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Neurotransmitter inactivation is important for the origin of nerve system in animal early evolution: A suggestion from genomic comparison

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Abstract

Metazoans possess complicated multicellular structure among the multicellularities of eukaryotes. One evolutionary pressure that permits such complexity relates to the directed and precise informational transmission performed by numerous synapses in neuron system. Neurotransmitter inactivations play essential roles in the termination of synaptic transmission and are thus crucial for precise synaptic transmission. Here, we performed a genomic comparison among 11 eukaryotic organisms including five bilaterian species and six pan-unicellular eukaryotes to search for genes related to metazoan multicellular function. The result showed that the majority of genes related to neurotransmitter inactivation in the synaptic cleft endured high and stable selective pressure and were specifically present in bilaterians, whereas genes related to transmitter release and postsynaptic transmitter receptors did not show these properties. From these data we conclude that neurotransmitter inaction may play a critical role in the origin of the nerve system encountered in the early evolution of metazoan. In addition, we suggest that neurotransmitter inactivation probably participates in the formation or refinement of the synapse, following the concept of ''ontogeny recapitulates phylogeny.'' Further experimental evidence is needed to support the suggestion and to explain the importance of neurotransmitter inactivation to metazoan multicellular function.

Keywords: Neurotransmitter inactivation; Metazoan multicellular function

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Abbreviations: AchE, acetylcholinesterase; AchRA2, Ach receptor alpha2; ADRB1, adrenergic beta 1 receptor; CNS, central neural system; COMT, catechol *O*-methyltransferase; DAT, dopamine transporter; DRD1, dopamine receptor D1; DRD2, dopamine receptor D2; GABA, gamma-aminobutyric acid; GABBR1, GABA receptor B1; GABRA1, GABA receptor subunit alpha 1; GAT, GABA transporter; GlyT, glycine transporter; MAO, monoamine oxidase; NAPA, *N*-ethylmaleimide-sensitive factor attachment protein; NET, norepinephrine transporter; ProT, proline transporter; SerT/SERT, serine transporter; SNAP25, synaptosomal-associated protein, 25 kDa; STX1A, syntaxin 1A; TaurT, taurine transporter

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The nerve system in metazoans plays a critical role in setting up and maintaining the overall coordination in these complicated multicellular organisms by allowing cell–cell signal transduction. In metazoan early evolution, a nerve cell system, even in a primordial form, was suggested to be one of the essential characteristics of the hypothetical ancestral animal, the Urmetazoa (Muller, 2001). In the neural system, neurons cooperate with each other and unite all the cells in the body to monitor a constant internal environment as well as to respond to an external environment (Wolpert and Szathmary, 2002; Bowers-Morrow et al., 2004). The synapse is a key functional unit between neurons or neurons and effector cells (Mattson and Bruce-Keller, 1999). It is generally accepted that neurons communicate with each other via dendritic and axonal contact to establish synaptic contacts by intimate communication between pre- and postsynaptic partners. Synaptic activity also plays an important role in these processes (Ziv and Garner, 2001; Cohen-Cory, 2002; Li and Sheng, 2003). After transmission to stimulate the target cell, neurotransmitter molecules should be removed or degraded from the synaptic cleft in time to maintain the normal function of synapse, the process of neurotransmitter inactivation. The molecular mechanism of neurotransmitter inactivation at synapse has been well characterized (Ruan and Shou, 1992; Nicholls et al., 2001). However, research of either development or evolution of the nerve system has focused mostly on the ''active'' components of synaptic transmission such as presynaptic transmitter release and postsynaptic transmitter receptors, while less has been done to characterize the role of neurotransmitter inactivation (Muller, 2001; Cohen-Cory, 2002).

Comparative studies to identify genes related to the nerve system as well as other molecular characters shared across the group of bilateria should be applied among widely divergent species within the group including descendants of the basal branches (Brooke and Holland, 2003). This includes data from Lophotrochozoa, Ecdysozoa and Deuterostomia. With the development of genomics, more eukaryotic genomes have become available. However, comparisons across metazoans were limited because the majority of sequenced animals were from Ecdysozoa and Deuterostomia. Sequencing of the transcriptome of schistosome in 2002 alleviates this problem (Hu et al., 2003), allowing a pan-bilaterian genomic comparison.

