Fungal Genomics & Biology
ISSN: 2165-8056

Potapova and Golyshev, Fungal Genom Biol 2016, 6:1
DOI: 10.4172/2165-8056.1000135

Review Article Open Access

Revisiting a Special Structural Order of a Growing Tip of the *Neurospora crassa* Hypha

Tatiana V Potapova* **and Sergei A Golyshev**

Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Russia

***Corresponding author:** Belozersky Tatiana V Potapova, Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119991 Moscow, Russia, Tel: 9329844585; Fax: (495) 939-3181; E-mail: potapova@genebee.msu.su

Received date: April 28, 2016; **Accepted date:** May 18, 2016; **Published date:** May 23, 2016

Abstract

Data are presented on the spatial arrangement of the intracellular structures in the apical end of the vegetative hyphae of the filamentous fungus *Neurospora crassa* and the involvement of these structures in the tip growth of the hyphae. On the basis of the comparison of the behavior of mitochondria and microtubules and the data on the electrical heterogeneity of the hyphal apex, a hypothesis is proposed about a possible supervisory role of the longitudinal electric field in the structural and functional organization of the growing tips of the *N. crassa* hyphae. New data are presented on the tip growth of *Neurospora crassa* in the conditions of the resource deficiency. Namely, impairments in the coherence of elongation, branching and septation were studied in isolated 400 μm long apical fragments of *N. crassa* hyphae growing for several hours without the influx of the nutrient materials from the mycelium. This experimental model can be used for the investigations of the molecular and genetic mechanisms regulating the interactions between intracellular structures involved in the tip growth of *Neurospora crassa*.

Keywords: *Neurospora crassa*; Tip growth; Interaction of intracellular structures; Electrical heterogeneity of hypha; Elongation; Branching; Septation; Intercellular interactions

Abbreviations:

TG: Tip Growth; Em: Membrane Potential

Introduction

The advances in modern experimental biology allowed accumulating a large amount of data on the structure and dynamics of intracellular systems involved in tip growth (TG) of *N. crassa* vegetative hyphae. The schematic maps of many metabolic pathways and descriptions of a large amount of participating molecules, including regulatory proteins, were assembled. But building of a holistic picture of the genetic regulation of the interaction between molecules and intracellular systems during TG is complicated by the fact that a growing *N. crassa* hyphal apex simultaneously performs several tasks – such as rapid elongation, spatial orientation, accumulation of matter resources, etc., all controlled by different genes [1].

Dozens of laboratories all over the world intensively study the connection between genome activity and *N. crassa* functions, and the TG analysis presents the most difficulties [2-4]. It became already clear that the activity of at least 50 genes is essential for TG to occur [5], but the exact correlations between separate processes behind cell morphogenesis- signal transmission, cytoskeleton building, polarized secretion and cell wall formation - are yet to be determined. There is certain hope concerning the new technological possibilities for the *N. crassa* cell biology research, such as vital fluorescent proteins [6-8].

Special Construction of the Anterior ~ 165 μm of Hyphal Apex

The latest research showed a special structural order of a 120-150 μm long hyphal apex, that demonstrates the following features:

- First septum develops no closer than ~ 165 μm from the growing tip of an adult hypha [9,10];
- *H+*-ATPases (main *Em* generators for *N. crassa*) are embedded into the plasma membrane no closer than ~120 μm from the growing tip [11,12];
- The value of E_m over the apical 120-150 μm is considerably lower than on a distal part of the hyphae [13,14];
- The motion pattern of microtubules in the apical 120-150 μm part also feature peculiarities, such as a parallel orientation along the hypha axis and ATP-dependent assembly-disassembly instead of movement with the cytoplasmic stream [5,15,16];
- Filamentous mitochondria are concentrated at a distance of 20-30 μm from the growing tip [17,18];
- Nuclei are absent in the area located at a distance of 20-30 μm from the growing tip ("nucleus-free zone") [7,19].

