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Protozoan Genes: As Viewed from Five Tetrahymena Genes

Yoshio Watanabe §, Masafumi Hirono, Tohru Takemasa and Osamu Numata
Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Introduction

Within the past few years, we hve succeeded in the cloning and squencing of *Tetrahymena* genes for actin (Hirono et al., 1987a), calmodulin (Takemasa, T., Takagi, T. and Watanabe Y., manuscript in preparation), two kinds of calmodulin family proteins which were designated as TCBP-25 (Takemasa et al., 1989) and TCBP-23 (Takemasa et al., 1990), and for a 14-nm filament forming protein (Numata, O., Takemasa, T., Takagi, I., Hirono, M., Chiba, J. and Watanabe, Y., manuscript in preparation). On the basis of the sequencing data obtained, here we intend to show some comprehensive features of protozoan genes.

Among them, genes for actin and calmodulin are ubiquitous in eukaryotes, so that their sequencing data will provide important clues for understanding the genealogical situation of protozoa among eukaryotes. The other genes for proteins specific to *Tetrahymena* are expected to provide information about the features and characteristics of ciliated protozoan genes.

In this review article, we will stress that although some properties of protozoan genes are common to, many other properties are diverged greatly from those of other eukaryotic genes. The latter properties are most important not only for understanding the genealogical status of protozoa, but also for elucidating the relationships between the structural and functional domains of multifunctional proteins, such as actin and calmodulin.

Protozoan Actin Genes

Actin is among the most biologically important proteins and is known to be ubiquitous, multifunctional and highly conserved in eukaryotic cells. The conservation of primary structure of actin held true for protozoan actins until very recently, that is, rhizopod actins, such as *Acanthamoeba* actin (Weihing and Korn, 1971; Vandekerckhove, et al., 1984; Nellen and Gallwits, 1982) and *Entamoeba* actin (Edman et al., 1987; Meza et al., 1983) which were isolated first shared about 90% identities with skeletal muscle actin and possessed many of the biochemical propeties common to those of eukaryotic actins known so far. However, it has now become clear that protozoan actins other than rhizopod actins diverge to a great extent from those of other eukaryotic actins (Amor et al., 1988; Cupples and Pearlman, 1986; Greslin et al., 1988; Hirono et al., 1987a; Wesseling et al., 1988).

Figure 1 shows the comparison of amino acid sequences of seven kinds of protozoan actins with those of other representative actins, such as actins from skeletal muscle, cytoplasmic β, sea urchin, *Physarum*, *Dictyostelium*, yeast and soybean. Among the seven kinds of protozoan actins, rhizopod actins (*Acanthamoeba* and *Entamoeba* actins) relatively conserve their primary structures. However, sporozoan *Plasmodium*, flagellate *Trypanosoma*, and ciliate *Tetrahymena* and *Oxytricha* share only 65-80% identity with rabbit skeletal muscle actin. Interestingly, similar low identity values are seen among these protozoan actins themselves (Table 1). In these actins, divergencies are seen not only in the so called "variable" regions (for example, the 6 N-terminal residues), but also in the "constant" regions. Especially, residues 39-60, residues 187-206, residues 221-239, residues 246-253, residues 263-284, and residues 307-320 tend to be diverged greatly (the 6 shaded regions shown in Fig. 1). Within these regions, diverged amino acids are not always conserved.

These data indicate that, though unicellular organisms with no chlorophyll have been proposed to be classified into the single phylum protozoa from traditional phylogenetic studies, the phylum includes various kinds of unicellular organisms which have evolved independently and diversely for a long period.

§ To whom all correspondence should be addressed

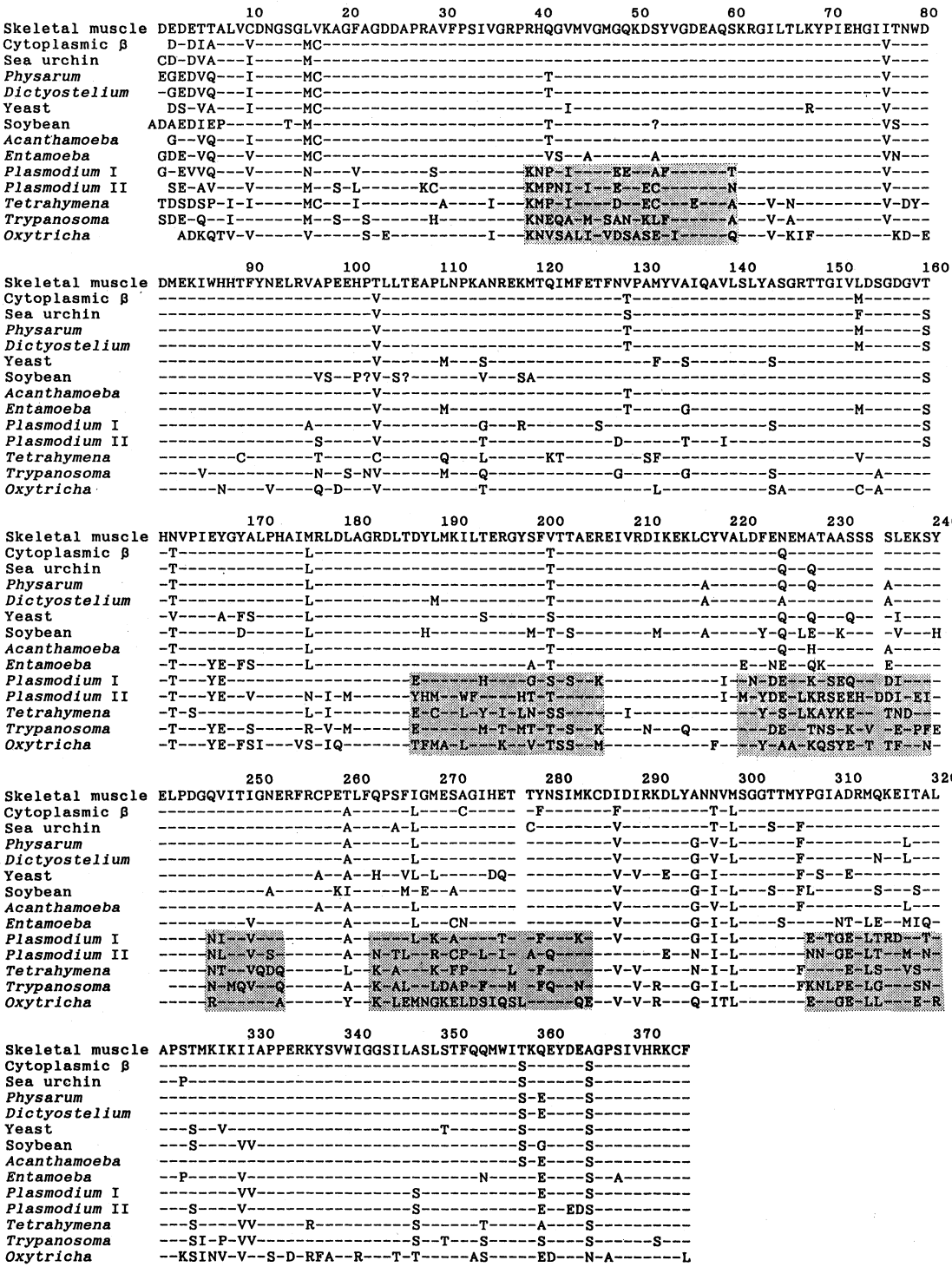

Figure 1. Comparison between actin amino acid sequences of rabbit skeletal muscle actin (Collins and Elzinga, 1975), bovine cytoplasmic β actin (Vandekerckhove and Weber, 1979), sea urchin actin (Cooper and Crain, 1982), *Physarum* actin (Vandekerchhove and Weber, 1978), *Dictyostelium* actin (Vandekerckhove and Weber,

1980; Romans et al., 1985), yeast actin (Gallwits and Sures, 1980; Ng and Abelson, 1980), soybean actin (Shah et al., 1982), *Acanthamoeba* actin (Nellen and Gallwits, 1982; Vandekerckhove et al., 1984), *Entamoeba* actin (Edman et al., 1987), *Plasmodium* actin (Wesseling et al., 1988), *Tetrahymena* actin (Hirono et al., 1987a), *Trypanosoma* actin (Amor et al., 1988) and *Oxytricha* actin (Greslin et al., 1988). Amino acid residues conserved with respect to those of skeletal muscle actin are represented by hyphens. Variable regions common to protozoan actins except for rhizopod actins are shaded.

Actins from the amoeba group are very similar to those from *Physarum* and *Dictyostelium* (Fig. 1 and Table 1) and are known to have various properties common to actins from higher organisms, implying that an ancestor of higher organisms might have evolved via an amoeba-type organisms. As stated before, actin is highly conserved in its primary structure. In fact, the amino acid sequences of actins from human skeletal muscle and chicken breast muscle are exactly identical. However, in protozoa, even within the same genus *Tetrahymena*, single type actins from *T. thermophila* and *T. pyriformis* differ 2.7% (10 residues) from each other (Cupples and Pearlman, 1986; Hirono et al., 1987a).

Protozoan actins other than amoeba actins are speculated to share some essential properties with those of other ubiquitous actins, but at the same time not to share certain other properties common to ubiquitous actins, because variations in the constant regions of their primary structure are evident. If this speculation is valid, we can evaluate the essential functions of actin from a genealogical standpoint and we can also understand the relationships between the structural and functional domains of the actin molecule. Previously, attempts to test the validity of this speculation could not be undertaken, because no protozoan actins except for amoeba actins had been isolated.

Recently, we succeeded in isolating protozoan actin (protein) from *Tetrahymena* for the first time (Hirono et al., 1989) and investigated its biochemical properties (Hirono et al., 1989; Hirono et al., 1990). Purified actin can polymerize into filaments under the same conditions as muscle actin does and the filaments are indistinguishable from those of rabbit skeletal muscle actin. They can be decorated by skeletal muscle heavy meromyosin or its subfragment 1 in the same manner as muscle actin filaments, and can stimulate skeletal muscle myosin Mg^{2+}.ATPase activity, though *Tetrahymena* actin had 8 times less activity than muscle actin (Hirono et al., 1989). Furthermore, *Tetrahymena* actin has such properties that it can copolymerize with skeletal muscle actin (Hirono et al., 1990). However, *Tet-*

Table 1. Percentage homologies between actin amino acid sequences

	Skeletal muscle	Cyto-plasmic β	Sea ur-chin	*Phys-arum*	*Dictyo-stelium*	Yeast	Soy-bean	*Acanth-amoeba*	*Ent-amoeba*	*Plasm-odium* I	*Plasm-odium* II	*Tetra-hymena*	*Trypano-somes*
Cytoplasmic β	93.6												
Sea urchin	92.3	95.7											
Physarum	91.2	94.9	95.2										
Dictyostelium	91.2	94.4	94.1	98.7									
Yeast	87.2	88.8	89.3	88.5	87.5								
Soybean	83.2	84.5	85.3	86.4	85.6	80.0							
Acanthamoeba	92.5	95.2	95.2	97.9	97.1	89.9	85.1						
Entamoeba	85.9	88.0	87.2	89.1	88.8	83.5	80.0	88.5					
Plasmodium I	80.5	81.3	80.8	82.1	82.1	79.2	76.8	81.6	81.3				
Plasmodium II	74.9	75.2	74.4	74.9	75.2	73.3	72.0	75.5	77.3	78.4			
Tetrahymena	74.6	76.5	76.0	76.5	76.3	74.9	72.8	76.3	73.3	73.9	72.5		
Trypanosoma	70.9	73.9	73.6	73.1	72.8	73.9	70.4	71.2	72.5	74.1	75.5	69.9	
Oxytricha	64.4	64.4	63.8	64.3	64.9	64.4	61.6	64.4	65.4	64.6	63.0	64.3	63.3

Each homology was determined from the same data as shown in Fig. 1.

Table 2. Comparison between properties of *Terahymena* actin and those of skeletal muscle actin

	Skeletal muscle actin	*Tetrahymena* actin
Polymerization	+	+
	Copolymerization with each other	
Myosin head binding	+	+
Activation of myosin Mg^{2+}-ATPase	+ (1)	+ (1/8)
Apparent molecular weight	42 kDa	43.5 kDa
and isoelectric point	5.2	5.4
Inhibition of DNase I activity	+	-
Phalloidin binding	+	-
Muscle α-actinin binding	+	-
Muscle tropomyosin binding	+	-

rahymena actin can not interact with either DNase I (Hirono et al., 1989), or with phalloidin (Hirono et al., 1989), with skeletal muscle α-actinin (Hirono et al., 1990) and tropomyosins from chicken gizzard and rabbit skeletal muscle (Hirono et al., 1990). These results are summarized in Table 2. In addition, *Tetrahymena* actin spot on a two dimensional gel electrophoretic plate clearly differed from that of muscle actin in both isoelectric point and apparent molecular weight (Hirono et al., 1987a).

To our knowledge, no other actin with such unusual properites has yet been reported. However, immunofluorescence for *Tetrahymena* actin was localized in the division furrow and in organellas considered to be involved in endocytosis and exocytosis, suggesting that *Tetrahymena* actin may play much the same biological roles as ubiquitous actins (Hirono et al., 1987b).

The comprehensive view of *Tetrahymena* actin is that it has indeed both usual and unusual properties. These unusual properties probably also hold true for actins from *Plasmodium*, *Trypanosoma* and *Oxytricha*, although they have not yet been isolated.

In non-muscle higher eukaryotic cells, it has been accepted that the dynamic physiological functions of actin involved in various life phenomena are regulated by many kinds of actin regulatory proteins, and that these actin functions are indispensable for all eukaryotic cells, so that the primary structure of actin is highly conserved to sustain these multifunctions.

