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DNA Fluorescence *in situ* Hybridization in *Paramecium*: Telomere Localization in Macronucleus

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ABSTRACT—In an attempt to develop a technique of fluorescence *in situ* hybridization (FISH) to detect DNA in *Paramecium*, we examined three different DNA probes, total genomic DNA, genomic DNA encoding C5 phagosomal membrane antigen, and telomere, prepared from *P. multimicronucleatum*. In accordance with the conventional method, total genomic DNA probe was denatured at 75–80°C for 2 min, and the cells were denatured at 75, 80, 85, or 90°C for 5 or 10 min. The homogeneous hybridization signal with the total genomic DNA probe was obtained at 85°C for 10 min, or at 90°C for 5 min or 10 min. This condition was applied for the smaller DNA probe, C5 (1. 3 kb, the size close to detection limits), in which the expected tiny signals throughout the macronuclear nucleoplasm was observed. However, the condition was not successful for the telomeric DNA probe. The hybridization signals of telomeric DNA were only detected when both cells and probes were denatured simultaneously in a same denaturation buffer. In the case of the simultaneously denatured samples, the preservation of the nuclear morphology was relatively poor, however, the signals of the telomeric DNA probe were observed in the periphery of the macronucleus. As a negative control, an irrelevant 40 kb human cosmid probe was examined by both conventional and simultaneous denaturation methods, and none of the hybridization signal was observed with this probe. These results suggest that the current methods allow us to follow localization of the specific sequences within the macronuclear compartment.

INTRODUCTION

Nuclear dimorphism is a characteristic feature of all ciliates. A vegetative cell of ciliate contains two types of nuclei with different structures and functions. Micronuclei are small and diploid. They remain transcriptionally inert through most of the life cycle. In contrast, macronuclei are large and highly polyploid. All vegetative transcription takes place in these macronuclei. In the course of sexual processes, the macronuclei stop replicating and eventually disappear, while micronuclei undergo meiosis, producing haploid gametic nuclei and transmit genetic information to the next sexual generation. After fertilization, new macronuclei differentiate from a half of mitotic products of the diploid zygotic nucleus while one of the other products becomes a new micronucleus. In *Paramecium*, the germ line genome is amplified to a high ploidy level (~1,000 n in *P. aurelia* species) during development of the macronucleus from a diploid nucleus, and extensively rearranged at specific sites (Reviewed by Prescott, 1994; Coyne *et al*., 1996). The chromosomes of *Paramecium* are fragmented into shorter, acentromeric molecules healed by the addition of telomeric repeats, and a number of internal sequences are eliminated from coding and noncoding sequences. The internal eliminated sequences (IESs) of *Paramecium* are short (28-882 bp), single-copy, AT-rich, noncoding elements that are invariably excised between 5'-TA-3' direct repeats, only one of which is left in the rearranged macronuclear sequence (Steele *et al*., 1994).

Paramecium DNAs, their spatial localization and orientation in the nucleus are, however, unclear. Even where DNA synthesis starts within their chromosome is unknown. It is also unclear how some tens of thousands of single-copy IESs is specifically recognized, and where the elimination of IESs occurs in the germ-line genomes. To ascertain these problems, a technique, the fluorescence *in situ* hybridization (FISH) of the DNA is necessary to be developed for *Paramecium*. Curtenaz and Beisson (1996) had already applied FISH for

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detecting mRNA (mRNA-FISH) transcribed from *Paramecium* β-tubulin gene. Their whole cell in situ hybridization methods took advantage of the fact that the different stages of the cell cycle are easily identifiable *in vivo* by the well-characterized morphological changes of the cell (Iftode, *et al*., 1989; Tucker *et al*., 1980), and allowed a better three-dimensional resolution and also quantitative analysis. While, FISH for detecting DNA (DNA-FISH) had never been applied to *Paramecium*. In DNA-FISH, the DNA has to be denatured to make partially single stranded allowing the FISH-probes to hybridize. We, therefore, adapted the method of cell pretreatment established by Curtnetz and Beisson (1996) and mainly examined the conditions of the denaturation process. To assess the validity of the method, we prepared biotin-16-dUTP labeled total genomic DNA probe as a positive control, and also examined the localization of the DNA, a telomeric DNA or a C5 antigen gene within the macronucleus of *P. multimicronucleatum*.

