Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2718–2722, May 1985
Biochemistry

Nucleotide sequence of two overlapping *myc*-related genes in avian carcinoma virus OK10 and their relation to the *myc* genes of other viruses and the cell

(proto-*myc* gene/nucleotide sequence analysis/*myc* gene polymorphism/helper *onc* gene/hybrid *onc* gene)

J. HAYFLICK*, P. H. SEEBURG*, R. OHLSSON†, S. PFEIFER-OHLSSON†, D. WATSON‡, T. PAPAS‡, AND P. H. DUESBERG§¶

*Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080; †Department of Oncology, University of Umea, S-901 87 Umea, Sweden; ‡Laboratory of Molecular Oncology, National Cancer Institute, Frederick, MD 21701; and §Department of Molecular Biology, University of California, Berkeley, CA 94720

Communicated by Heinz Fraenkel-Conrat, January 22, 1985

ABSTRACT **Avian carcinoma virus OK10 has the genetic structure *gag*-Δ*pol*-*myc*-Δ*env*. It shares the transformation-specific *myc* sequence with three other avian carcinoma viruses (MC29, MH2, CMII) and also with a normal chicken gene proto-*myc* and the *gag*, *pol*, and *env* elements with nontransforming retroviruses. Unlike the other *myc*-containing viruses, which synthesize singular *myc* proteins, OK10 synthesizes two different *myc*-related proteins of 200 and 57 kDa. Here we have sequenced the *myc* region of an infectious OK10 provirus to investigate how OK10 synthesizes two different proteins from the same *myc* domain and to identify characteristic differences between the normal proto-*myc* gene and the *myc*-related viral transforming genes. It was found that (*i*) the 1.6-kilobase *myc* domain of OK10 is colinear and coterminal with the *myc* domains of MC29, MH2, and the terminal two exons of proto-*myc*. It is preceded by the same splice acceptor as the *myc* sequence of MH2 and as the second proto-*myc* exon. From this and the known structure of retroviruses, it follows that the OK10 gene encoding the 57-kDa protein is discontinuous with a small 5′ exon that includes six *gag* codons and a large 3′ *myc* exon (δ*gag*-*myc*). This gene and the δ*gag*-*myc* gene of MH2 are isogenic. (*ii*) The proto-*myc*-derived intron preceding the *myc* domain of OK10 is in the same reading frame as the adjacent Δ*pol* and *myc* domains and, hence, is part of the *gag*-Δ*pol*-*myc* gene encoding the 200-kDa protein. (*iii*) Sequence comparisons with proto-*myc* and MC29 and MH2 indicate that there are no characteristic mutations that set apart the viral *myc* domains from proto-*myc*. It is concluded that transforming function of viral *myc*-related genes correlates with the lack of a viral equivalent of the first proto-*myc* exon(s) and conjugation of the viral *myc* domains with large or small retroviral genetic elements rather than with specific point mutations. Because OK10 and MH2 each contain two genes with potential transforming function (namely, δ*gag*-*myc* and *gag*-Δ*pol*-*myc* or Δ*gag*-*mht*, respectively), it remains to be determined whether the δ*gag*-*myc* genes have transforming function on their own or need helper genes. The possible helper requirement cannot be very specific because the two potential helper genes are very different.**

OK10, MH2, MC29, and CMII form a subgroup of avian retroviruses with transforming (*onc*) genes that share closely related *myc* sequences (1). These viruses have obligatory transforming function for susceptible avian cells in culture and cause carcinomas, acute leukemias, and solid tumors in animals (2). Each of these viruses consists of genetic elements from nontransforming retroviruses, also termed chronic leukemia viruses, and a normal chicken gene, termed proto-*myc* (1–3). Proto-*myc* is not known to have transforming function and is regularly expressed in normal chicken cells (4, 5). Nucleotide sequence analysis has identified at least three proto-*myc* exons; the borders of the 5′ exon(s) are as yet uncertain, while those of the two terminal exons are clearly defined (6–8).

