Study of multiple fibrillarin mRNAs reveals that 3' end formation in *Schizosaccharomyces pombe* is sensitive to cold shock

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Received December 22, 1992; Revised and Accepted March 16, 1993

EMBL accession no. X69930

ABSTRACT

Fibrillarin is a nucleolar protein which is associated with small nucleolar RNAs, and is required for pre-rRNA processing. We have cloned and characterized the gene encoding fibrillarin in the fission yeast *Schizosaccharomyces pombe* and we have followed its expression under various conditions. Fission yeast fibrillarin is a 305 amino-acid protein which appears to be highly conserved throughout evolution. In *Xenopus*, human or *Saccharomyces cerevisiae*, a single fibrillarin mRNA is detected while, in *S.pombe* a single copy gene encodes different mRNAs which differ at the 3' ends. Under normal growth conditions, two mRNAs of 1.1 and 1.35 kb are detected with the 1.1 kb being the most abundant. Both the total amount and relative abundance of these two mRNAs are strongly affected by exposure to low temperature, namely the 1.1 kb mRNA almost disappears while the 1.35 kb is less markedly diminished. A new species of 3.2 kb accumulates in the cell, which contains an unusually long 3' untranslated region of 2 kb. We have found that exposure of the cells to a cold shock has a profound effect on 3' end formation in *S.pombe* since the transcription of several other mRNAs is also capable of skipping the normal 3' end site to terminate at a further downstream site.

INTRODUCTION

Protein synthesis in eukaryotic cells takes place on cytoplasmic ribosomes, which are ribonucleoprotein particles formed by the assembly of about 80 different polypeptides with 4 different rRNA molecules. The major steps of ribosomes biogenesis in eukaryotes occur within a transient nuclear structure, the nucleolus. Ribosome synthesis requires a set of nucleolar molecules involved in the transcription of the pre-rRNA, its maturation, its assembly into pre-ribosomes and in the shuttling of the components back and forth between nucleus and cytoplasm (reviewed in 1,2). Among these nucleolar components, the small nucleolar RNPs (snoRNPs) are ribonucleoprotein complexes which, by analogy

with the nuclear snRNPs, are believed to be involved in pre-rRNA processing. In *S.cerevisiae*, in which many snoRNAs have been described, only U3, U14 and snR30 are essential for cell viability (3–5), and the deletion of U3, U14 or snR10 impairs, to different extents, pre-rRNA processing and production of 18S rRNA (6–8).

Until recently, little was known about the nucleolar proteins involved in pre-rRNA processing. In *S.cerevisiae*, the two nucleolar proteins fibrillarin (NOP1) and GAR1 have been shown to be required for pre-rRNA processing (9,10) and this is also true for fibrillarin in human and *Xenopus* (11,12). GAR1 is associated with snR10 and snR30 while anti-fibrillarin antibodies immunoprecipitate all the yeast snoRNAs so far characterized. The cDNAs or the genes encoding fibrillarin have been isolated from *Xenopus*, *S.cerevisiae* and human (11,13–16), and comparison of the deduced protein sequences has revealed that fibrillarin is highly conserved throughout evolution.

Regulation of the synthesis of ribosomes is still poorly understood since it requires the coordinate expression of many genes that are transcribed by the three types of RNA polymerases: I (rDNA genes), II (structural genes) and III (5S genes). The regulation of the transcription by each type of polymerase is now well documented, but it is clear that it is not the only way to regulate the level of each component (17,18). For instance, the amount of each ribosomal protein can be regulated post-transcriptionally (19) at the splicing level (20,21), at the translational level (22 and references therein), or by turnover of excess protein (23,24).

In addition to the ribosomal components themselves, the cell must also adapt the amount of the nucleolar components to the level of synthesis of ribosomes. Very little data are available concerning the regulation of the expression of these non-ribosomal components involved in ribosome synthesis. In *S.cerevisiae*, U3 is encoded by two different genes, *SNR17A* and *SNR17B*. Deletion of either of these genes is not lethal and in a *snr17A*[–] strain, the steady-state level of snR17B RNA is increased, while deletion of the two genes is lethal (4). In *Xenopus*, the expression of fibrillarin and nucleolin, the major nucleolar protein in vertebrate cells (25,26), has been studied during early embryo

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development. Fibrillarin mRNA accumulates during early oogenesis, as is the case for numerous mRNAs, but in contrast to nucleolin or ribosomal protein mRNAs, it is still present at a high level throughout fertilization and early development. This steady state is likely to be due to the presence of stabilizing sequences in its 3' untranslated region (27). Translation of this mRNA, which occurs during early embryogenesis, precedes the reformation of the nucleolus.