C5 antigen is a 33 kDa phagosomal membrane protein, involved in binding and/or fusion of the donor vesicles with the cytopharyngeal membrane that leads to digestive vacuole formation (Yamauchi *et al*., 1999). Telomeres, the specialized structures found at the end of chromosomes, consist of simple, tandem DNA repeats and the proteins which bind them (reviewed in Zakian, 1989; Biessmann and Mason, 1992). These structures function as a chromosome 'cap', protecting the termini from exonucleolytic cleavage and preventing illegitimate end-to-end chromosome fusions. Telomeres also ensure the complete replication of chromosomes and in this way prevent the loss of genetic information at the subtelomere. In addition, the telomeres have been proposed to help direct chromosome attachment to the nuclear envelope (Agard and Sedat, 1983; de Lange, 1992). Telomeric DNA from most eukaryotes consists of a variable number of short, tandemly repeated sequences. The sequences invariably have a strand bias, with a typically G-rich strand oriented 5' to 3' toward the chromosome terminus. The telomeric G-rich strand is synthesized by the ribonucleoprotein enzyme telomerase. In *Paramecium*, McCormick-Graham and Romero (1996) have cloned the telomerase RNA genes from four species, *P. tetraurelia*, *P. primaurelia*, *P. multimicronucleatum*, and *P. caudatum*, and reported that only a single telomerase gene was transcribed in all four species with templating nucleotides consistent with the synthesis of GGGGTT repeats. In the current study, the GGGGTT repeats and its complemental repeats, CCCCAA were, therefore, used as primer to obtain the specific probe for *Paramecium* telomeric DNA.

In the current study, all the probes prepared from *Paramecium* gene except for telomeric DNA was hybridized successfully in macronucleus by means of conventional methods. The hybridization of telomeric DNA was successful only when both cells and probes were denatured simultaneously in a same denaturation buffer. On the other hand, an irrelevant 40 kb human cosmid probe was examined as negative control, and none of the hybridization signal was detected neither by the conventional nor the simultaneous denaturation methods. Based on the results, we discuss about the optimum condition for the DNA-FISH and the localization of telomeric DNA in macronucleus of *P. multimicronucleatum*.

MATERIALS AND METHODS

Cells

The axenic culture of *P. multimicronucleatum*, syngen 2, was kindly supplied by Dr. A. K. Fok and Dr. R. D. Allen (University of Hawaii, USA; Fok and Allen, 1979). *Paramecium* were grown at room temperature (24±1°C) in axenic medium and harvested in mid-log phase of growth according to Fok and Allen (1979).

Polymerase chain reaction (PCR)

PCR is carried out in the absence of template using the primers $(CCCCAA)_5$ and $(TTGGGG)_5$ to obtain the probe specific for *Paramecium* telomeric DNA. PCR reactions were performed in 100 μl volumes containing 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, 0.05% W-1 (detergent), 200 μM of each dNTP, 0.1 or 1 μM of each primer and 2.5 units of Taq polymerase (Roche, GmbH, Germany) according to Ijdo *et al.* (1991). Cycling conditions of PCR were as follows: first 7 cycles, each consisting of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec; second 10 cycles, each consisting of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 45 sec; third 10 cycles, each consisting of 94°C for 60 sec, 60°C for 60 sec, and 92°C for 60 sec; final 30 cycles, each consisting of 94°C for 90 sec, 65°C for 90 sec, and 92°C for 90 sec. After PCR cycling, 4 μl of PCR products were analyzed by 1% agarose gel electrophoresis. 0.1 μM primers were applied on PCR according to Ijdo *et al.* (1991), then finally obtained 1–30 kb or longer probes.

Other Probes

For positive control experiments, total genomic DNA was extracted from 6.8×10^5 cells of *P. multimicronucleatum* by the conventional method for DNA isolation (Sambrook *et al*., 1989). The 1.3 kb genomic DNA probe for C5 antigen (1.3 μg/μl) was obtained as described elsewhere (Yamauchi *et al*., 1999). The 40 kb cosmid probe of human chromosome 22 q 11.2 was kindly supplied by Dr. A. Baldini (Baylor Collage of Medicine, Texas, USA; Lindsay *et al*., 1996) which was used as a negative control probe. All probes were subjected to nick translation.

Nick translation

The probes were non-isotopically labeled with biotin-16-dUTP (Roche, GmbH, Germany) by nick translation. Nick translation performed at 15°C for 2 hr in 100 μl volumes containing 50 mM Tris-HCl, 5 mM $MgCl_2$, 50 μg/ml BSA, 50 μM of each dATP, dGTP, dCTP, 50 μM biotin-16-dUTP, 10 mM 2-mercaptoethanol, 1 μg/ml DNase I (TaKaRa, Osaka, JAPAN), 2.5 units DNA polymerase (Roche, GmbH, Germany), and 2 μg of every DNA probes. After nick translation, the probes were subjected to 2% agarose gel electrophoresis to estimate their sizes. The obtained sizes of every biotin labeled probes were less than 200 bp.

