Reduced Incidence of Spontaneous Mammary Tumors in C3H/He Mice after Treatment with Polyadenylate-Polyuridylate

Abstract. *The effect of treatment with the double-stranded polynucleotide complex polyadenylate · polyuridylate [poly(A) · poly(U)] on tumor development in C3H/He mice was evaluated. Poly(A) · poly(U) was injected in newborn females, and mice were observed for 380 days. During this experimental period 42 percent of treated mice developed tumors, while the incidence in the control group was 63 percent. This difference was statistically significant.*

The double-stranded synthetic polyribonucleotide complex polyadenylic acid · polyuridylic acid [poly(A) · poly(U)] stimulates antibody formation and cell immune response (*1, 2*). Shortening of the induction period by injection of poly(A) · poly(U) together with a single injection of bovine γ-globulin as antigen has also been shown, and both 19*S* and 7*S* antibody titers were found to be raised (*3*).

The polynucleotide complex not only enhances antibody formation in normally responding animals, but it has also been demonstrated that the complex can act in genetically low responding strains to restore the normal levels of antibody production (*4*). The complex also acts on newborn mice to provoke high levels of antibody production at a time when such a response is normally very weak (*5*). In addition poly(A) · poly(U) has been able to restore responsiveness in aging C57 mice which display impaired antibody responses normally (*6*), and finally the complex has been used to drastically improve the capacity of neonatally thymectomized mice to respond to skin homografts (*2*).

Since the immune response is considered important in the control of tumors, the ability of this apparently nontoxic and nonpyrogenic homopolymer duplex to affect the growth of experimental tumors has been investigated. Some growth inhibitions of spontaneous mouse mammary tumors (*7*), of syngeneic transplantable mouse tumors (*8*), and of a transplantable hamster melanoma (*7*) have been reported.

The objective of our study was to determine whether administration of poly(A) · poly(U) to newborn C3H/He mice could reduce the percentage of the subsequently developing mammary tumors. The mammary tumors that appear in mature female mice represent the effect of a viral (mammary tumor virus, MTV) infection that occurred at birth. Poly(A) and poly(U) were each synthesized (by A. M. Michelson) with the use of polynucleotide phosphorylase (*9*). After being dissolved in 0.14*M* NaCl, *p*H 7, the two polynucleotides were mixed in equimolar ratios. Newborn C3H/He females received three intraperitoneal injections of 15 μg of poly(A) · poly(U) on days 1, 3, and 5 after birth. The mice were weaned 3 weeks later. To avoid any possible alteration of the normal environment necessary for development, the females were caged together with males. The animals were maintained on a diet of pellets and tap water and once a week they received carrots and lettuce.

A control group of untreated mice was kept under the same conditions as above. The animals in both groups were observed for 380 days, and autopsies were performed on all mice that died. The nature of the tumors found was confirmed by histological examinations.

During the 380-day experimental period 80 of the 127 control mice, or 63 percent, developed mammary tumors. Among the animals in the group that had received neonatal injections of poly(A) · poly(U), 35 of the 83 treated mice, or 42 percent, developed tumors in that same time period. The difference of 21 percent in tumor incidence between the experimental and control groups is statistically very significant, $\chi^2 = 8.8$; $P < .01$ (Table 1).

Not all the animals survived the full experimental period; however, all the tumors are recorded and have been considered in the calculations. In the treated group, there were 61 deaths prior to the end of the experiment; of these 30 had mammary tumors and 31 were free of tumors. Some of the latter had died from obvious extraneous infections. The 22 surviving mice at day 380 were killed, and upon autopsy 5 were found bearing tumors. The remaining 17 were tumor free.

Table 1. Analysis of autopsy observations of C3H/He mice during a 380-day experimental period.

Item	Mice (No.)		
	Total	With tumors	Without tumors
	Controls		
Deaths	84	55	29
Survivors	43	25	18
Total	127	80	47
	Poly(A)·poly(U) treated		
Deaths	61	30	31
Survivors	22	5	17
Total	83	35	48

In the control group 84 of the mice died before day 380; of these, 55 had mammary tumors and the remaining 29 died from unrelated causes. Among the surviving 43 mice, 25 were found at autopsy to be tumor bearing, and 18 were tumor free. The mortality rate in animals in which no tumor was found at the end of the observation or at death was essentially the same in controls (29/47) as in treated (31/48). The tumor incidence among the animals surviving 380 days is 58 percent in the control group and 23 percent in the treated group, a significant difference ($P < .01$).

From these experiments it can be concluded that a significant reduction in the occurrence of mammary tumors was obtained when poly(A) · poly(U) was given to newborn C3H/He female mice.

The mechanism of this effect has not yet been elucidated. The tumors occur as the result of infection of the young through the milk with RNA containing MTV. It was shown that exogenous interferon given early in life may cause a delay in mammary tumor development. It should be noted, however, that in the experiments with large doses of exogenous interferon the results indicated only a delay in the onset of the tumors rather than reduction in tumor frequency (*10*). Even though poly(A) · poly(U) is known to be a poor inducer of interferon, we cannot exclude the possibility that even a low dose of interferon was responsible for the reduction of mammary tumors. In contrast, evidence is now definitive that the C3H mouse can respond immunologically to MTV (*11*). But it is possible that poly(A) · poly(U) does induce a nonspecific stimulation of the immune response of the host to MTV.

