in man applies to both children and adults; that is, one 100-mg tablet of mebendazole, administered orally, morning and evening, on three consecutive days, regardless of body weight. Thus, for a standard 65-kg male the dose would be 1.5 mg/kg. The findings of the present study with mice, if they are applicable to man, suggest that children would get an effective dosage, but an average adult would receive only a partially effective dosage; in adults weighing 82 kg or more the dosage would be ineffective. It is possible that with timely diagnosis of the disease and a revised dosage regimen, mebendazole might give a consistent therapeutic response. Trial of the drug in severe trichinellosis in man should be considered.

RICHARD O. MCCRACKEN
DORIS D. TAYLOR
Department of Biology, Indiana University-Purdue University at Indianapolis 46205

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11. We thank W. C. Campbell of the Merck Institute for Therapeutic Research and M. J. Ulmer, Iowa State University, for helpful suggestions and critical review of the manuscript. This work was supported in part by PHS grant S07 RR 7031 to Indiana University under the Biomedical Research Support Grant Program and a summer faculty fellowship to R.O.McC. from Purdue University.

7 November 1979

Properties of a Normal Mouse Cell DNA Sequence (sarc) Homologous to the src Sequence of Moloney Sarcoma Virus

Abstract. *A 15.0-kilobase (kb)* Eco *RI DNA fragment from normal mouse Balb/c genomic DNA that contains sequences (sarc) homologous to the acquired cell sequences (src) of Moloney sarcoma virus (MSV) has been cloned in phage* λ. *The sarc region (1.2 to 1.3 kb) of the 15.0-kb cell fragment is indistinguishable from the src region of two isolates of MSV as judged by heteroduplex and restriction endonuclease analyses. The cellular sequences flanking sarc show no homology to other MSV sequences. Whereas cloned subgenomic portions of MSV that contain src transformed NIH-3T3 cells in vitro, the cloned sarc fragment is inactive.*

Sarcoma retroviruses provide a system for studying the contribution of normal cell DNA sequences to malignancy. The genomes of these viruses contain sequences, presumably acquired from the host cell, which are a prerequisite for their tumorigenic potential and their ability to morphologically transform cells in vitro (*1–4*). By isolating these sequences from normal cells with recombinant DNA cloning techniques, the specific molecular elements responsible for their oncogenic potential can be defined, and the contribution of other viral sequences to the transforming process can be determined. By convention, we use the term src for the acquired cell sequences found in sarcoma viruses and the term sarc for the normal cell DNA sequences homologous to them (*4*).

The src sequences of sarcoma viruses were acquired during putative recombination between parental leukemia viruses and host cell information (*4*). The Moloney murine sarcoma virus (MSV) arose spontaneously during passage of Moloney murine leukemia virus (MuLV) in Balb/c mice (*5*) and contains both MuLV and normal Balb/c mouse cell sequences (*2*). The mechanism by which the viral src sequence participates in cell transformation is unknown. Likewise, the physiological function of the normal cell sarc sequence is unknown. Sarc sequences are represented in the normal mouse genome from one to, at most, a few copies per cell, and their rate of evolutionary divergence parallels that of mouse globin DNA sequences (*6*). As a normal cell sequence, the tumorigenic potential of sarc is obviously of great interest. We have cloned the normal Balb/c sarc sequence in phage λ and present a comparative analysis of the physical and biological properties of sarc and src.

We previously cloned the integrated proviruses of two MSV isolates, m1 and HT1, into phage λ (*7*), and identified in each provirus the src portion as well as the sequences derived from MuLV (*8*). The src regions of both m1 and HT1 MSV are identical by heteroduplex analysis and restriction endonuclease mapping (*8*). To detect the cellular sarc sequence, we hybridized a src specific restriction fragment (4.5-kb *Kpn* I to 5.2-kb *Hind* III of λHT1) (Fig. 2) labeled by nick translation (*10*), to a Southern (*9*) trans-

