NOTEs

Dual-Color Imaging of Nuclear Division and Mitotic Spindle Elongation in Live Cells of Aspergillus nidulans

Wenqi Su,1,2 Shihe Li,1 Berl R. Oakley,3 and Xin Xiang1*

Department of Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences,1 and Walter Johnson High School,2 Bethesda, Maryland 20814, and Department of Molecular Genetics, The Ohio State University, Columbus, Ohio 43210

Received 10 December 2003/Accepted 16 December 2003

We have developed a dual-color imaging system based on cyan fluorescent protein-labeled histone H2A and green fluorescent protein-labeled alpha tubulin to visualize DNA and spindles simultaneously in the same living cell of Aspergillus nidulans. This system allows new details of mitosis and nuclear movement to be revealed.

The filamentous fungus Aspergillus nidulans is an excellent genetic system to study basic cellular processes, including mitosis and nuclear migration (1). Many mutants that are defective in mitotic regulation and nuclear migration have been isolated, and many genes involved in these two processes have been cloned and their products have been characterized (1, 2, 3, 6, 11, 18). Since both nuclear division and nuclear migration are microtubule dependent, mechanistic studies will clearly benefit from dual-color imaging of microtubules and nuclei simultaneously in the same living cell. Here we report the development of a dual-color imaging system that allows, for the first time, simultaneous observation of microtubules and chromosomes in the same live cell.

Because of the difference in codon preference, the commercially available green fluorescent protein (GFP) genes and variants do not express well in A. nidulans. To circumvent this problem, a codon-modified version of GFP has been constructed; this has facilitated living-cell imaging of many cellular structures, including those of microtubules and nuclei (4, 5, 15, 17). In this study, we first mutated the GFP-tubA plasmid that Han et al. had previously constructed (5) and changed GFP to cyan fluorescent protein (CFP). The change of GFP to CFP required six amino acid substitutions: F64L, Y66W, N146I, and SmaI, leaving the CFP-modified backbone of the LB01 vector that has been previously described (9). The region of the histone H2A gene in A. nidulans (10) was obtained from the genome by PCR using the following two primers: GGGCG GCGGCTGACCTGGCGGCAATCTG (the NotI site is underlined) and ATCCCAGCATATCTGGAGGGGACAG GCAG (the SmaI site is underlined). A 1.3-kb H2A genomic DNA that covers more than the entire coding region was digested with NotI and SmaI and ligated to the CFP vector obtained after removing the tubA gene. (Note that the ATG was changed to CTG right after the NotI site to allow in-frame fusion with CFP.) The resultant CFP-H2A plasmid was transformed into SO121 (pyrG89; nicB8) (a gift from Stephen A. Osmani). Multiple transformants were identified that contained CFP-labeled nuclei, and one transformant that contained a single integration of the plasmid and grew as well as the wild-type strain was crossed to the GFP-tubA strain whose genetic background was GR5 (pyrG89; pyroA4; w43). One strain that contained both the GFP-tubA fusion and the CFP-H2A fusion was identified by microscopic observation of cells (see below for details) and was named SL112 (CFP-H2A-pyr4; GFP-tubA-pyr4; pyrG89). In addition, we also made a similar strain that resulted from the cross of a GFP-tubA strain in the SO121 background with a GFP-H2A strain in the GR5 background; this strain is named SL118 (CFP-H2A-pyr4; GFP-tubA-pyr4; pyrG89; nicB8). Both the SL112 and SL118 strains used for image analysis form healthy colonies, suggesting that the fusion proteins did not interfere with cellular activities.

Imaging analysis was done using a system that has been described previously (5) but with the following modifications. Cells were grown at 32°C overnight and observed at 32°C in liquid minimal medium plus glycerol, because both fusions were driven by the alcA promoter. A PCO/Cooke Corporation Sensicam QE cooled charge-coupled device camera and Ludl Electronic Products dual individual excitation and emission motorized filter wheels were used. Chroma 8600 filters for CFP (430-nm peak excitation with a bandwidth of 25 nm and...
470-nm peak emission with a bandwidth of 30 nm) were used for observing CFP-H2A, and filters for YFP (500-nm peak excitation with a bandwidth of 20 nm and 535-nm peak emission with a bandwidth of 30 nm) were used for observing GFP microtubules. Custom macros in IPLab software were written by Jim Paladino of BioVision Technologies.

