

Insect Vitellogenesis in Juvenile Hormone-dependent Species

X. Bellés, M.D. Piulachs, J.L. Maestro and D. Martín

*Department of Physiology and Molecular Biodiversity
Institut de Biologia Molecular de Barcelona (CSIC), Barcelona, Spain*

Summary

In most insects, vitellogenesis is regulated by juvenile hormone (JH). The recent cloning of the vitellogenin gene in a number of JH-dependent species has allowed the study of the induction of vitellogenin production by JH at a molecular scale. Potential JH response elements have been described in the regulatory regions of vitellogenin and other JH-inducible genes. These elements may not only shed some light on the early molecular mechanisms of JH action, but also may help to characterise the JH receptor, which is the major goal in this research field.

Endocrine Regulation of Vitellogenesis in Insects

The vitellogenic role of juvenile hormone (JH) in insects was first reported by V.B. Wigglesworth on the blood-sucking bug *Rhodnius prolixus* in the nineteen-thirties. Removal of the corpora allata, the source of JH, followed by replacement therapy and monitoring of oocyte growth, led to description of the vitellogenic role of JH in other insect species. The study of insect vitellogenesis took a further step in 1975, when Hagedorn and associates showed that mosquito vitellogenesis is regulated by ecdysteroids, instead of JH (see Dhadialla and Raikhel, 1994). Although it is not easy to establish clear-cut generalities concerning the endocrine regulation of vitellogenesis in insects, it seems that in less specialized groups, e.g. dictyopterans, vitellogenesis is governed mainly by JH, whereas in more specialized ones, e.g. dipterans, it is regulated mainly by ecdysteroids.

Classical physiological data on the endocrinology of vitellogenesis in JH-dependent species have been provided by Wyatt and Davey (1996).

More recently, the cloning of vitellogenin genes or cDNAs has fostered expression studies, including mRNA monitoring, which have revealed good correlations between the pattern of JH production and that of vitellogenin mRNA, supporting the cause-effect relationship between JH and vitellogenesis in JH-dependent species.

Induction of Vitellogenin Gene Expression by Juvenile Hormone

In the African migratory locust, *Locusta migratoria*, JH and JH analogues (e.g. pyriproxyfen and methoprene) induce the transcription of vitellogenin genes *in vivo* with a lag time of 12–24 h (Glinka and Wyatt, 1996). This lag period can be shortened by prior administration of a dose of JH or JH analogue that is insufficient by itself to induce vitellogenin transcription, but primes fat body cells to an accelerated response to a subsequent effective dose (Wyatt *et al.*, 1996). On the other hand, the lag time can be extended by temporary inhibition of protein synthesis with cycloheximide (Edwards *et al.*, 1993). The results of these experiments carried out *in vivo* suggest that the effect of JH on vitellogenin genes requires the prior synthesis of protein factors involved in transcription.

In the German cockroach, *Blattella germanica*, the cloning of a vitellogenin cDNA (Martín *et al.*, 1998; Comas *et al.*, 2000) has facilitated the monitoring of vitellogenin mRNA. Experiments *in vivo* using allatectomized females have shown that vitellogenin mRNA can be detected as early as 2 h after the treatment with 1 µg of JH III, whereas vitellogenin can be detected 2 h later (Comas *et al.*, 1999). Experiments *in vitro* using periovaric fat bodies from allatectomized females and incubation periods of 7 h have revealed that a concentration as low as 0.1 nM JH III induces vitellogenin mRNA (Comas *et al.*, 2002). In addition, studies *in vitro* have shown that cycloheximide abolishes the vitellogenic effects of JH (Comas *et al.*, 2002), suggesting that the effect of JH on the vitellogenin gene involves the synthesis of protein transcription factors.

Potential Response Elements in Juvenile Hormone-dependent Genes

The characterization of the response elements of the JH-dependent genes that confer JH-inducibility may be a first step towards the study of JH action on gene transcription at molecular scale.

In *L. migratoria*, in the upstream region of the *jhp21* gene, which is inducible by JH and encodes for an ovarian-directed protein, the partially palindromic 15-nucleotide motif GAGGTTTCGAG^A/_TCCT^T/_C, was found in 3 copies at positions –1152, –1913 and –2028 from the transcription start point (Zhang and Wyatt, 1996). This motif is indicative

of a hormone response element, given that it is similar to the consensus ecdysteroid response element, as described by Jiang *et al.* (2000).

In *B. germanica*, within the 1200 bp sequenced upstream from the transcription start site of the vitellogenin gene (unpublished), there are a number of motifs reminiscent of hormone response elements, e.g., the sequence GTGTCAATGAACT at position -776, which fits the consensus of the ecdysteroid response element.