Cell preparation

Cells were fixed with 3% formaldehyde solution containing 50 mM Hepes (pH 7.0), 10 mM EGTA, and 2 mM $MgCl_2$ for 10 min at room temperature. Then cells were submitted to protainase K (Merck, Darmstadt, Germany) digestion for 8 min at 37°C, at the concentration of 50 μg/ml in TBT (0.1% Tween 20, 1% bovine serum albumin (BSA) in TBS (10 mM Tris-HCl (pH 7.2), 150 mM NaCl)) according to Curtenaz and Beisson (1996). Total volume of digestion buffer is 1 ml. Digestion was stopped by addition of 10 ml of cold (4°C) TBS, and the resultant cells were settled down for 20 min at 4°C, then rinsed 2 more times with TBS for 20 min each at room temperature. Cells were re-fixed with 3% formaldehyde solution in TBT for 30 min at room temperature, then, rinsed 3 times with TBS and once with

DDW. Cell suspension was mounted on the silanized slides (Dako, Kyoto, JAPAN) and air-dried at room temperature. The slides were stored at –20°C in a box with desiccant.

Hybridization

In the current study, following two different methods were used for denaturation: one is a conventional method, where probes and cells were denatured separately, and then hybridized; the other is a simultaneous denaturation method, where probes and cells were already coexisted in a same denaturation buffer, then denatured at the same time.

Conventional method: Prior to the hybridization, cells and probes were denatured separately according to Lichter *et al*., (1988). Cells were denatured with a buffer containing 70% formamide, 2×SSC (1×SSC: 150 mM NaCl, 15 mM sodium citrate, pH 7.0) for 5 or 10 minutes at different temperature, 70, 80, 85, or 90°C. After denaturation, cells were dehydrated first with cold 70% ethanol (–20°C) for 5 min, then progressively dehydrated with 90, 100% ethanol for 5 min each at room temperature. Cells were air-dried with aspiration in a dessicator. While, DNA probes were denatured with a buffer containing 50% formamide, 10% dextran sulfate, 2×SSC for 10 min at 75–80°C. Prior to the denaturation, ~80 ng of DNA probes were co-precipitated with 8 μg of salmon sperm DNA either with or without 2 μg of *Paramecium* competitor DNA (prepared by digesting total genomic DNA with 1 μg/ml DNase I at 15°C for 2 hr), then voltexed for 20 min. Denatured DNA probes were mounted on the cells (slides), and incubated in a moist chamber filled with 2×SSC over night at 37°C.

Simultaneous denaturation method: the 16 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2×SSC) containing ~80 ng of DNA probes, 8 μg of salmon sperm DNA and either with or without 2 μg of *Paramecium* competitor DNA, was mounted on the cells. Following 1 hr incubation at room temperature (in order to infiltrate the probe into the cells), denaturation was done at different temperature 75, 80, 85, or 90°C, for 5 or 10 min. Denatured probes and cells were cooled down then incubated over night in a moist chamber filled with 2×SSC at 37°C.

Blocking and Detection

After hybridization, the slides were rinsed first with 50% formamide in 2×SSC for 3 times at 43°C, then next with 0.8×SSC for 3 times at 61°C. Blocking was carried out with a blocking buffer containing 3% bovine serum albumin (BSA), 4×SSC, 0.1% Tween 20, incubated for 30 min at 37°C. Subsequently, cells were incubated with 5 μg/ml avidin-fluorescein (Roche, GmbH, Germany) in a buffer containing 1% BSA, 4×SSC, 0.1% Tween 20 for 30 min at 37°C for detection. After 3 times wash with 0.1% Tween 20 in 4×SSC at 43°C, cells were counter-stained with DAPI (20 ng/ml 4' 6-diamidino-2-phenylindole dihydrochloride in 2×SSC) for 5 min at room temperature. Anti-fade solution, 25 mg/ml DABCO (1.4 diazabycyclo-[222], octane), 20 mM Tris-HCl, and 90% glycerol was mounted on the cells and cells were examined in a Zeiss Axioplan 2 MOT equipped with epifluorescence illumination using an ×63 Plan-APOCHROMAT oil objective. The microscope was controlled by IPLab Spectrum (Signal Analytics Co., Vienna, Austria) running on a Macintosh 9600/200MP computer. Series of light optical sections of nuclei were captured and digitized with a cooled CCD camera (PentaMax 1317K-1, Princeton Instruments) and were deconvoluted by a software Haze Buster 2.0 (VayTek) to remove the unfocused haze images. The 50 layers sections for fluorescence images including DAPI and FITC were collected at every 0.5 μm steps on z-axis and the images were printed by Fuji Pictrography 3000.