Schizosaccharomyces pombe is a fission yeast which has been widely used for studying cell cycle, but in which ribosome biogenesis is still poorly documented, except for one of the two genes encoding the snoRNA U3 which has recently been cloned and sequenced (28,29). Since fibrillarin is highly conserved throughout evolution, human anti U3-sRNP autoimmune antisera are able to recognize fibrillarin from *S.pombe* and immunoprecipitate a subset of snRNAs, some of which possess a trimethyl CAP (30).

We have undertaken the characterization of the nucleolar components in *S.pombe* and have isolated, sequenced and studied the expression of the gene encoding the well conserved protein fibrillarin.

MATERIALS AND METHODS

Strains and recombinant DNA work

The haploid strain 972 (h⁻) used in this study was introduced by U. Leupold. The standard procedures to work with *S.pombe* were described previously (31). Plasmid DNA preparation, enzyme digestion and gel analysis were performed as described (32), or according to the supplier's instructions. DNA sequencing was carried out using a Sequenase kit (USB corp.). Oligonucleotides were synthesized using an Applied Biosystem synthesizer without further purification.

Southern and Northern blot analysis

Total yeast genomic DNA was extracted as described (33), then digested and submitted to electrophoresis. DNA was stained and transferred to charged nylon membrane (Zeta probe) under vacuum (vacu-blot Hoeffler sci.), and then air dried. For low stringency hybridization, the filters were pre-incubated at 37°C for 5 hours in denatured prehybridization buffer containing 25% formamide, 6× SSC, 5× Denhardt, 10% Dextran sulfate, 0.1% SDS, 0.1% PPI, 100 µg/ml of shared *E.coli* genomic DNA, 100 µg/ml of poly(A) and 100 µg/ml of tRNA. The denatured multiprime labeled (Amersham) probe, corresponding to the EcoRI-BstEII cDNA fragment encoding the GAR domain of *Xenopus* fibrillarin (15), was added to the same medium, at a final concentration of 2 to 4×10⁵ cpm per ml. Hybridization was performed for 15 hours at 37°C. Filters were then washed four times at room temperature and four times at 42°C in 2× SSC and 0.1% SDS before autoradiography at -70°C for 24 hours with intensifying screen. For high stringency hybridization of both Southern and Northern blots, standard conditions were used (32).

Isolation of the fibrillarin gene

To construct the partial genomic DNA library, 50 µg of *S.pombe* genomic DNA were digested with EcoRI and electrophoresed on a 18×25 cm 1% agarose TAE gel. After ethidium bromide staining, migration was measured using molecular weight standards (λ DNA cut by HindIII). 0.5 cm width bands were cut and DNA (about 0.5 µg) was purified using the 'GeneClean'

kit (Bio 101). The purified EcoRI DNA fragments were then inserted into the EcoRI site of the vector pBSK⁻ (Stratagene). The screening of the partial genomic library, thus obtained, was performed in one step using the probe and the low-stringency hybridization conditions described above.

Cold shock treatment of the yeast cells

Cells were grown in 50 ml of YES medium (BIO 101, La Jolla) in 250 ml flasks at 30°C to an OD_{600nm} of 1, then transferred to water baths equilibrated at the desired temperature. 5 ml samples were taken at different times and the cells quickly spun down at the same temperature used for the shock, and rapidly frozen in dry ice. Total RNA was then extracted as follows: 250 µl of cold LET buffer (100 mM LiCl, 1 mM EDTA, 100 mM Tris-Cl pH 7.5) was added to the frozen cell pellet, plus 0.5 g of 0.45 mm beads and vortexed 8 times for 30 sec., at maximum speed, alternating with 30 sec. on ice. Then 12.5 µl of 10% SDS was added to the mixture and vortexed for an additional 10 sec. Phenol extraction was performed with 3 cycles of vortexing as described above. The aqueous phase was recovered by centrifugation and extracted two times with phenol and once with phenol-chloroform. RNA was then recovered by precipitation with 3 vol. of ethanol. This miniprep protocol yields RNA sufficiently pure for northern analysis or cDNA synthesis.

