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The detection of *Jonah* gene transcripts in *Drosophila* by *in situ* hybridization

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The *Jonah* genes constitute a family of ~20 genes expressed at two periods during the development of *Drosophila melanogaster*. They are expressed only in the midgut, where they yield very abundant transcripts of ~900 bases. The function of their products is not known. We have used *in situ* hybridization to show that transcripts homologous to two members of the *Jonah* family have quite different distributions within the midgut. Transcripts closely homologous to *Jon65Aiv* are expressed throughout most of the anterior midgut and in the posterior section of the middle midgut. Transcripts closely homologous to *Jon99Cβ* are expressed only in the anterior region of the posterior midgut.

Key words: Drosophila/in situ hybridization/*Jonah*/midgut/RNA

Introduction

This paper describes the distribution in the larval gut of transcripts homologous to two members of the *Jonah* gene family. One purpose of the study was to investigate conditions for *in situ* hybridization. The *Jonah* RNAs are very abundant, and were known to be present in RNA extracted from midgut, but undetectable in RNA isolated from other tissues. They are thus excellent transcripts with which to test the specificity of *in situ* hybridization procedures.

A second aim was to investigate the function of the *Jonah* genes by mapping the precise distribution of their transcripts. The *Jonah* genes were discovered among cDNA clones prepared from larval RNA (Wolfner, 1980; Carlson, 1982). One of us has shown that there are ~20 sequences in the *Drosophila* genome that share homology and constitute the *Jonah* family, but these do not appear to be nomadic *copia*-like elements. Rather they would seem to be a family of genes encoding gut-specific proteins. The *Jonah* RNAs can be translated *in vitro*, but the function of the proteins for which they code is not known.

Here we show that the *Jonah* RNAs can be readily localized within the midgut by *in situ* hybridization. We find that expression of individual *Jonah* genes in larvae is limited to subsegments of midgut, and moreover that transcripts from different members of the *Jonah* family are abundant in different regions of the midgut.

The organization of the *Jonah* family is illustrated in Figure 1 and documented elsewhere (Carlson, 1982; Carlson and Hogness, 1985a, 1985b); only a summary is presented here.

Jonah genes are both clustered and dispersed: they are distributed in small clusters at eight or more widely dispersed chromosomal sites. The clusters exhibit a variety of configurations – direct repeats, inverted repeats and combinations of direct and inverted repeats (Figure 1). The family is defined on the basis of sequence homology, but restriction site heterogeneity is extensive within the family.

Members of the family are expressed twice in development (Carlson, 1982; Carlson and Hagness, 1985b). Filter hybridization experiments show that *Jonah* RNA is abundant during all three larval instars, declines at the end of the third instar, and is not detectable in the pupa. The RNA then appears a few hours after the eclosion of the adult, and remains present throughout adult life. At all of these stages *Jonah* RNA appears as a single band of size 900–920 bases. Filter hybridization to RNA isolated from dissected third instar larval and adult organs detects *Jonah* RNA exclusively in the midgut. Hafen *et al.* (1983) have detected low levels of *Jonah* RNA in

