The EMBO Journal vol.3 no.1 pp.165–173, 1984

Regulated expression of genes injected into early *Drosophila* embryos

H. Steller and V. Pirrotta*

European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG

*To whom reprint requests should be sent
Communicated by V. Pirrotta

DNA of cloned genes injected into 15–30 min *Drosophila* embryos reaches nearly all the cells of the later embryo and its expression can be detected by Northern blot hybridisation. The injected DNA continues to be expressed at least up to the first instar larval stage and some developmental control of expression is retained. Genes normally expressed in the embryo are strongly transcribed upon injection while genes characteristic of later stages are not, or only very weakly expressed. Some transcription was detected with a larval salivary gland secretion gene (*sgs-4*) and with the adult promoter of the alcohol dehydrogenase gene (*Adh*) but not from the *Adh* larval promoter or from larval cuticle genes. A foreign gene (Semliki Forest virus capsid protein) was used to monitor the activity of promoters attached to it. *In situ* immunofluorescence of thin sections showed that the *copia* promoter is active mostly in yolk cells. In contrast, the *hsp-70* heat-shock promoter is active, upon heat-shock, in nearly all cells in the embryo but most of the injected DNA is gradually lost so that in first instar larvae only 15% of the cells still express it.
Key words: Drosophila/embryos/gene expression

Introduction

In *Drosophila* as in many organisms, including vertebrates, early development is a period of rapid, synchronous nuclear (or cell) divisions which slow down abruptly after 12–13 cycles. In contrast to vertebrates, the *Drosophila* early embryo is syncytial: the rapidly dividing nuclei do not become enclosed in cell membranes until the blastoderm stage. Towards the end of the syncytial blastoderm stage, ~2.5–3 h after fertilization, genomic sequences begin to be actively transcribed (Zalokar, 1976; McKnight and Miller, 1976; Anderson and Lengyel, 1979). Many genes are developmentally regulated and are swtiched on and off at specific stages or in specific tissues. We do not know what kinds of signals effect these developmental programs of expression, how much of the transcriptional regulation is purely local, depending on the nucleotide sequence in the immediate vicinity of the genes affected, or how much is longer range, depending on the chromosomal or chromatin context in which the regulated genes find themselves. We have approached these questions by studying the expression of cloned genes microinjected into *Drosophila* embryos.

Because of the syncytial development, DNA injected into embryos in the first 30 min of development can in principle gain admittance to all the nuclei as the nuclear membrane dissolves during every division cycle. Furthermore, since at this stage the number of nuclei is still under 50, each nucleus that takes up DNA will impart it to an important fraction of the cells in the later embryo.

Elsewhere (Steller and Pirrotta, in preparation) we shall present evidence to show that the injected DNA survives, that ~20% reaches the nuclei where it becomes stable at least throughout embryonic development, that it reaches a wide variety of cells in the embryo and that, at least in some cases, it becomes incorporated into chromosomal sequences.

Here we consider the question of the expression and regulation of the injected genes. We were interested in particular in whether the injected DNA reaches, and can be expressed in, all parts of the embryo, whether expression is developmentally appropriate and whether regulated expression of a desired gene function can be achieved by driving the gene with an inducible promoter.

Results

Injection of copia DNA

To detect transcription from injected DNA, we first used the transposable element *copia*. The 276-bp direct terminal repeats of this element contain a promoter which has been sequenced (Flavell *et al.*, 1981), is transcriptionally active in the embryo and is likely to be independent of surrounding sequences. However, since the genome of *D. melanogaster* contains some 60 *copia* elements which would obscure the transcript from the injected *copia*, we used two alternative approaches: (i) we injected *copia* DNA into the embryos of *D. bipectinata*, a *Drosophila* species which contains no detectable *copia* sequences (H. Steller, unpublished) or (ii) we attached the *copia* promoter to a gene foreign to *D. melanogaster*, the capsid protein gene of Semliki Forest virus (SFV) (Kondor-Koch *et al.*, 1982). Expression of the injected DNA was detected by Northern blot hybridisation of the RNA extracted from the injected embryos. Figure 1 shows that the injected *copia* is transcribed in *D. bipectinata* at a level comparable with that of the endogenous *copia* in *D. melanogaster*. There is a response to the injection of increasing amounts of DNA. The injected pUC-*copia* clone (see Materials and methods) produces the normal *copia* RNA species found in *D. melanogaster* indicating that the different transcripts represent different ways to transcribe or process the same basic *copia* sequence.

The *copia* promoter can also drive the transcription of the SFV capsid gene in the pEV-*copia* plasmid shown in Figure 2. The RNA produced has the expected size indicating that the SV40 termination and processing signals provided in the pEV-*copia* expression plasmid are recognised in *D. melanogaster*. No SFV RNA is produced in embryos injected with pEV DNA (lacking the *copia* promoter) or with pEV-*copia* with the *copia* promoter in the wrong orientation. SFV capsid RNA is also produced if an entire *copia* element in the right orientation is placed at the 5′ end of the SFV capsid gene, indicating that both *copia* terminal repeats are active pro-

© IRL Press Limited, Oxford, England.