Using this dual-color imaging system, we observed mitosis in living *A. nidulans* cells. Our observations often started with short spindles that were surrounded by a chromatin mass (Fig. 1A). Although the resolution of individual chromosomes at this stage was not good, the shape of the chromatin mass suggested that it consisted of condensed chromosomes at prophase or prometaphase. Metaphase in higher eukaryotic cells is characterized by chromosomal congression to the metaphase plate midway between the poles of a spindle (13). In *A. nidulans*, a typical metaphase configuration in which all chromosomes align in the middle of the spindle was not observed even when images in many time-lapse sequences were captured at short intervals of 4 to 6 s. In some sequences, however, we did observe a chromatin mass located in the middle of the spindle (Fig. 1H). Whether this represents a metaphase plate-like configuration in *A. nidulans* would need further investigation. It is worthwhile to note that although no metaphase plate was observed in the budding yeast and other fungi (8, 16), a recent analysis on various regions of the budding yeast chromosomes indicates that while chromosome arms do not align at the metaphase plate, centromeres do have a metaphase conformation (12). Compared to the budding yeast in which chromosomes do not condense during mitosis, *A. nidulans* chromosomes do condense during mitosis (as seen with higher eukaryotic cells). Future work using this dual-color system to examine kinetochores and spindles should better address the issue of whether centromeres have a metaphase conformation in *A. nidulans*.

A very interesting feature of *A. nidulans* mitosis is that condensed chromosomes were seen to locate along the mitotic spindle (Fig. 1K to P and Fig. 2A and B). This mitotic configuration has been previously found in fixed cells (7, 14). Since this stage is very short, however, it is not easy to capture it in unsynchronized wild-type cells (14). Our present live-cell analysis allowed us to capture this configuration in almost every mitotic cell. We found that this configuration correlates with a spindle length of about 3.0 ± 0.5 μm (*n* = 10). Since this configuration is quickly followed by chromosome movement to the spindle poles (anaphase A) and spindle elongation (anaphase B), it is most likely that it represents the beginning part of anaphase A (during which chromosomes start to move towards the poles). In contrast to the results seen with budding yeast in which anaphase A starts with or right after anaphase B (12), *A. nidulans* has a relatively more discernible anaphase A during which chromosomes can be seen to move to the poles without a dramatic spindle elongation (Fig. 1K to R, Fig. 2, and Fig. 3B to D). As determined on the basis of data from 10 time-lapse sequences, we found that the time duration from the first appearance of chromosomes to spread along the spindle to the point at which daughter chromatin masses form at the poles differs from cell to cell and ranges from about 30 to about 120 s at 32°C. At the time when two daughter chromatin masses have just separated, the spindle is about 3.5 ± 0.6 μm in length (*n* = 10). After this stage, anaphase B spindle elongation continues, which results in a further separation of the daughter chromatin masses (Fig. 3E to H).

Spindles with associated chromatin often move along the hyphae. Analyses of images with obvious spindle movement
indicated that the average rate of the movement is about 3.8 ± 2.2 μm/minute (n = 16). Besides the movement of spindles, movement of the daughter nuclei after anaphase B spindle elongation was also observed, which is similar to what has been previously described as postmitotic nuclear movement in other filamentous fungi (1). In the particular sequence shown in Fig. 3, the two daughter chromosome masses move closer towards each other and then away from each other and then move back towards each other (Fig. 3I to T). The order and extent of movements differs among nuclei, and the movement rates of different nuclei are not identical either, but analyses of nuclei that showed obvious movement indicated that the average rate of movement is about 3.5 ± 1.6 μm/minute (n = 14). Some of the movements clearly occur after spindles are no longer apparent, suggesting that the forces for such movements most likely come from astral microtubules interacting with each other or with the cortex.

In summary, we have developed a dual-color imaging system that has allowed us to observe the behavior of both nuclei and microtubules in the same living cell. Future detailed analysis using this system in various mutant backgrounds should reveal more information about the mechanisms of mitosis and nuclear migration in filamentous fungi.

