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New telomere formation during the process of chromatin diminution in *Ascaris suum*

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ABSTRACT Chromatin diminution in the parasitic nematode *Ascaris suum* represents an interesting case of developmentally programmed DNA rearrangement in higher eukaryotes. At the molecular level, it is a rather complex event including chromosome breakage, new telomere formation and DNA degradation. Analysis of a cloned somatic telomere (pTel1) revealed that it has been newly created during the process of chromatin diminution by the addition of telomeric repeats $(TTAGGC)_n$ to a chromosomal breakage site (Müller *et al.,* 1991). However, telomere addition does not occur at a single chromosomal locus, but at many different sites within a short chromosomal region, termed CBR1 (chromosomal breakage region 1). Here we present the cloning and the analysis of 83 different PCR amplified telomere addition sites from the region of CBR1. The lack of any obvious sequence homology shared among them argues for a telomerase-mediated healing process, rather than for a recombinational event. This hypothesis is strongly supported by the existence of 1-6 nucleotides corresponding to and being in frame with the newly added telomeric repeats at almost all of the telomere addition sites. Furthermore, we show that telomeres are not only added to the ends of the retained chromosomal portions, but also to the eliminated part of the chromosomes, which later on become degraded in the cytoplasm. This result suggests that *de novo* telomere formation during the process of chromatin diminution represents a non-specific process which can heal any broken DNA end.

KEY WORDS: *Ascaris suum, nematodes, chromatin diminution, programmed DNA rearrangement*

Introduction

The phenomenon of chromatin diminution was discovered at the end of the 19th century in the horse intestinal parasite *Parascaris univalens* by Theodor Boveri during his classical developmental studies (Boveri, 1887). This unusual process that represents a developmentally regulated genome rearrangement, resulting in quantitative and qualitative differences in the DNA content between germ line and somatic cells, provided the first proof for the early segregation and independent development of the germ line and the somatic cell lines (reviewed in Tobler, 1986). Chromatin diminution was later on found to occur also in *Ascaris suum*, an intestinal parasite of the pig, and in a few other parasitic nematodes, most of them belonging to the family of *Ascarididae* (reviewed in Müller and Tobler, 2000). Unfortunately, the functional significance of this interesting phenomenon still remains an enigma.

Chromatin diminution in *A. suum,* like that in all other eliminating nematodes, takes place during early embryogenesis. The first two embryonic cleavages are normal and lead to the generation of four daughter cells with the same chromosomal complements. During the third cleavage division, however, the blastomeres S_{1a}, S_{1b} and S_2 undergo chromatin diminution (Fig. 1A), causing a fragmentation of the germ line chromosomes and the loss of all detectable heterochromatin. The somatic chromosomes, now reduced in size, segregate to the daughter nuclei of the elimination mitoses, whereas the eliminated heterochromatic material lacks kinetochore activity and is rendered to the cytoplasm (Fig. 1B) where it rapidly degrades. During the two following rounds of embryonic cell divisions, the process of chromatin diminution is repeated in the presomatic cells S_3 and S_4 (Fig. 1A). All cells undergoing chromatin diminution, and therefore containing less chromatin, become somatic cells, whereas nuclei maintaining the original integrity of the chromosomes and the full quantity of chromatin give rise to germ line cells (Fig. 1A). Thus, the process of chromatin diminution is linked to germ line - soma differentiation and leads to differences in the structural organization, the DNA content and the number of germ line vs. somatic chromosomes.

At the molecular level, chromatin diminution represents a rather complex event including DNA breakage, DNA degradation and new

Abbreviations used in this paper: CBR1, chromosomal breakage region 1; pTel1, cloned somatic telomere 1.

