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Intracellular reorganization and ionic signaling of the *Phycomyces* stage I sporangiophore in response to gravity and touch

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Unicellular zygomycete fungus ***Phycomyces blakesleeanus*** exhibits a typical apical growth that depends on a complex interaction of different physiological processes. Sensitivity to the light, wind, barriers, touch and gravity of ***Phycomyces*** sporangiophore implicate the existence of the cross-talk between different signaling pathways in the same cell. Touch and gravity in ***Phycomyces*** sporangiophore seem to share some common elements of Ca^{2+} and H^+ ion-based signal transduction systems. Apoplastic Ca^{2+} and H^+ ionic fluxes are important for establishing cell polarity in tip growing sporangiophore both in vertical and tilted position. Upon gravistimulation sporangiophores display asymmetric ionic distribution and intracellular reorganization leading to change in the growth pattern.

Intracellular Reorganization upon Gravistimulation

Although all living organisms on the Earth are continuously exposed to physical signal of the gravity, cellular mechanism of the gravitropism is far from being clear. Unicellular tip-growing cells are excellent experimental systems for dissecting the mechanisms of gravitropic signaling because cell extension, gravity sensing and gravity response are all placed in the apical zone of the single cell. *Phycomyces* sporangiophore is a suitable fungal model cell for investigation of the gravitropic signaling. As this vertically growing unbranched hypha is able to respond to light, wind, barriers, touch and gravity,[1] it is reasonable to assume that all these responses must be highly integrated to provide an appropriate growth response. *Phycomyces* sporangiophores grow in the air and display negative gravitropic bending upon displacement from vertical position.[2,3] Buoyant lipid globules in subapical region and sedimenting octahedral crystals as statoliths in central vacuole play an essential role in gravitropism in immature sporangiophore cell (stage I).[4-7] It has been earlier reported that density differences of the vacuoles and the cytoplasm may play a role for gravisensing of sporangiophores.[8] In this work we have observed the spatial relationship between the vacuole and the protoplasm of a living sporangiophore and revealed intracellular reorganization of sporangiophore upon vertical displacement. Sporangiophores responded to gravistimulation by bending growth, gradual dissolving lipid globules and sedimentation of octahedral crystals from central vacuole (**Figs. 1 and 2**). Bending growth is a very slow process which is accompanied by appearance of intensive vacuolization in the apical and subapical region of sporangiophores that could be associated with gravitropic response and/or with regular developmental processes (**Fig. 1**). Sporangiophores kept in vertical position showed gradual moving central vacuole to apical part of sporangiophore, but without significant vacuolization. These small vacuoles might be involved in preparation of some material for the next developmental stage and/or the site where the octahedral crystals are formed. Moreover, we have observed "Spitzenkörper like body" in the apical zone of *Phycomyces* sporangiophore, as previously also detected in apical region of *Phycomyces* sporangiophores[9] (**Fig. 2**). The appearance of accumulation of wall vesicles in the form of an organized apical body or "Spitzenkörper" just behind the apex is characteristic for almost all tip growing cells like hyphae of septate fungi,[10,11] pollen tubes,[12,13] root hairs[14] and algal rhizoids.[15] Although the exact role of these high-organized apical vesicles is still unclear, it might be speculated that they play an important role in directing growth and maintaining cell polarity.[16] Developmental processes of sporangiophore could interfere with gravity-triggered processes, so it is still unclear whether these processes are related or taking place in parallel. Thus, developmental changes would incidentally coincide with gravitropic response. Further ultrastructural investigation would be required in order to clarify whether the appearance of intensive vacuolization would be associated with gravisensing mechanisms.

