



Knockdown of the corazonin gene reveals its critical role in the control of gregarious characteristics in the desert locust



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ABSTRACT

The two plague locusts, *Schistocerca gregaria* and *Locusta migratoria*, exhibit density-dependent phase polyphenism. Nymphs occurring at low population densities (solitary forms) are uniformly colored and match their body color to the background color of their habitat, whereas those occurring at high population densities (gregarious) develop black patterns. An injection of the neuropeptide, corazonin (Crz) has been shown to induce black patterns in locusts and affect the classical morphometric ratio, F/C (F, hind femur length; C, maximum head width). We herein identified and cloned the CRZ genes from *S. gregaria* (SgCRZ) and *L. migratoria*. A comparative analysis of prepro-Crz sequences among insects showed that the functional peptide was well conserved; its conservation was limited to the peptide region. Silencing of the identified SgCRZ gene in gregarious *S. gregaria* nymphs markedly lightened their body color and shifted the adult F/C ratio toward the value typical of solitary forms. In addition, knockdown of the gene in solitary nymphs strongly inhibited darkening even after a transfer to crowded conditions; however, these individuals developed black patterns after being injected with the Crz as a rescue treatment. SgCRZ was constitutively expressed in the brains of *S. gregaria* during nymphal development in both phases. This gene was highly expressed not only in the brain in both phases, but also in the corpora allata in the gregarious phase. This conspicuous phase-dependent difference in SgCRZ gene expression may indicate a functional role in the control of phase polyphenism in this locust.

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1. Introduction

Many organisms have evolved phenotypic plasticity in morphology and behavior as adaptations to environmental changes and heterogeneity. In response to changes in population density, the desert locust, *Schistocerca gregaria*, and the migratory locust, *Locusta migratoria*, show phenotypic plasticity in behavior, physiology, and morphology, and this is referred to as density-dependent phase polyphenism (Uvarov, 1966; Pener and Yerushalmi, 1998; Pener and Simpson, 2009). Locusts that occur at low population densities are sedentary and avoid contact with other individuals, and are called the solitary phase (Uvarov, 1977). Solitary nymphs have been shown to develop various body colors

depending on the surrounding background (Faure, 1932; Tanaka et al., 2012). Locusts that occur at high population densities are attracted to one another and form aggregations, and are called the gregarious phase. Gregarious nymphs develop black patterns with a yellow or orange background in *S. gregaria* and with a dirty-orange background in *L. migratoria*. Intermediate forms between the two phases also occur at intermediate population densities or during a transition from one phase to another, and are called the transient phase. Adults in the different phases also exhibit different morphometric traits. For example, the classical morphometric ratio, F/C (F=length of the hind femur, C = maximum width of the head), which is often used to separate the different phases, was previously reported to be smaller in gregarious adults than in solitary adults (Dirsh, 1951; Tanaka et al., 2002a).

The underlying mechanisms that control locust phase polyphenism have attracted much attention. The functional roles of several biogenic amines have been suggested for *S. gregaria* and

Abbreviations: Crz, corazonin peptide; CRZ, Crz precursor-encoding gene; dsCRZ, double-stranded RNA for CRZ; qRT-PCR, quantitative reverse transcription-PCR.

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L. migratoria (Anstey et al., 2009; Guo et al., 2013; Ma et al., 2015). The green body color of these locusts is known to be induced by a juvenile hormone (Pener, 1991). The dark body color characteristic of gregarious forms was shown to be induced by a hormonal factor in the brain and corpora cardiaca (CC) in *L. migratoria* and *S. gregaria* nymphs (Tanaka, 1993; Tanaka and Pener, 1994; Tanaka and Yagi, 1997), which was identified later as [His⁷]-corazonin (Tawfik et al., 1999). Light-colored solitary nymphs developed black patterns after an injection of this neuropeptide in both *S. gregaria* (Tawfik et al., 1999) and *L. migratoria* (Tanaka, 2000b). [His⁷]-corazonin also influences the phase-dependent formation of antennal sensilla and morphometric traits in these locusts; an injection of the peptide into solitary nymphs caused them to shift the number of sensilla and the ratio toward the values typical for the gregarious locusts (Hoste et al., 2002; Tanaka et al., 2002a; Maeno et al., 2004; Yamamoto-Kihara et al., 2004).