PCR mapping of 3' mRNA termini

Mapping of the 3' ends was done as described (34) by a method which is based on PCR amplification (35). 1 µg of total RNA was mixed with 1 µg of a BamHI-oligo(dT)₁₇ oligonucleotide with the sequence CCCGGATCC(T)₁₇ and denatured for 10 min. at 70°C before returning to ice. Reverse transcription was then performed in a 20 µl volume, using a kit from BRL. After completing the reaction, cDNA was diluted 10 fold and 1 µl of this dilution was used in a 100 µl PCR reaction containing 1 µg of the above primer and 1 µg of the 18-mer primer P1 which has the sequence GCGCACGAATGTCATCCC. The reaction was performed using PFU polymerase enzyme (Stratagene) according to the supplier's conditions with a thermocycler PHCII (Techne) following the sequence: 95°C:4 min. + (95°C:1 min. + 37°C:1 min. + 72°C:1.5 min.)_{30 cycles} + 72°C:5 min. 1 µl of this product was then used in a second run of PCR, using the same conditions, except that 1 µg of primer P2 with the sequence CCCGGATCC-GTCGGAATGGTTGACGTT was used instead of primer P1. The two bands of 650 and 400 nucleotides obtained in this reaction were separated on a 1.6% agarose TAE gel and purified using glass beads (GeneClean: Bio 101) before digestion with BamHI, cloning and sequencing. The cloning of the 3' end of the 3.2 kb mRNA has been done essentially following the same strategy, excepted that only one run of PCR has been performed using primer P4 which has the sequence CCCGGATCCGGCC-AGATTTGATTCTTC. The reaction was less accurate but still contained a fragment of 700 nucleotides whose sequence corresponds to the 3' end of the 3.2 kb mRNA.

RESULTS

Cloning of the fibrillarin gene from *S.pombe*

We have recently reported the characterization of a family of nucleolar proteins in *S.cerevisiae* that contain a domain rich in glycine and arginine residues that we called the GAR domain (for Glycine Arginine Rich) (9). We investigated whether a similar class of proteins exists in *S.pombe* and we undertook to

characterize and isolate the corresponding genes. First, genomic DNA from *S.pombe* was analyzed by Southern blot and probed at low stringency with a cDNA fragment encoding the GAR domain of *Xenopus* fibrillarin. Four strong signals were observed, corresponding to EcoRI fragments of 13, 9, 6 and 5.5 kb (Fig. 1). We isolated the genes corresponding to the three smallest fragments, following a strategy that we previously described (9). Here we report the isolation and characterization of a recombinant clone corresponding to the 6 kb EcoRI fragment, which gave the strongest hybridization signal. The two other genes are designated *GAR1* and *GAR2* and detailed analyses of these will be described elsewhere (36,37). The 6 kb EcoRI insert has been mapped (Fig. 2A), and the region which hybridizes with the fibrillarin probe has been localized and sequenced (Fig. 2B). Sequence analysis indicates that the fragment contains a 915 bp open reading frame that could encode a 305-amino acid protein with a predicted molecular weight of 32,040 Da, and a deduced basic isoelectric point of 10.75. The major feature of this sequence is the presence at the N-terminus of a long GAR domain which is characteristic of the family of nucleolar GAR proteins. Sequence comparisons reveal that this fission yeast protein is highly homologous to fibrillarin from *S.cerevisiae*, *Xenopus* and human (77, 74 and 71 % of identity, respectively) (Fig. 3). This strongly suggests that the gene we isolated from *S.pombe* encodes fibrillarin and confirms the high degree of sequence conservation

of fibrillarin during evolution. In the central part of the protein, the atypical RNA binding domain (15,38) is particularly well conserved in these different species, with about 80% identity.

The alignment of the fibrillarin sequences from four different species reveals that the COOH terminal parts of the proteins match with a good score, while short gaps have been inserted at various places into the GAR domains in order to optimize the alignment. This is typical of GAR domains that evolve quickly from one species to another by duplication of short stretches of Gly, Arg and Phe residues (25,26). Nevertheless, short stretches of homology are found both at the amino-acid and at the nucleotide level, which allow cross-hybridization even between the genes encoding different GAR proteins. It is notable that certain residues like Ala are only found in the GAR domains of yeast fibrillarins while other like Asp are specific for fibrillarins from higher eukaryotes (11,15).

Sequence analysis reveals the presence of a motif -TAGTCA-CA-, located 67 nucleotides upstream from the AUG start codon within the promoter region of the *S.pombe* fibrillarin gene (Fig. 2B). This motif has strong homology with the <homol d> sequence element -CAGTCACA- which is located 70 nucleotides upstream of the AUG, in the promoter regions of nearly all the *S.pombe* ribosomal protein genes so far characterized (39).

A single fibrillarin gene encodes two different transcripts in *S.pombe*

Southern blot analysis using total genomic DNA digested either by EcoRI or HindIII was carried out. With either enzyme a single restriction fragment was detected when hybridization was performed under high stringency indicating that the fibrillarin gene is present at one copy per haploid genome (Fig. 4A).

