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Nucleotide Sequence of the Transforming Gene of Avian Myeloblastosis Virus

Abstract. Avian myeloblastosis virus is defective in reproductive capacity, requiring a helper virus to provide the viral proteins essential for synthesis of new infectious virus. This virus arose by recombination of the nondefective helper virus and host cellular sequences present within the normal avian genome. These latter sequences are essential for leukemogenic activity. The complete nucleotide sequence of this region is reported. Within the acquired cellular sequences there is an open reading frame of 795 nucleotides starting with the initiation codon ATG (adenine, thymine, guanine) and terminating with the triplet TAG. This open reading frame could code for the putative transforming protein of 265 amino acids with a molecular weight of approximately 30,000.

We present the complete nucleotide sequence of the transforming gene of avian myeloblastosis virus (AMV). This retrovirus is distinctive in that it causes acute myeloblastic leukemia in chickens (1). In vitro, AMV transforms a specific class of hematopoietic cells, but does not morphologically transform fibroblasts (2). Thus, it appears that only certain target cells are responsive to the AMV onc gene product (2).

The AMV provirus was isolated from a library of chicken recombinant λ phage constructed with a partial Eco RI digest of DNA from leukemic myeloblasts producing AMV (3, 4). The clone (λ 11A-1-1) contains the entire AMV provirus and adjacent chicken DNA sequences. Portions of the proviral genome were subcloned in the plasmid vector pBR322 and used for sequencing. One subclone contained AMV sequences located between the Kpn I site and the 3' proximal Xba I site. The other subclone contained AMV sequences located between the 3' proximal Eco RI site and the 3' viral terminus as well as the adjoining cellular DNA up to an Eco RI site.

The restriction map of the cloned AMV proviral genome and the strategy employed to determine the nucleotide sequence of the Kpn I to Bgl II DNA fragment containing the transforming gene (amv) and the long terminal repeat

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(LTR) at the 3' end of the AMV genome are shown in Fig. 1.

Within the nucleotide sequence of the 3' end of the integrated AMV provirus (Fig. 2) we can identify the following domains: (i) the terminal portion of the polymerase gene, identified by an open reading frame extending from position 1 and terminating with a TAG (thymine, adenine, guanine) codon at position 162; (ii) a region of 350 bases without an apparent open reading frame extending between positions 165 to 515; (iii) an open reading frame of 795 bases extending from positions 516 to 1310; and (iv) the 3' LTR adjacent to the host sequences.

Earlier studies have revealed that the AMV genome has undergone recombination in which the entire helper virus "env" gene have been replaced by cellular sequences (4, 5). In order to localize the points of recombination, we have compared the carboxyl terminal sequence of the AMV polymerase gene with that of the nondefective Prague strain of Rous sarcoma virus (PR-RSV) (6). From position 1 to position 78 of the AMV DNA fragment in the sequence reported here, the nucleotide sequence is identical to that of PR-RSV. From position 78 to the termination signal, TAG at position 1313, the sequences of AMV and PR-RSV are entirely different, thus localizing the 5' end of cellular insertion sequences.

The host-helper virus junction occurs at a region which constitutes a potential splice acceptor site. In general, splicing acceptor sites (at the 3' end of intervening sequence) contain a pyrimidine-rich nucleotide tract followed by the sequence AGG. The junction point between the cellular insertion sequence and the helper viral sequence fits the consensus acceptor splice sequence (7).

The product of the AMV transforming gene has yet to be identified. Examination of the cellular derived amv sequences (Fig. 2) reveals an open reading frame starting with the initiation codon ATG at position 516 and terminating with the triplet TAG at position 1310. This stretch of 795 nucleotides could



Fig. 1. Restriction enzyme map and strategy of sequencing. The Kpn I-Bgl II segment was fragmented and sequenced (12); the restriction sites are indicated. The 5' ends were labeled with $[\gamma^{-32}P]$ adenosine triphosphate and T4 polynucleotide kinase. The labeled end of each fragment is indicated by the closed circle. The numbers give the length of the fragments and the direction of sequencing is indicated by arrows.

code for a protein of 265 amino acids with a molecular weight of approximately 30,000 which has yet to be identified. The amino acid sequence predicted from this region is also shown in Fig. 2. The putative transforming protein would contain five methionine residues in addition to starting *N*-formyl methionine and cyanogen bromide cleavage would produce six peptides.

