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Aspergillus nidulans maintains short telomeres throughout development

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ABSTRACT

We report the identification and cloning of the telomeres of the filamentous fungus, *Aspergillus nidulans*. We have identified three classes of cloned chromosomal ends based on the telomere-associated sequences (TASs) and demonstrated that the telomeric repeat sequence is TTAGGG, identical to that found in vertebrates, including humans, and some lower eukaryotes. One category of telomere clones was found to contain internal, variant TAAGGG repeats. The *A.nidulans* telomeric tract length is strikingly short (4–22 repeats). We demonstrate that telomere length is remarkably stable in different cell types and at altered growth temperatures, suggesting a highly regulated mechanism for length control.

INTRODUCTION

The study of telomeres has seen a renaissance since the early cytological observations of eukaryotic chromosome termini in plants (1). The generally G/C-rich telomeric repeats found at chromosome ends are synthesized by the enzyme telomerase (reviewed in 2), providing a means for the complete replication of the chromosome. Telomeres were originally shown to be involved in preventing end-to-end chromosome fusion thereby increasing chromosome stability (1,3,4). They have also been shown to silence nearby genes (5), and increase chromosome stability (6). More recent evidence seems to implicate telomeres in a role in nuclear movement in pre-meiotic cells (7). However, the cellular roles of telomeres are still only poorly understood. Telomeres also define the boundaries of the genetic and physical maps of chromosomes and so are particularly important for the mapping of large genomes that is now being attempted.

The multinucleate ascomycete fungus *Aspergillus nidulans* has been used extensively for the study of eukaryotic gene structure, organization and regulation (8). *Aspergillus nidulans* has been especially valuable for investigating the genetic and molecular processes responsible for controlling development (8,9 and references therein) and mitotic regulation (10 and references therein).

Previous studies have shown that the *A.nidulans* telomeric repeat sequence is likely to be TTAGGG (11). Here we report the characterization of cloned *A.nidulans* telomeres, and demonstrate this organism has surprisingly short telomere tracts of 4–22 TTAGGG repeats. These tracts appear to be rigorously maintained in different cell types and under different growth temperatures.

MATERIALS AND METHODS

Growth media and conditions

Aspergillus nidulans (FGSC26, ATCC #24758) were grown in solid YAG medium (5 g/l yeast extract, 20 g/l dextrose, 20 g/l agar, 1 ml/l Cove's Trace Elements, 1.2 g/l $MgSO_4.7H_2O$) and in liquid YG medium (as described, excluding agar). Unless otherwise specified *A.nidulans* cells were grown at 37°C; on solid medium, cells were incubated for 24–48 h. In liquid culture, sterile YG media was inoculated with *A.nidulans* spores to a final concentration of 10^6 spores/ml and grown for typically 16–24 h with vigorous shaking/aeration (250–300 r.p.m.).

Escherichia coli cells (XL-1Blue MRF′) were grown in LB media containing 100 µg/ml ampicillin. Cells were grown for 12–16 h at 37°C, and in liquid culture shaken vigorously (250 r.p.m.) overnight.

Preparation of plasmid and *A.nidulans* genomic DNAs

Aspergillus nidulans cells (mycelia) were grown in liquid culture by inoculating 100 ml YG medium with a liquid spore stock (1.2×10^9 spores/ml) to a final concentration of 1.2×10^6 spores/ml. Cells were harvested by filtration over two sheets of sterile MiraCloth (CalBiochem), washed with 1 l sterile MilliQ water (MilliPore), and the resulting mat of cells transferred to a sterile 50 ml Falcon tube. Cells were lyophilized overnight, and subsequently ground to a fine powder with a pestle and mortar using 425–600 micron glass beads (Sigma). Twenty milligrams of this dry cell material was used to prepare DNA, using methods described by Raeder and Broda (12). Conidial spore genomic DNA was prepared as described in (13).

Plasmid DNA (Bluescript™-based, Stratagene) was prepared by inoculating 5–10 ml LB containing 100 µg/ml ampicillin with a single bacterial colony. Cells were grown to saturation, harvested and plasmid DNA isolated using Qiagen DNA columns, according to manufacturer's instructions (Qiagen).