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F Factor Promotes Turnover of Stable RNA in Escherichia coli

Abstract. *Male bacteria that contain an* srnA− *mutant allele degrade their "stable" RNA massively after RNA synthesis is blocked at 42°C; a normal F− female strain shows no such RNA breakdown unless both the* srnA− *allele and maleness (F factor) are introduced.*

In some conditions ribosomal RNA (rRNA) and transfer RNA (tRNA) are extensively degraded, but they are usually called "stable" because unknown regulatory mechanisms protect them against degradation in growing cells (*1, 2*). To try to clarify the mechanism of bulk RNA turnover, Ohnishi and Schlessinger isolated a mutant that grows normally at 30° or 42°C, but very rapidly degrades more than 80 percent of its rRNA and tRNA at 42°C after RNA synthesis is stopped (*2*). The *srn*− mutant was genetically analyzed, and the results suggested that two loci are involved in degradation of stable RNA in the mutant (*3*). One of them, *srnA*−, was closely linked to the *tsx*+ gene and mapped at about 10 minutes on the Taylor and Trotter map (*3, 4*). The other locus, *srnB*− or *srnB*+, was not completely mapped, but was probably in the region between 75 and 90 minutes (*3*). I now report that the *srnB* allele is associated with the F factor that produces maleness, and that a female strain can become *srn*− by the successive introduction of the *srnA*− allele and the F factor.

The original *srn*− mutant was a derivative of the male strain Hfr. All of the *srn*− strains obtained after conjugations and transductions were also found to be male strains, and in the course of mapping of *srnB*+, its site was always close to that of the integrated F DNA. Since an F− *srn*− strain never was found, three possibilities seemed open for the specification of the *srn*− phenotype: (i) *srnA*− and *srnB*− are necessary for the *srn*− phenotype, and *srnB*− is closely linked to integrated F DNA; (ii) *srnA*−, *srnB*−, and F DNA are all necessary, and *srnB*− is closely linked to integrated F DNA; (iii) *srnA*− and F DNA are necessary, and the F DNA bears one of the genes required for the extensive breakdown of stable RNA. In other words, alternative (iii) is that *srnB* is an F-specified or -regulated gene.

One of the lines of evidence suggesting the third possibility is shown in Table 1. The parent of the mutant, strain GP1 (*3*), already contains F DNA and, if it is thereby *srnB*+, should be able to donate it to recipients that contain *srnA*−, to produce strains capable of massive RNA breakdown. Consistent with this notion, not only were *srn*− recombinants always male, but the parental strain GP1 *srnA*+ was able to donate the *srn*− phenotype to a recipient F− *srnA*− strain (phenotypically *srn*+) after uninterrupted mating (Table 1). Thus the parental strain GP1 truly has one of the genetic loci required for the degradation of stable RNA.

Stronger evidence for the correlation of F factor and the capacity of strains for RNA breakdown was then obtained. Many Hfr strains show a less extreme, partial breakdown (*3*), and I checked to see whether F+ or F′ strains show a similar phenotype. Strains YS357 F+ *srn*+ (*5*) and W2241 F+ *srn*+ (*6*) did indeed show partial breakdown of stable RNA after treatment with rifampicin at 42°C, but not at 30°C (Fig. 1, a and c). When the F factor of strain YS357 or W2241 was put into the strain YS142 F− *srnA*− (which is phenotypically *srn*+), the strain showed the massive breakdown of stable RNA characteristic of the *srn*− phenotype (Fig. 1, b and c). The frequency of *srn*− and F factor donation to the F− *srnA*− strain was the same: 49 of 70 recipient clones.

Table 1. Conjugation of strain GP1 Hfr H (*srn*+) × YS142 F− *pyr*− *str*r *srnA*− (*srn*+) to select *pyr*+ *str*r recombinants.

Markers		Recombinants (No.)
srn	Sex	
−	M	16
−	F	0
+	M	15
+	F	19

Since the recipient strain YS142 was a recombinant after uninterrupted conjugation between strain YS105 Hfr H *srn*− and AB7N F− *srn*+ [table 5 in (*3*)], it was still possible that more than two mutant loci were involved in specifying the observed degradation of stable RNA. Another F− *srnA*− recipient therefore was constructed by crossing strain V64S Hfr H *srn*− (*2, 3*) with AT2535 F− *his*−*str*r*srn*+ (*3*) at 37°C for 80 minutes to permit selection of *his*+*str*r recombinants which contained *srnA*−; these were still phenotypically *srn*+. Typical was the strain YS31 F− *srnA*−: it showed complete stability of stable RNA, but the stable RNA broke down massively after an F factor was introduced from strain YS357 (Fig. 1d).

Proof that some feature of the F factor itself gives the *srn*− phenotype was obtained by introducing (or curing) various F factors in a YS31 F− *srnA*− (phenotypically *srn*+). When F *lac*+, F8, or KLF 12 (*7*) were introduced, all of the F′ recipients checked (4, 3, and 4 F ductants, respectively) showed massive degradation of stable RNA in the test condition (Fig. 1e). But when the newly isolated strains YS31/F *lac*+ were spontaneously cured of F *lac*+, the resultant derivatives showed no degradation of stable RNA (Fig. 1e). Therefore, the F factor is required for the *srn*− phenotype.

To show that only two loci are required for the *srn*− phenotype, I transferred both F factor and *srnA*− allele to an indifferent wild-type female starting strain, LC607 F− *proC*− *srnA*+ (phenotypically *srn*+) (*3*). First, the