With this data and other genomic data from model animals, we performed a comparison to identify genes related to the nerve system that were conserved across metazoan and, moreover, to find molecular changes accompanied with multicellularity and early evolution of the metazoan. Using a bioinformatics approach we show here that the genes related to the mechanism of neurotransmitter inactivation in the synaptic cleft share high and stable similarity and are specifically present in the metazoan, suggesting that neurotransmitter inactivation is one of the vital functional aspects of synaptic activity as well as presynaptic transmitter release and postsynaptic transmitter receptors. By extending the concept of synaptic activity, we attempt to put forward the essential role that neurotransmitter inactivation in synaptic cleft might play in the origin of nerve system in metazoan early evolution. Besides, following the concept of ''ontogeny recapitulates phylogeny,'' we suggest the possible role of neurotransmitter inactivation as a component of activity in formation or refinement of synapses. Therefore, as a cross-point of bioinformatics and experimental neurobiology, we hope this hypothesis may be constructive to experimental neurobiologists in the field.

1. Metazoan multicellular function and neurotransmitter transporters

1.1. Genomic comparative approach and metazoan multicellular function

Multicellularity has arisen many times in eukaryotic evolution, ranging from metazoa, green plants, fungi, and several other taxa (Whittaker, 1969; Kaiser, 2001). Several studies have initiated the study of molecular mechanism of multicellularity in metazoan (Li et al., 2004; Hazkani-Covo et al., 2004). A large fraction of the *Caenorhabditis elegans* interactome networks have been mapped with several thousand proteins that may relate to multicellular functions (Li et al., 2004), but their relative contributions remain largely unknown. Since proteins with increased conservation in metazoan may indicate more importance in function, we attempted to identify genes that are highly conserved in bilateria but absent in unicellular pan eukaryotes (King, 2004). We performed a comprehensive comparison in TOGA database (Lee et al., 2001), in which we selected five animal species to find the genes conserved in metazoan and six divergent unicellular organisms in eukaryotic kingdom to exclude conserved genes related to unicellular function. Because there was no genome data available for basal metazoan such as cnidarian and sponges, in this comparison we could only screen out the genes specifically conserved in bilateria instead of the whole metazoan taxon. The five metazoan species were *Ciona intestinalis* (Urochordata), *Homo sapiens* (Vertebrata), *C. elegans* and *Drosophila melanogaster* (Ecdysozoa), *Schistosoma mansoni* (Lophotrochozoa). These five species covered most of the main branches of metazoan except the branch of Echinodermata and Hemichordata and the non-bilateria at the root of the metazoan (Giribet, 2002). The six unicellular organisms included one alga, *Chlamydomonas reinhardtii*, two fungi, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and three protozoan, *Leishmania* sp., *Tetrahymena thermophila*, and *Plasmodium falciparum*, each of which characterized a main branch in eukaryotic kingdom at the phylum level (Baldauf et al., 2000).

We started from a *Schistosoma japonicum* cDNA resource (Hu et al., 2003), the data set from which included schistosome ESTs sharing similarity at different cutoff expectation value (*E*) in mammal, fruitfly and nematode (kindly provided by Dr. Zeguang Han in CHGC). The ESTs at cutoff *E* value of 10^{-50} were interrogated to TOGA human database in TIGR (Lee et al., 2001) and obtained complete genes conserved in human. The main part of the comparison was performed in TOGA. The human genes were interrogated to the five animal databases in TOGA to get genes conserved at cutoff E value of 10^{-50} in