In protozoan actins, such as *Tetrahymena* actin, the primary structure of actin as a whole is not highly conserved as we have shown. We interpret this to mean that, in *Tetrahymena* actin, the essential structural domains responsible for actin-actin interaction and actin-myosin interaction remain relatively unchanged, but other structural domains responsible for interacting with many different kinds of actin regulatory proteins are diverged significantly. In other words, most of *Tetrahymena* 's actin regulatory proteins would be largely diserged in accordance to the divergency of *Tetrahymena* actin. In support of this assumption, both *Tetrahymena* actin expressed in mammalian tissue culture cells by introduction of an expression vector (Hirono, M., Ohno, T., and Watanabe, Y. manuscript in preparation) and skeletal muscle actin injected into predivision *Tetrahymena* cells (Watanabe et al., 1990) exert harmful influences upon cell division. This indicates that heterogenus actin can copolymerize with endogenous actin, but can not interact with endogenous actin regulatory proteins involved in cytokinesis.

Protozoan Calmodulin Genes

Calmodulin is a calcium-modulated protein also known for ubiquity, multifunctionality and structural conservativeness. As for protozoan calmodulin, we succeeded in isolating it for the first time from *Tetrahymena pyriformis* in 1979 (Suzuki et al., 1979). Calmodulin possesses four EF-hand type calcium-binding domains and is composed of 148 amino acid residues. Even *Tetrahymena* calmodulin has an essential property common to calmodulins; calcium-dependent activation of mammalian brain 3′-5′-cyclic nucleotide phosphodiesterase activity (Suzuki et al., 1981; Kakiuchi et al., 1981). During the past ten years, determination of amino acid sequences of calmodulins from various organisms and cloning and sequencing of calmodulin genes have been done, showing that the primary structures of calmodulins from most of vertebrates are completely the same (Chien et al., 1984; Lagacé et al., 1983; Nojima and Sokabe, 1987; Putkey et al., 1983; Sasagawa et al., 1982; Watterson et al., 1980), and that of *Drosophila* calmodulin has only three amino acid substitutions (98% identity) (Smith et al., 1987). However, protozoan calmodulins, such as those from *Tetrahymena pyriformis* (Yazawa et al., 1981), *Tetrahymena thermophila* and *Paramecium tetraurelia* (Schaefer et al., 1987) are largely diverged from vertebrate calmodulin (Fig.2). *Paramecium tetraurelia* calmodulin shares only 88.5% identity with vertebrates calmodulin (17 amino acid substitutions), *Tetrahymena pyriformis* shares only 90.5% identity (13 substitutions and one deletion), and *Tetrahymena thermophila* shares only 91.9% identity (12 substitutions). Among the substitutions seen in the ciliated protozoan calmodulins, 10 amino acid substitutions are identical with one another. Previously we demonstrated that calmodulins from *Tetrahymena* (Nagao et al., 1979) and *Paramecium* (Kudo et al., 1981) have the special property of calcium-dependently activating *Tetrahymena*'s membrane-bound guanylate cyclase. Most probably, some of the 10 amino acid substitutions in *Tetrahymena* and *Paramecium* calmodulins are responsible for this guanylate cyclase activation, since vertebrate calmodulin does not demonstrate such an activity (Kakiuchi et al., 1981). These results indicate that ciliate protozoan calmodulins are diverged largely in their amino acid sequences as compared to higher eukaryotic calmodulins and also have a function unique to ciliated protozoa. However, 5 amino acid differences are seen between calmodulins from *Tetrahymena pyriformis* and *Tetrahymena thermophila* (96.6% identity). This suggests that even within the same genus, *Tetrahymena*, the two genes have evolved independently and significantly as compared with the fact that various vertebrate calmodulins which have the same amino acid sequence. In the flagellate *Trypanosoma*, calmodulin is also diverged greatly as compared with both vertebrate calmodulin and *Tetrahymena* calmodulin. The genealogical features of ciliated and flagellated protozoa viewed from *Tetrahymena* and *Trypanosoma* calmodulins appear to be very similar to those viewed from protozoan actins including *Tetrahymena* actins.

Gene Structures Common to Tetrahymena Genes for Five Different Proteins

Nucleotide sequence flanking the codon: Previously, Kozak established the initiation consensus sequence of ACCATGG by analyzing higher eukaryotic genes (Kozak, 1986). Whereas in *Tetrahymena*, Barahona et al. (1988) proposed a consensus sequence of PuAPuATGPu as flanking the initiation codon. As shown in Fig. 3, Barahona's consensus sequence holds true for actin, TCBP-23, calmodulin and 49K protein genes. However, in TCBP-25, the first Pu proposed by Barahona is T. As far as the five *Tetrahymena* genes are concerned, AAATGPu is conserved.

Junction consensus at the ends of intron: Among 5 genes, we analyzed the genomic sequences for *Tetrahymena* actin and TCBP-25 (Hirono et al., 1987a; Takemasa et al., 1989). The actin gene does not include any introns, but TCBP-25 includes at least two. The ends of the introns in TCBP-25 gene are in accordance with the universal junction consensus GT/AG rule proposed by Breathnach et al. in 1978. This rule is also the case for other *Tetrahymena* genes such as ribosomal protein gene, Histone H1 gene and conjugation specific gene (Martindale and Taylor 1988; Nielsen et al., 1986; Wu et al., 1986).

		Helix	Ca-binding loop	Helix
VERTEBRATES	ADQLTEEQIA	EFKEAFSLF	DKDGDGTITTKE	LGTVMRSL
Electric eel	----------	---------	----------	--------
Fruit fly	----------	---------	----------	--------
P.tetraurelia	-E--------	------A--	----------	--------
T.pyriformis	----------	---------	----------	--------
T.thermophila	----------	---------	----------	--------
Trypanosome	----SN---S	---------	----------	--------
VERTEBRATES	GQNPTEA	ELQDMINEV	DADGNGTIDFPE	FLTMMARK
Electric eel	-------	---------	----------	------K-
Fruit fly	-------	---------	----------	--------
P.tetraurelia	-------	---------	----------	--SL----
T.pyriformis	-------	---------	----D-------	--SL----
T.thermophila	-------	---------	----------	--SL----
Trypanosome	-------	---------	-Q--S-------	---L----
VERTEBRATES	MKDTDSEE	EIREAFRVF	DKDGNGYISAAE	LRHVMTNL
Electric eel	--------	---------	----------	--------
Fruit fly	--------	---------	------F-----	--------
P.tetraurelia	--EQ----	-LI---K--	-R----L-----	--------
T.pyriformis	--------	-LI---K--	-R--D-L-T---	--------
T.thermophila	-----T--	-LI---K--	-R----L-----	--------
Trypanosome	-Q-S----	--K------	------F-----	---I----
VERTEBRATES	GEKLTDE	EVDEMIREA	DIDGDGQVNYEE	FVQMMTAK
Electric eel	-------	---------	----------	--------
Fruit fly	-------	---------	----------	--T---S-
P.tetraurelia	------D	---------	------HI----	--R--VS-
T.pyriformis	-------	---------	------HI----	--R-- --
T.thermophila	-------	---------	------HI----	--R--M--
Trypanosome	-------	---------	-V-----I----	--K--MS-

Figure 2. Comparison between calmodulin amino acid sequences of vertebrate calmodulins [human (Sasagawa et al., 1982), cow (Watterson et al., 1980), rat (Nojima and Sokabe, 1987), chicken (Putkey et al., 1983), frog (Chien and David, 1984) electric eel (Lagacé et al., 1983)], fruit fly (*Drosophila melanogaster*) calmodulin (Smith et al., 1987), and protozoan calmodulins [*Paramecium tetraurelia* (Schaefer et al., 1987), *Tetrahymena pyriformis* (Yazawa et al., 1981), *Tetrahymena thermophila* (Takemasa et al., manuscript in preparation), *Trypanosoma brusei* (Tschudi et al., 1985)]. Recently, it was proved that electric eel has calmodulin identical to that of vertebrates (Toda et al., 1985). So the sequence shown here is one of the isoforms of electric eel calmodulin. The sequence of the first three amino acids (underlined) of *P. tetrauleria* has not yet been determined.

Actin	(*T. pyriformis*)	CTAAAAGAAAATGACTGACAGTG
TCBP-25	(*T. thermophila*)	AAATAAATAAATGGCTCAATACT
TCBP-23	(*T. thermophila*)	ACTAAAAGAAATGGAACACCAAA
Calmodulin	(*T. thermophila*)	AAATTAAGAAATGGCTGATCAAT
49K protein	(*T. thermophila*)	ATCAAAAAATGAGATCTATCA

Figure 3. Nucleotide sequences flanking initiation codons of *Tetrahymena* genes isolated in our laboratory. Initiation codons (ATG) are underlined.

Actin	(*T. pyriformis*)	AAAGTGCTTCTGATCAATTTAAA
TCBP-25	(*T. thermophila*)	CTTAGTTTTCTGAGCAAAATAAT
TCBP-23	(*T. thermophila*)	CACTCAAGCTTGATCTTATTTTA
Calmodulin	(*T. thermophila*)	GATGGCTAAGTGAGCGTGCAAAC
49K protein	(*T. thermophila*)	GTACAGAGAATGAACAAATTGAA

Figure 4. Nucleotide sequences flanking termination codons of *Tetrahymena* genes isolated in our laboratory. Termination codons (TGA) are underlined.

Therefore, the ciliated protozoan *Tetrahymena* appears to follow the universal junction rule.

Nucleotides in non-coding regions: In *Tetrahymena*, non-coding regions show an extreme abundance of A + T residues, for example, A + T make up 89% of the intron in TCBP-25 (Takemasa et al., 1989), and 75-80% in both the 5′ and 3′ non-coding regions of the five *Tetrahymena* genes we analyzed. On the other hand, their coding regions are not so A + T rich (Barahona et al., 1988; Hirono et al., 1987; Takemasa et al., 1989).

Termination codon: In the five *Tetrahymena* genes, only TGA is used for the termination codon (Fig. 4), and there is no flanking consensus sequence. In *Paramecium*, also only TGA is used as the stop codon (Preer et al., 1985). However, this is not true for all protozoan genes, that is, in *Acanthamoeba*, *Entamoeba*, *Plasmodium* and *Trypanosoma*, the universal termination codons, such as TAA, TAG and TGA are also used as in other eukaryotic cells (Nellen and Gallwits, 1982; Gallwitz and Sures, 1980; Wesseling et al., 1988; Tschudi et al., 1985). Even the ciliate, *Euplotes* is known to use TAA as a stop codon (Miceli et al., 1989; Harper and Jahn, 1989). This evidence also indicate that various unicellular organisms which have evolved diversely and independently are included in protozoa, even ciliated protozoa.

TAA and TAG condons: TAA and TAG codons are usually recognized as universal stop codons in both prokaryotes and eukaryotes. However, it has recently been proposed that TAA and TAG codons codes for glutamine in *Paramecium* and *Tetrahymena* from the results of the nucleotide sequencing data of several genes (Hirono et al., 1979a; Preer et al., 1985) and from the discovery of glutamine tRNA recognizing these stop codons (Hanyu et al., 1986). Recently, we directly proved that both TAA and TAG code for glutamine in *Tetrahymena* by determining parts of the amino acid sequences of TCBP-25 and TCBP-23 from both purified proteins and nucleotide analyses of their genes (Takemasa et al., 1989; Takemasa et al., 1990). In TCBP-25, 5 TAAs code for glutamine; in TCBP-23, 2 TAAs and 1 TAG code for glutamine; in calmodulin, 1 TAA codes for glutamine; in 49K protein, 15 TAAs code for glutamine; and in actin 1 TAG codes for glutamine. Although TAA and TAG are recognized as codons for glutamine and only TGA is used as the stop codon in *Tetrahymena* and *Paramecium*, this is not acceptable for all of the protozoan genes. As stated previously, in *Acanthamoeba*, *Entamoeba*, *Plasmodium* and *Trypanosoma*, TAA and TAG are not used for glutamine but for stop codons (Nellen and Gallwits, 1982; Gallwits and Sures, 1980; Wesseling et al., 1988; Tschudi et al., 1985). Even in the ciliate *Euplotes*, TAA is not used for glutamine but used as a stop codon (Harper and Jahn, 1989; Miceli et al., 1989). This evidence again indicates that cilites have evolved diversely and independently and they have extraordinarily deflected from the main stem of the genealogical tree.