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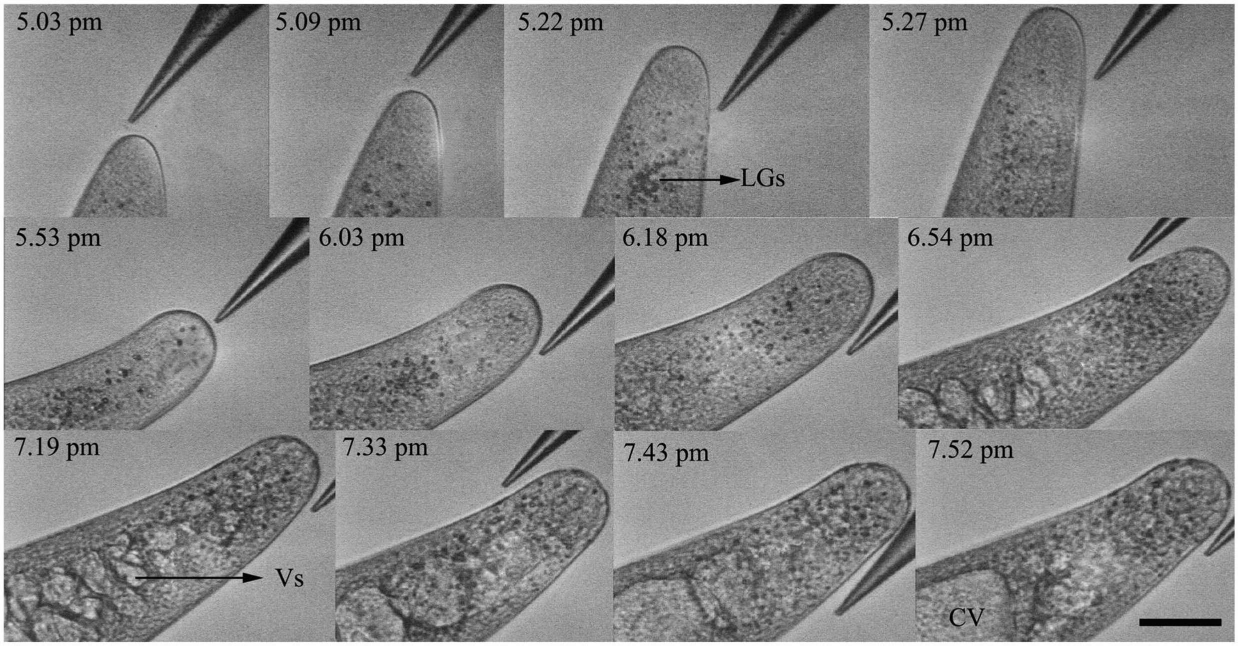

Figure 1. Original images of an intact wild-type strain *Phycomyces* sporangiophore (stage I) taken during measurements of net ion fluxes at different positions of Ca^{2+} and H^+ ion-selective microelectrode near the surface of the cell kept in vertical orientation for 24 min and subsequently tilted by 45° for 2 h 25 min. The numbers in left corner of images denote the real time when the images were taken. Apical part of sporangiophore displayed bending growth in tilted position. The appearance of intensive vacuolization in apical and subapical regions of sporangiophore was observed after 1h of gravistimulation. CV, central vacuole; LGs, -lipid globules; Vs, vacuoles. Bar marker = 50 μm.

