THE „SMALL" POLYDISPERSE CYTOPLASMIC EXTRACHROMOSOMAL DNA OF CHICKEN LEUKAEMIC MYELOBLASTS AND THE AVIAN MYELOBLASTOSIS VIRUS CORE-BOUND DNA SEEM TO DESCEND FROM ORIGIN REGIONS OF CHROMOSOMAL DNA REPLICATION

P. PAJER, J. ŘÍMAN*, M. DVOŘÁK

Institute of Molecular Genetics, Academy of Sciences of Czech Republic, Flemingovo nám. 2, 166 37 Prague 6, Czech Republic

Received November 17, 1998; accepted December 30, 1998

Summary. – Nucleotide sequences are presented for 12, 7 and 12 cloned extrachromosomal DNAs by nature harbored in nucleoprotein (NP) complexes forming chicken leukaemic myeloblast (CHLM) post microsomal sediment (POMS) components A, B and C, respectively, and for 11 cloned avian myeloblastosis virus (AMV) DNAs. Analysis of the abundance of sequence motifs significant for eukaryotic chromosomal DNA replication origin (ori) regions (and their initiation zones) has shown that these DNAs are reminiscent of cell DNA fragments enriched in ori sequences (Rao *et al.*, 1990) and/or sequence features of several eukaryotic chromosomal oris containing clusters of modular sequence elements (Dobbs *et al.*, 1994). Accordingly, these DNAs, with an (A+T) content prevalently higher than that of the total cell DNA, revealed the presence of asymmetrically distributed (A+T)-rich stretches, scaffold attachment region (SAR) T consensuses, polypyrimidine nucleotide (poly(Py)) tracts and minimal *Saccharomyces cerevisiae* autonomously replicating sequence (ARS) consensus, in abundance comparable with that of these sequences of DNA fragments enriched in oris. All these DNAs were found to be enriched also in sequence elements held as primase (Pr) attachment sites. Moreover, DNAs of POMS component B and those of AMV DNA were found to be enriched in the asymmetric pyrimidine (Py) heptanucleotide motif of Waltz *et al.* (1996) occurring in the initiation zones of ori region. Consequently, these extrachromosomal DNAs, portion of which represents a precursor of AMV DNA, seem to descent from initiation zones of various ori regions of an early replicating chromosomal myeloblast DNA. In addition, a possible explanation of the inclination of these DNAs to form multimers is presented.

Key words: extrachromosomal DNA; avian myeloblastosis virus DNA; origin regions of chromosomal DNA; modular sequence elements

Introduction

AMV, like other retroviruses so far studied, contains a „small" DNA of host origin (Říman and Beadreau, 1970; Levinson *et al.*, 1970; Biswal *et al.*, 1971; Weber *et al.*, 1973; Deeney *et al.*, 1976a). This virus core-bound DNA (Deeney *et al.*, 1976b; Dvořák and Říman, 1980a) designated as AMV DNA was found to represent actually metabolically active early replicative structures (Říman *et al.*, 1993a,b; Korb *et al.*, 1993) associated tightly in virus core isolates with Pr and Pr-alpha DNA polymerase (pol) activities (Říman *et al.*, 1995). These findings implicate accordingly that this DNA or the associated highly specialised cell proteins may participate in reactions accomplished by the virus core reaction machinery responsible for replication and integration of the retroviral information (Grandgenett and Mumm, 1990). Elucidation of this question needed, among others, to know more about the properties of NP complexes into which this DNA is organized

*Corresponding author.

Abbreviations: ARS = autonomouslly relicating sequence; AMV = avian myeloblastosis virus; CHLM = chicken leukaemic myeloblast; DUE = DNA unwinding element; ^{3}H-mTdR = [methyl-^{3}H] thymidine; MAR = matrix-associated region; MARs = MAR sequence; NA = nucleic acid; NP = nucleoprotein; nt = nucleotide; ori(s) = origin(s) of replication; poly(Py) = polypyrimidine; Pu = purine; Pr = primase; pol = polymerase; POMS = post-microsomal sediment; Py = pyrimidine; SAR = scaffold attachment region; SARs = SAR sequence

B2
ATGTATATAAACTGAGTGGAAAATTCCACTAAATGAGAGAACTCACAGTACTCTTCCAGGCTTATATCAGCTTTTACAAG
ACAGAAGAGTGAAGACATTCACTTTTAAGGCAGCAAGTGTGTTGCTTGTGGAGTTTCTACCAACTGTGCTGTTGTATTTC
AAGGGGAGTTTATGGTCTGTATTTTTTTACTTGTTAGAATACTAGCTTGAAAAAGTTAATTAACCGTGCATGGGTCCGCG
ATGTGACCATTGACTAACTTGAACACTTTGCCTTGCTGGAG

B9
TGCTTCTTCTATGCTGTGTTTTCAGAGTTAATAAAAAAAAATAAGATAAGGATGAATCAGAAATGTAATTCTTCTATCTTT
GTGCAGGAAAATAATACAGAGACGATTAAAAATATTTTCTGTTGCCTCTTTTTAGAAAAAAATAAGTTTCTACTTATACA
CACATTTAATGTAACATAATGTGTGTTTTTATTTTCAACAACAAAATGCTTCACTCACTGGAATGGGTGGCCCATCGGTG
GTGGTGGATGCTTTGTCCCTGCAGAACGTCAGGTCAGGCTGGATGGGGATCGAAGCAATGTATC

B10
CTTCAGGATGGNGNCAGGTCCACCTGCAAAGACATGATAAACTGATTTACAATGCACTTTCTTGAGGCTGCTCTTCAAGG
TCTGAAGTTCATATTCCACTGATCTTAAAATAATTTATGAATTAAGGA

BIR-CTY
CTTCAGGTTGCTGGAGTAAAGGGAGAATTACGTCCCCGTCTTTAAATGTAGCAAAGGCTGTAGCTAAGAACCAAATCAGT
ATTCACAGAATGTTAACTACTGACCTTTTGTGACTTAAAAACATCAGATGCCTGGAGGATGCTTTTCTCCATCTACAATT
AACATGGCTTTGCTTTGCTCTCTGTTTTCCAAAATAAAACCTTTATAAGGGCTGTTTTGTTCAGCGATGAATGTGGTTTG
TGACTGAC

B4 - tag
CACAAGATCTACCCTTCTCTTCCTTAGCTTTTAATATGGCTTCCAACAACAGAAGCATCTTGGTTTCTCTTGTGGATGCT
CCATGATCTGGAAGTCCCTCAGGTCATCACTTCAGCATGCTTTCCTAAATTAACAGCCACATAACTCGTGTGTACATTTG
TAGAAACTGTTGCAAAATGGAATAAAATGTGTCATCTAGGGTGTAGCAAAAAAATTGAGCAGATAATTCTGCGCTTGCCA
TGAAGCTGGGTTAATTACACTTCTATATGTAATTACTCTGCTTAGTTTACTAAAAGACAGTATGCTGAGGGCCAATTTTG
CAATTCAGTATTTACTGACGCTGAAGAACTTTTTGCCTGAGGAGATGTTGACAAGCAGAAGAGTTGAGTGG