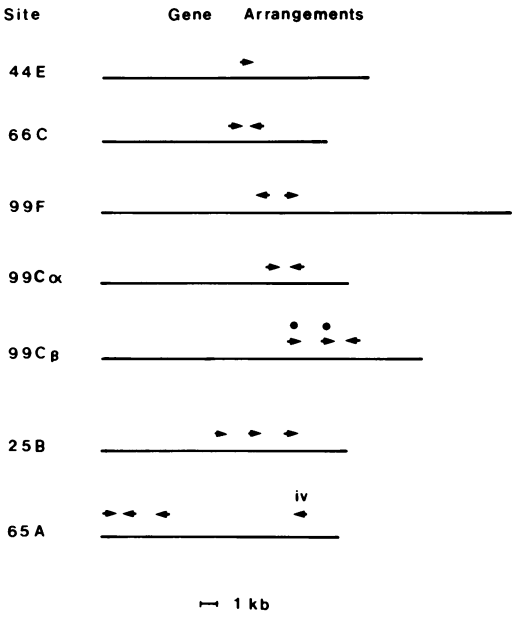

Fig. 1. *Jonah* gene arrangements at different chromosomal sites. Bars indicate cloned chromosomal segments containing regions homologous to a reference cDNA clone under standard filter hybridization conditions (Carlson, 1982; Carlson and Hogness, 1985a, 1985b). The chromosomal sites from which each segment derives are designated according to the lettered subdivisions of the standard polytene chromosome maps. The positions and orientations of the regions homologous to *Jonah* RNA are indicated by arrows, which have been drawn to correspond to the length of the mature *Jonah* RNA (910 bases), and to point in the direction of transcription. Positions of the arrows are approximate, and are based on restriction mapping, hybridization of cDNA probes to electrophoretically fractionated restriction fragments, isolation and analysis of inverted repeats, and heteroduplex analysis (Carlson, 1982). The cDNA plasmid adm135A8 derives from gene iv at 65A; the genomic fragment cloned in plasmid aDm3201 derives from one of the two genes marked ● located at 99Cβ (see text).

16–18 h embryos; at this stage the RNA is confined to presumptive midgut cells.

By synthesizing cDNA clones and exploiting the structural heterogeneity among different *Jonah* genes, Carlson has shown that at least five *Jonah* genes are expressed, and that at least one cluster of *Jonah* genes is active during both larval and adult stages (Carlson, 1982). At at least one *Jonah* site, two *Jonah* genes are expressed.

Results

Conditions for in situ hybridization

For the experiments described below we have used a modification of the hybridization procedure established by Brahic and Haase (1978), similar to that which we describe elsewhere for use with single-stranded probes (Akam, 1983).

In preliminary experiments to test the specificity of hybridization we compared patterns of labelling obtained with different probe sequences: a *Jonah* probe, expected to hybridize only to midgut cells; an *Sgs-3* probe, homologous to an abundant RNA encoding a salivary glue polypeptide which is expressed only in the salivary gland (Meyerowitz and Hogness, 1982), and probe prepared from plasmid vector sequences alone (pBR322) or from other plasmid clones which should have no homology to RNA in the *Drosophila* sections. These experiments confirm that the patterns of labelling observed are sequence specific (Figures 2,3). Moreover, the *Jonah* and *Sgs-3* probes label principally the cytoplasm, suggesting that hybridization is primarily to cytoplasmic RNA and not to nuclear DNA. Pre-treatment of the sections with ribonuclease eliminates specific labelling, confirming this suggestion.

