Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

The Use of cDNA Cloning Techniques to Isolate Genes Activated in Tumour Cells*

M. R. D. Scott, P. M. Brickell, D. S. Latchman, D. Murphy, K.-H. Westphal, and P. W. J. Rigby

A. Introduction

Oncogenic transformation by tumour viruses requires, in many cases, the function of only one viral gene. In the case of the papovavirus Simian virus 40 (SV40) it is the gene coding for large T-antigen [8, 16] while for most strongly transforming retroviruses it is a gene of cellular origin [1]. Recent work has shown that many murine and human tumours, for which there is no indication of viral involvement, contain genes which have been altered in such a way that they have acquired the ability to transform the NIH3T3 line of mouse cells [3, 5, 7, 10, 15]. In order to understand the molecular mechanisms of oncogenesis it is necessary to analyse in detail the biochemical functions of the protein products of these various types of transforming gene. However, even a total description of the activities of a transforming protein will not reveal the complete mechanism of oncogenesis. Transformed cells differ from their normal parents in a multitude of biological and biochemical properties and it is unlikely that all of these changes occur as a direct result of the action of the transforming protein. Rather, the product of the oncogene must reprogramme the cell's metabolism and/or gene expression so that having defined the transforming protein it is then necessary to identify its cellular targets.

The large T-antigen protein encoded by the early region of the SV40 genome is both necessary and sufficient for morphological transformation in vitro and for tumorigenesis in vivo [8, 16]. However, this protein possesses an extraordinary variety of biochemical activities [i.e. ref. 13], many of which are poorly understood. It binds with high affinity to the viral origin of DNA replication in a reaction required for the initiation of viral DNA replication. It also binds with lower affinity to cellular DNA and is capable of inducing a round of DNA replication in quiescent cells. The protein is displayed on the surface of transformed cells, although the vast majority of the antigen is nuclear, it has an ATPase activity and it can overcome the block to the productive infection of monkey cells by human adenoviruses. Large T-antigen represses its own synthesis by binding to the promoter of the viral early transcription unit and there is evidence that it can affect the transcription of cellular genes. Which of these activities are directly involved in transformation is not known.

There is evidence from solution hybridisation experiments that the cytoplasmic mRNA populations of cells transformed by SV40 or by Rous sarcoma virus (RSV) can be distinguished from those of their normal parents [6, 17]. SV40 provides a particularly attractive system in which to investigate this problem because the viral transforming protein has the properties expected of a transcriptional regulator. Two lines of evidence suggest that large T-antigen can di-

* P. M. B. and D. M. are supported by a Training Fellowship and a Research Studentship, respectively, from the Medical Research Council while K.-H. W. is supported by a Long Term Fellowship from the European Molecular Biology Organisation. P. W. J. R. holds a Career Development Award from the Cancer Research Campaign, which also paid for this work

rectly modulate the transcription of cellular genes. The first detectable event following the synthesis of large T-antigen in infected permissive or non-permissive cells is the induction of several cellular enzymes involved in nucleotide metabolism and DNA replication, for example, thymidine kinase [16]. Although there is no evidence that this induction is transcriptional, this seems to be a reasonable proposition. More directly, Baserga and his colleagues have shown that in human-mouse somatic cell hybrids only one of the two rRNA gene complements is active. Introduction of large T-antigen into such cells activates the previously quiescent rRNA gene complement at the level of transcription [14]. We therefore decided to seek to identify and isolate cellular genes which are switched on or off in SV40-transformed mouse cells.

B. Results

Our approach has been to use cDNA cloning techniques to construct libraries of plasmid clones representative of the polyadenylated, cytoplasmic mRNA populations of normal and transformed cells and then to screen these libraries for genes expressed at different levels in the two cell types. Such libraries must be truly representative and we have therefore developed techniques which produce cDNA clones containing large inserts at very high efficiency, 2×10^5 clones per microgram polyadenylated RNA. We constructed two such libraries, one from the normal Balb/c 3T3 mouse cell line and one from an SV40-transformed derivative SV3T3 C138 [2, 11, 12]. The libraries were plated at high colony density and then replica filters were

