博士論文（要約）

Calmodulin-binding transcription factor shapes the male courtship song in *Drosophila*

（雄の求愛歌の特性決定に関与する *croaker* 遺伝子の同定とその機能の解析）

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Introduction

Many animals utilize acoustic signals or songs to attract mates. During courtship, *Drosophila melanogaster* males vibrate a wing to produce characteristic sounds called pulse and sine song, respectively. However, genetic and neural mechanisms regulating courtship songs have not been fully elucidated. Males of the *Drosophila melanogaster* mutant *croaker* (*cro*) generate a polycyclic pulse song dissimilar to a monocyclic song typical of wild-type males during courtship. However, *cro* has not been molecularly mapped to any gene in the genome. In this study, I molecularly and functionally characterized the *cro* locus to understand how the nervous system shapes the male courtship song.

Results

In this study, I demonstrated that *cro* is a mutation in the gene encoding the Calmodulin-binding transcription activator (*Camta*). A previous study mapped *cro* to the chromosomal location 45DE on the 2nd chromosome. Based on this knowledge, I performed genetic complementation tests with chromosomal deficiencies located around this cytological region. Some of the tested deficiencies produced the *cro* like phenotype when placed in trans to *cro*. The deficiency mapping data placed the *cro* locus between the cytological positions 45D8 and 45E3. In this cytological interval, three genes have been annotated, i.e., bruchpilot (*brp*), *Wnt2* and *Camta*. To narrow down the gene responsible for the *cro* phenotype, I carried out a rescue experiment by introducing an intact *cro* locus into the mutant chromosome with a BAC clone (BAC-CH321-22B08) that contains the entire *Camta* locus, but neither *brp* nor *Wnt2* locus. The BAC clone rescued the *cro* mutant phenotype, suggesting that *cro* is allelic to *Camta*.

Since *cro* is a P-element insertion mutant, I next determined the P-element insertion point by two independent methods: plasmid rescue and inverse PCR. Both methods suggested that the *cro* mutant chromosome has a P-element insertion in

the fourth intron of the *Camta* gene. To further validate this result, I collected several available fly lines having a transposon insertion in the *Camta* locus to conduct genetic complementation tests with *cro*. Indeed, some of the chromosomes carrying a transposon insertion (e.g., $MiMIC^{MI04570}$) failed to rescue the *cro* phenotype, indicating that the *cro* phenotype is rescued from deficits of *Camta*. This notion is supported by the qPCR experiment that showed that the Camta mRNA amount is significantly reduced in the *cro* mutant.

I performed a knockdown experiment with *UAS-Camta RNAi* using several brain cell specific GAL4 drivers. Two pan-neural GAL4 (*elav-GAL4* and *nSyb-GAL4*) induced polycyclic songs typical of *cro* mutants whereas Glia specific *repo-GAL4* did not when used to drive *UAS-Camta RNAi*, indicating the role of *Camta* in neurons. To further narrow down the neural subset responsible for the *cro* phenotype, I performed *cro* knockdown in *fru-GAL4* or *dsx-GAL4* positive cells, because the core portion of the male courtship circuit is composed of *fru* and/or *dsx* expressing neurons. *fru-GAL4*-driven *Camta RNAi* expression induced polycyclic songs, indicating that *fru*[+] neurons are involved in inducing the *cro* phenotype. Cells positive for *dsx*-GAL4 had no effect on pulse song upon knocking down of *Camta*.

It has been postulated that courtship decision-making is made in the brain, whereas the motor pattern generator for courtship song resides in the ventral nerve cord (VNC). To determine where in the central nervous system (CNS) *cro* is required for normal song generation, I performed head specific knockdown of Camta in *fru*[+] cells with Otd^{FLP}, which releases GAL4 from GAL80-mediated repression by flipping the *STOP* sequence out from *tub>GAL80>* only in the head. Knockdown of Camta in *fru*[+] neurons in the head induced polycyclic pulse song, indicating their role in pulse song generation.

Taking into account the observation that the head-restricted *cro* knockdown in *fru-GAL4*-expressing neurons induced polycyclic pulse song, I tested whether *cro* knockdown in a few *fru*-expressing brain neurons known to have a pivotal

role in courtship song generation can induce polycyclic pulse song. I first examined pIP10 neurons, which represent laterally paired descending interneurons with the command fiber-like function to initiate male courtship upon artificial activation. These interneurons have been reported to trigger pulse song generation when forcibly activated. Notably, *cro* knockdown specifically targeted to pIP10 neurons by the *VT40556-GAL4* did not induce polycyclic pulse song during male courtship toward a female. Next, I examined the effect of *cro* knockdown in mcALa neurons, which have been implicated in the ordering of courtship elementary actions. Interestingly, the mcALa-specific intersectional combination *p52a-GAL4* and fru^{FLP} led to the generation of polycyclic pulse song, suggesting a new role for mcALa, i.e., shaping pulse song. In contrast, P1-targetted *cro* knockdown by the intersection of *NP2631-GAL4* and fru^{FLP} did not induce polycyclic pulse song during male courtship.

To decipher whether *cro* function is required for shaping pulse song during courtship actions or in the development of the courtship song circuit, I also conducted a stage-restricted *cro* knockdown experiment. Knockdown of *Camta* in developmental stages resulted in *cro* phenotype indicating its role in shaping the song circuit in developmental stages.

Conclusion

I demonstrated that the *cro* chromosome carries a P-element insertion in the fourth intron of the *Camta* locus, which decreases the amount of Camta mRNA in the cro mutant. *Camta* knockdown in a defined set of cells suggested that *cro* acts in *fru*[+] mcALa neurons in the brain to shape monocyclic pulse song. This finding points to the role of brain in pulse song shaping, which has been ascribed to the motor pattern generator for courtship song locating in the mesothoracic segment of the ventral nerve cord (e.g., the TN1 cluster). It would be thus interesting to examine the neural circuit mechanism whereby mcALa controls the action of the song pattern generator in the VNC to generate

monocyclic songs. Since *cro* has been implicated in the generation of the species difference in courtship songs between *D.sechellia* and *D.simulans*, it would also be interesting to examine an evolutionary divergence in the *cro* locus as a substrate of different courtship songs among species. This possibility can be tested by the experiment, where the entire Camta locus of one species replaces the counterpart in the other species to see whether this *cro* gene exchange is accompanied by the exchange of song characteristics.