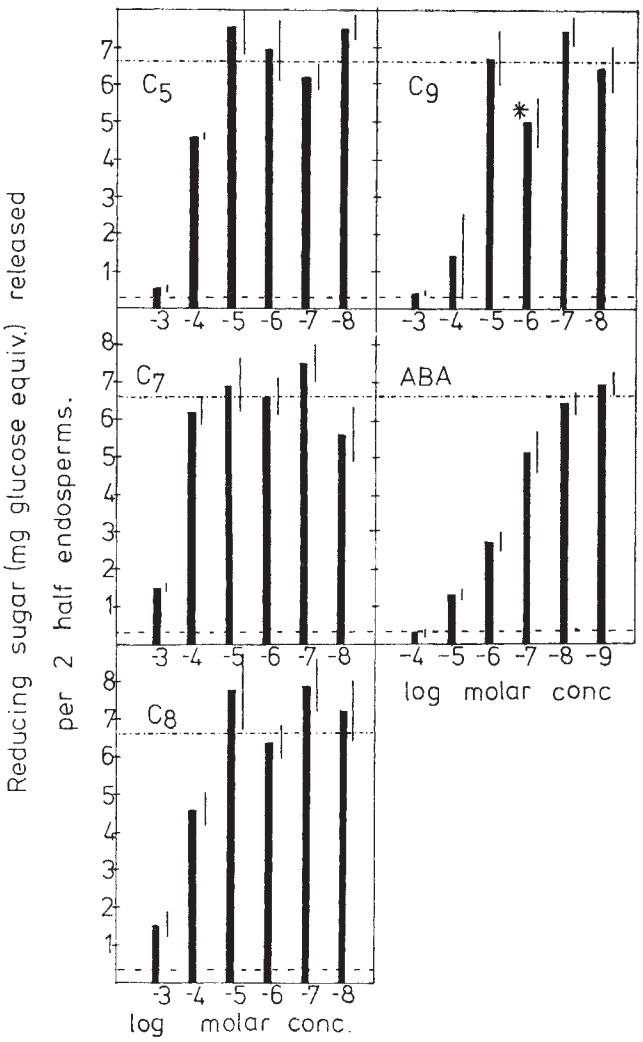

Fig. 2 Concentration dependence of fatty acid and ABA inhibition of GA-induced amylolysis in barley endosperm. Vertical bars represent reducing sugar released (see caption to Fig. 1) in presence of pentanoic (C_5), heptanoic (C_7), octanoic (C_8) and nonanoic (C_9) acids and ABA in the concentrations stated. All incubations were carried out at a constant GA concentration of 4.2×10^{-7} M, and *p*H 4.8. – – –, Mean blank value, that is, reducing sugar released in the absence of GA and inhibitors. · · · · · ·, Mean control value, that is, reducing, sugar released in the presence of GA and absence of inhibitor. *Not significantly different from control value at $P<0.10$.

acid levels and depth of dormancy in oat; aleurone layer activity in fenugreek seed; the mechanism of stomatal functioning, and leaf abscission and ethylene production. The *in vitro* inhibition of amylolysis described here represents yet another phenomenon which may have considerable physiological relevance.

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DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA

INFECTION of fibroblasts by avian sarcoma virus (ASV) leads to neoplastic transformation of the host cell. Genetic analyses have implicated specific viral genes in the transforming process[1–4], and recent results suggest that a single viral gene is responsible[4]. Normal chicken cells contain DNA homologous to part of the ASV genome[5–8]; moreover, embryonic fibroblasts from certain strains of chickens can produce low titres of infectious type C viruses either spontaneously[9] or in response to various inducing agents[10]. None of the viruses obtained from normal chicken cells, however, can transform fibroblasts, and results with molecular hybridisation indicate that the nucleotide sequences responsible for transformation by ASV are not part of the genetic complement of the normal cell[11]. We demonstrate here that the DNA of normal chicken cells contains nucleotide sequences closely related to at least a portion of the transforming gene(s) of ASV; in addition, we have found that similar sequences are widely distributed among DNA of avian species and that they have diverged roughly according to phylogenetic distances among the species. Our data are relevant to current hypotheses of the origin of the genomes of RNA tumour viruses[12] and the potential role of these genomes in oncogenesis[13].

