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Position-specific expression of β-galactosidase under heat shock conditions in the *hsp70-lacZ* insertion mutant of *Drosophila melanogaster*.

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Summary: Evidence suggests that P element vectors, which contain a bacterial β-galactosidase gene *lacZ*, often express β-galactosidase in a pattern that corresponds to the expression pattern of genes located near the insertion site. However, without having appropriate controls, it can be difficult to distinguish between a gene-specific expression pattern and the endogenous β-galactosidase expression. We have used a heat-shock inducible *lacZ* gene carried on a HBΔ-89 P element vector inserted in region 31B on the *Drosophila melanogaster* second chromosome to visualize the expression pattern of a nearby gene, *Dsur*, previously mapped to the same chromosomal region. The heat shock inducibility of this particular P element construct provides a unique opportunity for visualizing the tissue-specific expression of the *Dsur* gene at different stages of embryonic development.

Introduction

Extensive searches for cis-acting regulatory elements in the *Drosophila* genome have been carried out since the late 1980s. This approach was revolutionized with the introduction of a *Drosophila* transformation vector that carried a bacterial *lacZ* gene fused to a P-element promoter (O'Kane and Gehring, 1987). This method allowed the *in situ* visualization of gene expression in *Drosophila*. Upon the insertion into the genome, many vectors that carry the P-*lacZ* construct reveal position-specific expression patterns of β-galactosidase. It has been suggested that these patterns may reflect the expression of the genes neighbouring the insertion site (Bier *et al.*, 1989). On the other hand, extensive data show that when stained for β-galactosidase, some embryonic tissues reveal endogenous expression of *lacZ* (Bier *et al.*, 1989; O'Kane and Gehring, 1987). Nevertheless, some of what is perceived to be endogenous β-galactosidase expression in P-*lacZ* insertion mutants may, in fact, be true site-specific expression of P-*lacZ* (Bier *et al.*, 1989). The *hsp70-lacZ* construct (Lis *et al.*, 1983) provides a unique opportunity to differentiate between the position-specific expression of P-*lacZ* inserts and the endogenous β-galactosidase expression. In this model, the embryos carrying *hsp70-lacZ* can be heat shocked and stained for β-galactosidase, while non heat-shocked embryos from the same lay serve as a control.

The line of P-element vectors composed of *hsp70* promoter regions fused to a *lacZ* cassette was constructed to analyze the effect of altering the strength of the *hsp70* promoter on polytene chromosome puffing (Simon *et al.*, 1985). Previous reports indicate that the *hsp70-lacZ* construct responds well to heat shock regulation in transformant *Drosophila* adult flies and larvae (Lis *et al.*, 1983; Simon *et al.*, 1985). Under the heat shock, polytene chromosomes of the transformant flies undergo puffing at the site of *hsp70-lacZ* insertion (Simon *et al.*, 1985). However, there are no data available on the activation of this construct under heat shock conditions in the embryo. We present results that show tissue-specific activation of a *hsp70-lacZ* construct inserted in the 31B region on the *Drosophila* second chromosome, the genomic site for the *Drosophila* sulfonylurea receptor (*Dsur*) gene (Nasonkin, *et al.*, 1999).

Materials and Methods

Drosophila *stocks*: P{$ry^{+t7.2}$=HB-Δ89}31B Adh^{fn6} cn^{1}; ry^{502} stock was obtained from the Bloomington *Drosophila* Stock Center (stock #P210). This stock contains an insertion in region 31B of a P element that carries a *hsp70-lacZ* fusion gene (Simon *et al.*, 1985).

Heat shock: Yeast-apple juice-agar plates with overnight lay of embryos were placed in a Petri dish lined with moist tissue and kept in a 37°C incubator for 2-5 hours. After the heat shock, embryos were collected and fixed according to standard procedures (Goldstein and Fyrberg, 1994).

Immunohistochemistry: Mouse monoclonal antibody against β-galactosidase was obtained from Promega. β-galactosidase staining was performed essentially as described in the Staining Protocol for 2A12 Tracheal Antibodies from the University of Iowa Hybridoma Bank web site (http://www.uiowa.edu/~dshbwww/2a12.html), except that the Vector VIP Kit (Vector Laboratories, Inc.) was used as the enzyme substrate instead of the DAB Substrate Kit. β-galactosidase IgG antibody was used at 1:200 concentration. Biotinylated secondary antibody (from Vector laboratories) was used at 1:300. Overnight embryos were collected from yeast agar plates and fixed according to standard procedures.