Sequence analysis of MC29 (9, 10) and MH2 (11) has shown that the viral *myc*-related genes are structurally different from proto-*myc* (6, 7), consistent with their distinct transforming function. However, unexpectedly, the two viral genes were also found to be different from each other (12–14). The transforming gene of MC29 is a hybrid, sharing ≈1.5 kilobases (kb) with the retroviral *gag* gene and 1.6 kb with the proto-*myc* gene (Δ*gag*-*myc*), which encodes a 110-kDa protein (15). The *myc*-related gene of MH2 is discontiguous, with a *gag*-derived 5′ exon of only six codons (δ*gag*) and a 3′ 1.6-kg *myc* exon that is essentially colinear with the *myc* region of MC29 and the two terminal proto-*myc* exons (11). This viral *myc* exon is preceded by the same splice acceptor as the second proto-*myc* exon (6, 7, 11). However, it is as yet unclear whether the δ*gag*-*myc* gene of MH2 is sufficient for transforming function, because the virus contains a second potential *onc* gene, Δ*gag*-*mht*. This gene is not *myc*-related, and it may function as a helper gene of δ*gag*-*myc* (11–14, 16).

Here we have analyzed the nucleotide sequence of an infectious OK10 provirus to determine whether yet other conjugations of retroviral and proto-*myc* elements generate viral transforming genes and to answer the following open questions regarding the genetic structure of the *myc* region of OK10. The genome of OK10 measures 7.5 kb and is the most complex among defective transforming viruses (17, 18) (Fig. 1). It contains a complete *gag* gene (2 kb) that directs the synthesis of viral *gag* proteins, which allows the virus to form envelope-defective particles in the absence of helper virus; a near complete *pol* gene (1.7 kb); a *myc* sequence (2 kb); and a partial *env* gene (1.4 kb). These are linked *gag*-Δ*pol*-*myc*-Δ*env* (1, 17, 18). The *myc* sequence of OK10 is the only known *onc*-specific sequence that is part of two different genes, a large *gag*-Δ*pol*-*myc* hybrid, defined by a 200-kDa protein (19) that is translated from genomic RNA (1, 17) and a smaller gene, defined by a *myc*-related protein of ≈57-kDa. This size reflects an average among size estimates of 55 to 62 kDa reported in several independent investigations (20–23). This protein is translated from a spliced 3.5-kb mRNA with a leader sequence from the 5′ end of the viral genome and a *myc* domain from the 3′ half of the viral genome (24, 25).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: kb, kilobase(s); RSV, Rous sarcoma virus.

¶To whom reprint requests should be addressed.

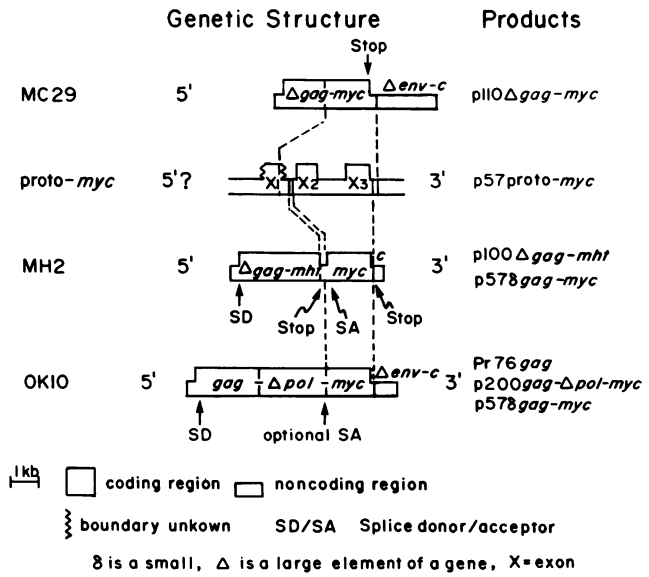


FIG. 1. Genetic structures and gene products of the avian *myc*-containing viruses MC29, MH2, and OK10, and of the chicken proto-*myc* gene.

The peculiar structure and products of OK10 raise the questions of how OK10 synthesizes two different proteins from the same *myc* region, how its *myc* region is related to those of MC29 and MH2, and how it is related to proto-*myc*. The sequence analysis described here has answered these questions.