Ionic Signaling in Response to Touch and Gravity

The thigmo responses are found over the entire range of the plant kingdom, but there is still unclear how the thigmo signal is transmitted to the interior of the cell and what the components are included in this signal transduction chain. The sensitivity of *Phycomyces* sporangiophores to touch as mechanical stimulus is additional adaptive mechanism for redirecting growth due to obstacle. *Phycomyces* sporangiophores responded to touch stimulus as other filamentous fungi where hyphal growing points usually serve as sensors.[17] The application of touch stimulus on *Phycomyces* sporangiophore caused transient influxes of Ca^{2+} and H^+ ions into the cell which appear likely to be important for avoiding obstacle by transiently increasing growth rate by inward-directed ion fluxes (**Fig. 3**). These transient apoplastic ionic changes along sporangiophores implicate that thigmo signals are probably received at the cell wall and transmitted through the plasma membrane via opening stretch-activated Ca^{2+} channels. Ionic signaling has been proposed to transduce both touch and gravity perception in plants.[18,19] The presence of stretch-activated channels as well as calcium-induced calcium release channels in plants suggests the possibility that mechano-perturbations cause increases in intracellular Ca^{2+} due to gating of these channels.[20-22] For highly polarized cells, such as root hairs and pollen tubes, control of expansion-related mechanical stresses seems to be a fundamental aspect of growth regulation.[23-25] The touch may result in perturbation of connections among the cell wall, plasma membrane and the cytoskeleton, thus conducting the extracellular stimulus into an intracellular signal.[17] Touch and gravity in *Phycomyces* sporangiophore seem to share some common elements of Ca^{2+} and H^+ ion-based signal transduction systems, as previously also reported for the plants.[27,28] It appears likely that in *Phycomyces* sporangiophores Ca^{2+} channels detected in plasma membrane of growing zone[29] are also involved in mechanosensing mechanisms. Although increase of cytosolic Ca^{2+} via Ca^{2+} channels in the plasma membrane in response to touch is well demonstrated,[26,30] the molecular basis for perception of a mechanical stimulus remains to be identified.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Bergman K, Burke PV, Cerdá-Olmedo E, David CN, Delbrück M, Foster KW, et al. Phycomyces. Bacteriol Rev 1969; 33:99-157; PMID:4889151.
2. Gamow RI, Ruiz-Herrera J, Fischer EP. The Cell Wall of *Phycomyces*. In: Cerda'-Olmedo, E, Lipson, ED eds. Phycomyces. Cold Spring Harbor Laboratory: New York, 1987; 223-46.
3. Cerda'-Olmedo E, Lipson ED. A Biography of Phycomyces. In: Cerda'-Olmedo, E, Lipson, ED eds. Phycomyces. Cold Spring Harbor Laboratory: New York, 1987; 7-26.