RESULTS

To determine the optimum condition for denaturation of cells, we used a biotin-labeled total genomic DNA probe of *P. multimicronucleatum*. In accordance with the conventional method, the total genomic DNA probe was denatured at 75–80°C for 2 min, then hybridized on sample cells. The cells were previously denatured at several different temperatures, 75, 80, 85 or 90°C for 5 or 10 min. The qualitative results were summarized in Table 1. At all denaturation temperature except for 75°C, the hybridization signals were detected. Hybridization signals increased with the increase in both temperature and time up to 85°C for 10 min, and no more strengthening of fluorescence was observed over 85°C for 10 min denaturation. In the cells denatured for 10 min at 85°C, the hybridization signals was observed every optical sections of macronucleus, and such homogeneous hybridization was also obtained either at 90°C for 5 min, or 10 min (Table 1).

Table 1. The extent of hybridization obtained at different denaturation conditions.

Denaturation time (min)	Denaturation temperature (°C)			
	75	80	85	90
5	–	+	++	+++
10	+/–	++	+++	+++

Figure 1 shows a typical appearance of the hybridization signals of total genomic DNA probe on a cell denatured for 10 min at 85°C. A series of four different focal planes (Fig. 1A–D) were picked up at every 5 steps (2.5 μm interval) of z-axis from periphery of the nucleus, and their corresponding DAPI images were shown in Fig. 1E-H, respectively. The sample cells were depressed by cover glass, so that the thickness of the macronucleus also depressed down within a range from 10 to 15 μm that was estimated from the scanning images of the DAPI staining. As seen in Fig. 1, a numbers of FISH signals were detected all over the macronuclear nucleoplasm, thus, the hybridization seemed to be occurred homogeneously throughout the macronucleus. Also, a numbers of spots-like brighter signals could be detected. The shape of these spots was irregular, seemed to be a mass of plural signals. The diameter of these brighter signals was 1.7±0.2 μm (n=15) and the number was around 50 (47.5±10, n=4) within a single macronucleus. While, the micronuclei are much smaller than macronuclei (the diameter is approximately 2–3 μm), and which can often be found in indentations of the macronucleus of the interphase cell, in *P. multimicronucleatum*. As shown in Fig. 1C, two small bumps (arrowheads) with fluorescent signals were found in an indentation of the macronucleus. The diameter of these bumps is within a range of the diameter of micronuclei, however, it is rather hard to determine whether these bumps to be real micronuclei or not, by light microscopic level.

We then applied this method to *Paramecium* genomic DNA probe of C5 antigen. Fig. 2A shows a merged image of the DAPI staining and typical appearance of C5 probe signals on macronucleus, which was taken by 10 times longer exposure time (10 sec). The images were captured at approximately 5 μm depth from the surface of macronucleus. Although the C5 probe is only 1.3 kb (which is rather short to detect whose

