

# Incorporation of a *Calliphora* Arylphorin Gene into the Germ Line of *Drosophila* by P-Element-Mediated Transformation

Martina Dorsch and Klaus Scheller

Zoologisches Institut der Universität Würzburg, Röntgenring 10, D-8700 Würzburg, Bundesrepublik Deutschland

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Two arylphorin gene clones were inserted into the transposable p-element vector *Carnegie 20* and injected into *Drosophila rosy*<sup>–</sup> mutants. Transposed animals were selected by their red eye colour and remated. We demonstrate by hybridization experiments the stable integration of the injected genes in the germ line of *Drosophila*. However, the incorporated genes are not expressed.

## Introduction

Arylphorins are the major serum proteins of insect larvae. Among dipterans these proteins are immunologically related and show similarities in their size, subunit and amino acid composition as well as in their developmental profile (for review, see [1]). In the blowfly *Calliphora vicina* arylphorin (= calliphorin) serves as a storage of aromatic amino acids [2] and as a carrier for ecdysteroid hormones [3]. The contribution of arylphorin to the sclerotizing system of the cuticle has also been reported [4, 5].

*Calliphora* arylphorin is composed of six closely related polypeptide subunits of about 80 kDa which are encoded by a multigene family clustered in region 4a of chromosome 2 [6]. The expression of the arylphorin genes is tissue specific, restricted to the fat body, and developmentally regulated [1, 7]. Therefore, arylphorin offers an appropriate system to study the mechanisms of stage specific gene activation through interaction of specific promotor elements with nucleic factors. This paper reports the incorporation of *Calliphora* arylphorin genes into *Drosophila* by P element-mediated germ line transformation as a prerequisite for further experiments with the aim of identifying promotor elements which are responsible for the activation and repression of the arylphorin gene family.

## Materials and Methods

### (a) P-element-mediated transformation

Germ-line transformation of *Drosophila* was carried out with some minor modifications as described [8]. After a 2 h precollection period the eggs were collected for 1/2 h after oviposition from a small population of the mutant strain *rosy*<sup>506</sup> to guarantee a synchronization of preblastoderm stages. After dechorionization in a 0.3% sodium hypochloride solution for 1 min the eggs were extensively washed with water and transferred individually to a strip of double-sided adhesive tape attached to a coverslip which was placed into a petri dish containing silica gel to desiccate the embryos. The embryos received injections into the posterior end with a mixture of recombinant (250 µg/ml) and helper plasmids p $\pi$  25.7 wc (50 µg/ml) in a volume between 0.1 and 0.3 nl using needles pulled from microcapillaries.

All surviving larvae were collected to a *Drosophila* standard medium, and those that pupated and enclosed (F<sub>0</sub> adults) were mated to several flies of the *rosy*<sup>506</sup> host strain. The phenotypically wild type offsprings (F<sub>1</sub> adults) of them were selfcrossed to establish transformed F<sub>2</sub> "isolines".

We established 3 transformed lines with the K 6.13 construct and 9 lines with the K 6.15 construct. DNA and RNA analyses were carried out as previously described [6, 7] in order to record transformation and expression of the recombinant DNA.

### (b) Injection of plasmids into *Calliphora vicina* embryos

*Calliphora* eggs were collected 45 min after oviposition on fresh beef meat and individually trans-

Reprint requests to Prof. K. Scheller.

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ferred to a double stick scotch tape. Dechorionization was performed by carefully rolling them with the tips of a forceps. The embryos were removed from their ruptured chorions and transferred to a taped slide. Following desiccation for 5–10 min in a petri dish containing silica gel the embryos were covered with heavy mineral oil. They were injected as described above for *Drosophila* with a mixture containing the transformation vector K 6.13 and the helper plasmid  $\pi$  25.7 wc.

RNA- and DNA-extractions were carried out 1 h after injection as described [6, 7]. A thymidine-kinase (= TK) promoter containing sequence from *Herpes simplex* virus [9] and the coding region of *Calliphora* arylphorin gene (pc 223, Fig. 1) were  $^{32}$ P-labelled by random-priming (Boehringer, Mannheim) and served as hybridization probes. The TK-promotor was a gift of Dr. F. Grummt, Würzburg.

