Drosophila Chorion Gene Amplification: A Model System for the Study of Chromosome Replication

Diane V. DeCicco, Barbara Wakimoto, Laura Kalfayan, J. Levine and A. C. Spradling

Email alerting service	Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

Drosophila chorion gene amplification: a model system for the study of chromosome replication

By Diane V. DeCicco, Barbara Wakimoto, Laura Kalfayan, J. Levine
and A. C. Spradling

*Department of Embryology, The Carnegie Institute of Washington, Baltimore,
Maryland* 21210, *U.S.A.*

Two chromosomal domains of 80–100 kilobases containing *Drosophila* chorion genes undergo tissue-specific amplification in ovarian follicle cells during oögenesis. We have investigated the ability of small segments of DNA from within these regions to induce amplification after insertion into new chromosomal sites by P element-mediated transformation. Certain transduced chorion DNA sequences initiated a pattern of tissue-specific differential replication that was identical to normal chorion amplification. Both the transformed chorion DNA as well as flanking *rosy* DNA sequences underwent amplification. Our results suggest that differential chorion DNA replication is mediated by specific origin-containing sequences located near the centre of the amplified domains. The possible role of such sequences in normal programmes of replication is discussed.

Introduction

Chromosomal DNA in eucaryotes generally replicates only once per cell cycle, as a result of controls that are poorly understood. Replication follows the same general pattern during each S phase, but it is unclear if the duplication of individual regions is precisely orchestrated. Mechanisms regulating chromosomal reproduction remain elusive in part because it is usually necessary to study an entire genome simultaneously. As a result, effort has been focused on viral (see, for example, Kornberg 1980), mitochondrial (Bogenhagen *et al.* 1979) or specialized extrachromosomal (Truett & Gall 1978; Jayaram *et al.* 1983) DNA replication. Although there are many differences in detail, these DNAs are copied according to the basic paradigm of procaryotic replicons; in particular, replication initiates at specific origin sequences. Since chromosomal DNA is known to initiate DNA synthesis at multiple points, one hypothesis is that the genome contains numerous dispersed origin sequences that are responsible for the initiation of replication during the S phase. Preliminary evidence for the existence of chromosomal origins has come from studies of the yeast *S. cerevisiae* (see Fangman & Zakian 1983). If such origins functioned only at particular times during the S phase, they might effectively programme genomic duplication.

Whether simple replicons can serve as useful models of chromosome replication is open to question, however (Harland & Laskey 1980). In early *Xenopus* embryos, injected DNAs undergo cell cycle-specific replication, even when they would be expected to lack origin sequences. Specific origins could have arisen in these molecules to facilitate rapid cell cycle independent replication. In chromosomes, DNA synthesis might begin at a wide diversity of sites, presumably according to some stochastic early event, such as a transient local melting of the DNA helix.

Another approach to studying chromosome replication involves investigating differential DNA replication, where cell cycle controls have been superseded within a limited genomic region. As in the case of viral molecules, this could indicate the evolution of a new independent mode of replication. However, in certain cases, only minor modifications of the normal process may have occurred. Study of such examples could provide information on normal control mechanisms.

Differential replication may lead to the amplification of specific genes (reviewed in Stark & Wahl 1984); furthermore, it is a normal event during the development of some organisms (reviewed in Tobler 1975; Spear 1977). During oögenesis in *Drosophila melanogaster*, genes encoding several chorion proteins are specifically amplified 16–64-fold in the ovarian follicle cells (Spradling & Mahowald 1980; Griffin-Shea *et al.* 1982).

Previous studies suggest that differential chorion gene replication utilizes specific chromosomal origins. Amplification occurs by the disproportionate replication of two chromosomal domains 80–100 kilobases in length containing multiple centrally-located chorion genes (Spradling 1981). Comparison of the restriction maps of normal with amplified domains suggests that amplification results from multiple rounds of initiation and bidirectional replication fork progression from a site or sites located near the centre of each region. The visualization of actively transcribed chorion genes associated with multiply-branched DNA molecules in chromatin from post-amplification egg chambers provides strong support for this model (Osheim & Miller 1983). The pattern of amplification, resulting from a chromosome inversion splitting one of the domains, strongly suggests that it contains a *cis*-acting region necessary and sufficient for amplification (Spradling & Mahowald 1981). This region might contain a specific replication origin, as well as elements that developmentally regulate its activity.