General codon usage: Codon usage in *Tetrahymena* is known to show (a strong) partiality (Martindale, 1989). Have we present the codon usage in five *Tetrahymena* genes that we cloned and sequenced (Table 3; A = actin, B = TCBP-25, C = TCBP-23, D = calmodulin and E = 49K protein). The codon usage displays a strong bias in *Tetrahymena*. Among 64 possible codons, 17 are not used in the five

Table 3. Comparison of codon usage in five genes from *Tetrahymena*

	A	B	C	D	E	Total	%
Phe							
TTT	0	1	3	0	1	5	9.4
TTC	14	7	5	8	14	48	90.6
Leu							
TTA	1	5	2	3	19	30	25.9
TTG	9	0	1	4	7	21	18.1
CTT	6	2	6	2	15	31	26.7
CTC	12	8	3	3	8	34	29.3
CTA	0	0	0	0	0	0	0
CTG	0	0	0	0	0	0	0
Ile							
ATT	10	6	4	8	17	45	52.9
ATC	19	8	3	1	9	40	47.1
ATA	0	0	0	0	0	0	0
Met							
ATG	13	5	9	10	13	50	100
Val							
GTT	7	7	11	1	16	42	45.7
GTC	16	7	8	5	13	49	53.3
GTA	0	0	1	0	0	1	1.0
GTG	0	0	0	0	0	0	0
Ser							
TCT	9	8	6	2	19	44	48.9
TCC	16	7	2	1	6	32	35.6
TCA	2	1	0	0	0	3	3.3
TCG	0	0	0	0	0	0	0
AGT	1	0	2	1	2	6	6.6
AGC	0	0	2	0	3	5	5.6

	A	B	C	D	E	Total	%
Pro							
CCT	5	2	2	1	11	21	41.2
CCC	16	3	1	1	9	30	58.8
CCA	0	0	0	0	0	0	0
CCG	0	0	0	0	0	0	0
Thr							
ACT	5	11	12	8	7	43	55.1
ACC	17	6	2	3	7	35	44.9
ACA	0	0	0	0	0	0	0
ACG	0	0	0	0	0	0	0
Ala							
GCT	11	9	13	8	24	65	70.7
GCC	13	3	3	3	5	27	29.3
GCA	0	0	0	0	0	0	0
GCG	0	0	0	0	0	0	0
Tyr							
TAT	1	1	3	0	7	12	20.7
TAC	16	9	3	1	17	46	79.3
His							
CAT	2	0	0	0	5	7	22.6
CAC	6	1	1	2	14	24	77.4
Gln							
CAA	6	4	3	3	10	26	51.0
CAG	0	0	0	0	0	0	0
TAA	0	5	2	1	15	23	45.1
TAG	1	0	1	0	0	2	3.9
Asn							
AAT	2	0	2	2	3	9	19.1
AAC	10	7	5	4	12	38	80.9

	A	B	C	D	E	Total	%
Lys							
AAA	2	1	3	1	4	11	9.5
AAG	22	18	22	7	36	105	90.5
Asp							
GAT	10	5	14	16	20	65	60.2
GAC	12	13	5	1	12	43	39.8
Glu							
GAA	28	17	18	21	20	104	98.1
GAG	1	0	0	0	1	2	1.9
Cys							
TGT	1	1	1	0	5	8	40.0
TGC	8	1	1	0	2	12	60.0
Trp							
TGG	3	1	1	0	7	11	100
Arg							
CGT	0	0	0	0	1	1	1.8
CGC	0	0	0	0	0	0	0
CGA	0	0	0	0	0	0	0
CGG	0	0	0	0	0	0	0
AGA	17	13	5	6	15	56	98.2
AGG	0	0	0	0	0	0	0
Gly							
GGT	25	11	8	11	26	81	87.1
GGC	0	3	1	0	3	7	7.5
GGA	1	0	2	0	2	5	5.4
GGG	0	0	0	0	0	0	0
Stop							
TGA	1	1	1	1	1	5	100

(A : Acti gene from *T. pyriformis*. B, C, D and E : TCBP-25, TCBP-23, calmodulin and 49K protein genes from *T. thermophira*, respectively)

genes (CTA and CTG for Leu, ATA for Ile, GTG for Val, TCG for Ser, CCA and CCG for Pro, ACA and ACG for Thr, GCA and GCG for Ala, CAG for Gln, CGC, CGA, CGG and AGG for Arg and GGG for Gly). Especially, among 6 codons for arginine, AGA is used exclusively. As seen in Table 3, 11 codons are preferentially used more than 70% for individual amino acids (TTC for Phe, 90.6%; GCT for Ala, 70.7%; TAC for Tyr, 79.3%; CAC for His, 77.4%; AAC for Asn, 80.9%; AAG for Lys, 90.5%; GAA for Glu, 98.1%; AGA for Arg, 98.2%; GGT for Gly, 87.1%). Such biased codon usage seems to be another remarkable characteristics of protozoa.

Putative poly(A) signal: In the 3'-flanking regions of cDNAs for TCBP-23 (Takemasa et al., 1990), calmodulin and 49K protein, we found a *AATAAA* consensus element (except for *TATAAA* in TCBP-25: Takemasa et al., 1989) which may be the poly (A) signal site shortly upstream of the poly (A) tail (Fig. 5). We also found *TCAACTCTTAA* and *TTTTTAAA* sequences in the genomic DNA for TCBP-25 (Takemasa et al., 1989). These sequences may correspond to the conserved recognition site for a poly (A) tail, namely, *ACAACTPyTCAPy* and *TTTTTAAAA* proposed by Barahona et al. (1988).

Conclusive Remarks

In the present review article, we deduced the genealogical aspects of protozoa from the view of five different kinds of *Tetrahymena* genes that we have recently analysed. One major parameter was the comparison of amino acid sequences of certain ubiquitous proteins from various organisms. For this, biologically important actin and calmodulin were selected, since they are widely distribrted in eukaryote and possess multifunctionality and structural conservativeness. We indicated that protozoan genes (except for rhizopod genes) are highly di-

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TCBP-25          AACTTTTCTTTATAAAATCATAAAAA

TCBP-23 (1)      ATCTTATTTTAATAAACATATTTCAA

TCBP-23 (2)      ACATATTTCAAATAAAAATTGAACTG

CALMODULIN       CAACTTTAAAAATAAAATATATATCA

49K PROTEIN      ATTAGTCTTTAATAAAAGTTGTATCA
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Figure 5. Nucleotide sequences flanking putative poly (A) signals of *Tetrahymena thermophila* genes isolated in our laboratory. Putative poly (A) signals (AATAAA or TATAAA) are underlined.

verged from those of higher eukaryotes and that, even between protozoans, these genes are evolved diversely and independently. For example, protozoan actin shares a few essential properties with those of eukaryotic actins known previously, but does not have other properties common to ubiquitous actins. This lack of the latter properties is considered to be important in understanding the relationships between structural and functional domains of the actin molecule. By using gene engineering techniques, such relationships are expected to be elucidated in the near furture.

We have also indicated that nucleotide sequence flanking the initiation codon, usage of the universal stop codons, TAA and TAG, and codon usage in general in *Tetrahymena* are considerably different from those of higher eukaryotes. Whereas junction consensus of introns and putative poly(A) signals in *Tetrahymena* are in accordance with those of higher eukaryotes. In these aspects, ciliated protozoa are considered to deflect largely from the main stem of genealogical tree.

In *Tetrahymena*, cloning and sequencing of the genes for a certain purified protein appears to be easy, since relatively accurate oligonucleotide probes can be synthesized referring to a part of its amino acid sequence and biased codon usage as shown in Table 3. If many more kinds of *Tetrahymena* genes were to be isolated, information of molecular evolution in protozoa would be much more substantiated.

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Invasive Sexually-Transmitted Amebiasis in Japan : Pathogenicity of Entamoeba histolytica Circulating among Male Homosexual Communities

Tsutomu Takeuchi, Seiki Kobayashi, Eiichi Okuzawa, Yasushi Miyahira, Severa R. N. Motta and Tomoyoshi Nozaki

Department of Parasitology, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160, Japan

Summary

Sexually-transmitted amebiasis due to transmission of *Entamoeba histolytica* cyst by oral-anal sexual contact of homosexual men seems to be primarily responsible for the recent increment in prevalence of amebic infection in the western countries and Japan. Numerous epidemiological studies clarified some unusual aspects of this novel sexually-transmitted disease (STD). The most striking feature was the fact that virtually all of the *E. histolytica* isolates from homosexual men in the western countries were judged to be non-pathogenic by clinical findings, serologic investigations and zymodeme analyses. Accordingly, the homosexual men with amebic infection in these countries were essentially asymptomatic cyst carriers. However, in Japan, a number of homosexual men have been found from the cases with invasive amebiasis. This led us to investigate the pathogenicity of *E. histolytica* strains circulating among Japanese including homosexual men. First, we found a high correlation between positive syphilis serology with invasive amebiasis. Subsequent studies indicated that the seropositivity of Japanese homosexual men for amebic infection was much higher than that of heterosexual males and female prostitutes, and that the ameba isolates from homosexual men had zymodeme II, XIV and XIX, all of which stood for pathogenic *E. histolytica*. These findings strongly suggest that pathogenic strains of *E. histolytica* are spread among male homosexual communities in Japan. Because pathogenicity of this protozoon appears to be the most important factor for development of symptomatic, invasive amebiasis, these data seem to be reasonable enough to explain why in Japan we have had a number of cases with invasive sexually-transmitted amebiasis. Zymodeme XIV was also frequently detectable from the cases without the history of homosexuality, which leads us to envision that the unique epidemiological aspects of amebic infection in Japan may be relevant with Indian Subcontinent, virtually the only region where this zymodeme has been frequently found.

Introduction

Since the World War Ⅱ, the prevalence of orally infectious diseases has generally been decreasing in Japan as well as in the western countries like the United States. Amebiasis, due to oral ingestion of *Entamoeba histolytica* cyst, was not exceptional. From around 1980, however, its prevaience suddenly increased in these developed countries (Marr, 1981; Takeuchi *et al.*, 1983a). Subsequent epidemiological studies conducted to clarify the reason for this increment indicated that the increased prevalence was primarily attributable to sexually-transmitted amebiasis caused by ingestion of *E. histolytica* cyst through oral-anal sexual contact of homosexual males (Marr, 1981; Phillips *et al.*, 1981; Keystone *et al.*, 1981; Quinn *et al.*, 1983; Takeuchi *et al.*, 1983a; 1983b). Further detailed investigations clarified some unusual, but interesting characteristics of this novel sexually-transmitted disease (STD). First of all, it was found most frequently in large cities like New York (Phillips *et al.*, 1981), Seattle (Quinn *et al.*, 1983), San Francisco (Markell *et al.*, 1984), Toronto (Keystone *et* al., 1980) and Edinburgh (McMillan *et al.*, 1984). Moreover, homosexual men with such an amebic infection were found to be frequently associated with a variety of concomitant infections with other pathogens of STD and orally communicable diseases. In particular, high rates of past or present concomitant infection with *Treponema pallidum* and / or *Neisseria gonorrhoeae* were noted (Quinn *et al.*, 1983; Markell *et al.*, 1984). Other such pathogens included *Shigella, Salmonella, Campylobacter, Mycoplasma, Chlamydia, Ureaplasma,* Herpes

simplex virus, Hepatitis A virus and Hepatitis B virus as well as parasitic protozoa like *Entamoeba coli* and *Giardia lamblia*.

These characteristics of sexually-transmitted amebiasis in the western countries seemed generally consistent with those of Japanese cases with amebic infection. In Japan, amebiasis has been primarily detected in large cities like Tokyo, Osaka and Nagoya (Masuda *et al.*, 1986), where a large number of homosexual men reside. In addition, approximately 40% of Japanese cases with invasive amebiasis showed positive syphilis serology as examined by *Treponema pallidum* hemagglutination test (TPHA) (Takeuchi *et al.*, 1983b; 1987). Thus, there seems little doubt that we have sexually-transmitted amebiasis in Japan. Indeed, the number of Japanese cases with this STD has been increasing for these several years. However, our recent investigations elucidated a distinct difference in this unusual type of amebic infection between Japan and the western countries concerning development of clinical symptoms, and eventually led us to envisage that pathogenicity of *E. histolytica* circulating among Japanese male homosexual population might be different from that in the western countries. The present review summarizes our recent efforts to clarify this as well as the epidemiological features in the western countries.

Pathogenicity of E. histolytica in Male Homosexual Communities in the Western Countries

The first significant observation concerning the pathogenicity of *E. histolytica* among male homosexual communities in the western countries was that there was no correlation between the presence or absence of gastrointestinal symptoms and amebic infection (Quinn *et al.*, 1983; Markell *et al.*, 1984). It is well known that homosexual men generally show a variety of gastrointestinal symptoms (Quinn *et al.*, 1983); however, amebic infection did not appear to contribute to development of their clinical symptoms. Such an observation strongly suggested that *E. histolytica* responsible for sexually-transmitted amebiasis in the western countries were non-pathogenic, as pathogenicity of this protozoon is undoubtedly the most important factor for development of symptomatic amebiasis (Ravdin, 1988). If this is true, the presence of only a few symptomatic cases with sexually-transmitted amebiasis (Schmerin *et al.*, 1977; Burham *et al.*, 1980; Ylvisaker and McDonald, 1980) can be explainable by the lack of pathogenic *E. histolytica* in the male homosexual populations in these countries.

This view was immediately supported by the observation of McMillan *et al.* (1984) that virtually all of the homosexual men with amebic infection were serologically negative for anti-amebic antibody in Edinburgh, the United Kingdom as examined by indirect hemagglutination test (IHA). Because IHA is very sensitive and one of the most reliable serologic tests for invasive amebiasis (Patterson *et al.*, 1980), it seems plausible that the homosexual men with amebic infection lack production of anti-amebic antibody, which probably means in these biased males amebae do not invade into intestinal tissues mainly because they are non-pathogenic.