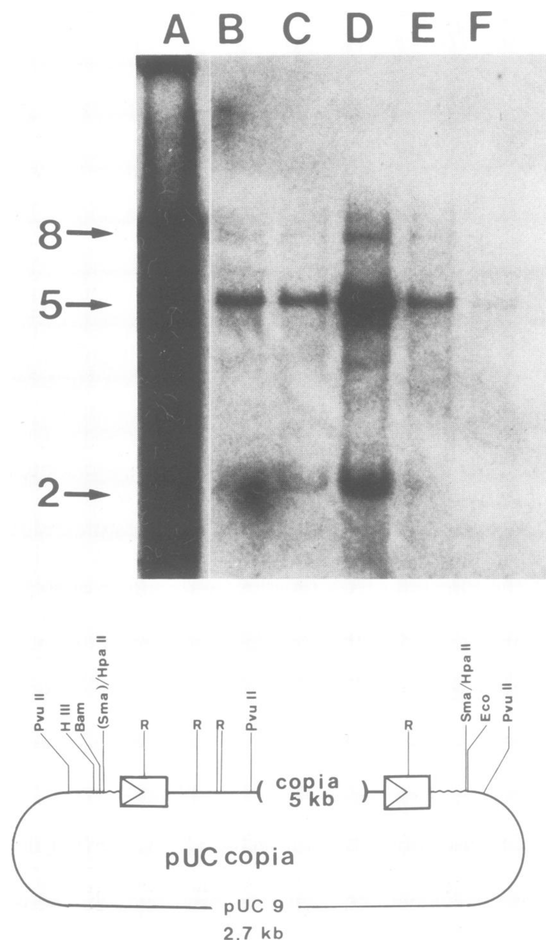


Fig. 1. (Top) Transcription from pUC-*copia*. Embryos injected with different amounts of pUC-*copia* DNA were grown to a physiological age of 12 h, their RNA was extracted and analysed on a 1% agarose-formaldehyde gel. After Northern blotting, the filter was hybridised to pUC-*copia* DNA labelled by nick translation. To verify that approximately equal amounts of RNA were applied to each slot, the filters shown in this and in subsequent figures were stained with methylene blue after the autoradiography. **(A)** 100 pg of pUC-*copia* plasmid DNA; **(B)** 30 uninjected embryos of *D. melanogaster*; **(C)** 30 embryos of *D. melanogaster* injected with 5 pg pUC-*copia* DNA; **(D)** 40 embryos of *D. melanogaster* injected with 50–100 pg pUC-*copia* DNA; **(E)** 40 embryos of *D. bipectinata* injected with 50 pg pUC-*copia* DNA; **(F)** 30 embryos of *D. bipectinata* injected with 5 pg pUC-*copia* DNA. **(Bottom)** Map of pUC-*copia*. A 5.2-kb *Hpa*II fragment from the pBR5002 *copia* clone was inserted into the *Sma* site of pUC9. This fragment contains only 80 bp of non-*copia* flanking sequences at the 5′ end and 230 bp at the 3′ end. Boxes represent the 276-bp *copia* terminal repeats with the promoter orientation indicated by the arrows. Only the restriction sites relevant to the construction are indicated. HIII: *Hind*III. Eco: *Eco*RI. Sma: *Sma*I. R: *Rsa*I.

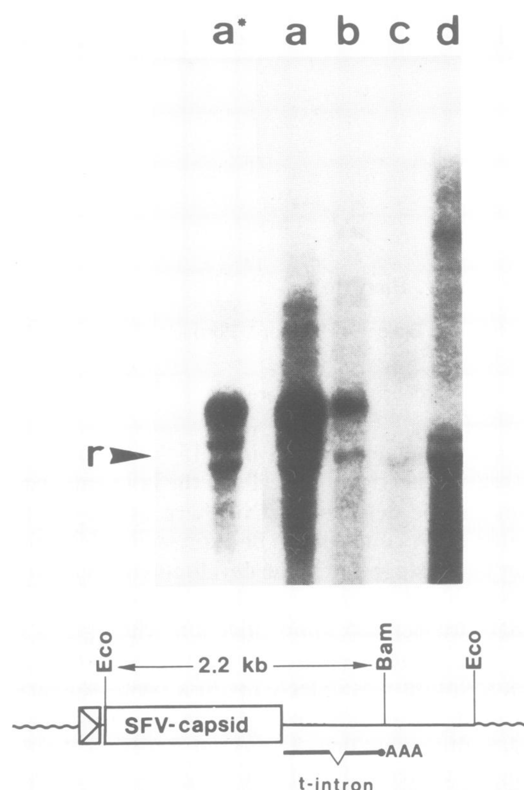


Fig. 2. (Top) Transcription of pEV-*copia*. This and all subsequent experiments were done with *D. melanogaster*. Embryos injected with pEV-*copia* or with pSV2 were grown to an age of 10 h. Their RNA was analysed on a 1.5% agarose formaldehyde gel and the Northern blots were hybridised with labelled pEV DNA. In this and other Northern blots from injected embyros, a background hybridisation is caused by residual plasmid DNA, as shown by hybridising the filter with the plasmid vector. When this background is displaced by the mass of rRNA, it may give rise to an artefactual double band of apparent hybridisation. **(a*)** and **(a)**: cytoplasmic fraction from 30 embryos injected with 50 pg pEV-*copia* DNA. **(a*)** is a short exposure of **(a)**; **(b)** nuclear fraction from the same embryos as in **(a)**; **(c)** total RNA from 30 embryos injected with 10 pg pSV2 DNA; **(d)** total RNA from 30 embryos injected with 50–100 pg pSV2 DNA. The arrowhead indicates the position of the rRNA visible in the stained filter. The pEV transcript of the expected 2.3 kb is visible above the ribosomal artefact. **(Bottom)** Map of pEV-*copia*. The *copia* promoter, indicated by a boxed arrow, was inserted in the polylinker preceding the SFV capsid gene which is followed by the SV40 t-intron and polyadenylation signal.

moters. Figure 2 also shows that RNA transcribed from the injected DNA is found both in the cytoplasm and in the nucleus. Since we can exclude contamination of the nuclear fraction with cytoplasm by monitoring the amount of rRNA present, we conclude that either the RNA can enter the nucleus from the cytoplasm or that the injected DNA has indeed found its way to the nucleus and at least some of the transcription is genuinely nuclear.

The analogous construction, pSV2, in which the SFV capsid gene is driven by the SV40 promoter instead of *copia* (Kondor-Koch *et al.*, 1982) functions in *Drosophila* tissue culture cells (H. Steller, unpublished) but is not active in the embryo as determined by production of a detectable SFV RNA species or by immunofluorescence.

Injection of B205 a clone specific for the early embryo

The preceding experiments indicate that the injected DNA can be expressed. However, *copia* elements are likely to be special cases. We therefore looked at the transcription from other cloned genes in *D. melanogaster*. B205 is a genomic clone containing unique sequences specifically active during early development in *Drosophila* (Scherer *et al.*, 1981). Figure 3 shows that although the endogenous gene is very active, the lowest amount of B205 DNA injected makes an equally strong contribution to the total B205 RNA detected. There is a strong response to increasing amounts of injected DNA. We note however that the injected DNA is not as efficiently transcribed as the endogenous DNA. As Figure 3 shows, the lowest dose injected increases the B205 transcription by a factor of two over the endogenous level but corresponds to the injection of about a million copies of the gene. This is ~100 times more copies than are present in the embryo after 10 h of development. Therefore, most of the injected DNA is

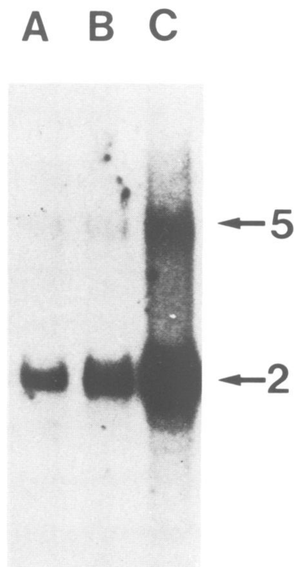


Fig. 3. Transcription of B205. Embryos injected with λB205, a clone specific for early development, were grown for 8 h and their RNA was then analysed on a 1% agarose-formaldehyde gel. After Northern blotting, the filter was hybridised with labelled pBR205E DNA, a subclone containing the transcribed region. The mol. wt. of the RNA species in kilobases is indicated. **(a)** RNA from 40 uninjected embyros; **(b)** RNA from 40 embryos injected with 10 pg λB205 DNA; **(c)** RNA from 40 embryos injected with 100 pg λB205 DNA.