B6R - tag
AGCTATCTTTTTTTTTCCCCCTCGAATTTAGTTTTAATACAGAGAATCATACTTAGTTGAACTCTTAAAGCTCTTTAAAG
GGGAACTTTTCAAATACTCATCTTTCTCTATATTTGTGTTTAATAATAGAGATTTCCCTCAATAGCTGGAGATACC

B6F - tag
CCTTGCCTGATGTGGGATTTACAGATGCCCAACTTTCTGGTTATTTGATTTTGGACAGGAAATGCTTTGCAATTAGGAAG
ATTGTTGCTGGACAAAAAAAAATAAATGAGTTTTTAAAAGTCACTTGATGCGATGCTGGAATATGCAAGTGCATGACACT
CGCTTTAGCTTGTTTAATCACATCTGTTCACATAGGTAGCAAGAACAAGTGAAAATGCTGAGTATTCAACTTTCCTAGCT
TCAAGAAAATACCAAATGTCATTCTAAAAAACCAAGCAAAGCAAAAATTGTGTGTGATTTTGTTTAATAAAAGGCTGAAT
ACCCGAACTGAGGCTCTGTGCTGAAG

Fig. 2
Sequence characteristics of cloned DNAs of POMS component B
B2-B6F: individual DNA clones. For the rest of legend see Fig. 1.

(b) The POMS DNAs were found to be organized into NP complexes intimately associated with NA synthesising activities significant for initiation of DNA synthesis in animals (Roth, 1987), i. e., with Pr and Pr-alpha DNA pol complex activities (Říman and Šulová, 1997b).

(c) It was demonstrated that these activities were leading in reactions accomplished *in vitro* to formation of initiator RNAs and Okazaki fragment precursors, and in cooperation with accompanying activity of the epsilon DNA pol to formation of full length Okazaki fragments (Říman and Šulová, 1997c).

(d) Accordingly, AMV DNA was found to be in virus core isolates tightly associated with Pr and Pr-alpha DNA pol complex acivities (Říman *et al.*, 1995).

(e) Early replicative nature of POMS NP complexes and their DNAs (Korb *et al.*, 1997) as well as of DNA of AMV DNA isolates (Korb *et al.*, 1993) was recorded also electronmicroscopically.

(f) Finally, the specific [methyl-^{3}H]thymidine (^{3}H-mTdR) radioactivity of AMV DNA and POMS DNA (DNA of POMS component B (Říman and Šulová, 1997a)) exceeded more than by one order that of total cellular DNA of CHLMs (Říman *et al.*, 1993b).

These common features of both kinds of these DNAs, as pointed out above, allowed to expect, consequently, that they might share similarly some common features at the level of their sequence characteristics indicating their common descent from initiation sites of chromosomal DNA replication. Since in animal chromosomal DNA the early replication events are taking place inside ori regions and their initiation zones (DePamphilis, 1993), we decided to analyse the sequence properties of these DNAs with the aim to search for modular sequence elements and motifs present in a higher abundance in „ori-enriched" (Kaufmann *et al.*, 1985) monkey DNA fragments (Rao *et al.*, 1990) or in ori regions (and their initiation zones) as described in different higher eukaryotes (Dobbs *et al.*, 1994).

AT-richness of the cloned DNAs

In eukaryotes the oris of chromosomal DNA are associated with nuclear skeleton proteins (Jackson and Cook, 1985;

C1
ACCACTAAGGCTTCCTGGTTTTACCACTAAGGCTTCCTGGTTTTGCCTTCAACCTTTGGAATAAGGGGCAAGGACTTCTG
GTCCAGCAACACTGTAAGAATGGTTTAAGGAAGAAAGGAATTTGAACTGTCTCATCTAAAACCCTCTGGGCAGAGCTGGC
ATTAGACCGAAGTAAAG

C3
CAGCTGCTCCTGTGACACTCGGAAGTCCTCAAAGGGCTGCTGGTACCGCACCTCGAAGGAGCCATCCCTGSTTGCTGGCC
AGCAGCTGGCCATGTGTCCCCGCCTCGCTCCCGCAGCACGGAGCCACCGAAGAGGCTGATCCTGCTGTTCATTCCATGAA
CACAATTTGTCAAGCAGTATTSTTTATTTCTTTGAAATCACCAAGCATCCTTGTACTCCAGATAGTTTCAATGCAACCAC
AGAATACAATGAGATCGACCTGTTAGAT

C5
TTTAAGCTCCATTTGTGAGGCATGGTGTCAAGTGAGATTTATTCCTTTACTCTCTGGAAACCTGTGATATGACTCCTTCA
GCCAAAGCATGCTTTTCCCTGGCCTCCCTTCAAAATGTACTTTTCAAGTTCGCAGTATTGGCTCAAC

C6
AACAACAATGGAAATCTACCTGAAAAAAAATTAACAACTCAGAGAAAAAACAAATTGCCAGCTTCGTTCCAAGGGCATCA
GCTTGGAATTTAGCTCTTGATTGGAAGAGCTTCACCTTTACTTCATTATTGACGGG

C7
AGTTGAACTGGCATTTGTGCATCTGGCTTTTTTTTCATGGGGCAGGACAGGGAAGCTTTTGAGACTTATGTCACATGTGA
ATCTTGGTCATGACAGTTCATTTGGGCCAGCACTTTGCATTGGCCCTTTCACATTTGCATAGCTCCTAACAAAGTCATTT
TCAAGACTGCA

C8
CAACAACTGGAGTTTCTGTTCGCTAAGATATTCCAGAACTTGAAATTGCCATTGTGTAGAATTATTACTCAAAGCTGAAT
TTCTGAGCGTCTTTGATTTGGCATCAGAGAATCTATTTGTTTTAGAAAGAAGAAAAATAGGTTTTCA

C9
ATCAATTAATGTAAAATGTATTTTAGTCTATTTTTGGCCAAATTGATGAGAAGCAGTTGTACAACCACACAGCAAGTTGT
CCTTACTGCTTTTGTTTACACCATTATTATTTTGGGGATATTCTCCAATTACTTACTCTAATTCCAACACAGCTGTCCCC
AGTTTAGTCCATTTGTGAAACGTGGCTTGACGACTGCTAAATACAAATAGCTTTTTGGCACTGCATATATTGCTACTGGG
AGCCTCTTTTGCAAATCCTATGAGCTGCAACGTTTGATGGGTGGCCAACACAGCCAAGTATGGTCGCAGTGCTGGAG

C10
CCATCAACCAGAGGTGACGGTGGATACGCTCACTACAAAGTACCACTATTTATTCACCTCAAATGCAAACACAGACCTTC
AATCTGAACATCTTATTCAAGCTTTATACCATTTTTTTTTATATTTTCAGTTTTCA

C12
CTTTTCTTTCGAAAATCACGCATTTTGCCCCGAAAATACCACCCGTCTCCCAGAAATTCTGCACTTCCTTCCCGAAAATA
CCACTTTTCGCTGCGAAATAACACATTTCTACCCCAAATATACCACGCTTCACTCACAAAGCACGCATTTTCA