To verify the existence of such amplification control elements, we have investigated the ability of defined segments of the amplified chorion domains to induce differential DNA replication in the tissues of flies into which they have been introduced by P element-mediated gene transfer. This approach should also allow the location and structure of amplification origins to be defined. In this report we demonstrate that *Drosophila* chorion gene clusters contain DNA sequences capable of specifying characteristic gene amplification at diverse chromosomal locations. Some of these studies have been described in detail elsewhere (DeCicco & Spradling 1984).

Results

Construction of Drosophila strains containing defined chorion DNA insertions

If chorion gene amplification occurs by disproportionate replication, the origin sequences must lie within the central, maximally-replicated part of each cluster (Spradling 1981). Consequently, sequences from within these two regions were used for these studies (figure 1). Individual genomic chorion DNA fragments (figure 1) were cloned into defective P transposable element vectors marked with the *rosy*+ gene (Rubin & Spradling 1983). The structure of two such constructs is given in more detail in figure 2. Plasmid p7710 contains a 7.7 kilobase *Eco*RI fragment from the third chromosome gene cluster (see figure 1), and p37512 contains the 1.9 kilobase and 2.0 kilobase *Eco*RI–*Sal*I fragments within the 7.7 kilobase fragment arranged back-to-back.

Transposons were introduced into *rosy*− M-strain embryos by microinjection as previously
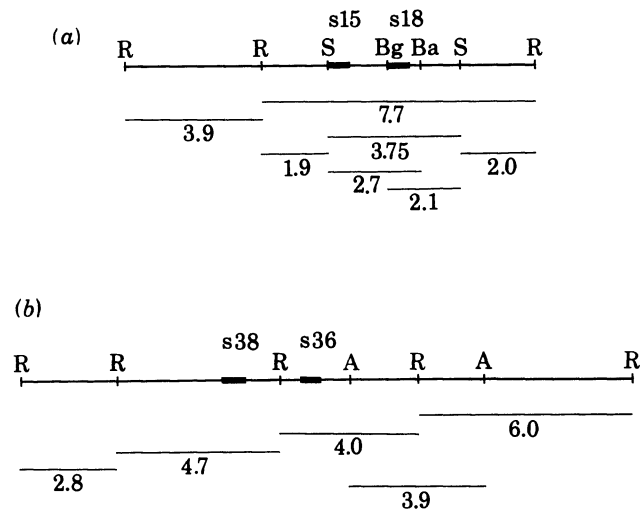
FIGURE 1. Chorion region DNA fragments tested. DNA fragments from the regions of the third (*a*) and X (*b*) chromosome chorion gene clusters (Spradling 1981) undergoing maximum levels of amplification which were cloned into P-element vectors are shown. Each fragment is depicted with its size in kilobases. The positions of the genes encoding four major chorion structural proteins on the chromosomes are indicated with a thick line. R = *Eco*RI; S = *Sal*I; Bg = *Bgl*II; Ba = *Bam*HI; A = *Ava*I.
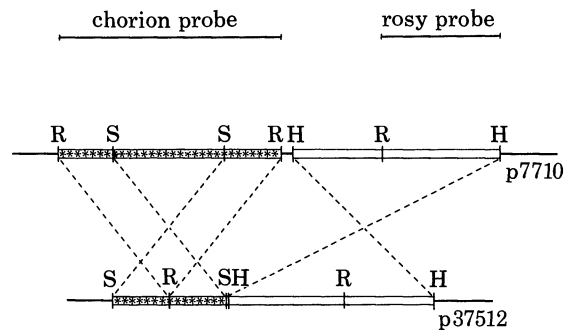
FIGURE 2. Constructs used for transformation. The plasmid p7710 contains the 7.7 kilobase *Eco*RI fragment from the third chromosome cluster (figure 1) cloned in the vector pV11 (Rubin & Spradling 1983). The plasmid p37512 contains the two *Eco*RI-*Sal*I fragments within the 7.7 kilobase *Eco*RI fragment cloned back-to-back as depicted into the vector Carnegie 20 (Rubin & Spradling 1983). The line drawing represents bacterial and defective P-element sequences (flanked by short stretches of *Drosophila* DNA from the *white* locus). The filled and blank blocks represent chorion and *rosy* DNA, respectively. The orientation of the *rosy*$^+$ sequences are opposite in pV11 and Carnegie 20, relative to the P-element sequences. The transposons contained in these plasmids are referred to as 7710 and 37512 respectively.

