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Original article

Aging and transcriptional activity in worker honey bees

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Summary — Age-specific variability in gene expression of worker honey bees was examined using *in vitro* translation of mRNA. An apparent pre-programmed regime of age-specific gene activity was observed as individual workers aged. The quantitative pattern of gene expression among young bees (aged 0–2 d) was more similar to that of older bees (aged ≥ 23 d) than to that of middle-aged bees (aged 6–20 d).

***Apis mellifera* / gene regulation / *in vitro* translation / aging**

INTRODUCTION

Temporal or age polyethism is well documented in the worker honey bee: individual workers display an ontogenetic sequence of physiological and behavioral changes relative to task performance (see Wilson, 1971; Michener, 1974; Seeley, 1985; Winston, 1987). Seeley (1982) recognized 4 broadly defined temporal castes: 1), cell cleaner caste (aged 0–2 d); 2), broodnest caste (aged 2–11 d); 3), food handler caste (aged 11–20 d); and 4), forager caste (aged 20 d or more). Still, considerable plasticity in exocrine activity and specific task performance is evident both within and between these temporal castes. Juvenile hormone, for example, plays a major role in the regulation of age polyethism (Robinson, 1987). Recent evidence indicates a genetic predisposition to perform at least some tasks within the colony (Calderone and Page, 1988; Frumhoff and Baker, 1988; Robinson and Page, 1988, 1989). Specificity of hormone activation in response to extrinsic stimuli appears to coordinate genotypic predispositions to task specialization (Jaycox, 1976; Jaycox *et al*, 1974).

Central to an understanding of temporal events in the life of a worker is a critical need to examine the aging process from the context of changes in gene expression. Some evidence suggests that aging and senescence in higher organisms reflects a preprogrammed (genetic) mechanism culminating in self-destruction (Pereira-Smith and Smith, 1983, 1988; Sugawara *et al*, 1990). Therefore, an *a priori* requirement for efforts to identify genes associated with temporal behavioral activities in workers is the necessity to

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determine if consistent patterns of change in mRNA (or protein) populations are evident as individual workers age. In this study, we provide evidence for temporal specificity in translatable RNA populations within cohorts of aging workers.

MATERIALS AND METHODS

Newly eclosed workers within a colony were marked (with a distinct paint dot on their thorax) at 2–4-d intervals which provided defined-age cohorts representing workers aged 0–27 d. Marked bees were allowed free flight and normal participation in colony activities. Individual bees representing each age cohort were collected on d 27 and immediately frozen in liquid N_2 and stored at –80 °C.

Individual workers were homogenized in equal volumes of phenol and a lysis solution (0.5% SDS, 0.2 M NaCl, 25 mM EDTA, pH 8.0). Homogenates were subjected to standard phenol/chloroform extraction procedures (Maniatis *et al,* 1982). Nucleic acids were initially precipitated in ethanol and RNA subsequently selectively precipitated in 3 M ammonium acetate.

Five μg of RNA from each sample were translated *in vitro* in the presence of [^{35}S]methionine (Amersham) using a rabbit reticulocyte system (Promega). One-dimensional SDS–12% polyacrylamide gel electrophoresis was conducted as previously described (Severson *et al,* 1989). A constant volume of 7 μl of the translation mixture was loaded directly onto each gel. A minimum of 2 individual workers at each age were examined. Autoradiography was performed at –80 °C with an intensifying screen.

RESULTS AND DISCUSSION

We compared *in vitro* translation products from workers at 2–4-d intervals as they aged from 0 (newly eclosed) to 27 d. Typical autoradiograms are shown in figure 1. These data indicate that changes in translatable RNA populations occurred as individual workers aged. Obvious changes in

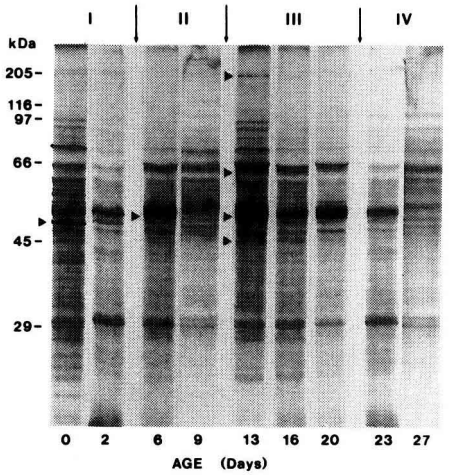

Fig 1. Typical autoradiograms of SDS–12% polyacrylamide gels showing *in vitro* translation products observed among adult worker bees as they aged from 0–27 d. Arrowheads indicate translation products which reflect age-associated quantitative changes discussed in the text. Vertical arrows delineate transition periods for temporal castes as defined by Seeley (1982): I), cell cleaner caste; II), broodnest caste; III), food handler caste; IV), forager caste. Molecular weight markers (kDa) are indicated.

