

Stress-Associated MAP Kinase Fills in the Map of Filamin-Mediated Neuronal Migration

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The gene encoding filamin-A (FLN-A), an actin filament-bundling protein, was found to be responsible for periventricular heterotopia—a disease characterized by the presence of abnormal neuronal nodules at the lateral ventricles. In a recent issue of *Neuron*, Sarkisian et al. revealed that the stress-associated mitogen-activated kinase kinase kinase MEKK4 appears to regulate neuronal migration by controlling the amount of FLN-A.

During the development of the forebrain, cortical neurons proliferate at the ventricular surface located along the cavity of lateral ventricle, and they then migrate to the outer layers including the cortical plate. Neurons born in the ventricular zone initiate migration and stop migrating at the appropriate place in response to “stop signals”. Extensive research has been performed on the “stop signal” after the discovery of the large extracellular protein reelin. Molecules downstream of reelin receptors such as integrin, VLDL receptor, and ApoE receptor 2 include mouse disabled-1 that transmits signals to stop neuronal migration (Tissir and Goffinet, 2003). However, molecules involved in the initiation of migration remain unclear, except FLN-A and ARFGF2 (Fox et al., 1998; Sheen et al., 2004).

Filamins form a large protein family (Stossel et al., 2001). They possess the activities of actin filament binding and bundling that are involved in actin cytoskeleton remodeling during cell migration. The gene encoding filamin-A (FLN-A) was found to be responsible for periventricular heterotopia. Loss-of-function mutations that cause stop-codon insertion or missplicing of *FLN-A* result in periventricular heterotopia (Fox et al., 1998). Interestingly, the expression of a filamin mutant that lacks the actin filament-binding region affects neuronal polarity and migration. Furthermore, FLN-A-inter-

acting protein (FILIP) appears to accelerate FLN-A degradation to stop cell migration at the ventricular zone; FILIP overexpression decreases the amount of FLN-A and suppresses neuronal migration (Nagano et al., 2002).

Surprisingly, in a recent issue of *Neuron*, Sarkisian et al. demonstrated that the MEKK4^{-/-} brain exhibits the phenotype of periventricular heterotopia and an increased amount of FLN-A (Sarkisian et al., 2006). Furthermore, they showed that the overexpression, not loss or loss-of-function, of FLN-A in the wild-type brain resulted in defective neuronal migration. Interestingly, the phosphorylation of Ser2152 in FLN-A was increased in MEKK4^{-/-} mice. This phosphorylation is thought to result in the resistance of FLN-A to calpain-mediated degradation, that in turn resulted in the accumulation of FLN-A (Vadlamudi et al., 2002). Although it is unknown whether a gain-of-function mutation in FLN-A affects neuronal migration, this mutation was found to be involved in other diseases including otopalatodigital syndrome, frontometaphyseal dysplasia, and Melnick-Needles syndrome (Robertson et al., 2003). Therefore, mechanisms other than loss-of-function mutations in FLN-A may be responsible for periventricular heterotopia in MEKK4^{-/-} mice. Alternatively, appropriate control of the FLN-A level in neurons may be a prerequisite for the control of neuronal migration.

MEKK4 was originally discovered as a MAP triple kinase in the p38/JNK pathway (Takekawa and Saito, 1998). In nonneuronal cells, MEKK4 is activated under conditions of environmental stress, such as exposure to methyl methanesulphonate (MMS), ultra-violet light, and γ radiations. The activation of this pathway leads to apoptosis and/or reparative responses. In MEKK4^{-/-} and MEKK4 siRNA-treated mice, the level of apoptosis in the forebrain was elevated (Chi et al., 2005; Sarkisian et al., 2006). The activity of p38 in the forebrains of these mice was higher than that in the forebrains of wild-type mice, but the activity of JNK was normal. The activity of the direct downstream kinase MKK4/SEK (MAP kinase kinase) was decreased. SEK1 mediated the increase in FLN-A phosphorylation, but it did not appear to directly phosphorylate FLN-A, though MEKK4 and FLN-A can form a protein complex via SEK1. Thus, the manner in which MEKK4 regulates FLN-A has not been clarified to date.

Ser2152 in FLN-A was previously identified as a site phosphorylated by p21-activated protein kinase (PAK), which is involved in membrane ruffle formation. The interaction of FLN-A with PAK and the phosphorylation of FLN-A were shown to be essential for ruffle formation in human breast adenocarcinoma and melanoma cell lines (Vadlamudi et al., 2002). Therefore, PAK may be involved in the

phosphorylation of FLN-A downstream of MEKK4.

Despite the presence of a large body of work on MAP kinases, little is known about whether MAP kinase signaling directly regulates the actin cytoskeleton or signaling molecules such as small GTPases or PAK that lead to cytoskeleton remodeling. However, MAP kinase is also involved in cell-substratum adhesion, presumably by regulating the assembly of focal complex proteins such as integrin, vinculin, and paxillin (Webb et al., 2004). Cell migration is a highly orchestrated process involving integrated membrane protrusion and membrane attachment to the substratum at the front of the cell and membrane retraction at the rear of the cell. In this respect, the distribution of the extracellular matrix (ECM) protein laminin was discontinuous in the brain of MEKK4^{-/-} mice (Sarkisian et al., 2006). Laminin is an integrin substrate and thus an

irregular ECM might affect cell migration.

The involvement of stress-associated MAP triple kinase in neuronal migration will shed light on a new layer of the mechanism of cell migration that may occur through the direct regulation of the actin cytoskeleton and/or through the indirect regulation of cell-substratum adhesion.

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TIRFing out Studies on Glut4 Trafficking

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Docking and fusion of Glut4 vesicles with the plasma membrane are essential but poorly understood steps during insulin-stimulated glucose transport. Recent studies utilizing TIRF microscopy shed light on these processes and map the sites of possible intervention by insulin from just underneath the plasma membrane.

Insulin stimulates glucose uptake into muscle and adipose cells by promoting the relocation of the glucose transporter Glut4 to the cell surface (Bryant et al., 2002). Despite the general notion that deficiencies in this process represent a primary lesion in the development of type 2 diabetes and related metabolic disorders, little is known about the molecular defects that underlie this pathology (Saltiel and Kahn, 2001). This is largely due to our limited knowledge of precisely how

insulin signaling influences the trafficking itinerary of Glut4.

In the basal state, Glut4 is retained in intracellular vesicles and then traffics to the plasma membrane upon insulin stimulation. This process involves multiple steps governed by insulin, including endocytosis into endosomes, sorting of Glut4 into specialized storage vesicles, their retention inside cells, transport along cytoskeletal tracks, tethering, docking, priming, and the final fusion with the plasma

membrane. However, the regulated steps in this complex process that are rate limiting, let alone the precise identity of the molecular targets that connect insulin signaling to cellular vesicle trafficking machineries, have yet to be defined.

Bai et al. (2007) have investigated the terminal stages of Glut4 trafficking by examining the motion of eGFP-tagged Glut4 molecules using time-lapsed total internal reflection fluorescence (TIRF) microscopy. This approach