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X-linkage of a vitellogenin gene in *Locusta migratoria*

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Abstract. Hybridization of a cloned DNA probe to blots of restriction digests of *Locusta migratoria* genomic DNA showed that a vitellogenin gene is present at one copy per haploid genome in females, and at only one-half that number in males. The genomic region encompassing the 3′-coding end of the gene is polymorphic, as revealed by blots of DNA from individual locusts. DNA from some female locusts yielded two variants in this region, whereas a series of male locusts showed one variant or the other, but never both. The results demonstrate that the vitellogenin gene, which is normally expressed only in females, is X-linked in *L. migratoria*.

Introduction

Among insects and oviparous vertebrates, vitellogenin synthesis generally occurs only in adult females as a result of developmental programming and hormonal modulation of gene activity (reviewed by Hagedorn and Kunkel 1979; Shapiro 1982). As part of an investigation of juvenile hormone regulation of vitellogenin gene expression in the fat body of the African migratory locust (Chen and Wyatt 1981; Chinzei et al. 1982; Dhadialla and Wyatt 1983), it was of interest to know the copy number and chromosomal localization of locust vitellogenin genes.

Until now, chromosomal localization of genes for vitellogenin has been established only for *Drosophila*. Protein electrophoretic variant analysis (Postlethwait and Jowett 1980) and in situ hybridization (Riddell et al. 1981) have demonstrated that the three vitellogenin genes in this insect are carried on the X chromosome. Here, we use two types of DNA blot hybridization experiments – gene copy number estimation and restriction fragment length polymorphism (RFLP) analysis – to show that a vitellogenin gene in the more primitive insect, *Locusta migratoria*, is also X-linked.

Materials and methods

Insects. The colony of *L. migratoria migratorioides*, descended from stock obtained from the Centre for Overseas Pest Research, London, has been maintained since 1973 as described by Chen et al. (1978).

DNA isolation. DNA was isolated from individual, newly emerged adult locusts by the method of Blin and Stafford (1976) with modifications. A locust was pulverized under liquid nitrogen, suspended in 100 mM EDTA pH 8.0, 0.5% n-lauroylsarcosine, 200 μg/ml proteinase K, and the mixture was incubated at 50° C for 3 h. After two phenol:chloroform:isoamyl alcohol (25:24:1) extractions, crude DNA was precipitated from the aqueous phase with 95% ethanol. The precipitate was dissolved in SSC and digested with ribonuclease A (100 μg/ml) for 1 h at 37° C. SDS was added to 0.5%, and the solution was incubated with 100 μg/ml proteinase K at 50° C for 1 h. After three additional phenol: chloroform:iso-amyl alcohol extractions, DNA was precipitated, dissolved in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, and dialyzed extensively against the same buffer. A typical yield was about 2 mg per locust.

Blot hybridization. The probe used in these studies, pLmVg144-EH4.6 (hereafter referred to as p 4.6), was a subclone from λLmVg144, a clone selected for vitellogenin gene sequences from an *L. migratoria* λ Charon 4 genomic library (Wyatt et al. 1981). The p 4.6 probe represents ~3.4 kb of the 3′-terminal coding region for vitellogenin mRNA along with 1.2 kb of 3′ flanking sequence. Details of construction and characterization of p 4.6 will be presented elsewhere (J. Locke, B.N. White, G.R. Wyatt, in preparation). Figure 1 shows relevant restriction sites in the genomic region encompassing p 4.6.

The p 4.6 probe was labeled by nick-translation (Rigby et al. 1977) with α-^{32}P-dCTP, (7,400 Ci/mmol; New England Nuclear, Boston, MA). Resulting specific activity was $\sim 8 \times 10^8$ cpm/μg, and the single-strand size of the labeled fragments was 300–1,000 nucleotides.

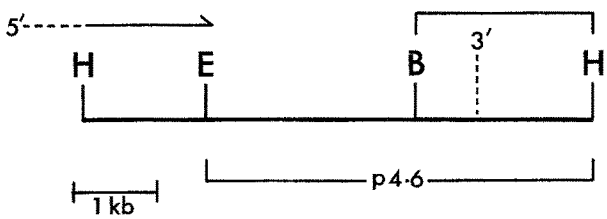

Fig. 1. Restriction map of the genomic region surrounding the 3′ end of the vitellogenin gene in *L. migratoria*. The *arrow* shows the direction of transcription, the 5′ end of the gene lying beyond the map. The *bracket* below the map delineates the sequence represented in probe p 4.6 (pLmVg144-EH4.6). Restriction sites are H: Hind III, E: Eco RI, B: Bam HI. The *bracket* above the map indicates the Bam HI/Hind III fragment showing length polymorphism (see Figs. 3, 4)