Cloning and DNA sequence analysis

To clone the *A.nidulans* telomeres, 1 µg genomic DNA was treated with 10 U T4 DNA polymerase for 10 min at 12°C in order to create blunt-ends. The genomic DNA was then ligated overnight at 15°C to an *Eco*RV-cut (blunt-ended) Bluescript™ vector (Stratagene). Subsequently, the genomic DNA was digested with *Eco*RI which cleaves the *A.nidulans* DNA and cuts once in the vector polylinker. The resulting vector joined to the putative terminal DNA fragments were ligated at 15°C overnight

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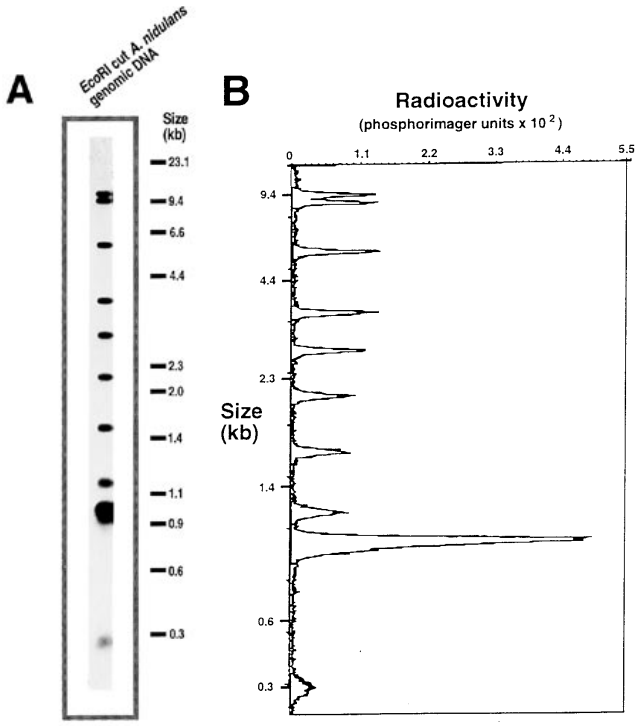

Figure 1. Southern blot analysis identifies putative telomeric DNA sequences in the *Aspergillus nidulans* genome. (**A**) *Aspergillus nidulans* genomic DNA derived from mycelium was cut with *Eco*RI, digestion products separated by 0.7% agarose gel electrophoresis and then subject to Southern blot hybridization with a $(TTAGGG)_4$ oligonucleotide probe. Hybridization was carried out under conditions described in Materials and Methods, and washed in 50 mM phosphate buffer (14) (3×5 min at 25°C; 2×5 min at 37°C). Black bars represent positions of DNA molecular weight size markers. (**B**) The radioactivity in the 10 probe-hybridizing bands observed was determined and quantitated by PhosphorImager analysis (Molecular Dynamics), and molecular weights assigned by correlation with the DNA size ladder used.

with T4 DNA ligase (New England Biolabs, NEB) to re-circularize, thus producing a library of telomeric DNA ends. A proportion of the library was transformed into *E.coli* (XL-1Blue MRF′, Stratagene) and plated onto selective media. The plated library was transferred to HyBondN+ (Amersham) membrane by performing colony lifts, and screened for putative telomeric DNA using a radiolabeled $(TTAGGG)_4$ oligonucleotide probe. Positive bacterial colonies were identified and Bluescript plasmids containing putative *A.nidulans* telomeric DNA were prepared. Unambiguous DNA sequencing data was obtained from the clones containing telomeric repeat sequences using a Sequenase™ DNA sequencing kit (US Biochemicals) using [α-^{35}S]ATP (1000 Ci/mmol) and primed by a 18 base T7 promoter oligonucleotide. All DNA sequence data collected was, therefore, derived by copying the C-rich telomeric strand.

Enzyme digestions

Bal31 nuclease (NEB) time course reactions were performed in a 35 µl volume of buffer (12 mM $CaCl_2$, 12 mM $MgCl_2$, 0.2 M NaCl, 20 mM Tris–HCl pH 8.0, 1 mM EDTA) on ~14 µg *A.nidulans* genomic DNA at 30°C. Aliquots of the reaction mix were taken at 5 min intervals, up to 65 min, and stopped by addition to 0.5 M EGTA to a final concentration of 20 mM in the reaction aliquot. An initial 5 µl aliquot of the reaction mix, prior to addition of nuclease, provided a zero time point for the time course. Terminated reactions were kept frozen on dry ice prior to digestion with *Eco*RI. Loading dyes (with RNAse A) were subsequently added and subject to agarose gel electrophoresis.

