type positive for probes from interval 1A2. The rarity of the postulated unequal recombination and the infertility of X^-Y females make it unlikely that XY gonadal dysgenesis and XX males (or hermaphrodites) would occur together among related individuals. This may be a reason why such families are not known.

X^-X females might not transmit the X^- chromosome because they, like X^-Y females, might be infertile.

Others have suggested that XX hermaphrodites and XX males might arise due to a shifting of *Tdf* to a region of the X that escapes inactivation[1,8]. The suggestion has also been made that dosage effects of the kind discussed here and earlier[3,4,9,10], might involve an entire 'gonad differentiation locus'[8].

Regulatory sequences controlling the activity of *Tdf* are likely to be species-specific, and unlikely to be found among the highly conserved sequences in interval 1A2. This argument is based on a point of view developed by Mayr[11], and supported among others by recent work on sex chromosome-linked genes in butterflies[12], that at the heart of species identity are species-specific differences in sex chromosome-linked regulatory genes concerned with sex determination and reproduction. If this view is correct, it may complicate attempts to test the function of structural genes in interval 1A2 in transgenic mice.

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ALTERED NUCLEASE AND PROTEASE ACTIVITY IN CYANOPHAGE LPP-1 INFECTED CYANOBACTERIA *PHORMIDIUM UNCINATUM* AND *PLECTONEMA BORYANUM*

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THE interaction of cyanophage with its host is modulated by various factors including enzymes of metabolic pathways, especially the nucleases and proteases. Cyanophage infection is supposed to be dependent upon the host cellular components[1-3]. Cyanobacteria have very low level of nucleic acid degrading enzymes[4] which increases drastically after phage infection until complete lysis occurs[5]. An increase in the DNase activity after cyanophage infection probably plays a key role in the breakdown of the host DNA and the resulting products are quickly reincorporated into newly synthesized phage DNA[6,7]. Similarly, it has been shown earlier that host protein synthesis ceases immediately after infection with cyanophage followed by an enhanced synthesis of phage protein[6,7]. Cyanophage infection may also affect the proteolytic activity of the host to meet the demand for amino acids from the pre-formed sources. However, such studies are confined to only *Escherichia coli*-T phage system[8]. The present study was aimed at determining the eventual changes in the activity of nucleases and proteases in cyanophage LPP-1 infected non-heterocystous cyanobacteria *P. uncinatum* and *P. boryanum* under light and dark conditions.

Pure cultures of *P. uncinatum* (CU 1462/7) and *P. boryanum* (IU 590) and cyanophage LPP-1 were obtained through the courtesy of Dr R. S. Safferman, National Environmental Protection Agency, Cincinnati, Ohio, USA. Cultures were maintained in modified Chu 10 medium containing trace elements as described earlier[2,3] at $26 \pm 2°C$ in an illuminated culture room fitted with fluorescent light at an intensity of 480 $ergs/cm^2/s$. Lysate of LPP-1 cyanophage was obtained by infecting batch cultures of the cyanobacteria (6.5×10^6 cells/ml) with the

Table 1 *Changes in DNase, RNase and protease activities in P. uncinatum and in P. boryanum after cyanophage LPP-1 infection (m.o.i = 5)*

	Time (h)											
	0				12				24			
	Healthy		Infected		Healthy		Infected		Healthy		Infected	
Enzymes	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Phormidium uncinatum												
DNase	0.333	ND	0.333	ND	0.686	0.642	2.0	1.87	0.866	0.821	3.40	1.50
RNase	7.33	5.10	7.33	5.10	8.64	8.11	32.66	26.66	8.79	8.22	33.64	42.05
Protease	7.20	4.10	7.20	4.10	7.20	4.10	6.20	3.60	7.58	4.30	9.20	6.20
Plectonema boryanum												
DNase	0.321	ND	0.321	ND	0.664	0.622	1.991	0.82	0.768	0.741	3.10	0.59
RNase	6.83	4.90	6.83	4.90	8.11	5.20	30.52	19.24	8.64	5.80	31.61	20.66
Protease	7.00	3.20	7.00	3.20	7.00	3.20	5.90	2.40	7.57	4.41	8.90	4.20

* Cells were preincubated for 24 h in dark before infection; ND, Not detectable; An average of three independent readings was taken into consideration.

phage at a multiplicity of infection (m.o.i) of 0.1. After lysis, cell debrises were removed by centrifugation at 4°C. Plaque assay technique[9,10] was performed to estimate the viral concentration. Lysates were always filtered through sintered glass membrane filter (2.5 cm GF/C Whatman, England) before being used in the experiment. Nuclease and protease activities were measured both in light and dark under phage infected and healthy cultures of the two cyanobacterial hosts at different time intervals. DNase and RNase activity was measured[4] in terms of absorbance at 260 nm (Bausch and Lomb 7100 spectrophotometer), using sodium salt of DNA and RNA as substrates respectively. The enzyme-less distilled water served as control during the experiments. Protease activity was estimated by the method described elsewhere[11]. The specific enzyme activity is defined as product/mg protein/min. Protein was estimated using Folin phenol reagent[12]. The result shown in table 1 is an average of three independent readings.

