Double abdomen induction by UV in Bradysia tritici (syn. Sciara ocellaris, Sciaridae): sensitive stages and conditions for photoreversal

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Summary. The pattern anomaly double abdomen was induced in embryos of Bradysia tritici (syn. Sciara ocellaris) by irradiation of the anterior egg pole with far UV (254 or 285 nm) using low UV fluences. The maximum yield of 18% of double abdomens was obtained when 2.5 h embryos were irradiated (late intravitelline cleavage stage); earlier irradiation failed to yield double abdomens, as did irradiations after the early syncytial blastoderm stage. Exposing irradiated embryos to photoreverting light (366 nm) reduced the yield of malformations. Most double abdomens were symmetrical and the number of segments ranged from 3 to 8 in each set, with the mean value at 6.4 segments.

Key words: Insect embryogenesis – Pattern formation – Double abdomen – UV irradiation – Photoreversal

Introduction

Current interpretations of longitudinal (antero-posterior) patterning in insect embryogenesis were strongly influenced by the double abdomen (DA) anomaly in which the anterior body regions are replaced by a posterior region of inverted axial polarity. The aberrant pattern can be induced by experimental interference with the egg system (see reviews by Sander 1976; Kalthoff 1983) and is also known to result from mutation (reviewed by Nüsslein-Volhard 1979; see also Mohler and Wieschaus 1986; Percy et al. 1986). The basic concept emerging from these findings involves “antero determinants” produced primarily during oogenesis and localized (or active) ultimately in the anterior region of the egg cell; if these determinants fail to reach or maintain certain thresholds (absolutely or in relation to some other, e.g. posterior, determinants), the anterior egg regions form posterior abdominal parts instead of the head and thorax, and this switch in patterning is accompanied by polarity reversal. However, many steps implied in this concept are still unknown, and the systems studied so far might not be optimal for revealing these steps. Therefore, further systems amenable to experimental induction of DA and related pattern anomalies should prove useful. We here report induction of the DA pattern in Bradysia tritici (syn. Sciara ocellaris), a sciarid species studied earlier for peculiarities of its polytene chromosomes (Metz 1938; Pavan and Perondini 1967; Perondini and Dessen 1985) and sex determination (Metz 1938; Berry 1941; Rieffel and Crouse 1966; Mori et al. 1979; Mori and Perondini 1980, 1984). As in some other species, the far UV radiation used for DA induction affects patterning in Bradysia only when applied during a rather brief period of development, and its effects can be reverted by subsequent irradiation with UV light of longer wavelengths.

Material and methods

Eggs. Eggs were collected from a Bradysia strain maintained in the laboratory since 1966 (Pavan and Perondini 1967). To obtain a large number of embryos at specific stages of development, aged females were induced to lay their eggs in a short time interval (Carson 1946). Only eggs deposited in a 30 min period were used in the experiments. The transparent eggs were maintained in boiled and filtered tap water and inspected with the stereomicroscope for staging and scoring. Rearing temperature was 22° C throughout.

Stages irradiated

Embryos at the following stages were used: intravitelline nuclear cleavage from 1 h up to 3.5 h, pole cell formation, early syncytial blastoderm (nuclei arrive in the periplasm at the anterior pole), and late syncytial blastoderm. A description of early embryonic stages can be found in Perondini et al. (1986).

Irradiation procedures. The eggs were lined up on the edge of a small block of agar and irradiated with the anterior pole facing the UV source. After irradiation most batches were kept in the dark for 24 h or longer. Some others, after identical irradiation, were exposed in the same orientation to near UV light (potentially photoreverting treatment) before being transferred to the dark. All treatments were carried out in a dark room under red safety light.

Source and measurement of UV radiation. Irradiation was done either with a desk-type germicidal UV lamp (Camag, Berlin), emitting mainly at 254 nm wavelength, or with monochromatic 254 nm UV radiation from a Xenon arc lamp (Osram XBO 450W) passed through a grating monochromator (Farrand Foci f/3.5) with a band width of 5 nm (for details see Kalthoff 1973). No significant differences in the yield of malformations or in the survival rate were...
Table 1. Induction of double abdomens by UV (254 nm wavelength)

<table>
<thead>
<tr>
<th>Stage</th>
<th>UV fluence (J·m⁻²)</th>
<th>Number of embryos</th>
<th>Developed embryos</th>
<th>Embryonic pattern n (% of developed embryos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC (1 h)</td>
<td>15</td>
<td>75</td>
<td>51 (70.9)</td>
<td>NL = normal larvae; MF = malformed; DA = double abdomens</td>
</tr>
<tr>
<td>IC (1.5 h)</td>
<td>25</td>
<td>90</td>
<td>77 (89.1)</td>
<td></td>
</tr>
<tr>
<td>IC (2 h)</td>
<td>40</td>
<td>80</td>
<td>64 (86.5)</td>
<td></td>
</tr>
<tr>
<td>IC (2.5 h)</td>
<td>80</td>
<td>60</td>
<td>56 (95.0)</td>
<td></td>
</tr>
<tr>
<td>IC (3 h)</td>
<td>120</td>
<td>84</td>
<td>54 (90.2)</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>95</td>
<td>76</td>
<td>58 (90.9)</td>
<td></td>
</tr>
<tr>
<td>ESB</td>
<td>15</td>
<td>74</td>
<td>50 (92.9)</td>
<td></td>
</tr>
<tr>
<td>LSB</td>
<td>20</td>
<td>99</td>
<td>49 (74.4)</td>
<td></td>
</tr>
</tbody>
</table>