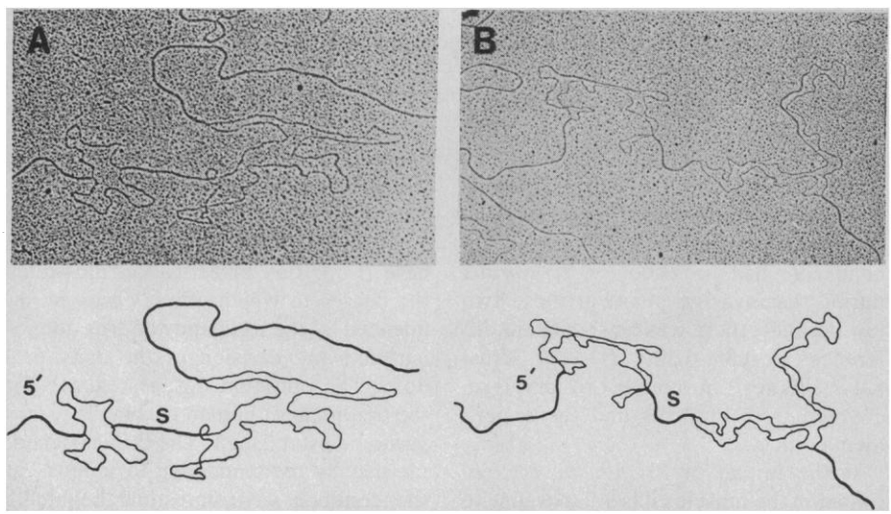

Fig. 1. Heteroduplex analyses of λMsarc with λm1 and λHT1. Heteroduplex analyses were performed on DNA extracted from λMsarc and λm1 (A) or λHT1 (B) as described (*11*). Restriction endonuclease mapping showed that the sarc sequence in λMsarc was in the same 5′ to 3′ orientation as the src in λHT1 and λm1 (*8*). The 5′ ends of the inserted fragments are adjacent to the left vector fragment of λgtWES (21.9 kb). Twenty-seven molecules were measured for (A) and 25 for (B). The measurements from the 5′ end to the 3′ end for the single-stranded, double-stranded, and single-stranded regions of the inserted fragments are, respectively, in (A) for λMsarc, 2.70 ± 0.34, 1.17 ± 0.18, and 10.84 ± 0.76 kb, and for λm1, 3.44 ± 0.34, 1.17 ± 0.18, and 2.19 ± 0.25 kb; in (B) for λMsarc, 3.0 ± 0.17, 1.30 ± 0.17, and 11.2 ± 0.5 kb; and for λHT1, 7.24 ± 0.42, 1.30 ± 0.17, and 3.34 ± 0.26 kb.

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fer of Balb/c *Eco* RI DNA fragments fractionated by RPC-5 (reverse phase) chromatography and gel electrophoresis (*7, 11, 12*). A single hybridizing region (15 kb) was enriched by preparative gel electrophoresis (*12*) and cloned into the EK2 vector λgtWES as previously described (*7*). Of 10,000 phages screened, only one hybridized to the HT1 MSV src specific probe and was denoted λ Msarc.

To visualize regions of homology between the Balb/c DNA fragment in λMsarc and MSV, we formed heteroduplexes between this clone and the recombinant phages λm1 and λHT1 (*7, 8*) (Fig. 1). In λm1 and λHT1, the 5′ end of both MSV proviruses are located proximal to the left arm of the phage λ vector (*8*). These analyses and concomitant restriction endonuclease mapping showed that the putative sarc sequence in λMsarc was in the same orientation. Only one region of homology was observed in the cloned fragments. The position of this double-stranded structure corresponds to the src region of both m1 and HT1 MSV. This region measures 1.17 ± 0.18 kb for λm1 and 1.30 ± 0.17 kb for λHT1 (*8*). In λMsarc the single-stranded regions of the heteroduplexes represent mouse sequences flanking sarc. In λm1 and λHT1 they represent mink host sequences and MSV sequences of MuLV origin flanking the src (*8*). These analyses position the sarc sequence approximately 3 kb from the 5′ end and 11 kb from the 3′ end of the cloned Balb/c fragment. At this level of resolution the Balb/c sarc and MSV src sequences were completely homologous and colinear.

We next compared a restriction endonuclease cleavage map of λMsarc with one derived for HT1 MSV (Fig. 2). These analyses demonstrated that the sarc and src regions possess the same series of nine restriction sites (from the *Pst* I site at the 5′ end of sarc to the *Hin*d III site at the 3′ end of the sarc) and positioned the HT1 MSV src sequence between the 4.1-kb *Xba* I and the 5.4-kb *Kpn* I MuLV sites. Other than the sarc/src region, no similarities in the restriction sites were observed between the Balb/c DNA fragment and the MSV sequences of MuLV origin. We also used the Southern blotting technique (*9*) to determine whether MuLV sequences could be detected in λMsarc. With a ^{32}P-labeled DNA transcript [complementary DNA (cDNA)] complementary to total MuLV virion RNA (*7*), no hybridization was detected in the 0- to 6-kb portion of the Balb/c fragment in the immediate vicinity of the sarc region (not shown). However, MuLV cDNA did hybridize strongly to fragments from the 6- to 10-kb region. Sequences in the probe responsible for this hybridization have not yet been characterized; heteroduplex (Fig. 1) and blot hybridization analyses (not shown) indicate that they are not homologous to the MuLV sequences in MSV.