Ribonuclease T1-resistant oligonucleotide analysis was carried out (5) on RNA isolated from the defective AMV particles. The RNA from these particles contains 14 distinctive T1 oligonucleotides that are unrelated to sequences present in nondefective avian retroviruses and to the transformation-specific sequences of other avian leukemia and sarcoma viruses. Duesberg *et al.* suggested that these RNA sequences belong to the leukemogenic region of the AMV genome (5). We have used the computer program devised by Queen and Korn (8)

r Kpn I GG TAC CGA AÅA GTT AAA CCĞ GAC ATC GCC ČAA AAG GAT GÅG GTG ACT AAĞ AAA GAT GAG ĞCG AGC CCT CŤT TTT GCA GĞ <u>C</u> Č TGG AGG CAC ÅTA GAT AAG AĞA ATT ATC Tyr Arg. Lys. Val. Lys. Pro. Asp. Ile. Ala: Gİn. Lys. Asp. Giu. Val. Thr. Lys. Lys. Asp. Giu. Ala. Ser. Pro. Leu. Pho. Ala: Giv. Tro. Arg. His. Ile. Asp. Lys. Arg. Ile. Ile.
150 ACŤ CTA CAT TCA ŤCT TTC TCA AÅG ATT AAT CTĂ CTT GTG TGT ŤTT ATA TTT CĂT TAG AATCGĞ ACAGATGTTČ AGTGCCAGCĂ CCGGTGGCAĞ AAAGTATTAĂ ACCCAGAACŤ Thr Leu His Ser Ser Phe Ser Ivs IIe Asn Leu Leu Val Cvs Phe IIe Phe His ***
250 TAACAAAGGŤ CCATGUA TĂ AAGAGGAGGĂ TCAAAGGGTĂ ATAGAACACĞ TGCAGAAATĂ CGGTCCAAAĞ CGCTGGTCGĞ ACATTGCTAĂ GCATTTGAAĞ GGAAGGATTĞ GAAAACAGTĞ CAGGGAGAGĞ
80 Region 400 Promoter 450 CAP box Iggcacaacc atctgaatc agaagtgaag aaaacctcct <u>ggacaga</u> aga ggaagataga at <u>tatttad</u> aggcacacaa gagactggba aacagatggg cagaaattgc aa <u>agttgctg cct</u> ggacgga
500 FEO RI CTGATAACGČ TGTCAAGAAČ CACTGGAA <mark>LT CCAD</mark> C ATG CĞC CGG AAG GTČ GAG CAG GAG ĞGT TAC CCG CĂG GAG TCC TCČ AAA GCC GGC ČCG CCC TCG GČA ACC ACC GGČ TTC
Hibosomal binding Met Arg Arg Lys Val Giu Gin Giu Giy Tyr Pro Gin Giu Ser Lys Ala Giy Pro Pro Ser Ala Thr Thr Giy Phe 600 CAG AAG ÅGC AGC CAT CTG ATG GCC TTT GCC CAC AAC ČCA CCT GCA GGC CCG CTC CCG GGG GCC GGC ČAG GCC CCT CTG GGC AGT GAČ TAC CCC TAC TAC CAC ATT GČT Gin Lys Sér Ser His Leu Met Ala Phe Ala His Asn Pro Pro Ala Giv Pro Leu Pro Giv Ala Giv Gin Ala Pro Leu Giv Ser Asn Tyr Pro Tyr Tyr His Ne Ala
750 GAG CCA CAĂ AAT GTC CCT ĜGT CAG ATC CĈA TAT CCA GTĂ GCA CTG CAT ĂTA AAT ATT AŤC AAT GTT CCŤ CAG CCA GCT ĜCT GCA GCT AŤT CAG AGA CAČ TAT ACT GAT Glu Pro Gln Asn Val Pro Gly Gln Ile Pro Tyr Pro Val Ala Leu His Ile Asn Ile Ile Asn Val Pro Gln Pro Ala Ala Ala Ile Gin Arg His Tyr Thr Asp
850. GAA GAC CCT GÅG AAA GAA AAÅ CGA ATA AAG ĜAA TTA GAG TTG CTA CTT ATĜ TCG ACT GAG ÅAT GAA CTG AÅA GGG CAG CAĜ GCA TTA CCA ÅCA CAG AAC CÅC ACA GCA Glu Asp Pro Glu Lys Glu Lys Arg Ile Lys Glu Leu Glu Leu Leu Leu Met Ser Thr Glu Asn Glu Leu Lys Gly Gln Gln Ala Leu Pro Thr Gln Asn His Thr Ala
950 AAČ TAC CCC GGC TGG CAC AGC AČC ACG GTT GCT GAC AAT ACC ÅGG ACC AGT GĞT GAC AAT GCĞ CCT GTT TCC TGT TTG GGG GÅA CAT CAC CAČ TGT ACT CCA TCT CCA Asn Tyr Pro Gly Trp His Ser Thr Thr Val Ala Asp Asn Thr Arg Thr Ser Gly Asp Asn Ala Pro Val Ser Cys Leu Gly Glu His His His Cys Thr Pro Ser Pro
