The EMBO Journal vol.4 no.6 pp.1551-1557, 1985

Molecular cloning of *lethal(2)giant larvae*, a recessive oncogene of *Drosophila melanogaster*

Bernard M.Mechler[1], William McGinnis[2] and Walter J.Gehring

Department of Cell Biology, Biozentrum der Universität, CH-4056 Basel, Switzerland

[1]Present address: Department of Genetics, Johannes Gutenberg-Universität, Postfach 3980, D-6500 Mainz, FRG
[2]Present address: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, USA

Communicated by W.J.Gehring

Recessive mutations at the *lethal(2)giant larvae* (*l(2)gl*) locus of *Drosophila melanogaster* cause a complex syndrome, which has as its most striking features the development of malignant neuroblastomas in the larval brain and tumors of the imaginal discs. A chromosomal segment containing the *l(2)gl* gene has been cloned. Within this segment a transcription unit has been localized which is structurally changed in all *l(2)gl* alleles examined. The developmental profile of expression of the two RNAs (6 and 4.5 kb) made by this transcription unit coincides with the two major terminal phases of cell proliferation in the developing fly, namely, early embryogenesis and late third instar larvae. Tumors are produced when both normal *l(2)gl* alleles are inactivated by deletion or insertional mutation. The normal function of the *l(2)gl* presumably controls the normal cell proliferation of the optic centers of the brain and the imaginal discs, as well as their post-mitotic differentiation.

Key words: recessive oncogene/chromosome walking/mutation changes in DNA/transcription/telomere organization

Introduction

In *Drosophila melanogaster* at least 24 recessive genes have been identified which can cause tissue-specific tumors when mutated (Gateff, 1978, 1982). Of these the *lethal (2) giant larvae (l(2)gl)* gene is best studied. The first mutant allele at this locus was discovered by Bridges in 1933, and was mapped to the extreme left end of the second chromosome, in region 21A/B (Bridges and Brehme, 1944; Lewis, 1945). Subsequently, many spontaneous mutant alleles were isolated from wild and laboratory populations (Golubovsky, 1978, 1980; Green and Shepherd, 1979; Gateff and Schneiderman, 1967; Ising and Block, 1980). Thirty-five mutant alleles have been obtained through mutagenesis of laboratory stocks (Gateff and Mechler, unpublished). Mutations of the *l(2)gl* locus result in the tumorous growth of the imaginal discs and of the presumptive adult optic centers of the larval brain (Gateff and Schneiderman, 1969, 1974; Gateff *et al.*, 1977). In contrast to the well-characterized retroviral oncogenes and their cellular counterparts, which have a dominant mode of expression (Land *et al.*, 1983; Yunis, 1983), the *Drosophila* tumor genes identified so far show a recessive mode of inheritance. This suggests that the development of neuroblastomas and imaginal disc tumor in *Drosophila* results from a lack of gene function rather than an enhanced expression of the oncogene or an altered oncogene product. Such genes have recently been designated as anti-oncogenes (Knudson, 1983). Recent evidence indicates that recessive oncogenes or anti-oncogenes may also exist in vertebrates (Murphee and Benedict, 1984; Koufos *et al.*, 1984; Orkin *et al.*, 1984; Revee *et al.*, 1984; Fearon *et al.*, 1984). Most of the *l(2)gl* alleles are fully penetrant so that homozygosity invariably leads to tumor formation and death of the animal at late larval or early pupal stages. Besides the imaginal discs and brain, the growth and differentiation of various other tissues is also affected (Hadorn, 1955; Gloor, 1943; Grob, 1952). In particular, the ring gland is defective so that pupariation is delayed considerably (Hadorn, 1937; Scharrer and Hadorn, 1938; Aggarwal and King, 1969). However, this does not seem to be the primary mutant defect since neither the transplantation of normal ring glands into mutant larvae nor the injection of ecdysone can fully rescue the mutant animals (Hadorn, 1937; Karlson and Hauser, 1952).

We report here the cloning of the *l(2)gl* locus of *D. melanogaster* and a preliminary molecular analysis of the gene.

Results

Localization of clone α8 near the 2L telomere region

During a search for pole cell-specific RNA sequences, a genomic segment from the chromosomal region 21A was isolated from a *Drosophila* Charon-4 recombinant library (Maniatis *et al.*, 1978). The insert from this recombinant clone, designated *α8*, was localized to the region 21A by *in situ* hybridization of cloned DNA to polytene chromosomes from salivary glands (Pardue and Gall, 1975; Langer-Safer *et al.*, 1981). The insert also hybridized with reduced intensity to region 61A at the left end of chromosome 3 (3L). Further *in situ* hybridization experiments with subcloned fragments of the *α8* sequence showed that the left-most *Eco*RI fragment (coordinates 0.0-4.5 in Figure 1A) hybridizes to both the 2L and 3L telomeres. On Southern blots the same probe exhibited hybridization to a complex set of repetitive sequences in the *Drosophila* genome (data not shown). The rest of the *α8* insert represents a unique sequence, hybridizing only to the 21A region as judged from *in situ* hybridization (coordinates 4.5-16, Figure 1A). However, some internally repeated sequences exist within the right part of the *α8* insert.

Overlapping clones extending to the right of *α8* and spanning a total of 40 kb were isolated by chromosome walking (Figure 1B). Our attempts to obtain clones with single copy DNA to the left of the *α8* insert were unsuccessful due to a long region of repetitive DNA. To test whether some of the cloned sequences were deleted in an *l(2)gl* mutant stock suspected of being a chromosome deficiency (Müller and Gateff, personal communication), we first hybridized the *α8* clone to polytene chromosomes heterozygous for $l(2)gl^{4}$. A hybridization signal was detected only over the chromosome carrying the wild-type allele (Figure 2) indicating that the *α8* sequences are deleted in $l(2)gl^{4}$. However, when the 8014 clone, from the right end of the chromosomal walk, was used as a probe, a hybridization signal was observed over both homologues indicating that one of the breakpoints of

