Journal of Insect Physiology 86 (2016) 1–10

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Divergent forms of endoplasmic reticulum stress trigger a robust unfolded protein response in honey bees

Brittany A. Johnston [a,d], Katarzyna B. Hooks [b,c], Mia McKinstry [a], Jonathan W. Snow [a,*]

[a] Biology Department, Barnard College, New York, NY 10027, USA
[b] Faculty of Life Sciences, University of Manchester, Manchester, UK
[c] U1053 INSERM, Université de Bordeaux, France
[d] Biology Department, The City College of New York – CUNY, New York, NY 10031, USA

ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form 4 December 2015
Accepted 12 December 2015
Available online 14 December 2015

Keywords:
Cellular stress response
Proteostasis
Endoplasmic reticulum
Unfolded protein response
IRE1
Xbp1
Honey bee

ABSTRACT

Honey bee colonies in the United States have suffered from an increased rate of die-off in recent years, stemming from a complex set of interacting stresses that remain poorly described. While we have some understanding of the physiological stress responses in the honey bee, our molecular understanding of honey bee cellular stress responses is incomplete. Thus, we sought to identify and began functional characterization of the components of the UPR in honey bees. The IRE1-dependent splicing of the mRNA for the transcription factor *Xbp1*, leading to translation of an isoform with more transactivation potential, represents the most conserved of the UPR pathways. Honey bees and other *Apoidea* possess unique features in the *Xbp1* mRNA splice site, which we reasoned could have functional consequences for the IRE1 pathway. However, we find robust induction of target genes upon UPR stimulation. In addition, the IRE1 pathway activation, as assessed by splicing of *Xbp1* mRNA upon UPR, is conserved. By providing foundational knowledge about the UPR in the honey bee and the relative sensitivity of this species to divergent stresses, this work stands to improve our understanding of the mechanistic underpinnings of honey bee health and disease.

1. Introduction

The Western Honey Bee, *Apis mellifera* provides critical pollination services of paramount importance to humans in both agricultural (Eilers et al., 2011; Gallai et al., 2009; Klein et al., 2007) and ecological settings (Potts et al., 2010). Honey bee colonies in the United States and Europe have suffered from an increased rate of die-off in recent years. The phenomenon, called Colony Collapse Disorder, is characterized by an abrupt disappearance of adult worker bees from a honey bee colony, and likely stems from a complex set of interacting stresses that remain poorly described (Ratnieks and Carreck, 2010). Key stresses thought to be involved include nutritional stress due to loss of appropriate forage, chemical poisoning from pesticides, changes to normal living conditions brought about through large-scale beekeeping practices, and infection by insect parasites and pathogenic microbes (Oldroyd, 2007; Potts et al., 2010; vanEnglesdorp et al., 2009).

As no single cause for the recent increase in honey bee disease is evident, there is increased focus on the impact of interactions between various stressors. Many studies have found synergistic effects of pesticides and microbial infection with each other (Alaux et al., 2010; Aufauvre et al., 2012; Boncristiani et al., 2012; Doublet et al., 2015; Pettis et al., 2013; Vidau et al., 2011) or other stresses such as nutritional stress (Di Pasquale et al., 2013; Huang, 2012). A critical first step in understanding these synergies involves defining specific common cellular processes that are impacted by multiple stressors and could therefore serve as links to cellular dysfunction, tissue pathology, disease, and mortality in honey bees. The various pathways that make up cellular stress responses provide logical and compelling processes to examine for such interactions. We have some appreciation of the physiological stress responses in the honey bee (Even et al., 2012). However, our molecular understanding of honey bee cellular stress responses is incomplete.

One critical cellular stress involves problems in proteostasis, which refers to the homeostasis of protein synthesis, folding, function, and degradation both within a cell and in an organism as a

Abbreviations: ER, endoplasmic reticulum; ERAD, endoplasmic-reticulum-associated protein degradation; UPR, unfolded protein response; HSR, Heat Shock Response; IRE1, inositol requiring enzyme 1; PERK, double-stranded RNA-activated protein kinase (PKR)-like ER kinase; ATF6, activating transcription factor 6; HSC, heat shock cognate; HSP, heat shock protein; XBP1, X-box binding protein 1.

* Corresponding author.
E-mail address: jsnow@barnard.edu (J.W. Snow).

http://dx.doi.org/10.1016/j.jinsphys.2015.12.004
0022-1910/

whole (Taylor et al., 2014). A number of normal and pathologic conditions can lead to disruption of proteostasis, leading to a build-up of unfolded proteins in the cell and triggering a suite of responses designed to limit damage to the cell from problems in protein folding and return the cell to homeostasis (Taylor et al., 2014). Within individual cells, proteostasis is maintained by the responses of the proteostatic network, including the Heat Shock Response (HSR) (Morimoto, 2012; Vabulas et al., 2010), responding to proteostatic disruption in the cytoplasm, and the unfolded protein response (UPR), responding to proteostatic perturbation in the endoplasmic reticulum (ER) (Walter and Ron, 2011).

In other species, the UPR has been shown to influence cellular and organismal outcomes to exposures to the very environmental stressors suspected to play a part in recent honey bee losses, including microbial attack (Wang and Kaufman, 2012), chemical toxicity (Lafleur et al., 2013), nutritional stress (Lee and Ozcan, 2014), and physiological stress (Taylor et al., 2014). A few highly conserved pathways, characterized by unique receptors and signal transduction machinery, are responsible for sensing unfolded proteins in the ER (Fig. S1, reviewed in (Samali et al., 2010; Walter and Ron, 2011)). They include the IRE1 (inositol requiring enzyme 1)-dependent pathway, the PERK [double-stranded RNA-activated protein kinase (PKR)-like ER kinase] pathway, and the ATF6 (activating transcription factor 6) pathway. Activation of these pathways results in short-term responses designed to limit the influx of new proteins and activation of three bZIP transcription factors, X-box binding protein 1 (XBP1) by IRE1, Activating transcription factor 4 (ATF4) by PERK, and activating transcription factor 6 (ATF6) itself, which participate in a medium-term adaptive response through transcriptional upregulation of proteins involved in multiple ER processes.

