A Structural Approach to Decipher the Neurexin and Neuroligin Splice Isoform Code

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The alternative splicing of neurexins (NRXs) and neuroligins (NLs) has been implicated in specifying synaptic connections. In this issue of *Neuron*, Koehnke et al. took a structural approach for assessing the contribution of alternative splice isoforms for β-NRX/NL-mediated synaptic recognition.

Neurexins (NRXs) and neuroligins (NLs) are probably the best-known *trans*-synaptic adhesion molecules in neurons (Craig and Kang, 2007; Südhof, 2008). Postsynaptic NLs function as ligands for presynaptically localized NRXs via interactions between their respective ectodomains (Figure 1A). Such *trans*-synaptic NRX/NL interactions have been shown to play critical roles in synaptic function, and mutations of the genes encoding NRXs and NLs have been linked to a variety of cognitive disorders, including autism, schizophrenia, and mental retardation in humans (Südhof, 2008). The mammalian genome contains three *NRX* genes (*NRX1-3*), each of which encodes a long α-NRX and a shorter β-NRX (Südhof, 2008). Extensive alternative splicing of the ectodomains of NRXs (α-NRX in particular) further diversifies NRX proteins (Südhof, 2008). Similarly, human NL exists in various alternative spliced isoforms derived from five genes and two spliced sites (Craig and Kang, 2007). Given the requirement of a huge number of specific synaptic connections in the brain, the diversity of the NRX and NL isoforms provides a tempting model for synaptic recognition where synaptic recognition is mediated in a lock and key fashion by differential pair-wise binding interactions between different splice isoforms of NRX and NL (e.g., NL-1 containing the splice insert B can only interact with β-NRX1, whereas NL-1 lacking the splice insert B binds to both β-NRX1 and α-NRX; Boucard et al., 2005) (Figure 1A). This splice-isoform-based model for NRX/NL interactions has received some experimental support (Boucard et al., 2005; Chih et al., 2006), although contradicting results are also present in the literature (see Reissner et al. [2008] for an example).

As a step toward further understanding the NRX/ML interactions, Koehnke et al. (Koehnke et al., 2010) took the heroic approach of using surface plasmon resonance (SPR), systematically measuring the quantitative binding affinities of all possible pair-wise combinations of alternatively spliced β-NRX1-3 and NL1-3 using purified recombinant protein produced in mammalian cells (i.e., with proper glycosylations). The most surprising finding of this systematic study is that alternative splicing has a very limited effect on the β-NRX/NL interactions, as the authors found that all NL family members with and without insert A or B can bind to all β-NRX isoforms regardless of whether they contain the splice insertion 4 (called "SS4"). Therefore, the study by Koehnke et al. challenges the splicing-specific *trans*-synaptic NRX/NL interaction code (Figure 1A), at least in the context of the interaction between NL1-3 and β-NRX1-3.

In parallel to these assessments of binding interactions, Koehnke et al. also determined the crystal structures of β-NRX1 and 2 containing the SS4 insertion (β-NRX+4). Although several crystal structures for β-NRX/NL complexes are available, Koehnke's approach was based on glycosylated proteins, and these new crystal structures of both the β-NRX1+4 and β-NRX2+4 ectodomains (referred to as β-NRX+4, as the two structures are essentially the same) reveal another unanticipated finding. As it turns out, the structure of β-NRX+4 and the previously determined structure of β-NRXΔ4 (Arac et al., 2007; Chen et al., 2008; Fabrichny et al., 2007) are very similar, which is surprising given the importance that has been ascribed to the SS4 insertion sequence. Based on their structural data, the authors propose that the high structural similarity between SS4-containing and SS4-lacking β-NRXs comes about due to a previously unknown, SS4-mediated structural rearrangement. In β-NRX+4, the C-terminal half of the 30 residue SS4 adopts a β strand structure and occupies the same position as the β10 in β-NRXΔ4. The displaced residues corresponding to the "old" β10 becomes a part of the elongated β9/β10 loop in the structure of β-NRX+4 (Figure 1A). Importantly, the residues corresponding to the β10 strand in β-NRX+4 and β-NRXΔ4 are highly homologous (i.e., the interfaces between β-NRX/NL complexes do not vary much between NRX containing and lacking SS4), and the elongated β9/β10 loop is located at the opposite face of the NRX/NL complex interface (i.e., the protrusion formed due to the β10 displacement does not interfere NRX's binding to NL) (Koehnke et al., 2010; Figure 1A).