The cloned integrated proviral DNA fragments of m1 and HT1 MSV produce foci of transformation on NIH-3T3 cells in a direct DNA transfection assay (*8, 13*). An obvious question is whether the Balb/c sarc sequence also transforms in this assay. For purposes of comparison, we first determined the efficiency of transformation by the intact HT1 MSV provirus and its subgenomic fragments (*14*) (Table 1). Whereas λHT1 DNA produces 4.7 × 10^4 focus forming units (ffu) per picomole of DNA, a subgenomic *Hin*d III fragment (3.1 to 5.2 kb) (Fig. 2) cloned in the EK2 plasmic vector

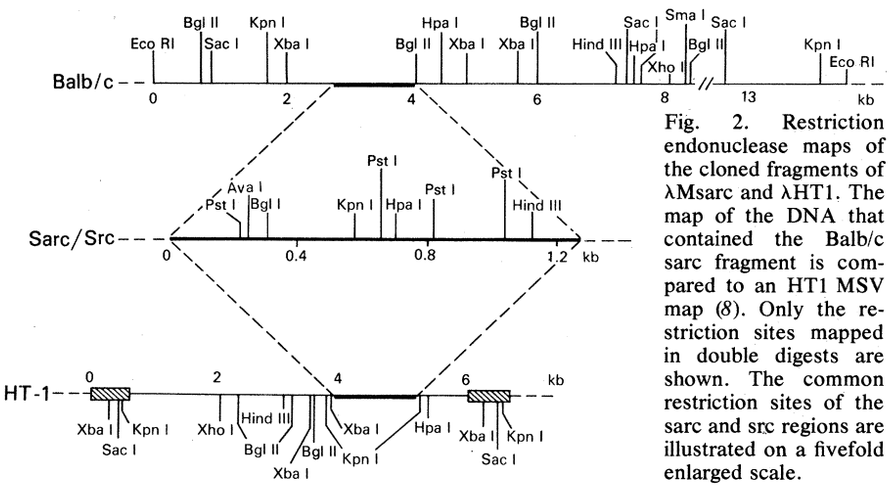

Fig. 2. Restriction endonuclease maps of the cloned fragments of λMsarc and λHT1. The map of the DNA that contained the Balb/c sarc fragment is compared to an HT1 MSV map (*8*). Only the restriction sites mapped in double digests are shown. The common restriction sites of the sarc and src regions are illustrated on a fivefold enlarged scale.

Table 1. Comparison of the biological activity of cloned Balb/c sarc and MSV proviral DNA fragments.

Clone designation	Schematic of fragment tested*	DNA (ng)	Foci (No.)	Activity (ffu/pmole DNA)
λHT1	R 5′ HB H 3′ R	10	61	47,000
pHT10		130	1	11
pHT13		1,500	0	< 1†
pHT22		58	175	6,000
λLS1		90	0	< 50
		810	0	< 6
		1,350	1	4
		1,800	1	3
λLS2		120	24	2,700
pMS1		420	0	< 5
		2,100	0	< 1
λMsarc	R B H B R	570	0	< 17
		1,000	0	< 10
		3,200	0	< 3

*To approximate scale: (····) normal mink sequences flanking the integrated proviral DNA; (∞∞) the 600 bp terminal repeat sequences (TRS) of the provirus (*8*); (—) MSV sequences of MuLV origin (*8*); (xxxx) src sequences (*8*); (- - - -) normal Balb/c sequence flanking sarc; (●●●●) sarc sequences. Plasmid pHT22 is recombined from pHT10 and pHT13, λLS1 is recombined from λMsarc and pHT13, and λLS2 is recombined from λm1 (*7, 8*) and pMS1. The approximate location of restriction endonuclease sites used in the construction of pHT22 and λLS1 are indicated; R (*Eco* RI); H (*Hin*d III); and B (*Bgl* II). †No foci were detected. The number given is a minimum value, assuming one focus was detected at the DNA concentration tested.

pBR322 and denoted pHT10, transforms cells with a 4000-fold lower efficiency (Table 1). The MSV sequences in pHT10 represent src and MuLV related sequences at the 5′ end of src. We have attributed the reduction in focus forming activity to the removal of the two 600-base pair (bp) terminal repeat sequences (TRS) bracketing the proviral genome (*13*). One example is given in Table 1. The plasmid subclone pHT13 of HT1 MSV (from the 5.3-kb *Hin*d III site to the 12.3-kb *Eco* R1 site) contains one TRS and is inactive in the transformation assay. We recombined these sequences at the *Hin*d III site to the 3.7- to 5.2-kb (*Bgl* II–*Hin*d III) MSV portion of pHT10 generating pHT22. This restored the transforming efficiency to within 10 percent of that obtained with λHT1 DNA.