described (Spradling & Rubin 1982). Transformed *rosy*$^+$ flies were recovered in the next (G1) generation by scoring for wild-type eye colour. Lines with single insertions were established as follows. Individual *ry*$^+$ G1 transformants were crossed to *ry*$^-$ host partners and DNA prepared from several flies from the resulting stock. Southern blots of such DNAs were hybridized with a *rosy*-specific probe, which allowed visualization of unique bands corresponding to transposon insertions. Lines containing single, non-identical insertions were retained. The chromosome location of the insertion in each line was then determined by *in situ* hybridization. By using this approach, from 1 to 13 single-insert lines were constructed in the case of each of the genomic fragments in figure 1 (as well as several additional subfragments).

Transformed sequences may undergo tissue-specific amplification

Transformation was done with transposon 7710 to create line 771030, which contains a single insertion at cytogenetic locus 68A. Similarly, line 37512Y was constructed containing a 37512 insertion. The replication of the transformed sequences was determined by the quantitative Southern blotting procedure used previously (Spradling & Mahowald 1980). DNA was prepared from egg chambers before (stages 1–8) or after (stages 12–13) completion of amplification, and from non-ovarian female adult tissues. Digested DNA was transferred to nitrocellulose and hybridized with probes specific for *rosy* or chorion sequences. By the appropriate choice of restriction enzyme, separate bands which derived from the transposon and from endogenous chorion or rosy sequences were visualized. Since the normal *rosy* gene does not undergo amplification, it could be used as an internal control in these experiments. Similarly, the endogenous chorion sequences provided a positive control since these sequences amplify in late stage egg chambers. Comparison of the relative intensity of the transposon-specific bands relative to the host rosy band in early and late stage egg chamber DNA allowed the level of amplification (if any) of the inserted sequences to be quantitated. The relative intensity of these bands in DNA from non-ovarian tissues indicated whether the transposon sequences underwent amplification in cells where the chorion regions ordinarily do not differentially replicate.

Figure 3 shows the results of amplification assays on lines 7711030 and 37512Y. Since endogenous *rosy* DNA does not amplify, the intensity of hybridization of the *rosy* probe to the host-specific *rosy* DNA fragment (B) in panels (*a*) and (*c*) gives a measure of the total amount of DNA present in each lane. It is clear for both lines that the amount of DNA from egg chambers of late stages is much lower than that from egg chambers of early stages or carcasses.
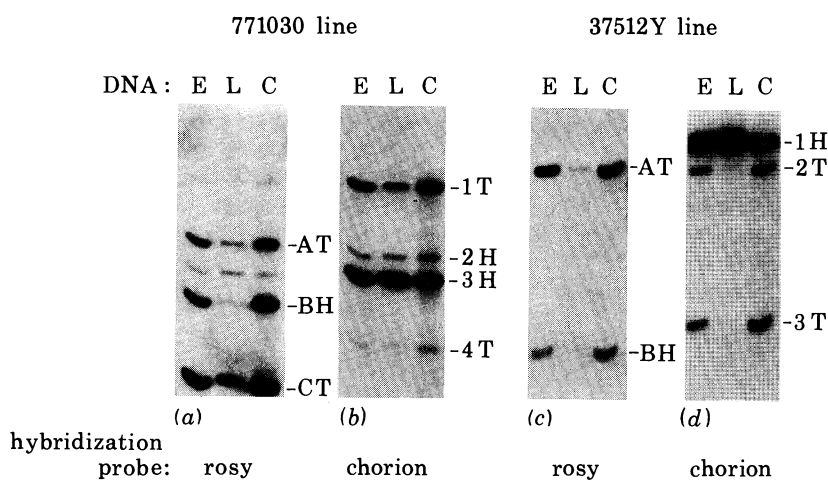