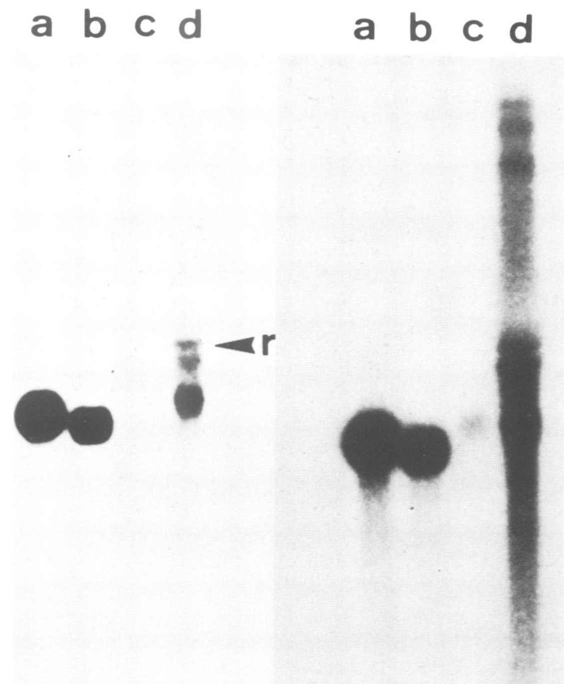


Fig. 4. Transcription of p*Adh* R1-2. Embryos were injected with p*Adh*2 R1-2, a subclone containing a 4.8-kb *Eco*RI fragment carrying the *Adh* gene (Benyajati *et al.*, 1981). After 10 h their RNA was analysed on a 1.5% agarose-formaldehyde gel, and the Northern blot hybridised with p*Adh* R1-2. The left panel is a short exposure and the right panel a long exposure of the same filter. **(a)** 5 μg of total RNA from adult flies; **(b)** 5 μg of total RNA from larvae; **(c)** total RNA from 40 uninjected embryos (~5 μg); **(d)** total RNA from 40 embryos (~5 μg) injected with 100 pg p*Adh* R1-2 DNA.

either degraded rapidly or fails to reach cellular sites active in transcription, e.g., the nucleus.

Developmentally controlled genes

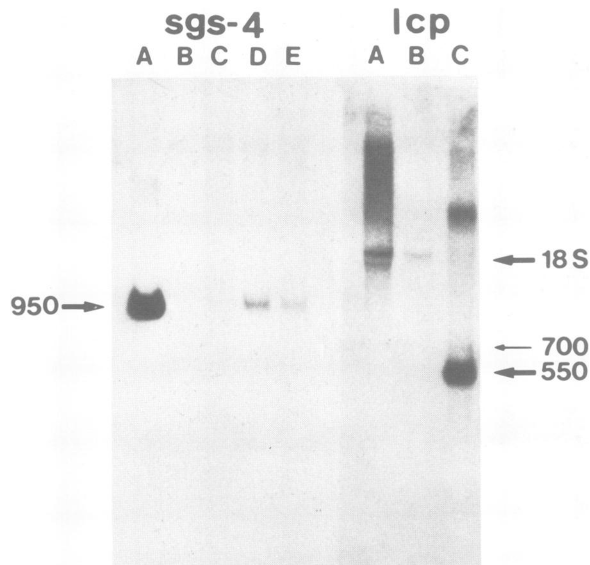
Are injected genes subject to normal developmental controls? To answer this question we injected three clones containing developmentally regulated genes: alcohol dehydrogenase (*Adh*) whose expression normally becomes detectable in the fat bodies of first instar larvae (Ursprung *et al.*, 1970); the *sgs*-4 gene which is specific for the salivary glands of third instar larvae (Muskavitch and Hogness, 1980), and *lcp*-1, a clone containing four larval cuticle protein genes specifically expressed in late third instar larvae (Snyder *et al.*, 1982). Figure 4 shows that the injected *Adh* gene is transcribed in the embryo at a modest rate but that uninjected embryos also contain a very low amount of *Adh* RNA. The *Adh* gene can be transcribed from two possible promoters, one active in larvae and the other in the adult (Benyajati *et al.*, 1983). The two are distinguishable by the slight difference in size of the RNA they produce. Figure 4 shows that both the residual endogenous RNA and that transcribed in injected embryos correspond in size to the adult variety and not the larval. We do not know if the endogenous *Adh* RNA is a maternal residue or if it is indicative of a very low constitutive activity. Within the limits of detection, however, the larval promoter appears to be tightly controlled. Whether transcribed from the adult or from the larval promoter, the *Adh* gene contains introns which must be spliced out to produce the mature RNA species. The *Adh* RNA detected after injection corresponds in size to the spliced adult *Adh* RNA although traces of the unspliced precursor may be detectable.

Figure 5 shows that the *sgs*-4 gene transcript was not detectable in uninjected embryos. We injected two different clones containing the entire *sgs*-4 gene plus 2 kb (λE4α21) or 8 kb (λE2α31) of 5′-flanking region. In both cases a low but detectable level of *sgs*-4 RNA was obtained which was at least an order of magnitude lower than that produced by the endogenous gene assayed in late third instar larvae.

In contrast, the cuticle protein genes contained in the *lcp*-1 clone remain silent. This clone contains four genes coding for small mRNAs which are divergently transcribed (Snyder *et al.*, 1982). We would expect that at least two of the genes present in the clone contain all the relevant regulatory signals for transcription. The total absence of detectable transcripts after injection of the *lcp*-1 clone indicates that developmental control in this case is strict.

Pre-blastoderm transcription

Transcription of nuclear genes in the *Drosophila* embryo has not been detected before the syncytial blastoderm stage around 2 h of physiological age (Zalokar, 1976). Are injected genes also silent before blastoderm? We resorted to *in vivo* labelling because, in the short time between injection and blastoderm, too little RNA might accumulate to be detected by Northern blot hybridisation. Embryos, either uninjected or injected with the pUC-*copia* DNA were allowed to develop for different times before being injected with [α-^{32}P]UTP and allowed to incorporate for 20 min. They were then fractionated into nuclear and cytoplamsic fractions. Figure 6 shows that in uninjected early embryos, labelled RNA is found only in the cytoplasm and is represented by tRNA and a few discrete bands of relatively low mol. wt. RNA, which are characteristically mitochondrial (Merton and Pardue, 1981). Nuclear species appear at 90–100 min, well before syncytial blastoderm, at about the time of pole cell formation. A few discrete species are detectable at this time, some of very high mol. wt. Massive nuclear transcription sets in at