1050 CCA GTG GAT CAT GGT TGC TTA CCT GAG GAA AGT GCG TCC CCC GCÀ CGG TGC ATG ÀTT GTT CAC CÀG AGC AAC ATC CTG GAT AAT GTT AAG AAT CTC TTA GAA TTT GCA Pro Val Asp His Giv Cys Leu Pro Giu Giu Ser Aia Ser Pro Aia Arg Cys Met Ne Vai His Gin Ser Asn Ne Leu Asp Asn Vai Lys Asn Leu Leu Giu Phe Aia
GAA ACA ČTC CAG TTA AŤA GAC TCC TTČ TTA AAC ACA ŤCG TCC AʿAT CẮC GAG AAT CIĞ AAC CIG GAC ẢAC CCT GCA CŤA ACC TCC ACĞ CCA GTG TGT ĜGC CAC AAG AŤC Glu Thr Leu Gln Leu Ile Asp Ser Phe Leu Asn Thr Ser Ser Asn His Glu Asn Leu Asn Leu Asp Asn Pro Ala Leu Thr Ser Thr Pro Val Cys Gly His Lys Met
TCT GTT ACČ ACC CCA TTC ČAC AAG GAC CÅG ACT TTC ACŤ GAA TAC AGG ÅAG ATG CAC GĞC GGA GCA GTČ TAG AGCTCAÅ TTATAATAAŤ CTTGCGAATČ GGGCTGTAAČ GGGGCAAGGČ Ser Val Thr Thr Pro Phe His Lys Asp Gin Th. Phe Thr Glu Tyr Arg Lys Met His Giy Gly Ala Val
1400 Plus strong, stop Start of LTR-Inverted repeat TTGACCGAGG GGACTATAAC ATGTATAGGC GAAAAGCGGG GTC+CGGTTG TAACGCGCTT AGGAAGTCCC CTCGAGGTAT GGCAGATATG CTTTTGCATA <u>GGGAGGGGGA AATGTAGTCT TAATG</u> GTAGC
1500 <u>CAT box</u> TTAACATGTĂ TATTACCAAĂ TAAGGGAATČ GCCTGATGCĂ CLAAATAAGĞ TATTATATGĂ TC <u>CCATT</u> GGİ GGTGAAGGAĞ CGACCTGAGĞ GCATATGGGČ GTTAACAGAĂ CTGTCTGTCČ TTGCGTCAT
1850 CAT_box 1700 Promoter 1750 CCTCATCGGĂ TCATGTACGČ GGCAGAGTAŤ GATTGGATAĂ CAGGATGGCĂ (<u>CCATT</u> CATCŚ IGGCGCATGČ IGATTGGTGČ ACTAAGGAGŤ IGTGTAACCČ ACGAATG <u>TAČ ITAAG</u> CIIGŤ AGTIGCTAAČ
Polyadenylation signal Poly A acceptor 1800 [<u>AATAAA</u> GT <mark>GČ CA</mark> ITTCTACCŤ CTCAC <mark>ČÁCA</mark> Ť TGGTGTGCAČ CTGGGTTGAŤ CGCCGGACCĞ TCGATTCCCŤ GACGACTGCĞ AACACCTGAĂ TGAA <mark>GCTGAĂ GGCTTCA</mark> ATĂ GTTGCATCAĞ TGCAGGTTAČ CAP acceptor ————————————————————————————————————
1900 Bgl # AACAGTGAAĞ AGACTTAGAŤ TCTGAATTGC TACGTAGGGC TGGĂGATC upper line shows the nucleotide sequence proceeding in the 5' to 3' direction and has the same polarity as AMV genomic RNA. The amino acid sequence deduced from the
open reading frame is given on the lower line. The major structural features of the genome are indicated. Alignment of T1 oligonucleotide is taker from (5) with the nucleotide sequence of the cellular insertion of the AMV genome. The AMV-specific T1 oligonucleotides and their position in the sequence [where D-101 (and so forth) indicates the number for the nucleotide chromatographic patterns in (9)]: D-101, ATTAATCTACTTG 132 to 144; D-102, AATTATCACTCTACATTCATCTTTCTCAAAG, 101 to 131; D-104, AATTATTTACCAG, 410 to 422; D-105, TTTTA-
TATTTCATTAG, 149 to 164; D-106, ACTACCCCTACTACCACATTG, 679 to 699; D-107, CCCACAACCCACCTG, 622 to 636; D-108, CATATAAATATTATCAATG, 747 to 766; D-113, CATTACCAACACAG, 892 to 905; D-110, CAAACTACCCCG, 916 to 927; D-111, ACTCCTTCTTAAACACATCG, 1153 to 1172; D-112, TACTCCATCTCCAACCACG, 1013 to 1029; D-103, CACTAACCTCCCACG, 1027 to 12207 to 1207
and D-114, 11ACCACCCCATTCCACAAG, 1246 to 1265. Common C region: D-51, CTCAATTATATATCTTG, 1516 to 1534. LTR (03 untranslated regions at 3'): D-52, TAATTACCAAATAAG, 1499 to 1514; D-53, CACCAAATAAG, 1529 to 1539; D-54, CTAACAATAAAG 1746 to 1757; D-56, TCATTCCTCATCG, 1616 to 1628; and D-57, CACCATTCATCG, 1669 to 1688. LTR (R, repeated region at both ends of the

