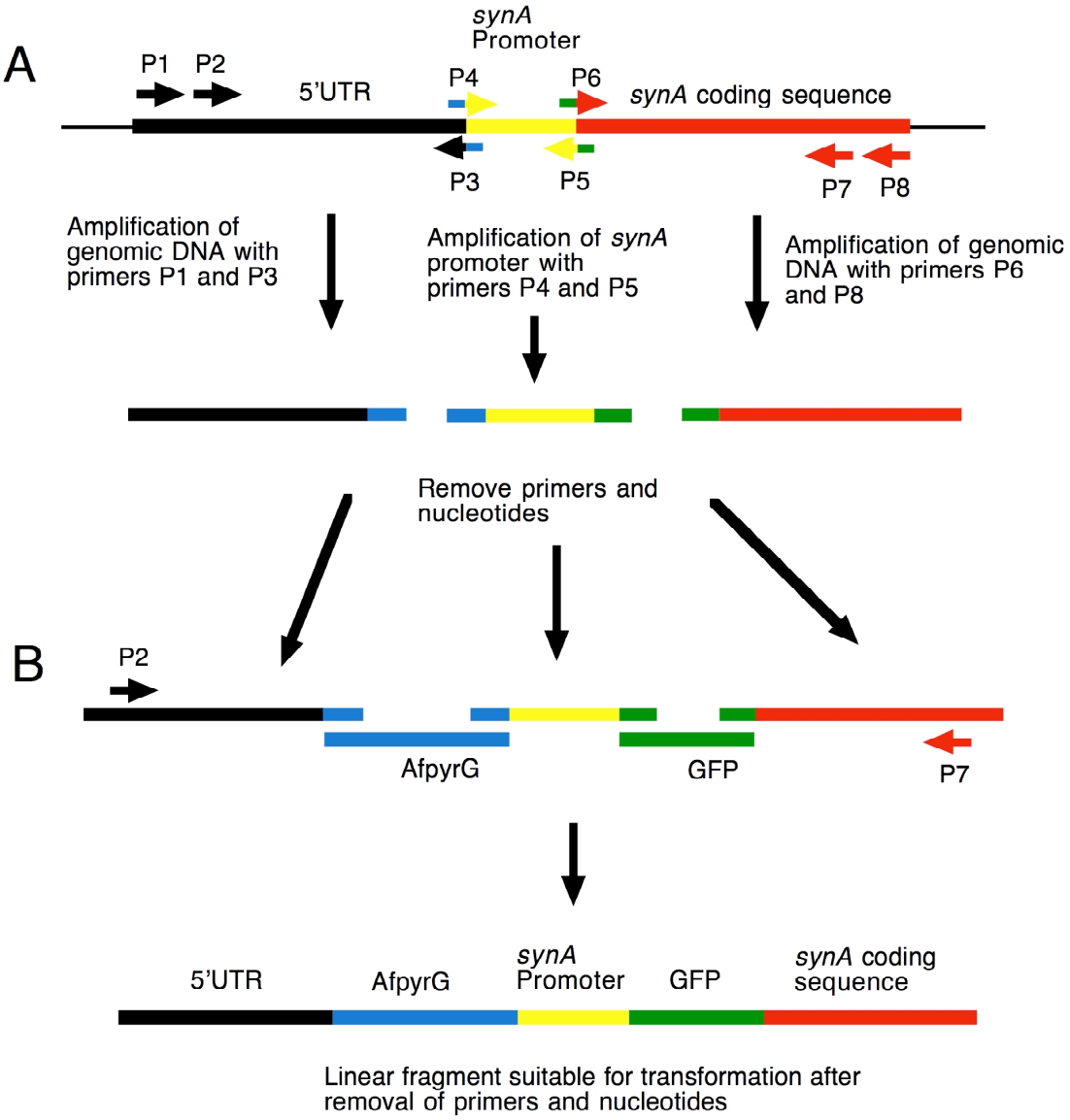
SUPPLEMENTAL FIGURES AND TABLE

FIGURE LEGENDS

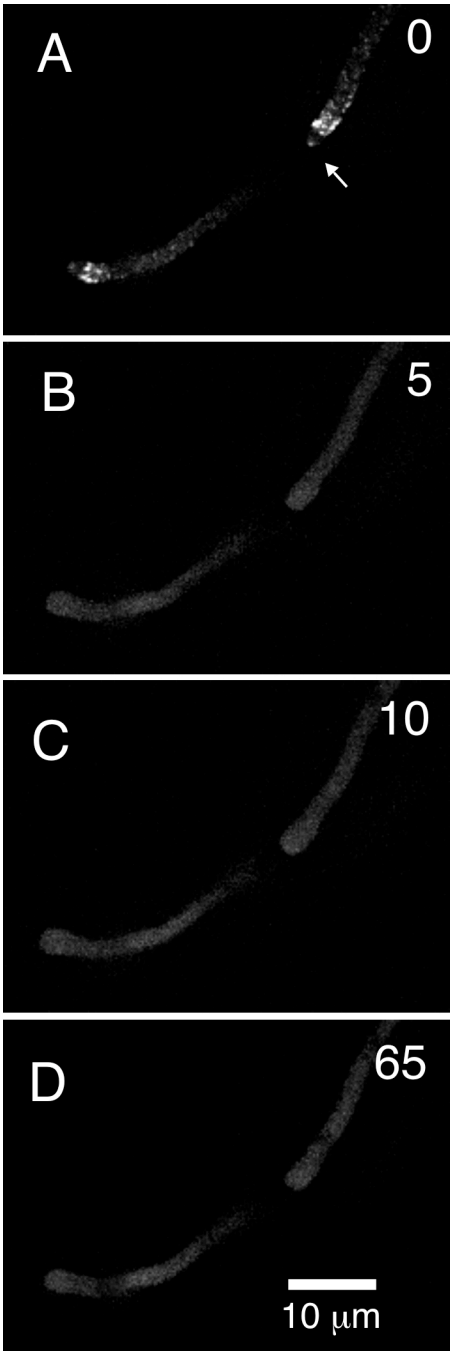
Supplementary Figure 1. Creation of a GFP-*synA* fusion by five-piece fusion PCR. (A) The 5´ untranslated region (5´ UTR), the *synA* promoter and approximately 1000 bp of the *synA* coding sequence are amplified in separate PCR reactions. Primers P3, P4, P5 and P6 have "tails" that do not anneal to the *synA* promoter or coding sequence but, rather, anneal to a fragment carrying the *A. fumigatus pyrG* (*AfpyrG*) gene (blue) or the GFP coding sequence (green). (B) The three fragments amplified in A are mixed with the *AfpyrG* fragment and the GFP coding sequence (which have been amplified separately) and all five fragments are fused together in a single PCR reaction using primers P2 and P7 generating the N-terminal GFP-*synA* replacement construct.

Supplementary Figure 2. Actin depolymerization and hyphal growth inhibition by latrunculin B. These images are projections of Z-series stacks taken at five min intervals (time in minutes at the upper right). Panel A shows the normal actin distribution in two hyphal tip cells. The actin patches are in collars behind the apex and there is an actin dot at the apex (arrow). Latrunculin B was added between panels A and B. The actin had depolymerized completely in less than 5 min. Hyphal tips bulged (B, C) and no additional hyphal growth occurred in more than an hour after latrunculin B addition. Bar in D = 10 μm

Supplementary Figure 1



Supplementary Figure 2



Supplementary Table 1. PCR Primers used in this study.