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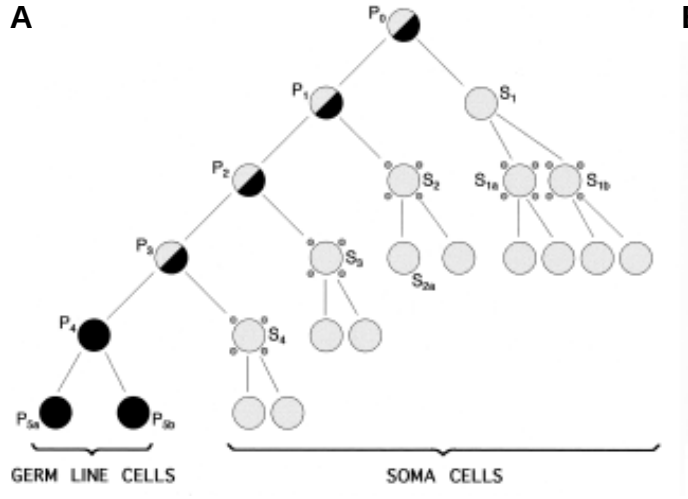


Fig.1. Chromatin diminution in *A. suum*. **(A).** *Cell lineage of the early* A. suum *embryo. Schematic representation of the cell lineage and segregation of germ line and somatic cells. The presumptive primordial germ cells (P_0-P_3) are represented by half-solid circles, the primordial germ cells (P_4, P_{5a}, P_{5b}) by solid circles. The presomatic cells (S_{1a}, S_{1b}, S_2-S_4) undergoing chromatin diminution are indicated by open circles surrounded by four small solid circles.* **(B)** *Phase contrast microscopy of an* A. suum *four cell-stage embryo in which the S1a and the S1b cells undergo chromatin diminution (from Tobler, 1986). The eliminated material (indicated by arrows) remains in the equatorial plate and is later on rendered to the cytoplasm where it is degraded.*

telomere formation. Since heterochromatin is not only located at the chromosomal ends but also at internal positions, its elimination leads to an increase of the chromosome number in somatic cells. Whereas the germ line genome of *A. suum* is contained in 2n = 38A + 10X (females) or 38A + 5X (males) chromosomes, the post-diminution cells have 2n = 58A and 12X (females) or 58A and 6X (males) chromosomes (Niedermayer and Moritz, 2000). Efficient healing of the truncated chromosomal ends is a particularly critical event, since it ensures the stable maintenance of the somatic chromosomes during further development. Previously, we have cloned and analyzed one somatic telomere (pTel1) from *A. suum* (Müller *et al.,* 1991), and found that it was newly formed during the process of chromatin diminution by the addition of 4-6 kb of nematode telomeric $(TTAGGC)_n$ repeats to the chromosomal breakage side (Müller *et al.,* 1991; Wicky *et al.,* 1996; Zetka and Müller, 1996). *De novo* formation of pTel1, however, does not take place at a single chromosomal locus, but can occur at many different sites within a specific several kb long chromosomal region, referred to as CBR1 (for Chromosomal Breakage Region1; see Fig. 2 and Müller *et al.,* 1991). In order to learn more about the molecular mechanisms involved in new telomere formation, we have PCR amplified and cloned 83 different telomere addition sites from the region of CBR1. Sequence comparison revealed that they do not share any obvious sequence homology or secondary structure, which could act as a signal for telomere addition. The existence of 1-6 nucleotides corresponding to and being in frame with the newly added telomeric repeats at almost all telomere additions sites, however, provides strong evidence for a telomerase mediated healing mechanism. Furthermore, we show that telomeres are not only added to the ends of the retained chromosomal portions, but also to the eliminated part of the chromosomes, which later on become degraded in the cytoplasm. This strongly suggests that *de novo* telomere