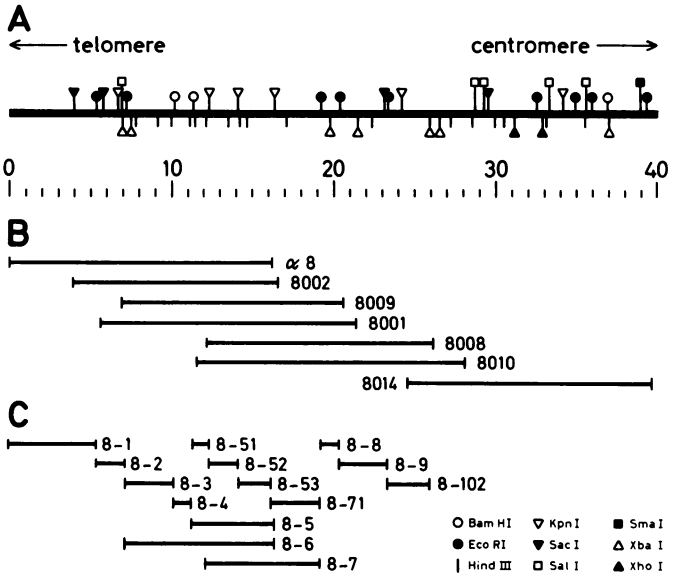

Fig. 1. Restriction map of the cloned *l(2)gl* region on chromosome 2. **(A)** A composite map of ~40 kb of DNA from the *l(2)gl* locus is shown. One unit in the coordinate scale below the map represents 1 kb. Coordinate 0 is chosen arbitrarily and lies at the left end of the cloned *Drosophila* DNA segment. The orientation of the physical map relative to the cytogenetic map is based upon the *l(2)gl U258* deletion whose left breakpoint is close to the telomere. **(B)** Overlapping array of *Drosophila* inserts found in recombinant phages isolated from the Canton-S library of Maniatis *et al.* (1978). Starting with the *α8* sequence, ~40 kb of genomic DNA were collected by chromosome walking (Bender *et al.*, 1983). **(C)** DNA fragments used as probes in Southern and Northern blot hybridization experiments and *in situ* hybridizations on polytene chromosomes of salivary glands.

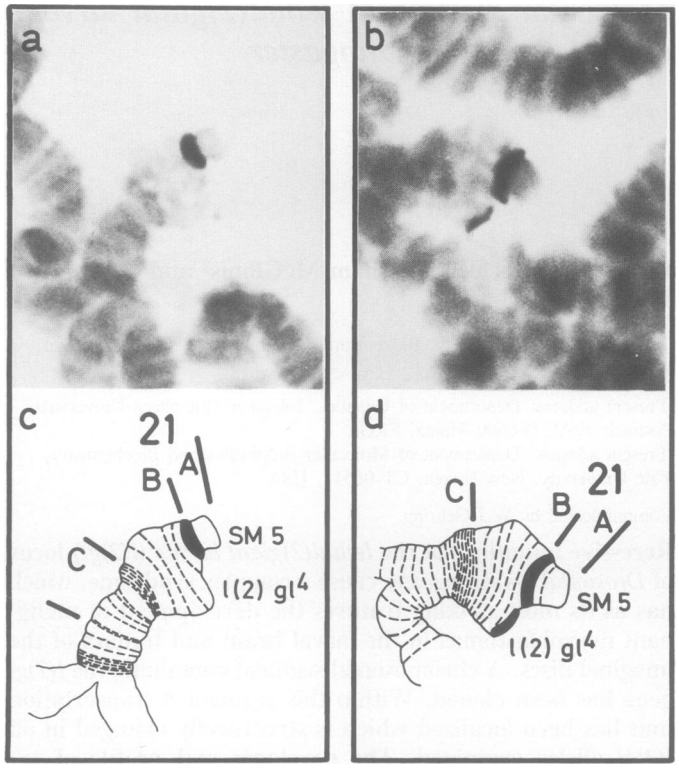

Fig. 2. *In situ* hybridization to the *l(2)gl*^{4} chromosome. **(A)** *α8* and **(B)** 8014 cloned DNAs were labelled with biotinylated nucleotide, hybridized to squash preparations of salivary gland chromosomes (Pardue and Gall, 1975) from heterozygote *l(2)gl*^{4}/*SM5* third instar larvae. The hybridizing site was visualized by an antibody procedure involving a final horseradish peroxidase enzyme reaction yielding dark deposits (see Materials and methods), the chromosomes stained with Giemsa and photographed. *SM5* is a multiple inverted second chromosome balancer (Lindsley and Grell, 1968). **(E)** and **(D)** Diagrammatic representation of the above **(A)** and **(B)** data, respectively, showing the sites of hybridization in relationship to the structure of the 21 region of the *l(2)gl*^{4}/*SM5* polytene chromosome.

the *l(2)gl*^{4} deletion has been passed (Figure 2).

Using either the clones *α8*, 8010, 8014 or subclones of these inserts (Figure 1C) a series of *in situ* hybridizations to polytene chromosomes of other *l(2)gl* alleles (listed in Table I) were performed. By these criteria each of the mutant alleles, *l(2)gl*^{1}, *l(2)glGB26* and *l(2)gl DV110*, lacks part of the cloned region. Furthermore, the entire cloned region was absent in the *Df(2)gl net 62, Df(2)gl net 78i30*, and *TE75* chromosomes. These *in situ* hybridization studies indicate that the cloned region in 21A is in or near the *l(2)gl* gene.

Southern blot analysis of l(2)gl mutant alleles

For a more detailed study of the *l(2)gl* region, DNA from homozygous *l(2)gl* mutant alleles and from the wild-type Ore-R stock was analyzed by the Southern blot method. Each DNA sample was analyzed with four different enzymes, *Eco*RI, *Hind*III, *Bam*HI and *Kpn*I. Nitrocellulose filters carrying digested and fractionated DNA were hybridized with various subfragments from the cloned region. A test of the above four enzymes on the DNA from Ore-R and Canton-S (another wild-type stock) showed no differences in restriction sites in this region (a map is shown in Figure 1A).

In contrast Southern blot analysis of the *l(2)gl* alleles showed large DNA aberrations in the cloned region. Almost all were deletions of various lengths. In the case of the *l(2)gl U314* mutant allele, the deletion was found to eliminate the entire cloned region, while 14 other *l(2)gl* chromosomes have one detectable breakpoint within the region (Figure 3). The *l(2)gl U334* chromosome represents the most distal deletion in the cloned area beginning with coordinates 10.2 and 10.4 and eliminating DNA to the left end of the cloned region. The identification of the left

