours, albeit with a much longer latent period. Such viruses, like their acutely transforming counterparts, integrate into the host cell chromosome. The structure of the retrovirus genome is such that it is repeated at both ends of the linear molecule. Thus, by integrating into the host cell chromosome randomly a retrovirus promotor will in a certain proportion of cases be positioned adjacent to a cellular gene, and in an even smaller proportion of cases adjacent to a cellular oncogene. Integration adjacent to and consequent activation of most cellular genes is probably of little consequence to growth control. By contrast, activation of transcription of a cellular oncogene might be deleterious. Thus, avian leukosis virus (ALV) induced tumours commonly contain viral DNA integrated adjacent to the c-myc gene, the cellular homologue of the transforming gene of MC29 virus. Presumably this juxtapositioning activates transcription of the c-myc gene, and this is ultimately involved in triggering tumourigenesis [8,9]. Thus it seems possible

that in this case an elevated level of a normal cellular oncogene product brings about transformation.

Another mechanism which results in overproduction of a cellular oncogene product but which in this case does not involve retroviruses is the amplification of the gene itself. Thus, the c-myc gene is estimated to be amplified 30–50 times in a human leukaemia cell line [10]. Other oncogenes are amplified in other tumour cell lines.

Experiments using mouse plasmocytoma cell lines and human Burkitt lymphoma-derived lines have revealed another mechanism of activation, again involving the c-myc gene [11]. Such cell lines often carry a chromosomal rearrangement in which parts of two chromosomes have been swapped. Studies to map genes adjacent to the cleavage sites have shown that the breakpoint often occurs within a limited region of an immunoglobulin gene on one chromosome and near to or within the c-myc gene on the other. The chromosomal rearrangement interrupts the c-myc gene but not within the presumed coding region. Various models to correlate chromosomal translocation with activation of the rearranged c-myc gene have been proposed, for example, involving immunoglobulin enhancers or escape from translational suppression. In general, the models would again imply that transformation is associated with increased expression of an apparently normal c-myc gene product.

Other results directly relating cellular oncogenes to human disease have come from experiments using DNA transfection techniques [12,13]. Here, DNA from human tumour material has been screened for the presence of genes able to transform the growth properties of established cells in tissue culture. Such genes have been detected, isolated, and characterised. Because the genes have the ability to transform cells in culture, they are also called cellular oncogenes. Some of the genes isolated in this way have subsequently been found to be related to known cellular oncogenes previously identified because they have a viral homologue. For example, the cellular oncogenes isolated from a number of human tumour cells lines have been shown to be members of the c-ras gene family, related to the oncogenes of the Harvey and Kirsten murine sarcoma viruses [14–16]. DNA sequence studies have shown that the predicted amino acid sequence of the gene product from tumour cells is slightly altered relative to normal [17–19]. Thus, the tumour-derived gene isolated from some bladder cancer-derived cell lines has an altered codon corresponding to amino acid 12, in an otherwise normal Ha-ras sequence. Presumably, therefore, the cellular oncogene is activated because the altered gene product has a different specificity or activity. Because the oncogenes associated with human tumours have so far been found to be altered from their normal counterparts, the transforming forms of the genes are usually referred to as being activated cellular oncogenes.

BIOCHEMICAL BASIS OF ONCOGENE ACTION

By contrast with the success in establishing the molecular basis of activation of cellular oncogenes and in associating such activation with a role in human cancer, until very recently relatively little progress has been made in answering the question, What are the products of cellular oncogenes? Some (for example, src; fps, the transforming gene of Fujinami sarcoma virus; abl, the transforming gene of the Abelson murine leukaemia virus) are protein kinases with a specificity for tyrosine [20]. This specificity is unusual and is associated with only a small group of other cellular enzymes, which include some growth factor receptors [21]. Almost all

tyrosine kinases so far described appear to be involved in some aspect of growth regulation and to be membrane associated enzymes. The outstanding questions regarding the tyrosine kinases coded by viral oncogenes are (a) which substrates, if any, are critical in triggering subsequent steps in the pathway leading to transformed growth properties, and (b) in what ways do the activities of viral tyrosine kinases differ from those coded by their normal counterparts? Although several substrates of the viral enzymes have been identified, it is not yet clear how their phosphorylation could act as a regulatory step, because many of the substrates identified are phosphorylated in vivo to only a very limited degree. A related question is, what substrates of cellular tyrosine kinases are crucial to their presumed role in normal cellular regulation?