For *N. crassa* germ tubes [20] it was demonstrated that the structural order of an apical end of hyphae is formed gradually: when a germ tube reaches ~ 150 μm in length, a *Spitzenkorper* (a vesicle aggregation center) appears on its apical end, intracellular organelles are positioned in a certain order, a "nucleus-free zone" emerges. A similar gradation in the formation of a hyphal apex structural order was described in the study of mitochondria and microtubules behavior during a lateral branching [5,15-18]. In *N. crassa* hyphae, mitochondria move along the microtubules, possessing the needed molecular genetic parts to interact with those structures [3,5,21-25].

As estimated by the mathematical model that quantifies the microtubule work on transporting vesicles to the apical end of *N.*

crassa hyphae [26], an ordered ensemble of ≈ 10 microtubules is capable of performing the task of delivering a sufficient quantity of vesicles containing materials necessary for hypha elongation to the growth zone at a necessary speed.

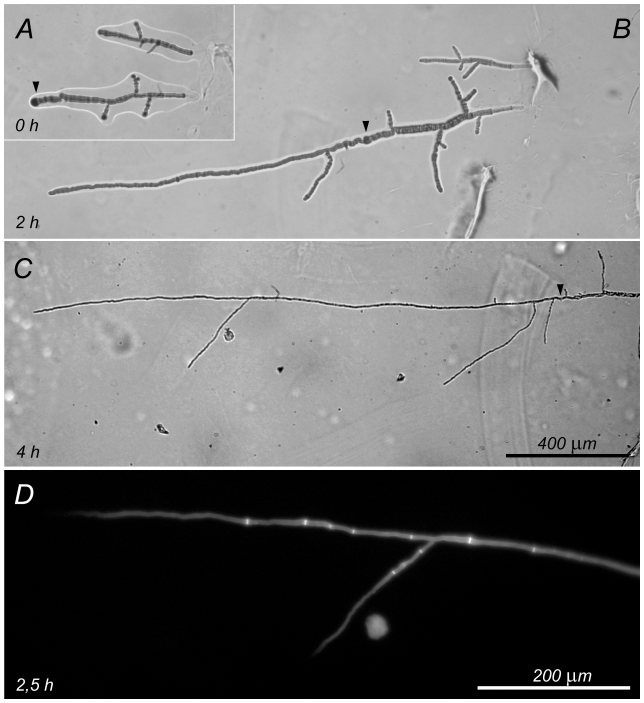

Figure 1: Growth and development of the apical fragments of *N. crassa* hyphae after isolation from maternal mycelium (details of method see in [27]). Black arrows show the point of hyphal tip immediately after isolation from mycelium. A. Two apical fragments (307 µm and 485 µm long) photographed immediately after isolation. B. The same fragments 2 h later. The shorter fragment stopped growth. The longer one continued to elongate with 6.6 µm/min rate but almost without developing the side branches. C. The successfully elongating fragments 4 h after isolation from mycelium. The 1st side branch is formed 693 µm away from the growing tip and 928 µm away from previously formed branch. Also there are some rudimental side branches between the normally developing ones. D. Fluorescence of Calcofluor White (standard marker of septa in *Neurospora crassa*) in the apical part the elongating fragments 2.5 h after isolation from maternal mycelium. At this moment the first septa was situated 267 µm away from the point of growth and the first side branch was 517 µm away. At the same time the length of segments did not differ from that in maternal mycelium.

Hyphal Fragments Isolated from Mycelium as an Experimental Mode and Its Properties

For decades one of the most effective instruments for researching molecular genetic foundations of *N. crassa* vegetative mycelium functional activity was using mutant strains of this fungus with certain defects within various functions. Interesting new possibilities were opened by a new experimental model developed recently, namely the tip growth of short *N. crassa* vegetative hypha fragments that are isolated from the maternal mycelium (Figure 1). Experimental procedures for preparation of such specimens were described in [27].