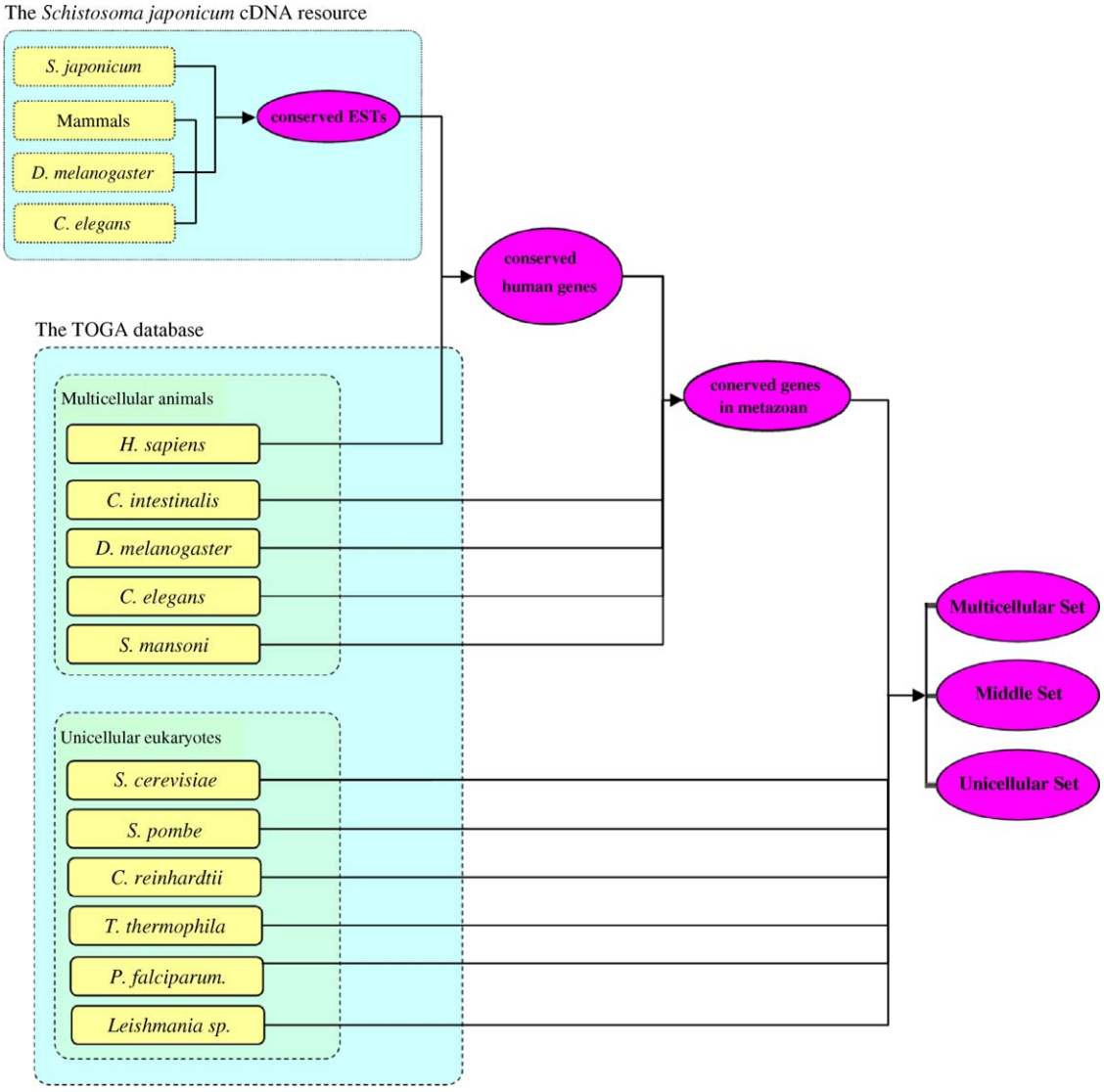

Fig. 1. The protocol of the comparison in eukaryotic organisms. The flow chart of the complete screening was shown. The two light-turquoise boxes show the two bioinformatic sources, in which every yellow box shows one organism database. The purple circles show the data obtained by each alignment. In the TOGA box, the two light-green boxes show multicellular organisms and unicellular organisms.

metazoan. This conserved gene set was compared with the six unicellular databases mentioned above. The resultant gene set was divided into three parts. The unicellular set included genes sharing similarity in all the 11 databases. The multicellular set included genes sharing no similarity at the E value of 10^{-10} in five of the six or all the six databases. The other genes fell in middle set (Fig. 1). In this study, we identified 501 human gene sequences sharing high similarity at the cutoff E value of 10^{-50} in five metazoan species, 40 genes in multicellular set, 67 genes in unicellular set and 394 genes in middle set.