The biochemical processes of cyanobacteria associated with replication of the specific cyanophage vary from those occurring in heterotrophic bacteria after bacteriophage infection[1-8]. It has been shown that the bacterial DNA serves as the major carbon source for the T-phage DNA whereas the carbon source of the cyanophage DNA is the product of photosynthesis [1,6,7]. The present findings reveal that there is an alteration in the enzyme activity of the DNA, RNA and protein metabolism (table 1) after cyanophage infection. This suggests that the proteolytic enzyme activity in *P. uncinatum* and *P. boryanum* gets accelerated following cyanophage LPP-1 infection in light as well as in dark incubated cultures. Further steps pertaining to the actual mechanism of the increase in proteolytic enzyme activity along with the role of these changes in host phage interaction still remain to be explored. As far as dehydrogenase activity is concerned, it is clear that oxido-reductive processes are responsible for the increase in the enzyme activity without the involvement of protein synthesis. The oxidative pentose phosphate pathway might have got activated after phage infection and served to synthesize the ribose residues required for the synthesis of phage DNA. However, the activation mechanism of protease remains to be elucidated.

On the basis of these findings, it is suggested that the increase in the enzyme activity in phage-infected cyanobacteria occurs in relation to the breakdown of host products to be utilized as building blocks for the synthesis of phage proteins and the proteolytic modification of the precursors of phage head proteins as already described for T-phages[13,14].

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STUDIES ON THE STEM EXUDATE OF *AZADIRACHTA INDICA* LINN.

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THE stem exudate of *Azadirachta indica* Linn. (Neem), a rare exudation of stem of older trees, has been reported to be a nutritive and an alternative tonic[1,2] and found useful in the treatment of atonic dyspepsia, general debility, chronic leprosy and skin diseases[1]. The present work was undertaken to study the chemical composition of the exudate and to ascertain the presence if any of active medicinal principle in it.

The neem exudate, collected from an old tree situated at Dasna, Ghaziabad had a b.p. 97°, $[\alpha]_D^{32}+46.87°$, pH of 3.5, sp. gr. of 0.997, moisture content of 92% and an ash content of 1.6% of the dry sap.

Free amino acids of concentrated exudate were determined by one-dimensional paper chromatography in n-BuOH-AcOH-H_2O (4:1:1) solvent system. The chromatograms, loaded with 25 μl of the extract and reference samples, were developed by spraying with ninhydrin and heating at 100°C for 5 min. The individual amino acids were eluted separately with 50% aqueous acetone and their optical densities were measured at 560 nm. In all, twelve amino acids were present in the exudate. The concentrations of γ-aminobutyric acid and glycine were the highest while arginine, glutamine, lysine and threonine were low (table 1).

The crude protein content (N × 6.25) (3.57 g/100 ml) of the exudate was determined by micro Kjeldhal's method as recommended by AOAC[3]. The protein, separated by adding ethanol (95%) to the concentrated exudate (50 ml), was washed several times with ethanol to remove the unbound amino acids and then hydrolysed with 20 ml of 6 N HCl in a sealed tube at 110°C for 24 h. The acid was removed in a rotatory film evaporator. The hydrolysed material was subjected to paper chromatography for analysing the bound amino acids. In all 14 amino acids were detected among which alanine, glycine and tyrosine were present in high concentrations (table 1).

The sugars glucose, fructose, mannose and xylose were detected by applying concentrated exudate on a paper chromatogram and developing

Table 1 *Free and protein bound amino acids of Azadirachta indica Linn. stem exudate*

Amino acids	Free amino acids (mg/l)	Protein amino acids (mg/100 g)
Alanine	328	486
γ-aminobutyric acid	486	–
Arginine	90	196
Asparagine	136	–
Aspartic acid	280	118
Cysteine	164	212
Glutamic acid	–	316
Glutamine	116	328
Glycine	542	416
Lysine	124	346
Methionine	–	142
Norleucine	–	294
Norvaline	254	112
Ornithine	–	248
Proline	266	–
Threonine	120	264
Tyrosine	–	432