Separation of apical fragments from maternal mycelium leads to a prominent discrepancy between such important tip growth parameters as elongation, septation and branching [27]. This experimental model is characterized by:

- Reduction of hypha diameter (by ~ 50%);
- Considerable reduction in hypha elongation speed with pre-existing orientation preserved;
- Disruption of a hypha branching rhythm combined with an occurrence of abnormally large intervals between adjacent branches as well as arrested development of some lateral branch rudiments;
- Preservation of a septation rhythm and a relative consistency of a hypha segment size;
- Formation of the first septa at about the same distance from the growing tip as in maternal hyphae;
- Irregularity of the distance between the first lateral branch and a growing tip compared to the maternal hyphae;
- Existence of a nucleus-free zone at a 5-33 µm distance to a growing point.

Because of a reduced diameter of growing daughter branches on isolated *N. crassa* fragments, it is possible to observe individual nuclei in them. According to observations, the shape of DAPI-stained nuclei in growing isolated fragment daughter branches varies from round to pear-shaped, and there are special more brightly fluorescent formations on their surface that correspond to a spindle pole bodies connecting a nucleus to microtubules [28-32]. Adjacent nuclei on the apical ends of growing isolated fragment daughter branches divide asynchronously, same as in hyphae connected to mycelium [20,29,30].

The Possible Regulatory Role of the Electrical Heterogeneity in the Apical End of Hypha

Considering different possible mechanisms of creating and maintaining an ordered microtubule and mitochondria ensemble on an apical end of a growing hypha we assumed that electric heterogeneity of an apical end of hyphae might play a certain part in this process [17,18,33].

According to the data from electrophysiological measurements and theoretical model analysis [14,34-36], hyphal apexes about 200 µm in length behave like segments devoid of Em generators. The main Em generators for *N. crassa* are H+-ATPases of plasma membranes [37], while plasma membranes of vegetative *N. crassa* hyphal apexes are devoid of H+-ATPases [11,12].

Higher Em values on hyphal apexes in the presence of electric connection between apexes and distal hyphae parts are created by Em generators of older cells, located at a distance of 800–1000 µm to a growing point [14]. According to our measurements conducted with the aid of intracellular microelectrodes, the approximate Em1 value at a distance of 100 µm (L1) from an N. crassa hyphal apex is –130 mV, and at a distance of 400 µm – approximately –160 mV (Em2) [14,38]. Using these values, we can determine the electric field intensity (E) along an apical end of hyphae:

$$E = (Em2 - Em1) / (L2 - L1) = 30 \text{ mV} / 300 \text{ µm} = 100 \text{ V/m}.$$

Isolated microtubules in a solution orient themselves and change their movement speed under the influence of a 2×10^3 V/m electric field [39,40]. It is possible that within a living cell a considerably weaker electric field can constantly affect the orientation, positioning and movement speed of a microtubule ensemble. A more precise analysis of this assumption requires developing an adequate mathematical model.

Experimental Impairments in the Coherence of Branching and Septation

There are still a lot of unknowns about the formation of new lateral branches on growing hyphae. For many years the correspondence between the emergence of a new septum on a growing hyphal apex and the subsequent formation of a new lateral branch led us to assume a cause-and-effect link between those events: it was believed that the emergence of a septum slows down the intracellular content streaming along the hypha and initiates lateral branching. By blocking different stages of a branching process on mutant lines it was demonstrated [41] that a branching process is regulated genetically and consists of at least four stages: 1) determining a new branching point; 2) developing this point into a growth zone; 3) initial formation of a new branch as a bud; 4) development of a branch involving microtubules.

During tip growth of *N. crassa* hyphal fragments the branching process is slowed down during the first 1-2 h after isolation from mycelium and is recovered during the next 2-3 h. At the same time the location of lateral branches in no way correlated with the distance to a growing point and the location and number of septs [27].

The specifics of branching and septation processes under the conditions of maternal mycelium resource shortage indicate the independence of branching and septation processes during TG, as well considerable automatism of a septation process.