1.2. Neurotransmitter transporters are strongly correlated to multicellular function in metazoan

We conducted several case studies in the multicellular set to reveal the molecular mechanisms of the metazoan multicellularity. Of the 40 genes defined in multicellular set, it was interesting that four of Na^+/Cl^--dependent neurotransmitter transporters, GAT, GlyT, DAT and NET, were screened out in multicellular set, while none of the parallel receptor was obtained. We then interrogated the human proteins of these genes into SMART (Schultz et al., 1998; Letunic et al., 2004). Resulting domain analysis showed that all the four proteins possess a SNF domain (accession No. PF00209 in SMART), which is confined to metazoan and absent from pan-unicellular species in the eukaryotic kingdom. These data suggested the strong correlation between the neurotransmitter transporter genes and the multicellular function of metazoan, which prompted us to search the TOGA and NCBI databases to check for the presence of genes related to the synaptic activity in eukaryotes.

2. Metazoan multicellular function and neurotransmitter inactivation

2.1. The remarkable phylogenic distribution of genes related to transmitter release, receptor binding and inactivation

In animals chemical neurotransmission involves five steps: synthesis, storage, release, receptor binding, and inactivation (Levintan and Kaczmarek, 1997). We compared genes from three of these processes: release, receptor binding, and inactivation. Abundant neurotransmitter molecules are removed from synaptic cleft in two ways, neurotransmitter reuptake and enzymatic metabolism. Of the classical neurotransmitters, only acetylcholine is mainly degraded via the latter, while the others are mainly inactivated by reuptake mechanism fulfilled by transmembrane transporter proteins. Therefore, we chose eight genes involved in neurotransmitter reuptake, *DAT*, *NET*, *SerT*, *GAT1*, *GlyT2*, *ProT*, *EAAT1* and *EAAT2* (Rothstein et al., 1996; Arriza et al., 1997; Zahniser and Doolen, 2001). Three genes related to enzymatic inactivation were also chosen, *AchE*, *MAO* and *COMT*. Also, we chose corresponding receptors in postsynaptic component to describe receptor binding, including ADRB1, DRD1, DRD2, GABRA1, GABBR1 and AchRA2. Another three genes, *SNAP25*, *STX1A*, *NAPA*, were chosen to represent genes related to transmitter release. The results (Table 1) indicated that the majority of genes performing neurotransmitter inactivation at synapse shared a high and conserved similarity ($E < 10^{-50}$) in selected metazoan species and were absent from pan-unicellular eukaryotes with the sporadic exception of AchE in *S. pombe* and *C. reinhardtii*. In comparison, the genes related to receptor binding were also confined to metazoan and absent from pan-unicellular eukaryotes but shared either a lower similarity (ADRB1, DRD1 and DRD2 in seven-transmembrane neurotransmitter receptor family) or a variable similarity in bilateria that keeps high similarity in some species but low similarity or even absent in some species (GABRA1, GABBR1 and AchRA2 in the ion channel receptor family). Besides, the genes related to transmitter release existed in both multicellular and the majority of unicellular organisms whose similarities in multicellular organisms were higher than that of unicellular eukaryotes. One exception was SNAP25 which specifically presented in bilateria. It appeared that the presence of all of the three steps had functional significance to synapse origin encountered in early evolution of metazoan. Therefore, the complete concept of synaptic activity should be a trinity including all the three aspects.

2.2. Transmitter inactivation should be a vital aspect of the extensive concept of synaptic activity in metazoan evolution

We proposed that transmitter inactivation might not be primarily involved in physiological defense mechanisms but rather in differentiation processes of mature synapses encountered during the construction and refinement of mature synapse. The origin and evolution of the synapse provides