* Normalized to the survival rate of non-irradiated control egg clusters: NL = normal larvae; MF = malformed; DA = double abdomens; IC = intravitelline nuclear cleavage; PC = pole cell; ESB = early syncytial blastoderm; LSB = late syncytial blastoderm

Results

Conditions for double abdomen induction

More than 600 eggs (deposited by 10 females) were divided into 16 batches. At each selected developmental stage 2 batches were taken: one was used as non-irradiated control, and the other for irradiation (254 nm) of the anterior egg poles using the germicidal lamp. The fluence rates were maintained at 2 J·m⁻²·s⁻¹ and the fluences adjusted for each stage to obtain a high frequency of developing embryos (Table 1). These experiments were carried out in duplicates; since in both cases the results were similar the data were pooled (Table 1). At the stages prior to blastoderm formation low fluences of far UV caused disturbances in the pattern of embryonic development. Although most resulting embryos showed multiple non-specific aberrations (MF in Table 1), some had formed a supernumerary abdomen instead of head and thorax (Fig. 1). These DA resulted from irradiations at the intravitelline nuclear cleavage stage (2.5 h) up to the early syncytial blastoderm stage with the maximum frequency of 18% at the former stage. The DA yield at the intravitelline nuclear cleavage stage (2.5 h) differed significantly from that obtained at the other stages (5% level, χ²-test).

In another series of experiments, embryos aged 2.5 h were irradiated with increasing UV-fluences (at 254 nm wavelength). DA resulted with fluences up to 260 J·m⁻² but the fraction of DA amongst the surviving embryos could not be increased (own data and those of Bischof 1983).

Kalthoff (1973) and Ripley and Kalthoff (1981) have shown that in Smittia irradiation is more effective in DA induction at 285 nm wavelength than at 254 nm. Our attempts to increase the DA yield by irradiating at a wavelength of 285 nm failed: we found only 2 DA amongst 27 embryos after irradiation at the intravitelline nuclear cleavage stage (3 h) and none after irradiation of 267 embryos at other stages (including the 2.5 h stage giving maximum DA yields with 254 nm). The photoreactivation experiments, too, furnished no evidence for an increased DA yield by irradiating at 285 nm.

Conditions for photoreversion

DA induction by far UV was found to be photoreversible in Smittia (Kalthoff 1973; Ripley and Kalthoff 1981). We checked for photoreversion in Bradysia eggs by exposing the anterior egg pole to near UV light at 366 nm wavelength (photoreverting light) after irradiation with monochromatic UV at 254 nm or 285 nm wavelength. Fluence of the PR light was 7.2 kJ·m⁻² which was given at the rate of 3 J·m⁻²·s⁻¹. This fluence is known to yield maximal photoreversion after lethal UV doses given to whole eggs at these developmental stages (Perondini 1984). For this experiment eggs deposited by several females were pooled and divided into four groups. When they had reached the intravitelline nuclear cleavage stage (3 h), two of these groups were irradiated with UV at 254 nm wavelength and the other two with UV at 285 nm. Fluences were adjusted to yield about 60% of developing embryos after incubation in the dark. Table 2 shows that PR treatment resulted in a significant increase of developed embryos, concomitant with a rise in the percentage of normal larvae. This is particularly clear among the embryos irradiated at 254 nm where PR treatment reduced the frequency of aberrant embryos (including DA) from 66% to 6%. With 285 nm, survival was increased from 60% to 90% but the rise in the percentage of normal larvae was small (from 80% to 93%).

Double abdomen larval cuticle pattern

The cuticle pattern of the freshly hatched larva has been described by Bischof et al. (1985). The segment borders are observed between these sources, provided the fluences were adequately adjusted. For photoreactivation, the irradiated embryos were exposed to monochromatic near UV light at 366 nm wavelength. Fluence rates were measured by a thermopile detector (Oriel Co., Stanford; microvoltmeter Keithley 150B) calibrated against a known UV source (see Kalthoff 1973).

Scoring of induced developmental defects. The treated embryos were grouped into two broad classes, one comprising those which showed no germ anlage differentiation, the other those which had reached late stages of embryonic development. Among the latter class three types were distinguished: hatched larvae with normal segment pattern, double abdomens (DA), and embryos showing multiple malformations other than DA. The cuticles of freshly hatched larvae or late embryos were prepared by the method of Van der Meer (1977).