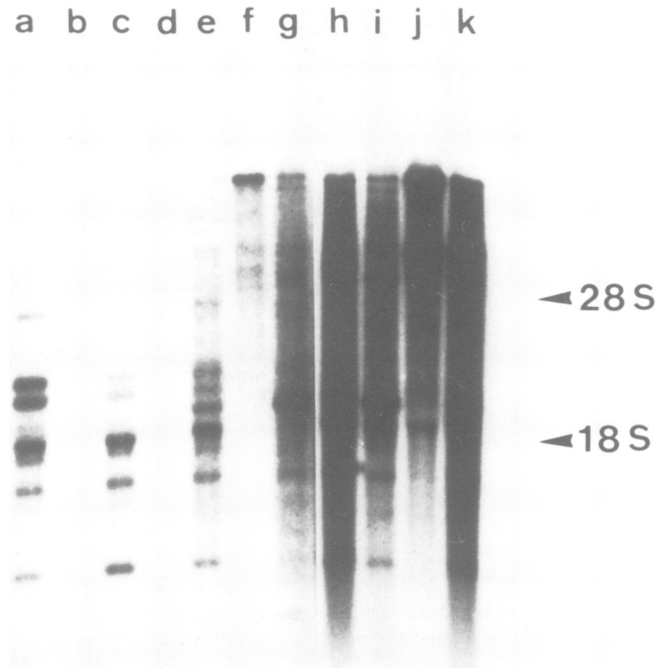


Fig. 5. (Left) Transcription of *sgs*-4. Embryos were injected with DNA from either λE2α31 or λE4α21, two λ clones containing the *sgs*-4 gene (C. Hadfield and V. Pirrotta, unpublished). After 10 h growth, the RNA was extracted and analysed on a 1% agarose-formaldehyde gel. The Northern blot was hybridised to the labelled *sgs*-4 DNA. The nucleotide chain length is indicated. **(a)** 4 μg total RNA from a single late third instar larva; **(b)** 4 μg total RNA from 40 uninjected embryos; **(c)** total RNA from 50 embryos injected with 100 pg λE2α31 DNA; **(d)** total RNA from 40 embryos injected with 100 pg λE4α21 DNA; **(e)** total RNA from 40 embryos injected with 20 pg λE4α21 DNA. **(Right)** Transcription of *lcp*-1. In an analogous experiment, embryos were injected with DNA from λ*lcp*-1 (Snyder *et al.*, 1982) and the Northern blot was hybridised with labelled λ*lcp*-1 DNA. **(a)** Total RNA from 60 embryos injected with 100 pg λ*lcp*-1 DNA; **(b)** total RNA from 40 uninjected embryos; **(c)** 4 μg total RNA from a single late third instar larva.

Fig. 6. *In vivo*-labelled RNA. 5–10 individually staged embryos, injected or uninjected with pUC-*copia* DNA were pulse labelled by injection of [α-^{32}P]UTP at the times indicated. The RNA was extracted after 20 min pulse labelling, analysed on a 1% agarose-formaldehyde gel, blotted on a nitrocellulose filter and autoradiographed. Due to the different rates of incorporation, different amounts of material were loaded for different stages. The arrowheads indicate the position of rat rRNA used as carrier.
(a) Cytoplamsic fraction from 30 min embryos; **(b)** nuclear fraction from the same embryos; **(c)** cytoplasmic fraction from 30 min embryos injected with 50 pg pUC-*copia* DNA; **(d)** nuclear fraction from the same embryos; **(e)** cytoplasmic fraction from 90 min embryos injected at 30 min with 50 pg pUC-*copia* DNA; **(f)** nuclear fraction from the same embryos; **(g)** cytoplasmic fraction from 2 h embryos; **(h)** nuclear fraction from the same embryos; **(i)** cytoplasmic fraction from 2 h embryos injected at 30 min with 50 pg pUC-*copia* DNA; **(j)** nuclear fraction from the same embryos; **(k)** nuclear fraction from embryos injected with label at 2 h and pulsed for 1 h.

2–2.5 h, when the embryos are still syncytial. Embryos which had received injections of *copia* DNA did not show any trace of the characteristic 2- and 5-kb *copia* transcripts before the 2 h stage, when the corresponding region of the gel is saturated by the bulk of nuclear transcripts.

Similar results were obtained when the experiment was repeated injecting DNA from B205, a clone which is strongly expressed at the blastoderm stage (Scherer *et al.*, 1981).

Both the incorporation and the specific activity of the RNA produced in this system are very high and the limits of detection of new transcripts should be correspondingly low. The UTP pool in the egg is 4–5 pmol (Zalokar, 1976; Anderson and Lengyel, 1979). The injected UTP (0.5 μCi at 3000 mCi/μmol) is only diluted by a factor of 30. A newly synthesised RNA molecule of 2 kb would incorporate some 2 x 10^{-4} d.p.m., and it would have been possible by pooling 10 embryos to detect as little as 500–1000 molecules per embryo.

Localisation of expression

To examine the localisation of expression in the injected embryo we returned to plasmid pEV-*copia* containing the SFV capsid gene driven by the *copia* promoter. Embryos were injected with DNA, allowed to develop for 10 h then fixed, sectioned and stained by indirect immunofluorescence. Figure 7 shows that fluorescence is localised mostly in the yolk cells and yolk mass. A few sporadic clusters of fluorescent cells are occasionally found also in the periphery. Yolk cells are known to be interconnected by cytoplasmic bridges, hence expression of the injected DNA in a few cells would cause the entire mass to fluoresce. Fluorescence was not detected in uninjected embryos, or in embryos injected with either pEV lacking the *copia* promoter or the *copia* promoter in inverted orientation. This result suggested either that the injected DNA remained localised and did not distribute to the entire embryo, or that the expression of pEV-*copia* was restricted to certain regions or cell types. The following experiment indicates that the injected DNA reaches virtually all cells.

In the construction illustrated in Figure 2 we replaced the *copia* promoter with the promoter of the *hsp*-70 gene coding for the major heat-shock inducible protein of *Drosophila*. The *Xba*I-*Xmn*I 456-bp fragment of the *hsp*-70 gene was inserted in front of the SFV capsid gene in pEV. The *hsp*-capsid RNA contains the first 198 nucleotides of the *hsp*-70 transcript resulting in an RNA of 2.4 kb. The heat-shock genes are thought to be expressible in most and perhaps all cell types. Furthermore, since the heat-shock promoter is inducible, it can be kept inactive to avoid the possibility that extended expression of the SFV capsid gene might be harmful. After injection with the pEV-hs plasmid, the embryos were allowed to develop to different stages at low temperature,