This work was supported by a National Science Foundation grant (MCB-0093106 to X.X.), a Uniformed Services University of the Health Sciences intramural grant (R071GO to X.X.), and a National Institutes of Health grant (GM31837 to B.R.O.)
REFERENCES

1. Aist, J. R., and N. R. Morris. 1999. Mitosis in filamentous fungi: how we got where we are. Fungal Genet Biol. 27:1–25.
2. De Souza, C. P., A. H. Osmani, L. P. Wu, J. L. Spotts, and S. A. Osmani. 2000. Mitotic histone H3 phosphorylation by the NIMA kinase in Aspergillus nidulans. Cell 102:293–302.
3. Efimov, V. P., and N. R. Morris. 1998. A screen for dynein synthetic lethals in Aspergillus nidulans identifies spindle assembly checkpoint genes and other genes involved in mitosis. Genetics 149:101–116.
4. Fernandez-Abalos, J. M., H. Fox, C. Pitt, B. Wells, and J. H. Doonan. 1998. Plant-adapted green fluorescent protein is a versatile vital reporter for gene expression, protein localization and mitosis in the filamentous fungus, Aspergillus nidulans. Mol. Microbiol. 27:121–130.
5. Han, G., B. Liu, J. Zhang, W. Zuo, N. R. Morris, and X. Xiang. 2001. The Aspergillus cytoplasmic dynein heavy chain and NUDF localize to microtubule ends and affect microtubule dynamics. Curr. Biol. 11:719–724.
6. Jung, M. K., N. Prigozhina, C. E. Oakley, E. Nogales, and B. R. Oakley. 2001. Alanine-scanning mutagenesis of Aspergillus gamma-tubulin yields diverse and novel phenotypes. Mol. Biol. Cell 12:2119–2136.
7. Jung, M. K., G. S. May, and B. R. Oakley. 1998. Mitosis in wild-type and beta-tubulin mutant strains of Aspergillus nidulans. Fungal Genet Biol. 24:146–160.
8. Kubai, D. F. 1975. The evolution of the mitotic spindle. Int. Rev. Cytol. 45:167–227.
9. Liu, B., and N. R. Morris. 2000. A spindle pole body-associated protein, SNAD, affects septation and conidiation in Aspergillus nidulans. Mol. Gen. Genet. 263:375–387.
10. May, G. S., and N. R. Morris. 1987. The unique histone H2A gene of Aspergillus nidulans contains three introns. Gene 58:59–66.
11. Morris, N. R. 2003. Nuclear positioning: the means is at the ends. Curr. Opin. Cell Biol. 15:4–59.
12. Pearson, C. G., P. S. Maddox, E. D. Salmon, and K. Bloom. 2001. Budding yeast chromosome structure and dynamics during mitosis. J. Cell Biol. 152:1255–1266.
13. Pollard, T. D., and W. C. Earnshaw. 2002. Cell biology, p. 725–740. Elsevier Science, New York, N.Y.
14. Prigozhina, N. L., C. E. Oakley, A. M. Lewis, T. Nayak, S. S. Osmani, and B. R. Oakley. 2004. y-Tubulin plays an essential role in the coordination of mitotic events. Mol. Biol. Cell 15:1374–1386.
15. Ramon, A., M. I. Muro-Pastor, C. Scazzocchio, and R. Gonzalez. 2000. Deletion of the unique gene encoding a typical histone H1 has no apparent phenotype in Aspergillus nidulans. Mol. Microbiol. 35:223–233.
16. Straight, A. F., W. F. Marshall, J. W. Sedat, and A. W. Murray. 1997. Mitosis in living budding yeast: anaphase A but no metaphase plate. Science 277:574–578.
17. Suelmann, R., N. Sievers, and R. Fischer. 1997. Nuclear traffic in fungal hyphae: in vivo study of nuclear migration and positioning in Aspergillus nidulans. Mol. Microbiol. 25:757–769.
18. Zhang, J., S. Li, R. Fischer, and X. Xiang. 2003. Accumulation of cytoplasmic dynein and dynactin at microtubule plus ends in Aspergillus nidulans is kinesin dependent. Mol. Biol. Cell 14:1479–1488.