Direct evidences to verify this view were obtained by zymodeme characterization of the ameba isolates from homosexual men. According to Sargeaunt (1987; 1988), zymodemes of *E. histolytica* are reliable markers for its pathogenicity, which are determined by isoenzyme profiles of hexokinase, phosphoglucomutase, malic enzyme and glucosephosphate isomerase on thin layer starch gel electrophoresis utilizing the lysate of ameba grown in Robinson's medium (Robinson, 1968). With this technique, Sargeaunt and his colleagues examined several thousands of subjects with amebic infection, and proposed 22 zymodemes. Nine of these originated from subjects with invasive amebiasis. Twelve were associated with asymptomatic cyst carriers, and the remaining zymodeme was produced by his laboratory enginnering (Sargeaunt, 1988). For instance, zymodeme II, XIV and XIX were designated to stand for pathogenic amebae, whereas zymodeme I was judged to be a representative one for non-pathogenic zymodeme (Fig. 1). Generally, pathogenic zymodemes were defined as amebae with following isoenzyme profiles; 1) presence of β-band of phosphoglucomutase in the absence of α-band, 2) presence of two fast-running isoenzymes of hexokinase except for zymodeme XIII, which does not have advanced bands of this enzyme. Isoenzyme profiles of

malic enzyme and glucosephosphate isomerase are used for other purposes like identification of *E. histolytica* and characterization of geographic features. Although some recent experimental data (Mirelman, 1987; Mirelman *et al.*, 1986a; 1986b) suggest that zymodemes of ameba may change during the process to produce axenic strains from the xenic culture by removing the bacterial associates, they still seem to be of a reasonable significance to determine the pathogenicity of ameba isolates from human sujects. Such studies conducted in the United States (Mathews *et al.*, 1986), Canada (Proctor *et al.*, 1987) and the United Kingdom (Goldmeirer *et al.*, 1986; Allason-Jones *et al.*, 1988) showed that vertually all of the ameba isolates from homosexual men had non-pathogenic zymodemes. Thus, all of the features of sexually-transmitted amebiasis in the western countries appear to well conform to the recent definition of asymptomatic cyst carrier of amebic infection proposed by Sargeaunt (1987), which can be summarized as follows; 1) asymtomatic cyst carriers have no clinical symptoms due to ameba, 2) they are serologically negative for anti-amebic antibody, 3) the ameba isolates from them show non-pathogenic zymodems. Sargeaunt and his colleagues (1986; 1987), on the basis of these zymodeme studies, insisted that asymtomatic cyst carriers with these characterisics may not need anti-amebic therapy. This eventually means that virtually all of the subjects with sexually-transmitted amebiasis are cosidered to be in no need of anti-amebic therapy in the western countries.

Pathogenicity of E. histolytica in Male Homosexual Communities in Japan

The occurrence of sexually-transmitted amebiasis in Japan was first suggested by analyses of the cases with invasive amebiasis (Takeuchi *et al.*, 1983a; 1983b). Because amebiasis is one of the infectious diseases which should be reported to local health departments, its incidence can roughly be known from the Statistics of Communicable Diseases issued by the Japanese Ministry of Health and Welfare. According to this statistics, the number of reported cases with invasive amebiasis increased from 1980 (Fig. 2). Interestingly, analyses of these cases indi-

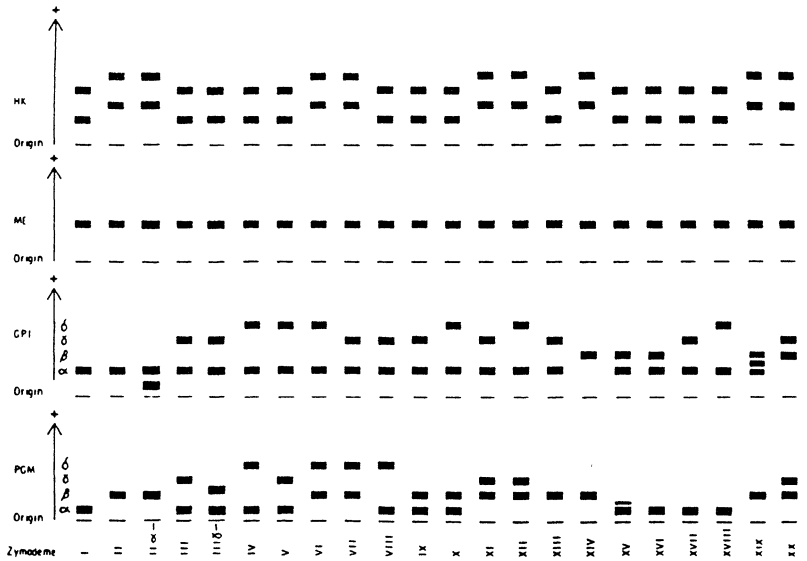

Figure 1. Twenty two zymodemes of *E.histolytica* isolated from human subjects as determined by isoenzyme patterns of hexokinase (HK), phosphoglucomutase (PGM), malic enzyme (ME) and glucosephosphate isomerase (GPI). Quoted from Sargeaunt (1988) (Ref. 23).

cated that most of them were adult males with no experience of oversea travels, and that approximately 40% of them were positive by TPHA, as mentioned above, half of whom were also positive by non-treponemal antigen tests like Venereal Disease Research Laboratory (VDRL) slide test (Takeuchi *et al.*, 1983b; 1987). Since these features seemed to be consistent with those of sexually-transmitted amebiasis in the western countries except that all of the Japanese cases had invasive amebiasis, we envisioned that pathogenic strains of *E. histolytica* might be spread among male homosexual populations in Japan. Similar environmental factors concerning transmission of orally infectious pathogens in Japan and the western countries also appeared to support our view.

As the first attempt to verify this view, we recruited approximately 300 homosexual men in Nagoya City, the 4th largest city in Japan, from two different groups belonging to a specifically isolated male homosexual community, and made a seroepidemiological investigation. What we found was, to our surprise, 14.3% and 20.3% of the 1st and 2nd group were positive for anti-amebic antibody, respectively, by enzyme-linked immunosorbent assay (ELISA), while gel diffusion precipitin test (GDP), which was conducted on only one of the groups, was positive at 7.6% (Takeuchi *et al.*, 1989) (Table 1). As controls, age-matched Japanese heterosexual men and female prostitutes were also examined; however, virtually none of them were positive by GDP and ELISA. Another similar study was also attempted on serum specimens from homosexual men collected by Department of Medicine, Teikyo University School of Medicine. About half of these homosexual men were from smaller cities of Japan, which means that the geographic background of this 3rd group is different from that of the 1st and 2nd group. Our serologic examination indicated that 220 specimens of this group included positive sera at 2.3% and 4.1% by GDP and ELISA, respectively (Takeuchi *et al.*, 1990) (Table 1). Judging from higher sensitivity of ELISA (Takeuchi *et al.*, 1988). It is reasonable that the positive rates by GDP are lower than those by ELISA. It is also acceptable that the 1st and 2nd group showed higher positive rates of anti-amebic serology than the 3rd one, as sexually-transmitted amebiasis has been

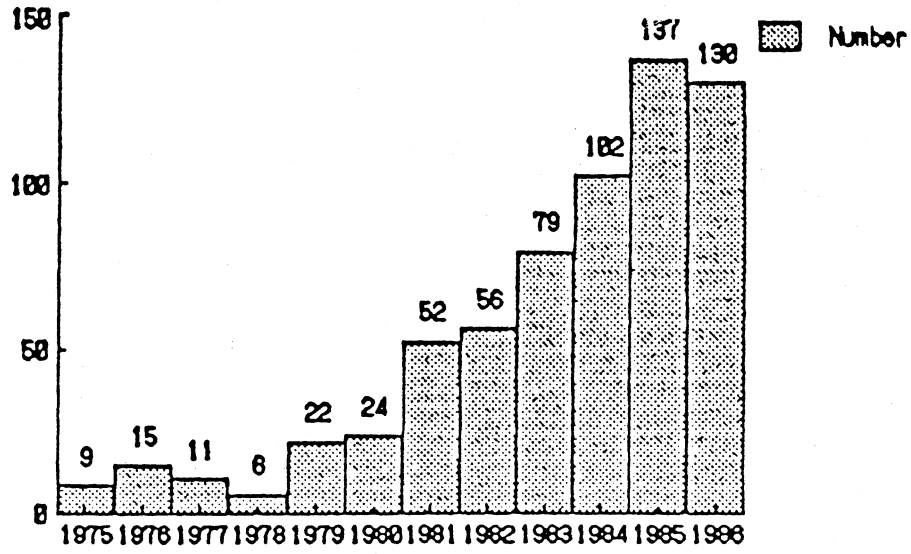

Figure 2. The number of cases with amebiasis reported to Japanese Ministry of Health and Welfare through local health departments from 1975 to 1986. Extracted from the Statistics of Communicable Diseases issued by the Ministry.

found in large cities in Japan (Masuda *et al.*, 1986). Concerning the correlation of serologic data with the pathogenicity of ameba, Jackson (1987) suggested that the positive GDP response appeared to reflect infection with pathogenic *E. histolytica*; accordingly, these serologic data are judged to support our view.

To further obtain this series of evidences, zymodeme analysis was attempted on the ameba isolates from Japanese homosexual men with amebic infection (Nozaki *et al.*, 1989). So far, we could isolate 49 strains of *E. histolytica*. Nine of them were isolated from homosexual males; 8 from Japanese and one from a residing Chinese. As summarized in Table 2, all of the ameba isolates from the biased males showed pathogenic zymodemes, i. e., zymodeme II (4 cases), zymodeme XIV (3 cases) and

Table 1 Seropositivity for amebic infection in Japanese homosexual men, prostitutes and heterosexual men.

Group	Seropositivity	
	GDP	ELISA
Heterosexual men	0% (0/100)	1.0% (1/100) *
Prostitutes	0% (0/120)	0.8% (1/120) *
Homosexual men		
Group 1	Not done	20.4% (44/216)
Group 2	7.6% (9/119)	13.4% (16/119)
Group 3	2.3% (5/220)	4.1% (9/220)

*Not significant as compared with negative controls. Data summarized in this table were extracted from Ref. 29 and 30.

Table 2 Zymodemes of *E. histolytica* isolated from homosexual men in Japan and the correlation with serology and clinical data

Subject No.	Age	Serology			Symptoms	Zymodemes	Nationality	Overser travell
		GDP	ELISA	IFA				
NOT-1	42	+	+		LA, D	XIX	Japan	−
NOT-3	46	+	+		LA, CF	XIV	Japan	−
NOT-4	38	+		+	D	II	Formosa	
NOT-8	62	+	+		LA	XIX	Japan	−
NOT-11	37	+			UC, D	II	Japan	−
NOT-16	33	+	+		LA, CF	II	Japan	−
NOT-24	37	+			BD, HB(+)	II	Japan	
NOT-43	29	+	+		CF, D	XIV	Japan	−
NOT-48	30	+	+		CF, D	XIV	Japan	−

LA: liver abscess; D: amebic dysentery; CF: *E. histolytica* cyst detected in feces; UC: ulcerative colitis; BD: bloody diarrhea; HB(+): hepatitis B antigen positive.

A part of this table was quoted from Nozaki *et al.* (1989) (Ref. 15).

zymodeme XIX (2 cases), which seemed compatible with their serologic and clinical findings. Thus, there seems little doubt that pathogenic strains of *E. histolytica* are spread among Japanese male homosexual populations. We have recently found a homosexual steady couple with pathogenic *E. histolytica* infection. Since they denied sexual activities with other homosexual men, this couple may also be an evidence to support transmission of pathogenic ameba among Japanese biased males.

Of particular significance concerning the zymodeme analysis was that we could demonstrate zymodeme XIV at a high percentage in the Japanese cases without a history of homosexuality as well as in homosexual men, since this pathogenic zymodeme has been primarily found around Indian Subcontinent. The present daga, terefore, may suggest that the unique epidemiological features of amebic infection in Japan is relevant with its close geographic correlation with Indian Subcontient and probably also Southeast Asia.

Conclusion

In conclusion, there certainly are pathogenic strains of *E. histolytica* circulating among male homosexual communities in Japan, which is evidently the most distinct difference in the epidemiological aspect of sexually-transmitted amebiasis between Japan and the western countries. The reason of this difference is not known, at present; however, this unique epidemiology of amebic infection in Japan may be relevant with amebiasis in Indian Subcontinent and probably also in Southeast Asia.

Acknowledgememts

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第8回国際原生動物学会議報告

樋渡　宏一，高橋三保子

1989年7月10日～17日に筑波大学大学会館で開催された上記国際会議については，日本人参加者が152名あったので，会員外の参加者を考慮しても，日本原生動物学会々員のほとんど8割の方が出席されたことになる。

したがって，日本から少数の参加者しかなかったこれまでの国際原生動物学会議の報告のような詳細な報告は必要ないと思われるが，日本原生動物学会が主催した歴史的な事業として，将来のために記録として残しておいた方がよいと考えられるいくつかのデータもあるので，それらを含めて学会の様子を報告する。

先ず登録者の国別の数を表1に示す。これによると，登録手続きを行った者は41ヶ国2地域（台湾と香港）413名，実際に出席した者は38ヶ国2地域371名である。アブストラクト集から計算した過去5回の登録者数の平均が453名であるから，原生動物学者の多い欧米からの距離が，これまでに比べて一番遠い場所で行われた会議としては，かなり良い参加者数ではないかと思う。

この中で，アジア地区からの参加者は10ヶ国2地域から53名で，中国20，インド18が目立って多い。日本を除くアジア地区からの過去5回の会議の登録者数平均が31名であるから（今回の登録手続者は71名），やはりアジア地区で開催した意義は充分にあったと考えられる。アジア地区からの参加者のかなりの部分は旅費滞在費の全部又は一部を援助した者である。

参加者に対する旅費滞在費の援助は，謝金的な意味をもった特別講演者への滞在費支給を除いて，途上国を主とした外貨入手の困難な国からの参加者に限定した。援助者の国別リスト（全額支給と一部支給の両者を含む）を表2に示す。このほかに登録料だけを免除した者がある。また，援助を決定したが出席できなかった者が6名ある。

国際会議の主催者になったときに一番頭を悩ます問題は資金の調達であろう。この会議でも当初は必要な金額が調達できるか危惧されたが，財務委員長と組織委員のこまめな努力により，最終的には余裕をもった経理を行うことができた。

収支の主な項目について述べると，登録料は予定をやゝ下まわり約600万円，各種財団からの補助金が約1000万円，業界からの寄付金が約1500万円，その他約200万円が収入で，支出は途上国出席者補助約480万円，印刷製本費約500万円，賃金約420万円，パーティーなどソシャルプログラム約570万円，国際委員会費約130万円，交通費旅費（委員会などの）約260万円，消耗品費約220万円，通信運搬費約120万円，募金経費（手数料等）約170万円，登録経費および事務依託費約120万円，その他で，支出の総額は約3350万円であった。