FIGURE 3. Analysis of amplification in two transformed lines. Line 771030 and 37512Y DNA was digested with *Bam*HI and *Eco*RI respectively, fractionated on 1 % agarose gels, transferred to nitrocellulose filters and hybridized successively with the chorion and *rosy* DNA probes p302.77 and pRR47. The former contains the 7.7 kilobase *Eco*RI fragment (figure 1) cloned in the *Eco*RI site of pBR322 (Spradling 1981); the latter contains a 4.7 kilobase *Eco*RI fragment of the chromosomal *rosy* gene (Bender *et al.* 1983) (which includes the 4.1 kilobase *Eco*RI-*Hind*III fragment labelled 'rosy probe' in figure 2) cloned into pAT153. E: early stage (1–8) egg chamber DNA; L: late stage (12–13) egg chamber DNA; C: ovarectomized female carcass DNA. The DNAs loaded in each track were prepared from 10–12 females (at least 2–3 days old) fed on fresh yeast for at least 24 h before use. The number of late-stage egg chambers obtained was 60–70. T: transformant-specific band; H: host-specific band.

The hybridization intensity of the chorion probe to the host-specific chorion DNA fragments 2 and 3 (panel (b)) for line 771030 and fragment 2 (panel (d)) for line 37512Y is much stronger than would be expected from the amount of stage 12–13 DNA (L) present, caused by amplification of these sequences. In line 771030, the intensity of the transformant-specific chorion DNA fragments 1 and 4 (panel (b)) is also increased relative to the *rosy* control, indicating that these fragments also undergo specific amplification. The level of transposon amplification is less than that of the host chorion DNA, however. In contrast, the transformant-specific chorion DNA fragments 2 and 3 of line 37512Y do not exhibit any amplification in late egg chamber DNA. Densitometric analysis of the bands reveals that the transformed DNA in line 771030 amplifies about 16-fold, a quarter of the level of the corresponding endogenous sequences (60-fold). The appearance of transposon amplification only in late stage egg chamber DNA and its failure to amplify detectably in carcass DNA, demonstrates that amplification of the transformed sequences occurs with the same developmental specificity as normal chorion gene amplification. Examination of figure 3 shows that the *rosy* sequences adjacent to the transformed chorion DNA (fragments A and C, panel (a)) also undergo specific amplification in line 771030 (but not in line 37512Y). This suggests that amplification which initiates within the 7.7 kilobase chorion segment spreads into adjacent *rosy* DNA, as expected if the induced amplification, like normal amplification, occurs by a re-initiation mechanism.

The results of numerous experiments similar to that described above allow the following conclusions to be drawn.

(1) Amplification occurs only when the transposon contains a specific DNA region from within the chorion gene amplified domain. In the case of the third chromosome, this region encompasses the 3.75 kilobase *Sal*I fragment from within the 7.7 kilobase *Eco*RI segment (see DeCicco & Spradling 1984). The only sequences from the X chromosome cluster to induce amplification derived from the 4.7 kilobase *Eco*RI fragment (figure 1).

(2) The level of amplification, but not its developmental specificity, is site dependent. At some sites, potentially active transposons do not differentially replicate. This position sensitivity creates experimental difficulties in mapping DNA segments capable of inducing amplification, particularly with X chromosome DNA. Perhaps because amplification of X chromosome chorion sequences is normally less than those on the third chromosome (16-fold as opposed to 60-fold), most transposons containing X-linked fragments did not induce amplification at their site of insertion, even those which could amplify at some locations. This made it difficult to rule out the possibility that other regions that were never observed to cause amplification nevertheless contained all the necessary sequences, but had simply not been tested at a permissive location.

Discussion

The results reported here and in DeCicco & Spradling (1984) demonstrate that the domains of chorion gene amplification contain control element sequences capable of autonomously inducing a characteristic developmentally-regulated pattern of differential replication at diverse chromosome locations. Non-chorion sequences adjacent to an active fragment also undergo amplification. This suggests that the control element acts as an origin for the initiation of multiple rounds of bidirectional DNA replication which spreads for an undetermined distance along the chromosome from its site of insertion.

