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Evidence for the existence of a single ubiquitin gene in *Giardia lamblia*

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Abstract

All eukaryotes investigated so far contain multiple copies of ubiquitin genes, most of which are arranged in fusions coding for either polyubiquitin or ubiquitin-ribosomal protein constructs; the former are normally under the control of a heat shock promoter. *Giardia lamblia*, an intestinal parasite, is the most primitive eukaryote known to date. We have investigated the arrangement and expression of ubiquitin genes in this organism by Southern and Northern blotting. Our data strongly suggest that *G. lamblia* contains just one ubiquitin gene, which consists of a single copy of the coding sequence and the expression of which is not enhanced by heat shock. By pulsed-field gel electrophoresis we localized this gene on the largest of the five giardial chromosomes. These data imply that the ubiquitin system in *Giardia* has probably been trapped at an original stage.

Key words: Ubiquitin; *Giardia lamblia*; Archezoon; Molecular evolution

1. Introduction

Ubiquitin is a small (8.5 kDa) protein that in higher eukaryotic cells plays an essential role in such diverse processes as cytoplasmic proteolysis, DNA repair and ribosome assembly [1,2]. All eukaryotic organisms investigated so far contain multiple copies of ubiquitin genes, with part of these genes organized in arrangements coding for either polyubiquitins or fusions of ubiquitin to a ribosomal protein. Polyubiquitin genes are normally under the control of a stress promoter [1,2]. Recently, ubiquitin has also been detected in archebacteria ([3] and Dr. J. Driscoll, personal communication); however, it appears to be absent from eubacteria. *G. lamblia*, an intestinal parasite of man, is a unicellular eukaryote that lacks mitochondria and peroxisomes [4]. Based on these and other properties, it has been proposed that *Giardia* is an Archezoon, i.e. an organism that branched off before eukaryotes captured protomitochondria [5]. This hypothesis has been supported by an evolutionary tree based on 16S RNA [6].

In the framework of our interest in the evolutionary development of the ubiquitin system [7,8] we have now had a look at ubiquitin genes and their expression in *G. lamblia*. Southern blotting showed that this organism has only one ubiquitin gene, which, judging from Northern blots, codes for one single copy of ubiquitin. Expression of the gene was not stimulated by heat shock. These data strongly suggest that the ubiquitin system in *Giardia* may have got stuck at a very early stage of its evolutionary development.

2. Experimental

2.1. Cells

Trophozoites of *G. lamblia* P-1 (ATCC 30888) were cultured in Keister's modified TYI-S33 medium supplemented with 5 mM arginine [9]. Cells were harvested at the end of the logarithmic phase and washed in PBS-8 [10].

2.2. Blotting and hybridizations

Genomic DNA was isolated according to Yee and Dennis [11], cut with restriction enzymes as indicated, electrophoretically separated on 0.7% agarose and blotted under vacuum onto a Nylon membrane (Hybond N. Amersham). For pulsed-field gel electrophoresis, intact trophozoites were embedded in agarose and lysed in a solution containing 0.5 M EDTA, 1% *N*-lauroylsarcosyl and 2 mg/ml proteinase K. Chromosomes were separated on a Rotaphor apparatus (Biometra) at the conditions specified by the producer for the separation of DNA between 1500 and 7000 kbp. Pulsed-field gels were blotted by capillary action. mRNA was isolated from solubilized cells with a magnetic kit (Dynabeads oligo dT), electrophoretically separated on 1.5% agarose containing 50% formaldehyde and blotted by capillary action on a nylon membrane. Hybridizations were carried out with a giardial ubiquitin gene amplified by PCR; the sequence of this PCR product has been deposited under EMBL Data Library no. X70050. Hybridizing bands were visualized by alkaline phosphatase (digoxygenin labeling kit, Boehringer).

