

## **Supplementary Material**

### **Single-molecule FISH reveals subcellular localization of $\alpha$ -amylase and actin mRNAs in the filamentous fungus *Aspergillus oryzae***

Yujiro Higuchi\*, Kaoru Takegawa

Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University,  
744 Motooka, Fukuoka 819-0395, Japan

\*Corresponding author. Tel/Fax: +81 92 802 4734, E-mail address:

y.higuchi@agr.kyushu-u.ac.jp

Word count: Abstract, 208; Main, 4390.

Number of figures: Main, 7; Supplementary, 4.

```

atgatggctcgcgtgggtctctatttctgtacggccttcaggctcgggcacctgctttg
cgaccaccagagataaaga
Probe # 1

gctgcaacgcctcggactggcgatcgcaatcatttatttctctcacggatcgattt
accgctagcgttaggtaaat gaggctcctagctaaa
Probe # 2 Probe # 3

gcaaggacggatgggtcgcgactgcgacttgaataactgcggatcagaatactgtggt
cgttc gacgctgaacattatgacgc ctttatgacacca
Probe # 4 Probe # 5

ggaacatggcagggcatcatcgacaagttggactatatacagggaaatggccttcacagcc
ccttgta gttcaactgatataggctc gaagtgtcgg
Probe # 6 Probe # 7

atctggatcaccctcgcttacagcccagctgccccagaccacgcatatggagatgcctac
tagacctagt gtatacctctacggatg
Probe # 8

catggctactggcagcaggatataactctctgaacaaaaactacggcactgcagatgac
gta gatgaccgtcgtcctatata gagagacttgcctttgatgc gtgacgtctactg
Probe # 9 Probe # 10 Probe # 11

ttgaagcgcctctctcggcccttcctgatgagaggggatgtatcttattgctgatgsgtt
aacttcc gagagaagccgggaagtact ccctacatagataaccagct
Probe # 12 Probe # 13

gctaaccatattgggctatgatggagcggtagctcagtcgattacagtggtttaaaccg
gattggtataccgatacta ccatcgagtcagctaagtgc ttggc
Probe # 14 Probe # 15 Probe

ttcagttcccaagactacttccaccgcttctgtttcattcaaaactatgaagatcagact
aagtcaagggttctg tgaaggtgggcaagacaaag atacttctagtctga
# 16 Probe # 17 Probe # 18

caggttgaggattgctgctgtaggagataacactgtctccttgctgatctcgataccacc
gtcca gatcctctattgtgacagag gactagactatgggtg
Probe # 19 Probe # 20

aaggatgtggtcaagaatgaatggtagactgggtgggatcattggtatcgaactactcc
ttc caccagttcttacttaccat caccctagtaaccatagctt
Probe # 21 Probe # 22

attgacggcctccgtatcgacacagtaaaacagctccagaaggacttctggccgggtac
ggaggcatagctgtgtcatt gtgcaggtcttctgaagac
Probe # 23 Probe # 24

aacaagccgcagcgtgtactgtatcggcgagggtctcgcggatcggcctact
atgtga
Probe

tgtccctaccagaacgtcatggacggcgtactgaaactatccattactatccactctc
acagggatggctt ccgcatgacttgatagggta atgataggtgaggag
# 25 Probe # 26 Probe # 27

aacgccttcaagtaacctccggcagcatggacgacctctacaacatgatcaaacaccgtc
ttcgc aagttcagttggaggccgtc tacctgctggagatggtgta tagttgtggcag
Probe # 28 Probe # 29 Probe # 30
aaatccgactgtccagactcaacactcctgggcacattcgtcgagaaccacgacaacca
tttaggct acaggtctgagttgtgaggga cctgtaagcagctcttgggt ctgttgggt
Probe # 31 Probe # 32 Probe # 33

cggttccttcttacaccaacacatagccctcgaagaacgtcgcagcattcatcatc
gccaagcgaag atgtggtgctgtatcggga cttgcagcgtgtaagtagt
3 Probe # 34 Probe # 35

ctcaacgacggaatccccatctactcaccggccaagaacagcactacgcccgggaaac
ctgccttagggtagtagat
Probe # 36

gacccccggaaccggaagcaacctgctctgggctaccggaccgacagcagctgtac

aagttaattgcctccggaacgcaatccggaactatgccattagcaaaagatacaggattc
ttcaattaacggagcgcctt gttaggccttgatacggtaa cgtttctatgtcctaag
Probe # 37 Probe # 38 Probe # 39
gtgacctacaagaactggccatctacaagagacacacacgatcgcattgcaagggcc
cac gatgttcttgaccgggtaga gtttctgctgtgtgctagc
Probe # 40 Probe # 41

acagatgggtcgcagatcgtgactatcttgcacaagggtgcttcgggtgattcgtat
cagcgtctagcactgataga acgaagcccactaagcata
Probe # 42 Probe # 43

accctctccttgagtggtcgggttacacagccggccagcaattgacggaggtcattggc
t cgttaactgcctccagtaac
Probe # 44

tgcacgaccgtgacggttggtcggatggaatgtgctgttctatggcaggtgggcta
cctttacagggacaaggata at
Probe # 45 Pr

cctagggtattgtatccgactgagaagttggcaggtagcaagatctgtagtagctg
ggatcccataacataggc tccatcgttctagacatcat
obe # 46 Probe # 47