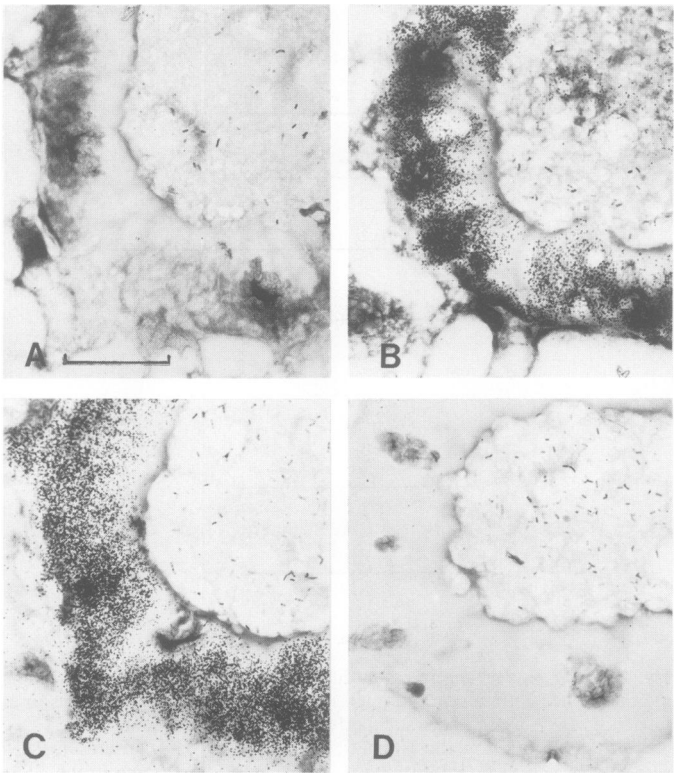

Fig. 2. *In situ* hybridization with *Jonah* probes. Adjacent sections from a single loop of midgut from an individual larva hybridized with probes prepared from pBR322 **(panel A)**; a *Drosophila* sequence encoding gene 99C cloned in pBR322 **(panel B)**; and the excised *Drosophila* sequence from the same plasmid **(panel C)**. **Panel D** was hybridized exactly as **panel B**, except that the section was pre-treated with ribonuclease before hybridization (100 μg/ml, 37°C for 1 hr). Probes: **(A)** pBR322 whole plasmid; 1.2 x 10^8 d.p.m./μg, 6 x 10^4 d.p.m./μl; **(B,D)** aDm3201 whole plasmid; 1 x 10^8 d.p.m./μg, 6 x 10^4 d.p.m./μl; **(C)** aDm3201 excised fragment, 9 x 10^7 d.p.m./μg, 6 x 10^4 d.p.m./μl. Autoradiographic exposure 4.5 days. NTB2 emulsion. Scale = 50 μm.

We have not optimized all aspects of the protocol used, but control experiments indicate one variable which is particularly important. Short probe fragments are essential for effective *in situ* hybridization under these conditions. Brahic and Haase (1978) observed that the optimum probe size was very short. We find an even greater dependence of hybridization intensity on probe size (Figure 4). The most effective probe fractions had a single strand length < 50 bases. We assume that the low efficiency of larger probes results from their inability to penetrate the tissues effectively.

Distribution of Jonah transcripts

We have examined the distribution in third instar larvae of transcripts homologous to two members of the *Jonah* gene family, genes located at chromosomal sites 99C and 65A.

The gene located at 99C, which we refer to as *Jon99Cβ*, (see Materials and methods for details of the *Jonah* nomenclature) is closely homologous to a large group of *Jonah* genes, as judged by their strong cross-hybridization under moderately stringent annealing conditions. Probe prepared from this one gene should therefore detect the distribution of RNAs transcribed from any one of the closely related genes.

The gene located at 65A, designated *Jon65Aiv*, is a divergent member of the *Jonah* family. It cross hybridizes to other *Jonah* genes only under conditions of low stringency (Carlson, 1982). From the results which we obtain it is clear that probes prepared from gene *Jon99Cβ* do not cross-hybridize efficiently *in situ* with RNA transcribed from gene *Jon65Aiv* and *vice versa*.

The regions of the gut containing transcripts homologous to each of these two probes are illustrated in Figures 5 and 6, and shown diagrammatically in Figure 7. *Jon65Aiv* hybridizes to two separate regions: one region includes most of the anterior midgut, the other covers the posterior part of the middle midgut. *Jon99Cβ* hybridizes to a single region, the most anterior part of the posterior midgut. This region overlaps slightly the most posterior section of the gut which is labelled with *Jon65Aiv*.

Since these two probes detect *Jonah* RNA in different regions of the midgut it is clear that they are hybridizing to different populations of *Jonah* RNA molecules. The maximum overlap in these RNA populations is indicated by the extent to which *Jon65Aiv* probe labels the regions of the gut which is maximally labelled with *Jon99Cβ* probe, and *vice versa*. In both cases such labelling is only a few fold above background levels, and amounts to < 5% of the signal observed in maximally labelled regions.