Table I. Origin of the *l(2)gl* mutant alleles

Allele	Origin		References
l(2)gl^{1}	Spontaneous, Lab. population	1933	1
l(2)gl^{4}	Spontaneous, Lab. population	1967	2
l(2)gl^{6}	EMS-induced, Lab. population	1982	3
l(2)gl M25	Magarach, Crimea, USSR	1967	4
l(2)gl GB26	Sonoma County, CA, USA	1978	5
l(2)gl GB52	Sonoma County, CA, USA	1978	5
l(2)gl DV110	Sedenka, Far East, USSR	1971	6
l(2)gl U138	Uman,Ukraine, USSR	1963	4,6
l(2)gl D150	Dilizhan,Armenia, USSR	1969	4,6
l(2)gl U258	Uman, Ukraine, USSR	1970	7
l(2)gl DV271	Sinii Gay, Far East, USSR	1971	4,6
l(2)gl DV275	Sinii Gay, Far East, USSR	1971	4,6
l(2)gl U314	Uman, Ukraine, USSR	1965	4,6
l(2)gl U334	Uman, Ukraine, USSR	1965	4,6
l(2)gl U353	Uman, Ukraine, USSR	1965	4,6
l(2)gl E432	Erevan, Armenia, USSR	1965	7
l(2)gl U558	Uman, Ukraine, USSR	1970	4
Df(2)lgl net^{62}	X-ray induced, Lab. population	1974	8
Df(2)lgl net$^{78i30(12)}$	Male recombination induced	1978	5
TE 75	Spontaneous, in TE, Lab. stock	1980	9

References: 1: Bridges and Brehme, 1944. 2: Gateff and Schneidermann, 1967. 3: E.Gateff, personal communication. 4: Sokolova and Golubovsky, 1979. 5: Green and Shepherd, 1979. 6: Gateff *et al.*, 1977. 7: Plus and Golubovsky, 1980. 8: Korochkina and Golubovsky, 1978. 9: Ising and Block, 1980.

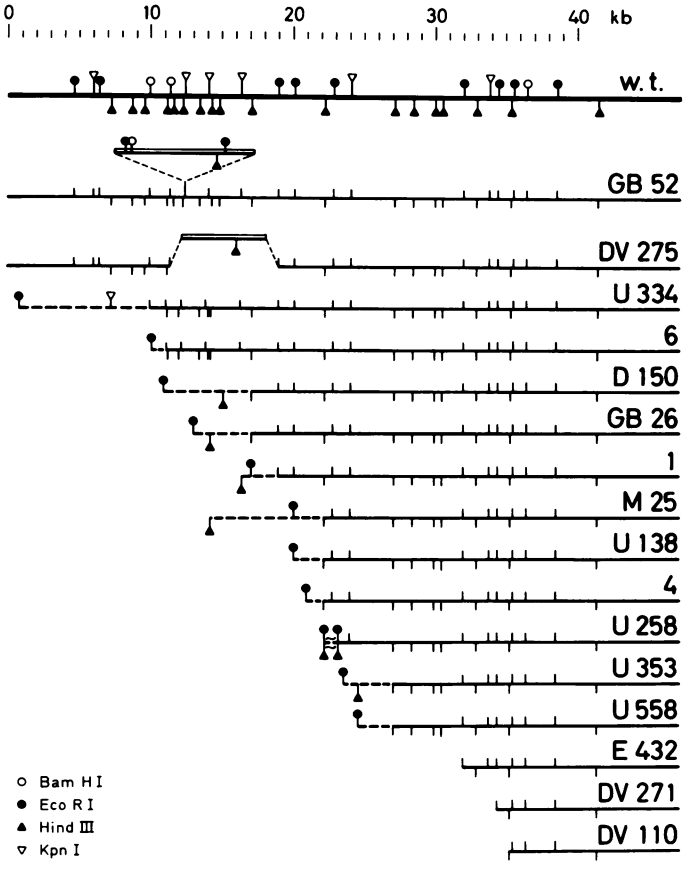

Fig. 3. Chromosomal DNA sequence organization of 16 mutant *l(2)gl* alleles. These molecular maps based on Southern genomic blotting experiments show the DNA aberrations characterizing the examined *l(2)gl* mutant alleles. *l(2)gl GB52* was found to contain only an insertion element of ~10 kb whereas *l(2)gl DV275*, in addition to an insertion of ~6.5 kb, is characterized by an 8-kb deletion. All the other *l(2)gl* mutant alleles are made of larger deletions for which only the presumed proximal breakpoint has been identified. In the upper part of the panel the wild-type coordinate restriction map of the *l(2)gl* locus is shown. In the lower part the restriction maps of the mutant *l(2)gl* alleles are presented. Restriction cleavage sites similar to the wild-type DNA are only indicated by a vertical bar whereas new restriction sites are indicated by symbols. Dashed lines represent non-homologous DNA that extend beyond the breakpoint of the deletions. The open bars in the *l(2)gl GB52* and *l(2)gl DV275* represent the insertions.

breakpoint of these deletions was not possible due to the failure to clone sequences to the left of the $\alpha 8$ insert. On the basis of the above structural evidence, we concluded that the *l(2)gl* locus resides either within the left part of the cloned region, eliminated by the smallest *l(2)gl* deficiencies, or else to the left of the cloned region.

A more precise molecular localization of the *l(2)gl* locus can be inferred from the structural alterations in the *l(2)gl DV275* and *l(2)gl GB52* chromosomes. The *l(2)gl DV275* allele (Figures 3 and 4) is associated with a relatively small deletion contained within the cloned region. This deletion eliminates DNA between coordinates 11.4 and 19.3. No hybridization signal was detected on Southern blots with either probe 8-7 (Figures 1C and 4, *Hind*III lane C1 and *Eco*RI lane C1) or probe 8-5 (not shown). As judged by the size of these probe sequences, the deletion extends over ~8 kb. The flanking sequences are unchanged (*Hind*III lanes A1 and 3; *Eco*RI lanes D1 and 3). However, in addition to this 8-kb deletion, there is also an insertion of heterologous DNA estimated of a minimal size of 6.5 kb. This is indicated by the size of the *Eco*RI fragment (shown in lane B1) which is only ~1.5 kb shorter than the respective fragment in the wild-type.

The *l(2)gl GB52* allele (Figures 3 and 4) is characterized by a 10-kb insertion of heterologous DNA between coordinates 12.2 and 12.4. The size of the insertion was deduced from the size of the new *Kpn*I fragment spanning this region which is 5.7 kb in wild-type DNA and ~16 kb in the *l(2)gl GB52* chromosome (Figure 4 *Kpn*I lanes E2 and 3). Southern blot analysis of *Bam*HI (Figure 4, *Bam*HI lanes E2 and 3) and *Hind*III sites in the region indicates that each enzyme cleaves within the inserted DNA. The sum of the two new DNA fragments is ~10 kb larger when compared with the wild-type fragment spanning the insertion site. The localization of the insertion was inferred from the Southern blot data showing that the insertion is contained within a 1.4-kb *Hind*III fragment (coordinates 12.4 – 14.2). On the basis of the structural alterations observed in the mutants *l(2)gl GB52* and *l(2)gl DV275*, we would conclude that the *l(2)gl* locus is contained in the cloned region.