TABLE 1

FORWARD PRIMERS USED FOR THE PCR AMPLIFICATION REACTIONS

Primers for the non-eliminated side			Primers for the eliminated side		
Name		**Sequence**	**Name**		**Sequence**
F1*	5'-	AGTGTTACCAACACCTG - 3'	R2115	5'-	CGTCGACCTGTTGTAGCTGA - 3'
F61*		CAGTACAAGCGTCTGGGAGT	R3813*		ccgggaTCCTTTATCATAAATG
F756		TCAGGAACTTCAGAACTGCC	R4920		aaagggCCCATAGAAAAGCGAGCTAA
F2109		ctagaGTCGACGGTATGCAA	R5197*		ATAGGTTGGTATCCTTATCGG
F2253		tatggatCCAACAGTACGCAC	R5816		GGCAGTGCGACAAGTAATCG
F3041		tatggatCCATCGCTGGCG	R6417		TGCCTGGAGGAATGGCCG
F3102		ATTAATTCGTACTGTAAATTCCC	R7026		CGAATTCTGCACAAAATAACAG
F4249		tatggatCCGTATTGAAAACAGAAATGT			
F4774		CGTACATCTCAATGTAGAGGG			
F5330		CTTCCGTCAGCTGCTGCTC			
F5891		CTGTAGCTAAACACTCCCGC			
F6533		CGTTCAATGTTGTGAGGTCG			
F7284		AGAAACAGTTGCCCCTAATC			

Uppercase letters indicate sequences homologous to CBR1, lowercase letters indicate linker sequences not homologous to the sequence of CBR1 that contain restriction sites for cloning. The names of the individual primers are derived from the position of the most 3' nucleotide that is homologous to the sequence of CBR1. Asterisk indicate primers that yielded no cloned telomere addition sites.

formation during the process of chromatin diminution may represent a non-specific process that can heal any free DNA end.

Results

Telomeres are Added to Many Different Sites within CBR1

Previously, we have shown that during chromatin diminution, new telomere formation at the chromosomal site corresponding to the cloned somatic telomere pTel1 can occur at many different sites within a short chromosomal region, termed CBR1 (Müller *et al.,* 1991). To learn more about the mechanisms and possible DNA signals involved in this process, we PCR amplified and cloned a number of different telomere addition sites from the chromosomal region of CBR1. For the PCR amplification step we used several forward primers derived from CBR1 and its flanking sequences (Table 1, Fig. 2), a reverse primer that was complementary to the newly added telomeric repeats and total DNA isolated from *A. suum* L2 larvae as template. An aliquot of each PCR reaction was analyzed on an agarose gel. The PCR products that appeared as smears on the agarose gels, were cloned in a Promega T-vector. For each PCR reaction, a few clones were picked, their DNA isolated and sequenced. Altogether, we have characterized 58 different telomere addition sites (Table 2A).

As a control, we performed two additional series of PCR reactions. In the first set of experiments we used cloned CBR1 DNA as template, either undigested or digested with *Sal*I or *Hin*fI and *Sca*I, together with the same CBR1 and telomere specific primer pairs as above. Since the DNA sequence of CBR1 does not encode telomeric repeats (Müller *et al.,* 1991), no PCR products should be obtained. For the second set of control reactions we