会期中に国際原生動物学委員会（IUBS原生動物学委員会）が2回あり，重要議題である会則及び附則を決定したほか，次回の第9回をベルリンで1993年に開催することを決定した。次回会議の会長はボッフム・ルール大学のH. Mehlhorn教授，事務局長はベルリン自由大学のK. Hausmann教授である。尚国際原生動物学委員会の会則と附則は文末に示す。

会議の翌日に行われたワークショップには50名以上の参加者があり，原生動物を取扱ういろいろな技術の実習が行われ盛会であった。

会期中および会終了後に外国からの参加者からいろいろな評判をきいたが，“extremely well organized”であるというのが一般的な評価で，特に何度も耳に入ったのは，会場がゆっくりしていて休憩室が快適であること，スライド係りがすばらしいエキスパートであること，会場周辺の食事が思ったより安いこと，開会式の時の琴の演奏と〝ツクバナイト〟の興奮が忘れられないこと，などなどであった。

梅雨期の後期にかゝっていたにもかゝわらずほとんど雨もなく天候に恵まれたのは幸であった。

最後に，共催によって会場使用料を免除していたゞいたほか，いろいろな面にわたって多大の援助を惜しまず会議成功の基盤を作っていただいた筑波大学当局に心から謝意を表する。

表1

国又は地域	A	B	C	国又は地域	A	B	C
Japan	152	2	1	Brazil	1		
U. S. A	52		3	Chad	1		
F. R. G.	22			Colombia	1		
P. R. China	20	3	1	Ghana	1		
India	18	1	9	Hong Kong	1		
Kenya	18			Hungary	1		
Italy	16	1		Indonesia	1		
U. K.	10			Iran	1		
France	5		2	Iraq	1		1
Philippines	5		2	Jordan	1		
Spain	4	1		Korea	1		
U. S. S. R	3	1	3	Malaysia	1	1	
Poland	3		4	Nicaragua	1		
Belgium	3	1		Norway	1		
Canada	3			Republic of Guinea	1		
Denmark	3			Thailand	1		
Netherland	3			Egypt			1
Switzerland	3			Uganda		1	
Austria	2			Yugoslavia			1
Australia	2						
Mexico	2	1					
South Africa	2						
Taiwan	2						
Tanzania	2		1				

A：参加者，B：登録料を支払ったか，又は免除されたが参加しなかった者，C：書類上の登録はしたが，登録料を支払わず参加もしなかった者.

表2

国　名	人　数
P. R. China	13
India	11（2）*
Philippines	4
Kenya	4（2）
Poland	3
U. S. S. R.	3
Colombia	1（1）
合　計	39（5）

*カッコ内の人数は国連環境計画（UNEP）からの援助者で内数，特別講演者への滞在費支給は含まれていない。

CONSTITUTION AND BY-LAWS OF THE INTERNATIONAL COMMISSION ON PROTOZOOLOGY

CONSTITUTION

ARTICLE Ⅰ—Aims

The International Commission on Protozoology shall, as a member organization of the International Union of Biological Sciences, promote the exchange of ideas among protozoologists throughout the world and aid in arranging periodic International Congresses of Protozoology.

ARTICLE Ⅱ—Membership

Section 1. There shall be two categories of membership: Regular and Honorary.

Section 2. Regular Members:

a. Regular members shall be appointed by each national protozoological organization, irrespective of its actual designation (e.g., Society, Affiliated Society, Section of a Society, or any other title) as described in the By-Laws (Article Ⅰ, Section 1).

Section 3. Honorary Members:

a. Honorary members may be elected by the Commission according to the procedure outlined in the By-Laws (Article Ⅰ, Section 2).

b. Honorary membership shall be limited to living persons who have been before or, at the time of their election, are still regular members of the Commission. It shall be conferred only upon those individuals who have rendered especially meritorious service to the Commission and to the cause of international cooperation among protozoologists.

c. If, at the time of their election, or at any future date, honorary members are not among the regular members appointed by their national organization, they shall not affect the number of regular members to which each group is entitled.

d. Honorary members shall be invited to all meetings of the Commission and be accorded all the rights and privileges of regular members except voting on issues before the Commission, unless he or she is replacing an absent regular member of their respective national organization.

ARTICLE Ⅲ—Officers

Section 1. There shall be two officers of the Commission: The President and the Secretary General.

a. The President of the Commission is the presideng officer of the International Congress.

1. The term of office of the President shall be four (4) years commencing at the end of the preceding International Congress with duties as described in Article Ⅱ, Section 1 of the By-Laws.

2. A vacancy in the office of President shall be filled (elected or appointed) by his / her national organization.

b. The Secretary General shall be the chief liaison officer, serving the President and all regular and honorary members of the Commission.

1. The term of office of the Secretary General shall be four (4) years, commencing at the end of the preceding International Congress.

2. The Secretary General shall be appointed or elected by his / her national organization with duties as outlined in Article Ⅱ, Section 2 of the By-Laws.

3. A vacancy in the office of Secretary General shall be filled (elected or appointed) by his / her national organization.

ARTICLE Ⅳ—Meetings

Section 1. The Commission shall meet two (2) years after the last Congress in the country that will be hosting the next Congress and at each Congress, as deemed appropriate by the President and Secretary General.

Section 2. Site selection for an International Congress shall be made by the regular members of the International Commission upon the acceptance of a bid submitted by a regular member of a national organization. This bid must be communicated to each regular commissioner at least six (6) months prior to the next Congress at which the bid will be presented. The bid will be voted upon at a regular meeting of the Commission. In the event of more than one bid, vote will be taken by secret ballot.

Section 3. The Commission shall use internationally recognized Rules of Parliamentary Procedure. Resolutions are adapted by majority vote of the Commissioners present at a regular meeting of the Commission.

ARTICLE Ⅴ—Amendments

Amendments to the constitution shall require a two-thirds majority vote of all regular members. Amendments must be submitted to the Commission at least six (6) months prior to an International Congress. The voting shall be conducted by secret ballot and may need to be carried out by mail.

BY-LAWS

ARTICLE Ⅰ—Membership

Section 1. The method of appointing regular members shall be determined independently by each national organization. This group may also limit the tenure of its regular members to a certain number of terms.

a. Each national organization consisting of between 20 and 99 members shall be entitled to one (1) voting delegate; a group with between 100 and 299 members shall have two (2) voting delegates; a group of 300 or more protozoologists shall be allowed to send three (3) delegates. A group with fewer than 20 members may be represented by an observer without a vote.

b. The eligibility of a national organization to join the Commission and appoint regular members shall be determined by a petition submitted to the Commission at a regular meeting of the Commission and approved by said body by a majority vote of the regular members or their substituted present.

c. The term of office of a regular member shall begin at the conclusion of the International Congress that immediately follows his / her appointment by his / her national organization. Length of service of a regular member on the Commission is at the discretion of each national organization.

d. If for any reason a regular member is unable to serve, his national organization shall appoint another person to fill the vacancy. The name of the substitute member who may be an honorary member (Article Ⅱ, Section 3d, Constitution) shall be transmitted immediately to the Secretary general of the Commission. The substitute member shall enjoy all the rights and privileges of a regular member.

Section 2. Honorary members may be nominated by any national organization by a petition submitted by a regular or honorary member to the Secretary General at least six (6) months prior to a regular meeting of the Commission.

ARTICLE Ⅱ—Officers

Section 1. The presdient shall preside at all meetings of the Commission and assist the new President during the transition of the presidency, particularly at the interim meeting of the Commission.

Section 2. The Secretary General has the responsibility, along with the President and the local organizing committee, for all aspects of the details of organizing and running the next Congress. Ordinarily, he / she is the officer who communicates with potential participants in the Congress and coordinates

activities of the Commission during the interim period (4 years) between Congresses.

a. Shall correspond with the International Union of Biological Sciences.

b. Shall work closely with the Secretary Generals of the two preceding Congresses during the period prior to the next interim meeting.

ARTICLE Ⅲ—Meetings

Section 1. At the interim meeting, the hosts shall outline their recommendations for the timing and tentative program of the Congress. The time and the tentative program of the Congress will be reviewed and finalized by the Commission. Traditionally, food and lodging for this meeting are subsidized by the organizing committee of the host country.

Section 2. Two meetings shall be held during the Congress; the first to take place early in the Congress and the second just before the final plenary session. During the second meetindg, new members shall be introduced; honorary members, if any, elected; and bids for the site of the next Congress voted upon. In the event more than one bid is submitted, a secret ballot will be taken. A simple majority of all regular members voting will select the site of the next Congress.

Section 3. The host Society shall guarantee each national organization participation in the Congress as set forth in the Constitution and By-Laws of the International Union of Biological Sciences.

ARTICLE Ⅳ—Amendments

Amendments may be made by simple majority of all regular members at a regular meeting of the Commission. The voting shall be conducted by secret ballot and may need to be carried out by mail.

第8回国際原生動物学会議プログラム

PLENARY LECTURES

P-1. THE KARYORELICTID NUCLEAR APPARATUS : A MODEL OF NUCLEAR DIFFERENTIATION
Igor B. Raikov (Chairperson : Hidemi Sato)

P-2. THE CONTRIBUTION OF HYPOTRICH CILIATES TO OUR UNDERSTANDING OF MOLECULAR BIOLOGY AND EVOLUTION OF CILIATES
Dieter Ammermann (Chairperson : Yoshio Watanabe)

P-3. CILIA IN CELL MOTILITY : MEMBRANE-CONTROLLED ROTARY ENGINES
Hans Machemer (Chairperson : Yutaka Naitoh)

P-4. MOLECULAR BASIS OF DIFFERENTIATION IN AFRICAN TRYPANOSOMES
George C. Hill (Chairperson : Toshio Nakabayashi)

P-5. ADVANCES IN THE BIOLOGY OF INTESTINAL PROTOZOA : *ENTOAMOEBA HISTOLYTICA* AND *GIARDIA LAMBLIA*
Victor Tsutsumi (Chairperson : Tsutomu Takeuchi)

P-6. THE ROLE OF PROTOZOA IN NATURE IN TERMS OF PHYSIOLOGICAL CONSTRAINTS OF PROTOZOAN ORAGANIZATION
Tom Fenchel (Chairperson : Akira Taniguchi)

SYMPOSIA

S1. GENOME ORGANIZATION AND REORGANIZATION IN CILIATED PROTOZOA
Chairperson : E. Orias
Vice-Chairperson : T. Higashinakagawa

S1-1. Ⅰ. OVERVIEW OF NUCLEAR DIMORPHISM AND PROGRAMMED GENOME REORGANIZATION DURING CILIATE DEVELOPMENT
Ⅱ. STOCHASTIC VARIATION IN THE RATIO OF ALLELIC rDNAs IN NEWLY DEVELOPED HETEROZYGOUS MACRONUCLEI OF *TETRAHYMENA THERMOPHILA*
E. Orias and A. D. Bradshaw

S1-2. CIS-ACTING SEQUENCES WHICH REGULATE CHROMOSOME BREAKAGE AND DNA DELETION IN *TETRAHYMENA*
M. -C. Yao, C. H. Yao and R. Godiska

S1-3. GENOME AND GENE ORGANIZATION AND REORGANIZATION IN HYPOTRICH CILIATES
D. Ammermann

S2. PROTOZOAN GENES : THEIR STRUCTURE, PRODUCTS AND REGULATION OF EXPRESSION
Chairperson : J. R. Preer, Jr.
Vice-Chairperson : Y. Watanabe

S2-1. PROTOZOAN GENES : WITH SPECIAL REFERENCE TO THE GENES FOR *TETRAHYMENA* ACTIN AND CALCIUM-BINDING PROTEINS
Y. Watanabe, M. Hirono and T. Takemasa

S2-2. STRUCTURE AND EXPRESSION OF *EUPLOTES RAIKOVI* MATING PHEROMONE GENES
C. Miceli, A. La Terza and P. Luporini

S2-3. THE DEVELOPMENT OF MOLECULAR BIOLOGY IN AFRICAN TRYPANOSOMES THROUGH STUDIES ON VARIANT SURFACE GLYCOPROTEIN (VSG) GENES
N. B. Murphy

S2-4. REMODELLING OF THE A SEROTYPE GENE DURING MACRONUCLEAR DEVELOPMENT IN

PARAMECIUM
J. R. Preer, Jr., B. M. Rudman and L. B. Preer

S3. REGULATION OF CELL STRUCTURE
Chairperson : M. Jerka-Dziadosz
Vice-Chairperson : J. Frankel

S3-1. FROM CILIATE ONTOGENY TO CILIATE PHYLOGENY
C. F. Bardele

S3-2. REVERSAL OF LARGE-SCALE CORTICAL ASYMMETRY IN CILIATES
J. Frankel

S3-3. DYNAMICS OF THE CORTICAL CYTOSKELETION DURING CELL DIVISION IN *PARAMECIUM*
F. Iftode, J. Cohen, F. Ruiz, A. T. Rueda, L. Chen-Shan, A. Adoutte and J. Beisson

S3-4. MOLECULAR MECHANISMS OF FURROW FORMATION DURING CELL DIVISION IN *TETRAHYMENA*
H. Ohba, M. Hirono and Y. Watanabe

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S4. CELL INTERACTIONS IN SEXUAL PHENOMENA
Chairperson : P. Luporini
Vice-Chairperson : K. Heckmann

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H. Urushihara and K. Yanagisawa