RESULTS

Nucleotide Sequence of the Δ*pol-myc*-Δ*env* Region of OK10. To determine the nucleotide sequence of the *myc* region and of the Δ*pol-myc* and *myc*-Δ*env* borders, an appropriate OK10 DNA fragment was prepared from an infectious 7.5-kb provirus cloned in a λ Charon 28 vector, termed λ401 (18). The *myc* region and retrovirus group-specific flanking regions of OK10 map on an *Eco*RI-resistant 4.1-kb OK10 DNA fragment that has been subcloned in pBR322 (18). From this subclone, termed pOK4.1, three adjacently mapping *myc* DNA fragments were prepared: a 0.6-kb *Bgl* II/*Pst* I-resistant Δ*pol-myc* fragment, a 0.6-kb *Pst* I/*Cla* I *myc* fragment, and a 0.8-kb *Cla* I/*Eco*RI *myc*-Δ*env* fragment. These fragments were cloned in M13 phage for sequence analysis by the dideoxy method as described (26, 27). Fig. 2 shows the complete nucleotide sequence of the 2-kb Δ*pol-myc*-Δ*env* region that has been sequenced.

Comparing the Nucleotide Sequence of the *myc*-Region of OK10 with Those of Proto-*myc* and the *myc*-Containing Viruses MH2 and MC29 and of Rous Sarcoma Virus. Comparison of the OK10 sequence with the known nucleotide sequence of the Prague C strain of Rous sarcoma virus (RSV) (28) and the proto-*myc* sequence of chicken (6, 7) identifies the Δ*pol-myc* border at a 2-base overlap between OK10 positions 224 and 225 and positions 4841 and 4842 in the *pol* gene of RSV (28). We found four mutations in the Δ*pol* sequence of OK10 compared to that of RSV at positions 101, 163, 166, and 216, which are marked in Fig. 2. The *myc*-Δ*env* border of OK10 lies at a 7-base overlap between OK10 positions 1798 and 1804 and positions 6124 and 6130 in the *env* gene of RSV (28). Thus, the recombination between the retrovirus and proto-*myc* that generated OK10 occurred probably at a 2-nucleotide overlap with the *pol* sequence and a 7-nucleotide overlap with the *env* sequence of this retrovirus and proto-*myc*. This deduction assumes that the respective *pol* and *env* sequences of the retrovirus that generated OK10 are the same as those of RSV and that the proto-*myc* sequence described by us (6, 7) is the same as that in the chicken in which OK10 was generated. The comparison between OK10 and RSV (28) also provides a definitive measure of the defectiveness of OK10; it shows that the Δ*pol* gene of OK10 lacks 348 nucleotides at its 3' end and that the Δ*env* gene lacks 1076 nucleotides at its 5' end.

Comparison with proto-*myc* indicates that (*i*) OK10 shares with proto-*myc* the 3' terminal 63 nucleotides of the proto-*myc* intron that precedes the second proto-*myc* exon. By contrast, MH2 contains 177 nucleotides of the same proto-*myc* intron (11), whereas MC29 contains instead 12 nucleotides from a possible upstream proto-*myc* exon (6, 7) (Fig. 2). (*ii*) OK10 shares with proto-*myc* the two 3' exons, but not the intervening intron of proto-*myc*, like MC29 and MH2. (*iii*) OK10 shares with proto-*myc* the translation stop codon and a noncoding region of 249 nucleotides between this stop codon and the polyadenylylation site of proto-*myc*. Instead, MH2 contains only 35 noncoding proto-*myc* nucleotides (11) and MC29 contains 286 noncoding proto-*myc* nucleotides (9) (Fig. 2). The presence of the proto-*myc* splice acceptor in OK10 virus explains the origin of the subgenomic 3.5-kb mRNA of OK10. Furthermore, it indicates that the *myc* region of OK10 is colinear with the two 3' exons of proto-*myc* and with the *myc*-related gene of MH2 (6, 7, 11). This mRNA is probably spliced from the known splice donor at the sixth *gag* codon and encodes a p57 protein. It is, thus, essentially colinear with the p57 *myc*-related protein encoded by the homologous subgenomic mRNA of MH2 except for a deletion of four OK10 *myc* codons at positions 910–921 (Fig. 2).

The presence of the proto-*myc* intron in OK10 answers the question of how the subgenomic mRNA is made but raises the question of whether the large *gag*-Δ*pol-myc* hybrid p200 protein of OK10 is translated from genomic OK10 RNA or from another spliced mRNA. If genomic RNA were the template, the same proto-*myc* region that serves as an intron in the virus and in proto-*myc* would also serve as a coding region in the virus. Inspection of the Δ*pol-myc* sequence shown in Fig. 2 indicates that the known reading frame of *pol* (28) extends without interruption into *myc*, through the region that functions as intron for the subgenomic mRNA. We conclude that the p200 *gag*-Δ*pol-myc* protein is probably translated from genomic OK10 RNA and that this particular *myc* region of OK10 has a dual function as intron and exon in OK10.