1746 to 1757; D-56, TCATTCCTCATCG, 1616 to 1628; and D-57, CACCATTCATCG, 1669 to 1688. L1R (R, repeated region at both ends of the molecule): D-1, CCATTCTACCTCTCACCACATTG, 1760 to 1782. Abbreviations: A, adenine; T, thymine; G, guanine; C, cytosine; Ala, alanine; Asn, asparagine; Asp, aspartic acid; Arg, arginine; Cys, cysteine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; and Val, valine.

and have positively identified 13 of the 14 oligonucleotides in the sequence presented in Fig. 2. The legend to Fig. 2 lists the T1 oligonucleotides identified by Duesberg et al. (5) and their position within our sequence. All but one of the AMV transformation-specific oligonucleotides fall within the cellular sequence inserted in AMV. Also, all but one of the helper-related oligonucleotides are found beyond the terminator signal TAG of the large open reading frame in the region shared with its helper virus. T1 oligonucleotide D-51, as in (5), located between positions 1316 and 1334 is present in both AMV and its helper virus. Comparison of our sequence with that of the RSV envelope region (6) reveals that the last 11 amino acids at the carboxyl terminus are shared by the two proteins, suggesting that the AMV gene is incomplete and utilizes the envelope terminator codon. This positions the 3' terminus of the recombination event at position 1277.

