Research Notes

Cuticle sclerotization in pharate adult and imago of *Drosophila melanogaster, Ceratitis capitata,* and *Haematobia irritans.*

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N-β-alanyldopamine synthase is the enzyme conjugating dopamine with β-alanine to generate N-β-alanyldopamine (NBAD) in the integument of insects (Pérez *et al.*, 2002). NBAD is the main cross-linking precursor of the brown insect cuticles (Hopkins and Kramer, 1992). NBAD is synthesized in the epidermis and apically exported to the cuticle to give rise to the corresponding quinone, which will crosslink proteins and eventually chitin (Hopkins and Kramer, 1992). In *Drosophila melanogaster* the locus *ebony* seems to be the coding gene for the enzyme, whereas in *Ceratitis capitata* the equivalent gene is *niger* (Pérez *et al.*, 2002). It is assumed that the enzyme expression is induced by 20-OH-ecdysone, the molt hormone, but only indirect demonstrations have been published (Pérez *et al.*, 2004). Data correlating ecdysone levels in haemolymph with epidermal NBAD synthase activity in the same insect are not available. On the other hand, we recently demonstrated that the enzyme is expressed in the neural system in a constitutive way (Pérez *et al.*, 2004). However, in terms of quantity of active enzyme, the amount in brain and ganglia is much lower than the enzyme amount in epidermis, when expressed. The knowledge of a novel brain activity, not detected previously (Pérez *et al.*, 2004), opened the possibility that different regulation of NBAD synthesis might exist in different tissues and/or insects. Since it is quite difficult to compare with precision the time of development in different insects, we focused our analysis of N-β-alanyldopamine synthase in three dipterans in which developmental time can be assessed with some accuracy, *i.e. Drosophila melanogaster,* the Mediterranean fruit fly (Medfly) *Ceratitis capitata* (Rabossi and Quesada-Allué, 1995), and *Haematobia irritans* (the horn fly). We first wanted to

Table 1. Developmental equivalence of *Drosophila melanogaster*, *Ceratitis capitata* and *Haematobia irritans* metamorphosis markers.

Metamorphosis events	*D. melanogaster*		*C. capitata*		*H. irritans*	
	hours	cumulative % *	hours	cumulative % *	hours	cumulative % *
1- Onset of pupariation, 'Time zero'. The shaping of the puparium is completed and cuticle is still white. [a-b-c-d]	0	0	0	0	0	0
2- Pupal – adult apolysis. [b-d]	50	52	160	51.3	90	53.6
3- Ocelli visible and colored. [d]	ND	ND	168	53.8	100	59.5
4- Bristle pigmentation begins dorsally on head and thorax.[b-c-e]	70	72.9	216	69.2	120	71.4
6- Wings, ptilinum and bristles darken. [b-c-e]	86	89.5	288	92.3	144	85.7
7- Ecdysis of the imago. [b-c-e]	96	100	312	100	168	100

* The progress of metamorphosis is indicated in terms of cumulative percentage of time within the puparium. *Ceratitis* and *Haematobia* were grown respectively at 23 and 29 °C. Letters correspond to citations below: a-c, *D. melanogaster* and d-e, *C. capitata*. a) Ashburner, 1989: b) Bainbridge and Bownes, 1981: c) Bodenstein, 1951; d) Rabossi *et al*., 1991; e) Rabossi *et al*., 1992.
ND: not determined

Table 2. Overall N-β-alanyldopamine synthase activity during pharate adult instar of *Drosophila melanogaster, Ceratitis capitat*a and *Haematobia irritans.*

	Pharate adult age		NBAD synthase activity (pmol/min.mg)**
	Hours, cumulative*	% of metamorphosis	
D. melanogaster	55 hs	57.3 %	13.6
D. melanogaster	70 hs	72.9 %	20.0
C. capitata	216 hs	69.2 %	9.5
H. irritans	120 hs	71.4 %	6.7
D. melanogaster	80 hs	83.3 %	12.3
C. capitata	288 hs	92.3 %	12.7
H. irritans	144 hs	85.7 %	11.9
D. melanogaster	95 hs	98.9 %	11.5
C. capitata	312 hs	100 %	17.5
H. irritans	168 hs	100 %	16.6

(*) Age within the puparium. (**) Average of not less than 3 determinations.