Table 1
The similarity of the homologue genes in neurotransmitter inactivation

M	Species	DAT	NET	GAT	GlyT	SerT	ProT	EAAT1	EAAT2	AChE	MAO	COMT	SNAP25	STX1A	NAPA	ADRB1	DRD1	DRD2	GABRA1	GABBR1	AchRA2
#	*H. sapiens*	0	0	0	0	0	0	0	0	0	0	0	0	10^{-76}	0	0	0	0	0	0	0
#	*C. intestinalis*	10^{-91}	10^{-97}	10^{-135}	10^{-105}	10^{-131}	10^{-150}	10^{-123}	10^{-78}	10^{-47}	10^{-7}	10^{-46}	10^{-72}	10^{-76}	10^{-85}	10^{-26}	10^{-19}	10^{-19}	10^{-27}	10^{-49}	10^{-108}
#	*C. elegans*	10^{-138}	10^{-151}	10^{-117}	10^{-132}	10^{-155}	10^{-109}	10^{-99}	10^{-68}	10^{-109}	10^{-5}	$<10^{-5}$	10^{-51}	10^{-87}	10^{-76}	10^{-49}	10^{-42}	10^{-22}	10^{-71}	10^{-154}	10^{-115}
#	*D. melanogaster*	10^{-163}	10^{-167}	10^{-145}	10^{-149}	10^{-179}	10^{-129}	10^{-85}	10^{-56}	10^{-95}	$<10^{-5}$	$<10^{-5}$	10^{-60}	10^{-93}	10^{-89}	10^{-57}	10^{-47}	10^{-47}	10^{-82}	10^{-172}	10^{-117}
#	*S. mansoni*	10^{-115}	10^{-114}	10^{-122}	10^{-143}	10^{-124}	10^{-118}	10^{-52}	10^{-37}	10^{-81}	$<10^{-5}$	$<10^{-5}$	10^{-54}	10^{-72}	10^{-82}	10^{-31}	10^{-25}	10^{-26}	10^{-6}	10^{-11}	10^{-22}
	Leishmania sp.	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	10^{-5}	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
	T. thermophila	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{7}$	$<10^{19}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
	P. falciparum	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{13}$	$<10^{33}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
	S. cerevisiae	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{23}$	$<10^{38}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
	S. pombe	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	10^{-20}	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{22}$	$<10^{45}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
	C. reinhardtii	10^{-6}	$<10^{-5}$	$<10^{-5}$	10^{-5}	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	10^{-8}	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{8}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	10^{-6}	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$

The M line in the left showed the multicellular structure of these organisms. The sharp (#) meant a metazoan organism. The blank meant a unicellular organism. The phylogenic relationship of the mentioned species was shown in Fig. 2. Note that when more than one parathologs exist, only the one with highest *E* value was listed here.

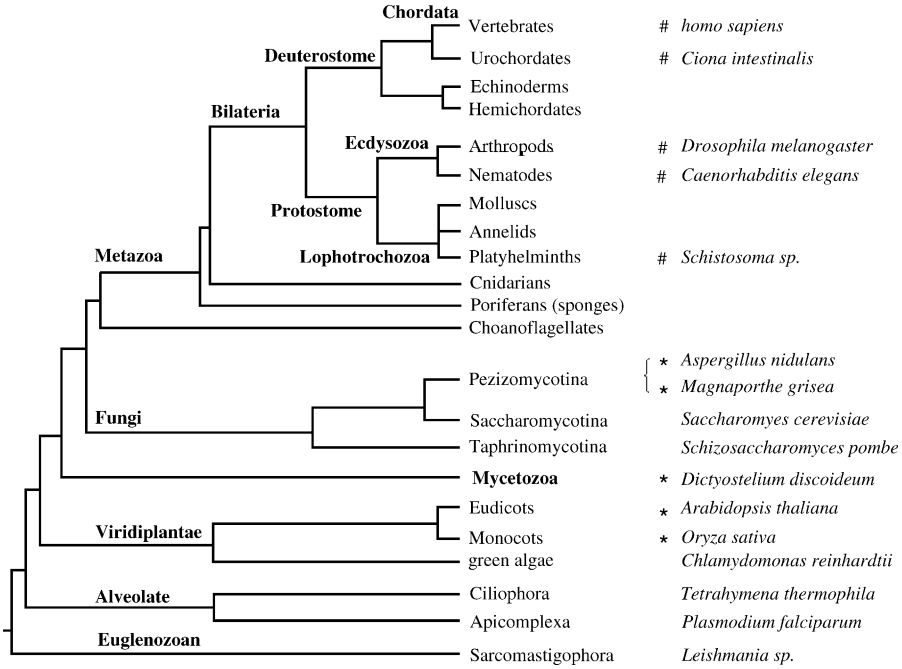

Fig. 2. The phylogenic tree of the mentioned eukaryotic organisms. The phylogenic tree of the mentioned taxa was shown with some higher taxa marked in the branch. Species names are listed on the right. Those with multicellular structure are marked with sharps (see Table 1). The branch lengths are not proportional to evolutionary distance (modified from Giribet, 2002; Baldauf et al., 2000; Hedges, 2002).