CIVUN
TTAAAAAAAAAAAAGAATGTGTATTGTATTAATATGTGTTTGGGTTTTCTGATGGTCTTGTCCAGCCACAGGAAATAACT
GTGTTAATGCCTGAGTTCACTAATAAGTGTATTTGGCTTTTAACGTGTTTTTATCCAGACCTCCCGTAGTATTTCTGACC
TCACACCTAACATGGAACTTATGGATGCCCTATTTTAAATCCTCTATTTTTTGTTTGTTTTTGAATGAGTTCATATTTTT
TTGGAAGAAGAAACCAGACCTAATTTTAATATTTGCAATGATAACCCTATACAACCTTAGGGACTGGAGAGAGTGTGAAA
TCGTTGATGTCACTGGAGGAGCCCCACGTTGGTGTCAATCACACAGATGTCTCTGACCGGCTTGCAGGCAAAATTTTGGA
TGTCAATGCTTTACCAACAGCGATGGTAGAAGTCAGTGCTCT

CIVRE
CTGACAATTTAGCAAGTTAAATTATATAGCGAAGAATTTACCATCTATTTGGTTACTGCCGTGACCCTCAGTGCCAAGTT
TTTTTTTCCAGCTAGCTTTCAAACAGTCTGCTTTTTCTTCAGCTACTGTGTTTCCATAAAATTGATTGCTTTCAATTGTA
GTTTAGCTAATGCAATACAT

CVU-CTY
TGCCAGTTCCAGGATCCCTTATCAGAGGGCAGGAGGCCCTCAGCAAATGAAGAGACATCCTACAGAGAACCACACATCAT
TGCCCTGGAAGATGAACAGGGCAGCCCTGGGAAGCTCCATCAGCTGCTGTACAGTTCTTGAGG

Fig. 3
Sequence characteristics of cloned DNAs of POMS component C
C1-CVU-CTY: individual DNA clones. For the rest of legend see Fig. 1.

Razin *et al.*, 1986) called nuclear matrix (Berezney and Coffey, 1974) or scaffold (Lebkowski and Laemmli, 1982). This non-covalent binding of DNA with nuclear skeleton proteins is mediated in this case by its AT-rich stretches which represent in turn the source of special matrix and scaffold-associated sequences, matrix-associated region sequences (MARs) (Cockerill and Garrard, 1986) and scaffold-associated region sequences (SARs) (Gasser and Laemmli, 1986), respectively. They are also source of ARS (Palzkill and Newton, 1988). Consequently, an AT-richness belongs to a general feature of eukaryotic DNAs derived from oris (DePamphilis, 1993). Such a feature was exhibited also by the cloned „ori-enriched" monkey DNA fragments (Rao *et al.*, 1990). Four of eight these DNA fragments revealed an (A+T) content from 60 to 66% with an overall (A+T) content of 57.9% in comparison to 56% of that of the total genomic monkey DNA. In contrast, one of these cloned „ori-enriched" DNA fragments was found to be especially poor (48.5%) in (A+T)

V1
GGCCTCATTAATTTAAGAAGTGCTTTCAGTTCCAAGTTTATTGTCTACAAAGTTCATGCGTATCTGCAAAAGCAAAGTTC
GCTTTTAAACAACAGTAGATAGAATCATAGAATTGTTTGAGTTGAAAGGGACATTTAAAAGTCACCCAGTCCAACT

V3
GCTCCTTTCCTTCCCAAACTGTCCAAAGTGAAAATGCATCTCAGTAATGCAGCACTTCAAGAGATTATGTCAAATACTCC
TGAGAAGAGTTTAGGAGAGAATGCAACAGACTCCACTCCTCCAAATATGTGGAGGGAAGATAAAATCAGTCAGGATATTC
CCAAAGGCAGA

V5
CCCATGATTGCTTGCATAGATACTGGTAAGCCACTGCCCTGCTCACATTTGATACTGGTTCTTAAGCCAGCTGTGTTAGG
GGCTGCTGTAGCAGCAGCAGGGAATGGGACATAATCTGCCTTTAGCTATATGCTTGGGGCAGACAGCAAGCCCTGTCTTC
AGAGCCTAAAATCCTGACTTCTGGAACAAAGACAGCAACAATGAGCAAAACAATTACAATACAGCTTTGTCAGCTCTTCA
GTCAATAAGGTGGG

V6
CCTAGAAGACATGTCAAGGCATAAGAAAGGTGAGTGGGTGAGCTGAGACAGCAACATGGCTTCACCAACAGAAGTCATGC
CTGACCAGTCTGGTGGCCTTCTGTGATGGAGTGAAAATGTTGGTGGACAAAGGCAAGGTGACCACTTGGA

V7
TTTTCTCTCCAAAAATAGCCCATTATCCCAAGAATCACACATTTTCTCTTGCAAAATGGCCCTTTTAAGCCCAAAATCTT
GCATTTTCTCTTGGAAATCGCCCATTTAACTCGGAAATCACACTTTTGCTCTTCAAAAATCCCCCGTTTGACC

V11
AACCCGCTTCACACGAATATCACGCATTGTCTACCGGAAAATACCACTTTTCTAACGAAAATNNCGCACTGTCCCGCCGA
AAAACACAAGTTNNNCCGAAAAATCACGCATTTTNNCCCAGAAAAAAAGCACCTCTGTMCACAAGTN

V15
GCTTTCCCTTCCTTCCCCTGATATAATGGGAACTGTGCAGCTACTCAAAAGTACGGACAGTTCAAGAGTTTTTATTGCTC
ACTGCTAATG

V18
ATTCATAACCTTGGGGCATCAAAGGAGTGCTTGGGAAACGGGTAGCTACCAGGGNCTTTTTTGTTTTGTTTTGTTTTACT
AGGGCTGAAATATCACTTGCTCAATAAGCTTATTTTCTACAGCTTATGTTTTCCTATGTATGTTTTGTCACTGTAGATGT
TACATATATGATAGWC

VIREV
CTAAGAATAAAACTTTCCTAATGGATTTTAAAAAGAAATGATTAATTATATGACTGGCAGTAACAATCTATGAAAAATAA
ACAAATAAGTAACTGATCAATTCTTATGCTTCATTGAATCTGTGAATGCAAAAGGATAGAACATTACGCAGTTGATAAGT
AAACAGTGTCGACTTCCCCATGTCCCCTCTGTTCCCCGTGTCCCAACAGAGGCCCATAGATCCCAATGGTTGCCATGGTT
ACCAACCACCCCGATGGTGGCBGCCATGTTGCTAGGCCTCATTCCCCATTTGTACCCNCAAATCTTCAATTTTTTGG

V4 - tag
TACACAGGTTCTTCTTGGTCAAGAGAAAGAAAACATCTGAGGAATATCCCTGTCTCCATTGCATCTATATGTTTACATGG
CCAGATGGGAACAGACATGAGTAACCCTGTCTCCCAATTGTCTCATGCATCCAAAAATCCATCAGCCAATGGATGGG

V25 - tag
NNNNNACTCCAGTTCGCCATTTGCTCCATTAACCTGCCTTCTGCWNCCCCATTCTTAAAGCAATCCATCCATCAATCCAC
GCTCAGCATCCATCACCCCACCCCACAAGAGCAGGTAGTCTTTGACCACCAGTGTAGGTGCCTACCATT