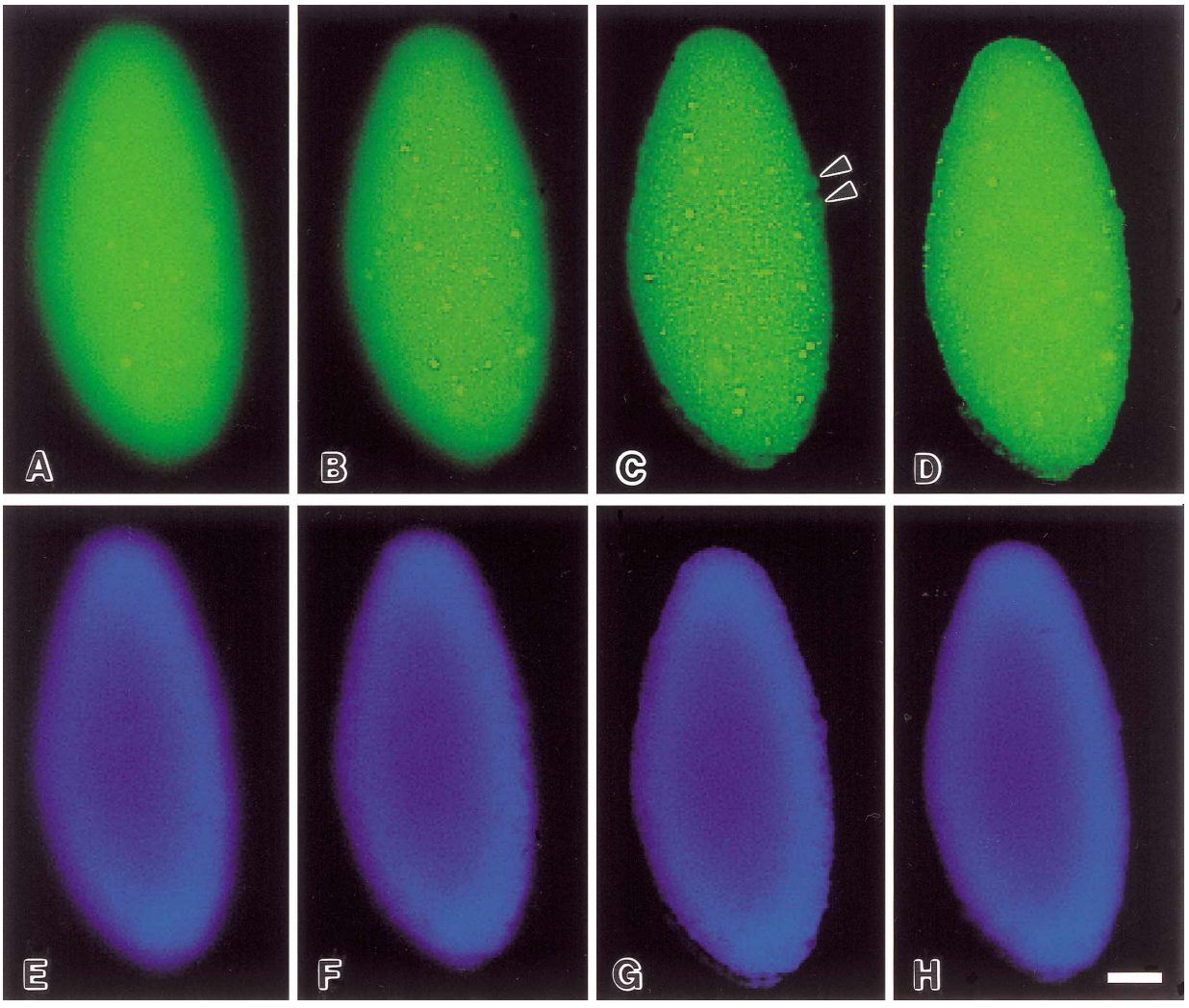

Fig. 1. FISH with *Paramecium* total genomic DNA probe in *Paramecium* nuclei. Cell was denatured at 85°C for 10 min in accordance with the conventional method. **A–D:** FITC images of FISH signals. **E–H:** corresponding DAPI staining images. A series of optical sections were captured at every 5 steps (2.5 μm) of z-axis from periphery of the nucleus. All images were deconvoluted to remove the unfocused haze images. Arrowheads, micronucleus; Bar, 10 μm.

signal), a number of tiny signals were detected as spreading all over the macronucleus. We also examined 10 min denature at different temperatures, 75, 80, or 90°C. As the result, C5 signals were detected at over 85°C (Data not shown) as well as total genomic DNA probe (Fig. 1). As a negative control, we hybridized a 40 kb cosmid probe from the human genome on the cells from the same preparation used in above. None of the signals were detected with this probe, as shown in Fig. 2B. Merged images of FITC and DAPI images of Fig. 2B, were captured at approximately 5 μm depth from the nuclear surface. As shown in Fig. 2B, the fluorescent signals could not detect with the human probe even at 10 times longer exposure time.

In the current study, a telomeric DNA probe was also examined. Unfortunately, the hybridization with a telomeric DNA probe was, however, not successful at any of the above described conditions, probably due to the faster annealing within the repeated telomere sequences. In order to obtain the FISH signal of the telomeric DNA probe, we therefore examined simultaneous denaturation methods (described in Materials and Methods). At every different denaturation temperature (75, 80, 85, or 90°C for 10 min each), the hybridization signals of the telomeric DNA were consistently observed on the periphery of the macronucleus, in which the extent of fluorescent signals did not change with increasing of denaturation temperature. However, DAPI staining demonstrated that the simultaneous denaturation induced the morphological damage on the macronucleus. Fig. 3 shows merged images of typical telomeric DNA probe signals and DAPI staining on the macronucleus. The images were picked up at every 5 steps (2.5 μm) of z-axis from the surface (A) to middle portion of the macronucleus. As shown in Fig. 3, a number of blister-like extrusions could be observed on the surface of the macronucleus (blue), and a large quantity of telomeric DNA signals (green) were detected on periphery of the macronucleus, in which the signals seemed to locate on the blister-like extru-