The finding that the receptors for the growth factors epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) have tyrosine kinase activity stimulated by ligand binding [21] suggested that the mechanism of action of some oncogene products resembled that of growth factors. Protein sequencing studies have recently revealed that there is a close structural relationship between the two. The c-sis gene appears likely to be closely related, if not identical, to platelet-derived growth factor, a protein factor previously isolated and characterised because of its ability to stimulate the growth of quiescent cells in culture [22,23]. Similarly, the v-erb B gene (part of the transforming sequences of avian erythroblastosis virus) is closely related to the putative intracellular domain of the epidermal growth factor receptor (that is, the cellular membrane protein known to be involved in the binding of EGF to cells) [24]. These results confirm beyond doubt the long suspected relationship among growth factors, their receptors, and cellular oncogenes.

Because growth factors and their receptors may be thought of as representing different steps on similar pathways, the discovery of the relationship between them and oncogenes raises another long-standing, unanswered question. Do all oncogene products act in a single or limited number of biochemical pathways and represent activities associated with different steps in the process, or are there many widely different mechanisms which lead to transformation? Clearly, the results presented above suggest that some oncogenes do act on similar pathways. The finding that the EGF receptor kinase has sequence similarities with other tyrosine kinases may mean that all such kinases trigger common events at the inner side of the plasma membrane and these converge on a limited number of key targets. Of course, other models are possible.

Further studies on the biochemical activities associated with transforming proteins and more especially on the physiological consequences of their action may well progress most rapidly in lower eukaryotes. Genes and proteins related to the ras family and its products have been characterised in yeast cells [25]. Other yeast genes involved in control of the cell cycle have been isolated and sequenced. At least one of the so-called cdc genes is related in sequence to oncogenes of the tyrosine kinase family [26].

COOPERATING ONCOGENES

Studies using DNA transfection techniques to transform the growth of primary cells rather than established cell lines has led to the idea of two complementary roles for oncogene products [27]. This work showed that activated cellular oncogenes of

the c-ras family are unable to transform primary mouse embryo cells, whereas they are able to in combination with the c-myc gene [28,29]. Such results are reminiscent of earlier studies using DNA tumour viruses where the E1A gene product of adenovirus has been associated with the ability to immortalise or establish primary cells and the E1b gene product with the ability to transform them [30]. Similar relationships hold for the large-T and middle-T of polyoma virus [31]. Mixing experiments have shown that the immortalising genes (myc), E1a and large-T) appear interchangeable with one another in allowing activated ras genes to transform. Indeed, limited sequence homology between the myc and E1a genes has been reported [32]. It is striking that all these gene products are predominantly localised in the nucleus.

In the same way, c-myc gene immortalisation of primary cells allows subsequent transformation by middle-T or E1b gene products as well as activated ras genes. Middle-T, p21 (the ras gene product), and some of the E1b proteins are cytoplasmic. The two-step model for transformation of cells in culture that developed from these studies is consistent with the current view that the genesis of neoplasias in humans is a multistep process [27].

The interchangeability of the transforming and immortalising genes of the DNA tumour viruses with cellular oncogene products leads directly to the subject of the remainder of the discussion here: Are the early genes of DNA tumour viruses related to known cellular oncogenes? For the purposes of this discussion the papovaviruses will be considered.