We have prepared radioactive DNA ($cDNA_{sarc}$) complementary to nucleotide sequences which represent most or all of the viral gene(s) required for transformation of fibroblasts by ASV[14]. Our procedure to isolate $cDNA_{sarc}$ exploited the existence of deletion mutants of ASV which lack 10–20% of the viral genome (transformation defective, or td viruses)[11,15–17]; results of genetic analyses indicate that the deleted nucleotide sequences include part or all of the gene(s) responsible for oncogenesis and cellular transformation[4,14]. In our procedure the genome of the Prague-C strain (Pr-C) of ASV was transcribed into complementary DNA by endogenous RNA-directed DNA polymerase activity; we then used molecular hybridisation to select DNA specific for the region missing from the genome of the td deletion mutants. The preparation of $cDNA_{sarc}$ used was a virtually uniform transcript from about 16% of the Pr-C ASV genome[14], a region equivalent in size to the entire deletion in the strain of td virus used in our experiments[11,15–17]. Since the unit genome of ASV contains about 10,000 nucleotides[18,19], the genetic complexity of $cDNA_{sarc}$ is about 1,600 nucleotides, sufficient to represent an entire cistron. Nucleotide sequences homologous to $cDNA_{sarc}$ seem to be ubiquitous in the genomes of ASVs, but are not present in the genomes of avian leukosis viruses (including the endogenous chicken virus, RAV-0) or sarcoma-leukosis viruses from other species[14].

DNAs from several avian species (chicken, turkey, quail, duck and emu) contain nucleotide sequences which can anneal with $cDNA_{sarc}$ (Fig. 1 and Table 1). In contrast, we detected no homology between $cDNA_{sarc}$ and DNA from mammals (mouse and calf thymus; Table 1). The kinetics of the reactions between $cDNA_{sarc}$ and DNAs from chicken, quail and duck (Fig. 1*a*, *c* and *d*) were similar to the kinetics for the reassociation of unique nucleotide sequences in avian DNAs (C_0t about 1,000 mol s^{-1})[20]; thus, nucleotide sequences homologous to $cDNA_{sarc}$ are present as single (or a few) copies in each haploid complement of the avian DNAs tested. This conclusion was substantiated by measuring in a single reaction

Table 1 Homology between $cDNA_{sarc}$ and normal DNAs

Assay	Hybridisation conditions		Extent of reaction between $cDNA_{sarc}$ and DNA from						
	$[Na^+]$	Temperature	Chicken	Quail	Turkey	Duck	Emu	Mouse	Calf
S1	0.9 M	68°	52%	46%	48%	45%	24%	<2%	<2%
HAP	0.9 M	68°					36%		<5%
HAP	1.5 M	59°					54%		

DNA was extracted from 10–11-d-old embryos of chickens, ducks and quails, 3-d-old mice (strain RIII), livers of adult turkeys, liver and heart of a 22-d-old emu, and calf thymus. Reaction mixtures contining denatured DNA (8 mg ml^{-1}) and ^3H-$cDNA_{sarc}$ (0.32 ng ml^{-1}, 7,000 c.p.m. ml^{-1}) in a final volume of 0.3 ml were incubated at either 59 or 68 °C for 48 h. Samples incubated at 59 °C were in 1.5 M NaCl (final C_0t = 40,000), those incubated at 68 °C were in 0.9 M NaCl (final C_0t = 32,000); all reactions also continued 0.001 M EDTA–0.02 M Tris-HCl, *p*H 7.4. Duplex formation was measured by either hydrolysis with S1 nuclease[28] (in 0.3 M NaCl at 50 °C) or fractionation on hydroxyapatite (HAP) (samples adsorbed in 0.14 M sodium phosphate at 50 °C).

mixture the rates of reassociation between chicken DNA and both $cDNA_{sarc}$ (labelled with ^3H) and unique nucleotide sequences purified from chicken DNA (labelled with ^{14}C) (Fig. 1*b*); the rates were similar for both labelled DNAs.

