Gene activity in the carnation-light synthetic lethal in *Drosophila melanogaster*

H. Nickla, T. Lilly and A. McCarthy

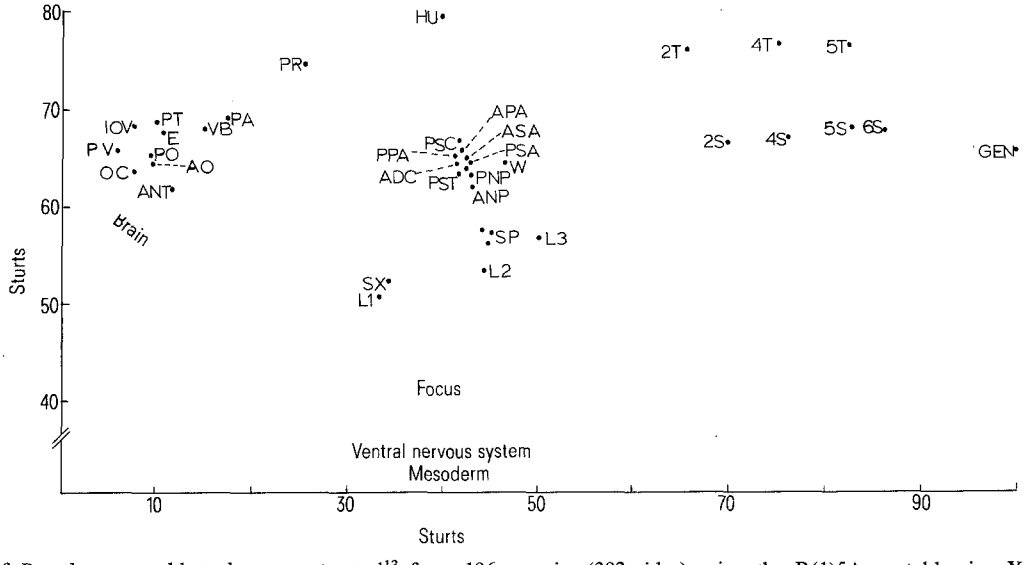
Department of Biology, Creighton University, Omaha (Nebraska 68178 USA), 2 July 1979

Summary. A gynandromorphic study of the carnation-light synthetic lethal revealed a site of lethal interaction (focus) in nerve tissue. Because both the carnation and light loci are known to be active in non-nervous tissue we have interpreted our results in terms of a model which accounts for diminution of developmental potential as a result of irreversible gene repression rather than selective gene activation.

Significant to an understanding of the mechanisms of development is a determination of the tissue specificity and developmental period of gene activity. The anatomical site(s) at which a function defined by a mutant gene exerts its primary effect is the focus for that gene[2]. A lethal focus defines an anatomical site at which a normal or lethal phenotype occurs depending upon whether cells at that site are genotypically normal or lethal respectively[3-5]. In chimeric[6] or mosaic[2-4,7] analyses a focus is definable in cases where a normal gene product, whether diffusible (non-autonomous) or restricted with respect to intercellular transport (autonomous), is produced in a particular cell type. Alternatively, a focus may not exist as such if a gene's normal product is produced by a variety of primary cell types. Among bilaterally symmetrical organisms 2 homologous foci could exist thereby providing the following possibilities for interaction: 1. a focus may be submissive such that a mutant phenotype results only when both homologous foci are genotypically mutant or 2. a focus may be domineering when presence of only 1 mutant focus (of a homologous pair) brings about a mutant phenotype[2]. It is generally accepted that a focus results from the inability of a 'tissue-specific gene' to provide a normal product[8].

By examining phenocritical phases (effective lethal phases) of a variety of lethal circumstances, the developmental period at which a normal gene product is required can be defined for a given focus[3,4,9]. We previously described maternally affected effective lethal phases of the carnation-light synthetic lethal in *Drosophila melanogaster*[10]. In this paper, we report the focus for the lethal interaction and interpret our findings in terms of gene activity during development. Interestingly, while the carnation and light gene products behave autonomously[11] and are known to be produced in a variety of tissues (eyes, Malpighian tubes, testis sheath, fat body) at times prior to the effective lethal phase, the lethal focus is in nerve tissue. This indicates that whereas cells of some tissues survive in the absence of normal (nonmutant) products of the carnation or light loci, the conditions of differentiation in nerve tissue are such that normal products of the carnation or light loci are required for a maintenance of cellular function. Our findings permit interpretations which contradict a generally accepted model which relates the time and place of gene activation with the phenocritical phase and focus of a genetic factor, respectively.

Carnation (car, position 62.5 on the X-chromosome) and light (lt, position 55.0 on the 2nd chromosome) are each fully viable eye-colour mutations which cause a general reduction in pteridines and ommochrome precursors in larvae and adults when compared to wild type[12]. A lethal interaction exists such that all car-lt double mutants (car/car; lt/lt and car/Y; lt/lt) die before eclosion. Maternal effects define effective lethal phases as larval or pupal depending on the number of maternal car^+ and/or lt^+ genes[10]. Mosaic analysis of the car-lt synthetic lethal is facilitated by the fact that car-lt pharate adults (fully developed adults dead with-



Fate map of *D. melanogaster* blastoderm constructed[13] from 196 mosaics (392 sides) using the R(1)5A unstable ring X-chromosome. Distances between paired sites are in sturts. Landmark and focus coordinates were computed using methods of Hotta and Benzer[2] and Flanagan[13]. Abbreviations: ADC, anterior dorsocentral bristle; ANP, anterior notopleural bristle; ANT, antenna; AO, anterior orbital bristle; APA, anterior postalar bristle; ASA, anterior supraalar bristle; E, eye; GEN, genital structures; HU, humeral bristles; IOV, vertical bristles; L, leg; OC, ocellar bristle; PA, palp; PNP, posterior notopleural bristle; PO, posterior orbital bristle; PPA, posterior postalar bristle; PR, proboscis; PSA, posterior supraalar bristle; PSC, posterior scutellar bristle; PST, presutural bristle; PT, postorbital bristle; PV, post-vertical bristle; SP, sternopleural bristles; SX, sex comb; S, sternite; T, tergite; Vb, vibrissae; W, wing.

in the puparium) are produced as offspring of car$^+$/car; lt$^+$/lt females, thereby allowing recovery of lethal (by dissection) and nonlethal mosaic organisms.