### (c) Plasmid constructs

**K 6.13:** This plasmid was constructed by cloning a 2.8 kb fragment of *Lambda* CV 50 [6] into the Sal I site of the *Carnegie* 20 vector [10]. The inserted *Lambda* fragment represents the 5'-end coding region (1.8 kb of the total 2.3 kb coding sequence) plus the promoter region up to -1 kb of the arylphorin gene (Fig. 1 a).

**K 6.15:** This construct contains an additional thymidine-kinase promoter of the TK-HSV I plasmid juxtapointed to the 5'-side of the arylphorin gene fragment (Fig. 1 b).

**$\pi$  25.7 wc:** This plasmid was used as a transformation helper that is itself unable to integrate but mediates the transposase activity [11].

## Results

### Transformation of *Drosophila* with the K 6.13 construct

About 2600 embryos were injected. The survival rate of first instar larvae was 14% and 3.5% of adults. Southern blot analysis revealed the stable integration of the 11 kb fragment f 13 (Fig. 1 a) into the germline of three *rosy*<sup>506</sup>. Due to a certain sequence-homology between the arylphorin genes from *Drosophila* and *Calliphora* [6] a hybridization signal could be observed in the uninjected *Drosophila* as well. The integration of the recombinant

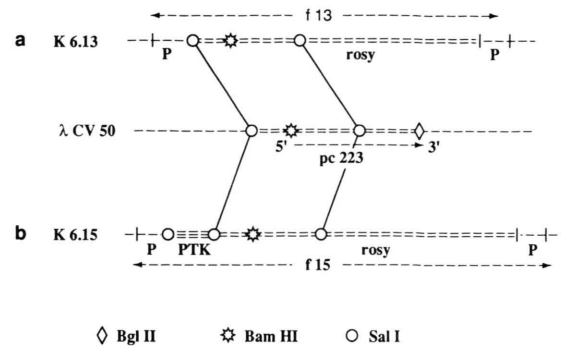


Fig. 1. Restriction maps of plasmids used for transformation of *Drosophila*. (a): K 6.13 plasmid with the Sal I-fragment of *Lambda* CV 50 inserted to the 5'-end of the *rosy* gene of the *Carnegie* 20 vector. P: Inverted terminal repeats responsible for the integration of f 13 into the genome. (b): K 6.15 plasmid containing the thymidine-kinase promoter (PTK) of *HSV* I near the 5'-end of the Sal I fragment. Only f 15 of the K 6.15 plasmid is integrated.

DNA is demonstrated by additional bands in the transformed lines (Lanes 2, 3 in Fig. 2).

Northern blot analyses of RNA from 3rd instar larvae of transformed flies showed no signals which indicate transcription of the inserted *Calliphora* gene fragment. In normal development the arylphorin biosynthesis is at a maximum during the early 3rd instar.

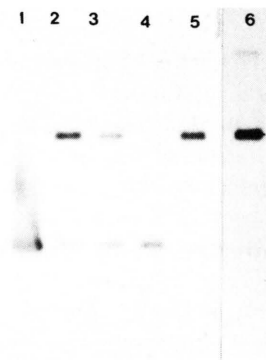


Fig. 2. Integration of *Calliphora* arylphorin genes into the germ-line of *Drosophila* by P-element transformation. Hybridization of DNA isolated from transgenic *F*<sub>2</sub> adults. Integration of f 13 (2, 3) and f 15 (5, 6) into Bam HI-restricted DNA of *F*<sub>2</sub> "isolines". (4): DNA from untreated *rosy*<sup>506</sup> mutants. (1): pc 233 control. Hybridization was carried out with the  $^{32}$ P labelled 5'-end of the pc 223 DNA fragment, including 1.8 kb of a *Calliphora* arylphorin gene and 1 kb 5'-end upstream sequences.

### Transformation of *Drosophila* with the K 6.15 construct

1700 embryos were injected. The survival rate of first instar larvae was 6.6% and 1.6% of adult flies. Southern blot analyses were carried out with the  $^{32}\text{P}$ -labelled coding region of the arylphorin gene. As can be seen from Fig. 2 (Lane 5 and 6) hybridization reveals the stable integration of the 12.9 kb DNA fragment f 15 of the K 6.15 construct (Fig. 1 b) into the germline of *rosy*<sup>506</sup>.