Fig. 4
Sequence characteristics of cloned AMV DNAs
V1-V25: individual DNA clones. For the rest of legend see Fig. 1.

content. As evident from Table 1, the cloned POMS DNAs are with their (A+T) content strongly reminiscent of the „ori-enriched" DNA fragments of Rao *et al.* (1990). The incidence of DNAs with (A+T) content over 60% was in the case of POMS DNAs even higher. This is also apparent from the mean values surpassing distinctly the (A+T) content of the total genomic DNA of CHLMs (57.20 ± 30%) (Table 1). Interestingly, like in the case of „ori-enriched" DNA fragments, they were found especially in the case of DNAs of POMS component C, DNAs evidently much poorer in their (A+T) content not surpassing 50% (Table 1). The DNAs with the highest (A+T) content belong to DNAs of POMS component B which is formed by NP complexes exhibiting the most active DNA synthesis (Říman and Šulová, 1997a,b). On the other hand, the DNAs with the relatively lowest (A+T) content are from POMS component C which is formed by NP complexes equipped with outstanding Pr and Pr-alpha DNA pol complex activities (Říman and Šulová, 1997a,b,c) and which are micromorphologically reminiscent of NP

Table 1. (A + T) content (%) of cloned DNA molecules originating from POMS components A, B and C, and from AMV

Source of cloned DNA	Maximal (A+T) content observed (%)	Minimal of (A+T) content observed (%)	Mean (A+T) content ± SD (%)
POMS component A	65.93	52.90	61.26±3.56
POMS component B	68.50	59.00	62.26±3.47
POMS component C	66.71	48.60	59.37±6.35
AMV DNA	64.70	46.19	56.15±5.15

(A+T) content was determined in 12, 7 and 12 cloned DNAs of POMS components A (Fig. 1), B (Fig. 2) and C (Fig. 3), respectively, and in 11 cloned DNAs (Fig. 4) of AMV. For comparison, the (A+T) content of the total CHLM DNA was found to be 57.20% (Trávníček *et al.*, 1964).

complexes accomplishing the initiation of DNA synthesis (Korb *et al.*, 1997). As regards the cloned AMV DNAs, two of the eleven DNAs (Fig. 4, V1, V18) revealed an (A+T) content substantially higher than 60%. In contrast, two of them (Fig. 4, V6, V25) revealed an (A+T) content lower than 50%. Since AMV DNA representing a heterogeneous polydisperse DNA consists of major and minor portions of DNA molecules descending from POMS components B and C, respectively (Říman and Šulová, 1997a,b), it is likely that a major part of these cloned molecules descended in this case from the minor portion of AMV DNA molecules originating from NP complexes of POMS component C. Such an explanation is supported by our previous data showing that AMV DNA molecules differ in their (A+T) content (Říman *et al.*, 1993a). In line with this explanation are also our previous data (Říman *et al.*, 1993b) showing that the (A+T) content of eight cloned DNAs of AMV DNA ranged from 54% to 80%.

Characteristic of the distribution of A and T residues

A and T residues are present in cloned DNAs of the POMS components or AMV as short stretches of oligo (dA)- or oligo(dT)-nucleotides composed of 3 – 8 residues. Their distribution along the individual DNA molecules is asymmetrical like it was observed in the case of the „ori-enriched" DNA fragments (Rao *et al.*, 1990). Longer oligo(dA) or oligo(dT) tracts were found to occur only twice in cloned DNAs of POMS component A (an oligo $(dA)_{16}$ tract in the A2 clone and oligo$(dT)_{10}$ tract in the A16 clone in Fig. 1). In contrast, a rather frequent occurrence was recorded in the case of short stretches composed from 4 – 6 A residues. An often flanking of them by C on 5'- and by T on 3'-sites, respectively, indicating sequence bending properties (Paleček, 1991), was noticed in all types of these cloned DNAs. Indeed, a constant occurrence of bent DNAs was demonstrated electron microscopically in AMV DNA as well as in POMS DNAs (Korb *et al.*, 1993; 1997). Moreover, it has been also shown in the case of AMV DNA that this bending is sequence-dependent (Korb *et al.*, 1993), because it was straightened with distamycin, a drug targeting the oligo(dA)-(dT) tracts (Käss *et al.*, 1988). The presence of sequences influencing DNA bending belongs to the set of sequence properties characteristic for ori regions of chromosomal DNA (DePampilis, 1993). To sequences with sequence-dependent bending belong, e.g., SARs (Amati and Gasser, 1988) as well as ARS and CEN sequences (Williams *et al.*, 1988), the curvature of which represents a conserved feature. These sequences interact efficiently with the nuclear scaffold (Käss *et al.*, 1988) and their abundance is significant for the initiation zones of ori regions. Later we will show in this paper that the DNAs analysed here are indeed rich in SARs.

Modular sequence elements and their abundance in POMS DNAs

The oris of chromosomal DNA in higher eukaryotes differ significantly from the well characterised fixed sites of initiation used by prokaryotes such as *E. coli* (ori C) and mammalian viruses such as the SV40 virus (ori sequence) (Benbow *et al.*, 1992). By analysis of sequences of oris at various loci of DNAs of six different eukaryotic species belonging to protozoa, protostomes and deuterostomes it has been shown that each of the ori regions analysed contains one or two initiation zones possessing clusters of common modular sequence elements represented by SARs, ARS and poly(Py) tracts (Dobbs *et al.*, 1994) aligned with a putative DNA unwinding element (DUE). Enrichment with these sequences was recorded also in the case of the „ori-enriched" monkey DNA fragments (Rao *et al.*, 1990). In accordance with these properties of the eukaryotic ori regions we analysed, consequently, in the cloned POMS and AMV DNAs the extent of the incidence of the consensus sequences of T- and A-SAR, MAR as well as ARS. Similarly, we evaluated in these DNAs the incidence of the poly(Py) tracts, the occurrence of which is monitoring, in general, the presence of the sites of initiation of the DNA synthesis by Pr activities of the Pr-alpha DNA pol complex (Roth, 1987). A tight association of Pr with alpha DNA pol implicates this enzyme complex in RNA-primed DNA synthesis at oris (Wang, 1991) as well as in the synthesis of the Okazaki fragment precursors on the lagging DNA strand (Nethanel *et al.*, 1989; Nethanel and Kaufmann, 1990).

However, not all Py-rich regions support RNA primer formation suggesting that a high Py content is not sufficient to constitute an RNA priming site (Harrington and Perrino, 1995). It has been demonstrated that Pr binds at specific positions within the poly(Py) tracts and initiates RNA synthesis at two different sequence motifs in dependence on ATP and/or GTP concentrations.