Crz was originally isolated from the cockroach *Periplaneta americana* as a cardioacceleratory peptide composed of eleven amino acids with a C-terminal amidated asparagine (Veenstra, 1989). Crz appears to be present in the brain and CC of many insects including crickets, stink bugs, silverfish, bees, moths, locusts, and flies, indicating that this neuropeptide is a widespread insect hormone (Hua et al., 2000; Hansen et al., 2001; Roller et al., 2003; Choi et al., 2005; Verleyen et al., 2006). Using an albino bioassay in which the implantation of brains and/or CC taken from various insects into albino locusts induces a dark color in the recipients if the implants contain Crz, a total of 18 insect orders were found to exhibit Crz activity, but no corazonin activity was detected in any of the coleopteran species tested (Tanaka, 2006). However, modulation of the heartbeat by Crz has only been observed in *P. americana* and the blood-feeding bug, *Rhodnius prolixus* (Veenstra, 1991; Hua et al., 2000; Patel et al., 2014).

The diverse physiological functions of Crz have been suggested for different arthropod species. For example, an injection of Crz into *Bombyx mori* larvae reduced the spinning rate and prolonged pupal development (Tanaka et al., 2002b). This peptide was shown to be involved in the initiation of ecdysis behaviors in the moth, *Manduca sexta*, (Kim et al., 2004). In the prawn, *Macrobrachium rosenbergii*, Crz affected testicular development, spermatogenesis, and androgen gland size (Siangcham et al., 2013). In contrast, the Crz signaling pathway is considered to be absent in coleopteran insects because the peptide and its coding gene were not found and its biological activity and immunoreactivity were undetectable in any of the species examined (Tanaka, 2000a, 2006; Roller et al., 2003; Gäde et al., 2008; Li et al., 2008). Accordingly, arthropods are not likely to have conserved the same function of the Crz pathway.

Crz is generated from the corazonin precursor, prepro-Crz, which can be divided into three parts, namely, a signal peptide, Crz, and the precursor-related peptide (CPRP) (Hansen et al., 2001; Boerjan et al., 2010). The signal peptide would target prepro-Crz to the endoplasmic reticulum, where the precursor is cleaved into the signal peptide and the other, pro-Crz. The CPRP is then removed from the pro-Crz, and the mature Crz is produced. The Crz gene has only been identified in a few species that include the fruit fly, *Drosophila melanogaster* and the great waxmoth, *Galleria mellonella* (Hansen et al., 2001; Choi et al., 2005). Although the importance of Crz in the control of locust phase polyphenism has been suggested (Tawfik et al., 1999; Boerjan et al., 2010), the gene encoding this peptide has not yet been identified in locusts. Therefore, the molecular mechanism of the Crz signaling pathway remains largely unknown.

In the present study, we identified the Crz precursor-encoding gene for *S. gregaria*, and *L. migratoria*; these genes were hereafter referred to as SgCRZ and LmCRZ, respectively. We herein examined the role of the SgCRZ gene in the control of body color and

morphometric traits in *S. gregaria*. The expression of the SgCRZ gene was also compared between gregarious and solitary desert locusts.

2. Results and discussion

2.1. Isolation of cDNAs coding for prepro-Crz in *S. gregaria* and *L. migratoria*

By searching sequencing data from the transcriptome of *S. gregaria* and genome of *L. migratoria*, we identified partial cDNA sequences for SgCrz and LmCrz, and carried out a 5' rapid amplification of cDNA ends (RACE) analysis using cDNA libraries from *S. gregaria* and *L. migratoria* to determine the translation initiation codons of the genes. The 3' end of the LmCRZ gene was determined by a 3'RACE analysis and full-length SgCRZ and LmCRZ ORFs were then amplified and sequenced as described in the Section 3. The full coding sequences of prepro-SgCrz and -LmCrz yielded polypeptides of 133 and 127 aa with calculated molecular masses of 14 kDa and 13 kDa, respectively.