Northern blot analysis showed again no expression of the integrated gene despite of the presence of a strong promotor.

Surprisingly the surviving rate of the injected animals depends on the size of the inserted DNA fragment and appears to be sex-specific as shown in Table I.

Table I. Surviving rate of males and females in dependence of the size of the DNA injected.

Plasmid injected	Size of integrated DNA fragment	Ratio ♂/♀
Carnegie 20	7.2 kb	1:1.3
K 6.13	11.0 kb	1:1.5
K 6.15	12.9 kb	1:2.75

### Fate of injected plasmids in *Calliphora* embryos

As we could not find transcription of the inserted genes in the heterologous system of *Drosophila* we switched to the homologous system of *Calliphora*. 200 eggs per experiment received injections of K 6.13 and helper plasmid into the posterior end as described above.

As can be seen from Fig. 3a the injected DNA is not degraded until the 1st larval instar. In contrast to the transgenic *Drosophila* the injected DNA is transcribed in 1st instar *Calliphora* larvae (Fig. 3b). However, from the DNA analysis of adults it can be suggested, that the genome of *Calliphora* does not contain transposable elements comparable to the P elements of *Drosophila* (data not shown). The DNA injected into *Calliphora* embryos is obviously lost during metamorphosis.

### Discussion

Our interest in the arylphorin genes lies in identifying the controlling elements that regulate their

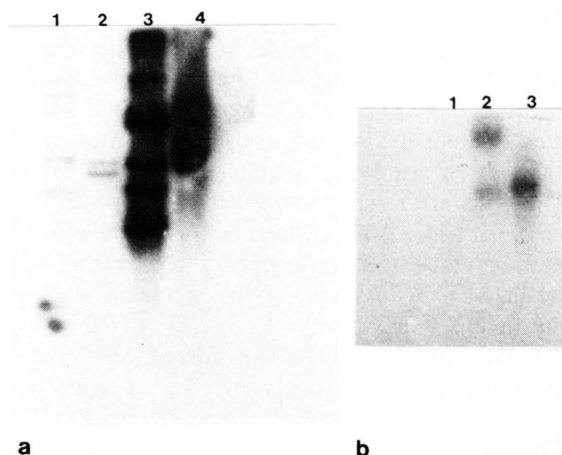


Fig. 3. Fate of plasmids injected into *Calliphora* embryos. (a): Hybridization of the  $^{32}\text{P}$ -labelled Carnegie 20 vector with Bam HI restricted DNA isolated from *Calliphora* larvae 1 h after ecdysis to the first larval instar (1, 2, 3). (4): DNA from uninjected embryos. (b): Transcription of the arylphorin gene construct K 6.13 after injection into *Calliphora* preblastoderm stages. RNA was extracted 1 h after emergence to the 1st larval instar (2). (1): RNA from uninjected embryos. (3): K 6.13 control. Hybridization was performed with the  $^{32}\text{P}$ -labelled Carnegie 20.

coordinate tissue- and developmental specific expression. The establishment of the methods for germ line transformation of *Drosophila* using P-element vectors offers a suitable means of studying *cis*-acting regulatory elements. The main advantage of this method compared with *in vitro* systems or cell cultures is the possibility of dissecting the function of genes in an intact organism. Although we were successful in creating transgenic *Drosophila* by integration of *Calliphora* arylphorin encoding genes into the germ-line and somatic cells of *rosy*<sup>506</sup> these genes were not transcribed. One crucial point in our experiments was, that we did not use endogenous regulatory elements of *Drosophila*, but those of *Calliphora*. So far we are not able to distinguish whether the non-expression of the transduced arylphorin genes depends on ineffective regulation sequences or the ability of the *Drosophila* genome to recognize DNA of a foreign organism.

The TK-promotor-arylphorin fusion gene was used to circumvent the mentioned difficulties. The

TK-promotor is a strong promotor, recognized by *Drosophila* in all tissues and all developmental stages [12]. Nevertheless, the integrated arylphorin gene of *Calliphora* is not expressed, indicating that either our constructs did not contain important transcription regulation sequences or the promotor regions are not recognized or suppressed by *Drosophila*. This question can be answered experimentally when the transcription controlling sequences of the *Calliphora* arylphorin genes will be identified by sequencing.