S4-2. GAMETE-GAMETE RECOGNITION IN *CHLAMYDOMONAS EUGAMETOS*
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S4-3. MOLECULAR SIGNALS IN SEXUAL INDUCTION OF THE GREEN ALGA *VOLVOX CARTERI*
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S4-4. AMINO ACID SEQUENCE ANALYSIS OF MATING PHEROMONES OF THE CILIATE *EUPLOTES RAIKOVI*
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S4-5. GAMONES AND GAMONE-SPECIFIC RECEPTORS IN *EUPLOTES OCTOCARINATUS*
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S5. ROLE OF CELL MEMBRANES AND CYTOSKELETAL ORGANIZATION IN CELL MOTILITY
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Vice-Chairperson : H. Machemer

S5-1. ELECTROPHYSIOLOGISTS' THOUGHTS ON *PARAMECIUM* MOVEMENT, EXCITATION AND ADAPTATION
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S5-6. BIOELECTRIC CONTROL OF THE TENTACLE MOVEMENT IN THE DINOFLAGELLATE *NOCTILUCA*
Y. Naitoh and K. Oami

S6. CYTOLOGY AND CYTOCHEMISTRY OF RUMEN PROTOZOA
Chairperson : J. Grain
Vice-Chairperson : W. van Hoven

S6-1. GENERAL SYSTEMATICS AND PLANS OF ORGANIZATION
J. Grain

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W. van Hoven

S6-3. PRINCIPAL ROLES OF THE RUMEN CILIATES
B. A. Dehority

S6-4. COMPARATIVE MORPHOLOGY BY NEW SCANNING ELECTRON MICROSCOPIC TECHNIQUES
S. Imai

S6-5. THE CYTOLOGY OF ENTODINIOMORPHID CILIATES
R. D. Butler

S6-6. A REVIEW OF THE IMMUNOBIOCHEMICAL AND IMMUNOLOGICAL STUDIES OF PROTEINS OF CORTICAL CYTOSKELETON IN RUMEN CILIATES
J. Grain, B. Vigues, M. Jadal and D. David

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S6-8. HYDROGENOSOMES IN RUMEN CILIATE PROTOZOA
R. G. Paul, R. D. Butler and A. G. Williams

S7. IMMUNOLOGY AND CHEMOTHERAPY OF PLASMODIUM
Chairperson : R. Nussenzweig
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S7-1. CALCIUM/CALMODULIN FUNCTIONS IN *PLASMODIUM FALCIPARUM IN VITRO* ; IMPLICATIONS FOR ANTIPROTOZOAL DRUG DESIGN
L. W. Scheibel

S7-2. MOLECULAR DIFFERENCES BETWEEN HIGH VIRULENCE *PLASMODIUM BERGHEI* (NK65) AND ITS PERMANENT ATTENUATED DERIVATIVE
M. Suzuki

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R. J. Howard

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S8-1. VARIABLE ANTIGEN TYPES OF *TRYPANOSOMA BRUCEI* AND PERSPECTIVES FOR VAC-

CINATION IN AFRICAN TRYPANOSOMIASIS
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S8-2. RECENT ADVANCES IN THE BIOLOGY OF *TRYPANOSOMA RANGELI*
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S8-4. INSTABILITY OF THE NUCLEAR CHROMATIN OF *TRYPANOSOMA BRUCEI BRUCEI* PROCYCLIC CULTURE FORMS
H. Hecker, K. Bender, U. -P. Modespacher and B. Betschart

S9. CYST-FORMING COCCIDIA
Chairperson : H. Mehlhorn
Vice-Chairperson : M. B. Markus

S9-1. FINE STRUCTURAL ASPECTS OF TISSUE CYST TYPES IN CYST FORMING COCCIDIA
H. Mehlhorn

S9-2. CYSTOISOPORA : RECENT ADVANCES
M. B. Markus

S9-3. CURRENT KNOWLEDGE OF CYST-FORMING COCCIDIA IN JAPAN
K. Shimura and S. Ito

S9-4. NEW APPROACHES TO LIFE CYCLES OF *BESNOITIA* AND *SARCOCYSTIS*
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S9-5. RECENT STUDIES ON THE BIOLOGY OF *CARYOSPORA BIGENETICA*
C. A. Sundermann

S9-6. IN VITRO CULTIVATION OF THE CYST-FORMING COCCIDIA
C. A. Speer

S9-7. MOLECULAR BIOLOGY OF THE CYST-FORMING COCCIDIA
A. M. Johnson

S10. SURVIVING MECHANISMS OF INTRACELLULAR PROTOZOA
Chairperson : L. H. Bannister
Vice-Chairperson : S. Takada

S10-1. MECHANISMS AND FUNCTIONAL ROLES OF CALCIUM ACQUISITION BY ERYTHROCYTIC MALARIA PARASITES
L. H. Bannister, S. Krishna, K. J. H. Robson and G. H. Mitchell

S10-2. CHARACTERIZATION OF THE 85 KILODALTON SURFACE ANTIGEN GENE OF *TRYPANOSOMA CRUZI*
D. L. Fouts and J. E. Manning

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S11. PIROPLASMIDS

Chairperson : A. Musoke
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S11-2. SUBUNIT IMMUNIZATION AGAINST BOVINE BABESIOSIS-EXPERIENCE WITH *BABESIA BIGEMINA* USING MEROZOITE SURFACE PROTEINS
T. F. McElwain, L. E. Perryman, W. C. Davis and T. C. McGuire

S11-3. IMMUNOGENIC ANTIGENS OF *BABESIA DIVERGENS*
C. M. Winger and E. U. Canning

S11-4. MOLECULAR APPROACHES TO THE DEVELOPMENT OF A VACCINE AGAINST EAST COAST FEVER
V. Nene, K. P. lams and A. J. Musoke

S12. DYNAMICS OF ECOLOGY OF FREE-LIVING PROTOZOA
Chairperson : W. Foissner
Vice-Chairperson : V. -F. Shen

S12-1. PROTOZOA IN ACTIVATED SLUDGE
P. Madoni

S12-2. PROTOZOAN ECOLOGY IN AQUACULTURE
M. Maeda

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W. Foissner

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Y. -F. Shen, M. -R. Gu, J. -P. Cai and W. -S. Feng

S13. ENDOSYMBIONTS OF PROTOZOA
Chairperson : K. W. Jeon
Vice-Chairperson : M. Fujishima

S13-1. OVERVIEW : ENDOSYMBIONTS (ENDOCYTOBIONTS, XENOSOMES) OF PROTOZOA
J. O. Corliss

S13-2. INFECTION AND MAINTENANCE MECHAINSMS OF ENDONUCLEAR SYMBIONT *HOLOSPORA OBTUSA* IN *PARAMECIUM CAUDATUM*
M. Fujishima

S13-3. CHLOROPLAST RETENTION IN ELPHIDIDS (FORAMINIFERA)
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A. T. Soldo

S13-5. BACTERIAL ENDOSYMBIONTS IN AMOEBAE
K. W. Jeon

S14. PHYLOGENY AND EVOLUTION OF PROTOZOA
Chairperson : A. M. Johnson
Vice-Chairperson : M. Suhama

S14-1. ARCHEZOA AND THE ORIGIN OF PROTOZOA
T. Cavalier-Smith

S14-2. A BROAD PHYLOGENY OF THE PROTISTS BASED ON PARTIAL SEQUENCES OF LARGE RIBOSOMAL RNA

A. Baroin, R. Perasso and A. Adoutte

S14-3. CILIATE SYSTEMATICS : ADAPTIVE ASPECTS AND EVOLUTION
D. H. Lynn

S14-4. PHYLOGENY OF THE APICOMPLEXA
A. M. Johnson

S14-5. ORIGIN AND DIVERSIFICATION OF PROTISTS WITH TUBULAR MITOCHONDRIAL CRISTAE
R. A. Andersen

S15. MARINE PROTOZOOPLANKTON
Chairperson : F. Rassoulzadegan
Vice-Chairperson : T. Fenchel

S15-1. BEHAVIOUR OF PROTOZOA BELONGING TO DIFFERENT TYPES OF HABITATS
T. Fenchel

S15-2. MIXOTROPHY IN THE MARINE PROTISTS
M. Laval-Peuto

S15-3. CHEMOSENSORY RESPONSES AND PARTICLE SELECTION IN MARINE PLANKTONIC CILIATES
P. G. Verity

S15-4. BACTERIA OR ALGAE AS MAJOR FOOD SOURCE FOR MICRO-ZOOPLANKTONIC CILIATES : A COMPARATIVE STUDY
F. Rassoulzadegan and C. Bernard

S16. ROLE OF BIOTECHNOLOGY IN RECOGNITION OF HOMOLOGY
Chairperson : J. O. Corliss
Vice-Chairperson : N. N. Sukhareva

S16-1. INTRODUCTORY REMARKS ON THE ROLE OF BIOTECHNOLOGY IN RECOGNITION OF HOMOLOGIES AMONG AND WITHIN PROTIST GROUPS
J. O. Corliss

S16-2. THE USE OF RAPID FREEZING AND FREEZE SUBSTITUTION TECHNIQUES FOR PREPARATION OF PROTISTS IN ULTRASTRUCTURAL AND IMMUNOCYTOLOGICAL STUDIES
M. A. Farmer

S16-3. NUCLEAR CONTROL OF CORTICAL DEVELOPMENT IN SEXUAL REPRODUCTION OF HYPOTRICHOUS CILIATES : AN EVOLUTIONARY INTERPRETATION
S. F. Ng

S16-4. EXPRESSIONS OF HOMOLOGY : FROM STRUCTURAL ORAGANIZATION TO RECOGNITION AND MOLECULAR COMPOSITION
J. J. Paulin and C. A. Sundermann

S16-5. MODELING ANCIENT MICROBIAL ECOSYTEMS : THE PHYSIOLOGY OF MODERN MICROBIAL MATS AS A HOMOLOG
L. J. Rothschild

S16-6. THE USE OF MONOCLONAL ANTIBODIES TO DETERMINE HOMOLOGIES IN PROTISTS
F. W. Spiegel

CONTRIBUTED PAPER SESSIONS (PLATFORM)

11B. MOLECULAR BIOLOGY AND GENETICS
Chairperson : G. Cleffmann
演題数：8題

11C. IMMUNOLOGICAL APPROACHES IN PARASITIC PROTOZOA
Chairperson : N. Suzuki
演題数：6題

11D. SYSTEMATICS AND TECHNOLOGIES IN THE IDENTIFICATION OF PROTOZOA-1
Chairperson : L. H. Otieno
演題数：4題

11E. CELL ORGANELLES AND ENDOSYMBIONTS OF PROTOZOA
Chairperson : A. T. Soldo
演題数：5題

12B. CYTOSKELETON, CELL MOTILITY AND BEHAVIOR-1
Chairperson : H. Asai
演題数：8題

12C. ACTIONS OF EXTERNAL AGENTS AND DRUGS
Chariperson : D. M. Saxena
演題数：10題

12D. ECOLOGY AND ADAPTATION OF PROTOZOA-1
Chairperson : D. Lynn
演題数：4題

12E. MORPHOGENESIS AND LIFE CYCLE
Chairperson : J. R. Nilsson
演題数：5題

15B. MITOSIS, MEIOSIS AND CONJUGATION
Chairperson : K. Heckmann
演題数：8題

15C. DISEASES CAUSED BY PROTOZOA-Ⅱ
Chairperson : H. G. Heidrich
演題数：7題

15D. BIOCHEMISTRY AND PHYSIOLOGY OF PROTOZOA
Chairperson : L. Kuźnicki
演題数：11題

15E. MORPHOGENESIS AND LIFE CYCLE-Ⅱ
Chairperson : T. Beyer
演題数：7題

17B. CYTOSKELETION, CELL MOTILITY AND BEHAVIOR-Ⅱ
Chairperson : C. Bardele
演題数：6題

17C. DISEASES CAUSED BY PROTOZOA-Ⅲ
Chairperson : H. Hirumi
演題数：7題

17D. SYSTEMATICS AND TECHNOLOGIES IN THE IDENTIFICATION OF PROTOZOA-Ⅱ
Chairperson : K. Hausmann
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17E. ECOLOGY AND ADAPTATION OF PROTOZOA-Ⅱ
Chairperson : M. Sudzuki
演題数：5題

CONTRIBUTED PAPER SESSION (POSTER Ⅰ)

演題数：49題

CONTRIBUTED PAPER SESSION (POSTER Ⅱ)

演題数：46題

POST-CONGRESS WORKSHOP

"Advances in Basic Techniques of Protozoology for Evaluation of Population Ecology and Pollution Abatement"

Chairperson : J. J. Lee

Coordinator : K. Ishii

平成元年度幹事会

日　時：平成元年12月９日

場　所：東京都私学会館

出席者：石井圭一，尾崎文雄，重中義信，竹内　勤，高田季久，中林敏夫，野沢義則，通渡宏一，藤田潯吉，渡辺良雄　(50音順)

議　題：総会提出議題の検討

1. **会長交代の件**

　藤田潯吉会長の辞任が承認され，渡辺良雄幹事が次期会長になることを全員一致で決定した。

2. **次期大会長及び開催地**

　金田良雅(東海大学医学部奇生虫学)，伊勢原市

第１回大会以来の開催地及び大会長

	開催地	開催年度	大会長
第１回	小平市	昭和42年	藤田　潯吉
第２回	吹田市	昭和43年	猪木　正三
第３回	広島市	昭和44年	尾崎　佳正
第４回	東京都	昭和45年	松林　久吉
第５回	徳島市	昭和46年	尾崎　文雄
第６回	仙台市	昭和47年	樋渡　宏一
第７回	奈良市	昭和48年	稲葉　文枝
第８回	東京都	昭和49年	石井　圭一
第９回	大阪市	昭和50年	高田　季久
第10回	東京都	昭和51年	盛下　　勇
第11回	岐阜市	昭和52年	野沢　義則
第12回	横浜市	昭和53年	斎藤　　実
第13回	吹田市	昭和54年	中林　敏夫
第14回	茨城県	昭和55年	渡辺　良雄
第15回	広島市	昭和56年	重中　義信
第16回	東京都	昭和57年	石井　俊雄
第17回	津　市	昭和58年	安達　六郎
第18回	東京都	昭和59年	浅見　敬三
第19回	大分県	昭和60年	山高　里盛
第20回	東京都	昭和61年	小山　　力
第21回	山口市	昭和62年	星出　一巳
第22回	つくば市	昭和63年	渡辺　良雄
第８回	国際原生動物学会		つくば市