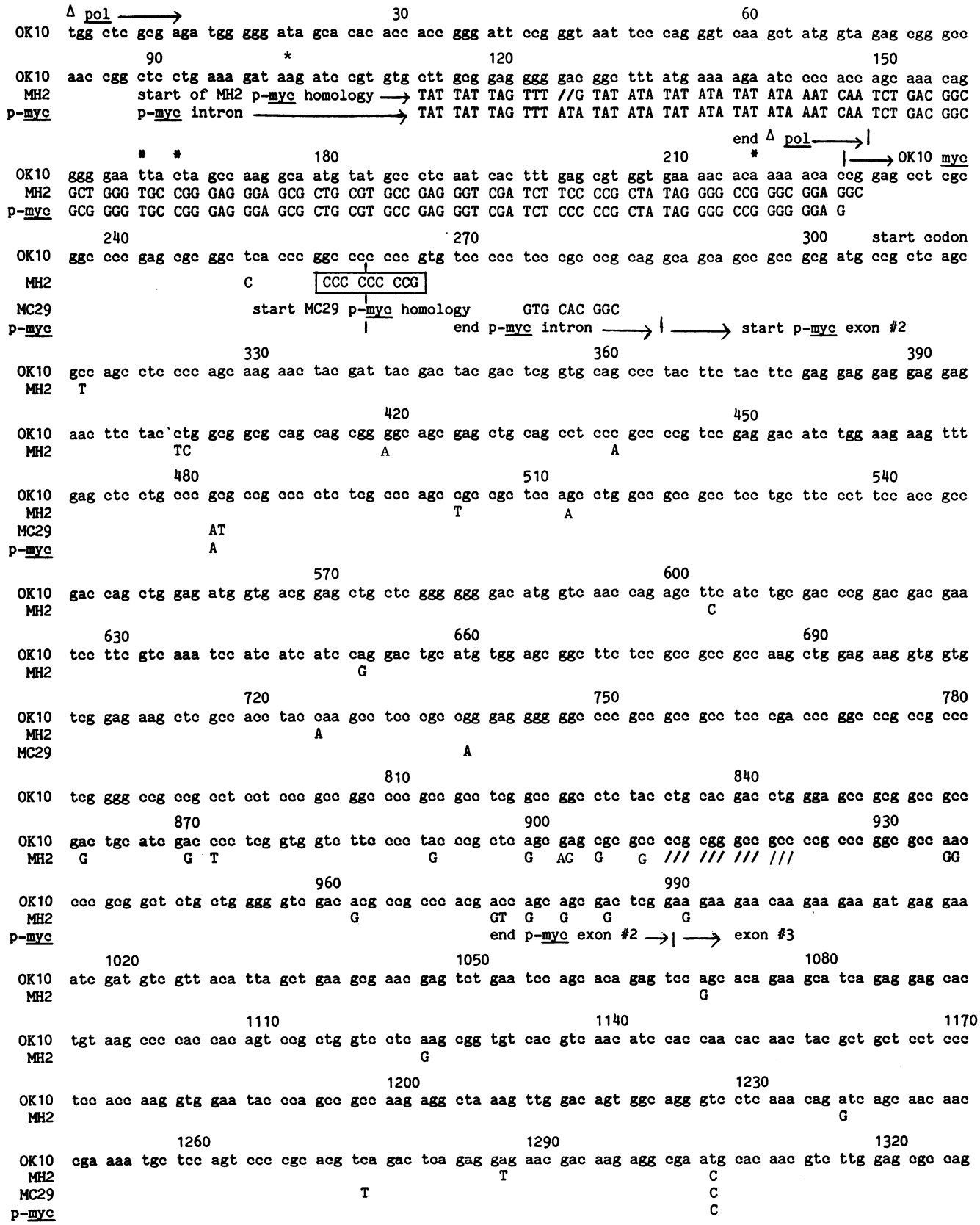
There Is No Characteristic Point Mutation That Sets Apart Proto-*myc* and the *myc* Regions of OK10, MC29, and MH2 Viruses. Two kinds of qualitative changes may be responsible for the obligate transforming function of OK10, MC29, and MH2 compared to proto-*myc*—namely, point mutations among *myc*-related genes or deletions of proto-*myc* sequences and substitutions by virus-specific sequences. Here we have compared the *myc* sequences of three viruses MC29 (9), MH2 (11), and OK10 with that of proto-*myc* (6, 7) (Fig. 2). Single base differences between the *myc* domains of the viruses and proto-*myc* are marked in Fig. 2. The data indicate that there is not one consistent point mutation that sets apart the three viral *myc* domains from proto-*myc*. We conclude that point mutations appear less likely to cause the functional differences between the viral *myc*-related genes and proto-*myc* than do the major deletions and substitutions.