Position of the telomere

Provided that we can identify a telomere in the vicinity of one of the *l(2)gl* deletion breakpoints, we should be able to orient the cloned DNA fragment. On the basis of both cytological and molecular analysis no definite orientation can be assigned to the cloned fragment for most of the mutant alleles. However, the *l(2)gl U258* mutant presents all the characteristics of a 'subterminal' deletion with the loss of all sequences between the cloned region and the telomere. The analysis of the restriction fragments, immediately adjacent to the deletion breakpoint, indicates first that all analyzed distal restriction sites were clustered at the same site. Furthermore, the distal restriction fragments showed an obvious size heterogeneity which is particularly visible when the fragments are small (see *Hind*III and *Xba*I digests in Figure 5) and also shows increased sensitivity to *Bal*31 exonuclease treatment (results not shown). Similar observations have been made with telomeres in trypanosomes (DeLange and Borst, 1982; Bernards *et al.*, 1983). Thus we believe that the 2L telomere lies adjacent to the left breakpoint of the *U258* deletion at a distance of 1 – 2 kb. This observation allows us to orient the cloned DNA fragment on the chromosome (Figure 1).

Transcription of the l(2)gl locus

On the basis of the *l(2)gl* mutant DNA rearrangements we tentatively assigned the locus to the region between coordinates 5 and 20. Consequently we examined the transcripts produced from this region and the surrounding sequences. A series of radioactively labelled DNA probes, spanning ~24 kb, were hybridized to Northern blots prepared from electrophoretically fractionated RNAs isolated from sequential stages of *Drosophila* development (Goldberg, 1980) (Figure 6A). These experiments showed two transcribed regions. The first mapping between the two *Eco*RI cleavage sites at 7.3 and 19.4 is in a region disrupted in all *l(2)gl* mutant chromosomes and thus appears to be the *l(2)gl* transcription unit. The second transcription region is disrupted in only some *l(2)gl* mutant chromosomes and is to the right of coordinate 21. This second region shows two transcripts of 4.7 and 4.0 kb. The analysis of the limit of the *l(2)gl* transcription unit is complicated by an internal repeated sequence occurring at least twice within the locus. The sequence is within the 71 probe (Figure 6A) and also in the right-most *Bam*HI-*Eco*RI fragment of $\alpha 8$ (coordinates 11.2 – 16.1, Figure 1). Therefore, the hybridization of the transcripts to the 71 probe could be due to cross-hybridization to the repeat sequences from the left part of the transcribed region.

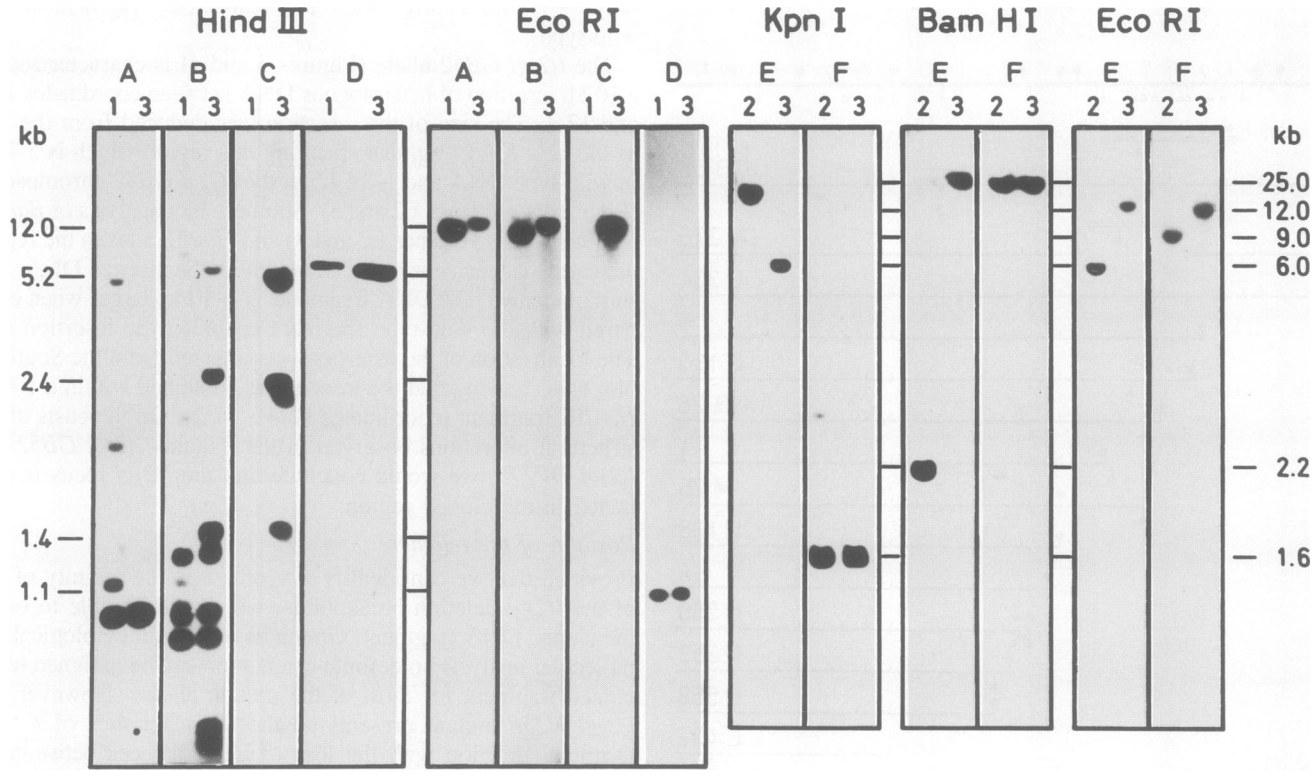

Fig. 4. Analysis of the DNA rearrangements associated with the *l(2)gl DV275* and *l(2)gl GB52* mutations. High mol. wt. DNAs (3 μg/lane) extracted from homozygous *l(2)gl DV275* **(lane 1)** and *l(2)gl GB52* **(lane 2)** giant larvae and from wild-type Oregon-R **(lane 3)** third instar larvae were digested with the indicated restriction enzymes, run on 0.7% agarose gel and analyzed by Southern blot hybridization using DNA fragments isolated from *α8* and 8001 as probes (see Figure 1). In **A:** 8-4; **B:** 8-6; **C:** 8-7; **D:** 8-8; **E:** 8-51 and **F:** 8-52. The minor hybridization bands detected with the 8-4 probe in the *l(2)gl DV275* DNA *Hind*III digest **(lane A1)** can be assigned to partially digested fragments. The 5.2-kb wild-type DNA-*Hind*III fragment weakly labelled by the 8-5 probe **(lane B3)** corresponds to a cross-hybridizing repeat sequence contained within the *l(2)gl* locus and present in 8-7 **(lane C3)**.