The car-lt lethal focus was determined by mosaic (gynandromorphic) fate mapping[2,13] using the R(1)5A, ywfB unstable ring X-chromosome[14,15]. Mosaics are generated when the R(1)5A chromosome (X_R) is not included in early mitotic nuclei of a developing female embryo. When such loss occurs 2 clones of nuclei result: one having a single X-chromosome thereby producing X/O male tissue and exposing recessive genes on the X-chromosome, the other containing nuclei with a X_R/X complement. An X-linked cuticular marker for twisted bristles and hairs (sn^3)[15] provides a method for determining the location of X/O tissue in pupal and adult mosaics. Mosaics eventually containing car-lt lethal tissue were produced by mating sn^{3}car/sn^{3}car; lt$^+$/lt females to X_R/B^{s}Y males[15]. Resulting sn^{3}car/X_R; lt$^+$/lt females were then mated to sn^{3}car/Y; lt$^+$/lt males thereby producing X_R-bearing female offspring of the control (C) and experimental (E) groups (table).

38 cuticular landmarks were scored to indicate X/O tissue on each side of each mosaic thereby yielding a total of 392 sides for fate mapping the blastoderm surface (figure). Positions of the landmarks and sturts (after Sturtevant)[2] are similar to other published fate maps[16]. Of the 196 mosaics examined, the average percentage of male tissue was 40.5% rather than 50% as is often observed[16]. Such a discrepancy may result from changes in genetic background in the X_R stock[13] induced by the initial cross needed to produce the sn^{3}car/X_R; lt$^+$/lt females. The focusing method of Flanagan[13] was used to mathematically compensate for a lack of typical 50:50 mosaics.

2 methods[2,13] were used to compute the focus coordinates for the car-lt lethal interaction (figure). Both methods provided a focus location in the ventral region of the blastoderm surface approximately 7 sturts from the blastodermal origins of the legs and 17 sturts from the midline. As indicated, the focus is in or near the origin of the ventral nervous system[13,17]. Our preliminary histological studies indicate abnormalities in the brain and ventral ganglion in that in 3rd-instar car-lt larvae the amount of fibrous core is reduced.

Discriminating between submissive or domineering aspects of the homologous foci was accomplished by comparing survival characteristics of mosaic organisms in which one or both sides of the ventral thorax contained sn^{3}car/O; lt/lt tissue. Adults were recovered in cases when both sides carried the nonlethal genotype whereas pupae (pharate adults) were recovered when one or both sides carried the lethal genotype. We therefore conclude that the car-lt interaction is domineering. The same interpretation was reached by comparing q-values using Flanagan's method[13].

Abnormal development is the result of the absence of a function provided by a particular gene product in a given tissue (or tissue array) and developmental period. What specifies these spatial and temporal requirements? Are tissue and time specific functions specified by attainment of a particular condition of differentiation such that a previously active gene attains functional significance? The latter interpretation is compatible with the irreversible gene repression model recently described by Caplan and Ordahl[18]. It is a model which accounts for diminution of developmental potential as a result of progressive irreversible repression rather than selective gene activation (derepression). Because car and lt loci are known to be active at the lethal focus in young 3rd-instar larvae, as evidenced by the survival of heterozygous types (car$^+$/car; lt/lt or car/car; lt$^+$/lt for example) from mothers with 1 car$^+$ or lt$^+$ locus[9], our results are incompatible with a model which relates the time of selective gene activation with the effective lethal phase.

Both car and lt loci are known to be active in cells derived from ectoderm (eyes)[15], mesoderm (fat body and gonad sheath)[19], and endoderm (Malpighian tubes)[20] at times prior to the effective lethal phase. In these tissues the state of differentiation must be such that car$^+$ and lt$^+$ gene products are spared. However, the focus determination for the car-lt lethal interaction indicates that nerve tissue uniquely requires car$^+$ or lt$^+$ gene products from the 3rd instar to late pupal interval[10]. An interpretation consistent with the gene repression model[18] would state that this unique requirement of nerve tissue is caused by progressive repression of genes capable of producing products which spare for the car$^+$ or lt$^+$ products. Thus, the phenocritical phase of this lethal circumstance is a developmental period at which a tissue (focus) reaches a state of genomic repression where a previously synthesized gene product attains vital significance by default.

X_R-bearing female zygotes from the cross: sn^{3}car/X_R; lt$^+$/lt females × sn^{3}car/Y; lt$^+$/lt males

Genotype	Non-mosaic or internal mosaic	Mosaic Pupal	Adult	Percent mosaic
Control: sn^{3}car/X_R; lt$^+$/lt sn^{3}car/X_R; lt$^+$/lt$^+$	962	10 (51.8)	138 (40.6)	13.3
Experimental: sn^{3}car/X_R; lt/lt	337	26 (57.9)	22 (14.7)	12.5*

Mean percentage of male tissue for each group is given in parentheses. * This value indicates that approximately 93% of the expected experimental mosaics were recovered.

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