TABLE 2

SEQUENCES OF THE DIFFERENT TELOMERE ADDITION SITES WITHIN CBR1

Telomere addition sites

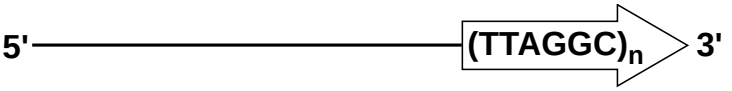
A) Non-eliminated side

Pos.	Primer	Sequence	Pos.	Primer	Sequence
784	F756	CCAAAAATATGGC	4328	F4249	TACCTTGATATT
891	F756	TGCTTCTTTTGCTTAG	4333	F4249	TGATATTTAGTA
1004	F756	GTCCCCGAGTTTA	4335	F4249	ATATTTAGTATA
2227	F2109	ACAGCAGCGTttgcttaggc	4348	F4249	GTATAACTTGA
2263	F2109	CCAACAGTACG	4817	F4774	CTAACAAGTTCTTA
2276	F2109	CCTTAGTACATT	4838	F4774	TTCTCCATATTTAG
2279	F2253	TAGTACATTGTTA	4841	F4774	TCCATATTTAGC
2289	F2253	TTACCTGCAAAGG	4855	F4774	TTCAGGTTGActtaggc
2297	F2253	AAAGGTGGCAG	4869	F4774	GCCTTTTCGATT
2331	F2253	CACTTGTGTTGG	4890	F4774	TTCAGTTGTATT
2490	F2253	CACAACGAAGggctta	4919	F4774	CTTTTCTATGGG
2493	F2253	AACGAAGACAGC	4932	F4774	AAAAATACAAC
2602	F2109	ACACCATACTC	4936	F4774	ATACAACCGATA
2697	F2253	CTAAATTTAGA	5356	F5330	CTCTTTCAATTT
2752	F2253	CAAATTTTTGA	5363	F5330	AATTTCTTCAT
3025	PTe11	GCAAGGAATTGG	5388	F5330	GATATCCATCT
3148	F3102	TTTCAAGTCAAGG	5393	F5330	CCATCTGTGCA
3153	F3102	AGTCAAGGAGG	5412	F5330	TAGTCTGTTTTT
3155	F3041	TCAAGGAGGAC	5524	F5330	GTGGATTTCGTT
3196	F3041	CCCTATAAATTTAG	5536	F5330	GGTGGCACAATT
3273	F3041	ACGATTTCTTA	5911	F5891	ACACTCCCGCTA
3282	F3102	TAAGAGCCATC	5940	F5891	TATTCGTTGActtaggc
3295	F3102	CATAATTACTG	6569	F6533	CAAGTGATTAG
3303	F3102	CTGCAATACAGC	6572	F6533	GTGATTAGTGA
3417	F3041	CCACTGATTTCT	6631	F6533	TTCCCTTCCGAGG
3448	F3102	ACCCCGTTCAGCT	6964	F6533	CTGTAAAGGTGG
4294	F4249	AATCGACAAACT	7355	F7284	CGTTAATTATG
4297	F4249	CGACAAACTAA	7356	F7284	GTTAATTATGC
4316	F4249	TCTAGTTTCATT	7377	F7284	CCTATGATTTGCTT
4326	F4149	TTTACCTTGAT			

B) Eliminated side

Pos.	Primer	Sequence
1983	R2115	GCATTGCTTGG
4438	R4920	CACTTTTTTAgcttaggc
4653	R4920	CGCATTATATG
4722	R4920	TTTCTTTATTG
4747	R4920	AAACCTCTATG
5312	R5816	AAGCAGTTAAAGGC
5601	R5816	CCCCCTCTCTCTT
5627	R5816	AAGAGTGGTACT
5693	R5816	ACTGCGCAAAGG
5702	R5816	TGTGCTTGTACT
5711	R5816	ATAAAGTTATG
5715	R5816	GTAGATAAAGTTA
5724	R5816	TGCAATGCAGTAG
5737	R5816	ATTAATAAAAA
5743	R5816	CGCCATATTAA
5748	R5816	ACCGTCGCCATA
5756	R5816	CATCCTTCACC
5758	R5816	CTCATCCTTCA
5763	R5816	TTCCACTCATC
5786	R5816	TGCTCACAGaC
5913	R6417	ACAAAAGCGTTTA
6229	R6417	TGATAACGAAgcttaggc
6241	R6417	CTCTTAAGACGG
6925	R7026	CGAGCGTAAGAG
6971	R7026	TTCTATTAACGCT

C)



The Table includes also the previously published pTel1 (Müller *et al.,* 1991). (A) Non-eliminated side. (B) Eliminated side of CBR1. The sequences are indicated 5'-to-3' towards the end of the chromosomes, so that the newly added telomeric repeats are to the right site as shown in (C). 78 out of 84 of the telomere addition sites contain at their chromosome-telomere junctions one to six nucleotides that correspond to, and are in frame with, the newly added telomeric repeats (indicated in bold). In the remaining 6 clones such nucleotides are missing, and the newly added telomeric sequences are shown in lowercase letters. The first column (Pos.) indicates position of the first telomeric nucleotide (underlined) at the chromosome-telomere junction relative to the first nucleotide upwnstream of AUG start codon of the non-eliminated gene *agp-1* (Huang *et al.,* 1996. The primers used for the PCR reactions are listed (Primer).

