

Thus, these changes may facilitate IMP mobility on the induction of cell fusion by Sendai virus, thereby making PE more prone to cell fusion. Whatever the explanation is, our study supports the concept that membranes of *Plasmodium*-infected erythrocytes are different from normal erythrocytes, and these differences can be detected by an increase in Sendai virus-induced cell fusion.

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Atypical base composition of foldback DNA¹

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Summary. Salmon, calf and human foldback DNAs all exhibited hypermethylation, reduced adenine plus thymine content and an excess of adenine over thymine when compared with their respective native DNAs. The only unusual feature of the base composition of wheat germ foldback DNA was the excess of thymine over adenine.

Foldback DNA forms a distinct class of repetitive sequences by virtue of its ability to renature instantaneously at low concentrations³. Apart from having perfect inverted repeat sequence arrangement of the type ABC...C'B'A'⁴, foldback DNA could contain interspersed single-stranded regions^{5,6}. Although foldback DNA occurs ubiquitously⁶⁻¹¹, its biological function remains unknown. Sequence analyses have shown that the recognition site for endonuclease R in *Haemophilus influenzae*¹² and the *lac* operon in *Escherichia coli*¹³ are made up of short inverted repeats of 6 and 24 nucleotide length respectively. Heterogeneous nuclear RNA of HeLa cells possesses regions that are self-complementary and are thought to have been transcribed from inverted repeat DNA^{14,15}.

While it is important to know the nucleotide sequence of specific foldback regions, it is equally important to study the base composition of foldback DNA isolated as a kinetic class. It is possible that the ease of renaturation might be associated with the preponderance of certain bases apart from the inverted repeat nucleotide arrangement. This point would be best clarified by comparing the base compositions of foldback DNAs obtained from a variety of organisms. To date, only rapidly reannealing DNA fractions from rodent tissues have been so analyzed. 5-Methylcytosine (5MC) enrichment was shown in these fractions from Ehrlich ascites carcinoma⁷ and mouse mastocytoma¹⁶, and an 8 moles percent of excess of A over T was observed⁷. The broader class of rapidly reannealing DNA in

Chinese hamster cells was also shown to be hypermethylated¹⁷. We report the analyses of the base composition of 4 eukaryotic foldback DNAs, all of which showed minor deviations from Watson-Crick base-pairing.

Materials and methods. All DNAs used were RNA-free. The preparation of foldback DNA from bovine and human DNAs employing nuclease S1-dioxane digestion and hydroxyapatite chromatography (60 °C) has been described¹⁰, and was applied without modification. The preparations of salmon and wheat foldback DNA were similarly obtained except that 40 °C was used as the temperature of nuclease S1 digestion. At least 3 foldback and 6 native preparations of each kind of DNA were analyzed in duplicate. 2–4 mg of native or foldback DNAs were hydrolyzed (70% HClO₄, 100 °C, 60 min) and neutralized with KOH.

The bases were separated by descending paper chromatography¹⁸ with the solvent system isopropanol:water:conc. HCl (170:36:44), and located under UV-light with reference to standards. Guanine was eluted with 1 M HCl, and the other bases with 0.1 M HCl, for quantitation with dual wavelength measurements¹⁹. In order to check for the possible contamination of 5MC fractions with C, eluates obtained from paper chromatograms were dried and reacted with 'Sil-Prep' (Applied Science Laboratories Inc., State College, Penn., USA). Gas chromatography modified from Razin and Sedat²⁰ was carried out on 1.5% OV-101 column (5' × 1/8") at 147 °C with nitrogen as the carrier gas.

Table 1. Temperature-absorbance characteristics of nuclease S1-resistant fractions as a probe for foldback DNA

Source of DNA	Native DNA T_m (°C)	Hyperchromic rise (%)	Enzymic digestion (°C)	Nuclease S1-resistant DNA* Yield (%)	T_m (°C)	Hyperchromic rise (%)
Salmon sperm	84.3	33.0	35	3.3	80.1	20.0
			38	2.8	79.3	32.5
			40	2.8	79.8	33.0
Wheat germ	87.2	35.0	38	4.0	79.7	30.0
			40	3.8	80.0	32.0
			42	2.8	80.5	31.5
Chicken blood	81.0	38.0	34	2.4	80.5	25.5
			38	2.4	81.1	28.0
			40	2.3	84.2	25.0

*Isolated from preparations renatured at $Cot \leq 10^{-3}$ moles sec l^{-1} according to procedures described¹⁰. Spectrophotometric measurements were made at 260 nm in 0.15 M NaCl-0.015 M sodium citrate, pH 7.4.