High ATP/GTP ratios promote initiation of RNA primer synthesis at 3'-CTTT-5' sites, while low ATP/GTP ratios do so at 3'-CCC-5' sites (Yamaguchi *et al.*, 1985). These specific motifs increase the binding affinity of Pr for Py-rich DNA template (Harrington and Perrino, 1995). Recently, the initiator site sequence 3'-CTTT-5' was found also as an integral part of an asymmetric Py heptanucleotide 5'-CTT-TC-Py Py-3' which has been shown to be a constant part of an initiation consensus sequence in the c-myc ori region (Waltz *et al.*, 1996). Moreover, there are also sequences, such as 3'-CC(A/C)-5', positioned in the DNA template few nucleotides downstream of Pr start site. These sequence motifs may interact with the Pr-alpha DNA pol complex (Davey and Faust, 1990) and this interaction may control site selection and frequency of initiation by Pr, which prefers in this case an initiation with ATP. On the basis of these

Table 2. Abundance of modular sequence elements characteristic for initiation regions among cloned DNA molecules of POMS component A

Function	Consensus sequence	Reference	Abundance	
			Expected[a] (mean±SD)	Found[b]
SAR-T (s)	TTWTWTTWTT	Gasser and Laemmli (1986)	0.36±0.60	2
SAR-T (l)	TWWTDTTWWW	Gasser and Laemmli (1986)	2.76±1.76	5
SAR-A (l)	WADAWAYAWW	Gasser and Laemmli (1986)	3.05±1.74	5
MAR	AATATTTTT	Dobbs *et al.* (1994)	0.15±0.38	0
ARS *S. cer.* (s)	WTTTATRTTTW	Palzkill and Newton (1988)	0.08±0.29	0
ARS *S. cer.* (l)	10/11 of above		1.90±1.40	2
$Poly(Py)_{12}$	YYYYYYYYYYYY	Roth (1987)	1.30±1.15	9
Pr motifs	CTTTCYY	Waltz *et al.* (1996)	1.50±1.23	2
	TTTC	Yamaguchi *et al.* (1985)	32.15±5.65	63
	YYCTTCYY	Harrington and Perrino (1995)	1.21±1.10	2
	YYCTTTCYY	Harrington and Perrino (1995)	0.37±0.37	1
	CCC	Yamaguchi *et al.* (1985)	31,50±5,60	36
	CCM	Davey and Faust (1990)	102.81±10.05	97
	YYCCCYY	Yamaguchi *et al* (1985)	2.40±1.58	5
	YYCTTTC	Harrington and Perrino (1995)	1.50±1.23	5

[a]The expected abundance, i.e. the mean number of times the given consensus sequence is expected in the sequence of given DNA, was estimated according to Rao *et al.* (1990). SD was calculated using the formula $SD = \sqrt{nP(1-P)}$.

[b]The found abundance, i.e. the number of times the given consensus sequence really occurs in the sequence of given DNA, was estimated by direct comparison of both sequences. Sequences of cloned DNAs were searched in both directions. Symbols and abbreviations: R = A or G; Y = C or T; M = A or C; W = A or T; D = A or G; N = any nucleotide; (s) and (l) = strict and loosened similarity to the original published consensus sequence, respectively; SD = standard deviation.

findings we complemented these analyses with the search for the abundance of sequence motifs more specific for Pr initiation sites as mentioned above. The evaluation of the abundance of the relevant modular sequence elements was done according to Rao *et al.* (1990). The results achieved in this way are summarised in Tables 2-5 depicting the abundance of modular sequence elements and the examined sequence motifs in the cloned DNAs of POMS components A, B and C, and AMV, respectively.

The results achieved by this analysis allow to make following conclusions: In general, the POMS DNAs are distinctly enriched in T-SAR sequences when analysed at strict (s) or loose (l) similarity to the SAR consensus sequence. T-SAR(s) and T-SAR(l) consensuses were found to be 5.5 and 1.8, 15.3 and 5.0, and 11.0 and 2.7 times , respectively, enriched in DNAs of POMS components A (Table 2), B (Table 3) and C (Table 4), respectively. In comparison, „ori-enriched" DNA fragments revealed an enrichment (4-fold) in T-SAR (l) consensus only (see Table II in Rao *et al.*, 1990). Here, in turn, a higher (8-fold) abundance of A-SAR (s) consensuses was revealed. A-SAR (l) consensuses in abundance similar (about 3-fold) to those in „ori-enriched" fragments were found in DNAs of POMS components A (Table 2) and C (Table 4). MAR sequence consensuses analysed among other sequence modular elements by Dobbs *et al.* (1994) were found in a higher (3-fold) abundance in DNAs of POMS component C (Table 4). This consensus sequence was not analysed in „ori-enriched" DNA fragments. As regards ARS *S. cerevisiae* consensuses, the frequency of incidence of ARS (s) in DNAs of POMS component C (Table 4) is reminiscent of that found in the case of „ori-enriched" DNA fragments (see Table II in Rao *et al.*, 1990). In DNAs of other POMS components an unnoticeable abundance of these sequences was recorded.

In our precedent work on this subject we have shown that the POMS DNAs represent actually early replicative structures (Říman and Šulová, 1997a) organized into NP complexes equipped with enzymes involved in the early replication events (Říman and Šulová, 1997b, c). On the basis of these findings we have suggested that the POMS DNAs seem to be descending from oris of CHLM chromosomal DNA. Besides the abundance sequence characteristic presented above, this suggestion is further strengthened by enrichment of these DNAs in modular sequence elements such as those represented by poly(Py) tracts (Dobbs *et al.*, 1994) as well as by special sequence motifs representing an integral part of them, responsible for Pr binding and, consequently, for initiation of RNA-primed DNA synthesis at ori as well as on the lagging DNA strand (Harrington and Perrino, 1995). The frequency of incidence of these sequences was not analysed in „ori-enriched" fragments (Rao *et al.*, 1990). The analysis of the presence of modular sequence elements in oris of eukaryotic chromosomal DNA accomplished by Dobbs *et al.* (1994) involved also the analysis of the occurrence of poly(Py) tracts. As regards analysis of the abundance of poly(Py) tracts in POMS DNAs

Table 3. Abundance of modular sequence elements characteristic for initiation regions among cloned DNA molecules of POMS component B

Function	Consensus sequence	Reference	Abundance	
			Expected[a] (mean±SD)	Found[b]
SAR-T (s)	TTWTWTTWTT	Gasser and Laemmli (1986)	0.26±0.51	4
SAR-T (l)	TWWTDTTWWW	Gasser and Laemmli (1986)	1.80±1.34	9
SAR-A (l)	WADAWAYAWW	Gasser and Laemmli (1986)	2.13±1.46	2
MAR	AATATTTTT	Dobbs *et al.* (1994)	0.10±0.32	1
ARS *S. cer.* (s)	WTTTATRTTTW	Palzkill and Newton (1988)	0.06±0.25	0
ARS *S. cer.* (l)	10/11 of above		1.50±1.20	2
Poly(Py)$_{12}$	YYYYYYYYYYYY	Roth (1987)	0.87±0.93	2
Pr motifs	CTTTCYY	Waltz *et al.* (1996)	0.98±0.99	4
	TTTC	Yamaguchi *et al.* (1985)	21.00±4.57	26
	YYCTTCYY	Harrington and Perrino (1995)	0.78±0.88	1
	YYCTTTCYY	Harrington and Perrino (1995)	0.24±0.49	0
	CCC	Yamaguchi *et al.* (1985)	19.67±4.42	21
	CCM	Davey and Faust (1990)	64.76±7.98	63
	YYCCCYY	Yamaguchi *et al.* (1985)	1.50±1.22	5
	YYCTTTC	Harrington and Perrino (1995)	0.98±0.99	0

For the legend see Table 2.