Restriction enzyme digestion (NEB) reactions and enzyme reactions required for cloning telomeric DNA, e.g. T4-derived DNA ligase and DNA polymerase (Boehringer), were carried out under conditions specified by the manufacturer.

Gel electrophoresis and Southern blot analysis

Agarose gels were typically electrophoresed at 1.2 V/cm for 12–16 h, and DNA visualized by staining in ethidium bromide solution (10 µg/ml) and photographed (Polaroid) under UV illumination.

Agarose gels used for Southern blot analysis were subsequently depurinated, denatured and transferred to HyBondN+ membrane (Amersham) in 0.4 M NaOH typically for 6–12 h. Transferred DNA was UV crosslinked to the membrane (StrataLinker 1800, Stratagene) prior to hybridization to ^{32}P-radiolabeled probes. Hybridization was carried out in SDS–Na_2PO_4 using the procedure of Church and Gilbert (14). Quantitation of radioactivity on Southern blots was performed using a PhosphorImager workstation (Molecular Dynamics, Sunnyvale, CA). The TAS probes used in Figure 4 were hybridized in 0.5 M phosphate buffer (14) at 37°C, overnight, and washed in 50 mM phosphate buffer 3×5 min at 25°C, followed by 2×5 min washes at 60°C.

DNA sequencing reactions (Sequenase, US Biochemicals) were analyzed on 0.4 mm thickness 6.0% polyacrylamide–7 M urea denaturing gels. Gels were dried down onto 3MM paper (Whatman) and subject to autoradiography.

Probes and radiolabeling

Oligonucleotide DNA probes (Only DNA™, TX) were generated by 5′-end-labeling with T4 polynucleotide kinase (NEB) and [γ-^{32}P]ATP (6000 Ci/mmol). The sequence of the 24 base 18S rDNA oligonucleotide probe (Fig. 3) used as a chromosome internal marker is 5′-AAGTCGTAACAAGGTTTCCGTAGG-3′.

Telomere-associated sequence (TAS) DNA fragments were produced using a GeneAmp kit (Perkin-Elmer Cetus) using standard protocols. TAS PCR products were agarose gel purified using GeneClean (Bio101, Inc.) and subsequently labeled to high specific radioactivity using a MultiPrime kit (Amersham) in the presence of [α-^{32}P]ATP and [α-^{32}P]CTP (3000 Ci/mmol). The sequence of the TAS A PCR primers is described in Figure 2B. The sequence of the TAS B primers is 5′-GAAGTGCTAGGA-TAGCTTTAATC-3′ and 5′-CGCAGGAATTCCTTTTAGAG-3′. The sequence of the TAS C PCR primers is 5′-GACGTGCCG-TACGTACGTAC-3′ and 5′-TCCTTGGATAATGTCGCAG-3′. The sizes of TAS A, B and C DNA fragments are ~240, ~200 and ~400 bp respectively.

^{32}P (and ^{35}S) radiolabeled DNA was visualized by autoradiography using Kodak X-OMat film at –80°C using fast-tungstate intensifier screens.

RESULTS

Identification of $(T_2AG_3)_n$-hybridizing DNA tracts

Previous studies suggested the telomeres of *A.nidulans* likely contain the sequence $(TTAGGG)_n$ (11). In order to map the

