

Japan-U.S. Brain Research Cooperation Program
Group Joint Study Project Report

Field: Cellular/ Molecular

1. Principal Researcher

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2. Project Title:

Novel mechanisms of actin regulation in the growth cones

3. Japanese Group

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4. U.S. Group

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5. Research Period, from/to (mm/dd/yyyy) and total number of years.

4/1/2009 –3/31/2012, total three years.

6. Abstract, Results, and Research Significance (300 words):

Molecular mechanisms of actin dynamics involved in the growth cone formation were investigated. We first demonstrated in vitro that dynamin 1 is implicated in actin assembly in brain cytosol. Depletion of dynamin 1 from the cytosol, addition of Dynasore, dynamin inhibitor, or addition of anti-dynamin 1 antibodies reduced actin polymerization. Dynamin 1 bound to cortactin by MALDI-MS, and

incubation of F-actins with these proteins resulted in the formation of actin bundles, along which dynamin 1 and cortactin were periodically presented. By EM, the actin bundles are composed of from several to a dozen of actin filaments, and decorated with clusters of protein complexes, which often appeared twining around a few actin filaments. Dynamin 1 and cortactin formed ring-shaped complexes, approximately 50 nm in outer diameter, which were widely open or linear-shaped in the presence of GTP γ S. Dynamin 1 and cortactin were also periodically presented and colocalized along growth cone filopodia of NG108 cell by immunofluorescence. Immuno-EM revealed that these proteins were present on actin bundles. Microinjection of anti dynamin 1 antibodies, or application of Dynasore resulted in acute retraction of the growth cone in SH – SY5Y cell. Cortactin RNAi also reduced growth cone formation.

Next, we found that PKC phosphorylation of cortactin is implicated in the actin dynamics in growth cones, and identified the phosphorylation sites by MALDI-MS. Cortactin was rapidly phosphorylated in NG108-15 cell during growth cone collapse by LPA, and the phosphorylation was inhibited by Ro-31-8220, a PKC inhibitor. Cortactin and PKC α colocalized along the growth cone filopodia of SH-SY5Y cell by immunofluorescence, and they interacted by immunoprecipitation. PMA, a PKC activator, increased cortactin-PKC α interaction in SH-SY5Y cells simultaneously with PMA-induced growth cone collapse. Consistently, cortactin-dependent actin bundle formation in vitro was reduced by cortactin phosphorylation via PKC α . Taken together, actin bundle formation by dynamin 1/ cortactin ring complex is important for stabilizing filopodia of growth ones.

7. Other (Research-related concerns, particular points of note):

Results of this study described above were presented at the following meetings.

Hiroshi Yamada, Tadashi Abe, Yoshihiro Kawada and Kohji Takei: Novel mechanism of actin bundle formation by dynamin/cortactin ring complex. 49th Annual meeting, American Society for Cell Biology, San Diego, CA, 2009

Kohji Takei, Tadashi Abe, Yoshihiro Kawada, and Hiroshi Yamada: Dynamin 1/cortactin complex mechanically bundles actin filaments and supports the formation of growth cone filopodia, Neuro 2010, Kobe, 2010

Kohji Takei, Tadashi Abe, and Hiroshi Yamada: Dynamin 1 / cortactin complex bundles actin filaments and supports growth cone formation. 50th Annual meeting, American Society for Cell Biology, Philadelphia, PA, 2010

Hiroshi Yamada, Tadashi Abe, Kohji Takei: Actin bundle formation by dynamin-cortactin complexes is important for filopodium stability of growth cone, The 131st Annual meeting, The Pharmaceutical Society of Japan, Shizuoka, 2011

Hiroshi Yamada, Mari Sei, Tadashi Abe, Toshio Masumoto, Tatsuya Kikuchi, Satoshi Ikeda, Kazuhito Tomizawa, Hideki Matsui, and Kohji Takei: PKC phosphorylation of cortactin and its possible role regulating actin dynamics in the formation of growth cone filopodia. The 84th Annual meeting, The Japanese Biochemistry Society, Kyoto, 2011

Hiroshi Yamada, Tadashi Abe, Toshio Masumoto, Teiichi Nishiki, Mari Sei, Tatsuya Kikuchi, Kazuhito Tomizawa, Satoshi Ikeda, Hideki Matsui, and Kohji Takei: PKC phosphorylation of cortactin is implicated in the regulation of actin dynamics: 51th Annual meeting, American Society for Cell Biology, Denver, CO, 2011

*Please attach any reference materials as necessary.