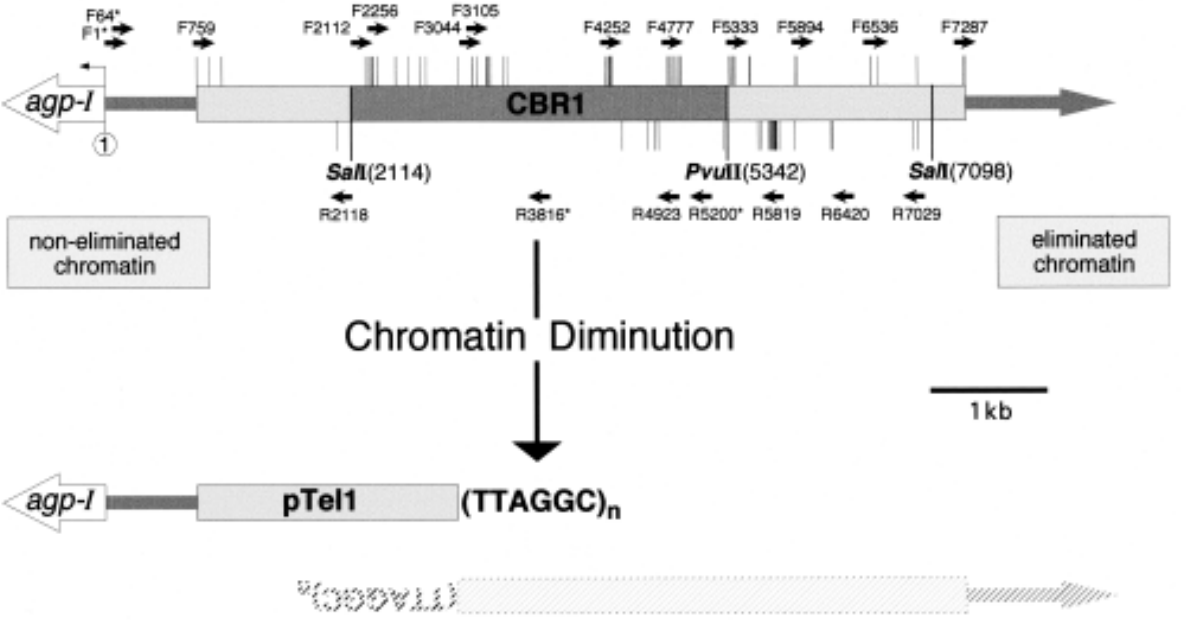


Fig. 2. PCR-mapping of telomere addition sites in CBR1. *Position of the PCR amplified telomere addition sites within CBR1. The 6593 bp long CBR1 is represented as a gray box with the eliminated chromatin on its right side. The previously determined extent of CBR1 (as determined by Southern blot hybridizations, Müller et al., 1991) is shown by a black box. The distribution and nucleotide positions of the* PvuII *and* SalI *recognition sites are shown. Primers complementary to CBR1 used for the PCR reaction are shown by arrows. Vertical lines above the CBR1 indicate telomere addition sites found at the end of the non-eliminated chromatin. The vertical lines below CBR1 indicate telomere addition sites to the eliminated chromatin.* agp-*1 is a non-eliminated gene encoding a putative GTP binding protein (Huang et al., 1994). The direction of transcription is indicated by an arrow. Nucleotide numbers are indicated with respect to the first nucleotide upstream of the AUG start codon of* agp-1 *and increase towards the telomere.*