In our experiments the transferred genes contained 1 kb upstream sequences. The *cis*-regulatory sequences of the *Drosophila* arylphorins LSP1 $\alpha$  and LSP1 $\beta$  are localized between -377 and -471 bp upstream of the transcription start point [13, 14]. The question arises whether *trans*-activating factors controlling the *Calliphora* arylphorin gene activity are lacking in *Drosophila*. Preliminary experiments when nucleic extracts of *Calliphora* fat bodies were co-injected with the plasmids did not result in transcription.

Another point of view is, that every transferred gene can be expressed only under the control of a *Drosophila* promotor, for example the bacterial CAT-gene with a LSP1-promotor [14] or Neomy-

cin-resistance transferred by a vector containing the resistance gene and the *Drosophila* heat shock promotor [15].

The genome of *Calliphora* does not contain P-element like factors, but this fact does not exclude the possibility that foreign DNA can be integrated into the genome by another transposing mechanism. An example for a stable integration of DNA into the germline of another organism than *Drosophila* has been demonstrated by the transfer of G-418 (= neomycin analogue) resistance to the mosquito *Anopheles gambiae* [16]. In this case the transformation seems to be independent of P-elements.

During the development of the blowfly arylphorin genes are never expressed in the embryonic stages. However, arylphorin genes injected into preblastoderm stages are transcribed in embryos and postembryonal stages. Therefore, this system as well as that of P-element transformation of *Drosophila* appears to be highly suitable to study the interaction of specific promotor elements with nucleic protein factors *in vivo*. Detailed analyses using specific nucleic fractions from different developmental stages are currently underway in our laboratory.

- [1] K. Scheller, B. Fischer, and H. Schenkel, Molecular Insect Science (H. H. Hagedorn, J. G. Hildebrand, M. G. Kidwell, and J. H. Law, eds.), Plenum Press, New York 1991 (in press).
- [2] L. Levenbook, Comprehensive Insect Physiology, Biochemistry and Pharmacology (G. A. Kerkut and L. I. Gilbert, eds.), Vol. 4, 307–346, Pergamon Press, New York 1985.
- [3] U. Enderle, G. Käuser, L. Reum, K. Scheller, and J. Koolman, The Larval Serum Proteins of Insects (K. Scheller, ed.), pp. 40–49, Thieme Verlag, Stuttgart 1983.
- [4] G. Peter and K. Scheller, The Physiology of Insect Epidermis (A. Retnakaran and K. Binnington, eds.), Inkata Press, North Carolina, Australia 1991 (in press).
- [5] O. P. Agrawal and K. Scheller, Z. Naturforsch. **45c**, 129–131 (1990).
- [6] H. Schenkel, J. Keizlarová-Lepesant, P. Berreur, J. Moreau, K. Scheller, F. Brégégère, and J.-A. Lepesant, EMBO J. **4**, 2983–2990 (1985).
- [7] H. Schenkel and K. Scheller, Roux's Arch. Dev. Biol. **195**, 290–295 (1986).
- [8] A. C. Spradling, *Drosophila*, a Practical Approach (D. B. Roberts, ed.), pp. 175–197, IRL Press, Oxford 1986.
- [9] F. Colbere-Garapin, S. Chousterman, F. Horodniceanu, P. Kourilski, and A.-C. Garapin, Proc. Natl. Sci. U.S.A. **76**, 3755–3759 (1979).
- [10] G. M. Rubin and A. C. Spradling, Nuc. Acid Res. **11**, 6341–6351 (1983).
- [11] M. G. Karess and G. M. Rubin, Cell **38**, 135–146 (1984).
- [12] C. Maisonneuve and G. Echalié, FEBS-Letters **197**, 45–49 (1986).
- [13] S. J. Delaney, D. F. Smith, A. Mc. Clelland, C. Sunkel, and D. M. Glover, J. Mol. Biol. **189**, 1–11 (1986).
- [14] J. A. Davies, C. F. Addison, S. J. Delaney, C. Sunkel, and D. M. Glover, J. Mol. Biol. **189**, 13–24 (1986).
- [15] H. Steller and V. Pirotta, EMBO J. **4**, 167–171 (1985).
- [16] L.-H. Miller, R. K. Sakai, P. Romans, and R. W. Gwadz, Science **237**, 779–781 (1987).