The extent of duplex formation with $cDNA_{sarc}$ varied with the amount of chicken DNA used in the reactions

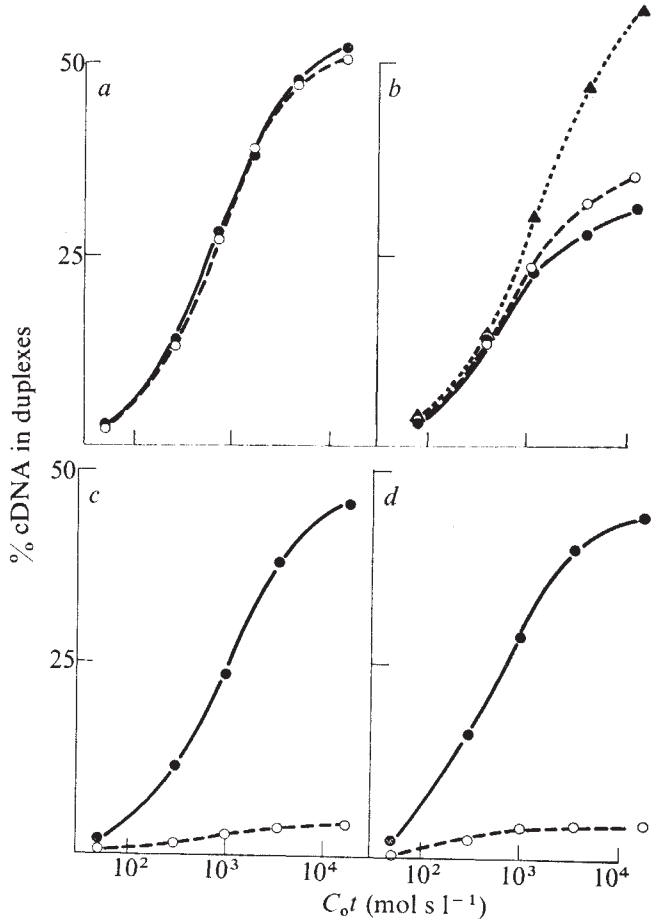

Fig. 1 Annealing of $cDNA_{sarc}$ to normal avian DNAs. DNA was prepared from 10–11-d-old embryos[7], denatured and incubated with ^3H-$cDNA_{sarc}$ and ^{32}P-$cDNA_{B77}$ in 0.6 M NaCl–0.001 EDTA–0.02 M Tris-HCl, *p*H 7.4 at 68 °C for 0.1 to 32 h. Samples (0.1–0.2 ml) were withdrawn at different times and the extent of duplex formation with $cDNA_{sarc}$ and $cDNA_{B77}$ was measured by hydrolysis with the single-strand specific nuclease, S1 (ref. 28). The preparation of $cDNA_{sarc}$ and $cDNA_{B77}$ has been described elsewhere[14] and is outlined in the text. Unique sequence chicken DNA was prepared by reannealing denatured ^{14}C-chicken DNA to a C_0t of 500; unique sequence DNA was eluted from a hydroxyapatite column at 60 °C in 0.14 M phosphate buffer[20]. *a*, Chicken DNA (9 mg ml^{-1}) with ^3H-$cDNA_{sarc}$ (0.5 ng ml^{-1}, 10,000 c.p.m. ml^{-1}) (●) and ^{32}P-$cDNA_{B77}$ (0.3 ng ml^{-1}, 10,000 c.p.m. ml^{-1}) (○). *b*, Chicken DNA (5 mg ml^{-1}) with ^3H-$cDNA_{sarc}$ (0.45 ng ml^{-1}, 9,000 cpm ml^{-1}) (●); ^{32}P-$cDNA_{B77}$ (0.5 ng ml^{-1}, 6,000 c.p.m. ml^{-1}) (○), and ^{14}C-chicken unique sequence DNA (▲). *c*, Quail DNA (9 mg ml^{-1}) with ^3H-$cDNA_{sarc}$ and ^{32}P-$cDNA_{B77}$ as in *a*. *d*, Duck DNA (9 mg ml^{-1}) with ^3H-$cDNA_{sarc}$ and ^{32}P-$cDNA_{B77}$ as in *a*.

(Fig. 1*a* and *b*), as expected in reactions where identical labelled and unlabelled DNA strands are competing for unlabelled complementary strands, and the reactions were incomplete (about 50%) at the highest value of C_0t (Fig. 1*a*, *c* and *d* and Table 1). Nevertheless, we believe that most or all of the nucleotide sequences of $cDNA_{sarc}$ are present in the DNA of quail since $cDNA_{sarc}$ anneals completely with RNA from certain quail cells (unpublished results of ourselves and C. Moscovici). Moreover, the rates and extents of annealing between $cDNA_{sarc}$ and DNA from chicken, duck and turkey are approximately the same as the rate and extent of annealing between $cDNA_{sarc}$ and quail DNA (Table 1 and Fig. 1). Thus, it is likely that most or all of the nucleotide sequences of $cDNA_{sarc}$ are present in the DNA of all these birds.