advantages for rapid intercellular communication over great distances and a high level of spatial specificity, both of which need the structural and functional aspects of synapses that set them apart from other cellular compartments (Mattson and Bruce-Keller, 1999). Therefore, transmitter inactivation must appear along with the transmitter release to ensure the origin of mature synapse performs precise transmission. One hypothesis suggests that the creation of a primordial nerve cell/receptor system is linked to the origin of the ancestral animal, the Urmetazoa (Muller, 2001). Our proposal defines transmitter inactivation as an important aspect of synaptic activity to nerve systems as well as transmitter release and receptor binding.

3. Synaptic activity and neurotransmitter inactivation in synaptogenesis

Following the view that ‘‘ontogeny recapitulates phylogeny,’’ we attempted to apply the extensive concept of synaptic activity to the development of the nerve system. This development involves a set-up of appropriate informational flow between neurons and from neurons to effector cells and requires accurately modulated synaptic activity. It is logical to deduce that the system of neurotransmitter inactivation should become functional at least before the triggering of neurotransmitter release in premature synapse. During development of inter neuronal synapses (reviewed by Cohen-Cory, 2002), the transmitter inactivation might be set up in either of the following conditions: (1) the mechanism of neurotransmitter inactivation is set up and contributes to the stage when an axon growth cone approaches and interacts dynamically with a developing dendrite through a two-way filopodial communication; (2) the mechanism is set up and participates in forming a morphologically unspecialized but functional contact; (3) the mechanism is set up accompanied with the neurotransmitter secretion at the presynaptic terminal and ensures the transmitter release inducing further synaptic differentiation.

Accumulating evidences of the developmental expression of several neurotransmitter transporters have suggested involvement in brain maturation or early synapse maturation (Hansson, 1998; Rosina et al., 1999; Kugler and Schleyer, 2004). Some indirect evidence has also indicated that some monoamine neurotransmitter transporters and AchE become operative long before synapse formation in embryogenesis in the mammal CNS (Layer, 1990; Bruning et al., 1997). However, there is a discrepancy about the role of transporters in synaptogenesis at least for glutamatergic synapses. On one side, the extent of glutamate transporter expression is intimately correlated with the formation of glutamatergic synapses (Bar-Peled, 1997) (Kugler and Schleyer, 2004). On the other side, these transporters appeared to be diffusely distributed throughout cell bodies and dendrites, which was quite unlike that of a molecule involved in synaptic termination highly concentrated at the synapse (O’Brien, 1997) (Coco, 1997). Clarification of the role of neurotransmitter inactivation in nerve system development requires additional sequence data from the root of metazoan and experimental documents in synaptogenesis. Further investigation is needed to give a possible explanation for the conserved function of neurotransmitter inactivation essential to the multicellular function of metazoan.

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References

Arriza, J.L., et al., 1997. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. Proc. Natl. Acad. Sci. U.S.A. 94, 4155–4160.

Baldauf, S.L., et al., 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290, 972–977.

Bar-Peled, O., 1997. Distribution of glutamate transporter subtypes during human brain development. J. Neurochem. 69 (6), 2571–2580.

Bowers-Morrow, V.M., et al., 2004. Comparison of molecular mechanisms mediating cell contact phenomena in model developmental systems: an exploration of universality. Biol. Rev. Camb. Philos. Soc. 79, 611–642.

Brooke, N.M., Holland, P., 2003. The evolution of multicellularity and early animal genomes. Curr. Opin. Gen. Dev. 13, 599–603.

Bruning, G., et al., 1997. Prenatal development of the serotonin transporter in mouse brain. Cell Tissue Res. 289 (2), 211–221.