3. Results

3.1. Determination of the number of ubiquitin genes in G. lamblia

Genomic DNA from *G. lamblia* was treated with three different restriction enzymes. These enzymes were cho-

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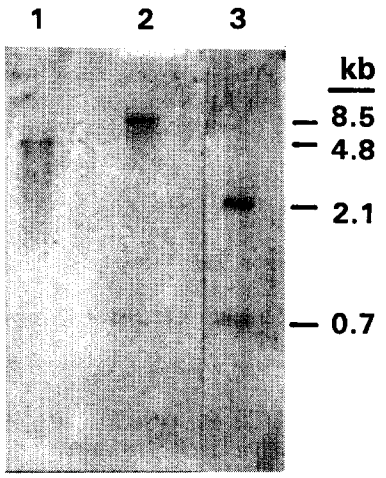

Fig. 1. Southern blot analysis of *G. lamblia*, using an amplified giardial ubiquitin gene as probe. Genomic DNA was digested with (1) *Hin*dIII, (2) *Pvu*I and (3) *Xho*I. The blot was hybridized at 68°C and washed with 0.1 × SSC. For further details, see section 2.

sen such that the first two had no restriction site-, the last had one restriction site within a giardial ubiquitin gene amplified by PCR; this gene was used as a hybridization probe (see section 2). As shown in Fig. 1, single hybridization bands were detected with the first two enzymes, whereas a doublet appeared with the latter. These data strongly suggest that *G. lamblia* possesses one single ubiquitin gene that is identical to the one amplified by PCR. A single hybridizing band was also found after pulsed-field gel electrophoresis (Fig. 2). This band coincided with the largest of the five giardial chromosomes [12].

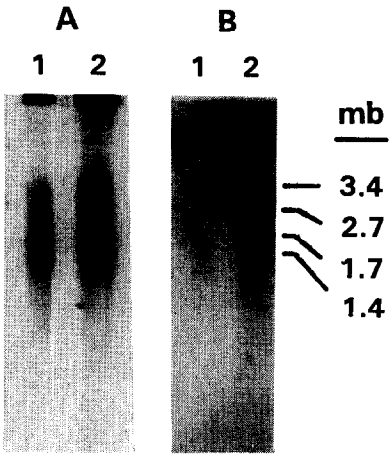

Fig. 2. Chromosomal localization of the giardial ubiquitin gene by pulsed-field gel electrophoresis. A, ethidium bromide-stained gel; B, blot hybridized with an amplified giardial ubiquitin gene. Lanes (1), 2×10^7 cells; lanes (2), 4×10^7 cells. The blot was hybridized at 60°C and washed with 1 × SSC. The values for chromosome size were taken from [12]; according to this reference, the band at 1.7 mb represents two chromosomes. For further details, see section 2.

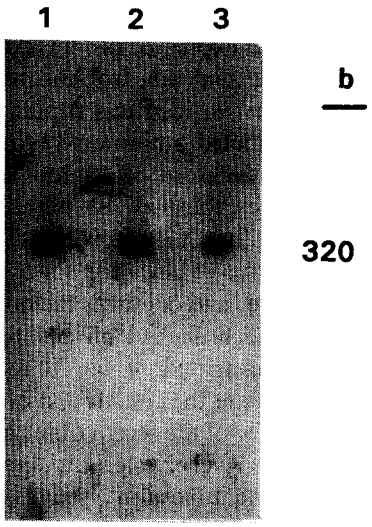

Fig. 3. Northern blot analysis of control and heat-shocked cells, using an amplified giardial ubiquitin gene as a probe. Cells (10^7 per incubation) were exposed to 43°C for (1) 0 min, (2) 10 min and (3) 30 min. mRNA was isolated from the three aliquots, separated electrophoretically and blotted according to section 2. The blot was hybridized with an amplified giardial ubiquitin gene at 60°C and washed with 1 × SSC.