Primer Name	Sequence 5′→3′
synA1-1 (P1)	GCAGTGGTGGCGCGATGTAAGAC
synA1-3 (P3)	CGAAGAGGGTGAAGAGCATTGATACTAGATTGCGGCTGGTGTC
synA1-4 (P4)	CATCAGTGCCTCCTCTCAGACAGTCCCTATTCCTGAAGCCATCAG
synA1-5 (P5, GFP)	GTGAAAAGTTCTTCTCCTTTACTCATGAGTCTCTGTGCGAAGAGCT
synA1-6 (P6, GFP)	GCTGGTGCAGGCGCTGGAGCCGGTGCCATGTCTGAGCAACCGTACG
synA1-8 (P8)	GAGCGCACCGACACTGACGACC
synA1-2 (P2)	CCTGCGAGATCCAATGACTC
synA1-7 (P7)	GTAGCACATGAGAAGGGAAAGTC
ssoA1-1 (P1)	GATATCATCACAAGCCAGCCAG
ssoA1-3 (P3)	CGAAGAGGGTGAAGAGCATTGCCGCAATTTAATCCGTTGTTTC
ssoA1-4 (P4)	CATCAGTGCCTCCTCTCAGACAGGATTCTCAAATCTCCATCATCAAG
ssoA1-5 (P5)	GTTCTTCTCCTTTACTCATCGTGAAGATATAATGGAATCAATG
ssoA1-6 (P6)	GGTGCAGGCGCTGGAGCCGGTGCCATGAGTGTATGTCCGCCGGCCTAA
ssoA1-8 (P8)	GCGGTAGGACAACTAATCGAAC
ssoA1-2 (P2)	CGACCGAGATACCTGTAGGAAG
ssoA1-7 (P7)	GACCATAGGCTTACTGAGGCG
sncA2-5 (P5, mCherry)	CCTCCTCGCCCTTGCTCACCATGATGCTCTGTGCGAAGAGCTTG
sncA2-6 (P6, mCherry)	GGTGCAGGCGCTGGAGCCGGTGCCATGTCTGAGCAACCGTACG
actA-P1	CAGAAGCTCGACGAGCGGCG
actA-P2	GAAAAGTTCTTCTCCTTTACTCCCCATGGTGTTTAGGGGTGGATTAGAATC
actA-P3	TTCTTCGTAGTTCGATTCTAATCCACCCCTAAACACCATGGGGAGTAAAGGAGAAGAACTTTTC
actA-P4	GAGAGCAGCAACTTCCTCCTCAGCACTAGCAGTACCTTTGTATAGTTCATCCATGCCATG
actA-P5	GGTACTGCTAGTGCTGAGGAGGAAGTTGCTGCTCTCT
actA-P6	CCCTGCAGTCGTTCTCGAAGATG
yA-P1	GAATCGGCCACGGACCTGAC
yA-P3	GACAACTACAGCCGAAGACTCCGGGGCTGGATCCCGGAGGAATC
tpmA-P4	GATTCCTCCGGGATCCAGCCCCGGAGTCTTCGGCTGTAGTTG
tpmA-P5	GTGAAAAGTTCTTCTCCTTTACTCATGGTGGAGGGCTAGCAGCGAATG
tpmA-P6	GCTGGTGCAGGCGCTGGAGCCGGTGCCATGGACAGAATCAAGGAGGTTTG
tpmA-7 (AfpyroA tail)	GTAATCCAGCATCTGATGTCCACTTTAGGGCGCTGGAAGGAC
3’ yA-forward (AfpyroA tail)	GTCCTTCATTATGTAGACACTCGCGTGAGCTCTCATATTCGTACTTAC
3’ yA-reverse	GCATCTGTCCTCATGCGCGGC
yA-P2	CGACAAGTTTCTCAGTGGAGCG

Primer Name	Sequence 5′→3′
yA-P7	GTTGAGCGCGTCATTGCCCTC
tpmA-P5 (GA5 tail)	GGCTCCAGCGCCTGCACCAGCTCCAACACTGTTCAAGGAGAGCTC
secC-P1	CAGGCTATGGACGAACTATATTCG
secC-P3	GGCTCCAGCGCCTGCACCAGCTCCTTTCCTAAACGTAGCGGCAACATC
secC-P4	GTCCTTCATTATGTAGACACTCGCTAATGTATCTTTCTATGTCTATCC
secC-P5	CAGTGATAGCTGAGTTCCTTAG
secC-P2	GGTCCATCAAAGCAGACCCTC
secC-P6	CAGAGACAACAATTAGCTTGAG

MOVIE LEGENDS

Video1.mov. GFP-actin dynamics in a germling. Z-series stacks (six images at 0.3 μm steps) were captured at four sec intervals. The germ tube is about 2.0 μm in diameter so most, but probably not all, patches are seen. Each frame is a maximum intensity projection of a single stack. To minimize fading, the images were captured with short exposures that do not allow the actin dot at the apex to be seen. Actin patches are extremely dynamic. They can be seen to move toward the apex, away from the apex and to appear and disappear.