PAPOVAVIRUS ONCOGENES AND CELLULAR GENES

Apart from some very limited and unexplained homologies in amino acid sequence between middle-T and small-t and the small polypeptide hormones—gastrin [33] and leutinising hormone [34], respectively—such evidence as exists does not suggest that the papovavirus early genes are related to cellular genes or proteins.

However, experiments to detect homology between viral and cellular DNA are not particularly sensitive, especially if only a limited degree of homology is expected and full length viral DNA is used as a probe. More sensitive assays using small cloned fragments of early region sequences have not yet been reported. Alternatively, homologies between papovavirus early proteins and cellular proteins may become more obvious when extensive comparison at the amino acid sequence level becomes possible.

In asking whether papovaviruses are related to known cellular oncogenes, one is forced to reexamine how such genes are defined. Originally, cellular oncogenes were recognised because of their relationship with retrovirus genes having the ability to cause tumours. Later work using DNA transfection defined another category of cellular gene with the potential to cause tumours, and although, as it transpired, these oncogenes are commonly related to known c-oncs with viral counterparts, some are not known to be carried by retroviruses—for example, the B-lym gene [35]. It is quite conceivable that there are yet further potential cellular oncogenes or proto-oncogenes that have not yet been detected either because a virus that has captured such an oncogene has not yet been described or because, for one reason or another, the oncogene is incapable of being captured. In the latter case, the oncogene would only be recognised as such if it were capable of detection by DNA transfection techniques.

Thus there may be other genes, the malfunction of which would result in immortalisation or transformation that, nevertheless, because they cannot be captured by virus or detected by DNA transfer do not come within the present definition of oncogene. An example of such genes might be the cellular int 1 and int 2 sequences. Mouse mammary tumour virus (MMTV) commonly integrates adjacent to these genes, suggesting that they may be associated with MMTV oncogenesis in the same way as c-myc is with ALV oncogenesis [36]. However, because int 1 and int 2 have no known viral counterparts and have not yet been shown to be active in DNA transfer experiments, it is not clear whether they should be referred to as oncogenes. Of course, the nomenclature is not important, what matters is that because a particular cellular gene cannot be called an oncogene does not necessarily exclude that gene and its product from a role in growth control nor mean that its mode of action is radically different from that of known cellular oncogenes. If and when more such genes are discovered it will be interesting to see if any are related to papovavirus early genes.

In spite of the foregoing discussion, it seems probable that the papovavirus transforming genes are not closely related to cellular genes, either known cellular oncogenes or those within the broader definition given above. The question then arises, how did the viruses acquire genes with the ability to interact with and transform normal cells, when there is no obvious selection for this property? Such reasoning might lead to the conclusion that like the acutely transforming retroviruses whose transforming potential might be considered an accident resulting from the capture of a cellular oncogene, the papovaviruses might transform as an accidental sequence of the activity of one of the gene products that has been selected to have a particular function in replication. Arguments of this type not only relate to the origins of papovavirus transforming genes but also to the mechanism of action of their gene products.

POLYOMA VIRUS

The early regions of the papovaviruses can be defined as a viral oncogene because it can give rise to tumours. As mentioned, they have no known cellular counterpart, but such a relationship cannot be excluded. Is it yet clear then how papovavirus oncogenes fit into the overall scheme?

The early region of polyoma virus codes for three proteins, large-T, middle-T, and small-t [3,37]. Large-T is essential for virus replication in tissue culture. Middle-T has the ability to transform established rodent cell lines and is therefore the transforming protein of the virus [38]. To transform primary cells, however, also requires the action of large-T, and, under some circumstances, small-t [31].

