

**P5: Biochemical purification of Arp2/3 complex from *Physcomitrella patens*.**

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The Arp2/3 complex is a seven subunit complex consisting of the actin related proteins Arp2 and Arp3 as well as five novel proteins (designated ARPC1- ARPC5). In organisms where the Arp2/3 complex has been well studied, it has been shown to be a critical regulator of actin dynamics. Arp2/3 complex is the main nucleator of actin filament polymerization and also acts to branch existing actin filaments. Arp2/3 complex nucleation activity stems from its ability to cap the slow growing pointed ends of actin filaments, leaving the fast growing barbed ends free to elongate. Such activities have not been demonstrated in plants but mutants in the *Arabidopsis* Arp2 and Arp3 as well as ARPC5, the smallest subunit of the complex, show aberrant cell shapes attributed to actin abnormalities (Mathur et al., 2003a & b). The Arp2/3 complex has been identified in a diverse array of eukaryotic organisms including the moss *Physcomitrella patens*. Although plant Arp2/3 complex sequences have been identified, it remains to be shown that these proteins interact as a complex in plants. We have proposed to utilize *Physcomitrella* in order to biochemically purify Arp2/3 complex and thereby ascertain the composition of the plant complex. We isolated ARPC1, the 40 kDa subunit of the Arp2/3 complex from *Physcomitrella*. Stable lines of ARPC1 with a triple 3' HA tag driven by the rice actin promoter were generated and screened by immunoprecipitation. The line with the strongest expression was used in subsequent experiments. We have utilized a three step chromatographic approach to obtain fractions enriched for *Physcomitrella* Arp2/3 complex. First, extracts from the ARPC1 HA line were passed over a DEAE anion exchange column. Fractions enriched for ARPC1-HA were then separated on a gel filtration column (Sephacryl S-300). The peak corresponding to molecular weights 158 – 350 kDa was then collected for further separation on MonoQ anion exchange. In each case the HA tag was followed by Western blot analysis using anti-HA antibody. Staining of select MonoQ fractions show potential enrichment of Arp2/3 components. Further identification of bands and additional fraction purification is currently in progress.

Mathur, J., N. Mathur, B. Kernebeck and M. Hulskamp. 2003a. Mutations in actin-related proteins 2 and 3 affect cell shape development in *Arabidopsis*. *Plant Cell* 15: 1632 -1645.

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Hulskamp. 2003b. *Arabidopsis* CROOKED encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. *Development* 130: 3137 - 3146.