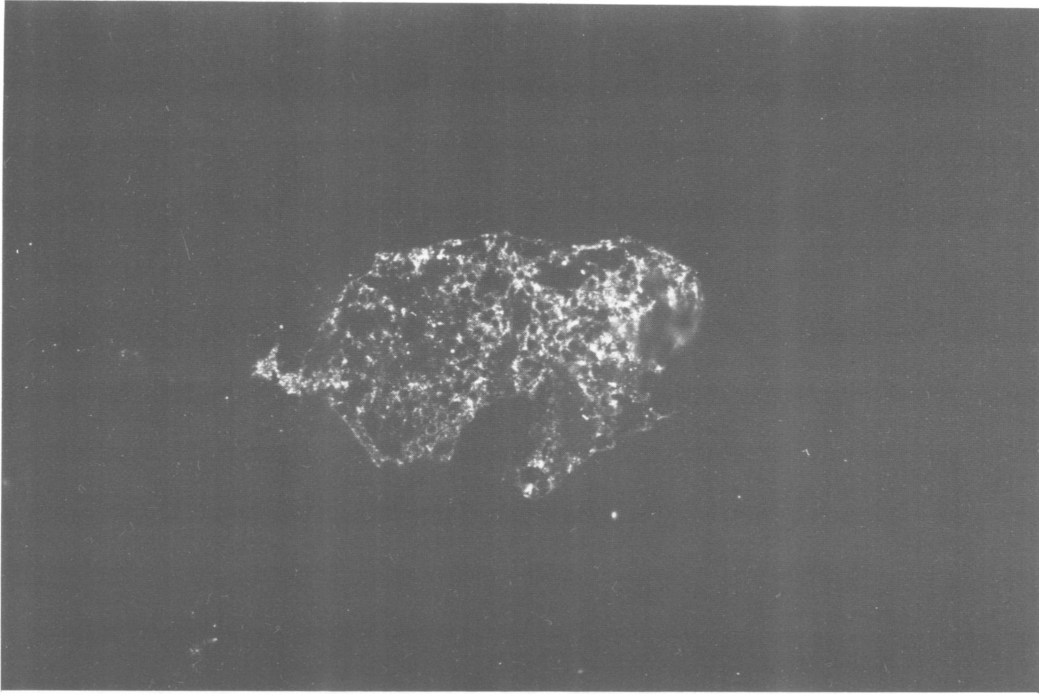


Fig. 7. Thin section immunofluorescence of embryos injected with pEV-*copia* DNA. Embryos injected with 100 pg pEV-*copia* DNA were allowed to develop for 10 h, then sectioned into 0.5 – 1 μm slices and stained by indirect immunofluorescence against SFV capsid protein. The fluorescing reticular structure is the yolk sac. Yolk droplets remain unstained.

then exposed to 37°C for 1 h, sectioned and stained. In the absence of heat-shock, or in uninjected but heat-shocked embryos, the expression of the SFV gene is undectable by Northern hybridisation (Figure 8) or by immunofluorescence (not shown). After heat-shock, expression is massive and universal (Figures 8 and 9). If sufficient DNA is injected (150 pg), essentially all distinguishable cells, including pole cells, fluoresce after heat-shock. Injection of lower quantities of DNA (50 pg) sometimes yields embryos containing dark regions, particularly at the posterior end of the embryo, with isolated, strongly fluorescing cells.

These experiments show that when sufficient DNA is injected, all the cells receive and maintain potentially functional DNA for at least 10 h. At later stages of development, a proportionally lower percentage of the cells can still express the injected DNA. Figure 10 shows injected embryos which have been allowed to grow at low temperature to first instar larvae (24 h) then heat-shocked for 1 h at 37°C before sectioning and staining. A much lower proportion of cells can still fluoresce strongly but many other cells still respond very weakly. We suppose that as development progresses much of the injected DNA is diluted out and is lost. Unfortunately we have no estimate of the fluorescence expected from a single copy of the injected DNA per cell. It is possible that the weak expression seen in many cells is due to a single residual copy of the gene.

Fig. 8. Transcription of pEV-hs. Embryos were injected with 100 pg pEV-hs DNA, grown for 10 h and heat-shocked (30 min, 37°C) or not shocked (30 min, 25°C). The RNA from 20 embryos was analysed in each case: shocked, not shocked and not injected, on a 1% agarose formaldehyde gel. The Northern blot was hybridised to pEV DNA. The arrowhead indicates the position of the ribosomal artefact.

Discussion

Injected DNA, from plasmid or phage clones, is expressed during embryonic development. Several experiments from the expression of *copia* DNA in *D. bipectinata* embryos to the expression of the SFV capsid gene show that it is the injected DNA that is transcribed and not endogenous sequences which are activated by titration of a regulatory substance. Transcription yields RNA of the correct mol. wt. and, in the case of the SFV capsid gene, we have shown that it is strictly dependent on the presence of the heat-shock or the *copia* promoter in the correct orientation, indicating that it originates