Table 4. Abundance of modular sequence elements characteristic for initiation regions among cloned DNA molecules of POMS component C

Function	Consensus sequence	Reference	Abundance	
			Expected[a] (mean±SD)	Found[b]
SAR-T (s)	TTWTWTTWTT	Gasser and Laemmli (1986)	0.17±0.41	3
SAR-T (l)	TWWTDTTWWW	Gasser and Laemmli (1986)	1.84±1.35	5
SAR-A (l)	WADAWAYAWW	Gasser and Laemmli (1986)	1.57±1.25	5
MAR	AATATTTTT	Dobbs *et al.* (1994)	0.07±0.27	0
ARS *S. cer.* (s)	WTTTATRTTTW	Palzkill and Newton (1988)	0.04±0.21	1
ARS *S. cer.* (l)	10/11 of above		1.00±1.00	2
Poly(Py)$_{12}$	YYYYYYYYYYYY	Roth (1987)	1.12±1.06	3
Pr motifs	CTTTCYY	Waltz *et al.* (1996)	1.26±1.12	1
	TTTC	Yamaguchi *et al.* (1985)	24.70±4.96	42
	YYCTTCYY	Harrington and Perrino (1995)	1.07±1.04	4
	YYCTTTCYY	Harrington and Perrino (1995)	0.31±0.56	1
	CCC	Yamaguchi *et al.* (1985)	33.70±5.79	38
	CCM	Davey and Faust (1990)	103.03±10.04	126
	YYCCCYY	Yamaguchi *et al.* (1985)	2.62±1.62	1
	YYCTTTC	Harrington and Perrino (1995)	1.26±1.12	4

For the legend see Table 2.

we chose for these purposes the search for abundance of poly(Py)$_{12}$ tracts as the most representative. Table 2 shows that the DNAs of the POMS component A reveal the highest (7-fold) abundance of these consensuses while the DNAs of the other POMS components (Tables 3 and 4) exhibit a moderate (2-fold) abundance of these consensuses only. An integral part of poly(Py) tracts composed of various number of Py nucleotides might be formed, in general, by sequence motifs specific for Pr binding sites. These motifs are further designated as Pr motifs. An overall abundance characteristic of Pr motifs shows that the majority of them exhibit in POMS DNAs a moderate (2-fold) enrichment. However, some of Pr motifs reveal in the DNAs of the individual POMS components a significantly higher abundance. In this respect, e.g., in DNAs of POMS component A (Table 2), the 5'-YYCTTTCYY-3' and 5'-YYCTTTC-3' motifs were enriched 2.7 and 3.3 times, respectively. Similarly, in DNAs of POMS component B, the 5'-YYCCCYY-3' and 5'-CTTTCYY-3' motifs were enriched 3.3 and 4.0 times, respectively. In this case, interestingly, the latter motif, an asymmetric Py heptanucleotide, has been found only recently as an integral part of the initiation consensus in c-myc initiation zone of the ori region (Waltz *et al.*, 1996). Finally, in DNAs of POMS component C, three Pr motifs exhibited a high abundance: 3.0-, 3.0- and 4.0-fold abundance was observed in the motifs 5'-YYCTTTCYY-3', 5'-YYCTTTC-3' and 5'-YYCTTCYY-3', respectively (Table 4). All these motifs represent an integral part of a 40 b

sequence of a special Pr DNA template (Harrington and Perrino, 1995). Such sequence properties complement accordingly our previous findings that the NP complexes, in which the POMS component C DNAs are organized, are equipped with outstanding Pr- and Pr-alpha DNA pol activities (Říman and Šulová, 1997b,c). Interestingly, these NP complexes are micromorphologically (Korb *et al.*, 1997) reminiscent of the so-called O-somes (Dodson *et al.*, 1985), i.e. the NP complexes associated with initiation of DNA synthesis.

In conclusion of this section it is possible to say that the abundance of modular sequence elements found in DNAs of the all three POMS components of CHLMs is strongly reminiscent of the similar sequence characteristics performed in the case of „ori-enriched" DNA fragments (Rao *et al.*, 1990). These characteristics of POMS DNAs resemble also the sequence properties of the ori regions (and their initiation zones) significant for chromosomal DNA replication in higher eukaryotes (Dobbs *et al.*, 1994). Nevertheless, in the case of POMS DNAs it is possible to record besides the general sequence features differences in abundance of certain sequence modular elements and motifs in dependence on POMS component descent (A, B, C). This was found valid not only for DNAs of POMS component C as mentioned above but also for DNAs of POMS components B and A. This is in line with our previous findings that the NP complexes, in which these DNAs are organized, are equipped with activities of an early DNA synthesis minimally, medium and maximally advanced in NP complexes of POMS components C, B and A, respectively (Říman and Šulová, 1997a,b,c; Korb *et al.*, 1997). In this respect it seems to be of importance to stress that the DNAs harbored by NP complexes of POMS component B endowed with the highest DNA synthesising activity revealed the highest abundance of consensuses (SARs and MARs) which are supposed to be nuclear scaffold attachment sites (Gasser and Laemmli, 1986). Moreover, these DNAs possess also abundance of asymmetric Py heptanucleotide sequence, characteristic for initiation zones of the ori regions (Waltz *et al.*, 1996).

Sequence properties of the cloned AMV DNAs

Isolates of AMV DNA represent collections of minute early replicative structures (Říman *et al.*, 1993a,b), the (A+T) content of majority of which is about 60% and higher while that of the minor part of them is equal to that of the total CHLM DNA (57.2%) and even lower (Říman *et al.*, 1993a). According to physico-chemical properties, length, radioactive labelling for DNA and RNA and other features, the major and minor portions of AMV DNA are descending from DNAs of POMS components B and C, respectively (Říman and Šulová, 1997a,b; Korb *et al.*, 1997). Accordingly, the sequence properties of the individual cloned AMV DNAs should reflect, consequently, their descent from the relevant portion of AMV DNA molecules as well as those of the individual POMS components. This suggestion helps us to explain our past and present results obtained by studying the sequence properties of AMV DNA molecules. In our first attempt to evaluate sequence properties of cloned AMV DNAs, the eight for these purposes selected DNA clones exhibited an (A+T) content of a mean value of 67.4% (Říman *et al.*, 1993b). These findings suggest, consequently, that a majority of these DNAs were in this case picked up by cloning from the major portion of molecules of AMV DNA isolate. Suggesting already by that time a chromosomal ori descent of AMV DNA, we searched in its cloned DNAs for occurrence (not abundance) of well defined consensuses, the presence of which may indicate ori regions (Diffley and Stillman, 1990; Hamlin, 1992). In this respect, scanning these DNAs for homology and using the matrix method (Pustell and Kafatos, 1984) we found in three cloned DNAs ARS-like sequences with 77.0, 88.9 and 90.9% homology with the yeast ARS „core" consensus. Moreover, a distinct ARS-like motif (5'-AAATATAAT-3') was found also in the shortest (29 bp) sequence of these cloned DNAs (see Figs. 4-6 in Říman *et al.*, 1993b). The presence of topoisomerase II consensuses, the occurrence of which we also analysed in these DNAs, was not much significant. However, these DNAs contained frequently A-stretches composed of 4 – 6 residues and SAR- and MAR-like sequences, the abundance of which was not analysed this time. Such properties were in line with our findings that AMV DNA exhibits a significant homology with CHLM nuclear scaffold-bound DNA (Říman *et al.*, 1993b).