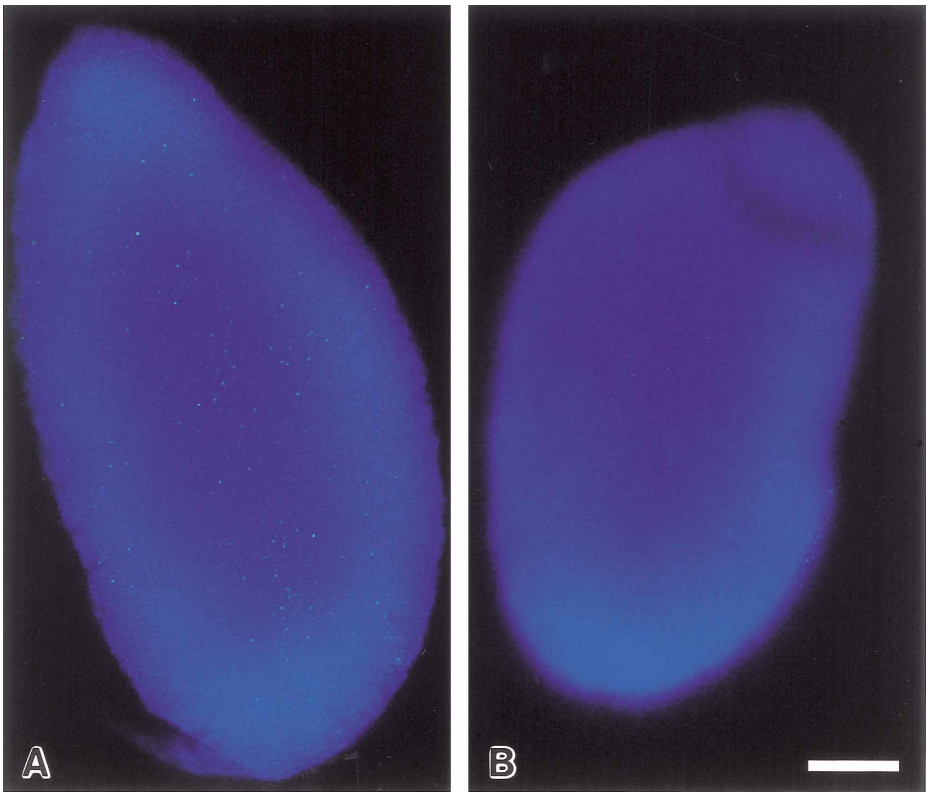

Fig. 2. FISH with *Paramecium* genomic DNA probe for C5 antigen or a cosmid probe from human in *Paramecium* nuclei. Cells were denatured at 85°C for 10 min in accordance with the conventional. FISH signals (green) and its corresponding DAPI staining image (blue) were merged. **A:** FISH with 1.3 kb paramecium genomic DNA probe for C5 antigen. **B:** FISH with 40 kb cosmid probe of human chromosome 22q11.2. Sections were captured at 5 μm depth from the nuclear surface. All images were deconvoluted to remove the unfocused haze. Bar, 10 μm.

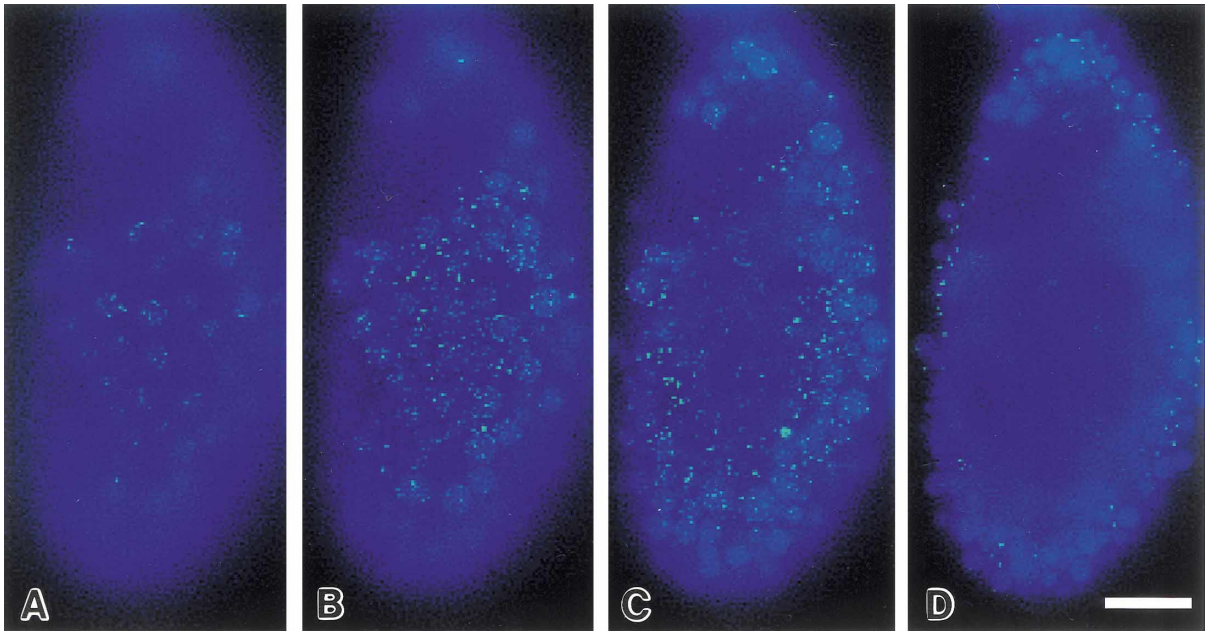

Fig. 3. FISH with a telomeric DNA probe in *Paramecium* nuclei. Telomeric DNA FISH signals (green) and DAPI staining images (blue) were merged. Cell was prepared by the simultaneous denaturation method as described in Materials and Methods. A series of optical sections (**A–D**) were captured at every 5 steps (2.5 μm) of z-axis from surface of the nucleus. All images were deconvoluted to remove the unfocused haze images. Bar, 10 μm.