At the molecular level there are three stages of septum formation described for *N. crassa* [10,42,43]:

- Accumulation of a septal actin-myosin tangle (SAT) in a particular place;
- Transforming this tangle into a contractile actomyosin ring (CAR);
- CAR contraction and simultaneous invagination of plasma membrane and transverse cell wall formation.

According to [27], a new segment forms twice as fast in adult hyphae as in daughter branches of isolated fragments, but explaining this phenomena requires additional research.

Conclusion

Based on the analysis of elongation, branching and septation specifics for *N. crassa* hyphal apexes under the conditions of resource shortage (isolated fragments) and full resource support from the maternal mycelium (hyphae connected to mycelium) the following hierarchy of structural tasks that sustain TG [27] is proposed:

- Presence of an organized intercellular structure ensemble on a 100—150 μm long hyphal apex that prevents the formation of the first septum closer than ~ 150 μm from a growing tip;
- Septation rhythm with a distance between septum remaining relatively constant;
- Obvious correlation between the formation of new lateral branches and the influx of resources from older hyphal parts.

A growing *N. crassa* hyphal apex simultaneously performs several tasks: elongation, orientation, accumulation of resources, etc. The particular importance of the elongation function is supported by the fact that a highly organized intracellular structure ensemble on the 150 μm long apical segment responsible for elongation remains fully functional even under the resource shortage conditions (when a growing hypha is isolated from the mycelium), limiting the location of the septa closest to a growing point.

Neurospora crassa is one of the first mycelial fungi with completely decoded genome (details of this work can found at: http://fungalgenomes.org/wiki/Fungul_Genome_Links). In many laboratories of the World, the relations of genome activity with functions of *N. crassa* are intensively studied, and analysis of TG is the most difficult [2,3]. It is already clear that the activity of at least 50 genes is essential for TG [5], numerous details of TG at the molecular and genetic levels are known, and however, the entire technological scheme of this process is still unavailable. F. Harold, an authority in the field of cell growth and development, is sure that the program for organization of behaviour of intracellular structures, especially a program changing with such high rates as observed during TG of *N. crassa*, cannot be described in the genome [22]. From Harold's viewpoint, any molecule arising in the cell according to genetic instructions enters the already organized space under the influence of pre-existent forces and fields, and this itself is a rather complicated problem to analyze.

The proposed experimental model of the discrepancy in branching and septation processes during TG that allows one to measure morphology and locations of single nuclei on apical end of hyphae can prove useful in the experimental analysis of molecular genetic mechanisms of interaction regulation in intracellular structures sustaining apical growth of *N. crassa* vegetative hypha.

Acknowledgement

The authors are sincerely grateful for help in the preparing this manuscript for publication A. Ya. Dunina-Barkovskaya and N. O. Sventsitskaya.

This work was performed in the framework of the theme "Local Intercellular Interactions" (State registration No. 01201353079).

References

1. Brand A, Gow NA (2009) Mechanisms of hypha orientation of fungi. Curr Opin Microbiol 12: 350-357.
2. Seiler S, Plamann M (2003) The genetic basis of cellular morphogenesis in the filamentous fungus *Neurospora crassa*. Mol Biol Cell 14: 4352-4364.
3. Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, et al. (2004) Lessons from the genome sequence of *Neurospora crassa*: Tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68: 1-108.
4. Potapova TV (2014) Structural and functional organization of growing tips of *Neurospora crassa* Hyphae. Biochemistry (Mosc) 79: 593-607.
5. Riquelme M, Yarden O, Bartnicki-Garcia S, Bowman B, Castro-Longoria E, et al. (2011) Architecture and development of the Neurospora crassa hypha – a model cell for polarized growth. Fungal Biology 115: 446–474.
6. Freitag M, Giuffetti LM (2001) Expression and visualization of Green Fluorescent Protein (GFP) in *Neurospora crassa*. Fungal Genetics Newsletter 48: 15–19.