In contrast to AMV DNAs cloned previously, as referred to above, eight of eleven in total cloned AMV DNAs presented in this study revealed an (A+T) content equal to that of the total CHLM DNA or even distinctly lower (Table 1). Only two of these cloned DNAs exhibited a high (distinctly over 60%) (A+T) content (see cloned DNAs V1 and V18 in Fig. 4). The (A+T) content of one of these DNA clones was 60%. By these sequence properties these eight DNAs with a lower (A+T) content are reminiscent of DNAs of POMS component C (Table 1). Consequently, they may descend from DNAs of this POMS component and thus from DNAs forming the minor portion of AMV DNA molecules. These molecules were found to exhibit *in vivo* the presence of RNA-DNA molecules, which are Okazaki fragment precursors and unmature Okazaki fragments with a length up to 100 b or bp (Říman *et al.*, 1993a,b). As regards the two or three AMV DNAs with a higher (A+T) content, they represent evidently an admixture of DNAs of the major portion of AMV DNA molecules descending, consequently, from POMS component B. These DNAs were most probably picked up by cloning from DNAs belonging to the major portion of AMV DNA molecules.

Table 5. Abundance of modular sequence elements characteristic for initiation regions among cloned molecules of AMV core-bound DNA

Function	Consensus sequence	Reference	Abundance	
			Expected[a] (mean±SD)	Found[b]
SAR-T (s)	TTWTWTTWTT	Gasser and Laemmli (1986)	0.10±0.32	0
SAR-T (l)	TWWTDTTWWW	Gasser and Laemmli (1986)	1.13±1.06	1
SAR-A (l)	WADAWAYAWW	Gasser and Laemmli (1986)	0.99±1.00	1
MAR	AATATTTTT	Dobbs *et al.* (1994)	0.05±0.21	0
ARS *S. cer.* (s)	WTTTATRTTTW	Palzkill and Newton (1988)	0.03±0.16	0
ARS *S. cer.* (l)	10/11 of above		1.00±1.00	2
$Poly(Py)_{12}$	YYYYYYYYYYYY	Roth (1987)	0.88±0.94	3
Pr motifs	CTTTCYY	Waltz *et al.* (1996)	0.99±0.99	5
	TTTC	Yamaguchi *et al.* (1985)	18.70±4.30	27
	YYCTTCYY	Harrington and Perrino (1995)	0.86±0.93	4
	YYCTTTCYY	Harrington and Perrino (1995)	0.24±0.25	2
	CCC	Yamaguchi *et al.* (1985)	29.30±5.40	48
	CCM	Davey and Faust (1990)	87.46±9.24	111
	YYCCCYY	Yamaguchi *et al.* (1985)	2.30±1.51	4
	YYCTTTC	Harrington and Perrino (1995)	0.99±0.99	3

For the legend see Table 2.

Like in the case of DNAs of POMS component C these cloned AMV DNAs were found to be enriched in sequence motifs indicating the presence of Pr- and Pr-alpha DNA pol complex binding sites. This is in accord on one hand with the findings that NP complexes, in which the DNAs of POMS component C are organized, are equipped with distinct Pr- and Pr-alpha DNA pol complex activities (Říman and Šulová, 1997b) and on the other hand with the findings showing that these enzymatic activities are tightly bound to AMV DNA (Říman *et al.*, 1995). In the case of these cloned AMV DNAs the abundance of poly(Py) tracts was found to be even higher (3.4-fold) than that in POMS component C DNAs (2.6-fold) (Tables 4 and 5). This holds equally for abundance of Pr motifs such as 5'-YYCTTTC-3' (3-fold), 5'-YYCTTCYY-3' (4.6-fold) and 5'-YYCTTTCYY-3' which exhibits even an 8-fold abundance. Interestingly, these DNAs were found to be substantially enriched (5-fold) also in the asymmetric Py heptanucleotide sequence (5'-CTTTCYY-3') significant for initiation zones (Waltz *et al.*, 1996). An enrichment (4-fold) in this sequence was found in DNAs of POMS component B (Table 4) as mentioned in the precedent section. It seems to be unlikely that the high abundance of this special Pr binding site consensus in these cloned AMV DNAs, the majority of which has a low (A+T) content (Table 1 and Fig. 4), was due to an admixture of two or three DNAs belonging to the POMS component B. An explanation of the abundance of this sequence motif in these cloned AMV DNAs can be found rather in the context with some other sequence properties. These AMV DNAs are in contrast to the AMV DNAs cloned previously (Říman *et al.*, 1993b) poor in some sequence modular elements. In comparison to DNAs of POMS component C (Tables 4 and 5) they revealed only an inexpressive evidence of *S. cerevisiae* ARS (l) consensus and were void of SAR and MAR sequence motifs. Such sequence properties may indicate that in this case the cloning picked up from the minor portion of AMV DNA molecules (descending from DNAs of POMS component C), which were primarily cut out during the chromosomal DNA replication from zones positioned outside the nuclear scaffold attachment sites. This could be responsible for scarcity of these DNAs in some relevant modular sequence elements. Such an explanation of the above mentioned differences recorded in sequence properties of these cloned AMV DNAs and POMS component C DNAs is in line with high heterogeneity of these „small" polydisperse DNAs. Nevertheless, the data obtained in this study complement accordingly our previous findings dealing with the physico-chemical properties of these DNAs (Říman *et al.*, 1993a,b; Říman and Šulová, 1997a) as well as with enzymatic activities, with which these DNAs are associated (Říman *et al.*, 1995; Říman and Šulová, 1997b,c). They show equally on the basis of sequence characteristic that the CHLM cytoplasmic „small" polydisperse extrachromosomal DNA represented by the POMS DNAs (the part of which is the AMV DNA) descends from the oris of chromosomal DNA as we suspected already earlier. If so, then some or possibly all of these DNAs could serve, albeit inefficiently, as oris *in vivo*, as it was shown in the case of the „ori-enriched" monkey DNA fragments (Frappier and Zannis-Hadjopoulos, 1987).

Unexplained properties and sequence peculiarities of AMV and POMS components DNAs

In our previous studies on AMV DNA we have observed that this DNA at native but not denaturing conditions in-

Table 6. Length of cloned and uncloned DNAs of POMS components and AMV expressed in the number of bases (b) and base pairs (bp), respectively

Source of cloned DNAs	Maximal length of cloned DNAs (b)	Minimal length of cloned DNAs (b)	Mean length ± SD of cloned DNAs (b)	Mean length of uncloned DNAs (bp)
POMS component A	516	119	235±135	205[a]
POMS component B	384	126	264±72	160[a]
POMS component C	441	127	210±74	100[a]
AMV DNA	317	90	174±56	150[b]

Analysis was performed with 12, 7 and 12 cloned DNAs of POMS components A, B and C, respectively, and with 11 cloned AMV DNAs. Length of the uncloned DNAs was obtained electron microscopically.
[a]Korb *et al.* (1997).
[b]Korb *et al.* (1993).