Video2.mov. GFP-SYNA and ABPA-mRFP in a rapidly growing hypha. Z-series stacks (12 images at 0.5 μm steps) were captured at five min intervals. Each stack was deconvolved and each frame is a maximum intensity projection of a Z-series stack. The image intensity thresholds were chosen to make the Spitzenkörper clear at the expense of the dome of GFP-SYNA between the ABPA-mRFP patches and the hyphal apex. The Spitzenkörper is at the apex and the position of the Spitzenkörper correlates with the direction of tip growth.

Video3.mov. Cytochalasin addition and washout in a strain expressing GFP-actin. Each frame is a projection of a Z-series stack. The first nine frames were taken at five min intervals and the remaining frames were taken at 10 min intervals. Cytochalasin A was added after frame five and removed after frame nine. The collar of actin patches dissolves after cytochalasin A addition and the tip stops growing and swells slightly. After cytochalasin A washout, the tip swells further and eventually a hyphal tip emerges and an actin patch collar eventually reforms.

Video4.mov. Latrunculin B addition to a strain expressing mRFP-ABPA and GFP-SYNA. Figure 5 is from the same data set, but the panels in Figure 5 are single focal plane images or a projection of only two focal planes whereas each frame in this movie is a maximum intensity projection of the entire stack. Stacks were taken at five min intervals. Latrunculin B was added after frame five. After latrunculin B addition, the Spitzenkörper disappears, tip growth stops, the mRFP-ABPA patches disappear and the GFP-SYNA occupies a larger area of the membrane at the tip.

Video5.mov. Cytochalasin A addition and washout in a strain expressing ABPA-mRFP and GFP-SYNA. Each frame is a projection of a Z-series stack. The first 10 stacks were taken at five-min intervals and the remaining stacks were taken at 10 min intervals. Cytochalasin A was added after frame five and washed out after frame 10. After cytochalasin A addition, tip growth stops, the Spitzenkörper disappears and the mRFP-ABPA patches initially collapse to the hyphal tip before becoming delocalized. After washout, the tip swells further and a hypha with Spitzenkörper an mRFP-ABPA collar emerges. The Spitzenkörper reappears shortly before the mRFP-ABPA collar re-forms. The thresholds were chosen to allow the Spitzenkörper to be seen clearly do not reveal the GFP-SYNA at the membrane of the growth-arrested tip very well.

Video6.mov. Latrunculin B addition and washout in a strain expressing ABPA-mRFP and GFP-SYNA. Each frame is a projection of a Z-series stack. The first 10 stacks were taken at five min intervals and the remaining stacks were taken at 10 min intervals. Latrunculin B was added after frame six and washed out after frame 10. After latrunculin B addition, the Spitzenkörper disappears as do the ABPA patches. The GFP-SYNA occupies a larger area of the plasma

membrane at the tip. After washout the hyphal tip swells then the Spitzenkörper re-forms and the APBA patches re-form and re-organize as a hyphal tip re-grows.

Video7.mov. GFP-α-tubulin and ABPA-mRFP in a strain treated with benomyl. Each frame is a projection of a Z-series stack and stacks were taken at five min intervals. Benomyl was added after frame five. In this case, the ABPA-mRFP collar remains intact for about 50 min after the addition of benomyl. This was among the longest times the tip growth apparatus remained intact after benomyl addition. A portion then collapses toward the hyphal tip while other ABPA-mRFP patches relocate to a forming lateral branch.