It is not possible to discriminate exactly which *Jonah* genes are being transcribed in each labelled region. Probes from *Jonah* genes located at 25B (clone adm2371) and 66C (clone aDm2352) show the same distribution of hybridization as probe from 99C (results not shown); these probes cross-hybridize strongly with the 99C probe under conditions more stringent than those used here, and probe from each probably hybridizes *in situ* to transcripts from all active members of this group of genes. Hence any or all of them may be active in the region to which probe *Jon99Cβ* hybridizes. Similarly, transcripts homologous to gene *Jon65Aiv* may be derived

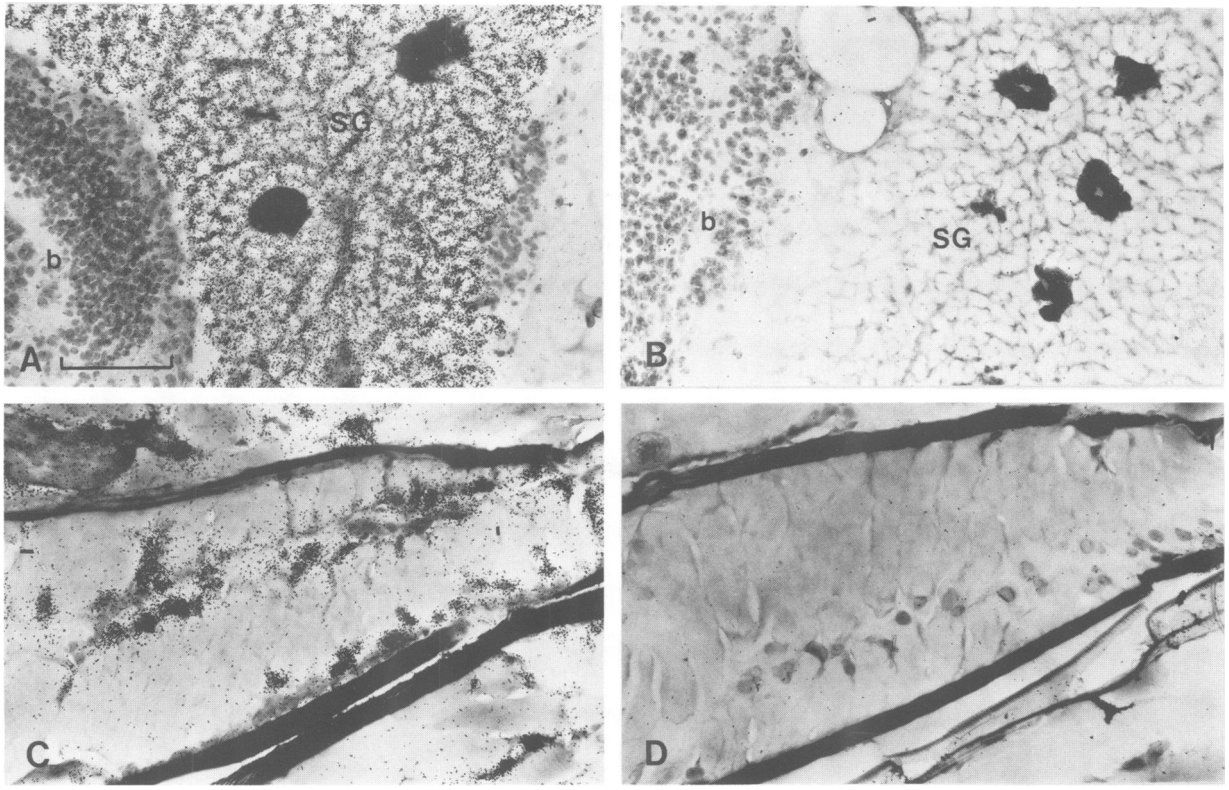

Fig. 3. Specific hybridization to RNA in other tissues. **Panels A** and **B:** salivary gland (SG) with large polytene nuclei and adjacent brain (b) tissue with diploid nuclei. Both sections hybridized with probes prepared from cDNA clones of similar construction, but carrying different *Drosophila* sequences, one of which is homologous to a transcript which is abundant in the salivary gland **(A)**, the other rare or absent **(B). Panels C** and **D:** sections through the pharyngeal musculature of larvae, heat shocked for 1 h at 37°C before sectioning. **Panel C:** probed with plasmid containing an insert of the gene encoding the 70 kd heat shock protein (Artavanis-Tsakonas *et al.*, 1979); **panel D:** probed with plasmid containing an insert of the histone repeat unit of *D. melanogaster* (Lifton *et al.*, 1978). Note that only very weak hybridization is evident to the nuclei in panel D, despite the ~100 x reiteration of the histone repeat unit in the genome. **Probes: (A)** plasmid adm124E8, insert homologous to the salivary glue protein gene *Sgs-3*. 