会 員 名 簿

賛助会員（順不同）　　（〒・所在地・住所）

武田薬品工業㈱畜産事業部	532	大阪市淀川区十三本町２丁目17－85
第一製薬㈱特薬部動薬課	103	東京都中央区日本橋３の14の10

正 会 員

	（所　属）	（〒・所在地・住所）	（電話）
【A】			
朝日博子	国立予防衛生研究所	141 東京都品川区上大崎2-10-35	(03)444-2181
安達六郎	三重大学生物資源学部	514 津市上浜町1515	
阿部弘和	山口大学教育学部生物教室	753 山口市大字吉田	(08392)2-6111
阿倍正史	昭和大学医学部医動物学教室	142 東京都品川区旗の台1-5-8	(03)784-8000
青木　孝	順天堂大学医学部寄生虫学教室	113 東京都文京区本郷2-1-1	(03)813-3111
赤尾信吉	防衛医科大学寄生虫学教室	359 所沢市所沢500	(0429)95-1211
浅井　博	早稲田大学理工学部	160 東京都新宿区大久保3-4-1	(03)209-3211
浅井良紀	㈱環境調査技術研究所技術研究部	108 東京都港区三田1丁目4-28 三田国際ビル23Ｆ	(03)452-6461
荒川　皓	大阪府立大学農学部獣医学科 家畜内科学教室	591 堺市百舌鳥梅町4-804	(0722)52-1161
荒木恒治	奈良県立医科大学寄生虫学教室	634 奈良県橿原市四条町840	(07442)2-3051
麻布大学附属図書館		229 相模原市渕野辺1丁目17-71	(0427)54-7111
安　泳謙	Dept. of Parasitology, Yonsei Univ. Medical College,	Shindhon-dong, Seodaimoon-ku Seoul, Korea	
安藤博司		283 千葉県東金市山口509-27	
安藤元紀	広島大学総合科学部	730 広島市中区東千田町1-1-89	(082)241-1221
【B】			
坂野喜子	岐阜大学医学部生化学教室	500 岐阜市司町40	(0582)65-1241
【C】			
趙　基穆		595 大阪府泉大津市旭町23-57	(0725)22-7584
【E】			
遠藤卓郎	国立予防衛生研究所寄生虫部	141 東京都品川区上大崎2-10-35	(03)444-2181
【F】			
福井啓二	㈱メイテック東京美術開発センター	160 東京都新宿区西新宿4-15-3 三省堂新宿ビル4Ｆ	
福間利英	長崎大学熱帯医学研究所原虫学部門	852 長崎市坂本町12-4	(0958)47-2111
福森義弘	東京工業大学理学部生命理学科	152 東京都目黒区大岡山2-12-1	(03)726-1111
藤岡　寿	名古屋大学医学部医動物学教室	466 名古屋市昭和区鶴舞町65	(052)741-2111
藤崎幸蔵	農林水産省家畜衛生試験場	305 茨城県つくば市観音台3-1-1	
藤島政博	山口大学理学部生物学教室	753 山口市大字吉田1677-1	(0839)22-6111
藤田滓吉	日本獣医畜産大学	180 武蔵野市境南町1-7-1	(0422)31-4151
古谷正人	高知医科大学附属動物実験施設	781-51 南国市岡豊町小蓮	(0888)66-5811
【H】			
萩原博光	国立科学博物館植物研究部微生物研究室	205 茨城県つくば市 並木4-410-303	
林　弘三	徳島大学総合科学部保健科学教室	770 徳島市南常三島町1丁目	(0886)23-2311
林　芳生	三重県立上野高等学校	518 三重県上野市丸之内107	
林　新治	藤女子大学生物学教室	001 札幌市北区北16条西2丁目	

姫野國祐	徳島大学医学部寄生虫学教室	770	徳島市蔵本町3	(0886)31-3111
比留木武雄	島根医科大学微生物免疫学教室	693	出雲市塩治町89-1	(0853)23-2111
広野雅文	筑波大学生物科学系	305	茨城県つくば市天王台1-1-1	(0298)53-2111
樋渡宏一	石巻専修大学理工学部	986	宮城県石巻市南境新水戸1	(0225)22-7716
星出一巳	山口大学教育学部生物学教室	753	山口市吉田	(0839)22-6111
堀江秀光	微生物化学研究所	141	東京都品川区上大崎3-14-23	(03)441-4173
堀上英紀	法政大学教養学部生物学教室	102	東京都千代田区富士見2-17-1	(03)264-9111

【I】

五十嵐慎	大阪大学微生物病研究所原虫学部門	565	吹田市山田丘3番1号	(06)877-5121
井関基弘	大阪市立大学医学部医動物学教室	545	大阪市阿倍野区旭町1-4-54	(06)633-1221
伊藤進午	農林水産省家畜衛生試験場	305	茨城県つくば市観音台3-1-1	(02975)6-7753
伊藤義博	徳島大学医学部寄生虫学教室	770	徳島市蔵本町3丁目	(0886)31-3111
石井明	岡山大学医学部寄生虫学教室	700	岡山市鹿田町2-5-1	(0862)23-7151
石井圭一	法政大学教養学部生物学教室	102	東京都千代田区富士見2-17-1	(03)264-9111
石井俊雄	日本獣医畜産大学寄生虫学教室	180	武蔵野市境南町1-7-1	(0422)31-4151
石田正樹	広島大学総合科学部	730	広島市中区東千田町1-1-89	(082)241-1211
磯部尚	農林水産省家畜衛生試験場鶏病支場	501-32	岐阜県関市倉地4909-58	(0575)22-7125
今井壮一	日本獣医畜産大学寄生虫学教室	180	武蔵野市境南町1-7-1	(0422)31-4151
岩月謙司	日本石油化学㈱筑波生命科学研究所	300-26	つくば市東光台5-9-9	
岩渕功		285	佐倉市鏑木町631（自宅）	
岩本哲人		673	明石市松が丘2丁目5-1-502	

【K】

加藤定吉		671-22	姫路市白鳥台1-7-10	
彼谷邦光	国立公害研究所	305	茨城県つくば市小野川	(0298)51-6111
柿市徳英	日本獣医畜産大学寄生虫学教室	180	武蔵野市境南町1-7-1	(0422)31-4151
角本正明	㈱環境調査技術研究所	253	茅ケ崎市中海岸3-2-14(自宅)	(03)452-6461
金田良雅	東海大学医学部寄生虫学教室	259-11	神奈川県伊勢原市望星台	(0463)93-1121
神谷正男	北海道大学獣医学部家畜寄生虫病学教室	060	札幌市北区北18条西9丁目	(011)716-2111
亀谷了	目黒寄生虫館	153	東京都目黒区下目黒4-1-1	(03)716-1264
亀山泰永	朝日大学歯学部口腔生化学教室	501-02	岐阜県本巣郡穂積町大字穂積1851-1	(05832)6-6131
河村信夫	東海大学医学部秘尿器科学教室	259-11	神奈川県伊勢原市望星台	(0473)93-1121
川上久子	鈴峯女子短期大学	733	広島市西区井口4丁目6-18	
川端善一郎	愛媛大学農学部生物資源学科生物環境保全	790	松山市樽味3-5-7	(0899)24-4171
神立誠		300-42	茨城県つくば市泉847-1	
神原広二	長崎大学熱帯医学研究所原虫学部門	852	長崎市坂本町12-4	(0958)47-2111
菅野康則	麻布大学獣医学部伝染病学教室	229	神奈川県相模原市渕野辺1-17-71	(0427)54-7111
韓載琴	釜山女子大学		釜山市東萊区蓮山洞822-64	
木原章	法政大学第1教養学部生物学教室	102	東京都千代田区富士見2-17-1	(03)264-9111
北潔	順天堂大学医学部寄生虫学教室	113	東京都文京区本郷2-1-1	(03)813-3111

久保龍三		660	尼崎市杭瀬本町1丁目9番6号	(06)489-3532
熊田三由	国立予防衛生研究所寄生虫部	141	東京都品川区上大崎2-10-35	(03)444-2181
熊沢秀雄	高知医科大学寄生虫学教室	781-51	高知県南国市岡豊町小蓮	(0888)66-5811
栗原康		982	仙台市八木山本町1-16-22	
肥沼昭	信州大学理学部生物学教室	390	長野県松本市旭3-1-1	(0263)35-4600
小泉貞明		982	仙台市太白区鈎取1-8-7	
小長谷史朗	水産庁中央水産研究所	104	東京都中央区勝どき5-5	(03)531-1221
小阪敏和	広島大学理学部動物学教室	730	広島市中区東千田町1-1-89	(082)241-1221
小林昭夫	東京慈恵会医科大学寄生虫学教室	105	東京都港区西新橋3-25-8	(03)433-1111
小山力		181	東京都三鷹市野崎1-4-2	(0422)43-5793
【L】				
季原在	釜山水産大学海洋学科	608	韓国釜山市南区 大渕洞5p9-1	
【M】				
前田昌調	水産庁養殖研究所環境管理部 餌料生物研究室	516-01	三重県度会郡南勢町 中津浜浦422-1	(05996)6-1830
牧岡朝夫	東京慈恵会医科大学寄生虫学教室	105	東京都港区西新橋3-25-8	(03)433-1111
町田昌昭	国立科学博物館動物研究部	160	東京都新宿区百人町3-23-1	(03)364-2311
松井利博	杏林大学医学部寄生虫学教室	181	三鷹市新川6丁目20-2	(0422)47-5511
松坂理夫	熊本大学理学部寄生虫学教室	860	熊本市黒髪2-39-1	(0963)44-2111
松林隆房	大阪府立万代診療所	558	大阪市住吉区万代東3-1-45	
丸山正	東亜燃料工業㈱総合研究所 技術開発研究所	354	埼玉県入間郡大井町 鶴ヶ岡175	(0492)64-8140
見上一幸	宮城教育大学理科教育研究施設	980	仙台市荒巻字青葉	(0222)22-1021
三島祥二	茨城大学教養部	310	水戸市文京2-1-1	
南哲郎	農林省家畜衛生試験場	305	茨城県つくば市観音台3-1-1	(02975)6-7749
宮田彬	大分医科大学生物学教室	879-56	大分県大分郡挾間町 医大ヶ丘1-1506	(0975)49-4411
三輪五十二	茨城大学教養部生物学教室	310	水戸市文京2-1-1	
盛下勇	㈱環境調査技術研究所技術研究部	108	東京都港区三田1-4-28 三田国際ビル23F	(03)452-6461
森田順一	県立弥栄西高等学校	222	横浜市港北区篠原町2829	(045)433-8051
【N】				
内藤豊	筑波大学生物科学系	305	茨城県つくば市天王台1-1-1	(0298)53-2111
永倉貢一	東海大学医学部寄生虫学教室	259-11	神奈川県伊勢原市望星台	(0473)93-1121
中林敏夫	藤田学園保健衛生大学医学部寄生虫学教室	470-11	豊明市沓掛町田楽ヶ窪1-98	(0562)93-2435
中村和郎	Dept. of Bilogical Sciences, University of Lethbridge		Lethbridge, Alberta, Canada	
西出昌之	㈱環境保健生物研究センター検疫部	528	滋賀県甲賀郡水口町 大字川字稲場555	(07486)2-2316
沼田治	筑波大学生物科学系	305	茨城県つくば市天王台1-1-1	(0298)53-4530
沼田静香	北鎌倉高等学校	251	藤沢市辻堂1192-1	
野田亮二		248	鎌倉市腰越1718-93	
野沢義則	岐阜大学医学部生化学教室	500	岐阜市司町40	(0582)65-1241
【O】				
大場浩美	東京理科大学基礎工学部生物工学科	278	千葉県野田市山崎2641	(0471)24-1501
小野忠相	大阪府立大学農学部家畜病理学内疫学	591	堺市百舌鳥梅町4-804	
小野寺良次	宮崎大学農学部畜産学科家畜飼養学講座	889-21	宮崎市学園木花台西1-1	(0985)58-1116