These results have interesting potential implications for understanding both regular and

differential replication. For example, maturing larvae of *Rhynchosciara* amplify the genes encoding some abundant salivary gland secretory proteins (Glover *et al.* 1982) before their expression during cacoon formation. Amplification occurs only in salivary gland cells, where it is associated cytologically with the appearance of DNA puffs in the polytene chromosomes (Breuer & Pavan 1955). Genes for these secretory proteins may be located near amplification control elements that are structurally similar to those regulating chorion gene amplification. This element would differ, however, in the timing (late larval stages instead of adult stages) and tissue-specificity (salivary gland cells instead of ovarian follicle cells) of activation.

How might such developmental differences in origin activation be regulated? One model postulates that disproportionate replication at an amplification control element is controlled by a process of transcriptional activation (Furth *et al.* 1982; Itoh & Tomizawa 1980). The developmental specificity of the promoter which activates replication would then determine the timing and tissue-specificity of amplification. Transient transcription within the amplification control region of the third chromosome chorion gene cluster at about the time amplification begins in stage 8 has been reported (Thireos *et al.* 1980). Alternatively, initiation could be regulated by *trans*-acting factors.

Differential replication of Drosophila rDNA genes occurs under a variety of circumstances (see Ritossa 1976). During development, rDNA replication is controlled independently in polyploid cells such as those of the larval salivary gland (Hennig & Meer 1971; Spear & Gall 1973). Only a small fraction of the approximately 450 genes present in normal diploid flies replicate extensively during salivary gland polytenization (Endow & Glover 1979), and these appear to be derived from a single nucleolus organizer (Endow & Glover 1979; Endow 1980). Active rDNA genes lacking inserts appear to be preferentially replicated. Our results suggest that the rDNA genes which replicate in salivary gland cells may be clustered in the vicinity of one or more amplification control sequences where rounds of DNA synthesis initiate. Procunier & Tartof (1978) described a genetic locus, cr^+, located in heterochromatin distal to the rDNA genes on the *D. melanogaster* X chromosome which influenced the total rDNA content measured in the DNA of whole flies. The cr^+ locus has some of the properties expected for an amplification control element for rDNA replication as postulated above. However, the effects of chromosomal changes involving cr^+ on rDNA replication in salivary gland cells have not been reported.

Finally, chorion region amplification control elements may provide a model for replication origins that do not function differentially. If origin sequences *per se* can be separated from *cis*-acting sequences that control their activation, origins from other regions in the genome might be able to substitute for them in promoting chorion gene amplification. This would provide an assay for identifying such origin sequences in other genomic regions.

References

Bender, W., Spierer, P. & Hogness, D. S. 1983 Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the bithorax complex in *Drosophila melanogaster*. *J. molec. Biol.* **168**, 17–23.

Bogenhagen, D., Gillum, A. M., Martens, P. A. & Clayton, D. A. 1979 Replication of mouse L-cell mitochondrial DNA. *Cold Spring Harb. Symp. quant. Biol.* **43**, 253–262.

Breuer, M. E. & Pavan, D. 1955 Behavior of polytene chromosomes of *Rhyncosciara angelae* at different stages of larval development. *Chromosoma* **7**, 371–386.

DeCicco, D. & Spradling, A. 1984 Localization of *cis*-acting element responsible for the developmentally-regulated amplification of *Drosophila* chorion genes. *Cell* **38**, 45–54.

Endow, S. A. 1980 On ribosomal gene compensation in *Drosophila*. *Cell* **22**, 149–155.

Endow, S. A. & Glover, D. M. 1979 Differential replication of ribosomal gene repeats in polytene nuclei of *Drosophila*. *Cell* **17**, 597–605.

Fangman, W. L. & Zakian, V. A. 1981 Genome structure and replication. In *Molecular biology of the yeast Saccharomyces: life cycle and inheritance* (ed. J. N. Strathern, E. W. Jones & J. R. Broach), pp. 27–58. Cold Spring Harbor Laboratory Press.

Furth, M., Dove, W. & Meyer, B. 1982 Specificity determinants for bacteriophage L DNA replication. III. Activation of replication of Lri^c mutants by transcription outside of *ori*. *J. Molec. Biol.* **154**, 65–83.

Glover, D. M., Zaha, A., Stocker, A. J., Santelli, R. V., Pueyo, M. T., de Toledo, S. M. & Lara, F. J. S. 1982 Gene amplification in *Rhynchosciara* salivary gland chromosomes. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2947–2951.