1.2 x 10^8 d.p.m./μg; 6 x 10^4 d.p.m./μl; **(B)** plasmid adm129E7, insert homologous to very rare *Drosophila* transcript. 1.8 x 10^8 d.p.m./μg; 4.5 x 10^4 d.p.m./μl; **(C)** plasmid 132E3, 3 x 10^7 d.p.m./μg; 7 x 10^4 d.p.m./μl; **(D)** plasmid aDm500, 1.6 x 10^8 d.p.m./μg; 1 x 10^5 d.p.m./μl. Autoradiographic exposure: **A** and **B**, 15 days; **C** and **D**, 12 days. Scale = 50 μm.

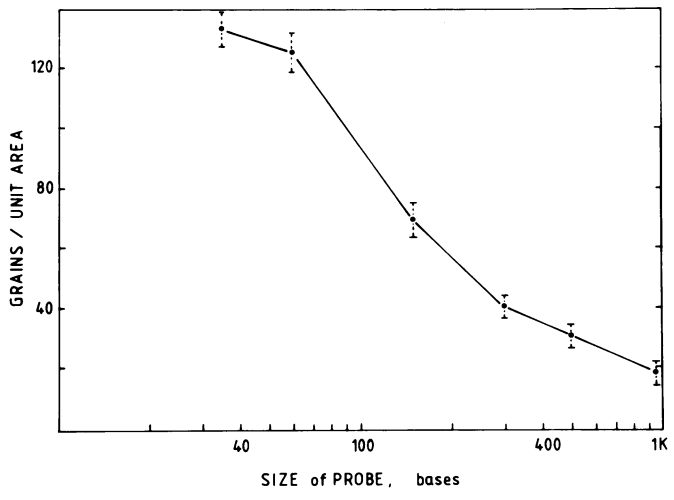

Fig. 4. Effect of probe size on hybridization efficiency. Sized probe fractions from plasmid aDm3201 were hybridized to serial sections from a single loop of larval midgut. The size distribution of each probe fraction was estimated by electrophoresis on denaturing gels; the size distribution of each fraction overlapped with that of adjacent fractions but not with alternate fractions. Probe specific activity 1.5 x 10^8 d.p.m./μg at 5 ± 1.5 x 10^4 d.p.m./μl. Exposure 3.5 days. Grain counts represent means (with their standard errors) of counts on five areas taken from three different sections hybridized with each probe fraction.

from this gene itself, or from *Jonah* sequences located at 74E to which it is closely homologous.

Histological limits of Jonah-positive regions

The *Drosophila* larval midgut may be somewhat imprecisely subdivided into three regions: anterior, middle and posterior, (Strasburger, 1932; Poulson and Waterhouse, 1960; Filshie *et al.*, 1971). These correspond approximately with regions derived from different embryonic primordia, and with three major functional zones, but the correlation between developmentally and functionally defined regions has been established only in part.