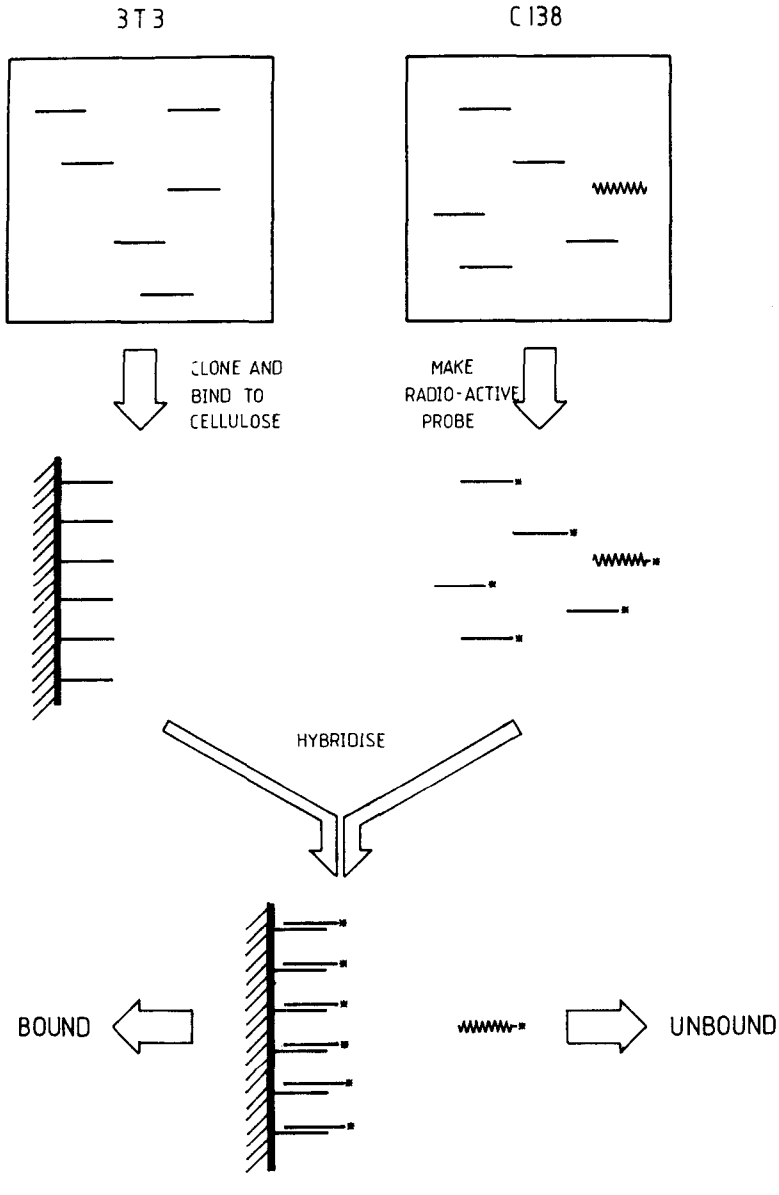

Fig. 1. Procedure for preparing a cDNA probe specific for genes expressed at higher levels in tumour cells than in normal cells. The Balb/c 3T3 cDNA library was pooled and grown in mass culture; plasmid DNA was isolated and covalently coupled to cellulose [9]. ^{32}P-labelled cDNA was prepared using SV3T3 C138 mRNA as the template and then hybridised to the immobilised normal cell cDNA. Sequences held in common between the two cell types will hybridise; sequences expressed only in the transformed cell will not