Molecular Mechanisms in Juvenile Hormone Action

The motifs described in the previous section remain putative response elements, unless functional identification is afforded. In this regard, progress has been made for the motif GAGGTTTCGAG^A/_TCCT^T/_C, of the *jhp21* gene of *L. migratoria*. The relevance of this sequence in JH regulation of the *jhp21* gene was tested in a cell-free transcription system. Transcription from the *jhp21* promoter was observed with nuclear extracts from JH-exposed, but not from JH-deprived, fat body. Then, using truncated constructs of the promoter region of the *jhp21* gene, it was shown that specific transcription was reduced by deletion of the region containing this sequence, and restored by addition of two tandem copies of it (Zhang *et al.*, 1996). In addition, electrophoretic band-shift experiments with fat body nuclear extracts have revealed a protein that specifically binds to the sequence GAGGTTTCGAG^A/_TCCT^T/_C (Zhang *et al.*, 1996). Finally, Zhou *et al.* (2002) have described that this binding shows a preference for the inverted repeat GAGGTTTC in the left half-site and that it is abolished by phosphorylation catalyzed by a C-type protein kinase present in the nuclear extracts. These results suggest that the sequence GAGGTTTCGAG^A/_TCCT^T/_C is a JH response element, and that the protein that binds to it is a transcription factor brought to an active state by JH.

Juvenile Hormone Receptor Urgently Needed

The analogies with the ecdysteroid-regulated vitellogenin transcription in mosquitoes (Dhadialla and Raikhel, 1994) suggest that JH may use a receptor belonging to nuclear receptor superfamily. The JH receptor complex could include the ultraspiracle (USP) protein, as occurs in the ecdysone receptor complex in *Drosophila melanogaster* and other dipterans. In this regard, Jones and Sharp (1997) reported that JH binds to *D. melanogaster* USP and modify its conformation. In addition, yeast two-hybrid assays indicate that JH may promote USP homodimerization. More recently, Jones *et al.* (2001) have described that recombinant USP binds to a DR12 hormone response element in gel shift assays, whereas the same DR12 element confers enhanced transcriptional JH-responsiveness to a transfected JH esterase core promoter in transfected cells.

Nevertheless, the data provided by Jones and coworkers are still inconclusive, in part because the K_d of the binding of JH III to USP is in the order of 0.5 μM , while a K_d of 10^{-9}M is generally considered the upper limit for a hormone receptor, at least in vertebrates.

In any case, the hypothesis that USP may behave as a JH receptor, either as a homodimer or as the partner of a hypothetical heterodimer with a JH receptor, is still appealing. Indeed, the formation of such dimers would compete with the heterodimerization of USP with the ecdysteroid receptor, thus explaining, at least in some instances, the interaction between JH and ecdysone. In fact, USP mediates the action of JH, as shown by the production of a supernumerary larval cuticle during the final instar by *USP* mutants of *D. melanogaster*, suggesting that loss of *USP* function perturbs the balance between JH and ecdysone signaling (Hall and Thummel, 1998). Studies on USP in species depending on JH for vitellogenesis would also shed light on the involvement of this nuclear receptor in the molecular action of JH in vitellogenin gene activation. Recently, we have cloned the USP of *B. germanica* (unpublished), and the sequence analysis reveals that the ligand binding domain of it is closer to vertebrate RXR and *L. migratoria* and *Tenebrio molitor* orthologues, than to dipterans or lepidopterans USP. This raises the question of whether RXR orthologues of lower insects, where vitellogenesis is directed by JH, might be better candidates for a JH receptor role than the USP orthologues of higher insects, where vitellogenesis is mainly governed by ecdysteroids. Crystal structure analysis of the ligand binding domain of USP has been carried out on the lepidopteran *Heliothis virescens* (Billas *et al.*, 2001) and on *D. melanogaster* (Clayton *et al.*, 2001), and it would be also interesting to follow this structural studies on more primitive insect species, reproductively dependent on JH.

The *Methoprene-resistant* (*Met*) gene of *D. melanogaster* may be also a useful model for gaining insight into the molecular action of JH. The first relation between JH and *Met* was established when it was observed that *Met* mutants had higher resistance to the lethal effect of JH (or several analogues) by 100-fold when applied to the last larval instar of *D. melanogaster*. *Met* is a member of the bHLH-PAS family of proteins that behave as transcriptional regulators (Ashok *et al.*, 1998). These proteins show a basic helix-loop-helix domain (bHLH), which is characteristic of a number of transcription factors, and a PAS domain. Although the role of *Met* as a JH receptor has not been ruled out, loss of function of *Met* mutants show no serious problems during embryonic and larval development or during metamorphosis (Wilson and Ashok, 1998).

Among the methods that may allow us to elucidate the identity of the JH receptor, the identification of response elements is specially promising. In addition to data on *L. migratoria* and *B. germanica*, studies in

D. melanogaster may greatly facilitate this approach, since they allow the use of powerful genetic tools and background. However, the absence of JH-target genes in this species has deterred the development of reliable functional assays to assess the identification of the receptor. In this regard, Dubrovsky *et al.* (2000, 2002), using a differential display-screening for JH-inducible genes in a *D. melanogaster* embryonic cell line, described the occurrence of several genes: *JH-inducible (JhI)-1*, *JhI-26*, *JhI-21* and *minidisc (mnd)*, a previously identified gene. These authors clearly showed that one of these genes, *mnd*, behaves as a primary responsive gene to JH, making it a useful model for analysing its promoter region and finding response elements for the JH receptor. The occurrence of a JH target gene in *D. melanogaster* offers great potential for the establishment of genetic strategies aimed at isolating the so needed JH receptor.

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