Equipment: Images were taken with the Nikon Microphot-SA fluorescence microscope, Nikon Corporation, equipped with SPOT Cooled Color Digital Camera, Diagnostic Instruments, Inc. Images were processed on Power Macintosh 7500/ 100 using the SPOT Twain 2.1.3 software and Adobe Photoshop 5.0, Adobe Systems, Inc. for PowerPC.

Results and Discussion

A transformant line that carries the *hsp70-lacZ* construct inserted in the region 31B on the *Drosophila* second chromosome (Simon *et al.*, 1985) received a designation P210. Unlike similar insertions of this construct at other chromosomal locations, this particular insertion does not induce chromosomal puffing in salivary glands under heat shock conditions (Simon *et al.*, 1985). Furthermore, this line exhibits reduced levels of endogenous β-galactosidase expression in various larval tissues and an exceptional tissue-specific β-galactosidase expression under heat shock conditions (Simon *et al.*, 1985). P210 was originally our candidate for the *Dsur* mutant, a gene from the ABC transporter family that is expressed in the dorsal vessel of the developing *Drosophila* embryo and is thought to be involved in cell migration (Nasonkin *et al.*, 1999). Based on the P-element insertion site in the chromosomal region 31B of the P210 mutant, we originally postulated P210 to be a *Dsur* mutant. Further investigation did not confirm this assumption and further revealed normal *Dsur* expression in the dorsal vessel by *in situ* hybridization (data not shown). The fact that *lacZ* in this construct is under control of a heat shock promoter prompted us to analyse the pattern of

β-galactosidase expression under the heat shock conditions in the developing embryo. The results are presented in Figure 1. At embryonic stages 13-17, β-galactosidase expression in the dorsal vessel was clearly visible. In addition, β-galactosidase expression was apparent in tricoid sensillas (Figure 1, C),