When total RNA was extracted from exponentially growing cells and analyzed in a northern blot experiment, using a fragment corresponding to the coding sequence as a probe (Fig. 2A), two mRNAs of 1.35 and 1.1 kb were detected (Fig. 4B). Since the fibrillarin gene contains no introns within the coding sequence, as deduced from the sequence of a cDNA clone (data not shown), we investigated whether the differences between these two mRNAs were due to additional sequences located on the 5' or on the 3' side of the mRNA. To achieve this, small fragments corresponding to the two flanking regions of the gene were used to probe identical northern blots (data not shown). The results indicate that the two mRNAs have very similar, if not identical, 5' ends while the 1.35 kb mRNA possesses a longer 3' untranslated region (UTR).

In order to define precisely the 3'-ends of the two mRNAs, we decided to clone the two corresponding cDNAs, using a PCR-based procedure to amplify short fragments encompassing the 3'-ends of the cDNAs (34). The first cDNA strand was primed with an oligonucleotide which contains a track of dT₍₁₇₎ plus a BamHI site on its 5' side. Then cyclic amplification was performed using this oligonucleotide and a primer P1, corresponding to the coding sequence of fibrillarin (Fig. 2B: position 582 to 599). To increase the specificity of the reaction, a second step of PCR was carried out with the same oligonucleotide BamHI-dT₍₁₇₎ and a primer P2, also specific for the coding region (position 643 to 660). After the second run of PCR, two fragments of about 650 and 400 bp were detected, which correlates with the size of the two mRNAs detected in the northern blot experiment (data not shown). These PCR products were cloned and sequenced in order to determine precisely the location of the two sites of addition of poly(A)

Figure 1. *S.pombe* genomic DNA contains sequences related to the cDNA encoding the GAR domain of *Xenopus* fibrillarin. EcoRI fragments of genomic DNA were separated by electrophoresis prior to transfer onto charged nylon membrane. Hybridization of the filter was then performed under low stringency conditions, with a [³²P] labeled fragment of cDNA encoding the GAR domain of *Xenopus* fibrillarin as a probe. Lane 1, λ DNA digested with HindIII as radioactive molecular weight standards. Lane 2, genomic DNA digested with EcoRI. Four fragments give strong signals that are indicated by arrows, the sizes in kb are shown on the right.

Figure 2. A: Physical map of the 6 kb EcoRI insert containing the fibrillarlin gene. The positions of the main restriction sites are indicated (H₂ for HincII), as well as the position of the fibrillarlin ORF (Fib Sp). The 3' ends of the three cDNAs are indicated by (I). The region of 3506 nucleotides for which the sequence is presented below is indicated by the box with diagonal lines. **B:** Nucleotide sequence of the *S.pombe* fibrillarlin gene and its predicted product. Number 1 refers to the putative initiation codon, which is followed by an ORF of 915 nt. A putative <homol d> box situated in the promoter region centered on the position -70, is underlined. The amino-acid translation is given below the nucleotide sequence. The GAR domain, which is located on the N-terminus of the protein, is boxed with a thick line. A putative RNA binding domain, situated in the middle of the protein, is boxed with a thin line, with the RNP1 consensus site underlined. The three polyadenylation sites, which are deduced from the sequence of the three types of cDNAs, are indicated within the 3' UTR: the last nucleotide of each cDNA preceding the poly(A) tract has been boxed at positions 1032, 1278 and 2998. The putative tripartite signals have been underlined.

Figure 3. Alignment of the four fibrillarin sequences. The sequence of the *S.pombe* (Sp) protein, deduced from the nucleotide sequence of the gene, has been compared to the sequences available: (Sc) for *NOP1* from *S.cerevisiae*, (Xl) for *X.laëvis*, and (Hu) for human. The GAR domain and the well conserved proline residue situated just downstream are boxed with double lines, while the putative RNA binding domain is boxed with a single line. Identical residues are denoted by short horizontal double lines while gaps, which have been inserted to obtain best matches, are indicated by dots. Numbers on the top refer to the sequence of *S.pombe*.

(Fig. 2B). As expected from the results of the northern blot, the 3'-ends of the two mRNAs differ by 248 nucleotides. Surprisingly, while a recent study has reported that the higher eukaryotic consensus signal AAUAAA would not be functional in *S. pombe* (40), the sequence AATAAA is found just upstream of the two polyadenylation sites (Fig. 2B and Fig. 7) as is the

case for the cDNAs encoding the nucleolar proteins GAR1 and GAR2 that we have isolated in this species (36,37). Interestingly, the two *S.pombe* fibrillarin mRNA 3' UTRs are AT rich and possess sequences resembling the tripartite elements described in *S.cerevisiae* (41), although the one present in the 1.1 kb mRNA differs slightly from the consensus (Fig. 7).