Restriction enzymes were purchased from Bethesda Research Laboratories (Gaithersburg, MD) and used as recommended by the supplier. Samples of digested DNA (15 µg each) were applied to 0.8% agarose gels and subjected to electrophoresis at 40 V overnight in 40 mM Tris-acetate pH 7.8, 1 mM EDTA. Restriction fragments were transferred to diazobenzyloxymethyl paper as described by Wahl et al. (1979). Transfers were hybridized for 20–24 h at 42° C with $\sim 10^7$ cpm/ml (15 ng/ml) labeled p 4.6 in 50% formamide, 5 × SSC, 50 mM phosphate buffer pH 6.5, 2 × Denhardt's solution (Denhardt 1966), 50 µg/ml denatured heterologous DNA. The papers were washed at 32° C in 50% formamide, 2 × SSC, at room temperature in 2 × SSC, and then repeatedly in 0.1 × SSC, 0.1% SDS at 55° C. Autoradiography was for 5 days at −70° C with pre-flashed Dupont Cronex 4 film and Lightning Plus Intensifying screens.

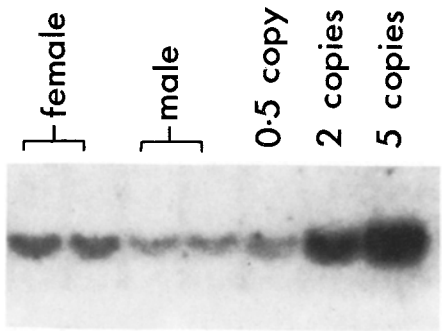

Fig. 2. Copy-number estimation for the vitellogenin gene in *L. migratoria*. Samples of DNA (15 µg each) from individual locusts were digested with Eco RI/Hind III (see Fig. 1) and probed with p 4.6. The three lanes on the right contain a cloned vitellogenin gene fragment co-digested and electrophoresed with 15 µg human placental DNA. The amounts of the cloned fragment in these lanes were based on a haploid genome size of 6.5 pg

Results

Copy number in males and females

For estimation of vitellogenin gene-copy number in male and female locusts, 15 µg samples of DNA were digested with Eco RI/Hind III, separated by electrophoresis, and probed with p 4.6 (Fig. 2). As quantitative reference standards, three samples were included containing DNA of the parent λ genomic clone, λLmVg144, co-digested and electrophoresed with 15 µg human placental DNA. The amounts of the cloned fragment in these lanes, corresponding to 0.5, 2, and 5 copies per haploid genome, were calculated as follows. The haploid genome size of *L. migratoria* is 6.5 pg (Fox 1970; Rees et al. 1978), or 6.5×10^9 base pairs, and λLmVg144 is 4.1×10^4 base pairs. Therefore, 1 copy of the vitellogenin gene per haploid genome in 15 µg genomic DNA is represented by $15 \times (4.1 \times 10^4 / 6.5 \times 10^9) = 9.5 \times 10^{-5}$ µg or 95 pg λLmVg144. The intensity of signal in the lanes containing DNA of males closely corresponds to that for 0.5 copy per genome, while the signal for females is about twice that, or 1 copy per genome. Since *L. migratoria* has the XX female – XO male sex chromosome system (references in Makino 1951), we inferred from these results that the vitellogenin gene was X-linked. Corroboration was sought from an additional line of evidence.

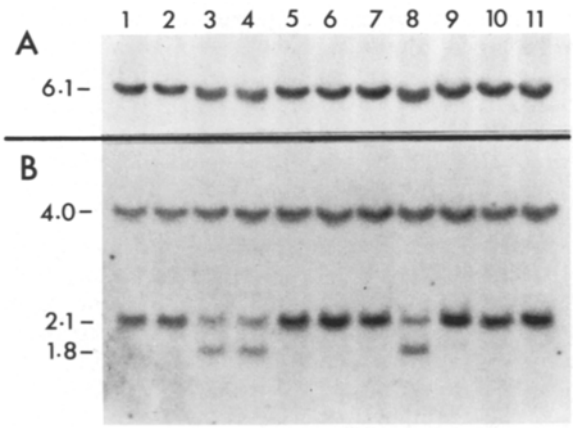

Fig. 3. Restriction fragment length polymorphism, shown by hybridization of p 4.6 to digests of individual female locust DNA samples (15 µg each). **A** Hind III digests; **B** same samples digested with Bam HI/Hind III. The scale on left indicates sizes, in kb, of the hybridizing fragments (see Fig. 1)

X-linkage confirmed by RFLP analysis

If the genomic region carrying the vitellogenin gene existed in the population in forms distinguishable by restriction fragment length, then RFLP analysis could be used to confirm X-linkage of the gene. Accordingly, we hybridized p 4.6 to digests of DNA from individual locusts. In the initial experiment we examined Hind III digests of DNA samples from 11 females (Fig. 3A). All of these showed a band at 6.1 kb, but in three (lanes 3, 4, 8) the band was broader, suggesting the additional presence of a slightly faster migrating fragment. In Bam HI/Hind III digests of the same DNA samples (Fig. 3B), polymorphism became quite evident by the appearance of an extra fragment at 1.8 kb in lanes 3, 4, and 8. The combined results in Figure 3 demonstrate that the sequence change giving rise to the 1.8 kb fragment occurs within the Bam HI/Hind III fragment carrying the 3′-coding end of the vitellogenin gene (see Fig. 1). Of the 22 haploid genomic sequences, obtained from randomly selected females, examined in Figure 3, only 3 carry the 1.8 kb fragment, while 19 have the larger fragment at 2.1 kb. Animals homozygous for the smaller fragment should therefore be scarce in the population, with a predicted frequency from the combined data from females and males (below) of $(10/44)^2 = 0.052$.

