Striking changes in polyubiquitin genes of *Tetrahymena pyriformis*

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The presence of ubiquitin genes in *Tetrahymena pyriformis* has already been demonstrated (1). To further analyse the organization of this multigene family a partial genomic library consisting of *EcoRI* DNA fragments cloned into the pBR322 vector, was screened using the 434-bp *AluI/HindIII* DNA fragment of pTU2 (1). Hybridization was performed at 65°C in 6 × SSC, 5 × Denhart's solution.

Here we present some striking features of pTU10, a putative polyubiquitin clone containing an *EcoRI* fragment of 2.6kb (Fig. 1). The 5' end of the DNA insert reveals a 353-nucleotide long open reading frame (ORF), corresponding to one and a half ubiquitin repeats. At the opposite end of this insert, another 792-nucleotide long ORF contains three and a half ubiquitin repeats. The two coding regions are separated by a 1433-nucleotide long sequence, where unlike pTU2 no heat-shock elements are present. The putative polyubiquitin encoded by the

second ORF is the most divergent ubiquitin gene so far detected (Fig. 2). The first and third complete repeats (B1, B3) present only 85.5% homology with the human and pTU2 ubiquitin amino acid sequence (1, 2) and B2 is 89.5% homologous. This is the first example of an ubiquitin gene containing repeats that differ so strikingly in amino acid sequence. In respect to the first ORF the absence of the second Gly residue and also two extra amino acid residues upstream of the stop codon are interesting features the meaning of which should be clarified.

REFERENCES

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Figure 1. Restriction map of pTU10 genomic clone. Restriction map of *EcoRI* DNA fragment inserted in pBR322 was determined using the following restriction enzymes: A, *AluI*; Bg, *BglIII*; C, *ClaI*; E, *EcoRI*; Hc, *HincII*; P, *PstI*; Sc, *ScaI*; X, *XbaI*. Boxed regions represent polyubiquitin genes A and B; A1–2 are ubiquitin repeats of gene A and B1–4 are ubiquitin repeats of gene B.

	1	10	20	30	40	50	60	70	76
Human	MQIFVKTLTG	KTITLEVEPS	DTIENVKAKI	QDKEGIPPDQ	QRLIFAGKQL	EDGRTLSDYN	IQKESTLHLV	LRLGG	
pTU2		D..A.							
pTU10-A2		NST	.N.....			SIA...	.S.....	*	
pTU10-B1		..V....AST	.N...I.Q..	S..		..E...V...	V...		
pTU10-B2	..V.....	AQT	.N...I....	S..		V...			
pTU10-B3	..V.....	E.NS.	.N..SI.S..	S..		NI....			

Figure 2. Comparison of the predicted amino acid sequences of human and *T. pyriformis* (pTU2) ubiquitins and complete ubiquitin repeats of pTU10. ONLY divergent amino acids from human ubiquitin sequence are shown. *Indicates the absence of the second Gly residue.

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