The extent of the reaction between $cDNA_{sarc}$ and DNA from emu, a relatively primitive Australian bird, was limited (24%) when tested by hydrolysis with a single strand-specific nuclease (Table 1); the extent of the reaction was greater when analysed on hydroxyapatite, a less stringent procedure than the nuclease test (Table 1), and was further augmented when the annealings were performed in conditions which facilitate pairing of partially matched nucleotide sequences (1.5 M NaCl, 59 °C, ref. 21) (Table 1). These date indicate that the nucleotide sequences homologous to $cDNA_{sarc}$ in emu DNA are substantially diverged from the homologous sequences in the other avian DNAs; we have obtained further data to sustain this conclusion by analysing the thermal stability of the duplexes formed between $cDNA_{sarc}$ and various avian DNAs (see below, Table 2 and Fig. 2).

Avian DNAs were also tested with ^{32}P-labelled single-stranded DNA, complementary to the RNA genome of the B77 strain of ASV ($cDNA_{B77}$), synthesised with detergent-disrupted virions, and purified as described previously[14]. The virus used to prepare $cDNA_{B77}$ consisted mainly of td variants (about 90% of the particles; unpublished observations). Consequently, DNA synthesised with the virus was deficient in nucleotide sequences homologous to $cDNA_{sarc}$ and served principally to detect other portions of the ASV genome. A substantial fraction (about 50%) of $cDNA_{B77}$ reacted with normal chicken DNA, but there was little or no reaction with quail, duck, turkey and emu DNAs (Fig. 1 and unpublished data); these results conform to previous reports[20,22,23]. We conclude that the DNAs from widely divergent avian species all contain nucleotide sequences which are at least partially related to transforming gene(s) of ASV, whereas only chicken DNA has appreciable homology with the remainder of the ASV genome.

The relatedness of DNA sequences homologous to $cDNA_{sarc}$ in different avian species was analysed by denaturing duplexes formed between $cDNA_{sarc}$ and normal avian DNAs (Fig. 2 and Table 2). In addition, we denatured duplexes between $cDNA_{sarc}$ and DNA from XC cells (rat cells transformed by Pr-C ASV) to test completely matched duplexes containing $cDNA_{sarc}$ sequences. The mammalian DNAs we have tested (calf and mouse) contain no nucleotide sequences homologous to $cDNA_{sarc}$ before in-

