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CIN85 is a Key Mediator in the Regulation of Behavior and Metabolism

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Adaptor proteins are non-catalytic polypeptides that contain one or more domains that are capable of binding to other proteins or nonprotein ligands [1]. These molecules are essential for intracellular signal transduction involved in the regulation of endocrine action, metabolic activity, neuronal function, and cell growth. Recently, there has been growing evidence that adaptor proteins play critical roles in behavioral control and metabolic regulation. Glutamate receptor interacting protein 1 (GRIP1) regulates social behavior and modulates the autistic phenotype [2]. Maiya et al. [3] reported that a Lin11, Isl-1 and Mec-3 (LIM) adaptor protein, LIM domain only protein 4 (LMO4), regulates fear learning. The G protein-coupled receptor kinase-interacting protein-1 (GIT1) deficiency in mice causes psychostimulant-responsive attention deficit hyperactivity disorder (ADHD)-like phenotypes [4]. On the other hand, the hepatic tumor necrosis factor receptor-associated factor 2 (TRAF2) autonomously promotes hepatic gluconeogenesis by enhancing the hyperglycemic response to glucagon and other factors that increase cyclic adenosine monophosphate (cAMP) level, thus contributing to hyperglycemia in obesity [5]. In the present editorial, I briefly review a novel function of the adaptor/scaffold protein Cbl-interacting protein of 85 kDa (CIN85) in the regulation of behavior and metabolism.

CIN85 was independently identified as CIN85 [6], regulator of ubiquitous kinase (Ruk) [7], SH3 domain-containing gene expressed in tumorigenic astrocytes (SETA) [8] and SH3 domain kinase binding protein 1 (SH3KBP1) [9]. These genes were isolated from either human (CIN85), rat (Ruk and SETA) or mouse (SH3KBP1) sources and show between 92% and 97% sequence identities, suggesting that they represent homologues of one gene. The CIN85 gene is localized on the distal arm of the X chromosome (Xp22.1-p21.3) and its length is approximately 353.7 kb in humans (http://www.ncbi.nlm.nih.gov/sites/entrez?db=geneandcmd=retrieveanddopt=full_reportandlist_uids=30011). The main 3.2 kb CIN85 mRNA is expressed in all adult and newborn tissues [6,7]. Owing to alternative splicing and the use of different promoters, multiple CIN85 mRNA signals have been detected, which showed a more restricted pattern of expression [7]. CIN85 is composed of three N-terminal SH3 domains, followed by a centrally located proline-rich region and a C-terminal coiled coil domain [10]. Initially, CIN85 was identified as a negative regulator of epidermal growth factor receptor (EGFR) signaling and phosphoinositide 3-kinase (PI 3-kinase) signaling pathways via its interaction with c-Cbl [6,7]. Then, CIN85 was identified as a central adaptor molecule involved in the recruitment of the endocytic machinery required for the internalization of various cell surface receptors, including receptor tyrosine kinases such as EGFR [11,12], hepatocyte growth factor receptor (HGFR, Met) [13], and vascular endothelial growth factor receptor (VEGFR) [14], and also immunoglobulin IgE receptors in mast cells [15]. Recently, it has been reported that CIN85 is involved in the regulation of the immune system and cytokinesis. Using B cell-specific CIN85 knockout mice, Kometani et al. [16] found that CIN85 links the B cell receptor to IκB kinase-β/nuclear factor-kappa B (IKK-β/NFκB) activation, thereby contributing to T cell-independent immune responses. Haglund et al. [17] reported that Cindr, a Drosophila CD2AP/CIN85 ortholog, interacts with Anillin and that depletion of either Cindr or Anillin gives rise to binucleate cells and fewer intercellular bridges *in vivo*, therefore, Cindr is involved in complete and incomplete cytokinesis in Drosophila. In the future, as these reports, a novel function of CIN85 might be identified since CIN85 is expressed ubiquitously.

Recently, we have found a novel function of CIN85 in the regulation of the signaling of behavior and metabolism [18]. In the mouse brain, both of the major isoforms expressed, CIN85-xl and CIN85-l, were found to be abundant in most brain regions examined [18,19]. Interestingly, CIN85-xl is expressed only in the central nervous system (CNS). Furthermore, CIN85 colocalizes with postsynaptic density protein 95 (PSD-95) at postsynaptic sites in the somatodendritic compartment, in which it frequently clustered in dendritic shafts, as well as within dendritic spines [18]. Dendritic spines are small protrusions extending from the surface of dendrites, which are believed to be the main sites of excitatory synapses and are thus vital centers for synaptic transmission in the brain [20]. To investigate the function of CIN85 in the CNS, we generated mice deficient in the two major CIN85 isoforms expressed in the brain (CIN85-xl and CIN85-l) [18]. By homologous recombination, we deleted exon 2 of the CIN85 genomic locus (CIN85$^{\Delta ex2}$). As expected, all CIN85 protein variants encoded by transcripts initiated from promoter #1 (CIN85-xl, CIN85-l, and the shorter CIN85-ΔCP) were abolished in CIN85$^{\Delta ex2}$ mice.