3.2. *Expression of the ubiquitin gene in* G. lamblia

A single hybridizing band was detected after Northern blotting (Fig. 3). The size of the hybridizing mRNA corresponded to about 320 nt, which just suffices to account for a single copy of ubiquitin (76 or 77 triplets for the coding region and the remaining part for 5′ and 3′ noncoding parts and a poly A-tail [4]). To investigate whether expression of this messenger was increased by heat shock, we isolated mRNA under conditions that have been shown to effectively induce the production of other heat-shock proteins in *G. lamblia* [13]. However, as shown in Fig. 3, lanes 2 and 3, these conditions failed to lead to an increase in the expression of the ubiquitin messenger. The slight reduction in intensity in lane 3 is probably due to cell death [13].

4. Discussion

In higher eukaryotic organisms such as yeast or man the ubiquitin system consists of at least 50 proteins and plays an essential role in an astonishing variety of partly unrelated processes. Ubiquitin genes in these organisms come in different classes, including polyubiquitin genes and heterologous fusion genes; typically, part of the former are under the control of a stress promoter [1,2]. It seems reasonable to assume that this complex configuration has gradually evolved in the course of evolution, and that 'primitive' organisms such as *G. lamblia* can provide clues as to its roots. Our data confirm this notion by indicating that *G. lamblia* has only one ubiquitin gene that is expressed independent of heat shock. As *G. lam-*

blia possesses two identical nuclei, the genetic information of both of which is expressed [4], this conclusion has to be qualified to the extent that trophozoites of *G. lamblia* probably contain two identical copies of the gene. Interestingly, the predicted amino acid sequence of the giardial ubiquitin deviates in 10 amino acid residues from the consensus established for higher eukaryotic cells (compare EMBL no. X70050 with refs. 7 and 14). This is the highest degree of deviation for any eukaryotic ubiquitin characterized, and confirms the notion that the ancestor of *G. lamblia* branched off before any of the other eukaryotes investigated so far. It remains to be established what is or are the function(s) of ubiquitin in *G. lamblia*. Also, in view of the recent observation [3] that ubiquitin is present in Archebacteria it should be interesting to compare the ubiquitin systems of the two groups of organisms.

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References

[1] Finley, D. and Chau, V. (1991) Annu. Rev. Cell Biol. 7, 25–69.
[2] Jentsch, S. (1992) Annu. Rev. Genet. 26, 179–207.
[3] Wolf, S., Lottspeich, F. and Baumeister, W. (1993) FEBS Lett. 326, 42–44.
[4] Adam, R.D. (1991) Microbiol. Rev. 55, 706–732.
[5] Cavalier-Smith, T. (1983) in: Endocytobiology II (Schenk, H.E.A. and Schwemmler, W., Eds.) pp. 1027–1034, de Gruyter, Berlin.
[6] Sogin, M.L., Gunderson, J.H., Elwood, H.J., Alonso, R.A. and Peattie, D.A. (1989) Science 243, 75–77.
[7] Wöstmann, C., Tannich, E. and Bakker-Grunwald, T. (1992) FEBS Lett. 308, 54–58.
[8] Bakker-Grunwald, T. and Wöstmann, C. (1993) Parasitol. Today 9, 27–31.
[9] Edwards, M.R., Schofield, P.J., O'Sullivan, W.J. and Costello, M. (1992) Mol. Biochem. Parasitol. 53, 97–103.
[10] Diamond, L.S. (1988) in: Amebiasis. Human infection by *Entamoeba histolytica* (Ravdin, J.I., Ed.) pp. 27–40, Wiley, New York.
[11] Yee, J. and Dennis, P.P. (1992) J. Biol. Chem. 267, 7539–7544.
[12] Fan, J.B., Korman, S.H., Cantor, C.R. and Smith, C.L. (1991) Nucleic Acids Res. 19, 1905–1908.
[13] Lindlay, T.A., Chakraborty, P.R. and Edlind, T.D. (1988) Mol. Biochem. Parasitol. 28, 135–144.
[14] Jentsch, S., Seufert, W. and Hauser, H.P. (1991) Biochim. Biophys. Acta 1089, 127–139.