The anterior midgut (AMG) is taken to include the outer cell layer of the proventriculus, all of the stomach, the gastric caecae, and the first loop of gut caudal to the stomach. The gut epithelium throughout this region is composed of large cuboidal polytene cells with strongly basophilic cytoplasm (Strasburger, 1932; Bodenstein, 1950). Virtually the whole of this region is labelled with the *Jon65Aiv* probe. The only cells which do not label are those in the outer cell layer of the proventriculus. At the neck of the proventriculus there is an abrupt transition between cells which are unlabelled, and those which are maximally labelled by *Jon65Aiv* (Figure 6). Cells on either side of this boundary are not otherwise distinguishable in our preparations.

Caudally, the anterior midgut is bounded by a short tran-

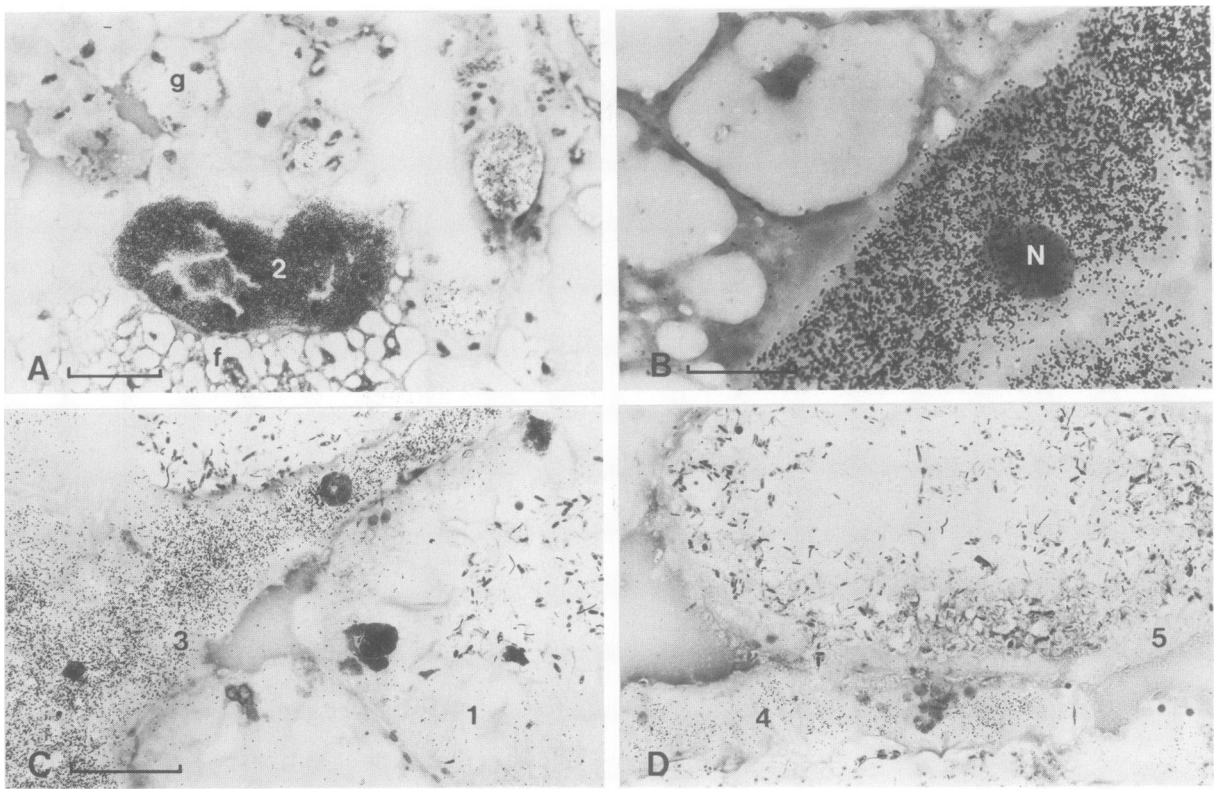

Fig. 5. Distribution of RNA homologous to *Jon99Cβ*. **Panels A – D** show regions of the midgut of a single larva probed with *Jon99Cβ*. **Panel A** shows a loop of gut (2) in the region of the midgut which labels most strongly with this probe. It is surrounded by fat body (f), gastric caecae (g), and other loops of gut. A region of the labelled gut is enlarged in **panel B**. Label is distributed throughout the cytoplasm of these large polytene cells; the nucleus (N) is labelled less heavily. **Panel C** includes a transverse section through the gut at a more anterior level, near the base of the stomach (1). This region labels strongly with *Jon65Aiv*, but only very weakly with *Jon99Cβ*. Regions of the midgut 3, 4 and 5 **(panels C** and **D)** are increasingly caudal to the maximally labelled region, and show the gradual decrease in the intensity of hybridization. Section hybridized with aDm3201 plasmid, 1 x 10^8 d.p.m./μg; 7 x 10^4 d.p.m./μl. Autoradiographic exposure 12 days. Scales: **A** = 100 μm; **B** = 20 μm; **C,D** = 50 μm.

sitional zone leading into the middle midgut (MMG). The MMG can be functionally defined as a zone of low gut luminal pH (pH 3 – 4) between the neutral to alkaline regions of the anterior and posterior midguts. It extends through at least three regions which can be distinguished histologically. The epithelium in the first of these zones is a mosaic of two principal polytene cell types, goblet cells (cuprophilic cells or calycocytes) and interstitial cells (Filshie *et al.*, 1971). In sections prepared for *in situ* hybridization this zone is clearly distinguishable by the vacuolated appearance of the goblet cells and by the weak staining of the whole epithelium (Figures 5,6). No cells in this region are labelled by either *Jonah* probe. At the junction of the AMG and the MMG there is a rapid decline in the concentration of *Jonah* RNA which parallels the transition in staining characteristics of the cells.