Griffin-Shea, R., Thireos, G. & Kafatos, F. C. 1982 Organization of a cluster of four chorion genes in *Drosophila* and its relationship to a developmental expression and amplification. *Devl Biol.* **91**, 325–336.

Harland, R. M. & Laskey, R. A. 1980 Regulated replication of DNA microinjected into eggs of *Xenopus laevis*. *Cell* **21**, 761–771.

Hennig, W. & Meer, B. 1971 Reduced polytomy of ribosomal RNA cistrons in giant chromosomes of *Drosphila hydei*. *Nature, New Biol.* **233**, 70–72.

Itoh, T. & Tomizawa, J. 1980 Formation of an RNA primer for initiation of replication of Co E1 DNA by ribonuclease H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2450–2454.

Jayaram, M., Li, Y. Y. & Broach, J. R. 1983 The yeast plasmid 2u circle encodes components required for its high copy propagation. *Cell* **34**, 95–104.

Kornberg, A. 1980 *DNA replication*. New York: Academic Press.

Osheim, Y. N. & Miller, O. L. Jr 1983 Novel amplification and transcriptional activity of chorion genes in *Drosophila* melanogaster follicle cells. *Cell* **33**, 543–553.

Procunier, J. D. & Tartof, K. D. 1978 A genetic locus having *trans* and contiguous *cis* functions that control the disproportionate replication of ribosomal RNA genes in *Drosophila melanogaster*. *Genetics, Princeton* **88**, 67–79.

Ritossa, F. 1976 The bobbed locus. In *The genetics and biology of Drosophila* (ed. M. Ashburner & E. Novitski), vol. 1b, pp. 801–846. London: Academic Press.

Rubin, G. M. & Spradling, A. C. 1983 Vectors for P element-mediated transformation in *Drosophila*. *Nucl. Acids Res.* **11**, 6341–6351.

Spear, B. B. 1977 Differential replication of DNA sequences in *Drosophila* chromosomes. *Am. Zool.* **17**, 695–706.

Spear, B. B. & Gall, J. E. 1973 Independent control of ribosomal gene replication in polytene chromosomes of *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1359–1363.

Spradling, A. 1981 The organization and amplification of two clusters of *Drosophila* chorion genes. *Cell* **27**, 193–202.

Spradling, A. C. & Mahowald, A. P. 1980 Amplification of genes for chorion proteins during oogenesis in *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1096–1100.

Spradling, A. C. & Mahowald, A. P. 1981 A chromosome inversion alters the pattern of specific DNA replication in *Drosophila* follicle cells. *Cell* **27**, 203–210.

Spradling, A. C. & Rubin, G. M. 1982 Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science, Wash.* **218**, 341–347.

Stark, G. R. & Wahl, G. M. 1984 Gene amplification. *A. Rev. Biochem.* **53**. (In the press.)

Thireos, G., Griffin-Shea, R. & Kafatos, F. C. 1980 Untranslated mRNA for a chorion protein of *Drosophila melanogaster* accumulates transiently at the onset of specific gene amplification. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5789–5793.

Tobler, H. 1975 Occurrence and developmental significance of gene amplification. *Biochem. animal Dev.* **3**, 91–143.