DISCUSSION

Relationship Among the *myc*-Related Genes of OK10 and Other *myc*-Containing Viruses. Our results indicate that the *myc* domain of OK10 virus, unlike that of other *myc*-containing viruses, is part of two overlapping viral genes. It is colinear and coterminal with the two 3' exons of proto-*myc* and with the end of the intron preceding these two exons of

proto-*myc* (see Fig. 1). One *myc*-related gene of OK10 is a contiguous *gag-Δpol-myc* hybrid, and the other is a discontiguous *δgag-myc* gene. The *δgag-myc* gene includes the same splice acceptor as the second proto-*myc* exon and as the *δgag-myc* gene of MH2. Thus, the *δgag-myc* genes of OK10 and MH2 are essentially isogenic, differing in several point mutations and in a 4-codon deletion in the *myc* sequence of MH2 compared to that of OK10.

The *myc*-related *onc* genes with large *gag* complements of MC29 and CMII are probably singular viral oncogenes. By contrast, the *myc*-related genes of OK10 and MH2 with small *gag* complements may not be autonomous *onc* genes. In-



(Fig. 2 continues on the following page.)

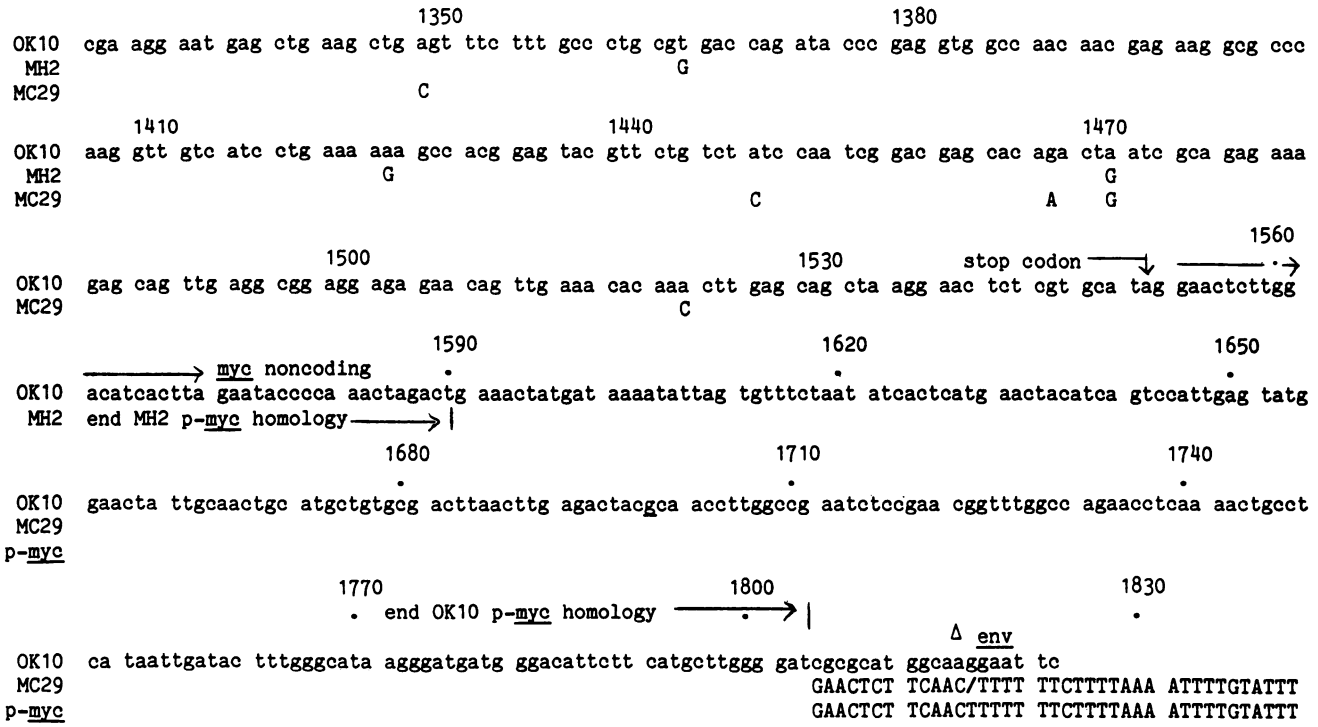


FIG. 2. Nucleotide sequence of the Δ*pol-myc-env* region of the OK10 provirus compared to the *myc* regions of MH2 and MC29 viruses and to the chicken proto-*myc* gene (p-*myc*). OK10 sequences are presented in lower case letters. Non-OK10 sequences are presented in capital letters and only where they differ from OK10. / denotes deletions, and □ denotes insertions compared to the OK10 sequence. * denotes base changes in the *pol* region of OK10 compared to that of RSV (28).

stead, they may need helper genes for transforming function, because OK10 contains a *gag-*Δ*pol-myc* and MH2 contains a Δ*gag-mht* gene as a second potential *onc* gene. If the *myc*-related genes of OK10 and MH2 are indeed helper gene-dependent for oncogenic function, the requirements for a hypothetical helper gene cannot be very specific. This follows, because the δ*gag-myc* genes of OK10 and MH2 are isogenic and, thus, probably functionally identical, whereas the respective potential helper genes are very different—namely, *gag-*Δ*pol-myc* and Δ*gag-mht*. Further work studying genetic variants of MH2 and OK10 is necessary to define complementary functions of the two potential *onc* genes of OK10 and MH2.