Koehnke et al. also suggest that the conformational differences of SS4 between the previous bacterial expressed β-NRX+4 structure (Shen et al., 2008) and the structure determined by them is likely due to the effects of posttranslational glycosylation. In the glycosylated version of β-NRX+4, the carbohydrate moiety packs with a bulky Trp in SS4 to stabilize the β10 strand formed by the insertion sequence. Without this carbohydrate/Trp interaction (i.e., in the nonglycosylated β-NRX+4), a part of SS4 forms an α helix protruding from the surface of the

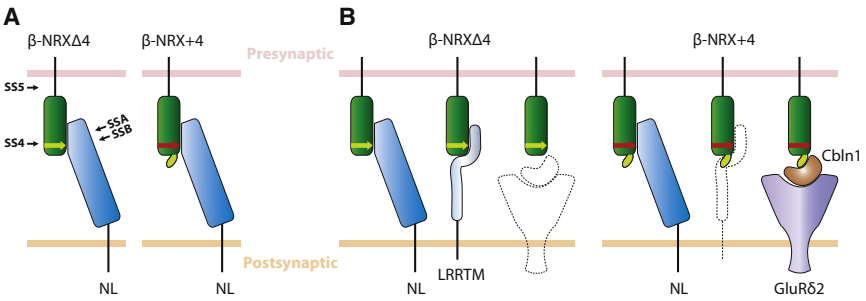

Figure 1. The NRX/NL-Mediated Synaptic Connections
(A) The binary NRX/NL interaction model hypothesized to mediate specific synapse connections.
(B) Some of the newly discovered *trans*-synaptic connections involving NRXs with LRRTMs or Cbln1/GluRδ2. The β10 strand in β-NRXΔ4 and the new β10 formed by the SS4 insertion in β-NRX+4 are indicated by greenish yellow and red arrows, respectively.

NRX+4 that forms an interface with NLs (Shen et al., 2008). Therefore, the diminished interaction observed between nonglycosylated β-NRX+4 and NL could be an experimental artifact. That being said, it should be pointed out that the β9/β10 region of β-NRXs is structurally dynamic and the SS4 sequence further modifies the dynamic properties of β-NRXs (Koehnke et al., 2008). Therefore, the crystal structures of β-NRXs determined by Koehnke et al. (2010) and by others earlier may represent only a few snapshots among many possible conformational states of the proteins, and it is possible that the conformational dynamics of β-NRXs may play a critical role in how β-NRXs decode signals presented by various postsynaptic ligands.