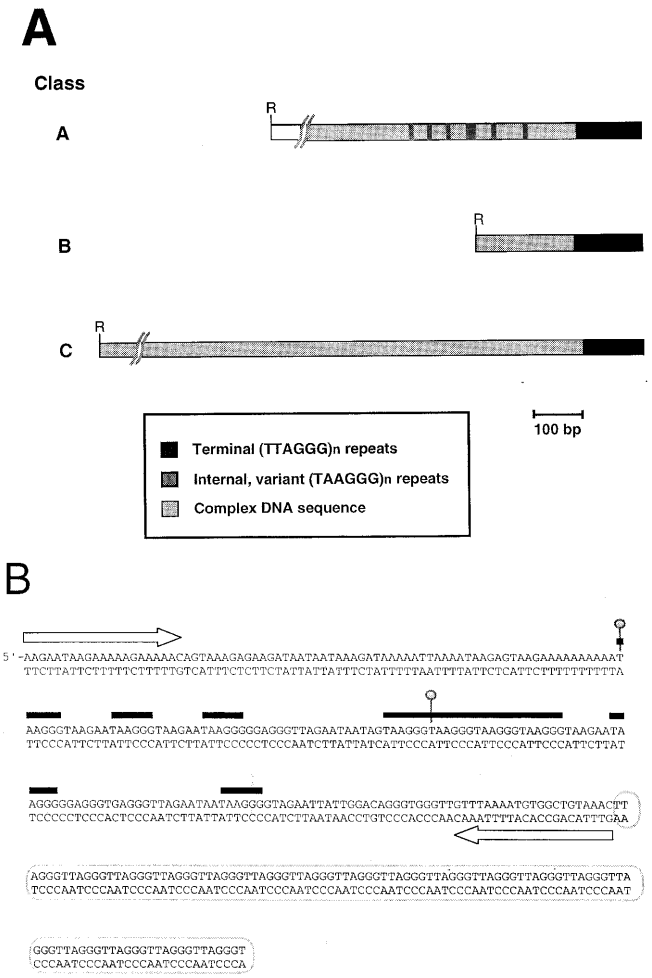

Figure 2. Cloning of the *Aspergillus nidulans* telomeres. (**A**) Schematic representation of the organization of three classes of telomere clones identified from *A.nidulans* (FGSC26) are indicated. Classification was assigned based on DNA sequencing of the telomere-associated sequences (TASs) of the telomere clones isolated, such that TAS A, B and C defined classes A, B and C respectively. The left terminal boundary of each class of telomere clone shown, ended in an *Eco*RI (R) restriction site. There is a ~650 bp of TAS A sequence which has not yet been analyzed (unshaded) and it is unclear if this region is also conserved for this telomere class. (**B**) DNA sequence from class A telomere clones revealed a complex telomere-associated sequence. Shaded areas identify terminal $(TTAGGG)_n$ repeat stretches. Solid black bars indicate positions of perfect, variant $(TAAGGG)_n$ repeats in the telomere-associated sequence. The sequence between the shaded circular markers represents a telomere clone containing a deletion of this enclosed region. Large open arrows indicate the sequence and hybridization position of PCR primers employed to generate a 238 bp TAS DNA fragment from class A telomere clones (referred to as TAS A probe), employed later, see Figure 4. The TAS A DNA sequence shown reflects the extent of DNA sequencing analysis performed, thus only part of the full TAS sequence from this class of telomere clones is known to date.

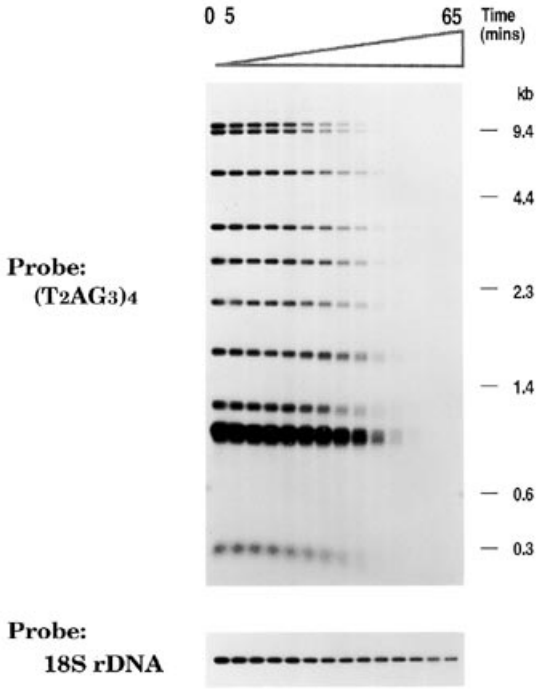

Figure 3. Southern blot analysis reveals Bal31 nuclease sensitivity of *A.nidulans* telomeres. Genomic DNA, prepared from vegetatively grown *A.nidulans* mycelium, was digested with Bal31 exonuclease, and aliquots were removed and enzymatic activity stopped at 5 min intervals up to 65 min. DNA was then digested with *Eco*RI. DNA fragments were separated on 0.7% agarose gel, and Southern blots were probed using an oligonucleotide with the telomeric repeat sequence $(TTAGGG)_4$ under conditions described in Materials and Methods. **Inset** shows the result of re-probing the blot with a radiolabeled oligonucleotide to the chromosome-internal 18S rDNA gene, as a control. Some overall loss of DNA occurred towards the later time points because of the endonucleolytic activity of Bal31.