Figure 4. A single fibrillarin gene encodes two transcripts. **A:** Genomic DNA from *S.pombe* was analyzed in a Southern experiment with an EcoRI-HindIII fragment of 2.5 kb containing the whole *S.pombe* fibrillarin gene as a probe (Fig. 2A). Hybridization was performed under high stringency conditions. Lane 1, 5 μ g of genomic DNA digested by EcoRI and, Lane 2, digested by HindIII. The sizes of the fragments are indicated on the left. **B:** Total RNA are separated on a 1% formamide-formaldehyde agarose denaturing gel, electrotransferred onto nitrocellulose and then hybridized to a HincII-HincII fragment of 0.8 kb, used as a probe (Fig. 2A). The sizes of the two species which are detected are indicated on the right.

Effect of cold shock on the fibrillarin gene expression

In order to determine the functional significance of the two mRNAs for fibrillarin in *S.pombe*, we have determined their levels in various situations. In exponentially growing cells, the 1.1 kb was the most prominent form and corresponded to about two thirds of the total amount of fibrillarin mRNA, while the 1.35 kb corresponded to about one third (Fig. 4B). When RNA was prepared from cells entering stationary phase, no significant difference in the total amount of fibrillarin mRNA, nor in the relative amount of each mRNA were detected. The cell cycle conditional mutant of *S.pombe*, *cdc25.22* (42,43), was used to synchronize the cell culture. No significant changes in the pattern of accumulation of the two mRNAs were observed during the progression through the cell cycle (data not shown). Similarly, we did not detect any differences when the cells were grown in medium containing glucose or galactose. In another set of experiments, we investigated the effect of exposing the culture to different temperatures. A shift to 40°C had no effect on the pattern of accumulation of fibrillarin mRNAs, while exposure to lower temperatures induced a dramatic change in this pattern (Fig. 5A). The strongest effect was observed when the cells were exposed to 10°C, while exposure to 15°C and 20°C had respectively a reduced and no effect. The amount of the 1.1 kb mRNA decreased rapidly when the cells were incubated at low temperature, while the accumulation of the 1.35 kb transcript was less strongly affected. More strikingly, as early as two hours after shifting the culture to low temperature, a novel mRNA of 3.2 kb is synthesized and is accumulated over long periods. This novel RNA is also polyadenylated, since we obtained similar results when using poly(A)⁺ RNA instead of total RNA (data not shown). To determine whether the 3.2 kb mRNA was extended on the 5' or 3' side, we used the sequences flanking the gene on both sides as hybridization probes in Northern

Figure 5. Effect of cold shock on the accumulation of fibrillarin mRNAs. **A:** Cells have been exposed to 10°C for 0, 2, 4 or 16 hours, as indicated. Total RNA was extracted and analyzed in Northern blot using the fragment HincII-HincII of 0.8 kb as a probe. **B:** An identical blot was tested with riboprobes corresponding to the PstI-NheI fragment of 450 nucleotides (antisense: position 2260 to 1810; numbering is according to Fig. 2B). The sizes of the three mRNAs are indicated on the right.

Figure 6. Effect of cold shock on various mRNAs of *S.pombe*. Northern blots identical to those of figure 5 were hybridized to the different probes indicated below each panel: **GAR1** detects only a single mRNA of about 900 nucleotides; **GAR2** reveals the normal 1.8 kb mRNA and a 2.2 kb form after induction; **URA4** hybridizes to the 850 nucleotides mRNA, and to two additional forms of 2.1 and 3.3 kb after cold shock; and **CDC25** allows the detection of a 3.2 kb mRNA in non-treated cells and of a 7 kb mRNA in cold shock cells.

experiments, as described above. We found that the three mRNAs have similar 5' ends, while the 3.2 kb possesses a very long 3' end extension. To test whether this larger mRNA was transcribed from the same DNA strand as the two other mRNAs, we used single strand specific probes (riboprobes). A riboprobe corresponding to the fragment PstI-NheI (antisense), detects readily a single species of 3.2 kb mRNA in the cold shocked cells but not in the non stressed cells (Fig. 5B), while a riboprobe corresponding to the opposite strand does not hybridize with this mRNA (data not shown). Since the difference between the 3.2 kb mRNA and the two shorter ones was apparently only due to the presence of a longer 3' UTR, we isolated a cDNA clone by the PCR-based method indicated above, using another primer P4 (Fig. 2B: position 2318 to 2335) and total RNA prepared from cells which had been exposed to 10°C for 16 hours. Comparison of the sequence of this cDNA with the one of the genomic clone identifies unambiguously the site of addition of poly(A) (position

Figure 7. Schematic representation of the 3' UTR consensus signals. The six rows correspond to the different consensus sequences found downstream the stop codon. For the first five rows, only the sequence of the upper DNA strand is given, while for the sixth row, the sequence of the two DNA strands is represented. The higher eukaryotic consensus signal for polyadenylation (aataaa) or its derivatives (tataaa and attaaa) are underlined with a thick line, and the three motifs of the tripartite element from *S.cerevisiae* are in upper case letters with an arrow on it. The numbers on the left refer to the position of the first nucleotide on the sequence (see Fig. 2B). The first, the second and the last rows correspond to the three mRNAs whose the lengths are indicated on the right, with the polyadenylation sites indicated by the sign -(A). The third, fourth and fifth rows correspond to the three signals found in the 3' UTR extension of the 3.2 kb mRNA, which do not appear to be used to direct 3' end formation *in vivo*.