Table 3. N-β-alanyldopamine synthase activity in adults of *Drosophila melanogaster*, *Ceratitis capitata* and *Haematobia irritans.*

Exarate adults		NBAD synthase activity (pmol/min.mg)*
Young imago (whole body)		
D. melanogaster	(4 hs after ecdysis)	24.6
C. capitata)	(4 hs after ecdysis	36.4
H. irritans)	(1 hs after ecdysis	30.7
Old imago (head)		
D. melanogaster	(more than 1 week after ecdysis)	43.0
C. capitata.	(more than 1 week after ecdysis)	21.1
H. irritans	(more than 3 days after ecdysis)	33.4
Old imago (thorax + abdomen)		
D. melanogaster	(more than 1 week after ecdysis)	0.0
C. capitata	(more than 1 week after ecdysis)	0.7
H. irritans.	(more than 3 days after ecdysis)	0.8

(*) Average of not less than 3 determinations

know the degree of equivalence of the three corresponding life cycles during the stages within the puparium. As shown in Table 1, using reliable markers to follow the progress of metamorphosis, the occurrence of four events differs less than 8% of the studied portion of the life cycle (from the onset of pupariation to imago ecdysis). Therefore, we may assume that a good equivalence in the time of development exists among the three flies and, therefore, that metamorphosis events may be correlated with the enzymatic activity, also assuming similarity of physiological events. NBAD synthase activity is measured and NBAD analyzed as described in Pérez *et al.* (2004). Table 2 shows that NBAD synthase activety at the time of bristles sclerotization and pigmentation in *Drosophila* is higher than during early or late metamorphosis. This is different from activity in the medfly or in the horn fly, which showed increasing activity until ecdysis. The data in Table 2 must be compared with the maximum specific activities attained by the NBAD in just-emerged imagos, as shown in Table 3. For comparison, activity values at puparium peak sclerotization are 45.2 pmoles/min.mg for *Drosophila* and 23.11 pmoles/min.mg for the medfly. As expected, the overall activity measured in epidermis of old adults is very low (see thorax + abdomen in Table 3) whereas the (constitutive) brain activity is in the range of that in recently ecdysed young imagos. Thus the apparent expression (and probably regulation) of NBAD synthase activity seems very similar in the three flies, thus allowing us to switch certain experiments from small size, short life-cycle *Drosophila* to much bigger size, longer life-cycle flies. It seems appropriate to assume that robust biochemical data obtained in such a way might be very helpful when planning the study of *ebony* expression in *Drosophila*, since little is known on gene regulation in *Ceratitis* and even much less in *Haematobia.*

References: Ashburner, M., 1989, Drosophila. *A Laboratory Handbook*, Chapter 8. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, MA. 179 pp; Bainbridge, S.P., and M. Bownes 1981, J. Embryol. Exp. Morphol. 66: 57-80; Bodenstein, D., 1951, *In*: *Biology of* Drosophila, Chapter 4, 275-367; Hopkins, T.L., and K.J. Kramer 1992, Ann. Rev. Entomol. 37: 273-302; Pérez, M., J. Schachter, and L.A Quesada-Allué 2004, Neuroscience Letters 368: 186-191; Pérez, M., P.

Wappner, and L.A. Quesada-Allué 2002, Insect. Biochem. and Mol. Biol. 32: 617-625**;** Rabossi, A., G.L. Boccaccio, P. Wappner, and L.A. Quesada Allué 1991, Entomol. Exp. Appl. 60: 135-141; Rabossi, A., and L.A. Quesada-Allué 1995, Dros. Inf. Serv. 76: 148-149; Rabossi, A., P. Wappner, and L.A. Quesada Allué 1992, Can. Entomol. 124: 1139-1147.

The identification of a silencer element in the *cut* locus of *Drosophila melanogaster* located 73 and 96 kb upstream from the promoters of the locus.

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Abstract

Homeotic genes are important for early development, but they are subjected to silencing during the later stages. It is known that Polycomb group proteins are involved in this type of distant regulation. The *cut* locus, a hierarchal regulator of external sensory organs, spans about 220 kb in 7B region on X chromosome. It is important for the normal development at different stages of *Drosophila* development from embryo to imago. It follows that the chromatin organization and the regulation of the transcription inside the locus are very complex. Up-to-date silencer elements were not identified in the locus. In the present study we detected a functional silencer in the distal part of the locus. The silencer is located inside the locus control region (LCR), has long protein binding region, and possesses sequences characteristic for PRE/TRE.

Introduction

The *cut* locus of *Drosophila* is the neural selector locus encoding a homeodomain-containing protein. It is known that silencing or activation of such loci are performed through interaction between certain DNA sequences, so-called Polycomb Response Elements/Trithorax Response Elements (PRE/TRE), and proteins belonging to Polycomb group (PcG) or Trithorax group (trx), respectively (Grewal and Elgin, 2002).

However, the PRE/TRE sequences have not yet been identified in the *cut* locus. It is known that the distal area of the locus contains numerous enhancers (Jack *et al*., 1991) and an important regulatory region termed as the Locus Control Region (LCR) (Churikov *et al.*, 1998). LCR has a long region binding proteins (Churikov *et al.*, 1998). Insertion of *burdock* LTR-element about 1 kb downstream from LCR leads to *ct*-lethal phenotype (Tchurikov *et al*., 1989).

In this study we investigated the nature of regulatory sequences within LCR using the reporter genetic construct and the transfection assays on *Drosophila* Schneider 2 cell line. *Sau10* fragment from LCR was inserted about 2 kb upstream from the promoter in this construct. Our data indicate that this small proximal fragment from LCR binds abundant nuclear proteins, has a silencer that is active in Schneider 2 cells, and possesses PRE/TRE sequences.