telomeres for cloning purposes, *A.nidulans* genomic DNA from vegetative mycelium was prepared and then cleaved with *Eco*RI. The resulting genomic DNA fragments were separated by agarose gel electrophoresis and subjected to Southern blot analysis using a $(TTAGGG)_4$ oligonucleotide probe. Figure 1 shows result of this Southern blot hybridization and confirm the previous findings. Ten DNA fragments were observed to hybridize strongly to the probe (Fig. 1A). Since *A.nidulans* possesses eight chromosomes we would predict the presence of 16 chromosomal ends or telomeres. To analyze the representation of chromosome termini in each hybridizing band, we quantitated the amount of radioactivity in each band (Fig. 1B). This revealed that the ~1 kb signal was ~7–8 times stronger than the signal from the other nine bands. Therefore, all 16 chromosome ends can be accounted for on this Southern blot: seven bands which co-migrate at ~1 kb, and nine independent fragments.

Cloning and DNA sequence analysis of *A.nidulans* telomeres

To further characterize the *A.nidulans* telomeres, we cloned the telomeres into a Bluescript vector using techniques that enrich and preserve full telomere length (15–17). DNA sequence was obtained from 11 telomeric clones, and all clones were found to contain a terminal tract of contiguous TTAGGG repeat sequence. The number of repeats varied from 4 to 22 (Table 1). We note that it is a possibility, though unclear, that the clone with the smallest telomeric repeat number may have deleted some telomeric repeat sequence due to possible sequence instability during cloning/propagation in *E.coli*. Analyzing the frequency of clones by repeat number, the most frequently obtained clones (7/11) contained 15–20 repeats (90–120 bp of repeat tract). A mean telomere length of 84 bp of TTAGGG sequence was found in the clones studied (Table 1), with the G-rich repeat strand oriented as expected for a telomere 5′ to 3′ toward the chromosome terminus.

The telomere clones identified were classified according to the sequence of their telomere-associated sequences (TASs; Fig. 2A).

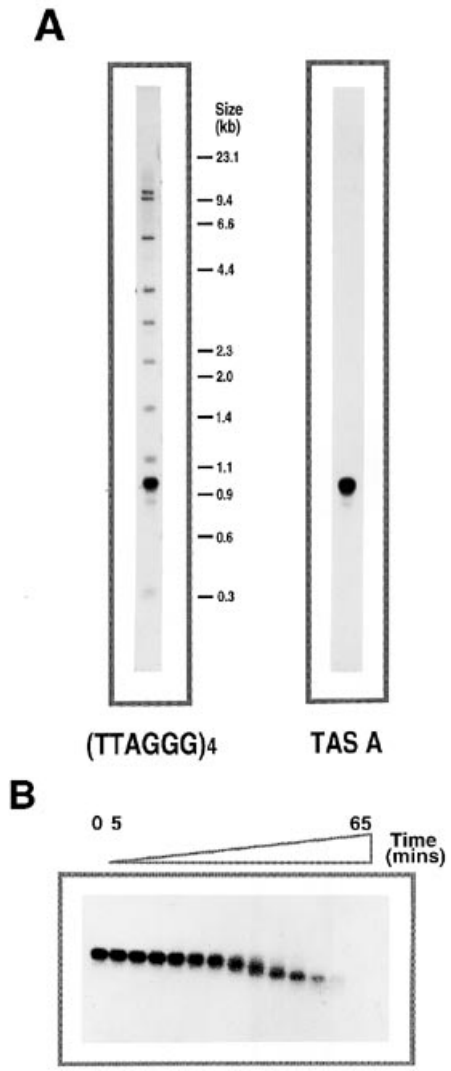

Figure 4. Southern blot analysis identifies a telomere-associated sequence (TAS A) localized to a chromosomal end. (**A**) Duplicate samples of *A.nidulans* genomic DNA from mycelia were cut with *Eco*RI and loaded onto to duplicate lanes of a 0.7% agarose gel. After Southern blotting, the blot was cut into duplicate membrane strips. One blot (left panel) was probed with the radiolabeled telomeric $(TTAGGG)_4$ oligonucleotide probe, while the second (right panel) was probed with a radiolabeled 238 bp TAS A PCR product (under conditions noted in Materials and Methods). The DNA fragment hybridized to by the TAS A probe co-migrates with and correlates to the ~1 kb telomeric DNA fragment. (**B**) The TAS A probe employed above was then used to re-probe the Bal31 digestion time course blot (employed in Figure 3). The DNA fragment hybridizing to the ^{32}P-labeled TAS A probe shortened in size and eventually disappeared with time, indicative of a telomere localization.