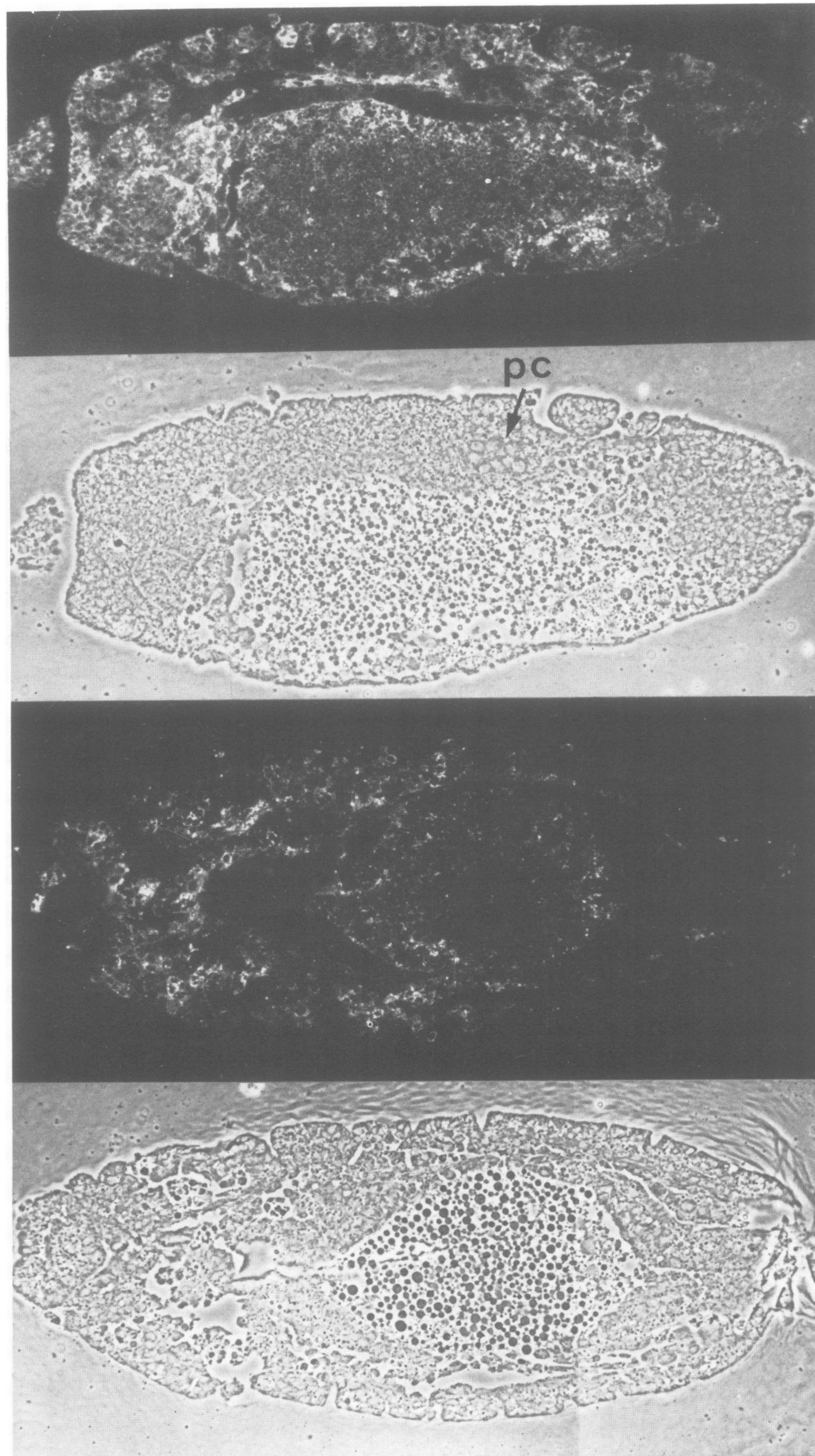


Fig. 9. Thin section immunofluorescence of embryos injected with pEV-hs DNA. Embryos injected with pEV-hs DNA were allowed to develop for 10 h at 25°C, then heat-shocked for 1 h at 37°C. They were then sectioned and stained by indirect immunofluorescence against SFV capsid protein. Cell nuclei remain dark as the capsid protein is exclusively cytoplasmic. **(a)** Embryo injected with 150 pg pEV-hs DNA. Fluorescence image; **(b)** same section viewed under phase contrast. The arrow labelled pc indicates the invaginated pole cells, whose cytoplasm fluoresces but whose large nucleus remains dark; **(c)** embryo injected with 50 pg pEV-hs DNA. Fluorescence image; **(d)** same section viewed under phase contrast. In all cases the anterior end is to the left of the picture.

from the promoter.

The *copia* terminal repeat sequences function as promoters during embryonic development but were not very efficient in our experiments. This may reflect the normal *in vivo* situation since, although the transcripts from endogenous *copia* have been detected in 2–5 h embryos, most are apparently degraded in the nucleus (Young and Schwartz, 1981) and do not accumulate in the cytoplasm until after 10 h (Scherer *et al.*, 1981). Both *copia* terminal repeats contain promoters which are functional in the embryo. In an intact *copia* ele-

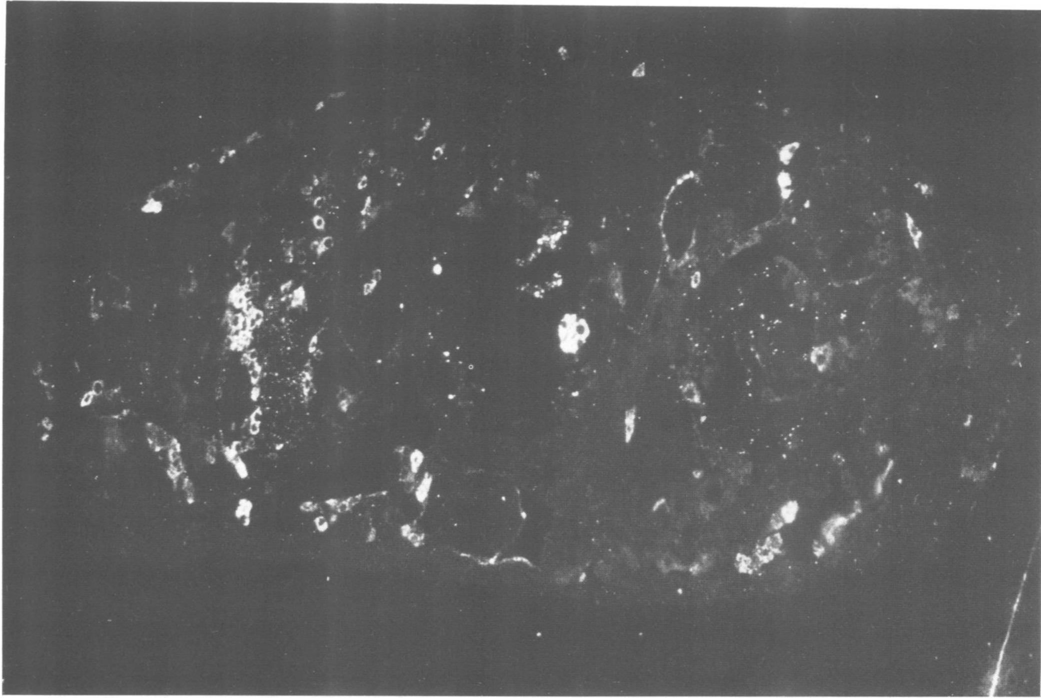


Fig. 10. Thin section immunofluorescence of a first instar larva. Embryos injected with 150 pg pEV-hs DNA were allowed to develop at 25°C until they hatched as first instar larvae (24 h), then heat-shocked 1 h at 37°C, sectioned and stained by immunofluorescence again SFV capsid protein. Only some 15% of the cells fluoresce strongly but many others fluoresce weakly or intermediately. A pattern of fine grains of fluorescence is reproducibly found in the region of the larval brain and nervous system. The anterior end is to the left.

ment transcription extends outside the right terminal repeat and into flanking sequences. It would be possible therefore for a *copia* element to activate the expression of a gene by inserting upstream of it.

We do not know whether the transcription we observe from injected DNA is 'normal'. That is, whether it reflects a normal nuclear mechanism of promoter recognition within a template assembled in the form of chromatin. In favor of 'normal' transcription are the following observations. (i) No transcription of injected DNA is observable before blastoderm. (ii) An important proportion of the transcripts detected are found in the nuclear fraction. (iii) The transcripts seen are consistent with normal splicing and maturation. (iv) Transcription is at least partially subject to developmental control. These observations do not suffice to prove that the transcription takes place in nuclei.

In another set of experiments (Steller and Pirrotta, in preparation) we have shown that up to 80% of the injected DNA, whether plasmid or phage, remains in the cytoplasm where it is slowly degraded and only 20% enters the nucleus. However, even allowing for the gradual loss of the injected DNA, transcription from the injected genes is at least an order of magnitude less efficient than that from endogenous genes. It is possible therefore that only a fraction of the injected molecules is able to enter the proper cellular compartment and be transcribed.

Developmental control

The results obtained with embryos injected with developmentally regulated genes suggest that at least some of the corresponding promoters respond to developmental controls. Transcription of the B205 gene which is normally specific for the early embryonic stages, is very strongly enhanced by injection of B205 DNA. We do not see a band corresponding to the larval *Adh* RNA or to larval cuticle protein RNAs, which should not be expressed in the embryo. We do observe a band of the size of the adult type *Adh* RNA and one of the size of *sgs*-4 RNA. This may mean that the corresponding promoters can function in the embryo. There is in fact a low but detectable level of endogenous adult-type *Adh* RNA in the embryo. Similarly, although it was not detected in our experiments, a very low level of *sgs*-4 RNA has been observed in embryos (O. Pongs, personal communication).

These low amounts may be due to a basal level of expression of the endogenous *Adh* and *sgs*-4 genes. A possible interpretation of our results is that genes which have at least a basal level of expression in the embryo also function when injected, while genes not detectably expressed in the embryo do not function when injected.