2998). Sequence analysis reveals that the 3' end of the 3.2 kb mRNA, which is induced after cold-shock, has the sequence TAA-N₁₄-TAGT-N₁₁-TTT-N₅-A_n, which deviates slightly from the consensus. Nevertheless, a perfect tripartite consensus sequence is found in the same region, but on the other strand (Fig. 7). The motif TAG-N₁₉-TATGT-N₁₈-TTT, which is found between positions 2999 and 2952, is identical to the tripartite element which has been shown to function in both orientations in *S.cerevisiae* (44). More strikingly, the extension of 1720 nucleotides of the 3' UTR of the 3.2 kb mRNA, compared to the 1.35 kb one, contains three perfect tripartite motifs: TAGTA-G-N₂₂-TATGT-N₄-TTT, TAG-N₂₄-TAGT-N_(1 or 15)-TTT, and TAG-N₈-TAGT-N₉-TTT starting at positions 1287, 1666 and 2808 respectively. None of these three signals are efficiently used, even after cold shock treatment, since overexposure of the northern blots failed to detect significant amount of the corresponding mRNAs (data not shown).

We then tested whether 3' end processing of other mRNAs was also responsive to cold shock. Identical northern blots were probed for the mRNAs encoding two other nucleolar proteins, GAR1 and GAR2 (36,37), and for the mRNAs encoding URA4 and CDC25 (45,46). GAR2, URA4 and CDC25 probes revealed the accumulation of longer mRNAs (respectively, 1, 2 and 1 additional species), while the probe for GAR1 did not detect any additional forms after cold shock treatment (Fig. 6).

DISCUSSION

We report here the cloning of the gene encoding the nucleolar protein fibrillarin in *S.pombe* and the characterization of its multiple transcripts which are differentially expressed after cold shock. The *S.pombe* fibrillarin, as deduced from its gene sequence, is highly conserved to its homologs in *S.cerevisiae*, *Xenopus* or human, and appears to have the same properties in these different organisms, i.e. localization within the nucleolus (47) and association with U3 snoRNA (30). Since fibrillarin has been well conserved throughout evolution, it is interesting to investigate how the regulation of its synthesis is achieved in different organisms. The promoter of the fibrillarin gene in *S.pombe* contains a sequence <homol d>, which is present in many promoters of the ribosomal protein genes (39). This suggests that this could be a regulatory element in the expression of the nucleolar components, in coordination with the synthesis of the ribosomal proteins.

In *Xenopus*, human or *S.cerevisiae*, the gene is transcribed into a single mRNA while in *S.pombe* grown under normal conditions, we found that a single gene gives rise to two mRNAs of 1.1 and 1.35 kb which represent two thirds and one third, respectively, of fibrillarin mRNAs. Among the numerous mechanisms which control gene expression, an increasing interest has been taken in those which govern termination of transcription and polyadenylation (48,49). For instance, the length of the poly(A) tail can greatly influence the stability of the mRNA and the efficiency of its translation (50,51). There are now several examples of regulation of gene expression by multiple polyadenylation of a single gene transcript. The choice of a particular polyadenylation site among several can either affect the sequence of the ORF, or can lead to additional 3' UTR sequences that can modulate stability and/or translation efficiency of the mRNA (reviewed, 52). This choice can depend upon tissue-specific transcription (53), stage of differentiation (54) or specific carbon source (55 and references therein).

To investigate further the reason for the presence of two mRNAs for fibrillarin in *S.pombe*, we have looked for a situation which could affect the ratio between these two species. While cell cycle progression, carbon source, exponentially growing phase or resting state, or heat shock do not affect significantly the pattern of accumulation of these two mRNAs, we found that cold shock has a striking effect on the ratio between the two. The 1.1 kb mRNA, which is normally the most abundant form, progressively diminishes, more rapidly than the 1.35 kb mRNA. Even more surprisingly, a new species of 3.2 kb is detected, as soon as two hours after shifting the culture to cold temperature. The cloning of the cDNAs corresponding to the two mRNAs of 1.1 and 1.35 kb, which are produced under normal growth conditions and the third mRNA of 3.2 kb, which is cold shock-inducible, has revealed that the difference in size among these mRNAs is due to the use of three different polyadenylation sites.

