Synaptic Specificity Between Embryonic Motoneurons and Muscles Are Mediated by "Boolean" Combinatorial Mechanisms Among Recognition Molecules

Yoshiki Hotta

Department of Physics, Graduate School of Science
The University of Tokyo, Tokyo 113 Japan

We are exploiting refined molecular genetic techniques of Drosophila (Fruit fly: a geneticists' pet) to unravel the molecular bases of neuronal and synaptic specificity. As is described below, we succeeded in changing a neuronal circuit in a defined manner by manipulating a gene. The results revealed clearly that neuronal circuit is programmed genetically, but it is based on rather complex combinatorial interactions among cell recognition molecules. To understand the nature of the "combinatorial coding", we need a theoretical and logical formulation to extract all the possible "models" from the experimental data. Without it, current biologists have to rely on their instinct to design a model from data, without knowing how many other (and/or better) models are also possible. In this symposium, I would like to explain the experimental data and invite computer scientists and theoreticians to try opening a new paradigm in theoretical biology. I consider that such a formulation will be a general one which is also applicable to understanding complex interactions among genes, among neurons in the brain and many others. For more details and data, see Nature 374, 166-168 (1995).

Motoneuron RP3 consistently innervates muscle 6/7 in Drosophila embryos. During RP3 synaptogenesis, the cell surface glycoprotein fasciclinIII appears in both RP3 growth cone and muscles 6/7. The chemical nature of fasciclinIII and its timely presence on the RP3 and its target muscles strongly suggest its role in the "specific target recognition" mechanism. We examined if fasciclinIII is necessary and/or sufficient for the RP3 target recognition by testing the effects of deleting and misexpressing the gene. The effects are assessed by intracellular dye injection into RP3 and by immunohistochemistry. Firstly, we have found that fasciclinIII null mutant can make correct synapse between RP3 and muscle 6/7, demonstrating that fasciclinIII is not necessary for the target recognition.

There are two possibilities; fasciclinIII is either entirely irrelevant for the process, or it is playing a positive role but its absence can be compensated for by another redundant mechanism

("X"). We now have clearly demonstrated that the latter is the case. This was shown by generating transgenic flies which misexpress fasciclinIII ectopically in all the skeletal muscles during the period of synaptogenesis. The construct of the transgene has a fasciclinIII gene under the control of the myosin heavy chain gene promoter. If the former is the case, such a transgenic fly will consistently make correct synapse between RP3 and muscle 6/7. However, we observed that RP3 often innervates illegitimate (abnormal) target muscles, especially with those (e.g. muscle 15) that are close to RP3 exit site from ventral CNS. In some cases, RP3 axon reaches and synapses with muscle 13 which are normally innervated by motoneuron RP1. In contrast, RP1 innervates its correct target, muscle 13, in both fasciclinIII mutant and in fasciclinIII misexpressed transgenic flies.

The experimental data described above prove at the single identified-cell level that fasciclinIII functions as a specific "synaptic target recognition molecule". It is most likely that the homophilic fasciclinIII expression at right time on both RP3 and muscle 6/7 provides a sufficient condition for synaptogenesis. Our data can be taken as a proof that fasciclinIII ("F") is an authentic target recognition molecule. Its function, however, is redundant with another unknown recognition molecule ("X") that is also sufficient to make correct RP3: muscle 6/7 synapse. The two works in "OR" manner ("F + X"), so that absence of either one does not cause any abnormality in the synaptogenesis. In order to find "X" gene, we should look for mutants in which the RP3 target recognition is disrupted under the background of fasciclinIII null mutation. However, it may not be possible when "X" is again made up of multiple redundant "OR" mechanism ("X"="X1 + X2").

A question still remains about the behavior of RP1. RP1 also expresses fasciclinIII in normal embryos, but it can synapse consistently with muscle 13 under all the circumstances described above. In normal embryos, RP1 growth cone touches muscle 6/7 about 30 minutes before RP3 axon arrives. When RP1 axon passes by muscle 6/7, the two muscles are not mature enough so that they are not yet expressing fasciclinIII. 30 minutes later, both RP3 and RP1 reach their correct target when muscle 6/7 express fasciclinIII. Therefore, the timing difference of growth cone arrival can be used as an explanation why fasciclinIII expressing RP1 never makes synapse with muscle 6/7. However, this interpretation meet some difficulties when we looked at fasciclinIII transgenic flies. Myosin gene promoter cause early enough expression of fasciclinIII in all the muscles so that RP1 growth cone has to travel through the "jungle" of fasciclinIII expressing muscle fibers. RP1 nonetheless is nonchalant to reach muscle 13 to make the correct synapse. We, therefore, have to conclude that the simple presence of fasciclinIII on both surface of motoneuron and muscle at their encounter is not sufficient for the "target recognition". They need at least one additional factor ("Y") to make fasciclinIII functional (or active). This "Y" is not present in RP3, making it non-responsive to fasciclinIII expressing non-target muscle. If we assume that the homophilic fasciclinIII recognition property to be symmetrical (same set of factors functioning both in motoneuron and muscle), the "Y" must exist in all the muscles including 13, since RP3 can make synapse with most of them as long as muscles misexpress fasciclinIII. In this sense, fasciclinIII ("F") and "Y" act in an "AND" manner ("F x Y"). We can further infer that there is another factor ("Z") both in RP1 and in muscle 13 to ensure target recognition between them. "Z" may be absent in other muscles to make 100mechanism. By combining all the inference, the neuromuscular synapse specificity mechanism can be represented either as

"X + F x Y + Z" or (F + X) x Y + Z.

This discussion is based on my instinctive inference. Indeed, there are other equally plausible models. To deduce all the possible models, I propose to represent each model by a Boolean equation, and count all the possible equations that fit well with the experimental data. In my talk, I would like to ask the theoretically minded biologists to consider better ways of dealing with such "complex system". At the same time, I wish to point out important requirements such theory should fulfill in order to be useful in guiding future experiments.