小國昭信	神戸常盤短期大学	655	神戸市長田区大谷町2-6-2
尾崎文雄		655	神戸市垂水区瑞ヶ丘5番18号 (078)707-2390
扇元敬司	東北大学農学部	980	仙台市堤通雨宮町1-1 (0222)72-4321
大島慧	田辺製薬㈱特約業務室	541	大阪市東区道修町3-21
大島範子	東邦大学理学部生物学科	274	船橋市三山2-2-1 (0474)72-1141
大村浣	警友総合病院	231	横浜市旭区川島町2950-100
大家裕	順天堂大学医学部寄生虫学教室	113	東京都文京区本郷2-1-1 (03)813-3111
岡健司	㈱日本海洋生物研究所	142	東京都品川区豊町4-3-16 (03)787-2471
岡三希生	徳島大学医学部寄生虫学教室	770	徳島市蔵本町3丁目 (0886)31-3111
長田信		238	横須賀市佐野町6-35
落合勉		376	桐生市広沢町5-248 フジハイツ502 (0277)53-4066
【R】			
林漢鐘	Dept. of Parasitology, College of Medicine, Seoul National University		Seoul, Korea
【S】			
佐伯英治	日本獣医畜産大学寄生虫学教室	180	武蔵野市境南町1-7-1 (0422)31-4151
佐藤忠文	香川医科大学生物学教室	761-07	香川県木田郡三木町 (0878)98-5111
佐藤勝幸	鳴門教育大学学校教育学部 自然系教育講座	772	徳島県鳴門市鳴門町高島 (0886)87-1311
斉藤実		251	藤沢市鵠沼藤が谷4-14-29
坂本司	岩手大学農学部獣医学科 家畜寄生虫学教室	020	盛岡市上田3-18-8 (0196)23-5171
志賀正男	青山学院高等部	178	東京都練馬区南大泉2-31-10 (自宅)
島村初太郎	法政大学教養学部生物研究室	102	東京都千代田区富士見2-17-1 (03)264-9111
重中義信	広島大学総合科学部細胞生物学研究室	730	広島市中区東千田町1-1-89 (082)241-1221
志村亀夫	農林水産省家畜衛生試験場鶏病支場	501-32	岐阜県関市倉知4909-58
末広誠一		270	松戸市金ケ作364-11
末広誠之	生物活性科学研究所	673-14	兵庫県加東郡社町木梨川北山442-5
菅井俊郎	茨城大学理学部生物学教室	310	水戸市文京2-1-1 (0292)26-1621
菅沼美子	奈良佐保女子学院短期大学	630	奈良市鹿野園806
洲崎敏伸	広島大学総合科学部	730	広島市中区東千田町1-1-89 (082)241-1221
洲浜幹雄	広島大学理学部動物学教室	730	広島市中区東千田町1-1-89 (082)241-1221
鈴木實	日本大学法学部一般教育研究室	330	埼玉県大宮市東新井
鈴木直義	帯広畜産大学獣医生理学教室	080	帯広市稲田町 (0155)48-5111
鈴木守	群馬大学医学部寄生虫学教室	371	前橋市昭和町 (0272)31-7221
鈴木了司	高知医科大学寄生虫学教室	781-51	南国市岡豊町小蓮 (0888)66-5811
芹沢直樹		193	東京都八王子市台町2-20-16（自宅）
【T】			
田辺和裕	大阪工業大学生物学研究室	535	大阪市旭区大宮5丁目16-1
高木由臣	奈良女子大学理学部生物学教室	630	奈良市北魚屋西町 (0742)23-1131
高田季久	大阪市立大学医学部医動物学教室	545	大阪市阿倍野区旭町1-4-54 (06)633-1221
高橋永治	山形大学理学部生物学科	990	山形市小白山町1-4 (0236)31-1421
高橋忠夫	広島大学理学部動物学教室	730	広島市中区東千田町1-1-89 (082)241-1221
高橋三保子	筑波大学生物科学系	305	茨城県つくば市天王台1-1-1 (0298)53-2111

竹内　勤	慶応義塾大学医学部寄生虫学教室	160	東京都新宿区信濃町35	(03)353-1211
武政　徹	筑波大学生物科学系	305	茨城県つくば市天王台1-1-1	(0298)53-2111
橘　裕司	東海大学医学部寄生虫学教室	259-11	神奈川県伊勢原市望星台	(0473)93-1121
谷口　旭	東北大学農学部水産学科	980	仙台市堤通雨宮町1-1	(0222)72-4321
月井雄二	法政大学教養学部生物学教室	102	東京都千代田区富士見2-17-1	(03)264-9111
鳥海三郎		247	横浜市栄区本郷台4-12-6	

【U】

上村春樹	長崎大学熱帯医学研究所原虫学部門	852	長崎市坂本町12-4	(0958)47-2111
宇仁茂彦	大阪市立大学医学部医動物学教室	545	大阪市阿倍野区旭町1-4-54	(06)645-2066

【W】

渡辺定博	岡山大学医学部第一解剖学教室	700	岡山市鹿田町2-5-1	(0862)23-7151
渡辺　彊	東北大学理学部生物学科	980	仙台市荒巻字青葉	(0222)22-1800
渡辺良雄	筑波大学生物科学系	305	茨城県つくば市天王台1-1-1	(0298)53-2111

【Y】

八木田健司	国立予防衛生研究所	141	東京都品川区上大崎2-10-35	(03)444-2181
柳生亮三		733	広島市西区高須4-88-4(自宅)	
保田友義	国立予防衛生研究所技術部	141	東京都品川区上大崎2-10-35	(03)444-2181
矢野健一	大阪大学微生物病研究所原虫学教室	565	大阪府吹田市山田丘3番1号	(06)877-5121
八島洋一	岩手医科大学教養部生物学教室	020	盛岡市本町通3-61-1	(0196)51-5111
山浦　常	東京女子医科大学寄生虫学教室	162	東京都新宿区河田町8-1	
山高里盛	大分医科大学生物学教室	879-55	大分県大分郡狭間町医大ヶ丘1506	(0975)49-4411
山本一彦	麒麟麦酒㈱開発科学研究所	370-12	高崎市宮原町3	(0273)46-1561
吉田幸雄	京都府衛生公害研究所	612	京都府伏見区村上町395	

（退会者）

大林正士，唐崎　正，長尾清治，高柳　坦，河合　清，田中朝雄，
稲葉文枝，石崎　達，佐々木　昇，荒井久人，岡　好万，堀　学

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岐阜市司町40
岐阜大学医学部生化学教室
日本原生動物学会事務局
電話（0582）65-1241（内線2230）

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8．単語，数式等が2行にわたるものをハイフンで分ける場合，もともとハイフンを要するものは，ハイフンの下に片仮名で **トルナ** と朱書する．

図，表及び写真等

1．図，表及び写真等は，いづれも1件毎に別紙とし，用紙の右肩部に刷り上がりの横幅（cm）を指定（本誌は横2段組みで，1段の幅は約7cm）して，右下端に**著者**（共著の場合は代表者）の**姓**を朱書する．

2．図及び表は白ケント紙あるいは淡青色方眼紙を用いる．凸版用のものは墨書きにする．説明文字はタイプトーン（市販）を用い適当な位置に張り付けるか，活字組込みを要する場合は軟質鉛筆書きにする．

3．図のトレースを要するものは右肩部に **トレース**（実費著者負担）と朱書する．

4．写真はのり付けすることなく，アルバム用コーナーで厚紙にとめること．

5．写真等を色刷りにする場合及びアート紙を使用する必要のあるときは，指定横幅の下に**色刷，アート**（実費著者負担）等朱書する．

6．本文中に挿入する写真は図として通算し，図表等の見出しの表現はTable 1，Fig. 1の如くする．

7．本文中の図，表，写真等の挿入位置は，原稿の左欄外に **Table 1，Fig. 1** 等朱書して示し，配列に希望のある場合は Table 1 | Table 2 | Table 3 の如く指示する．

8．図，表及び写真の用語などはすべて本文に準じ，説明一件ごとに別の紙にタイプする．

文　献

1．引用文献はA，B，C，順に一連番号を付け，

本文該当箇所には著者の姓(2名までは省略せずに)と年号を記載例4の如くしるす.

2. 文献は,著者氏名(共著者も全部),年号,表題,雑誌名,巻数,ページ数(原則として,はじめとおわり)を,単行本の分担執筆者のものを引用の場合は,表題の次に書名,著者名,巻数,発行者,発行地,ページ数の順にしるす(記載例5).

脚 注

脚注を用いる場合は,本文中の該当箇所を右肩に[1),2)]等を付け,その内容は各ページ毎に別紙に,符号と共にしるし,それぞれ冒頭に,**第○ページ脚注**と朱書,一括して本文の末尾,抄録の前につづり,本文該当ページの左下端に **注** と朱書しておく.

(例 1)

(論文表題)
(著 者 名)
(所 属)

(本 文) 30枚
(図) 3枚
(表) 3枚
(写 真) 9枚(1～6アート;2,2色刷) 枚 (原稿と印画紙であっても写真版を必要としないものは図の方に入れること)
(別刷数) 150部
(連絡先)

(例 2)

↑
約4cm
↓

STUDIES ON MAMMALIAN CELLES IN VITRO 2

↑
ダブルダブルスペース
↓

Quantinative studies on mammalian cells in vitro
2 An effective single-cell plating technique

↑
ダブルダブルスペース
↓

Taro Ueno, Jiro Nakayama and Saburo Hanaki
Department of Genetics, School of Medicine, Tokushima University,
Tokushima

↑
ダブルダブルスペース
↓

SUMMARY

→約3cm→××××××××××××××××××××××××, ××××××××××××××→約3cm→

↑
ダブルダブルスペース
↓

INTRODUCTION

××××××××××××××××××××××××××
××××××××××××××××××××××××××
××××××××××, ××××××××××××××××, ××××××××××

↑
約4cm
↓

（例　3）

1　△△△
1・1△△△
1・1・1　△△△
(1)　△△△
(a)　△△△
××××××××××××××××××××××
××××.
(b)　△△△
××××××××××××××××××××××
××××.
(2)　△△△
××××××××××××××××××××××
××××.
1・1・2　△△△
(1)　△△△
(a)　△△△
××××××××××××××××××××.
(b)　△△△
××××××××××××××××××××.
(2)　△△△

△　見出し題　　×　本文

（例　4）

1. According to the earlier papers (Hanson, 1960 ; Nielsen et al.,* 1962a ; Robinson and Smith, 1963 ; Corliss, 1966a, b), …………
2. Frenkel (1955) reported that ………………………………………
3. The two forms are readily distinguishable structurally Taliaferro, 1929 ; D'Alesandro, 1962).
4. Cooper et al. (1965 ; 1966) described lymphoid centers in the spleen, including ………

＊3人以上の場合

（例　5）

1　Taliaferro, W. H. and Pizzi. T. (1960) The inhibition of nucleic acid and protein synthesis in *Trypanosoma lewisi* by the antibody ablastin. Proc. Natl. Acad. Sci. USA,
←→ 46, 737-745
4字

単行本（全体）　2　Schnitzer, R. J. and Grunderg, E. (1975) Drug Resistance of Microorganisms, Academic Press. N. Y., pp. 395

単行本（一部）　3　Trager, V. (1967) Morphogenesis in Protozoa, Research in Protozoology (Chen,T. T., editor), Vol. 2, Pergamon Press, Oxford, 1-116

和 文 引 用　4　Kobayashi, A., Kumada, M. and Tsunematsu, Y. (1968) Standarization of the dye test for toxoplasmosis (2). On the use of plasma as the accessory factor. Jap. J. Parasit. 17, 81-85 (in Japanese with English Summary)

投 稿 規 定

1. 原稿は欧文で書き，原生動物に関係ある原著，総説，短報とする．
2. 投稿は日本原生動物学会会員に限る，共著の場合は少なくとも内1名は会員であることを要する．
3. 発行は年1回（各巻第2号）とし，他に大会抄録号（各巻第1号）を発行する．
4. 論文は刷り上り3ページを無料とし，超過ページおよび図版などは著者負担とする．
5. 別刷は50部までを無料とし，以上50部単位で著者負担とする．

日本原生動物学会会則

第１条　本会は日本原生動物学会（Japan Society of Protozoology）と称する．

第２条　本会は原生動物に関する研究をすすめ，その知識の普及，向上を図ることを目的とする．

第３条　第２条の目的を達成するために，年１回大会を開催し，会誌を発行するほか必要な事業を行う．
大会においては，本会の運営を議する総会および学術講演を行なう．総会および学術講演会は，それぞれ臨時にまたは別個に開くことができる．これらの会合は会長の委嘱する委員が運営する．
会誌は原則として年１回発行する．このほか，不定期に別刊を発行することがある．但し，別刊は実費をもって会員に配付する．

第４条　本会会員は正会員，賛助会員および名誉会員とする．正会員は年会費4,500円（学生の場合は2,000円）を前納するものとし，会の主催する各種の会合に参加し，幹事を互選し，会誌に投稿し，会誌の無料配付をうけることができる．賛助会員は本会の主旨に賛同し，会の維持発展のため１口10,000円以上を納入するものとし，会誌の無料配付をうけることができる．名誉会員は幹事会の推薦により総会において決定する．

第５条　本会運営のため，会長１名，幹事及び監事それぞれ若干名をおく，幹事は会員の互選により，監事は幹事会の議を経て幹事以外の会員から会長が依嘱し，会長は幹事の選挙によって決定する．
会長，幹事及び監事の任期は３年とし，会長及び幹事は重任を妨げない．
会長は会を代表して会務を統轄する．幹事は幹事会によって会務を処理し，庶務，会計および編集の業務を分担する．監事は会計を監査する．

第６条　本会の事務年度は暦年とする．

第７条　本会に入会を希望するものは，別に定める手続きによって申し込むものとする．

第８条　会則の変更は幹事会の議をへて，総会において行なう．

付　　則

1．本会の事務所は当分の間，岐阜大学医学部生化学教室内におく．

2．入会手続きは所定の申し込み用紙を用い，会費１年分以上を添えて会長あて事務局に提出するものとする．

3．納入した会費は返却しない．会費を２年間滞納した場合は退会とみなす．

編集後記

早いもので第８回国際原生動物学会（樋渡宏一会長）からすでに１年が経ちました。会員の先生方をはじめ関連の方々の御協力のお陰で成功裡に終えることができました。なお，名誉会長として御活躍いただきました藤田尋吉先生が国際学会終了を機会に日本原生動物学会会長を辞任され，新会長に渡辺良雄先生が推薦されました。藤田先生はかねてより新旧交代を申しておられ，国際学会までという条件で無理にお引受けいただいておりました。故猪木正三前会長についで会長として日本原生動物学会の発展に多大な尽力をされました。ここに会員一同厚くお礼申し上げます。また，新会長は筑波大学副会長の要職にあり，非常に多忙ななかを本学会の新しい発展のために頑張っておられます。

（野澤）

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Office of the Editorial Board
c/o Department of Biochemistry, Gifu University
School of Medicine
Tsukasamachi 40, Gifu 500, Japan