If the region carrying the vitellogenin gene is X-linked, individual males can have either the 2.1 kb or the 1.8 kb Bam HI/Hind III fragment, but not both. We therefore probed digests of DNA samples from 22 males. Results for 11 of these are depicted in Figure 4. Four samples (lanes 1, 2, 4, 6) have the 1.8 kb fragment only, while the remaining seven show only the 2.1 kb fragment. Among the other 11 samples from males (not shown), three had the fragment at 1.8 kb and eight the 2.1 kb fragment. In no DNA sample from a male were fragments of 2.1 and 1.8 kb present together. These results show that the vitellogenin gene is X-linked in *L. migratoria*.

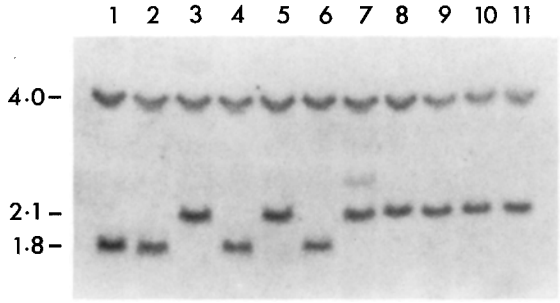

Fig. 4. Hybridization of p 4.6 to individual male *L. migratoria* DNA samples. Compare with Figure 3B as to distribution of the hybridizing fragments at 2.1 and 1.8 kb. The faint band at about 3 kb in lane 7 is probably due to a trace of plasmid contamination

Discussion

RFLP analysis is probably best known as a potential tool in prenatal diagnoses of inherited human disease (Kan and Dozy 1978; Botstein et al. 1980). Here we use this procedure, together with sequence copy number estimation, to show that a vitellogenin gene is X-linked in *Locusta migratoria*. Controlled mating experiments, to support this conclusion further, would be laborious and time-consuming with this species. The mode of inheritance of unique DNA sequences can be determined for many organisms using RFLP analysis, provided only the chromosome system is known.

At least two coordinately expressed genes for vitellogenin exist in *L. migratoria* (Chen et al. 1978; Chen 1980; Locke and Wyatt, unpublished results). The X chromosome location of the gene examined here is fortunate since, when suitable probes become available, further blot hybridization experiments will allow us to determine if the vitellogenin genes are linked in this insect. Analyses of translation products suggest that the vitellogenin genes in most insects have a common ancestral origin (Harnish and White 1982). On the basis of the observations that the vitellogenin genes of *Drosophila* (Postlethwait and Jowett 1980; Riddell et al. 1981) and *Locusta*, two insects separated by considerable evolutionary distance, are both X-linked, one may expect that X-linkage of vitellogenin genes is widespread among insects.

While larval fat body from both sexes of *L. migratoria* can be stimulated by a juvenile hormone analog to synthesize vitellogenin, after metamorphosis vitellogenin production is limited strictly to females (Dhadialla and Wyatt 1983), even though adult males exhibit comparable circulating levels of juvenile hormone (Johnson and Hill 1973) and specific responses to the hormone by fat body cells (e.g. DNA replication; Nair et al. 1981). The sex-limited differentiation during metamorphosis leading to female-specific vitellogenesis is due to differences intrinsic to fat body cells, evoked by hormonal signals common to both sexes (Dhadialla and Wyatt 1983), and is thus attributable to fat body genotype. It is generally considered that sexual phenotype in animals with the XX, XO system is due to the autosome/X ratio, with male-determining factors on the autosomes and female determinants on the X chromosome (White 1973). It is reasonable to suppose that some of these factors operate in locust fat body cells in determination of sexual phenotype with respect to vitellogenesis. The two-fold dosage difference of the vitellogenin gene observed here between the sexes of *L. migratoria* may facilitate the required level of gene expression.

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Note added in proof

Recent experiments with a cloned genomic DNA probe for a second locust vitellogenin gene (gene B) indicate one copy per female and 0.5 copy per male haploid genome. This complements the results for gene A, reported above, to show that two vitellogenin genes are linked on the X chromosome. In a recent publication, James et al. (1982) have reported 1–2 copies per female haploid genome, on the basis of liquid hybridization with a cloned *L. migratoria* cDNA probe.