The deduced gene products of SgCRZ and LmCRZ were in a complete agreement with the sequence of [His⁷]-corazonin that has been detected in both locust species by the mass spectrometric analysis, instead of other homologs such as [Arg⁷]- or [Thr⁴]-corazonin (Fig. 1) (Hua et al., 2000; Baggerman et al., 2001). As reported previously for the CRZ gene of other insects (Hansen et al., 2001; Verleyen et al., 2006), prepro-SgCrz and -LmCrz both had the signal peptide at the N-terminus, which may be associated with transmembrane transport and export into the hemolymph. Sequence comparisons showed that the amino acid sequence of prepro-SgCrz exhibited 70% and 24% identities to prepro-LmCrz and -BmCrz, respectively. The corazonin-precursor-related peptide (CPRP) sequence, which is coded after the corazonin peptide, shared 69% identity with the locust prepro-corazonins. Prepro-SgCrz and -LmCrz both conserved a glycine residue that corresponded to the substrates for amidation, followed by Arg-Lys-Arg residues that were presumably cleaved by endopeptidases (Docherty and Steiner, 1982; Hook et al., 1994; Hansen et al., 2001).

	Signal peptide	Corazonin ▼	
Sg	1 MMRPWVSVVLLLVACWCLGAL-VHCQTFQYSHGWTNGR	KKAGSSS-----APG	47
Lm	1 MLRPWVSVALLAVACWVLSAVVWSGQTFQYSHGWTNGR	KKAGS-----G	44
Bm	1 -MVTNITLILTLMLTAS----VTAQTFQYSRGWTNG- KK DGH	----- 33	
Gm	1 -MATNITMFLIVITLTS----VAAQTFQYSRGWTNG- KK DGH	----- 33	
Am	1 -MVNSQILILFILSLTIT---IVMCQTFYSHGWTNG- KK STS	----- 35	
Dm	1 -MLRLLLPFLFLFLLSM----CMGQTFQYSRGWTNG RR SFNAASPLLANGHLH	48	
Sg	48 ALLPPGRLPPPAAS--DMDAQPCRVRLRLLQLGGAVPQLYVPELWQQVDE	98	
Lm	45 ALVPAARLPLPAAADLEGGQQTQPCRVRLRLLQLAAALPQVYVPEAWQLEE	94	
Bm	34 -----KRDELREDEV--LERILTPCQLDKLVYLEGKPLNDRFLVPCDY--IEE	80	
Gm	34 -----KTEDIRDLTNNLERILSPQMNKLVYLEGKPLNERLLGPCDTSKTRS	84	
Am	36 -----LEELANRNAIQSDNVFANCELQKRLRLQLGNNINQLFQTPCELLNFPK	86	
Dm	49 RASELGLTDLYLDQDSSDRRLERELCSQLQRSLIARNCVPGSDFNANRVDPPE	102	
Sg	99 EGDNMAARQGGGARLRHALPPPGAAAADSDSDM-----	133	
Lm	95 E----SASQGGVRLRHALPPPGAAA--DSDSDM-----	127	
Bm	81 EVNQPKRYKGERNHLEDFVFQ-----	101	
Gm	85 TTNPSDNTSAVKTPCSTHFNKHCYSFSY-----	113	
Am	87 RFSFSENMINDHRQPAPTNNNY-----	107	
Dm	103 NSAHPRLSNSNGENVLYSSANI PNRRHQSNELLELSAAGGASAEPNVFGKH	154	

Fig. 1. Alignment of amino acid sequences of insect Crz precursors. The amino acid sequences of prepro-SgCrz and -LmCrz were aligned together with the sequences of prepro-BmCrz (BAC66443), -GmCrz (AAF87082), -AmCrz (NP_001012981.1), and -DmCrz (CG3302) for comparison. The prepro-Crzs included a putative signal peptide at the N-terminus. The Crz sequences are highlighted in light gray; putative cleavage sites (Lys-Arg) are shaded in black. The arrowhead indicates a glycine residue corresponding to the substrates for amidation. Abbreviations: Sg, *Schistocerca gregaria*; Lm, *Locusta migratoria*; Bm, *Bombyx mori*; Gm, *Galleria mellonella*; Am, *Apis mellifera*; Dm, *Drosophila melanogaster*.

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