used a cloned genomic *A. suum* DNA fragment encoding the ribosomal protein RPS19S (Etter *et al.,* 1990; Etter *et al.,* 1994) as template together with different forward primers derived from the *rps19S* gene and a reverse primer complementary to the *A. suum* telomeric repeats. Again, since the genomic *rps19S* fragment does not encode telomeric sequences, no PCR products should be obtained. Both sets of PCR control experiments were performed exactly as indicated above. As expected, no PCR products were detected on agarose gels, and after ligating the PCR reaction mix into a Promega T-vector, no false positive "telomeric" clones were obtained (data not shown). These results confirmed that our PCR reactions were specific for newly formed telomeres and suggested that all of the analyzed PCR clones represent true telomere addition sites, rather than resulting from cloning or PCR artefacts.

The different telomere addition sites (including 58 PCR amplified sides and the previously cloned somatic telomere pTel; see Table 2A) were scattered throughout a region of about 6.5 kb length (Fig. 2), but their distribution was not uniform. A majority of them (40/59) was concentrated between the *Sal*I site at nucleotide position 2111 and the *Pvu*II site at nucleotide position 5339, a region that corresponds to CBR1 as it has been determined previously based on Southern blot hybridizations (Müller *et al.,* 1991). The PCR products with forward primers derived from this region produced strong and clearly visible signals on agarose gels and, upon ligation into a Promega T-vector, gave rise to many telomeric clones. We found some additional telomere addition sites with primers from outside of that region. Their yield, however, was significantly lower and in order to see the corresponding PCR products on agarose gels, a second amplification step had to be performed. Two primers from the region between the start codon of the non-eliminated gene *agp-1* (Huang *et al.,* 1996) and the left-handed *Sal*I site of CBR1 (nucleotide position 2111), did not yield a positive clone (Table 1 and Fig. 2). The telomere addition sites at nucleotide position 784, obtained with primer F756, and at nucleotide position 7377, obtained with primer F7284, were the two most extreme ones found on either side (Table 2 and Fig. 2). The distance between them is 6593 bp, which therefore represents a minimal size for CBR1.

Telomeres are also Added to the Eliminated Chromatin

To test whether telomeres can also be added to the ends of the eliminated chromatin we have performed a PCR experiment with total genomic DNA isolated from synchronised 4-cell embryos as template. At this developmental stage, three of four blastomeres undergo chromatin diminution and the eliminated DNA can be found in the cytoplasm for a short period before it becomes degraded (see Fig. 1A). Like in the previous set of experiments, we used a series of different CBR1 specific primers, but in the opposite orientation, and a telomere complementary reverse primer (Fig. 2). The experiments were performed as described above, and 25 different telomere addition sites were cloned and mapped. Thus, our results demonstrate that telomeres are not only added to the ends of the non-eliminated DNA, but also to the ends of the eliminated part of CBR1. Most of the telomere addition sites at the eliminated DNA are located within two kbs of genomic DNA between the *Pvu*II and the *Sal*I sites (nucleotide positions 5339 to 7095; see Fig. 2). Only six telomere addition sites were found outside of this region. The telomere addition site at nucleotide position 6971 is the most extreme one for the eliminated chromatin at the right end of CBR1. We did, however, not

perform rigorous PCR experiments with primers complementary to the genomic region to the right of the *Sal*I site, and therefore it is possible that we may have missed some of the most extreme telomere addition sites located on this side. The telomere addition site at position 1983 is the most extreme left telomere addition event we found in the eliminated chromatin. Even by repeating the PCR experiments with several primers derived from this region or from the region further to the left, we could not find any other telomere addition site. The distance between the two most extreme telomere addition sites on either side is 4988 bp.