Although biochemical and structural studies like the one presented by Koehnke et al. (2010) often reduce a highly complicated in vivo biological question to a much simpler, in vitro amenable system, their systematic binding study underscores an important message: *trans*-synaptic protein-protein interaction "codes" are much more complicated than the simple, binary NRX/NL complexes anticipated in the past. In line with this idea, more recently other synaptic proteins have been shown to interact with NRXs, in a manner that is splice form specific. leucine-rich repeat transmembrane neuronal proteins (LRRTMs) are recently identified NRX ligands that regulate synapse formation (de Wit et al., 2009; Ko et al., 2009; Siddiqui et al., 2010). Interestingly, LRRTM1 and -2 specifically bind to β-NRXΔ4 but not β-NRX+4, and the binding of LRRTMs and NLs to β-NRXΔ4 are mutually exclusive (Ko et al., 2009; Siddiqui et al., 2010). Although the displacement of β10 in β-NRX by the SS4 insertion keeps the β-NRX/NL interface essentially unchanged, it is possible that the new protrusion formed by the displaced "old" β10 could block LRRTMs from accessing β-NRX+4 (Figure 1B). The exact molecular basis governing the LRRTMs/NRX interactions await future structural studies. In addition to *trans*-synaptic cell adhesion molecules, diffusible factors secreted by neurons or glia and even ion channels can also interact with NRXs or NLs in regulating synaptic formation and functions. For example, Zhang et al. have shown that presynaptic neurexins interact with postsynaptic GABA(A) receptors (Zhang et al., 2010). Another example of this is the recent finding that the secreted glycoprotein cerebellin 1 precursor protein (Cbln1), which is important for synaptic development in the cerebellum, functions as an obligatory factor in bridging the extracellular N-terminal domain of GluRδ2 with β-NRXs (Uemura et al., 2010). Cbln1 interacts directly with both GluRδ2 NTD and β-NRXs, although it is not known whether GluRδ2 NTD and β-NRXs physically contact with each other in the GluRδ2/Cbln1/β-NRXs tripartite complex. Remarkably, only β-NRXs with SS4 are capable of forming GluRδ2/Cbln1/β-NRXs (Figure 1B). These studies of the interactions between NRXs and LRRTMs and between NRXs and Cbln1 further highlight the importance of alternative splicing of NRXs and NLs, and the work here by Koehnke et al. (2010) suggest the possibility that the main function of this alternative splicing may not be to regulate interaction between NRXs and NLs but rather to modulate interactions with other binding partners. Future biochemical and functional studies will be critical for testing this possibility.

REFERENCES

Arac, D., Boucard, A.A., Ozkan, E., Strop, P., Newell, E., Südhof, T.C., and Brunger, A.T. (2007). Neuron *56*, 992–1003.

Boucard, A.A., Chubykin, A.A., Comoletti, D., Taylor, P., and Südhof, T.C. (2005). Neuron *48*, 229–236.

Chen, X., Liu, H., Shim, A.H., Focia, P.J., and He, X. (2008). Nat. Struct. Mol. Biol. *15*, 50–56.

Chih, B., Gollan, L., and Scheiffele, P. (2006). Neuron *51*, 171–178.

Craig, A.M., and Kang, Y. (2007). Curr. Opin. Neurobiol. *17*, 43–52.

de Wit, J., Sylwestrak, E., O'Sullivan, M.L., Otto, S., Tiglio, K., Savas, J.N., Yates, J.R., 3rd, Comoletti, D., Taylor, P., and Ghosh, A. (2009). Neuron *64*, 799–806.

Fabrichny, I.P., Leone, P., Sulzenbacher, G., Comoletti, D., Miller, M.T., Taylor, P., Bourne, Y., and Marchot, P. (2007). Neuron *56*, 979–991.

Ko, J., Fuccillo, M.V., Malenka, R.C., and Südhof, T.C. (2009). Neuron *64*, 791–798.

Koehnke, J., Jin, X., Trbovic, N., Katsamba, P.S., Brasch, J., Ahlsen, G., Scheiffele, P., Honig, B., Palmer, A.G., 3rd, and Shapiro, L. (2008). Structure *16*, 410–421.

Koehnke, J., Katsamba, P.S., Ahlsen, G., Bahna, F., Vendome, J., Honig, B., Shapiro, L., and Jin, X. (2010). Neuron *67*, this issue, 61–74.

Reissner, C., Klose, M., Fairless, R., and Missler, M. (2008). Proc. Natl. Acad. Sci. USA *105*, 15124–15129.

Shen, K.C., Kuczynska, D.A., Wu, I.J., Murray, B.H., Sheckler, L.R., and Rudenko, G. (2008). Structure *16*, 422–431.

Siddiqui, T.J., Pancaroglu, R., Kang, Y., Rooyakkers, A., and Craig, A.M. (2010). J. Neurosci. *30*, 7495–7506.

Südhof, T.C. (2008). Nature *455*, 903–911.

Uemura, T., Lee, S.J., Yasumura, M., Takeuchi, T., Yoshida, T., Ra, M., Taguchi, R., Sakimura, K., and Mishina, M. (2010). Cell *141*, 1068–1079.

Zhang, C., Atasoy, D., Araç, D., Yang, X., Fucillo, M.V., Robison, A.J., Ko, J., Brunger, A.T., and Südhof, T.C. (2010). Neuron *66*, 403–416.