When the cloned 15-kb Balb/c sarc fragment in λMsarc was tested in the transformation assay, no foci were detected (Table 1). pMS1, a sarc containing subclone of this fragment (0.7- to 4.1-kb *Bgl* II) in pBR322 produced no foci at the highest DNA concentrations tested. The Balb/c sarc sequence may fail to transform cells for several reasons. For example, a mutation in sarc could have occurred during the generation of MSV engendering it with tumorigenic activity. However, the MuLV sequences bracketing src may be responsible for activating its transforming activity (*13*). We therefore replaced the normal mouse sequences flanking the sarc region of the λMsarc insert with subgenomic portions of MSV. The stimulation of focus forming activity by the addition of MSV sequences in pHT13 to the 3′ end of the src sequence in pHT10 (pHT22, Table 1) raised the question of whether the transforming activity of Balb/c sarc could be induced in an analogous fashion. For this purpose a hybrid DNA recombinant was constructed in λgtWES and denoted λLS1; it contained λMsarc sequences from the left *Eco* RI sites to the sarc *Hin*d III site linked at the *Hin*d III site to the MSV sequences in pHT13. In contrast to the high transforming activity of pHT22, the λLS1 fragment produced only single foci at the highest DNA concentrations tested (Table 1). The most apparent difference between the inserted fragments of the inactive λLS1 and the active pHT22 resides at the 5′ end of the src sequence. The latter contains MuLV-related sequences at the 5′ end of src as does the subgenomic transforming fragment of MSV 124 (*15*). To test whether sarc would transform when attached to these MuLV-related sequences, we constructed the recombinant λLS2. The inserted fragment in this phage consists of the entire 5′ end of the MSV provirus joined at the *Bgl* I site in src to the *Bgl* I–*Hin*d III fragment from sarc (Table 1). This fragment transformed with an efficiency similar to pHT22. While we cannot exclude other reasons for the transforming activity of λLS2, these analyses suggest that MuLV sequences at the 5′ end of src are necessary for its activity (*13*).

MARIANNE OSKARSSON
WILLIAM L. MCCLEMENTS
Laboratory of Molecular Virology, National Cancer Institute, Bethesda, Maryland 20205
DONALD G. BLAIR
Laboratory of Viral Carcinogenesis, National Cancer Institute
J. V. MAIZEL
Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland 20205
GEORGE F. VANDE WOUDE
Laboratory of Molecular Virology, National Cancer Institute

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8 November 1979; revised 2 January 1980

Vomeronasal Pump: Significance for Male Hamster Sexual Behavior

Abstract. *Vomeronasal chemoreceptors segregated within the vomeronasal organ are important for male hamster sexual behavior. An autonomically controlled vascular pump, previously demonstrated in anesthetized animals, can transport stimuli to the receptors. Interruption of the efferent nerves controlling the pump results in behavioral deficits similar to those produced by interruption of the afferent nerves carrying information from the vomeronasal organ to the brain. Pump activation is thus a prerequisite for normal vomeronasal stimulation in behaving animals.*

Powers, Winans, and their colleagues (*1–3*) have shown that the vomeronasal organ (VNO) is involved in male hamster sexual behavior. They cut the afferent vomeronasal (VN) nerves and observed deficits in mounting and ejaculation. The effects of cutting the VN nerve were best revealed when animals were also treated with intranasal infusions of zinc sulfate to destroy or damage the main olfactory receptors (*2, 4*). Under these conditions, all animals showed deficits, whereas animals treated with $ZnSO_4$ alone showed no deficit.

We have previously investigated the physiological mechanisms by which stimulus molecules are transported to VN receptor neurons. In the hamster these neurons are sequestered inside the VNO, a tubular sac which opens at one end only through a long narrow duct onto the floor of the nasal cavity. In anesthetized animals, fluid can be moved in and out of the duct by local vasomotor

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