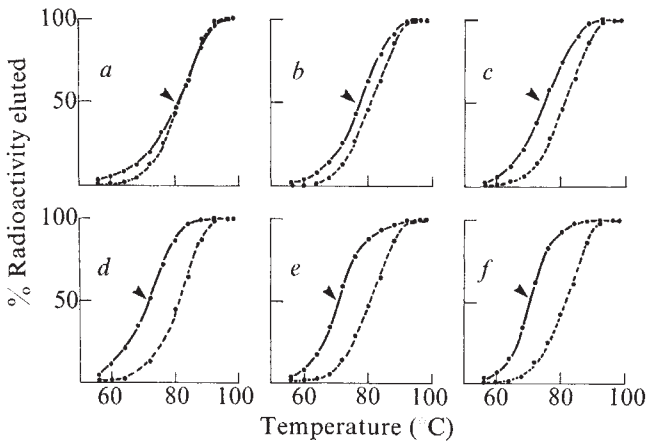

Fig. 2 Thermal denaturation of duplexes formed between $cDNA_{sarc}$ and cellular DNAs. DNA was extracted from XC cells grown in culture, from 10–11-d-old embryos of chickens, ducks and quails, from the livers of adult turkeys, and from liver and heart of a 22-d-old emu. Denatured DNAs (0.5 mg) were incubated with ^{3}H-$cDNA_{sarc}$ (0.25 ng, 5,000 c.p.m) in 0.1 ml of 0.9 M NaCl–0.001 M EDTA–0.02 M Tris-HCl, *p*H 7.4, for 100 h at 68 °C, final $C_0t = 40{,}000$. In a separate reaction, ^{32}P-$cDNA_{B77}$ (2.5 ng, 100,000 c.p.m.) was annealed with 7 mg of denatured chicken DNA (final $C_0t = 80{,}000$). Samples (0.4 mg of chicken DNA and 6,000 c.p.m. of $cDNA_{B77}$) of this reaction were added to the DNAs which had been annealed with $cDNA_{sarc}$. The mixtures were then passed through a column of hydroxyapatite (3 ml packed vol) in 0.12 M sodium phosphate, *p*H 6.8, at 56 °C; in these conditions, stable duplexes bind to the hydroxyapatite, whereas single-stranded DNA does not. The columns were then washed continuously with 0.12 M sodium phosphate (0.4 ml min^{-1}) while the temperature of the column was raised in increments of 4 °C every 10 min. Fractions (4 ml) were collected and analysed for acid-precipitable radioactivity. ●——●, Duplexes with $cDNA_{sarc}$; ●– – –●, duplexes with $cDNA_{B77}$ and chicken DNA (internal standard). *a*, Provirus (XC); *b*, chicken; *c*, quail; *d*, turkey; *e*, duck; *f*, emu.

fection by ASV; consequently, the DNA homologous to $cDNA_{sarc}$ in rat cells after infection presumably represents nucleotide sequences contained entirely in recently integrated provirus for ASV rather than related sequences present in the DNA of uninfected cells. Denaturation was standardised internally by adding duplexes formed in a separate annealing reaction between normal chicken DNA and ^{32}P-labelled $cDNA_{B77}$; these duplexes denatured with a T_m of 81 ± 1 °C.

Duplexes between $cDNA_{sarc}$ and DNA from XC cells had the highest T_m (81 °C; Table 2), as expected for completely matched complementary nucleotide sequences. Duplexes with chicken DNA were slightly less stable (T_m=77 °C); the nucleotide sequences of $cDNA_{sarc}$ in the ASV genome are apparently diverged from the homologous sequences in the avian host. The reduction in the T_m is consistent with about 3% mismatching of bases[24]. The stability of duplexes with other avian DNAs (T_m=70–74 °C) decreased roughly in accord with phylogenetic distances among the species tested (Table 2); the T_ms suggest 5–8% mismatching of base pairs[24]. We made no effort in these tests to obtain maximum duplex formation with $cDNA_{sarc}$, and the reactions with quail, turkey, duck and emu were relatively limited in extent (15–37%). In other experiments where at least 50% of $cDNA_{sarc}$ was annealed into duplexes with chicken, quail and duck DNAs, we observed T_ms similar to those given in Table 2. $cDNA_{sarc}$ seems to represent nucleotide sequences which arose and diverged during the course of avian speciation.

We have shown previously that the nucleotide sequences of $cDNA_{sarc}$ include genetic information required for transformation of fibroblasts by ASV[14]; the data reported here indicate that similar or partially related information is present in the genome of the normal chicken cell and widely distributed among the avian species. We suggest that part or all of the transforming gene(s) of ASV was derived from the chicken genome or a species closely related to chicken, either by a process akin to transduction[25] or by other events, including recombination, which are alleged to have generated type C viruses from normal cellular genes[12]. If the entire ASV genome originated from cellular genes[12], then nucleotide sequences in the transforming gene(s) have been conserved relative to other viral genes, because the nucleotide sequences of $cDNA_{sarc}$ are the only portion of the ASV genome which we can detect in DNA from birds other than chickens. We cannot exclude, however, the possibility that viral genes other than those represented by $cDNA_{sarc}$ have been introduced into the germ line of chickens after speciation. The sequences homologous to $cDNA_{sarc}$ in the genome of ASV are slightly diverged from the analogous sequences in chicken genome; this could be the consequence of either the process which generated viral genes from cellular genes[12] or mutations during the course of repeated viral propagation.

Others have reported evidence concerning the genetic divergence during possible spread of unidentified portions of type C viral genomes during evolution[26,27] and the apparent transduction of cellular nucleotide sequences by type C viruses[25], but our study provides the first data on the origins of a genetically identified set of viral nucleotide sequences. It should be possible to carry out similar studies for at least one other gene of ASV (the gene coding for the type-specific glycoprotein), using techniques similar to those reported here.

Neiman and his colleagues have reported that the hybridisation of ASV 70S RNA to normal chicken DNA was competed completely by RNA from non-transforming viruses[11]. They therefore concluded that the genes responsible for transformation by ASV were present in the avian genome only after viral infection. We cannot explain the discrepancy between their results and ours.