Thus, TASs A, B and C defined telomere classes A, B and C respectively (Fig. 2A). Sequences of the cloned TAS and telomeric DNA tracts from these three classes of clones have been entered into the GenBank database. The class A telomere clones contained chromosome-internal variant $(TAAGGG)_n$ telomere-like DNA repeats, sometimes flanked by degenerate telomere repeat-like sequences, for example, 5′-..TAAGAA.. (Fig. 2A and B). The distal $(TTAGGG)_n$ repeats were segregated in a solely terminal domain. One telomere clone (class A) lacked a 53 bp region containing variant repeat tracts within the TAS A sequence (schematized in Fig. 2B).

Table 1. Cloned telomeric DNA tracts from *A.nidulans*

Clone and telomere class	No. of clones	No. of T_2AG_3 repeats	Telomeric DNA tract length (bp)
A3	1	4.7	28
A4	1	20.3	122
A5	1	20.2	121
A6	1	15.2	91
A8	1	18.0	108
A9	1	19.7	118
A10	1	18.2	109
B5.1	2	22.3	134
C8.1	2	15.8	95

Eleven telomeric DNA clones were analyzed by DNA sequence analysis to determine the telomeric repeat number and sequence. The mean cloned telomeric tract length from all clones studied was determined to be 84 bp. Telomere clones from classes A, B and C were derived from ~1, ~0.3 and ~1.5 kb *Eco*RI-cut telomeric fragments respectively. Telomere clones from classes B and C represent duplicate clones from the same telomeric DNA fragment.

Bal31 nuclease sensitivity of *A.nidulans* telomeres

To unequivocally demonstrate that we had indeed cloned the telomeric DNA sequences from *A.nidulans*, we utilized the preferential sensitivity of telomeres to the exonuclease Bal31 (15 and references therein). We performed a Bal31 digestion time course on uncut *A.nidulans* genomic DNA. The digestion products of the Bal31 reaction were digested with *Eco*RI, separated by agarose gel electrophoresis and analyzed by Southern blotting using a $(TTAGGG)_4$ oligonucleotide probe (Fig. 3). In contrast to the 18S rDNA chromosome-internal marker (inset, Fig. 3), the signal from the TTAGGG hybridizing bands diminished in size and eventually disappeared with time, indicating these sequences were indeed telomeric. Moreover, there was very little hybridization to any chromosome-internal TTAGGG hybridizing tracts under these conditions. This suggests there are few, if any, telomere-like repetitive sequences at internal positions in the *A.nidulans* genome.

The discrete nature of these TTAGGG hybridizing bands (Fig. 3) suggests that rather than being heterogeneous in length, *A.nidulans* native telomeres are relatively uniform and defined in length. Furthermore, at the time of near disappearance of the telomeric DNA bands there is only a small decrease in size compared with the zero time point, indicating that *A.nidulans* native telomeres are relatively short. From these data, we estimate that the average telomere length is ~30–100 bp. This correlates well to the mean length of 84 bp obtained from the cloned telomere tracts.

Terminal localization of telomere-associated sequence

In order to verify the telomeric position of the cloned TAS A sequence, we constructed a hybridization probe containing no TTAGGG telomeric repeats using PCR (Fig. 2B). The 238 bp TAS A PCR product was then used as a probe for the Bal31 Southern blot employed in Figure 3. Under these conditions, there was specific hybridization of the probe to the ~1 kb telomeric DNA fragments (Fig. 4A and B). There was also a small amount of hybridization to the ~1.5 kb telomeric band which, however, washed off under more stringent wash conditions (data not shown). As predicted for a telomere-located sequence, the 1 kb DNA fragment hybridizing to the TAS A probe was shortened

and then lost during the Bal31 time course (Fig. 4B), indicating that this sequence does reside at a telomere. However, it is possible that the TAS A probe is hybridizing to more than one chromosomal end in the 1 kb class of telomeres. Additionally, we noted earlier that the TAS A DNA sequence, now identified in the 1 kb telomeric band (Fig. 4A), contained the variant TAAGGG repeats (Fig. 2B). Cross hybridization of the $(TTAGGG)_4$ probe to the variant repeats could account for the slightly higher than expected signal obtained from quantitation of this band in Figure 1B.