4. Horie T, Schimek C, Ootaki T. Gravitropic responses of Phycomyces and Pilobulus sporangiophores and possible mode of action. Abstract. 6th International Mycological Congress 1998, Jerusalem, Israel, p. 103.
5. Schimek C, Eibel P, Grolig F, Horie T, Ootaki T, Galland P. Gravitropism in Phycomyces: a role for sedimenting protein crystals and floating lipid globules. Planta 1999a; 210:132-42; PMID:10592041; http://dx.doi.org/10.1007/s004250050662.
6. Schimek C, Eibel P, Horie T, Galland P, Ootaki T. Protein crystals in Phycomyces sporangiophores are involved in graviperception. Adv Space Res 1999b; 24:687-96; PMID:11542610; http://dx.doi.org/10.1016/S0273-1177(99)00400-7.
7. Eibel P, Schimek C, Fries V, Grolig F, Schapat T, Schmidt W, et al. Statoliths in Phycomyces: characterization of octahedral protein crystals. Fungal Genet Biol 2000; 29:211-20; PMID:10882537; http://dx.doi.org/10.1006/fgbi.2000.1199.
8. Dennison DS, Shropshire W Jr. The gravireceptor of Phycomyces. Its development following gravity exposure. J Gen Physiol 1984; 84:845-59; PMID:6520601; http://dx.doi.org/10.1085/jgp.84.6.845.
9. Girbardt M. Die Ultrastruktur der Apikal region von Pilzhyphen. Protoplasma 1969; 67:413-41; http://dx.doi.org/10.1007/BF01254905.
10. Brunswik H. Untersuchungen über Geschlechts und Kernverhältnisse bei der Hymenomycetengattung *Coprinus*. In: K Goebel (ed) Botanische Abhandlungen; Gustav Fisher, Jena, 1924; 1-152.
11. Löpez-Franco R, Bracker CE. Diversity and dynamics of the Spitzenkörper in growing hyphal tips of higher fungi. Protoplasma 1996; 195:90-111; http://dx.doi.org/10.1007/BF01279189.
12. Rosen WG, Gawlik SR, Dashek WV, Siegesmund KA. Fine structure and cytochemistry of Lilium pollen tubes. Am J Bot 1964; 51:61-71; http://dx.doi.org/10.2307/2440065.
13. Lancelle SA, Hepler PK. Ultrastructure of freeze-substituted pollen tubes of *Lilium longiforum.* Protoplasma 1992; 167:215-30; http://dx.doi.org/10.1007/BF01403385.
14. Emons AM. The cytoskeleton and secretory vesicles in root hairs of Equisetum and Limnobium. Cytoplasmic streaming in root hairs of Equisetum. Ann Bot (Lond) 1987; 60:625-32.
15. Leitz G, Schnepf E, Greulich KO. Micromanipulation of statoliths in gravity-sensing Chara rhizoids by optical tweezers. Planta 1995; 197:278-88; PMID:11540723; http://dx.doi.org/10.1007/BF00202648.
16. Bartnicki-García S. Hyphal tip growth: Outstanding questions. In: Osiewacz HD ed. Molecular biology of fungal development. Marcel Dekker, New York and Basel, 2002; 42-71.
17. Jaffe MJ, Leopold AC, Staples RC. Thigmo responses in plants and fungi. Am J Bot 2002; 89:375-82; PMID:21665632; http://dx.doi.org/10.3732/ajb.89.3.375.
18. Fasano JM, Swanson SJ, Blancaflor EB, Dowd PE, Kao TH, Gilroy S. Changes in root cap pH are required for the gravity response of the Arabidopsis root. Plant Cell 2001; 13:907-21; PMID:11283344.