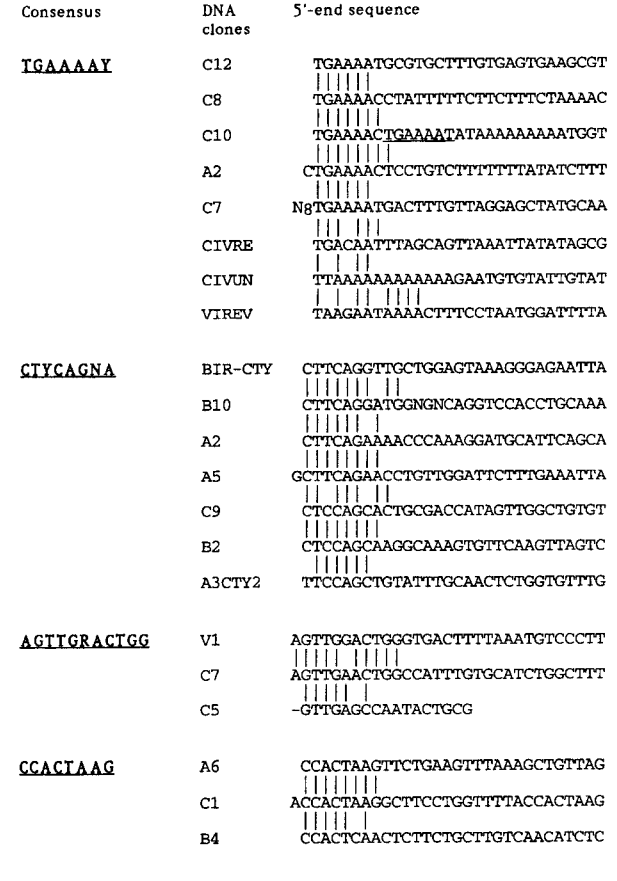

Fig. 5
Common sequence motifs situated on the ends of cloned DNAs of POMS components A, B and C, and AMV

Both ends of all cloned DNAs were checked manually for sequence similarity. Clones exhibiting such similarity were assorted into groups which enabled to devise a relevant consensus sequence. The incidence of each of these consensuses on the entire (±1 nt) end of the cloned DNAs was compared with that in the total (9059 nt) sequence of all cloned (POMS and AMV) DNAs. As evident from this figure, the end sequence similarities of particular motifs present in the relevant cloned DNAs were strict as well loosened. Interestingly, in the case of the end sequence motif TGAAAAY the cloned C10 DNA exhibited twice tandem repetition of this motif. Presented sequences of DNA clones exhibiting a mutual similarity are positioned from the left to the right in 5'-3' direction.

Table 7. Poly(Py) tracts present near the 5'-ends of cloned DNAs of POMS components A, B and C, and AMV

DNA clones	Poly(Py) tracts
C12	$--Y_{10}$
V7	$--Y_{10}$
V15	$--Y_{18}$
V3	$-GY_{15}$
B6	$AGY_{20/21}$
B9	TGY_{8}
C8	$N_6Y_{17/18}$
B4	$N_7Y_{17/18}$
A2	$N_7Y_{39/42}$
I-2[a]	AY_{15}
I-2'[a]	CY_{10}
CIVUN	TTR_{15}

All sequenced DNA clones of POMS components A, B and C, and AMV were searched for the presence of poly(Py) tracts composed of 8 nts starting at least up to 8 nts of the end of the given clone. Note that only in the CIVUN clone a poly(Py) tract fulfilling the criteria mentioned above was found. Consequently, the 5'-poly(Py)-3' : 5'-poly(Pu)-3' ratio at either 5'-end was found to be 11:1.
[a]Five previously published AMV DNA clones were involved in this analysis (Říman *et al.*, 1993b).

clines to form multimers (up to pentamers) of its molecules of the basic size unit (150 – 180 bp). Consequently, the isolates of the native AMV DNA molecules except for the major (about 50%) and minor (about 30%) portions of 150 – 180 bp and up to 80 bp in size, respectively, consist from a portion (up to 25%) of AMV DNA multimers (most frequently dimers and trimers). This phenomenon was recorded electrophoretically (Říman *et al.*, 1993b) and micromorphologically (Korb *et al.*, 1993) by analysis of radioactively labelled AMV DNA isolated from 7-hr-old virions present in tissue cultures of CHLMs and unlabelled virions in chicken leukaemic blood plasma, respectively. The phenomenon of multimer formation was also evidently present in isolates of POMS components and AMV DNA used in this study for cloning purposes. This is apparent from maximal, mean and minimal length of these cloned DNAs (Table 6)

in comparison with the mean length of the main portion of the relevant uncloned DNAs. The ability to form multimers of NA molecules suggests, in general, the availability of cohesive ends. To evaluate this suggestion in the case of POMS components and AMV DNAs, we searched in these cloned DNAs for the occurrence of sequence motifs positioned on the ends of these molecules and exhibiting a mutual interclonal sequence similarity, which may be responsible for the cohesiveness of some POMS and AMV DNAs. Suggesting a common descent of DNAs of POMS components and AMV we involved in this analysis DNAs of all the clones described in this study. The results achieved in this direction are presented in Fig. 5, which shows that some of the cloned DNAs regardless of their POMS or AMV DNA origin contain at their ends mutually identical or closely similar motifs composed from 7 to 13 nts. In addition to these sequence motifs we found a further particular motif (5'- CCTGTGNNNAGRG-3') on the ends of the clones A3, A18 and V11 (Figs. 1and 4). The occurrence of these motifs on the ends of these DNAs is not easy to explain, but it seems to be not incidental and might be responsible for the mutual cohesiveness of these molecules. Besides these motifs these cloned DNA possessed also near their ends the poly(Py) tracts (Table 7) which may represent another type of sequence motifs involved in the mutual cohesivity of these DNAs. Finally, these sequence characteristics contributed further accordingly to the concept of the common descent of AMV and POMS DNAs.

Remark. The first two authors (P. Pajer and J. Říman) contributed to this work in an equivalent manner.

Additional remarks. Studying the sequence properties of these DNAs we were able also to record some other similarities with the already known consensuses stored in the GenBank. In this respect, e.g., the clones V1 and V6 were found to be homologous with the chicken dispersed repetitive sequences, cr1 family, of a retrotransposon nature. The clones A6, C12, V7 and V11 were found, for a change, homologous (70 – 90%) with the chicken W chromosome repetitive sequence. Finally, the C3 clone was found to be homologous with sequence coding for the phospholipase C-gamma.

Acknowledgement. The authors are indebted to Dr. A. Šulová, for specialised assistance.

Dedication. The authors are dedicating this paper to the great successor of the Kanehiro Tanaki's work, Prof. Kunio Yagi at the occasion of his 80th birthday for the achievements he reached in the fields of flavoprotein, senescence and alimentation biochemistry.

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