As yet, no mammalian *myc*-containing retroviruses have been isolated. However, *myc*-containing feline provirus-like DNAs with unknown biological activity have recently been detected by hybridization of lymphoma DNA from feline leukemia virus-infected cats (29–31).

Differences Between Viral *myc* Genes and Proto-*myc* Genes from Normal Cells and Cancer Cells. The conversion of cellular proto-*myc* to a viral cancer gene could be due to gross structural changes or to point mutations or both.

Clearly, the known *myc*-containing viral *onc* genes are all different from normal or activated proto-*myc* genes (37) (Fig. 1). Each of the four *myc*-related avian carcinoma viruses identified to date lack the 5′ proto-*myc* exon(s) and contain instead *myc*-related genes with large (MC29, CMII) or small (OK10, MH2) *gag* complements. However, the apparently close structural similarity between the cellular p57 proto-*myc* gene and the p57 δ*gag-myc* genes of OK10 and MH2, suggests that very minor changes may be sufficient to convert a proto-*myc* product into a transforming protein. Assuming that the cellular p57 is initiated not in the first proto-*myc* exon(s) but at the sixth codon of the second proto-*myc* exon (position 301 in Fig. 2), the two protein species might differ by as little as 11 amino-terminal codons. The viral species may contain 6 *gag* codons up to the splice donor and the 5 *myc* codons up to the first methionine in the second proto-*myc* exon that are not shared with the cellular counterparts. It is also possible that the viral and the cellular proteins are colinear and that both are initiated at position 301. However, coding or noncoding information of the upstream proto-*myc* exon(s) may also differentially affect the function of cellular and viral *myc*-related genes.

It is shown in Fig. 2 that the *myc* domains of each virus also differ from proto-*myc* in private point mutations. Each of these could be necessary to convert proto-*myc* to a viral cancer gene. However, since a common mutation was not found in all viral *myc* genes, we conclude that mutations may not be essential for oncogenicity. A similar comparison between the *myb* sequences of avian myeloblastosis virus and erythroblastosis virus E26 and proto-*myb* also failed to identify a characteristic point mutation common to viral *myb* sequences (38).

It would appear that deletions and conjugations of proto-*myc* elements with viral genetic elements, rather than specific mutations, are necessary to convert proto-*myc* to an active cancer gene.