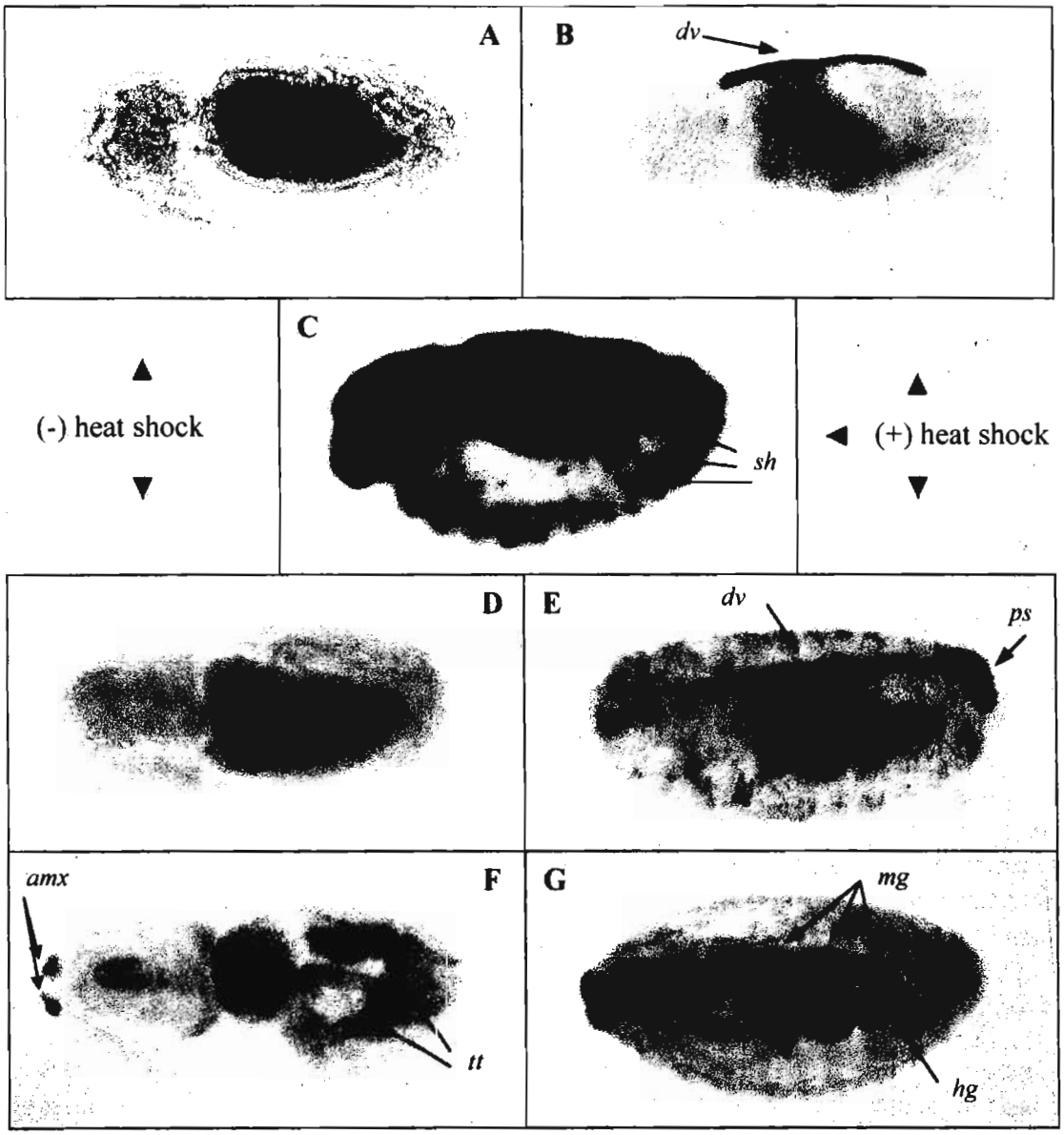

Figure 1. Exposure of the P210 embryos to heat shock results in β-galactosidase activity in dorsal vessel, midgut, hindgut, and sensory structures (visualized with antibodies against β-galactosidase). A-C, Stage 14. A, (-) heat shock, B, C, (+) heat shock. B, at early stage 14, β-galactosidase expression in dorsal vessel (*dv*) becomes clearly visible. C, β-galactosidase expression in tricoid sensillas (*sh*) is apparent. D, E, Stage 15. D, (-) heat shock, E, (+) heat shock. E, at stage 15, β-galactosidase expression in posterior spiracles (*ps*) becomes visible after heat shock treatment. F, G, Stage 16. F, (-) heat shock, G, (+) heat shock. F, the endogenous expression of β-galactosidase in tracheal trunk (*tt*) and the antenno-maxillary complex (*amx*) is visible. G, β-galactosidase activity is present in midgut and hindgut after heat-shock treatment.

posterior spiracles (Figure 1, E), as well as in the midgut and the hindgut (Figure 1, G). The hindgut and midgut expression of β-galactosidase had incomplete penetrance at early stages, but was very prominent at later stages (Figure 1, G). The endogenous β-galactosidase expression was visible in the tracheal system and the antenno-maxillary complex (Figure 1, F) of the control embryos.

Previous reports indicated a remarkable tissue-specific β-galactosidase expression in larval and adult tissues of the P210 flies, suggesting that the *lacZ* expression is affected by a gene located near the *hsp70-lacZ* insertion site in the P210 mutant (Simon *et al.*, 1985). The heat-shock induced β-galactosidase expression in the dorsal vessel of the P210 embryos and the positioning of the insert in the 31B chromosomal region of the P210 flies indicate that this particular P-element construct is inserted in the vicinity of the *Dsur* gene. In the majority of cases, the insertions of P-*lacZ* do not disrupt the structure or function of the nearby genes (O'Kane and Gehring, 1987). It has also been reported that P elements have an insertional preference to the promoter regions and that, in some cases, such insertions can influence adjacent gene expression without abolishing its function (Kelley *et al.*, 1987; Tsubota *et al.*, 1985). Taken together, these data suggest that the P210 strain carries an insertion of the P-*lacZ* element in or near the promoter region of the *Dsur* gene.

The observed heat-shock induced expression of β-galactosidase in both the dorsal vessel and the gut of the P210 embryos may indicate that *Dsur* is in the pathway of pattern formation of various embryonic tissues. Previous reports demonstrated that many *Drosophila* lines transformed with the P-*lacZ* fusion gene express β-galactosidase activity in the gut (Bier *et al.*, 1989). On one hand, the persistent gut-specific expression of *lacZ* in various insertion backgrounds may reflect the high complexity of the tissue structure of that particular organ. On the other hand, β-galactosidase expression in the gut of the P210 line may also indicate that *Dsur* is involved in the genetic mechanism of pattern formation that is shared among different organs during *Drosophila* development (Sun *et al.*, 1995), such as the gut and the embryonic heart. Thus, the tissue-specific activation of the *hsp70-lacZ* construct in various tissues of the developing embryo of the P210 line strengthens our hypothesis that *Dsur* is involved in pattern formation during *Drosophila* embryogenesis.

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