Different physiological conditions can influence the choice of a specific polyadenylation signal, but no example has yet been reported concerning an effect of a temperature shock on 3' end formation. We found that cold shock affects not only the length of fibrillarin mRNAs, but also those of *URA4*, *CDC25* and *GAR2*. On the other hand, *GAR1* mRNA does not extend beyond its normal 3' end site in the stressed cells. This result reveals the differences among the 3' end signals of different mRNAs, because they do not all behave similarly during this specific stress. The functional relevance of this mechanism is still unknown.

Nevertheless, the addition of 3' untranslated sequences can influence either the stability of the mRNAs or the efficiency of their translation, as recently shown for the amyloid mRNA in human (56). Further work will focus on the characterization of the *cis*-sequences and the *trans*-factors which determine 3' end formation for fibrillarin and other genes in *S.pombe* in cold shock response. Another area of interest is the relative stabilities and translational efficiencies of the different length mRNA transcripts.

ACKNOWLEDGEMENTS

We thank D.Tollervey, E.Sun and D.Ritter for critical reading of the manuscript, and other members of our group for fruitful discussions. We are indebted to F.Amalric for his continuous support. We thank B.Ducommun and G.Bassi for the gift of plasmids carrying respectively the CDC25 and the URA4 genes. We are grateful to Y.De Preval for oligonucleotide synthesis. JPG had a fellowship from the Ministère de la Recherche et de la Technologie. This research was carried out under contract SC1*-0259-C of the Science Programme of the Commission of the European Communities and also supported by the CNRS, by the Association pour la Recherche sur le Cancer, and by the Université Paul Sabatier.