There remains the theoretical possibility that the transcription observed comes not from the injected DNA but from the endogenous genes which are derepressed when sufficient DNA is injected to titrate a hypothetical repressor. Although we consider it unlikely, our results do not rule this out in the case of the *Adh* and *sgs*-4 genes.

Expression of injected genes before blastoderm

We have been unable to detect transcription of injected DNA in the early embryo although we should have been able to see as little as 1000 RNA molecules per embryo or one transcript per 1000 injected template molecules. Newport and Kirschner (1982) have reported that a tRNA gene injected into fertilised *Xenopus* eggs gives rise to a short burst of transcription before becoming silent until the onset of nuclear transcription at the mid-blastula transition. It is possible that in *Drosophila* embryos such transient expression is very brief or absent and injected DNA is rapidly converted to a repressed form. In our experiment the block to nuclear gene expression is gradually

released, beginning around 90 min of development when pole cells are formed. The nuclear transcripts appearing at this time are qualitatively different from the bulk of transcription which is turned on around 2 h. It is possible that these early RNA species represent transcriptional activity of pole cells which form ~30 min before blastoderm and might initiate transcription correspondingly earlier.

Localisation of expression

Injected *copia*-SFV DNA produces detectable SFV capsid protein mostly in the central yolk mass, with a few isolated exceptions. However, autoradiography of radioactive injected DNA (Steller and Pirrotta, in preparation) as well as the expression of injected *hsp*-70-SFV DNA show that the injected DNA reaches all parts of the embryos. We conclude that the expression of *copia* promoted genes is localised by regulating the transcription, the processing or the translation of the RNA. Most *copia* RNA is reportedly turned over in the nucleus while a majority of cytoplasmic *copia* RNA is not associated with polysomes (Young and Schwartz, 1981; Falkenthal *et al.*, 1982). It is possible that such controls are dependent on the 5′ end of the *copia* RNA and are retained in our *copia*-SFV hybrid. However, it is also possible that strong expression of the SFV capsid protein is harmful and selectively kills the cells that indulge in it.

Of particular interest are the results obtained with the *hsp* 70-SFV construction. In this case possible harmful effects of expression were avoided by allowing development to proceed at low temperature. We can conclude that the control of the *hps*-70 promoter is very tight: neither fluorescence nor transcription were detected at 25°C. After heat-shock, the distribution of fluorescence shows that the injected DNA reaches virtually all cells and persists in them at least for 12 h of embryonic development. By the first instar stage, however, as many as 80% of the cells are no longer able to express strongly the injected *hsp*-70-SFV DNA although we do not yet know the significance of the weakly fluorescing cells.

Heat-shock in *Drosophila* affects both transcription and translation of normal genes (Storti *et al.*, 1980). Normal RNAs do not initiate translation and those already on polysomes are inhibited by a block to elongation (Ballinger and Pardue, 1982). Since the expression we observed was obtained at 37°C, we conclude that some *hsp*-70-SFV RNA escapes the translational block, or that the sequences which permit the bypass of the block are contained in the first 180 nucleotides of the *hsp*-70 RNA.

The *hsp*-70-SFV construction opens the door to a range of interesting applications. Since it shows that virtually all cells receive and express the injected *hsp* promoter, it should be possible to rescue embryos carrying mutations affecting early development or to alter development by causing premature or inappropriate expression of certain developmental genes. It may also be possible to alter the tissue specificity or localisation of the expression of certain genes. The strict temperature dependence of the *hsp*-70 promoter would allow normal development until the temperature pulse.

The ease and rapidity of the injection and transient expression technique should permit the screening of recombinant clones from clone libraries or chromosomal walks for their effects on the embryo or early larva.

Materials and methods

Construction of expression vectors

Plasmid pUC-*copia* was constructed by inserting a *Hpa*II fragment containing the entire *copia* element from clone pBR5002 (Levis *et al.*, 1980) into the *Sma* site of pUC9 (Vieira and Messing, 1982). A vector designed for the expression of the SFV capsid protein was constructed by subcloning the 3.0-kb fragment from the pSV2 capsid clone of Kondor-Koch *et al.* (1982) into the *Eco*RI site of pUC9. This construction, now called pEV, contains the capsid protein gene immediately preceded by the polylinker of pUC9 and followed by the polyadenylation signal from SV40 extracted from the mammalian expression vector of Mulligan and Berg (1980).

Using the polylinker which precedes the SFV capsid gene in pEV we inserted a 420-bp fragment containing the first 140 bp of the *copia* terminal repeat in the proper orientation to transcribe the capsid gene. This construction we call pEV *copia* and is diagrammed in Figure 2.

The pEV plasmid was also used to construct pEV-hs by inserting in the polylinker a *Xba*I-*Xmn*I 456-bp fragment containing the promoter and the first 198 nucleotides of the *hsp*-70 major heat-shock gene (Ingolia *et al.*, 1980).

Microinjection

After a 3 h pre-collection period, eggs were collected at 15–30 min intervals at 25°C. All other operations were carried out at 18°C. After hand dechorionating, the eggs were mounted in a row on the edge of a microscope slide covered with double sided scotch tape. The embryos were dehydrated over silica gel for 15–20 min then covered with 10–20 S Voltalef oil. Injection needles were made with an electrode puller from thin walled glass capillaries and were mounted on a de Fonbrune micromanipulator. The needles, with an opening of 1–3 μm diameter, were filled with a pressure system connecting them either to a 10 ml syringe (for filling) or to a 100 μl Hamilton syringe controlled by a micrometer screw (for injecting).

The DNA solutions (50–1000 μg/ml in 5 mM KCl, 0.1 mM sodium phosphate pH 6.8) were injected by inserting the needle into the posterior end of the embryos. The pressure system was adjusted so that flow initiated spontaneously as soon as the needle was inserted in the embryo and stopped when it was withdrawn into the oil. To ensure even distribution, the needle was inserted nearly the full length of the egg and slowly withdrawn. The volume injected by this procedure depends on the degree of dehydration of the embryo and was usually 0.1–0.3 nl. After injection the embryos were transferred to a humidified Petri dish and incubated at 18°C for the desired time. Here incubation times are always referred to in terms of physiological temperature, 25°C. Development at 18°C takes approximately twice as long.

With care, up to 200 embryos could be injected per hour with a survival rate of 80% to first instar larvae and 50–60% to adult flies.

To label newly synthesized RNA, embryos were injected with [α-^{32}P]UTP at 3000 mCi/μmol and ~1 mCi/μl to give 0.1–0.3 μCi/embryo.

RNA extraction

Injected embryos were removed from the scotch tape by dissolving the adhesive with heptane. Thirty to fifty embryos were homogenised in 40 μl of GHCl buffer (7.5 M guanidine-HCl, 0.025 M sodium citrate pH 7.0, 5 mM dithiothreitol) containing 0.5% lauryl sarcosine, 0.2–0.5% diethylpyrocarbonate. After addition of 1 μl 1 M acetic acid, the RNA was precipitated with 25 μl ethanol at −20°C. The pellet was resuspended in 40 μl GHCl buffer and re-precipitated with 1 μl 1 M acetic acid and 25 μl ethanol. Three precipitations from GHCl are usually sufficient to remove the DNA (Chirgwin *et al.*, 1979).