Because viral *onc* genes contain genetic elements derived from cellular proto-*onc* genes, it has been proposed that proto-*onc* genes can also be converted, or activated, to cellular cancer genes without becoming viral *onc* genes (5). Examples are the hypotheses that activated proto-*myc* is the cause of avian B-cell lymphoma and human Burkitt lymphoma (32–34). In the case of the avian lymphoma, it has been suggested that enhanced transcription by the promoter or enhancer of a retrovirus without an *onc* gene, also termed chronic leukemia virus, integrated within several kilobases of proto-*myc* may be sufficient to activate proto-*myc* to a cancer gene (8, 32, 35). In the case of Burkitt lymphoma, deregulation of proto-*myc* expression by chromosome translocation is thought to be a sufficient or, at least, a necessary cause (33, 34, 36). Alternatively, it has been postulated that point mutations may convert cellular proto-*myc* genes to active cancer genes in B-cell lymphomas (39–41).

If the viral models apply to proto-*myc* activation in such tumors, one would expect the upstream exon(s) of proto-*myc*

to be separated from exons 2 and 3 and possibly some of the point mutations found in viruses. Yet, in several cancers in which *myc* is suspected to be an active carcinogen, proto-*myc* is not rearranged (34, 37) and no *myc* mutations were found (37, 42, 43). There is as yet neither functional proof for these hypotheses nor are there consistent correlations between specific structural or transcriptional proto-*myc* activations and these tumors (37).

Further work is necessary to determine whether the *δgag-myc* genes of OK10 and MH2 are sufficient for transforming function or require helper genes and whether the p57 viral and proto-*myc* proteins are colinear or contain specific 5′ elements and whether the first proto-*myc* exon(s) may suppress potential transforming function, perhaps by encoding proteins not shared with the viruses.

If the *δgag-myc* genes of OK10 and MH2 are indeed helper gene dependent for transforming function, they could serve as models of *myc* genes that are necessary but not sufficient for cancer, whereas MC29 is a model for a *myc* gene that is sufficient for carcinogenesis.

We thank N. Kan, K. Kobalter, and S. Pfaff for advice and critical discussions. This research was supported in part by National Institutes of Health Research Grant CA 11426 from the National Cancer Institute to P.H.D.