REFERENCES

- Hadjiolov, A.A. (1985) The Nucleolus and Ribosome Biogenesis. Cell Biology Monographs, Vol. 12. Springer, New-York.
- Warner, J.R. (1990) *Curr. Op. Cell Biol.*, **2**, 521–527.
- Bally, M., Hughes, J.M.X. and Cesareni, G. (1988) *Nucleic Acids Res.*, **16**, 5291–5303.
- Hughes, J.M.X., Konings, D.A.M. and Cesareni, G. (1987) *EMBO J.*, **6**, 2145–2155.
- Zagorski, J., Tollervey, D. and Fournier, M. J. (1988) *Mol. Cell. Biol.*, **8**, 3282–3290.
- Hughes, J.M.X. and Ares Jr., M. (1991) *EMBO J.*, **10**, 4231–4239.
- Li, H.V., Zagorski, J. and Fournier, M.J. (1990) *Mol. Cell. Biol.*, **10**, 1145–1152.
- Tollervey, D. (1987) *EMBO J.*, **6**, 4169–4175.
- Girard, J.-P., Lehtonen, H., Caizergues-Ferrer, M., Amalric, F., Tollervey, D. and Lapeyre, B. (1992) *EMBO J.*, **11**, 673–682.
- Tollervey, D., Lehtonen, H., Carmo-Fonseca, M. and Hurt, E.C. (1991) *EMBO J.*, **10**, 573–583.
- Jansen, R.P., Hurt, E. C., Kern, H., Lehtonen, H., Carmo-Fonseca, M., Lapeyre, B. and Tollervey, D. (1991) *J. Cell Biol.*, **113**, 715–729.
- Kass, S., Tyc, K., Steitz, J.A. and Sollner-Webb, B. (1990) *Cell*, **60**, 897–908.
- Aris, J.P. and Blobel, G. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 931–935.
- Henriquez, R., Blobel, G. and Aris, J.P. (1990) *J. Biol. Chem.*, **265**, 2209–2215.
- Lapeyre, B., Mariottini, P., Mathieu, C., Ferrer, P., Amaldi, F., Amalric, F. and Caizergues-Ferrer, M. (1990) *Mol. Cell. Biol.*, **10**, 430–434.
- Schimmang, T., Tollervey, D., Kern, H., Frank, R. and Hurt, E.C. (1989) *EMBO J.*, **8**, 4015–4024.
- Raué, H.A. and Planta, R.J. (1991) *Prog. Nucl. Ac. Res. Mol. Biol.*, **41**, 89–129.
- Warner, J.R. (1989) *Microbiol. Rev.*, **53**, 256–271.
- Warner, J.R., Mitra, G., Schwindinger, W.F., Studeny, M. and Fried, H.M. (1985) *Mol. Cell. Biol.*, **5**, 1512–1521.
- Eng, F.J. and Warner, J.R. (1991) *Cell*, **65**, 797–804.
- Presutti, C., Ciafrè, S.A. and Bozzoni, I. (1991) *EMBO J.*, **10**, 2215–2221.
- Amaldi, F. and Pierandrei-Amaldi, P. (1990) *Enzyme*, **44**, 93–105.
- Maicas, E., Pluthero, F.G. and Friesen, J.D. (1988) *Mol. Cell. Biol.*, **8**, 169–175.
- Tsay, Y.-F., Thompson, J.R., Rotenberg, M. O., Larkin, J.C. and Woolford, J.L. (1988) *Genes and Dev.*, **2**, 664–676.
- Caizergues-Ferrer, M., Mariottini, P., Curie, C., Lapeyre, B., Gas, N., Amalric, F. and Amaldi, F. (1989) *Genes and Dev.*, **3**, 324–333.
- Lapeyre, B., Bourbon, H.M. and Amalric, F. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 1472–1476.
- Caizergues-Ferrer, M., Mathieu, C., Mariottini, P., Amalric, F. and Amaldi, F. (1991) *Development*, **112**, 317–326.
- Dandekar, T. and Tollervey, D. (1989) *Gene*, **81**, 227–235.
- Porter, G.L., Brennwald, P.J., Holm, K.A. and Wise, J.A. (1988) *Nucl. Acids Res.*, **16**, 10131–10152.
- Tollervey, D., Tessars, G. and Lüthmann, R. (1990) *Nucleic Acids Res.*, **18**, 5207–5212.
- Moreno, S., Klar, A. and Nurse, P. (1991) In Guthrie, C. and Fink, G.R. (eds.), Guide to yeast genetics and molecular biology. Meth. Enzym., Vol. 194. pp. 795–823. Academic Press, San Diego, California.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor University Press, Cold Spring Harbor.
- Johnston, J.R. (1988) In Campbell, I. and Duffus, J.H. (eds.), Yeast-A Practical Approach. IRL Press, Oxford, pp. 107–123.
- Frohman, M.A., Dush, M.K. and Martin, G.R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8998–9002.
- Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988) *Science*, **239**, 487–491.
- Girard, J.-P., Caizergues-Ferrer, M. and Lapeyre, B. (submitted).
- Girard, J.-P., Xue, Z., Caizergues-Ferrer, M., Méléze, T. and Lapeyre, B. (unpublished).
- Kenan, D.J., Query, C.C. and Keene, J.D. (1991) *Trends Biochem. Sci.*, **16**, 214–220.
- Gateman, K.B., Teletski, C., Grob, T. and Käufer, N.F. (1989) *Curr. Genet.*, **16**, 361–367.
- Humphrey, T., Sadhale, P., Platt, T. and Proudfoot, N. (1991) *EMBO J.*, **10**, 3503–3511.
- Zaret, K.S. and Sherman, F. (1982) *Cell*, **28**, 563–573.
- Fantes, P. (1979) *Nature (London)*, **279**, 428–430.
- Thuriaux, P., Sipiczki, M. and Fantes, P. (unpublished).
- Irniiger, S., Egli, C.M. and Braus, G.H. (1991) *Mol. Cell. Biol.*, **11**, 3060–3069.
- Grimm, C., Kohli, J., Murray, J. and Maundrell, K. (1988) *Mol. Gen. Genet.*, **215**, 81–86.
- Russell, P. and Nurse, P. (1986) *Cell*, **45**, 145–153.
- Potashkin, J.A., Derby, R.J. and Spector, D.L. (1990) *Mol. Cell. Biol.*, **10**, 3524–3534.
- Manley, J.L., Proudfoot, N.J. and Platt, T. (1989) *Genes and Dev.*, **3**, 2218–2222.
- Wickens, M. (1990) *Trends Biochem. Sci.*, **15**, 277–281.
- Bachvarova, R. F. (1992) *Cell*, **69**, 895–897.
- Sachs, A. (1990) *Curr. Op. Cell Biol.*, **2**, 1092–1098.
- Jacob, S.T., Terns, M.P., Hengst-Zhang, J.A. and Vulapalli, R.S. (1990) *Gene Exp.*, **1**, 49–59.
- Thekkumkara, T.J., Livingston, W.III, Kumar, R.S. and Sen, G.C. (1992) *Nucl. Acids Res.*, **20**, 683–687.
- Wang, Y.-C. and Rubenstein, P.A. (1992) *J. Biol. Chem.*, **267**, 2728–2736.
- Mayer, S. A., and Dieckmann, C.L. (1991) *Mol. Cell. Biol.*, **11**, 813–821.
- de Sauvage, F., Kruys, V., Marinx, O., Huez, G. and Octave, J.N. (1992) *EMBO J.*, **11**, 3099–3103.