7. Freitag M, Hickey PC, Raju NB, Selker EU, Read ND (2004) GFP as a tool to analyze the organization, dynamics and function of nuclei and microtubules in *Neurospora crassa*. Fungal Genetics Biology 41: 897–910.
8. Fuchs F, Prokisch H, Neupert W, Westermann B (2002) Interaction of mitochondria with microtubules in the filamentous fungus *Neurospora crassa*. J Cell Sci 115: 1931-1937.
9. Trinci APJ, Wiebe MG, Robson GD (1994) The mycelium as an integrated entity. In: The Mycota I. Growth, differentiation and sexuality. Berlin-Heidelberg, Springer-Verlag.
10. Delgado-Álvarez DL, Bartnicki-García S, Seiler S, Mouriño-Pérez RR (2014) Septum development in *Neurospora crassa*: the septal actomyosin tangle. PLoS One 9: e96744.
11. Riquelme M, Freitag M, Leon-Hing ES, Bowman B (2005) Live imaging of the secretory pathway in hyphae of *Neurospora crassa*. Fungal Genet Newsl 52: 53.
12. Fajardo-Somera RA, Bowman B, Riquelme M (2013) The plasma membrane proton pump PMA-1 is incorporated into distal parts of the hyphae independently of the Spitzenkorper in *Neurospora crassa*. Eukaryotic Cell 12: 1097-1105.
13. Slayman CL, Slayman CW (1962) Measurement of membrane potentials in Neurospora. Science 136: 876-877.
14. Potapova TV, Aslanidi KB, Belozerskaya TA, Levina NN (1988) Transcellular ionic currents studied by intracellular potential recordings in *Neurospora crassa* hyphae. (Transfer of energy from proximal to apical cells). FEBS Lett 241: 173-176.
15. Mourino-Perez RR, Robertson RW, Bartnicki-Garcia S (2006) Microtubule dynamics and organization during hyphal growth and branching in *Neurospora crassa*. Fungal Gen Biol 43: 389-400.
16. Held M, Edwards C, Nicolau DV (2010) Temporal and spatial *in vivo* optical analysis of microtubules in *Neurospora crassa*. In: Imaging, manipulation, and analysis of biomolecules, cells, and tissues. VIII. San Francisco: SPIE, pp: 75680V.
17. Potapova TV, Boitzova LJu, Golyshev SA (2011) Problem of Interactions between intracellular structures during the *Neurospora crassa* tip growth. Doklady Biochemistry and Biophysics 436: 44-48.
18. Potapova TV, Boitzova LJu, Golyshev SA, Popinako AV (2014) The organization of mitochondria in growing hyphae of *Neurospora crassa*. Cell Tiss Biol 8: 166-174.
19. Davis RH (2000) Neurospora: contributions of a model organism. Oxf. Univ. Press.
20. Roca MG, Kuo HC, Lichius A, Freitag M, Read ND (2010) Nuclear dynamics, mitosis, and the cytoskeleton during the early stages of colony initiation in *Neurospora crassa*. Eukaryot Cell 9: 1171-1183.
21. Davis RH, Perkins DD (2002) Timeline: Neurospora: A model of model microbes. Nat Rev Genet 3: 397-403.
22. Harold FM (2005) Molecules into cells: Specifying spatial architecture. microbiology and molecular biology reviews 69: 544-564.
23. Prokisch H, Neupert W, Westermann B (2000) Role of MMM1 in maintaining mitochondrial morphology in *Neurospora crassa*. Mol Biol Cell 11: 2961-2971.
24. Kato F, Kurashima K, Chae M, Sawada S, Hatakeyama S, et al. (2010) Deletion of a novel F-Box protein, MUS-10, in *Neurospora crassa* leads to altered mitochondrial morphology, instability of mtDNA and senescence. Genetics 185: 1257-1269.