A message generated from the AMV transforming region should direct the synthesis of the transforming protein with the predicted amino acid sequence (Fig. 2). This messenger RNA (mRNA) could be generated either by splicing with the leader sequence derived from the 5' terminus of genomic RNA or by independent promotion. Splicing is generally used in the synthesis of viral subgenomic messages. Leader sequences identified in MC29 (9) and RSV (6) cloned proviruses contain the 5' LTR, a noncoding region, and 18 nucleotides coding for six amino acids of the aminoterminal portion of the viral protein p19 (6, 9). The splice donor portion of these sequences agrees with the consensus splice sequence of eukaryotic genes (7).

The alternative model for controlling the expression of the transforming gene would use the transcriptional signals found within the cellular insertion sequences in the region which lies between the polymerase gene and the open reading frame. This type of independent promotion would not utilize the transcription controls of the viral 5' LTR. Within the regions containing 350 base pairs (bp) in front of the putative leukemogenic sequence we have identified transcriptional signals similar to those present in other eukaryotic genes (10, 11). A sixbase AT-rich sequence characteristic of eukarvotic promoters was identified at position 413 to 417, -56 bp from the capping site. Similarly, signals such as the -80 bp region and ribosomal binding sites have also been identified within this region (Fig. 2). The presence of these regulatory signals for transcription implies that the *amv* insert was generated by recombination directly with host DNA and not with a reverse transcript (complementary DNA) of the mRNA coded by chicken sequences homologous to amv.

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Schizophrenia: Dopamine B-Hydroxylase Activity and **Treatment Response**

Abstract. Cerebrospinal fluid levels of dopamine β -hydroxylase, found to be relatively constant over time in individual patients, were significantly lower in schizophrenic patients who became nonpsychotic during neuroleptic treatment than in those who remained psychotic. Dopamine β -hydroxylase activity may delineate a subgroup of patients who have a dopamine-sensitive brain disorder.

Many hypotheses about the etiology of schizophrenia have focused on changes in central catecholamine and indoleamine metabolism, and most evidence has indicated a role for dopamine (1). Evidence supporting the dopamine hypothesis of schizophrenia, which suggests that there is an increase in dopamine activity in specific central nervous system sites in schizophrenic patients, includes the pharmacological observations that the ability of neuroleptic drugs to block dopamine receptors correlates significantly with their antipsychotic potency (2) and that drugs that increase central dopamine function exacerbate schizophrenia (3). Many investigators have concluded from such data that the neuroleptic medications act by decreasing the central transmission of dopamine.

Alterations in brain dopamine in schizophrenia could derive from variations in its enzymes of synthesis or degradation. Dopamine β-hydroxylase (DBH), the enzyme that catalyzes the synthesis of norepinephrine from dopamine, has been localized in brain noradrenergic neurons, peripheral sympathetic nerves, and chromaffin granules of the adrenal medulla (4). Studies of the adrenal medulla and sympathetic nerve endings show that the enzyme is bound to storage vesicles and is released along with catecholamines by exocytosis (5). The serum of humans and other mammals contains DBH activity that arises from the DBH of sympathetic nerve endings (6). The DBH activity in cerebrospinal fluid (CSF) may originate from DBH released from brain noradrenergic neurons (7). A significant correlation between levels of DBH activity in the CSF and brain has been found in animals (8). In any individual, serum DBH activity is extremely constant over time (9), and it is likely that DBH activity is under significant genetic control (10).

Dopamine β -hydroxylase activity has been studied in serum (11), CSF (12, 13), and postmortem brain tissue (14) of schizophrenic patients and controls, but differences between these two groups have not been consistently found. The heterogeneity of the clinical syndrome of schizophrenia itself may be the source of such discrepant results, since schizophrenia probably represents a variety of