Middle-T is present in the membrane fraction of cells [39] and it has an associated kinase activity that phosphorylates tyrosine residues in vitro [40–42]. Superficially, it appears to fall into the group of transforming proteins, like $pp60^{v\text{-}src}$ the product of the v-src gene, which are membrane associated tyrosine kinases. However, this view is almost certainly an oversimplification. By contrast with $pp60^{v\text{-}src}$, tyrosine kinase activity has never been shown to be an intrinsic property of middle-T. Similarly, there is not gross alteration in phosphotyrosine metabolism nor in phosphorylation of specific proteins on tyrosine in cells transformed by polyoma virus, whereas there is in cells transformed by RSV [43]. Whilst these results do not

rule out a role for subtle changes in phosphotyrosine metabolism in polyoma virus transformation, they do indicate that the action of middle-T does not directly parallel the action of $pp60^{v\text{-}src}$, as once seemed possible.

Courtneidge and Smith [44] have proposed a model for the action of middle-T based on the observation that middle-T forms a stable complex with a cellular tyrosine kinase, identified as $pp60^{c\text{-}src}$ (the cellular homologue of $pp60^{v\text{-}src}$). The presence of the complex provides an explanation of the middle-T-associated kinase. It is not a property of middle-T itself but of the bound $pp60^{c\text{-}src}$. The existence of the complex between a transforming protein and a cellular oncogene product suggests a role for the complex in the transformation process in vivo. Although such a role has yet to be proven, there is a correlation between the ability of different mutant middle-T species to transform and the presence of the complex [45]. All transformation-positive mutants retain the complex, whereas some transformation-defective mutants lack it. A possible mechanism for transformation by polyoma virus consistent with these findings is that middle-T interacts with $pp60^{c\text{-}src}$ and thereby alters its stability, activity, or specificity. In the absence of data to indicate increased stability, it is possible that transformation by middle-T is brought about in much the same way as activation of $pp60^{c\text{-}src}$ by addition of a slightly altered carboxy terminal amino acid sequence to produce RSV $pp60^{v\text{-}src}$ or of cellular oncogenes with slight changes in amino acid sequence. Admittedly, there is a difference in that one type of change is at the DNA level, whereas the other is at the protein level. However, it is possible that the presence of middle-T bound to $pp60^{c\text{-}src}$, the altered carboxy terminus in $pp60^{v\text{-}src}$ and the altered amino acid in the ras gene product, p21, all lead to conformational changes in the normal oncogene product, which, in turn, lead to altered enzyme activity.

If the middle-T:$pp60^{c\text{-}src}$ complex model for transformation is correct, it remains to be seen whether this is reflected in a change in phosphotyrosine metabolism in polyoma virus-transformed cells. Certainly, no gross changes in levels of phosphotyrosine similar to those seen in RSV-transformed cells are observed [43]. Thus, as mentioned above, there is a difference in the action of $pp60^{v\text{-}src}$ and $pp60^{c\text{-}src}$ middle-T, even if the activation process is analogous. It still remains possible that the differences are secondary and transformation is triggered in both cases by phosphorylation of some minor but crucial cellular factor.

SV40

The early region of SV40 DNA codes for only two proteins, large-T and small-t [3]. There is no equivalent of polyoma virus middle-T. Mutants lacking a full-sized small-t gene are still able to transform, implying that large-T is the transforming protein of SV40, but experiments to deduce the molecular mechanism of transformation by large-T are complicated because the protein also plays an essential role in viral replication and in immortalisation. Large-T exists in nuclear and membrane-associated forms [3,46]. The protein has high affinity for DNA, particularly for SV40 sequences around the origin of DNA replication that play a central role in replication [3,47]. Thus it is not yet established whether the DNA binding activity of large-T also has a role in transformation or immortalisation. Mutants lacking the ability to bind to the origin region of SV40 DNA have been reported, and these retain the ability to transform [47a]. However, such mutants still have the ability to bind to

DNA cellulose. If large-T has been selected largely for its role in viral replication, it is conceivable that its transforming activity is merely a by-product of its ability to bind to DNA. Perhaps the specificity of binding is such that certain cellular genes are directly activated by binding of large-T. Perhaps immortalisation is likewise caused by a mitogenic effect of large-T that is necessarily required to promote viral replication, particularly in growth-arrested permissive cells, but which in nonpermissive cells gives rise to a sustained mitogenic stimulus. These models would envisage transformation and immortalisation of nonpermissive cells as accidental, nonselected properties that are only manifest because cell killing in this case is inefficient.