Telomere Addition Sites Contain One to Six Nucleotides which Correspond to, and are In Frame with, the Newly Added Telomeric Repeats

A comparison between the 84 telomere addition sites isolated from the eliminated and the non-eliminated sides of CBR1 (including the previously published pTel1) revealed no obvious sequence homologies or shared secondary structures that may act as a signal for telomere addition. In 78 out of the 84 cloned telomere addition sites, however, we found one to six nucleotides that correspond to, and are in frame with, the newly added telomeric repeats (bold nucleotides in Table 2). This result strongly argues for a telomerase meditated healing mechanism, in which the free 3'end of the truncated DNA pairs with the RNA template and initiates the synthesis of new telomeric repeats (see discussion). Six of the 84 cloned telomere addition sites, however, did not show the expected boundary and thus do not reconcile with the current model of *de novo* telomere formation by telomerase. One possible explanation is that the DNA used for our experiments has heterogeneities in the CBR sequences relative to the cloned and sequenced CBR1 fragment, since it was extracted from wild animals. Therefore, nucleotide substitutions, deletions or insertions in individual animals, located in the region of the telomere addition sites, may explain at least some of these exceptions. Alternatively, a non-stringent telomerase enzyme activity could be responsible for telomere addition at these sites.

Discussion

Here we show the results of a fine-scale PCR mapping of many different telomere additions sites within CBR1. Including the previously cloned somatic telomere pTel1 (Müller *et al.,* 1991), we have mapped 59 positions where telomeres are added to the non-eliminated chromosomal ends. These sites are scattered throughout a chromosomal region of several kb length (Fig. 2), but they are not evenly distributed. Rather, a majority of them (39/59) accumulates in a central region located between the proximal *Sal*I and the *Pvu*II sites (nucleotide positions 2111 to 5339; see Fig. 2). The PCR data are in agreement with the results of earlier Southern blot experiments that positioned the majority of the telomere addition sites within the same region (Müller *et al.,* 1991). Outside of that region, telomere addition sites are scarcer, explaining why they have not been detected in the previous Southern blot experiments. The PCR approach is more sensitive and allows the detection of rare telomere addition sites at the periphery of CBR1. The most proximal telomere addition site found by PCR amplification is located only 784 bp upstream of the initiator codon of *agp-1*, a non-eliminated gene encoding a putative GTP binding protein (Fig. 2; Huang *et al.,* 1996). Since this gene is expressed in all somatic cells at all developmental stages tested (Huang *et al.,* 1996), and since the region between the initiator codon and the telomere addition site at position 784 is likely to contain important regulatory sequences that can not be eliminated, we think that this site must be located very close to or at the proximal end of CBR1. The most distal telomere addition site on the other end of CBR1 was found at position 7377, thus determining a new minimal size for CBR1 of 6593 bp.

To our surprise, we found that telomeres are also added to the newly formed ends of the eliminated part of the chromosomes. The fact that the mechanism of *de novo* telomere addition does not discriminate between eliminated and non-eliminated chromatin, suggests that it may represent a general and non-specific process capable of healing any newly created free DNA end. Alternatively, however, it may be important that all ends of the eliminated chromatin are repaired, since any chromosomal breakage, even if degraded later, could cause a cell cycle arrest (reviewed in Shore, 2001).