Table 2. Base composition of foldback and native DNA from cattle, man, salmon and wheat

Source of DNA	Nature of DNA	Mole percent of bases					(A + T) (G + C + 5MC)
		A	T	C	G	5MC*	
Calf thymus	Foldback	23.7	20.9	26.4	25.7	3.3 ± 0.1	0.82
	Native	27.6	27.7	21.4	21.5	1.8 ± 0.1	1.24
Human placenta	Foldback	26.0	24.1	24.6	24.0	1.3 ± 0.1	1.00
	Native	29.1	28.8	20.9	20.7	0.6 ± 0.01	1.37
Salmon sperm	Foldback	28.7	24.4	21.5	23.3	2.1 ± 0.1	1.13
	Native	27.3	27.5	21.6	21.9	1.7 ± 0.01	1.21
Wheat germ	Foldback	24.6	29.8	16.0	23.3	6.2 ± 0.1	1.19
	Native	26.4	26.8	18.1	22.6	6.0 ± 0.01	1.14

*Mean ± SD.

Contamination with C was less than 5% in all 5MC eluates.

Results and discussion. The working definition of foldback DNA was the fraction which renatured at Cot (the molar DNA concentration multiplied by the time allowed for reassociation in seconds) $\leq 10^{-3}$ moles sec l^{-1} in 0.2 M phosphate (Na^+ , 0.3 M), that exhibited a hyperchromic rise and T_m (temperature at which the midpoint of hyperchromic rise is attained) within 10% of the values obtained in the native DNA. At this low Cot -value, only those inverted repetitive sequences within a single strand should be able to foldback⁷ and contamination from fast-reannealing DNA duplexes arising from bimolecular reactions would be very unlikely. Salmon, wheat and chicken DNAs were renatured as specified, and subjected to nuclease S1-dioxane digestion at various temperatures. We examined the temperature-absorbance characteristics of the nuclease S1-resistant fractions in order to detect the presence of foldback sequences in these organisms (table 1).

Both salmon and wheat DNA contained foldback fractions which could be isolated following S1 digestion at temperatures of 38°C or slightly higher. At the temperature adopted for large-scale preparations of these 2 foldback DNAs (40°C), the S1-resistant fraction showed no absorbance rises below 65°C, and the melting curves obtained from 2 heating cycles were similar to those reported for human and bovine foldback DNAs¹⁰.

Nuclease S1-resistant DNA from chicken blood exhibited anomalous temperature-absorbance characteristics of low hyperchromicity with $T_m \geq$ that found in the native preparations, and was excluded in subsequent studies.

Table 2 summarizes the base composition of foldback and native DNAs isolated from 3 different animals and a plant. Many of the regularities which characterized native DNAs were absent or altered in the foldback fractions. In all 3 animal foldback DNAs, there was more A than T. The

molar proportions of 5MC were significantly higher than those found in the corresponding native DNAs ($p < 0.05$). Thymine content was lowered in every foldback DNA and G was increased. The overall effect of these differences was to reduce the (A + T):(G + C + 5MC) ratio, which were most pronounced in the bovine and human foldback fractions. These fractions failed to show the preponderance of AT base pairs which is characteristic of animal DNAs. Such unusual features of base composition were, however, not exhibited by wheat germ foldback DNA. The inequality of A and T was present, but T was in excess. 5MC was not significantly increased in foldback DNA and the (A + T):(G + C + 5MC) ratios in both foldback and native DNA preparations were similar. The GC content of both salmon and wheat foldback DNAs were found to resemble those obtained from the native preparations. This excluded the possibility that GC-rich fractions were preferentially selected out by the prescribed method. The ability to renature instantaneously would appear, then, to be independent of the overall base composition.