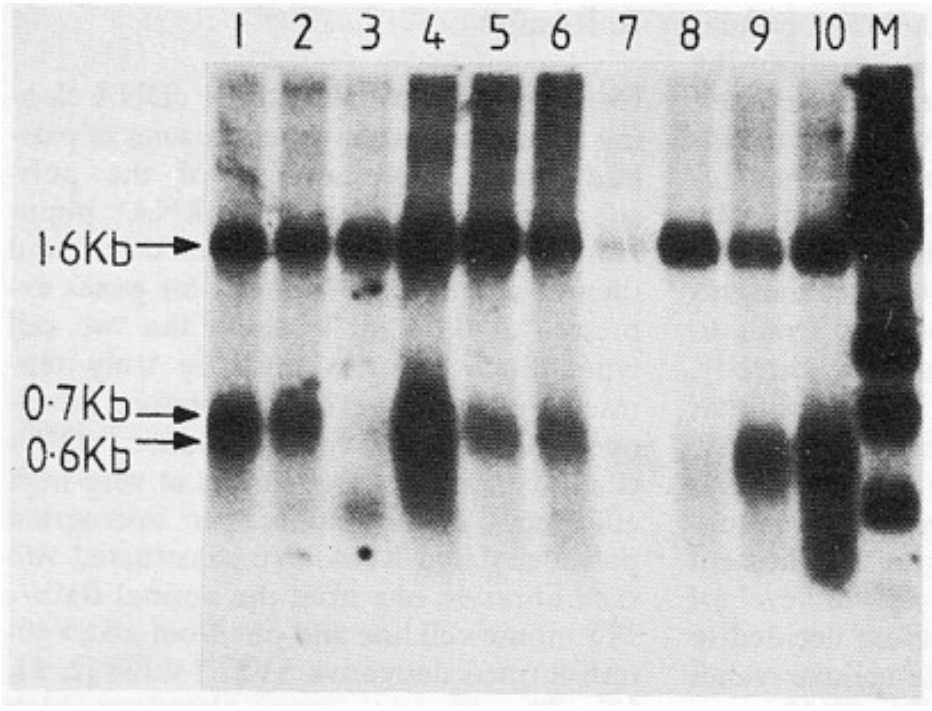

Fig. 2. Transfer hybridisation analysis of mRNAs from mouse cells transformed by a variety of agents. Polyadenylated, cytoplasmic RNA was fractionated by electrophoresis in an agarose gel containing formaldehyde and then transferred to a nitrocellulose filter [4]. The filter was then hybridised with ^{32}P-labelled pAG64, a plasmid containing a cDNA insert which hybridises to mRNAs of 1.6 kb, 0.7 kb and 0.6 kb present at elevated levels in SV3T3 C138 relative to Balb/c 3T3. The tracks are: *1,* Balb/c mouse fibroblasts transformed by methylcholanthrene epoxide; *2,* Balb/c mouse fibroblasts transformed by methylcholanthrene; *3,* NIH3T3 cells transformed by A-MuLV; *4,* a second line of NIH3T3 cells transformed by A-MuLV; *5,* NIH3T3 cells transformed by RSV; *6,* Swiss 3T3 cells transformed by RSV; *7,* normal Balb/c mouse fibroblasts; *8,* Balb/c 3T3 cells transformed by polyoma virus; *9,* a second line of Balb/c 3T3 cells transformed by polyoma virus; *10,* SV3T3 C138. The track labelled *M* contains DNA size markers

prepared and screened by hybridisation with labeled mRNA from the two cell types. In such a colony screening the intensity of the autoradiographic signal is a measure of the abundance of a particular sequence within the total RNA population and we could thus readily search for clones corresponding to mRNAs present at higher or lower levels in the transformed cell line. However, this approach failed to reveal any differentially hybridising colonies. Such colony screening detects only those RNAs of an abundance of 0.1% or above. Our data therefore indicate, in agreement with those of Williams et al. [17], that despite their very different biological and biochemical properties normal and transformed cells do not differ in their abundant mRNAs.

We remained convinced that there must be differentially expressed genes and therefore sought to develop procedures for enriching the corresponding mRNAs. The procedure we adopted is shown in Fig. 1. The Balb/c 3T3 cDNA library was pooled and grown in mass culture; plasmid DNA was then isolated and covalently coupled to a cellulose support. ^{32}P-labelled cDNA was prepared using SV3T3 C138 mRNA as the template and hybridised repeatedly to the immobilised normal cell cDNA. This procedure should remove those RNA sequences held in common between the two cell types and thus the cDNA which fails to hybridise should represent the differentially expressed genes. We analysed the enriched cDNA by hybridising it in solution to vast excesses of either normal or transformed cell mRNA and thus showed that it did indeed detect sequences present in the transformed cell but not in the normal cell. We used this cDNA to screen the SV3T3 C138 cDNA library and thus isolated a number of clones. We have used these cDNA clones as probes in transfer hybridisation experiments [4] to analyse the polyadenylated, cytoplasmic mRNAs of Balb/c 3T3 and SV3T3 C138 cells. Our data con-

firm that the clones correspond to mRNAs present at higher levels in SV40-transformed cells than in normal cells and characterise the relevant transcripts. We have also isolated the corresponding genomic DNA sequences from bacteriophage λ libraries of mouse DNA and are presently determining the detailed structures of these genes and of their RNA products.