How do the broken chromosome ends of *A. suum* acquire new telomeres? No pre-existing telomeric sequences are encoded by the germ line genome in the region of CBR1 (Müller *et al.,* 1991). Furthermore, no obvious sequence homologies or conserved secondary structures, that may act as a signal for the telomere addition mechanism, are shared between the different telomere addition sites at the eliminated and the non-eliminated site of CBR1. Telomere addition during the process of chromatin diminution does not seem to have any particular sequence requirement and occurs without a specific permutation at any position within the telomeric hexamer TTAGGC. Thus, a recombination-mediated telomere acquisition or an illegitimate or non-homologous recombination process is unlikely to explain telomere addition during chromatin diminution in *A. suum*. The existence of one to six nucleotides that correspond to, and are in frame with, the newly added telomeric repeats at 78 out of 84 cloned somatic telomeres, however, provides strong evidence that the new telomeres are added by the activity of an *A. suum* telomerase. This hypothesis is supported by the recent finding that cell free extracts of *A. suum* elimination stages contain a telomerase activity that *in vitro* can efficiently elongate nontelomeric primers with telomere repeats (Magnenat *et al.,* 1999). The activity of this enzyme is developmentally regulated, and correlates temporally with the phenomenon of chromatin diminution. The telomerase activity is up-regulated during the first two rounds of embryonic cell divisions, and reaches a peak in 4-cell-stage embryos, where three presomatic blastomeres prepare for chromatin diminution. *In vitro*, three bases of permutated telomeric sequences at the 3' end of non-telomeric primers are recognized and elongated correctly by the *A. suum* telomerase (Magnenat *et al.,* 1999). Our findings here suggest that *in vivo*, even one single nucleotide may be sufficient to pair efficiently with the RNA template and to initiate the synthesis of telomeric repeats. Since all four bases are represented in the telomeric repeats (and hence in the putative telomerase template), any free 3' end of DNA can potentially be healed by the *A. suum* telomerase. Telomerase-mediated chromosomal healing of spontaneous chromosomal breakages, requiring minimal 3'-terminal sequences, has been shown to occur in *Plasmodium* sp. (Pologe and Ravetch, 1988; Scherf and Mattei, 1992; Mattei and Scherf, 1994), humans (Wilkie *et al.,* 1990; Lamb *et al.,* 1993; Flint *et al.,* 1994) and *C. elegans* (Wicky *et al.,* 1996).

Nothing is yet known about the chromosomal breakage that precedes telomere addition. The fact that the region containing telomere addition sites for the eliminated chromatin overlaps with

that for the non-eliminated chromatin, suggests that chromatin breakage does not occur at a single site, but (like telomere addition), takes place at different sites within CBR1. Furthermore, chromosome breakage may be followed by some degree of exonuclease degradation before the DNA ends are sealed by the addition of new telomeres. Some weak evidence for that is provided by our finding, that the CBR1 regions accumulating most of the telomere addition sites at the eliminated and the non-eliminated chromatin seem not to overlap, but are displaced for some kbs (see above and Fig. 2).

Materials and Methods

Isolation of Genomic DNA

Adult *A. suum* worms were collected from infected pigs in a local slaughterhouse. Females were dissected and eggs were collected (Spicher *et al.,* 1994). For development, the eggs were incubated at 30°C in water containing 0.1% H_2SO_4. Four-cell stage embryos were harvested after 60-72 hours and L2 larval stages after 20-30 days. Removal of the chitinous layer (peeling) and the DNA isolation was performed as described (Müller *et al.,* 1982; Spicher *et al.,* 1994).

DNA Sequencing and Sequence Analysis

Sequencing was performed by the chain termination method (Sanger *et al.,* 1977) with Sequenase 2.0 (U.S. Biochemicals).

Cloning of Telomere Addition Sites

Different primers homologous to CBR1 (indicated in Fig. 2) and a telomere complementary primer (5'-CCT AAG CCT AAG CCT AAG CCT AAG CC-3') were used to amplify the newly formed somatic telomeres by PCR. Best results were obtained by using the Taq polymerase from Appligene with 35 cycles (94°C, 40 sec; 54°C, 1 min; 72°C, 1 min), followed by a terminal elongation step (5 min at 72°C). For analysis, 5 µl of a 25 µl PCR reaction was loaded on a 1% agarose gel. The remaining 20 µl of reaction mixture was used for cloning by adding 80 µl of TE and 10 µl of 3 M NaAc, pH5.2. The DNA was extracted once with phenol, saturated with sodium acetate, and once with chloroform/isoamylalcohol 24:1 and finally precipitated with ethanol. The DNA was ligated in a Promega T-Vector, amplified in XL1 blue cells and sequenced as described above.

Accession Number

The sequence of CBR1 and its surrounding region is available from GenBank under the accession number AY048884.

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