```

**Supplementary Figure S1.** smFISH probe for *amyB*. In 1,497 b of *amyB* sequence, 47 regions (indicated as Probe #) of 18-22 nt were selected for the smFISH probe, each attached with FAM fluorescence molecule.

```

atggaagaggaaagtgtcgtctctcgtcattgacaatggttcgggtatgtgcaaggccgggt
    cttcaacgacgagagcagta    gttaccaagcccatacacgt
    Probe # 1                Probe # 2

ttcggcgggtgacgatgccccccgtgctgtcttccccctcattgtcggctgccccgcat
    cagcagacaagggggaggtaa    agcagggggcagta
    Probe # 3                Probe # 4

catggtatcatgattggtatgggtcagaaggactcttatgtcgggtgataggcacagtc
gtaccat    tactaaccatacccagttt    gagaatacagcccactactcc    cagg
    Probe # 5                Probe # 6                Prob

aagcgtggtatctcaccctcagatatcctatcgagcacgggtgctcacaactgggat
ttcgaccataggaggt    gtctataggatagctcgtgc    cagtgtttgacccta
e # 7                Probe # 8                Probe # 9

gacatggagaagatttggcatcacacattctacaacgagctccgtgttgetcccaggagg
ctgta    tcttctaaccgtagtgtgt    gatgttctcagggcacaac
    Probe # 10               Probe # 11

caccggttctctcgtacggaagccccctatcaaccctaagtccaaccgtgagaagatgacc
    gggatagttgggattcaggt    cactcttactagg
    Probe # 12               Probe # 13

cagattgtcttcgagacctcaacgccccgccttctacgtctccatccaggccgtcctg
gtctaa    gaagctctggaagttgcccc    cggaaagatcgagggtaggt    caggac
    Probe # 14               Probe # 15               Probe

tcactgtatgcctccggtctaccaccgggtatcgttctggattcgggtgacgggtcacc
agtacatacggag    cagcatgggtggccatagcaa    ctaagaccactgccacagtg
# 16                Probe # 17                Probe # 18

cacgttgtccccatctacgaggggtttcggcctccccacgccatctcccgtgttgacatg
tgcaacaggggtagatgctc    cggtagagggcacaactgta
    Probe # 19                Probe # 20

gctggccgtgatttgaccgattacctcatgaagatcctggctgagcgcgggttacacttc