quantitative patterns of transcriptional activity occurred between bees aged 2–6 d as well as between bees aged 20–23 d. The observed increase in a 51 kDa translation product was characteristic of bees aged 6–20 d (middle-aged). Quantitative evidence of variability in transcriptional activity was evident with bees aged ≈ 13 d; obvious increases in at least 3 additional RNA populations were observed within this age cohort. The increase in a 48 kDa translation product was always observed among bees aged 0–1 d. The quantitative pattern of gene expression among bees aged 0–2 d (young) was, however, more similar to those aged 23 or more days (old) than to middle-aged bees. We consistently

observed this general pattern of transcriptional activity in workers aged 0, 9 and 27 d in previous studies (Severson *et al,* 1990). Observations of similar patterns of gene expression among young and old workers and an increase in the complexity of gene expression among middle-aged workers is similar to that reported for *Drosophila* (Fleming *et al,* 1986).

A new model is developing for age polyethism in workers which incorporates both genetic and epigenetic determinants for specific task performance (Robinson, 1987). At the organismal level, we are presented with a complex mosaic reflecting genome and consequences of various environmental stimuli. Separation and ultimate identification of the individual components involved in determination of worker behavior will therefore only be accomplished through a combination of research at both the holo-organismal and molecular levels. The apparent pre-programmed regime of age-specific gene activity observed in this study provides the first insight at a molecular level as to the chronological sequence of gene expression as individual workers age.

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Résumé — Vieillissement et activité transcriptionnelle chez les ouvrières d'abeilles (*Apis mellifera* L). Nous avons étudié la succession chronologique de l'expression des gènes chez des ouvrières individuellement au cours de leur vie. Des ouvrières fraîchement écloses d'une même colonie ont été marquées à intervalles de 2 à 4 j, jusqu'à obtenir des groupes d'ouvrières d'âge défini allant de 0 à 27 j. Nous avons prélevé des individus de chaque groupe pour en extraire l'ARN individu par individu. Cet ARN a été traduit *in vitro* avec marquage au ^{35}S. Les produits de traduction ont été séparés par électrophorèse sur gel de polyacrylamide-SDS.

Les autoradiogrammes (fig 1) indiquent que des changements dans les populations d'ARN traductibles s'opèrent au cours du vieillissement des ouvrières. Des changements évidents de l'activité de transcription se produisent chez les ouvrières de 2 à 6 j, ainsi qu'entre 20 et 23 j. Une augmentation d'un produit de traduction de 51 kDa est caractéristique des abeilles âgées de 6 à 20 j (moyennement âgées). Le schéma d'expression des gènes des abeilles jeunes (0 à 2 j) s'apparente plus à celui des vieilles abeilles (23 j et plus) qu'à celui des abeilles moyennement âgées. Ces données indiquent l'existence, chez l'ouvrière, d'une préprogrammation de l'activité génique qui est fonction de l'âge.

***Apis mellifera* / régulation des gènes / traduction *in vitro* / vieillissement**

Zusammenfassung — Altern und Transkriptions-Aktivität bei Arbeitsbienen. Wir untersuchten die zeitliche Abfolge der Gen-Expression im Verlauf des Alterungsvorganges von einzelnen Arbeitsbienen. Frisch geschlüpfte Arbeitsbienen in einem einzigen Volk wurden in Abständen von 2 bis 4 Tagen makiert, bis definierte Altersgruppen mit Arbeiterinnen in einem Alter von 0 bis 27 Tagen zur Verfügung standen. Es wurden Individuen von jeder Altersgruppe gesammelt und anschließend die RNA von individuellen Bienen isoliert. Dann wurde die *in vitro* Translation der RNA-Proben durchgeführt, begleitet von einer Inkorporation eines ^{35}S-Labels. Die Translations-Produkte wurden mittels einer SDS-Polyakrylamidgel-Elektrophorese aufgetrennt.

Die typischen Autoradiogramme (Fig 1) weisen darauf hin, daß im Verlaufe der Alterung der individuellen Arbeiterinnen Veränderungen in den übersetzbaren RNA-Populationen erfolgten. Deutliche Veränderungen im den quantitativen Mustern der Transkriptions-Aktivität erfolgten bei Bienen der Altersgruppen von 2 bis 6 Tagen und ebenso bei Bienen im Alter von 20 bis 23 Tagen. Eine Vermehrung eines 51 kDa Translations-Produkts war für Bienen im Alter von 6 bis 20 Tagen (mittleres Alter) charakteristisch. Das quantitative Muster der Gen-Expression bei Bienen mit einem Alter von 0 bis 2 Tagen (junge Bienen) war dem von alten Bienen (mit 23 und mehr Tagen) ähnlicher als solchen von mittlerem Alter. Diese Daten bringen ein vorprogrammiertes Regime einer altersspezifischen Genaktivität im Verlaufe des Alterungsvorganges der einzelnen Biene zum Ausdruck.

***Apis mellifera* / Genregulierung / *in vitro* Translation / Altern**

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