25. Wideman JG, Go NE, Klein A, Redmond E, Lackey SW, et al. (2010) Roles of the Mdm10, Tom7, Mdm12 and Mmm1 proteins in the assembly of mitochondrial outer membrane proteins in *Neurospora crassa*. Mol Biol Cell 21: 1725-1736.
26. Sugden KE, Evans MR, Poon WC, Read ND (2007) Model of hyphal tip growth involving microtubule-based transport. Phys Rev E Stat Nonlin Soft Matter Phys 75: 031909.
27. Potapova TV, Boitsova LYu, Golyshev SA, Dunina-Barkovskaya AYa (2016) Tip growth of *Neurospora crassa* in the conditions of the resource deficiency: Impairments in the coherence of elongation, branching, and septation. Cell Tiss Biol (in press).
28. Thompson-Cofee C, Zicker D (1994) How the cytoskeleton recognizes and sorts nuclei of opposite mating type during the sexual cycle of filamentous ascomycetes. Dev Biol 165: 257-271.
29. Minke PF, Lee IH, Plamann M (1999) Microscopic analysis of Neurospora ropy mutants defective in nuclear distribution. Fungal Genet Biol 28: 55-67.
30. Minke PF, Lee IH, Tinsley JH, Bruno KS, Plamann M (1999) *Neurospora crassa* ro-10 and ro-11 genes encode novel proteins required for nuclear distribution. Mol Microbiol 32: 1065-1076.
31. Riquelme M, Roberson RW, McDaniel DP, Bartnicki-García S (2002) The effects of ropy-1 mutation on cytoplasmic organization and intracellular motility in mature hyphae of *Neurospora crassa*. Fungal Genet Biol 37: 171-179.
32. Ramos-García SL, Roberson RW, Freitag M, Bartnicki-García S, Mouriño-Pérez RR (2009) Cytoplasmic bulk flow propels nuclei in mature hyphae of *Neurospora crassa*. Eukaryot Cell 8: 1880-1890.
33. Potapova TV (2012) Cell-to-cell communication in the tip growth of mycelial fungi. In Biocommunication of Fungi. Berlin-Heidelberg, Springer-Verlag.
34. Takeuchi Y, Schmid J, Caldwell JH, Harold FM (1988) Transcellular ion currents and extension of *Neurospora crassa* hyphae. J Membr Biol 101: 33-41.
35. Aslanidi KB, Aslanidi OV, Vachadze DM, Mornev OA, Potapova TV, et al. (1997) Analysis of electrical phenomena accompanying the growth of *Neurospora crassa* hyphae: theory and experiment. Membr Cell Biol 11: 349-365.
36. Smolianinov VV, Potapova TV (2003) Estimation of the critical length of the growing *Neurospora crassa* hyphae. Biologicheskie membrani (in Russian) 20: 304-312.
37. Slayman CL (1987) The plasma membrane ATPase of Neurospora: A proton-pumping electroenzyme. J Bioenerget Biomemb 19: 1-20.
38. Potapova TV, Boitzova LJu (1998) Structure, function, regulation: Experimental analysis in groups of non-exitable cells coupled via permeable junctions. Membr Cell Biol 11: 817-829.
39. Böhm KJ, Nikolaos E, Mavromatos NE , Michette A, Stracke R, et al. (2005) Movement and alignment of microtubules in electric fields and electric-dipole-moment estimates. Electromag Biol Med 24: 319–330.
40. Dujovne I, van den Heuvel M, Shen Y, de Graaff M, Dekker C (2008) Velocity modulation of microtubules in electric fields. Nano Lett 8: 4217-4220.
41. Dunlap JC, Borkovich KA, Henn MR, Turner GE, Sachs MS, et al. (2007) Enabling a community to dissect an organism: Overview of the Neurospora functional genomics project. Adv Genet 57: 49-96.
42. Mouriño-Pérez RR (2013) Septum development in filamentous ascomycetes. Fungal Biol. Rev. 27: 1-9.
43. Mouriño-Pérez RR, Riquelme M (2013) Recent advances in septum biogenesis in *Neurospora crassa*. Adv Genet 83: 99-134.