gaccggcactaaactggcta    ggagtacttctaggaccgac    gcgccaatgtgaaag
    Probe # 21                Probe # 22                Probe # 23

tccaccaccgctgagcgtgaaattgtccgtgacatcaaggagaagctttgctacgtgcc
aggtg    ggcgactcgcactttaacag    actgtagttcctcttcgaaa    atgcagcgg
    Probe # 24                Probe # 25                Probe # 26

ctcgacttcgagcaggagattcagaccgcttctcagagctccagcctcgagaagtcctat
gagctgaagct    tctaagtctggcgaagagtc    gtcggagctcttcaggata
6                Probe # 27                Probe # 28

gagcttctgatggccagggtcatcaccatcgtaacgagcgtttccgtgctcctgaggtc
c    gaaggactaccggccagta    ggtagccattgtctcgaag    cgaggactccga
    Probe # 29                Probe # 30                Probe # 31

ctcttcagcctagcgttctgggtctgaaagcggtggatccacgttaccacctcaac
gagaaggt    gatcgcaagaccagacctt    ccataggtgcaatggtggaa    tg
    Probe # 32                Probe # 33                Pr

tccatcatgaagtgtgatgttgatgtccgtaagatctctacggtaacatcgtcatgtct
aggtagtacttcacacta    actacaggcattcctagaga    ccattgtagcagtacaga
obe # 34                Probe # 35                Probe # 36

gggtgactaccatgtaccctggcatctccgatcgtatgcagaagaaatcaccgccctt
cc    gatggtacatgggaccgtag    ctacatacgtcttctctta    gaa
    Probe # 37                Probe # 38                Pro

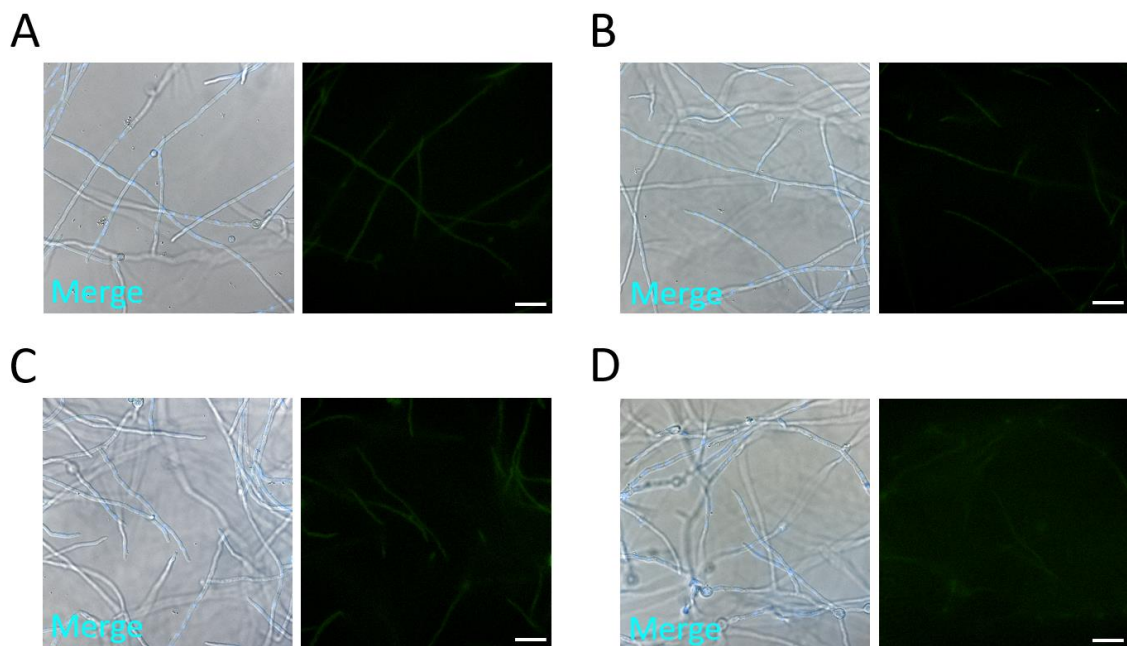
gccccctcgtccatgaaagtcgaagatcattgctcctcctgagcgtaaatactcctctgg
cgggggagcaggtactt    gttctagtaacgaggggac    gcatttatgaggcagacc
be # 39                Probe # 40                Probe # 41

atcgggtgtccatcttggcttccctgtccacctccagcagatgtgatctccaagcag
ta    caccaaggtagaaccgaagg    caggtggaaggtcgtctaca    tagagggtcgtc
    Probe # 42                Probe # 43                Probe # 44

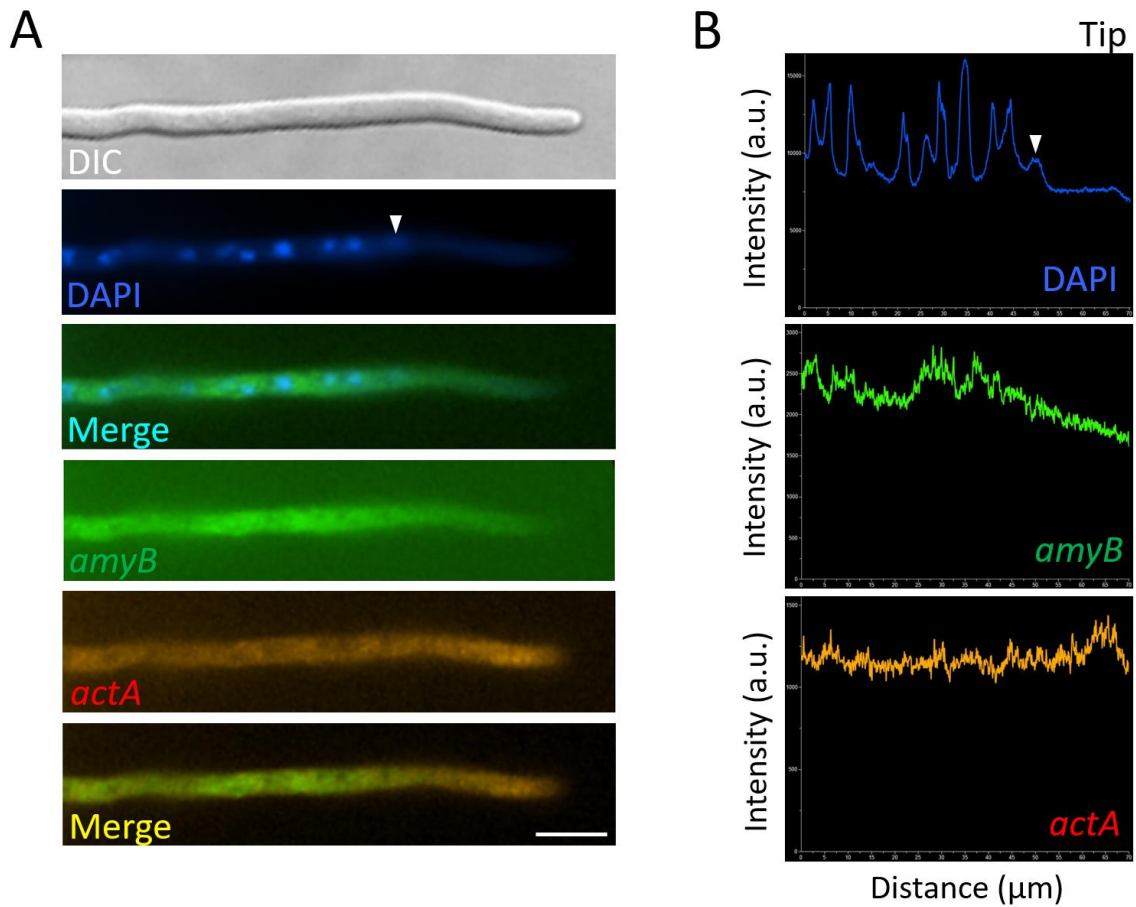
gagtagcagcagagcgggtccttcgatcgttcaccgcaagtgcttctaa
ctcatgct    ggaagctagcaagtggcgtt
    Probe # 45