An alternative model would argue that perhaps the immortalising function of SV40 large-T, like those of adenovirus E1a and c-myc, is nuclear, and the transforming function, like those of *ras* and middle-T, is cytoplasmic. In this case, perhaps the subfraction of large-T present in the cytoplasm and reported to be membrane associated, is the transforming agent. This view is supported by the isolation of mutant large-T species that appear unable to locate to the nucleus and yet are still able to transform [48]. The membrane-associated form of large-T is only very poorly characterised, except that it is reported to be acylated, and this may distinguish it from nuclear large-T. Whether it has activities distinct from nuclear large-T is unknown. Perhaps the membrane-associated form mimics the activity of or associates with a cellular membrane protein, possibly an oncogene product, and thereby alters membrane activity. These hypotheses are all testable.

Large-T does associate with a cellular protein called NVT or p53 [49]. It is not yet clear whether this association is essential for immortalisation or transformation. The cDNA for p53 has recently been cloned and sequence analysis reveals no extensive homology with a known cellular oncogene [50]. Whether p53 alone is able to transform cells following DNA transfer is currently being tested.

Recent data shows that transcription of specific cellular genes becomes activated in SV40-transformed cells, and that similar transcripts can be detected in cells transformed by other agents, including retroviruses and in embryonic cells [51]. The activated genes have been characterised in some cases, but none are thought to be known oncogenes. At present neither activation of these genes nor transformation by SV40 is thought to be similar to the promoter insertion scheme described above for ALV, since common sites of integration of SV40 DNA in the chromosome have not been detected.

The finding of a common set of activated transcripts in SV40- and retrovirus-transformed cells revives the question, Do all transforming proteins act to switch on at different points a common pathway of events in which growth factors, their receptors and intracellular tyrosine kinases all might play a role, and which might ultimately switch on a common set of genes? Such a model would mean that, say, v-sis acted early in the pathway, v-erb. B at an intermediate stage, v-src at a later stage, and so on. SV40 might act at a very late stage by interacting directly with DNA. In support of this idea, it is known that the action of normal growth factors such as PDGF (or the c-sis product) whose initial site of action is the plasma membrane, nevertheless switch on transcription of specific cellular genes including [52] the c-myc gene. Whilst this model has some attractions, it is naively simple and it remains almost entirely speculative. Activated transcription of genes may be a consequence of the increased rate of proliferation rather than a cause. It remains to be seen whether activation results directly (but accidentally) from the DNA binding

activity of large-T in SV40-transformed cells and from the activity of related cellular proteins in cells transformed by other agents, or whether it is a consequence of events distant from the initial activity of large-T. Again these different possibilities are testable.

CONCLUSIONS

Recent results point to the central role that oncogenes and their products play in normal and abnormal cellular regulation. Here the question addressed has been, How do papovavirus early proteins fit into this pattern? The discussion has emphasised a model for transformation by polyoma virus that suggests that it transforms by a novel mode involving direct binding of a normal cellular oncogene product. If this model is correct, it will provide a further example of cellular oncogene activation in transformation. The discussion of transformation by SV40 has reached no particular conclusions but does suggest some hypotheses that can be tested, particularly the hypotheses that the transforming function is cytoplasmic. Some of the hypotheses involve interactions with cellular oncogenes, but it was emphasised that the present definition of oncogene may exclude many other cellular genes with an important role in control of cellular growth. SV40 large-T could be related to, interact with, or mimic the product of one of these genes or of a known oncogene. An alternative mechanism is that transformation is an accidental by-product of the ability of large-T to bind to cellular DNA and caused by the direct activation of cellular genes. The discussion of immortalisation by papovaviruses was minimal. This reflects the paucity of results on this subject which in turn reflects the lack of suitable experimental systems for study. This is an area where future work may prove fruitful.

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