Transcripts from the *l(2)gl* unit fall into two size classes of ~6 and 4.5 kb. The *l(2)gl* transcripts are developmentally regulated, as shown in Figure 6B, with the most abundant expression occurring during early embryogenesis (0–6 h) and in late third instar larvae. Although both transcripts were detected during these two periods, their relative proportion varies considerably. The 4.5-kb sequence represents the major form transcribed during early embryogenesis whereas the 6-kb transcript is predominantly synthesized during late third instar larvae. During embryogenesis, a consistent smear of hybridization signal is observed in the lower molecular range. This smear may represent some specific endogenous degradation since probing the same filter with cDNAs complementary to *fushi tarazu* (1.9 kb) and *Antennapedia* (3.4 and 5.0 kb) (Kuroiwa *et al.*, 1984) did not show any smearing.

We have also isolated a cDNA clone homologous to the left end of the transcription unit. It was isolated from the pupal cDNA library of M.Goldschmidt-Clermont which was constructed using a poly(dT) primer on a poly(A)$^+$ RNA template. The 1.3-kb cDNA probe detected only the 6-kb RNA from the assigned *l(2)gl* transcription unit and is entirely contained within the 1.4-kb *Hind*III fragment between coordinates 7.8 and 9.2.

This cDNA clone allows us to place tentatively the distal limit of the *l(2)gl* gene in the proximity of the *Hind*III cleavage site at coordinate 7.8 assuming that the cDNA represents the 3′ end of the 6-kb transcript.

Discussion

On the basis of the structural disruptions of the cloned region in all the *l(2)gl* mutant chromosomes we would conclude that it contains the *l(2)gl* locus. The 6- and 4.5-kb transcripts homologous to the region, which is deleted or interrupted in all *l(2)gl* chromosomes, presumably represent the major RNA products of the locus. We have recently obtained (M.Opper, G.Schuler and B.Mechler, in preparation) final proof that the cloned region contains the entire *l(2)gl* gene using the method of germ line transformation (Rubin and Spradling, 1982), with a P-transposon containing the 12.1-kb *Eco*RI fragment between coordinates 7.2 and 19.3.

One striking property of all *l(2)gl* mutant chromosomes was the gross nature of the DNA alterations, almost all being large deletions. The only exception is *l(2)gl GB52* which has an insertion of heterologous DNA. It is well known that the *l(2)gl* locus is easily mutable in wild populations (Golubovsky, 1980). Recent results showed also a high mutation rate after ethyl methanesulfonate mutagenesis in laboratory stocks (Gateff and Mechler, unpublished). The nature of the *l(2)gl* mutant chromosomes that we have described, suggests that almost all these mutants are deletions occurring near the telomere. This could be explained if we assume that the *l(2)gl* gene region was a preferred site for transposable element integration with a successive uni- or bi-directional excision as has been proposed by Green (1982). A second possibility is that the telomeric region itself is unstable and undergoes spontaneous deletions at a frequency higher than that of more proximal regions.

It is known that the cytology of the *Drosophila* telomeres varies in different stocks, presumably indicating chromosomal variability at the DNA level (Roberts, 1979). On a molecular scale the telomeric regions of trypanosomes also undergo periodic expansion and diminution (Bernards *et al.*, 1983). This process may not

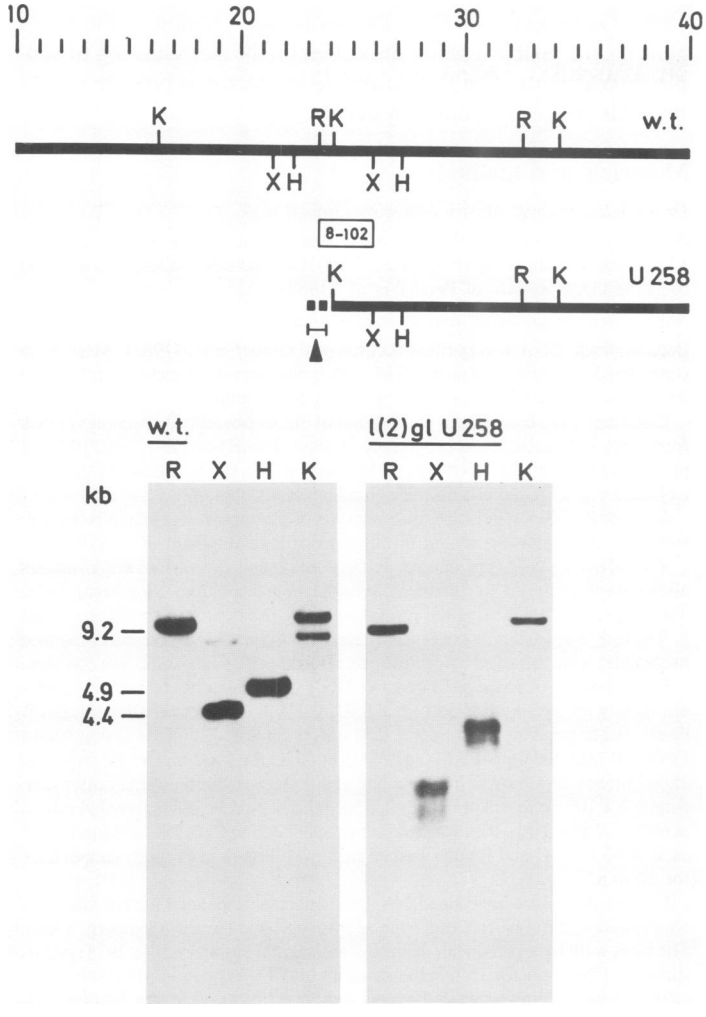

Fig. 5. Evidence that the *l(2)gl U258* deletion maps close to the telomere. High mol. wt. DNA (3 μg/lane) extracted from homozygous *l(2)gl U258* giant larvae and from wild-type Oregon-R third instar larvae were digested with *Eco*RI (R), *Xba*I (X), *Hind*III (H) or *Kpn*I (K) restriction enzymes, run on a 0.7% agarose gel and analyzed by Southern blot hybridization (as described in Figure 4) using the 8-102 DNA fragment isolated from 8008 (see Figure 1C). In the upper part of the panel the *l(2)gl U258* restriction map and the coordinates of the $l(2)gl^+$ locus are represented. The deletion breakpoint characterizing *l(2)gl U258* can be placed in the immediate vicinity of the *Kpn*I cleavage site (coordinate 24.3) or just to the left of it, since no second hybridizing *Kpn*I fragment could be identified. The arrow indicates a cluster of restriction sites (*Bam*HI, *Eco*RI, *Hind*III and *Xba*I) which are contained within a 0.8-kb stretch of DNA (dashed line). When the terminal restriction fragments are small enough (*Xba*I or *Hind*III), they show a size heterogeneity which is characteristic of telomeric sequences (DeLange and Borst, 1982; Bernards *et al.*, 1983; Raibaud *et al.*, 1983).