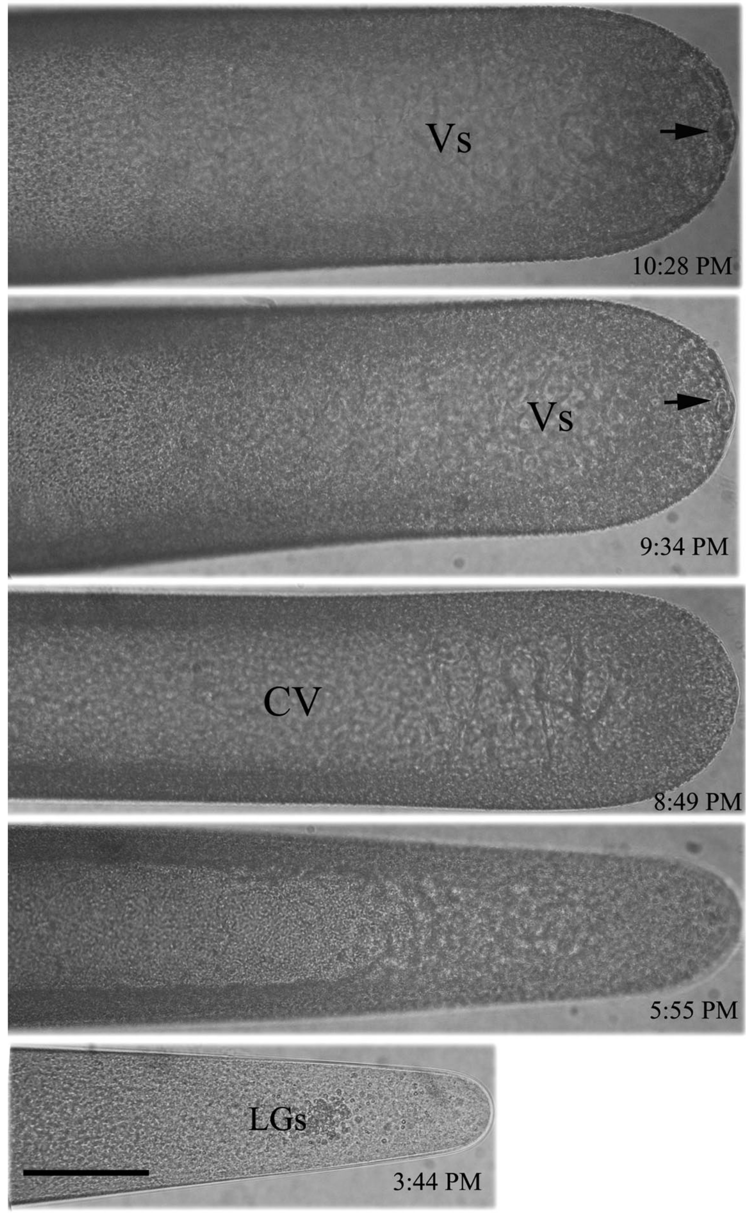

Figure 2. Original images of an intact wild-type strain *Phycomyces* sporangiophore (stage I) kept in horizontal position (3.44 p.m.–10.28 p.m.). The gradual intracellular reorganization was observed upon displacement from vertical position: the position of lipid globules was changed, intensive vacuolization in apical and subapical regions, and "Spitzenkörper like body" (vesicle clusters) in the apical zone of the sporangiophore (arrow) appeared. The numbers in right corner of images denote the real time when the images were taken. CV, central vacuole; LGs, lipid globules; Vs, vacuoles. Bar marker = 100 μm.

19. Sievers A, Sondag C, Trebacz K, Hejnowicz Z. Gravity induced changes in intracellular potentials in statocytes of cress roots. Planta 1995; 197:392-8; PMID:11540724; http://dx.doi.org/10.1007/BF00202662.
20. Edwards KL, Pickard BG. Detection and transduction of physical stimuli in plants. In: Wagner E, Greppin H, Biller B eds. The Cell Surface in Signal Transduction. NATO AS1 Series H12 (Heidelberg: Springer-Verlag), 1987; 41-66.
21. Ding JP, Pickard BG. Mechanosensory calcium-selective cation channels in epidermal cells. Plant J 1993; 3:83-110; http://dx.doi.org/10.1111/j.1365-313X.1993.tb00013.x.
22. Ward JM, Schroeder JI. Calcium-activated K^+ channels and calcium-induced calcium release by slow vacuolar ion channels in guard cell vacuoles implicated in the control of stomatal closure. Plant Cell 1994; 6:669-83; PMID:12244253.