The most highly conserved pathway of the UPR is the IRE1 pathway. IRE1 is a transmembrane receptor that contains both kinase and endonuclease activity that are important for its function. IRE1 is usually bound to the ER chaperone HSC70-3 and maintained in a monomeric, inactive form. Upon increase of unfolded proteins in the lumen of the ER, IRE1 is activated by loss of Hsc70-3 binding (as this molecule is sequestered by unfolded proteins) or by binding of unfolded proteins themselves. This leads to IRE1 dimerization, autophosphorylation of the kinase domain, further multimerization, and ultimately to activation of the endonuclease domain. The endonuclease participates in the activation of the UPR thru the non-canonical splicing of the mRNA encoding the bZIP transcription factor XBP1. In its unspliced form, the *Xbp1* mRNA (*Xbp1u*) encodes a truncated protein (XBP1u) with low transactivation activity. Splicing removes a short sequence containing an in-frame stop codon, leading to the translation of the new transcript (*Xbp1s*), which encodes a longer form of XBP1 (XBP1s), with robust transactivation activity that upregulates the transcription of UPR target genes. In a recent paper (Hooks and Griffiths-Jones, 2011), the non-canonical intron structures in *Xbp1* mRNA were predicted for 128 eukaryotes, including the honey bee. Interestingly, the honey bee *Xbp1* mRNA possess a rather unique deviation from the CNGCNG site found at the 5′ splice site of the intron, instead having a CNACNG sequence.

We hypothesized that this pronounced divergence in the *Xbp1* mRNA non-canonical intron could have important consequences for honey bee UPR function and proteostasis. Thus, we began functional characterization of the UPR in this species, focusing on the IRE1 pathway, to answer this question and further our molecular understanding of honey bee cellular stress responses. We found that the core components of the UPR were conserved in the honey bee. In addition, we characterized a number of gene targets of the UPR that are robustly induced upon UPR activation in response to multiple known triggers. While the *Xbp1* mRNA splice site has unique features in this species and other related bees, IRE1-dependent splicing of *Xbp1* mRNA upon UPR stimulation is conserved.

2. Materials and methods

2.1. Honey bee tissue collection

Honey bees were collected from the landing board of outbred colonies in New York, New York consisting of a typical mix of *A. mellifera* subspecies found in North America. Only visibly healthy bees were collected and all source colonies were visually inspected for symptoms of common bacterial, fungal, and viral diseases of honey bees.

2.2. Ortholog screening of the honey bee genome

UPR pathway gene candidates from *Drosophila melanogaster* and *Caenorhabditis elegans* were used to find orthologs in the honey bee genome using the BLAST family of search functions (www.ncbi.nlm.nih.gov). Honey bee orthologs were identified by searching honey bee genome assemblies 4.5 directly using TBLASTN. In addition, the KEGG (Kyoto Encyclopedia of Genes and Genomes) database was used as a guide for comparing pathways between species (Kanehisa and Goto, 2000). The alignment of unconventional intron sites was created using INFERNAL 1.1.1 (Nawrocki and Eddy, 2013) cmalign command with an covariance model based on full alignment from (Hooks and Griffiths-Jones, 2011) and then manually adjusted in RALEE (Griffiths-Jones, 2005). Secondary RNA structures were drawn using VARNA (Darty et al., 2009).

2.3. Chemical treatments

For all caged experiments, honey bees were selected as above and kept in 177.4 mL (6 oz.) Square-bottomed Drosophila Stock Bottles (Fisher Scientific, Pittsburg, PA) plugged with modified foam tube plugs (Jaece Industries, North Tonawanda, NY). Bees were maintained in incubators at 35 °C (unless otherwise stated) in the presence of PseudoQueen (Contech, Victoria, British Columbia, Canada) as a source of Queen Mandibular Phermone (QMP) and used as per manufacturer's instructions. Bees were fed 30% sucrose via a modified 1.5 ml screw-cap tube with or without the following chemicals at the doses indicated in the figure legends and text: 12–24 μM tunicamycin (Sigma, St. Louis, MO) (Chow et al., 2013; Kang et al., 2012) and 10–25 mM dithiothreitol (DTT) (Sigma, St. Louis, MO) (Caruso et al., 2008). The solute for tunicamycin is DMSO, so equivalent amounts of DMSO were added to the food of the control group in tunicamycin experiments. Water is the solute for DTT, so equivalent amounts of water were added to the food of the control group in DTT experiments. UPR stimulation experiments with tunicamycin were performed a minimum of four times and UPR stimulation experiments with DTT were also performed a minimum of four times. Survival experiments with Tunicamycin and DTT were each performed twice with similar results.

2.4. RNA isolation, reverse-transcription and quantitative PCR for gene expression analysis

Midguts, and sometimes abdomen tissue with all gut tissue removed, were placed into RNAlater (Invitrogen, San Diego, CA) for storage prior to gene expression analysis of individual workers. RNA was prepared from bees from the described populations by manually crushing the tissue of interest with a disposable pestle in Trizol Reagent (Invitrogen, San Diego, CA) and extracting the RNA as per the manufacturer's instructions. RNA was subsequently DNAseI treated by RQ1 RNase-Free DNase (Promega, Madison, WI)