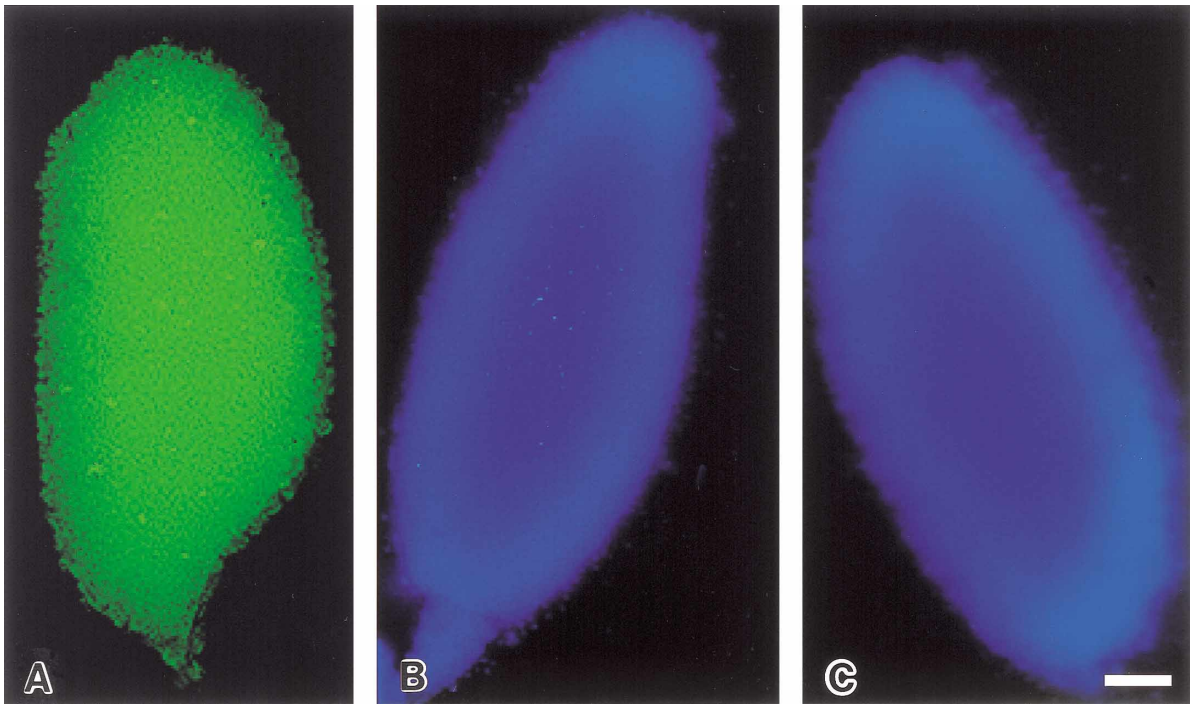

Fig. 4. FISH with total genomic DNA, C5, or human DNA in *Paramecium* nuclei. Cells were prepared by the simultaneous denaturation method as described in Materials and Methods. FISH signals (green) and its corresponding DAPI staining image (blue) were merged. **A:** FISH with *Paramecium* total genomic DNA probe. **B:** FISH with 1.3 kb paramecium genomic DNA probe for C5 antigen. **C:** FISH with 40 kb cosmid probe of human chromosome 22q11.2. Sections were captured at 5 μm depth from the nuclear surface. All images were deconvoluted to remove the unfocused haze images. Bar, 10 μm.

sion of the macronucleus.