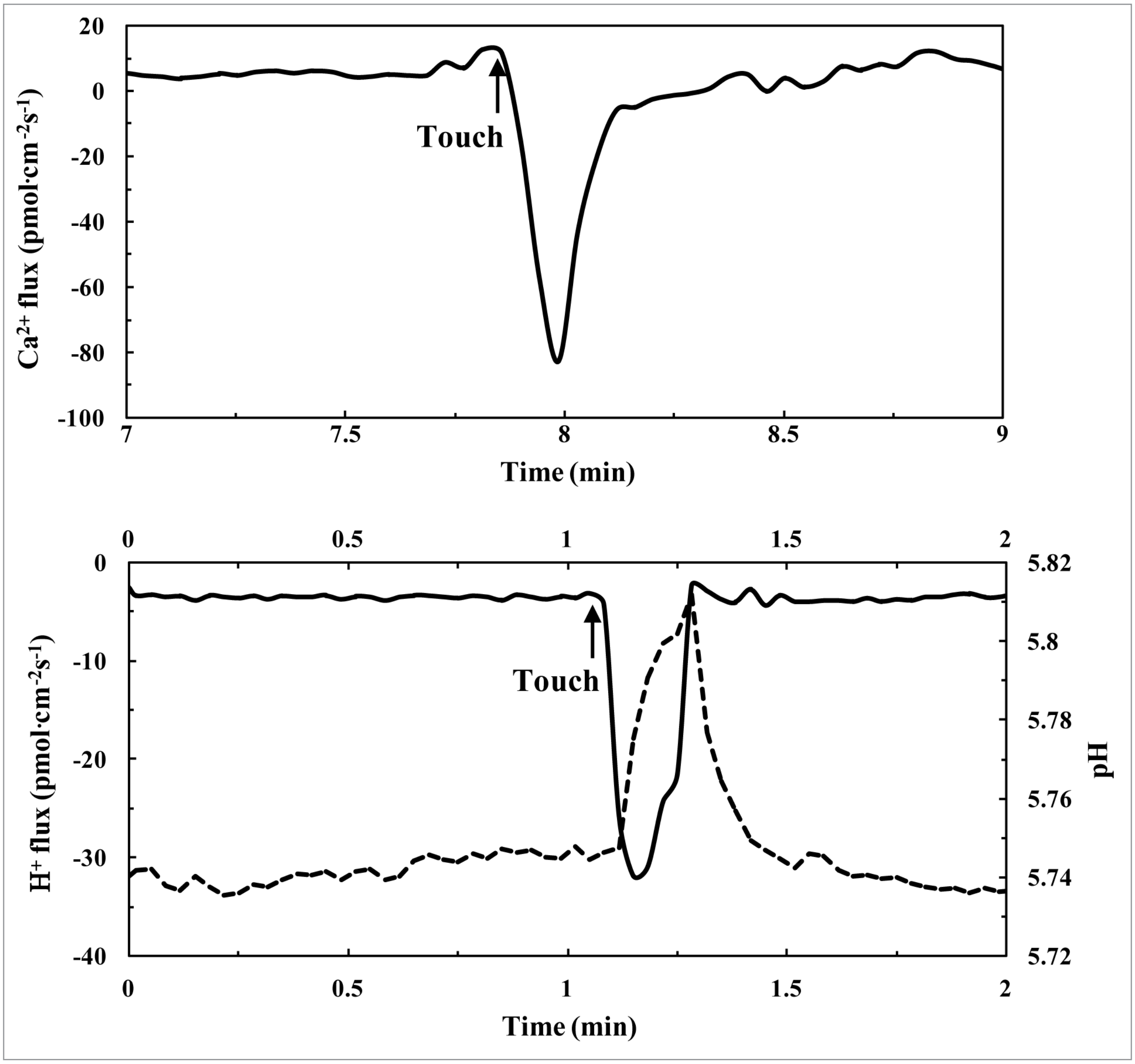

Figure 3. Representative records of touch-induced transient changes of net Ca^2 and $^+H^+$ ion fluxes (outward-efflux as positive, and inward-influx as negative values) recorded from intact *Phycomyces* sporangiophores. Thigmo stimulus caused transient alkalization at the surface of sporangiophore and influxes of both ions measured.

23. Dutta R, Robinson KR. Identification and characterization of stretch-activated ion channels in pollen protoplasts. Plant Physiol 2004; 135:1398-406; PMID:15247410; http://dx.doi.org/10.1104/pp.104.041483.
24. Monshausen GB, Bibikova TN, Messerli MA, Shi C, Gilroy S. Oscillations in extracellular pH and reactive oxygen species modulate tip growth of Arabidopsis root hairs. Proc Natl Acad Sci USA 2007; 104:20996-1001; PMID:18079291; http://dx.doi.org/10.1073/pnas.0708586104.
25. Monshausen GB, Swanson SJ, Gilroy S. Touch sensing and thigmotropism. In: Gilroy S, Masson P eds. Plant Tropisms, 2008, Ames, IA, Oxford, UK: Blackwell Publishing, 2008: 91-122.
26. Monshausen GB, Messerli MA, Gilroy S. Imaging of the Yellow Cameleon 3.6 indicator reveals that elevations in cytosolic Ca^{2+} follow oscillating increases in growth in root hairs of Arabidopsis. Plant Physiol 2008; 147:1690-8; PMID:18583529; http://dx.doi.org/10.1104/pp.108.123638.
27. Mullen JL, Wolverton C, Ishikawa H, Evans ML. Kinetics of constant gravitropic stimulus responses in Arabidopsis roots using a feedback system. Plant Physiol 2000; 123:665-70; PMID:10859196; http://dx.doi.org/10.1104/pp.123.2.665.
28. Fasano JM, Massa GD, Gilroy S. Ionic signaling in plant responses to gravity and touch. J Plant Growth Regul 2002; 21:71-88; PMID:12016507; http://dx.doi.org/10.1007/s003440010049.
29. Mitiku G, Edwards KL. Ion channels of membrane vesicles from gravity-sensitive sporangiophores of *Phycomyces*. Amer Soc Gravitational Space Bio Bull 1991; 5:47.
30. Monshausen GB, Gilroy S. Feeling green: mechanosensing in plants. Trends Cell Biol 2009; 19:228-35; PMID:19342240; http://dx.doi.org/10.1016/j.tcb.2009.02.005.