```

**Supplementary Figure S2.** smFISH probe for *actA*. In 1,128 b of *actA* sequence, 45 regions (indicated as Probe #) of 18-22 nt were selected for the smFISH probe, each attached with CAL Fluor Red 610 fluorescence molecule.



**Supplementary Figure S3.** smFISH procedures were performed without the *amyB* probe in cultures of CD before shifting (**A**) and after shifting to CDmal, at 30 min (**B**), 60 min (**C**) and 90 min (**D**). Note that almost no fluorescent signals associated with *amyB* were observed (each right panel). Scale bars, 20  $\mu$ m.



**Supplementary Figure S4.** Localization of *amyB* and *actA* mRNAs in an *A. oryzae* hypha cultured in complete medium containing maltose. **(A)** smFISH was performed with *amyB* and *actA* probes added to an overnight culture in MPY. The white arrowhead indicates the nucleus located closed to the apex. **(B)** Line scan analyses of DAPI, *amyB* and *actA* mRNAs fluorescent signals were conducted through the hypha of **(A)**. The white arrowhead indicates the nucleus located closed to the apex. Note that the fluorescent signals of *amyB* mRNAs are not seen in the apical region, whereas those of *actA* mRNAs appeared more intense in the apical region. Scale bar, 10  $\mu\text{m}$ .