Truett, M. & Gall, J. G. 1978 The replication of rDNA in the macronucleus of Tetraheymna. *Chromosoma* **64**, 295–304.
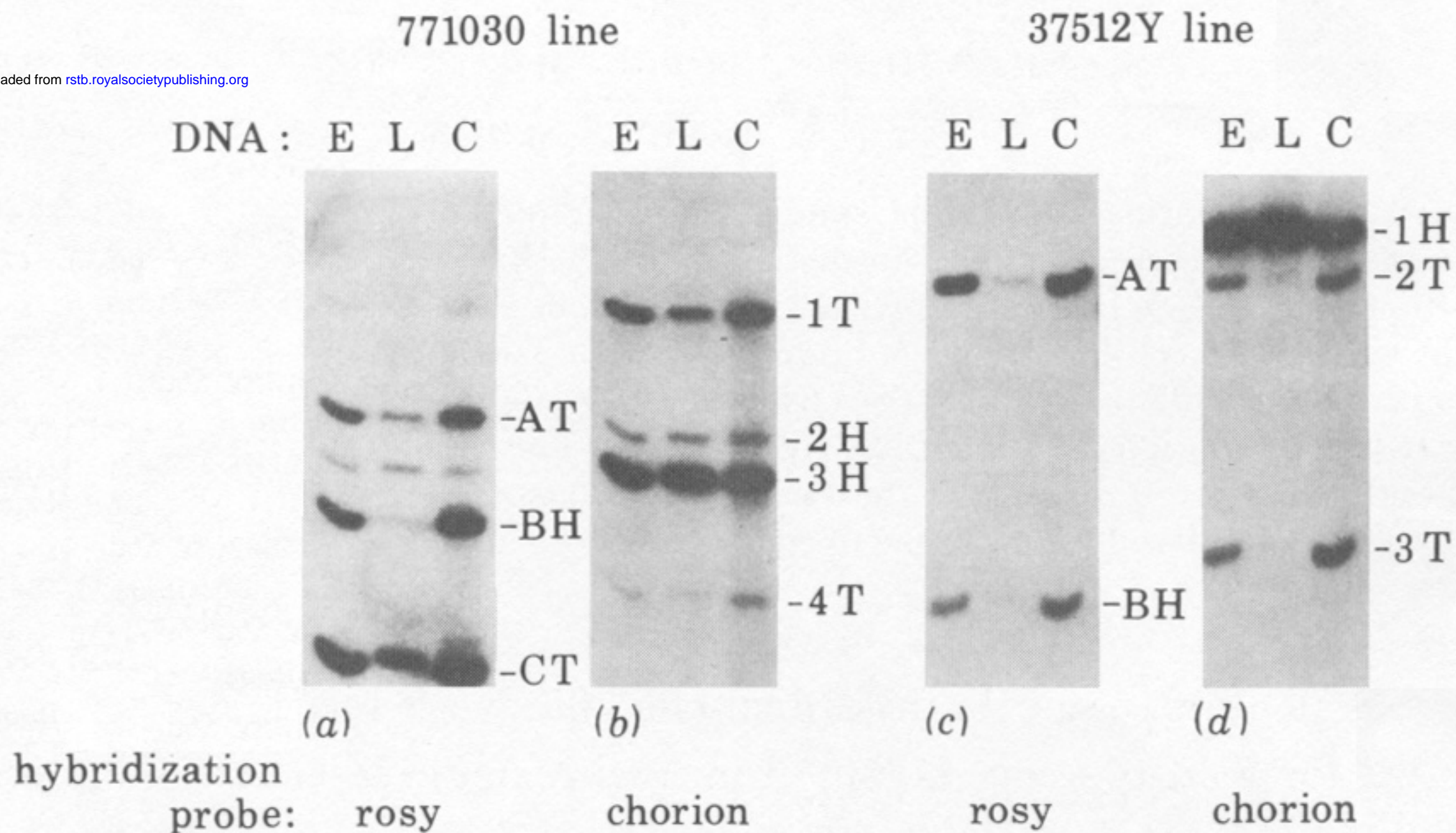
FIGURE 3. Analysis of amplification in two transformed lines. Line 771030 and 37512Y DNA was digested with *Bam*HI and *Eco*RI respectively, fractionated on 1% agarose gels, transferred to nitrocellulose filters and hybridized successively with the chorion and *rosy* DNA probes p302.77 and pRR47. The former contains the 7.7 kilobase *Eco*RI fragment (figure 1) cloned in the *Eco*RI site of pBR322 (Spradling 1981); the latter contains a 4.7 kilobase *Eco*RI fragment of the chromosomal *rosy* gene (Bender *et al.* 1983) (which includes the 4.1 kilobase *Eco*RI-*Hind*III fragment labelled 'rosy probe' in figure 2) cloned into pAT153. E: early stage (1–8) egg chamber DNA; L: late stage (12–13) egg chamber DNA; C: ovarectomized female carcass DNA. The DNAs loaded in each track were prepared from 10–12 females (at least 2–3 days old) fed on fresh yeast for at least 24 h before use. The number of late-stage egg chambers obtained was 60–70. T: transformant-specific band; H: host-specific band.