PCR probes were also made from TAS B and C (described in Materials and Methods) and used to probe similar Southern blots. The TAS B probe hybridized to four DNA fragments (~0.3, ~1 and ~2.2 kb telomeric bands, and ~0.5 kb DNA fragment) with varying signal intensities (data not shown). The TAS C probe hybridized to >30 DNA fragments of generally higher molecular weight than detected by TAS A and B probes (data not shown). The class B and C telomere clones contained terminal telomeric arrays adjacent to TAS B and C respectively, which each consisted of a complex DNA sequence, lacking any variant telomere-like or degenerate repeats as seen in TAS A (data not shown).

Uniformity of native telomere length with cell-type and growth temperature

As discussed above, the Southern blot analysis of the *A.nidulans* telomeres (Figs 1A and 3) showed the telomeric bands were strikingly discrete and uniform in length. This is in contrast to the broadness of telomeric DNA bands observed with some other eukaryotic telomeres, which is generally indicative of heterogeneous telomere repeat tract length (reviewed in 2,18). This suggests that telomere length in *A.nidulans* may be tightly regulated. To search for conditions which might perturb the telomere length or homogeneity, we first studied the length of the telomeres in cells from a different developmental stage, asexual spores. Genomic DNA was prepared from *A.nidulans* spores (described in Materials and Methods) and native telomere length was analyzed by Southern blotting (Fig. 5). No difference was seen in the native telomere lengths in the dormant spore stage compared with the vegetatively grown mycelium (Fig. 5, compare lane b with d). Furthermore, a Bal31 digestion time course performed on spore genomic DNA also gave an estimated telomere length of 30–100 bp (data not shown).

Studies on the yeast *Candida albicans* have shown that telomere length changes with growth temperature, such that longer telomeres are observed at higher temperatures (15). To determine if variation of growth temperature could alter the telomere length in *A.nidulans*, cells were grown at 30, 37 and 42°C (described in Materials and Methods) and genomic DNA prepared from these vegetatively grown mycelial cells. Again, by Southern blot analysis (Fig. 5) no difference was visible in telomere length of *A.nidulans* cells grown at these three temperatures. Thus, our results indicate that telomere length in *A.nidulans* remains the same irrespective of the cell types and temperatures studied.

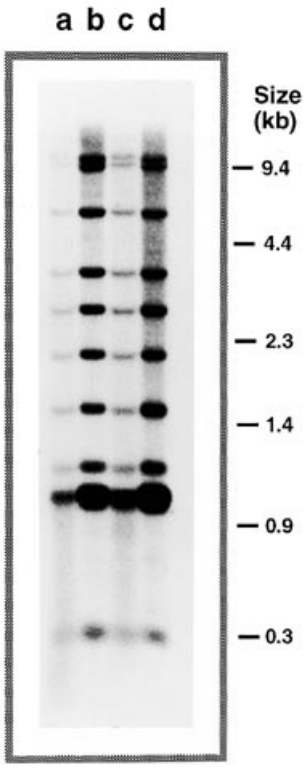

Figure 5. Invariance of telomere length as a function of cell type and growth temperature. The effect of growth temperature and cell type on telomere length was investigated first by preparing *A.nidulans* genomic DNA derived from vegetative mycelia grown at 30, 37 and 42°C, and from the conidiospores. DNA was cut with *Eco*RI, separated by agarose gel electrophoresis, Southern blotted and probed with a ^{32}P-labeled telomeric $(TTAGGG)_4$ oligonucleotide. Lanes a–d, genomic DNA derived either from vegetative mycelia grown at (a) 30, (b) 37, (c) 42°C or (d) the asexual conidiospore stage of *A.nidulans* grown at 37°C. Variations in signal intensity between lanes is due to differences in DNA loaded onto the gel, as determined from the ethidium bromide staining (data not shown).