As the control, three probes, total genomic DNA, C5 or irrelevant human probes were tested with the simultaneous denaturation method (at 75°C for 10 min). Although the blister-like extrusions on the surface of the macronucleus was also observed (Fig. 4B and C), the signals were detected with total genomic DNA probe or C5 probe in either case (Fig. 4). Fig. 4A shows a single optical section obtained from the middle portion (approximately 5 μm depth from surface) of the macronucleus, demonstrating FITC signals of total genomic DNA probe. The signals of total genomic DNA probe were homogeneously detected in the macronucleus in which a number of brighter spots-like signals were detected as well as Fig. 1C (the number of these spots-like signals counted by scanning throughout the macronucleus was within a same range). We also examined higher denaturation temperature (80, 85, 90°C) for 10 min, in which homogeneous hybridization was observed with total genomic DNA, as well as the sample denatured for 10 min at 75°C. Thus, the denaturation with 75°C for 10 min seemed to be enough to obtain the homogeneous hybridization by this simultaneous denaturation method. Figure 4B shows a merged image of the FITC signals of C5 probe and DAPI staining, in which tiny dots-like signals were detected in the nucleoplasm of the macronucleus, as well as in Fig. 2A. On the other hand, none of fluorescent signals were detected with the irrelevant human DNA probe (Fig. 4C).

DISCUSSION

By using total genomic DNA probe, both temperature and time for denaturation were determined in accordance with a conventional method. The probe was non-isotopically labeled with biotin-16-dUTP, and which were detected with avidin-fluorescein. FISH signals of total genomic DNA probe were detected all over the nucleoplasm of macronucleus. As shown in Fig. 1, a homogeneous hybridization was obtained in the sample denatured for 10 min at 85°C. Higher temperature and longer denaturation time also showed consistent and representative results with this probe (Table 1), but neither additional non-specific nor strengthening of fluorescence was observed. Denaturation for 10 min at 85°C is, therefore, the minimum to obtain the homogeneous hybridization with total genomic DNA probe (Fig. 1). By using this denaturation condition, the hybridization signals of C5 probe were also detected on the macronucleus (Fig. 2A). Although 1.3 kb C5 probe is rather short to detect, tiny signals were reproducibly detected by this method. In contrast, the negative control probe did not hybridize at all (Fig. 2B). These results indicated the appropriateness of this denaturation condition in *P. multimicronucleatum*. Thus, we applied this method to the telomeric DNA probe, however, the probe was not hybridized in the cells denatured at 85°C for 10 min. We further examined with the sample denatured for 10 min at higher temperature 90°C, but the hybridization signals of telomeric DNA could not be detected at all. Fast annealing within the repeated telomere

sequences seemed to disturb the hybridization of the probe in this case.

The hybridization of the telomeric DNA probe was observed only in the sample denatured simultaneously, in which the hybridization signals were homogeneously detected throughout the macronucleus. Under this condition, the probes and the cells were denatured at the same time, so that freshly-denatured both probe and cellular DNA might be able to hybridize each other soon after denaturation. In the case of simultaneously denatured samples, the 75°C for 10 min treatment is already enough to cause denaturation of *Paramecium* DNA, which is, however, inconsistent with the temperature that we determined by conventional method. Judging from the appearance at the light microscopic level, the preservation of nuclear structure in the simultaneously denatured sample was relatively poor than that of the sample prepared by the conventional method. DAPI staining showed that the macronucleus has blister-like extrusion of the nuclear contents all over the cytosolic surface of the nuclear envelope. Thus, the simultaneous denaturation method seemed to cause a disruption of either nuclear envelope or macronucleus itself, and which probably leads exposing denatured DNA to the probes. While, in the case of the sample prepared by the conventional methods (85°C for 10 min), relatively preserved membrane structures or the nuclear protein structures of macronucleus probably affected on either the denaturation of the macronuclear DNA or the invasion of the probes into the nucleus. Higher temperature, 85°C, might be therefore required to obtain the homogeneous hybridization pattern in the conventional method.

The poor preservation of the nuclear structure and the hybridization of the telomeric DNA probe were obtained only when the denaturation was done with the 10% dextran sulfate in the denaturing buffer of the simultaneous denaturation method. We examined the simultaneous denaturation without dextran, but neither the damage in the morphology of the macronucleus nor the hybridization signals with telomeric DNA probe could be detected in all preparations at different temperature (75, 80, 85, or 90°C). Therefore, the denaturation under the existence of the dextran in a hybridization buffer seemed to cause the morphological damage of the nucleus, and only in this case, the hybridization signals of the telomeric DNA probe were detected. Unfortunately we failed to figure out why the denaturation with dextran sulfate caused morphological damage on macronucleus, however, these results support the idea that the simultaneous denaturation method cause a disruption of either nuclear envelope or macronucleus itself, and which probably leads either exposing denatured DNA to the probes.

As shown in Fig. 3, the hybridization signal of telomeric DNA was detected in the macronucleus in which the signals seemed to be located on the periphery of the macronucleus where the blister like extrusion located. The results obtained from the hybridization experiments either with total genomic DNA or C5 probes suggested that the probes were penetrated into the macronucleus and hybridization occurred throughout the nucleoplasm. These results suggest that the *Paramecium* telomere gene might localize on the periphery of the macronucleus as reported in higher animals (Agard and Sedat, 1983; deLange, 1992).

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