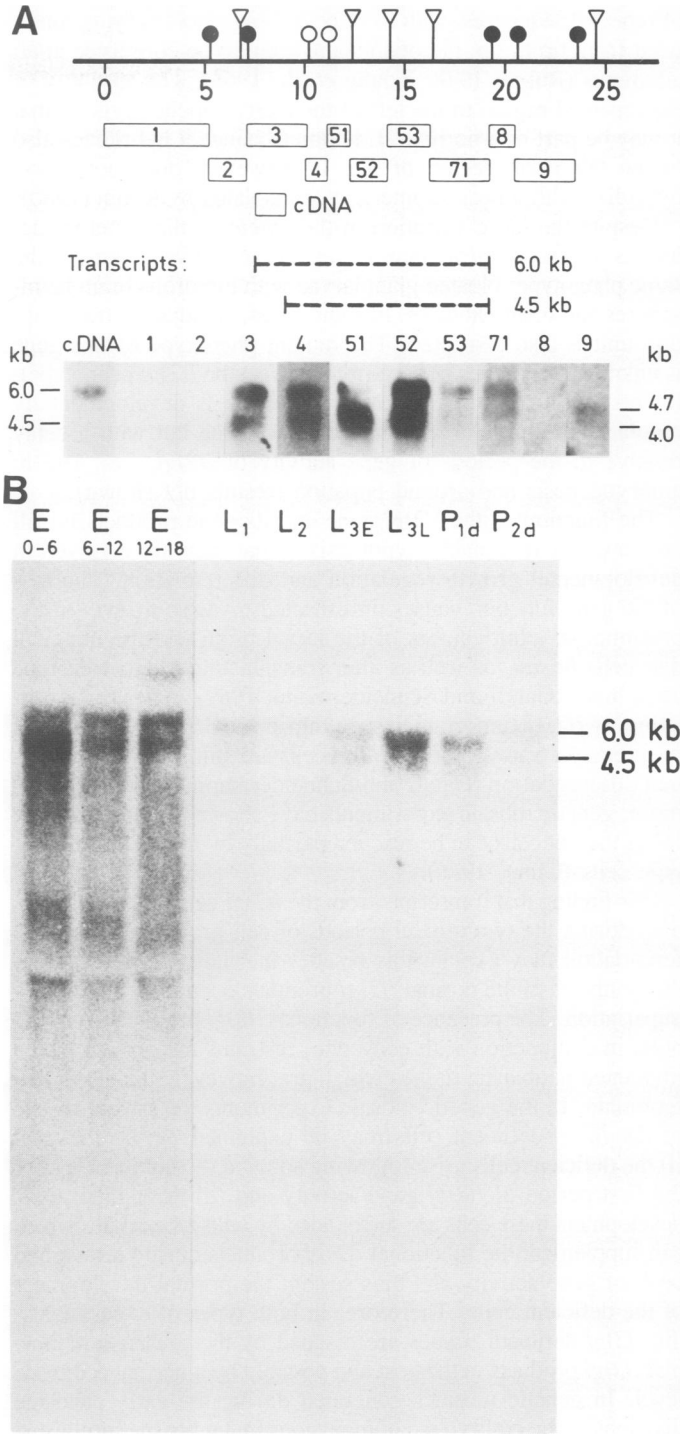

Fig. 6. Transcripts from the *l(2)gl* locus. A map of the *l(2)gl* locus is shown in **(A)**. Positioned below the map are the restriction fragment subclones used as probes of Northern blots of late third instar larval RNA. Autoradiographic exposures of the blots are shown at the bottom of **(A)**. The map position of a cDNA clone described in the text is also indicated. The approximate size of the transcripts from the locus is indicated alongside the lanes. The apparent hybridization of the 71 region to trancripts from the locus may be due to a repeated sequence within this fragment that is also present in the left part of the locus in the 8-5 clone (Figure 1). **(B)** This panel shows a developmental Northern blot probed with the 8-6 subclone (Figure 1) which includes most or all of the RNA coding sequence at the locus. Embryonic stages (0–6 h, 6–12 h and 12–18 h) are designated by (E). Larval stages, 1st, 2nd, early 3rd and late 3rd are designated by (L). Pupal stages (1 day and 2 day old) are designated by (P). Each lane contained 10 μg of total RNA. The largest band in the 12–18-h-old embryonic RNA is due to DNA contamination of the RNA.

be precise, occasionally eliminating nearby single copy coding sequences. We envisage that this telomeric trimming may also take place on *Drosophila* chromosomes, thus creating deletions near the telomere of chromosome 2 which may remove all or part of the *l(2)gl* gene. In this case, one must question the evolutionary strategy of retaining a gene which can mutate to a lethal phenotype at the chromosome tip. However, some authors have suggested that the *l(2)gl* heterozygotes are more fit for certain ecological niches (Plus and Golubovsky, 1980; Golubovsky and Sokolava, 1973).

The telomeric sequence adjacent to the *l(2)gl U258* deletion breakpoint suggests that a *Drosophila* telomere need not be an extremely complicated structure. Most studies on the sequences of *Drosophila* telomeres or near-telomeres suggest long stretches

of repeated sequences, with the repeated sequences varying somewhat from tip to tip, although some seem to be conserved at all telomeres (Rubin, 1978; Young *et al.*, 1983). The structure of the repeated region to the left of the $l(2)gl^+$ locus suggests that it may be part of a normal 2L telomere, since it hybridizes also to the telomeric region of 3L. It, however, does not cross-hybridize with a near telomeric clone isolated by Rubin (1980).

Despite the large variation in the extent of the different deletions all the *l(2)gl* mutant alleles so far analyzed present the same phenotype: bloated giant larvae with tumorous brain hemispheres and imaginal discs. In some cases, an adjacent transcription unit is also disrupted. The mutant phenotype of this gene is unknown (and does not apparently affect the *l(2)gl* phenotype). Interestingly the transcription of this gene is developmentally regulated in a similar way to the *l(2)gl* gene but with a delay relative to the periods of gene activity of *l(2)gl*, i.e., late in embryogenesis and around pupation (results not shown).