It was clearly of interest to ask whether these same genes are activated in mouse cells transformed by agents other than SV40. We have used transfer hybridisation techniques to answer this question and an example of our data is shown in Fig. 2. The plasmid pAG64 contains a cDNA insert which hybridises to three RNAs, of 1.6 kb, 0.7 kb and 0.6 kb, in SV3T3 C138 cells. These RNAs are present in Balb/c 3T3 cells but at a much lower level. Figure 2 shows clearly that the 1.6 kb RNA is also present at a higher abundance in mouse cells transformed by polyoma virus, by RSV, by Abelson murine leukaemia virus (A-MuLV) and by the chemical carcinogens methylcholanthrene and methylcholanthrene epoxide. The 0.7 kb and 0.6 kb RNAs are also present in several of the transformed cell lines.

C. Discussion

Our work has shown that the application of recombinant DNA technology makes it possible to analyse very sensitively the differences in gene expression between normal and tumour cells. We have isolated a number of cDNA clones which correspond to mRNAs present at a higher level in SV40-transformed mouse cells than in their normal parents and we are presently analysing the mechanism by which viral transformation affects the expression of these genes. At least one of these genes is also expressed in fibroblasts transformed by a wide variety of agents including a murine leukaemia virus. The transforming proteins of A-MuLV and RSV are not thought to be capable of interacting directly with the genome and thus it seems likely that there are several mechanisms by which viral transforming proteins can affect the expression of these genes.

The technology that we have developed is clearly applicable to other transformation systems and it should be possible to use it to analyse the differences in gene expression which distinguish leukaemic cells from their normal counterparts. In this way will be able to build up a detailed picture of the changes in gene expression which occur during the induction and progression of leukaemia and thus further our understanding of this disease.

Our studies of the genes we have isolated would be greatly facilitated if we knew their function in a normal mouse. In this regard our recent data are of considerable interest. We have shown that the plasmid pAG64 detects no RNAs in a variety of adult mouse tissues but that there are a large number of transcripts homologous to it in mouse embryos and in cell lines derived from embryos, for example teratocarcinoma cells. These observations raise the interesting possibility that the viral transforming proteins act upon genes normally involved in embryonic development and we are currently exploring this idea in detail.

References

1. Bishop JM, Varmus H (1982) In: Weiss et al. (eds) Molecular biology of tumor viruses: RNA tumor viruses, 2nd ed. pp 999–1108. Cold Spring Harbor Laboratory, New York
2. Clayton CE, Rigby PWJ (1981) Cell 25:547–559
3. Der CJ, Krontiris TG, Cooper GM (1982) Proc Natl Acad Sci USA 79:3637–3640
4. Derman E, Krauter K, Walling L, Weinberger C, Ray M, Darnell JE Jr. (1981) Cell 23:731–739
5. Goldfarb M, Shimizu K, Perucho M, Wigler M (1982) Nature 296:404–409
6. Groudine M, Weintraub H (1980) Proc Natl Acad Sci USA 77:5351–5354
7. Lane M-A, Sainten A, Cooper GM (1982) Cell 28:873–880
8. Martin RG (1981) Adv Cancer Res 34:1–68
9. Noyes BE, Stark GR (1975) Cell 5:301–310
10. Reddy EP, Reynolds RK, Santos E, Barbacid M (1982) Nature 300:149–152
11. Rigby PWJ (1979) Biochem Soc Symposium 44:89–101
12. Rigby PWJ, Chia W, Clayton CE, Lovett M (1980) Proc R Soc Lond Ser B 210:437–450
13. Rigby PWJ, Lane DP (1983) Adv Viral Oncol 3 (in press)

14. Soprano KJ, Jonak GJ, Galanti N, Floros J, Baserga R (1981) Virology 109:127–136
15. Tabin CJ, Bradley SM, Bargmann CI, Weinberg RA, Papageorge AG, Scolnick EM, Dhar R, Lowy DR, Chang EH (1982) Nature 300:143–149
16. Tooze J (1981) Molecular biology of tumor viruses. Part 2. DNA tumor viruses. Revised 2nd Edn. Cold Spring Harbor Laboratory, New York
17. Williams JG, Hoffman R, Penman S (1977) Cell 11:901–907