The 4 foldback DNAs had one point in common, the inequality $A \neq T$. This could not be attributed to the presence of mismatched sequences resulting from incomplete digestion with nuclease S1 as this has been estimated to be small¹⁰. Unconventional base-pairing²⁰ and single-stranded loops^{5,6} present in foldback DNA might have accounted for the observed inequalities. Alternatively, since some strands in the native DNA may be only half-methylated²² and methylation of cytosine in the 5-position results in greater stability of the GC pair²³, fully methylated foldback strands which renature faster than complementary sequences methylated to a lesser degree, might be preferentially selected in the foldback preparations leading to the base-pairing discrepancy. The question why there should be a selective excess of adenine in the 3 animal DNAs and of thymine in wheat DNA, however, remains to be investigated.

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Location of a lure by the drumming insect *Pimpla instigator* (Hymenoptera, Ichneumonidae)

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Summary. Females of the parasitoid *Pimpla instigator* (Hymenoptera, Ichneumonidae) are able to locate a lure hidden in a paper sheath. The lure is located without having been touched or sighted, and without any olfactory stimuli. We propose that the ability to locate the lure is linked with the drumming behavior of the females.

Most studies on host invasion by hymenopteran endoparasites have been concerned with its 1st step, host selection¹. The general conclusion is that olfactory, visual and tactile stimuli may all be involved in the process. Some additional information has been obtained by Carton²⁻⁵, working on *Pimpla instigator*, a large ichneumonid which parasitizes the pupae of Lepidoptera. *Pimpla* females were shown to be attracted by protruding objects, and they tend to perforate any cylindrical object with a diameter close to that of a pupa. Also, they can locate a *Pieris brassicae* pupa even when it is hidden behind sheet of paper.

Under these conditions, host selection is likely to have involved the interaction of several sensorial functions. We have attempted to evade this obstacle by replacing the pupal host by a substitute as neutral as possible. Cigarette filter-tips are actively perforated by *Pimpla* females (fig. 1): we tried to find out whether this lure could be located as

well as a pupa. (It should be noted that eggs were never laid into the filter.)

In a 1st experiment (fig. 2, A), the filter was placed in the middle of a sheath made of thin paper (32 g/m²). Most perforations were observed to be associated with the filter. The next series of tests (fig. 2, B) involved 2 filters, each of them at a distance of 4 cm from 1 extremity of the sheath. Most perforations were again found to be associated with the lures. So the females were able to locate the filter whatever its position was in the sheath.

In a 3rd experiment, females were presented with a sheath made of thick paper (80 g/m²) which had a filter in the middle (fig. 2, C). The perforations were now observed to be uniformly distributed along the sheath (the females utilized were checked both before and after these experiments, and found to be fully able to locate the lure when it was hidden behind thin paper).

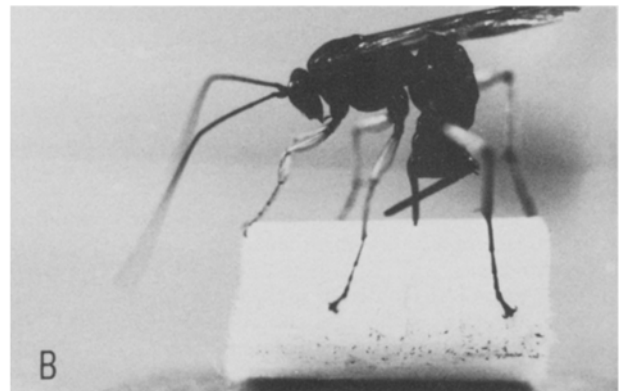
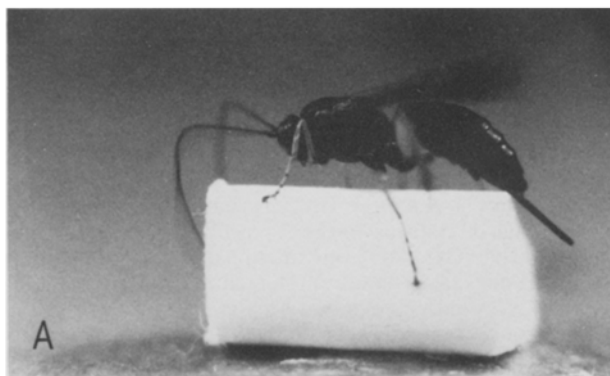


Figure 1. 2 behavioural elements of *Pimpla instigator*. A *Pimpla* female explores (A) and perforates (B) a cigarette filter.