CIN85$^{\Delta ex2}$ mice are viable and fertile, and display no obvious abnormalities in appearance. We subjected the CIN85$^{\Delta ex2}$ mice to extensive analyses of a broad range of parameters in accordance with the physiological screens defined by the German Mouse Clinic (http://www.mouseclinic.de/). Among the parameters tested, the mice showed a clear knockout-specific phenotype in behavior and energy metabolism. When subjected to the modified hole-board test [21], which assays spontaneous behavior such as forward and vertical locomotor activity, speed of movement, and exploratory behavior in a novel environment, the CIN85$^{\Delta ex2}$ mice showed significantly increased activities, as compared with the wild type. Specifically, the CIN85$^{\Delta ex2}$ mice exhibited increased forward locomotor activity, as manifested by increases in total distance travelled, number of line crossings, mean and maximum velocities, as well as turning frequency. In addition, CIN85$^{\Delta ex2}$ mice showed enhanced exploratory behavior, namely, entering the board more frequently and exploring a larger number of holes on the board than the wild-type mice.

Interestingly, the CIN85$^{\Delta ex2}$ mice display abnormally high levels of dopamine and D2 dopamine receptors (D2DRs) in the striatum, an important center for the coordination of animal behavior. Importantly, CIN85 localizes to the postsynaptic compartment of striatal neurons, in which it co-clusters with D2DRs. Moreover, it interacts with endocytic regulators such as dynamin and endophilins in the striatum. In neurons of the wild-type mice, CIN85 resides postsynaptically and associates with endocytic regulators, such as dynamin and endophilins, and it clearly has a crucial function in stabilizing endophilin binding

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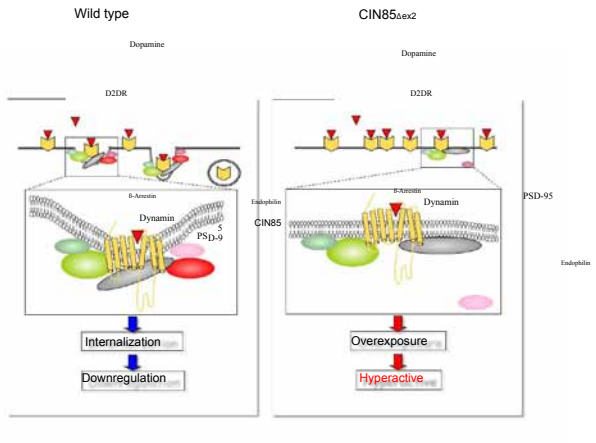
to D2DRs in the striatum. The internalization of D2DRs is caused by the coordination of these endocytic proteins. As a result, dopamine signals are attenuated, and then the appropriate locomotor activity is maintained (Figure 1). As a consequence, the absence of CIN85 gives rise to insufficient endocytic internalization of D2DRs owing to the defect of endophilin recruitment to the endocytic complex after dopamine stimulation, increasing striatal dopamine receptor levels, which can, at least in part, explain the enhanced locomotor and exploratory behavior we observe in the CIN85$^{\Delta ex2}$ mice (Figure 1). The resulting increase in the expression levels of surface-associated D2DRs in CIN85$^{\Delta ex2}$ mouse striatal neurons and the ensuing hyperactivity phenotype are in line with earlier findings, showing that activation of postsynaptic D2DRs results in increased locomotor activity and that D2DR knockout mice display reduced spontaneous movements [22,23].

When comparing CIN85$^{\Delta ex2}$ mice with wild-type animals, we found that the mice deficient in CIN85-xl and CIN85-l showed abnormalities in several metabolic parameters, including higher energy uptake level, higher lean mass, and lower fat content. Both male and female CIN85$^{\Delta ex2}$ mice showed a higher lean mass, as well as lower total and subcutaneous fat contents than their wild-type littermates. Female CIN85$^{\Delta ex2}$ mice also showed a significantly increased energy uptake level compared with their wild-type littermates. However, CIN85$^{\Delta ex2}$ mice did not show any significant alterations in insulin metabolism. The explanation for this phenotypical trait may be directly linked to the observed alterations in dopaminergic activity, given the previously reported involvement of D2DR-mediated signaling in the regulation of appetite, energy intake, and obesity. Multiple studies have shown correlations between striatal D2DR expression level and body composition and between low D2DR expression level and obesity [24]. A recent report has furthermore showed a strong link between certain D2DR allelic variations and obesity, suggesting that individuals with certain genotypes resulting in dopaminergic hypofunction are prone to obesity [25]. An enhanced dopaminergic signaling is, therefore, consistent with the slim appearance of CIN85$^{\Delta ex2}$ mice.



D2DR: D2 dopamine receptor; PSD-95: postsynaptic density protein-95.

Figure 1: Model for involvement of CIN85 in internalization of D2DR. CIN85 localizes to the postsynaptic compartment of striatal neurons where it co-clusters with D2DRs. CIN85 interacts with endocytic regulators such as dynamin, β-arrestin, and endophilins in the striatum (left panel). The absence of striatal CIN85 causes insufficient complex formation of endophilins with D2DRs in the striatum and ultimately attenuates D2DR endocytosis in striatal neurons in response to dopamine stimulation. The defect of D2DR endocytosis induces hyperactivity (right panel) [18].

The involvement of dopaminergic signaling in the regulation of movement, emotion, reward feelings, and obesity is well established [26,27]. In agreement with this idea, aberrations in dopaminergic pathways have been strongly linked to various neurological or metabolic disorders, including Parkinson's disease, schizophrenia, ADHD, Huntington's disease, and obesity [26-28]. The molecular defects underlying these pathologies have not been fully characterized, but may include alterations in the expression levels of dopamine ligands and/or receptors, as well as defects in downstream signaling events [29,30].

CIN85 is a novel regulator of D2DR endocytosis, involved in controlling behavior as well as metabolism, and the use of CIN85$^{\Delta ex2}$ mice enables new developments in ADHD or obesity research.

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