DISCUSSION

We have cloned the telomeres of the multinucleate filamentous fungus *Aspergillus nidulans*. As previously predicted (11), the cloned telomeres comprise the repeated sequence TTAGGG. This is the same repeat sequence found in vertebrates, including mammals (e.g. mice and humans), *Neurospora crassa*, slime molds (e.g. *Physarum*) and trypanosomes (2,18,19). The size, sequence and uniformity of the telomeric repeats of the filamentous fungi resemble those of higher eukaryotes rather than some budding yeasts, which in *S.cerevisiae* contains short, degenerate repeats, while *Candida albicans* and *Kluyveramyces lactis* have very large (23 and 25 bp respectively) repeat units (15,20).

Although the distal *A.nidulans* telomeric repeats are uniform, some degeneracy was found centromere proximally. In many organisms, variant telomeric repeats are found in subtelomeres, but the number and degeneracy of the variant repeats depends on the species (21,22). In humans, several variant repeats are interspersed in the more inner repeats (23). In one class of *A.nidulans* telomeric clones (TAS A) variant repeats were buried within a tract containing degenerate repeats. The TAS sequence containing variant repeats was localized to the ~1 kb telomeric DNA fragments shown to contain 6–7 chromosomal ends. It is thus possible that nearly half the *A.nidulans* telomeres contain the variant repeats. The other telomeres, and the remainder of the *A.nidulans* genome is largely devoid of such variant telomere-like DNA sequences. Though the exact function of the internal variant repeats is unclear, they likely arose from a mutation that occurred in a telomeric tract and subsequent recombination, rather than through the action of a second, variant-template telomerase (21 and references therein).

The *A.nidulans* telomeres are dramatically different from vertebrate telomeres in one respect: the length of the telomere tract. The telomeric TTAGGG repeat tracts of human somatic tissues are ~10 kb, and those of some mice are up to ~150 kb (24). Thus, the telomeres constitute ~0.1% of a human chromosome. In contrast, we demonstrate that the *A.nidulans* telomeres are ~100 bp (0.003% of an average *A.nidulans* chromosome), only ≤3% of the vertebrate length. The only shorter known telomeres are found on the subchromosomal fragments of some ciliated protozoa. For example, the hypotrichous ciliates *Oxytricha* and *Stylonychia* contain 20 bp of telomeric duplex DNA, with 16 bases of 3′ single-stranded overhang (25). Similarly, *Euplotes* telomeres comprise 28 bp of duplex telomeric sequence with a 14 base single-stranded 3′ overhang (25). Although short relative to other organisms, the telomeric DNA of the hypotrichous ciliates constitutes 1% of the chromosome, since the total length of the hypotrichous ciliate subchromosome is only ~2 kb (25). Interestingly, the hypotrichous ciliate telomere length is extremely tightly regulated. Perhaps the short telomeres in these organisms and in *A.nidulans* define the minimum length required to maintain function, and therefore must be rigorously controlled. Conversely, perhaps *A.nidulans* exhibits a strong selection against longer telomeres due to an unknown deleterious nature of longer telomeres.

Variation in telomere length in eukaryotes is common (26). In fact, one of the characteristic identifying features of telomeres of most organisms is that they are usually heterogeneous in length, producing 'fuzzy' bands on Southern blots. However, the telomeres of *A.nidulans* appeared as more discrete bands on Southern analysis, indicating a greater degree of uniformity in native telomere length. These results suggest that telomere length is tightly controlled in *A.nidulans*. This is further supported by the observation that *A.nidulans* telomere length remains the same irrespective of cell types studied and changes in growth temperature. By comparison, studies in the yeast, *Candida albicans*, revealed that telomere length increased with increase in growth temperature (15). One possible explanation is that disruption of telomeric chromatin at higher growth temperatures would likely render the 3′ chromosomal end accessible to extension by telomerase. In fact, recent studies in the yeast, *Kluyveramyces lactis*, indicate that this temperature effect on telomere length may indeed result as a consequence of disruption of telomeric chromatin structure, in particular, involving the protein Rap1p (27). Krauskopf and Blackburn (27) propose Rap1p participates in telomere length control in *K.lactis* via formation of a DNA tertiary structure mediated by protein–DNA and protein–protein interactions involving Rap1p molecules. Such interactions are likely strongly temperature dependent (28,29). However, in *A.nidulans* the invariance in telomere length suggests there may be a different or less easily perturbed mechanism for telomere length control.

The studies presented in this paper will provide better landmarks for the physical and genetic characterization of the *A.nidulans* genome. We also envision that further investigation into the tenacious control of *A.nidulans* telomeres will reveal new insights into telomere homeostasis.

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