1. Bister, K. & Duesberg, P. H. (1982) in *Advances in Viral Oncology*, ed. Klein, G. (Raven, New York), Vol. 1, pp. 3–42.
2. Weiss, R. A., Teich, N. M., Varmus, H. & Coffin, J. M., eds. (1982) *RNA Tumor Viruses: The Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), 2nd Ed.
3. Robins, T., Bister, K., Garon, C., Papas, T. & Duesberg, P. (1982) *J. Virol.* **41**, 635–642.
4. Gonda, T. J., Sheiness, D. K. & Bishop, J. M. (1982) *J. Mol. Cell. Biol.* **2**, 612–624.
5. Duesberg, P. H. (1983) *Nature (London)* **304**, 219–226.
6. Watson, D. K., Reddy, E. P., Duesberg, P. H. & Papas, T. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2146–2150.
7. Papas, T. S., Kan, N. K., Watson, D. K., Flordellis, C. S., Psallidopoulos, M. C., Lautenberger, J., Samuel, K. P. & Duesberg, P. (1984) in *Cancer Cells 2/Oncogenes and Viral Genes*, eds. Vande Woude, G. F., Levine, A. J., Topp, W. C. & Watson, J. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 153–163.
8. Shih, C. K., Linial, M., Goodenow, M. M. & Hayward, W. S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4697–4701.
9. Reddy, E. P., Reynolds, D. K., Watson, D. K., Schulz, R. A., Lautenberger, J. & Papas, T. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2500–2504.
10. Alitalo, K., Bishop, J. M., Smith, D. H., Chen, E. Y., Colby, W. W. & Levinson, A. D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 100–104.
11. Kan, N. C., Flordellis, C. S., Garon, C. F., Duesberg, P. & Papas, T. S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3000–3004.
12. Kan, N. C., Flordellis, C. S., Garon, C. F., Duesberg, P. & Papas, T. S. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6566–6570.
13. Jansen, H. W., Rückert, B., Lurz, R. & Bister, K. (1983) *EMBO J.* **2**, 1969–1975.
14. Galibert, F., de Dinechin, S. D., Righi, M. & Stehelin, D. (1984) *EMBO J.* **3**, 1333–1338.
15. Mellon, P., Pawson, A., Bister, K., Martin, G. S. & Duesberg, P. H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5874–5878.
16. Kan, N. C., Flordellis, C. S., Mark, G. E., Duesberg, P. H. & Papas, T. S. (1984) *Science* **223**, 813–816.
17. Bister, K., Ramsay, G., Hayman, M. J. & Duesberg, P. H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7142–7146.
18. Pfeifer, S., Zabielski, J., Ohlsson, R., Frykberg, L., Knowles, J., Pettersson, R., Oker-Blom, N., Philipson, L., Vaheri, A. & Vennström, B. (1983) *J. Virol.* **46**, 347–354.
19. Ramsay, G. & Hayman, M. J. (1980) *Virology* **106**, 71–81.
20. Hann, S. R., Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. (1983) *Cell* **34**, 781-798.
21. Pachl, C., Biegalke, B. & Linial, M. (1983) *J. Virol.* **45**, 133–139.
22. Alitalo, K., Ramsay, G., Bishop, J. M., Pfeifer, S. O., Colby, W. W. & Levinson, A. D. (1983) *Nature (London)* **306**, 274–277.
23. Moelling, K., Bunte, T., Greiser-Wilke, I. & Donner, P. (1984) in *Cancer Cells 2/Oncogenes and Viral Genes*, eds. Vande Woude, G. F., Levine, A. J., Topp, W. C. & Watson, J. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 173–180.
24. Chiswell, D. J., Ramsay, G. & Hayman, M. J. (1981) *J. Virol.* **40**, 301–304.
25. Saule, S., Sergeant, A., Torpier, G., Raes, M. B., Pfeifer, S. & Stehelin, D. (1982) *J. Virol.* **42**, 71–82.
26. Messing, J., Crea, R. & Seeburg, P. H. (1981) *Nucleic Acids Res.* **9**, 309–321.
27. Seeburg, P. H., Lee, W.-H., Nunn, M. F. & Duesberg, P. H. (1984) *Virology* **133**, 460–463.
28. Schwartz, D. E., Tizard, R. & Gilbert, W. (1983) *Cell* **32**, 853–869.
29. Levy, L. S., Gardner, M. B. & Casey, J. W. (1984) *Nature (London)* **308**, 853–856.
30. Neil, J. C., Hughes, D., McFarlane, R., Wilkie, N. M., Onions, D. E., Lees, G. & Jarrett, O. (1984) *Nature (London)* **308**, 814–820.
31. Hardy, W. D., Jr. (1984) *Nature (London)* **308**, 775.
32. Hayward, W. S., Neel, B. G. & Astrin, S. M. (1981) *Nature (London)* **209**, 475–480.
33. Klein, G. (1983) *Cell* **32**, 311–315.
34. Leder, P., Battey, J., Lenoir, G., Moulding, C., Murphy, W., Potter, H., Stewart, T. & Taub, R. (1983) *Science* **222**, 765–771.
35. Payne, G. S., Bishop, J. M. & Varmus, H. E. (1982) *Nature (London)* **295**, 209–214.
36. Klein, G. & Klein, E. (1984) *Carcinogenesis* **5**, 429–435.
37. Duesberg, P. (1985) *Science*, in press.
38. Nunn, M., Weiher, H., Bullock, P. & Duesberg, P. (1984) *Virology* **139**, 330–339.
39. Westaway, D., Payne, G. & Varmus, H. E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 843–847.
40. Rabbits, T. H., Forster, A., Hamlyn, P. & Baer, R. (1984) *Nature (London)* **309**, 592–597.
41. Taub, R., Moulding, C., Battey, J., Murphy, W., Vasicek, T., Lenoir, G. M. & Leder, P. (1984) *Cell* **36**, 339–348.
42. Battey J., Moulding, C., Taub, R., Murphy, W., Stewart, T., Potter, H., Lenoir, G. & Leder, P. (1983) *Cell* **34**, 779–787.
43. Stanton, L. W., Fahrlander, P. D., Tesser, P. M. & Marcu, K. B. (1984) *Nature (London)* **310**, 423–425.