The function of the *l(2)gl* gene and its gene products is still unknown. A reasonable hypothesis is that they are involved in developmental growth regulation and differentiation. The loss of the gene function causes unrestricted invasive growth of the presumptive adult centers of the larval brain and the imaginal disc cells *in situ*, as well as after transplantation into wild-type adult hosts (Gateff and Schneidermann, 1969). After transplantation of *l(2)gl* tumorous tissues into normal larvae, abnormal proliferation ceases, but the *l(2)gl* cells are still incapable of normal differentiation (Gateff and Schneidermann, 1974). Furthermore, genetic mosaic experiments have shown that the deficient *l(2)gl* mutant cells can be rescued partially by neighboring wild-type cells (Cline, 1976).

Our finding that transcripts from the *l(2)gl* gene are most abundant during the two critical periods of cell growth and cell differentiation may explain this rescue. In wild-type host larvae, the synthesis of the normal *l(2)gl* products occurs shortly before pupariation. The presence of functional *l(2)gl* products from the host, in conjunction with ecdysone, restrains the growth of the implanted neoplastic tissues which are, however, unable to differentiate. In the genetic mosaic experiments the partial rescue of the *l(2)gl*-deficient cells may be explained by the fact that (i) the deficient cells arise following somatic recombination after the first period of *l(2)gl* gene activity and (ii) throughout their development these cells are surrounded by wild-type tissues which can supplement the functional *l(2)gl* products during the second peak of gene activity and thus sustain the normal development of the deficient cells. Therefore, in both types of experiments, the *l(2)gl*-deficient tissues are rescued by the presence of normal *l(2)gl* products in the host and possibly by a normal ecdysone level. In genetic mosaics generated during the early cleavage divisions in the embryo the homozygous mutant tissue rarely survives (Cline, 1976).

It was assumed that the *l(2)gl* phenotype results in part from an ecdysone deficiency since the ring gland of *l(2)gl* homozygotes appears hypoplastic (Hadorn, 1937; Scharrer and Hadorn, 1938; Aggarwal and King, 1969). Moreover, implantation of wild-type ring glands (Hadorn, 1937) or injection of ecdysone extracts (Karlson and Hauser, 1952) accelerates the puparium formation of *l(2)gl* larvae. However, the hypoplasia of the ring gland is probably a secondary effect of the *l(2)gl* mutation, resulting from the morphological and cellular disorganization of the brain in which the neurosecretory cells and their axons become disconnected from the prothoracic cells of the ring gland (Akai, 1975; Klose *et al.*, 1980). Furthermore, ecdysone deficiency alone is not sufficient to induce neoplastic transformation (Klose *et al.*, 1980; Garen *et al.*, 1977). The molecular cloning of the *l(2)gl* gene should enable a better dissection on the steps leading to neoplastic transformation in *Drosophila* and perhaps provide insight into this process in other systems.

Materials and methods

Drosophilia genomic library and chromosomal walk

A *Drosophila* Canton-S DNA library cloned in Charon-4 was obtained from J.Lauer and T.Maniatis (Maniatis *et al.*, 1978). Chromosome walking was achieved by using the procedure of Bender *et al.* (1983).

Nucleic acid preparations and molecular analysis

Bacteriophage DNA was purified according to Garber *et al.* (1983). Maps were constructed by single, double and triple DNA digestions with enzymes purchased from New England Biolabs and Boehringer Mannheim.

Subclones were constructed by isolation of electrophoretically separated DNA fragments and ligation to appropriately cleaved (*Bam*HI and/or *Eco*RI) pBR322 plasmid DNA with T4 DNA ligase (Maniatis *et al.*, 1982). Plasmid DNA was isolated by CsCl/ethidium bromide gradient centrifugation.

Drosophila DNA was isolated from larvae of the appropriate genotype by collecting ~300 larvae according to the procedure of Bingham *et al.* (1981).

The DNAs of wild-type Oregon R *Drosophila* and the various *l(2)gl* mutant alleles were analyzed by Southern hybridization techniques (Southern, 1975; Maniatis *et al.*, 1982) using the probes and enzymes described in Figures 4 and 5. The nick-translated protocol of Rigby *et al.* (1977) was used, and unincorporated nucleotides were removed by centrifugation through Sephadex G50 fine columns.

Hybridizations of *Drosophila* genomic DNA blots were done as follows. Southern blots were pre-hybridized in 5 x SSC, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 250 μg/ml sonicated, boiled herring sperm DNA, 50 mM $NaPO_4$ pH 7.0, 0.1% SDS at 68°C for 3–4 h. The pre-hybridization buffer was removed from the bag and replaced with the same buffer containing 2 x 10^6 c.p.m./ml of ^{32}P-labelled hybridization probe. Blots were hybridized at 68°C for 18–20 h, then washed four times in 2 x SSC, 0.1% SDS for 15 min each at 65–70°C and finally washed in 3 mM Tris-base at room temperature for 30 min.

For Northern blot analysis, RNA from successive developmental stages of *Drosophila* was run on formaldehyde agarose gels and blotted (Goldberg, 1980). The blots were hybridized with the nick-translated (Weinstock *et al.*, 1978) probes shown in Figure 6A for 36 h in the stringent buffer described in McGinnes *et al.* (1984). After a stringent wash the blots were used to expose X-ray film for 24 h at −70°C with an intensifying screen.

Drosophila culturing and chromosome preparations

Flies were raised on a cornmeal, sucrose, dried yeast and agar medium at 25°C for normal crosses and mass growth and at 18°C for obtaining larval salivary glands to prepare polytene chromosomes.

Cloned DNA sequences were mapped to cytological positions of normal and mutant polytene chromosomes by *in situ* hybridization (Pardue and Gall, 1975) using DNA probes containing biotinylated dUTP. The hybridizing sites were immunologically detected in association with a diaminobenzine staining (Langer-Safer *et al.*, 1981).

Acknowledgements

We thank Drs Michael Golubovsky, Mel Green, Elisabeth Gateff and Nadine Plus for providing the *l(2)gl* mutant stocks; Elisabeth Gateff and Iain Mattaj for critical reading of the manuscript and constructive suggestions; Dr Michael Levine for stimulating discussions; Mrs Ingrid Schuster for preparation of the illustrations and Erika Wenger-Marquardt for assistance in preparing the manuscript. This work was supported by the Swiss National Science Foundation and the Kanton Basel-Stadt. W.McG. was supported by a Jane Coffin Childs fellowship.