We anticipate that cellular DNA homologous to $cDNA_{sarc}$ serves some function which accounts for its conservation during avian speciation. The nucleotide sequences which anneal with $cDNA_{sarc}$ are part of the

Table 2 Thermal stabilities of duplexes between $cDNA_{sarc}$ and normal DNAs

DNA	%$cDNA_{sarc}$ in duplexes	T_m	ΔT_m	Phylogenetic distance from chicken (Myr)
Provirus (XC)	56	81	0	
Chicken	52	77	− 4	0
Quail	37	74	− 7	35–40
Turkey	30	72	− 9	40
Duck	16	71	−10	80
Emu	15	70	−11	100

Denatured DNAs (0.5 mg) were annealed with ^{3}H-$cDNA_{sarc}$ (0.25 ng, 5,000 c.p.m.), adsorbed to hydroxyapatite in 0.12 M sodium phosphate at 56 °C, and denatured with a thermal gradient ,all as described for Fig. 2. Duplexes between ^{32}P-$cDNA_{B77}$ and normal chicken DNA, included in each analysis as an internal standard, denatured with T_m = 8 1 ± 1 °C. The estimates of phylogenetic distance, deduced from fossil records and antigenic relationships among proteins[29], were provided by Professor Allan Wilson (personal communication).

unique fraction of cellular DNA and could represent either structural or regulatory genes. But the function of those sequences is unknown. We are testing the possibilities that they are involved in the normal regulation of cell growth and development or in the transformation of cell behaviour by physical, chemical or viral agents.

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Raman spectra of transfer RNAs with ultraviolet lasers

We suggest here a new use for Raman spectroscopy in structural studies of nucleic acids. We demonstrate that an enhanced vibrational Raman spectrum of a certain limited portion of an amino acid transfer RNA (tRNA) macromolecule can be observed, if a proper ultraviolet laser beam is chosen for excitation.

Until now, Raman spectra of tRNAs have been observed with laser beams of visible light[1–6]. They all show a number of Raman lines characteristic of the four common bases, namely, adenine, uracil, guanine, and cytosine. For such observations concentrated solutions (usually about 5 %) or considerable amounts (10 mg) of tRNA are required. Some of the minor constituent nucleosides in tRNAs have strong absorption bands in the 300–350-nm region, whereas the common bases have them at 260 nm. Using the correct ultraviolet laser, therefore, one would expect to observe resonance Raman effects of minor nucleoside bases. Such effects would be valuable, first, because they would provide information on one particular base residue of the 70–80 bases in tRNA and second, because this could be done with much smaller amount samples of tRNA this usual.

We have observed a Raman spectrum of formylmethionine tRNA of *Escherichia coli* with the 363.8-nm beam of an argon ion laser (Coherent Radiation 52 GA). The power of the laser beam was 5 mW at the sample. The tRNA concentration of the sample solution was only 0.3 %, in cacodylate buffer, *p*H 7.2. The total volume of the sample solution used was 0.2 ml, contained in a cylindrical cell of silica glass. A JRS-U1 Raman spectrophotometer (Japan Electron Optics) was used. The recorded curve is shown in Fig. 1*a*. The curve can be reproduced more than ten times from one sample solution. The aminoacylation activity of the tRNA remained undegraded after 5 h irradiation with the ultraviolet laser beam. When the wavelength of the laser beam was changed from 363.8 to 351.1 nm, almost the same Raman spectrum was observed.

All of the observed Raman lines are attributable to the 4-thiouridine residue (at position 8) subjected to photochemical modification (probably cross linking[7,8] with the cytidine residue at position 13 of the tRNA). This can be concluded because, first, at that concentration and with that laser power, on the basis of the available data, none of the Raman lines of the common base residues can be expected to appear. Moreover, none of the

Fig. 1 Raman spectra excited by the 3,638 Å beam of an argon ion laser. *a*, $tRNA^{fMet}$ from *E. coli;* concentration, 0.3 %; solvent, 10^{-1} M NaCl + 10^{-2} M Na-cacodylate (*p*H 7.2) + 10^{-3} M $MgCl_2$. *b*, $tRNA^{Gly}$ from *E. coli;* concentration, 0.3 %; solvent as in *a*.