Coco, S., 1997. Non-synaptic localization of the glutamate transporter EAAC1 in cultured hippocampal neurons. Eur. J. Neurosci. 9 (9), 1902–1910.

Cohen-Cory, S., 2002. The developing synapse: construction and modulation of synaptic structures and circuits. Science 298, 770–776.

Giribet, G., 2002. Current advances in the phylogenetic reconstruction of metazoan evolution: a new paradigm for the Cambrian explosion? Mol. Phylogenet. Evol. 24, 345H–357H.

Hansson, S.R., 1998. Ontogeny of vesicular monoamine transporter mRNAs VMAT1 and VMAT2. Part I. The developing rat central nervous system. Dev. Brain Res. 110 (1), 135–158.

Hazkani-Covo, E., et al., 2004. Evolution of multicellularity in metazoa: comparative analysis of the subcellular localization of proteins in *Saccharomyces*, *Drosophila* and *Caenorhabditis*. Cell Biol. Int. 28, 171–178.

Hedges, S.B., 2002. The origin and evolution of model organisms. Nat. Rev. Genet. 3, 838–849.

Hu, W., et al., 2003. Evolutionary and biomedical implications of a *Schistosoma japonicum* complementary DNA resource. Nat. genet. 35, 139–147.

Kaiser, D., 2001. Building a multicellular organism. Annu. Rev. Genet. 35, 103–123.

King, N., 2004. The unicellular ancestry of animal development. Dev. Cell (7), 313–325.

Kugler, P., Schleyer, V., 2004. Developmental expression of glutamate transporters and glutamate dehydrogenase in astrocytes of the postnatal rat hippocampus. Hippocampus 14 (8), 975–985.

Layer, P.G., 1990. Cholinesterases preceding major tracts in vertebrate neurogenesis. Bioessays 12, 415–420.

Lee, Y., et al., 2001. Cross referencing eukaryotic genomes: TIGR orthologous gene alignments (TOGA). Genome Res. 12, 493–502.

Letunic, I., et al., 2004. SMART 4.0: towards genomic data integration. Nucleic Acids Res. 32, D142–D144.

Levintan, I.B., Kaczmarek, L.K., 1997. The Neuron Cell and molecular Biology, 2nd ed. Oxford University Press Inc., New York, USA.

Li, S., et al., 2004. A map of the interactome network of the metazoan *C. elegans*. Science 303, 540–543.

Li, Z., Sheng, M., 2003. Some assembly required: the development of neuronal synapses. Nat. Rev. Mol. Cell Biol. 4, 833–841.

Mattson, M.P., Bruce-Keller, A.J., 1999. Compartmentalization of signaling in neurons: evolution and deployment. J. Neurosci. Res. 58 (1), 2–9.

Muller, W.E., 2001. Review: how was metazoan threshold crossed? The hypothetical Urmetazoa. Comp. Biochem. Physiol. Part A 129, 433–460.

Nicholls, J.B., et al., 2001. From Neuron to Brain, 4th ed. Sinauer Associates Inc., Sunderland, England.

O'Brisen, R.J., 1997. The development of excitatory synapses in cultured spinal neurons. J. Neurosci. 17 (19), 7339–7350.

Rosina, A., et al., 1999. GAT-1 developmental expression in the rat cerebellar cortex: basket and pinceau formation. Neuroreport 10 (1), 27–32.

Rothstein, J.D., et al., 1996. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16, 675–686.

Ruan, D., Shou, T., 1992. Neural Physiology. USTC Press, Hefei, China.

Schultz, J., et al., 1998. SMART, a simple modular architecture research tool: Identification of signaling domains. Proc. Natl. Acad. Sci. U.S.A. 95 (11), 5857–5864.

Whittaker, R.H., 1969. New concepts of kingdoms of organisms. Science 163, 150–160.

Wolpert, L., Szathmary, E., 2002. Evolution and the egg. Nature 420, 745.

Zahniser, N.R., Doolen, S., 2001. Chronic and acute regulation of Na^+/Cl^- dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors, and signaling systems. Pharmacol. Ther. 92, 21–55.

Ziv, N.E., Garner, C.C., 2001. Principles of glutamatergic synapse formation: seeing the forest for the trees. Curr. Opin. Neurobiol. 11, 536–543.