References

Aggarwal,S.K. and King,R.C. (1969) *J. Morphol.*, **129**, 171-200.
Akai,H. (1975) *The Cell (Japan)*, **4**, 38-41.
Bender,W., Akam,M., Karch,F., Beachy,P.A., Pfeifer,M., Spierer,P., Lewis, E.B. and Hogness,D.S. (1983) *Science (Wash.)*, **221**, 23-29.
Bernards,A., Michels,P.A.M., Linke,C.R. and Borst,P. (1983) *Nature*, **303**, 592-597.
Bingham,P.M., Lewis,R. and Rubin,G.M. (1981) *Cell*, **25**, 995-1004.
Bridges,C.B. and Brehme,K.S. (1944) *Carnegie Inst. Wash. Publ.*, **552**.
Cline,T.W. (1976) *Genetics*, **83**, s16.
DeLange,T. and Borst,P. (1982) *Nature*, **299**, 451-453.
Fearon,E.R., Vogelstein,B. and Feinberg,A.P. (1984) *Nature*, **309**, 176-178.

Garber,R.L., Kuroiwa,A. and Gehring,W.J. (1983) *EMBO J.*, **2**, 2027-2036.
Garen,A., Kauvar,L. and Lepesant,J.A. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5099-5102.
Gateff,E. (1978) *Science (Wash.)*, **200**, 1448-1459.
Gateff,E. (1982) *Adv. Cancer Res.*, **37**, 33-74.
Gateff,E. and Schneidermann,H.A. (1967) *Am. Zool.*, **7**, 760.
Gateff,E. and Schneidermann,H.A. (1969) *Natl. Cancer Inst. Monogr.*, **31**, 365-397.
Gateff,E. and Schneidermann,H.A. (1974) *Whilhelm Roux's Arch. Dev. Biol.*, **176**, 23-65.
Gateff,E., Golubovsky,M.D. and Sokolova,K. (1977) *Dros. Inf. Serv.*, **52**, 128-129.
Gloor,H. (1943) *Rev. Suisse Zool.*, **50**, 339-394.
Goldberg,D.A. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5794-5799.
Golubovsky,M.D. (1978) *Dros. Inf. Serv.*, **53**, 179.
Golubovsky,M.D. (1980) *Genetica*, **52/53**, 139-149.
Golubovsky,M.D. and Sokolova,K.B. (1973) *Dros. Inf. Serv.*, **50**, 124.
Green,M.M. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 5367-5369.
Green,M.M. and Shepherd,S.H.Y. (1979) *Genetics*, **92**, 823-832.
Grob,H. (1952) *Z. Ind. Abst. Vererb. lehre*, **84**, 320-360.
Hadorn,E. (1937) *Proc. Natl. Acad. Sci. USA*, **23**, 478-484.
Hadorn,E. (1955) in *Letalfaktoren*, Thieme, Stuttgart.
Ising,G. and Block,K. (1980) *Cold Spring Harb. Symp. Quant. Biol.*, **45**, 527-544.
Karlson,P. and Hauser,G. (1952) *Z. Naturforsch.*, **7b**, 80-83.
Klose,W., Gateff,E., Emmerich,H. and Beikirch,H. (1980) *Wilhelm Roux's Arch. Dev. Biol.*, **189**, 57-67.
Knudson,A.G. (1983) *Prog. Nucleic Acid Res. Mol. Biol.*, **29**, 17-25.
Korochkina,L.S. and Golubovsky,M.D. (1978) *Dros. Inf. Serv.*, **53**, 197-200.
Koufos,A., Hansen,M.F., Lampkin,B.C., Workman,M.L., Copeland,N.G., Jenkins,N.A. and Cavenee,W.K. (198) *Nature*, **309**, 170-172.
Kuroiwa,A., Hafen,E. and Gehring,W.J. (1984) *Cell*, **37**, 825-831.
Land,H., Parada,L.F. and Weinberg,R.A. (1983) *Science (Wash.)*, **222**, 771-778.
Langer-Safer,P.R., Levine,M. and Ward,D.C. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6633-6637.
Lewis,E.B. (1945) *Genetics*, **30**, 137-166.
Lindsley,D.L. and Grell,E.H. (1968) *Carnegie Inst. Wash. Publ.*, **627**.
Maniatis,T., Hardison,R.C., Lacy,E., Lauer,J., O'Connell,C., Quon,D., Sim,G.K. and Efstratiadis,A. (1978) *Cell*, **15**, 687-701.
Maniatis,T., Fritsch,E.F. and Sambrook,J. *Molecular Cloning, A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
McGinnis,W., Levine,M.S., Hafen,E., Kuroiwa,A. and Gehring,W.J. (1984) *Nature*, **308**, 428-433.
Murphee,A.L. and Benedict,W.F. (1984) *Science (Wash.)*, **223**, 1028-1033.
Orkin,S.H., Goldman,D.S. and Salen,S.E. (1984) *Nature*, **309**, 172-174.
Pardue,M.L. and Gall,J.G. (1975), *Methods Cell Biol.*, **10**, 1-16.
Plus,N. and Golubovsky,M.D. (1980) *Genetika (Yugoslavia)*, **12**, 227-231.
Raibaud,A., Gaillard,C., Longacres,S., Hibner,U., Buck,A., Bernardi,G. and Eisen,H. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 4306-4310.
Revee,A.E., Housiaux,P.J., Gardner,R.J.M., Chewings,W.E., Grindley,R.S. and Millow,L.J. (1984) *Nature*, **309**, 174-176.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.
Roberts,P.A. (1979) *Genetics*, **92**, 861-878.
Rubin,G.M. (1978) *Cold Spring Har. Symp. Quant. Biol.*, **42**, 1041-1046.
Rubin,G.M. and Spradling,A. (1982) *Science (Wash.)*, **218**, 348-353.
Scharrer,B. and Hadorn,E. (1938) *Proc. Natl. Acad. Sci. USA*, **24**. 236-242.
Sokolova,K. and Golubovsky,M.D. (1979) *Genetika*, **15**, 233-243.
Southern,E. (1975) *J. Mol. Biol.*, **98**, 503-517.
Weinstock,R., Sweet,R., Weiss,M., Cedar,H. and Axel,R. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1299-1303.
Young,B.S., Pession,A., Traverse,K.L., French,C. and Pardue,M.L. (1983) *Cell*, **34**, 85-94.
Yunis,J.J. (1983) *Science (Wash.)*, **221**, 227-236.

Received on 18 September 1984; revised on 27 March 1985