To separate cytoplasmic and nuclear RNA, 30–50 embryos were homogenised in 30 μl ice-cold lysis buffer containing 0.14 M NaCl 1.5 mM $MgCl_2$ 10 mM Tris-HCl, pH 8.6 0.5% NP-40 and 1000 units/ml placental RNase inhibitor.

The suspension was underlayered with 30 μl of the same buffer containing 24% (w/v) sucrose and 1% NP-40. The nuclei were sedimented by centrifugation at 10 000 *g* for 20 min. The supernatant (cytoplasmic fraction) was removed and added to 60 μl of PK buffer (0.2 M Tris pH 7.5, 25 mM EDTA, 0.3 M NaCl and 2% SDS) containing 5 μg pupal RNA as carrier. The nuclear fraction was resuspended in 30 μl lysis buffer and lysed by adding an equal volume of PK buffer containing 5 μg rat rRNA as carrier. Both fractions were extracted twice with phenol-chloroform isoamyl alcohol (25:25:1) and precipitated with 2.5 volumes of ethanol. For some Northern blot analyses, the RNA was treated with 1.8 μg DNase, RNase free (a gift of V. Esposito) for 30 min at 37°C.

Northern blot hybridisation

RNA samples were analysed by formaldehyde-agarose gel electrophoresis (Goldberg, 1980). After electrophoresis the gels were saturated with 20 x SSC buffer and blotted onto nitrocellulose filters.

After baking at 80°C in a vacuum oven, the filters were pre-hybridised at 42°C in 50% deionized formamide, 5 x Denhardt's solution (Denhardt, 1966), 5 x SSPE buffer (1 x SSPE is 0.18 M NaCl, 0.01 M NaH_2PO_4 pH 7.4, 1 mM EDTA) and 100 μg/ml denatured, sonicated salmon sperm DNA. Hybridization was done under the same conditions but with 1 x

Denhardt's solution and with the addition of 0.3% SDS and 2 x 10^6 c.p.m./ml of a DNA probe labelled by nick translation. After hybridisation, filters were rinsed and finally washed in 0.1 – 0.5 x SSPE, 0.2% SDS at 65°C for one or more hours. After autoradiogaphy to verify that different lanes had received the same amount of RNA, the filters were washed for 15 min with 5% acetic acid then the RNA was stained with 0.04% methylene blue in 0.5 M sodium acetate pH 5.2.

Thin sections and immunofluorescence staining of embryos

Embryos were permeabilized by treating the heptane for 10 min, then fixed for 15 – 30 min in 3% paraformaldehyde in PBS (1.86 mM NaH_2PO_4, 12.6 mM Na_2HPO_4, 0.15 M NaCl). The embryos were freed from the vitelline membrane by dissection with a fine needle. After two additional hours in the fixative, the embryos were washed 2 x 20 min in PBS plus 10% sucrose. They were then infiltrated with molten 15% gelatine in PBS for 1 h at 37°C, allowed to set at 4°C and fixed with the fixative solution for 4 – 12 h at 4°C. The gelatine blocks were washed in PBS plus 10% sucrose for 30 min, and then in 2 M sucrose for 1 h. Sections 0.5 – 1 μm thick were cut on a cryo-microtome at –50°C, applied to acid-washed slides and treated with rabbit anti-SFV capsid antibody (a gift from K. Simons) followed by rhodamine-conjugated goat anti-rabbit serum as described by Timm *et al.* (1983). the stained sections were photographed under a fluorescence microscope with a rhodamine filter.

Acknowledgements

We are grateful to K. Simons and A.M. Ohlsen for advice on thin section immunofluorescence, to C. Christensen, J. Stanger and A. Weischer for their patience with photographic reproduction and to W. Moses for typing the manuscript. H. Steller was the recipient of an EMBL predoctoral fellowship.

References

Anderson,K.V. and Lengyel,J.A. (1979) *Dev. Biol.*, **70**, 217-231.
Ballinger,D. and Pardue,M.L. (1982) in Schlesinger,M.J., Ashburner,M., and Tissieres,A. (eds.), *Heat Shock from Bacteria to Man*, Cold Spring Harbor Laboratory Press, NY, pp. 183-190.
Benyajati,C., Place,A.R., Powers,D.A. and Sofer,W. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 2717-2721.
Benyajati,C., Spoerel,N., Haymerle,H. and Ashburner,M. (1983) *Cell*, **33**, 125-133.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry (Wash.)*, **18**, 5294-5299.
Denhardt,D.T. (1966) *Biochem. Biophys. Res. Commun.*, **23**, 641-646.
Falkenthal,S., Graham,M.L., Korn,E.L. and Lengyel,J.A. (1982) *Dev. Biol.*, **92**, 294-305.
Flavell,A., Levis,R., Simon,M. and Rubin,G. (1981) *Nucleic Acids Res.*, **9**, 6279-6291.
Goldberg,D.A. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5794-5798.
Ingolia,T.D., Craig,E.A. and McCarthy,B.J. (1980) *Cell*, **21**, 669-679.
Kondor-Koch,C., Riedel,H., Söderberg,K. and Garoff,H. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4525-4529.
Levis,R., Dunsmuir,P. and Rubin,G.M. (1980) *Cell*, **21**, 281-288.
McKnight,S.L. and Miller,O.L. (1976) *Cell*, **8**, 305-319.
Merton,S.H. and Pardue,M. (1981) *J. Mol. Biol.*, **153**, 1-21.
Mulligan,R.C. and Berg,P. (1980) *Science (Wash.)*, **209**, 1422-1427.
Muskavitch,M.A.T. and Hogness,D. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 7362-7366.
Newport,J. and Kirschner,M. (1982) *Cell*, **306**, 687-696.
Scherer,G., Telford,J., Baldari,C. and Pirrotta,V. (1981) *Dev. Biol.*, **86**, 438-447.
Snyder,M., Hunkapiller,M., Yuen,D., Silvert,D., Fristrom,J. and Davidson, N. (1982) *Cell*, **29**, 1027-1040.
Storti,R., Scott,M., Rich,A. and Pardue,M. (1980) *Cell*, **22**, 825-834.
Timm,B., Kondor-Koch,C., Lehrach,H., Riedel,H., Edström,J.-E. and Garoff,H. (1983) *Methods Enzymol.*, **96**, 496-511.
Ursprung,H., Sofer,W.H. and Burroughs,N. (1970) *Wilhelm Roux's Arch. Dev. Biol.*, **164**, 201-208.
Vieira,J. and Messing,J. (1982) *Gene*, **19**, 259-268.
Young,M. and Schwartz,H.E. (1981) *Cold Spring Harbor Symp. Quant. Biol.*, **45**, 629-640.
Zalokar,M. (1976) *Dev. Biol.*, **52**, 31-42.

Received on 28 September 1983; revised on 2 November 1983