In the most posterior part of the MMG a short section of large flat cells is followed by a region of cuboidal, basophilic cells, distinguished by their ability to accumulate iron from the food media (Poulson and Waterhouse, 1960). Cells throughout both of these regions are labelled by the *Jon65Aiv* probe, though occasional unlabelled polytene cells have been observed in an otherwise uniformly labelled epithelium. The transition between unlabelled cells in the first zone of the MMG and labelled cells in these following zones is abrupt.

The posterior midgut is not a homogeneous region, but no clearly demarcated zones can be distinguished within it. The anterior part of the posterior midgut has a thick, strongly basophilic cuboidal epithelium, with large polytene cells somewhat resembling those of the AMG. This reduces gradually to a thin weakly staining squamous epithelium in the posterior part.

The *Jon99Cβ* probe hybridizes very strongly to the anterior part of the PMG. The intensity of hybridization falls gradually towards the hindgut, and neither *Jonah* probe labels the more posterior half of the PMG (Figures 5,6).

There is no abrupt transition in the expression of the *Jonah* genes in the region where the MMG is considered to give way to the PMG (Filshie *et al.*, 1971). Homology to both *Jon65Aiv* and *Jon99Cβ* is detected in a transitional zone covering ~10 cell diameters along the length of the gut. The intensity of hybridization to *Jon65Aiv* falls in the caudal direction, while labelling with *Jon99Cβ* rises. We are not able to distinguish a cytological discontinuity in this region which might independently locate the boundary between the posterior region of the MMG as defined above and the PMG.

In most regions of the midgut, diploid imaginal cells can be distinguished, located between the polytene cells of the larval gut and the underlying basement membranes. Where small clusters of these cells are visible in section it is clear that they are unlabelled by the *Jonah* probes, even in those regions of the gut where the polytene cells are most strongly labelled.

Discussion

The number of copies of *Jonah* genes in the genome is similar to that of the *copia*-like elements, but no other features of this gene family suggest that they are transposable elements. *Jonah* genes are present at conserved sites in several strains of

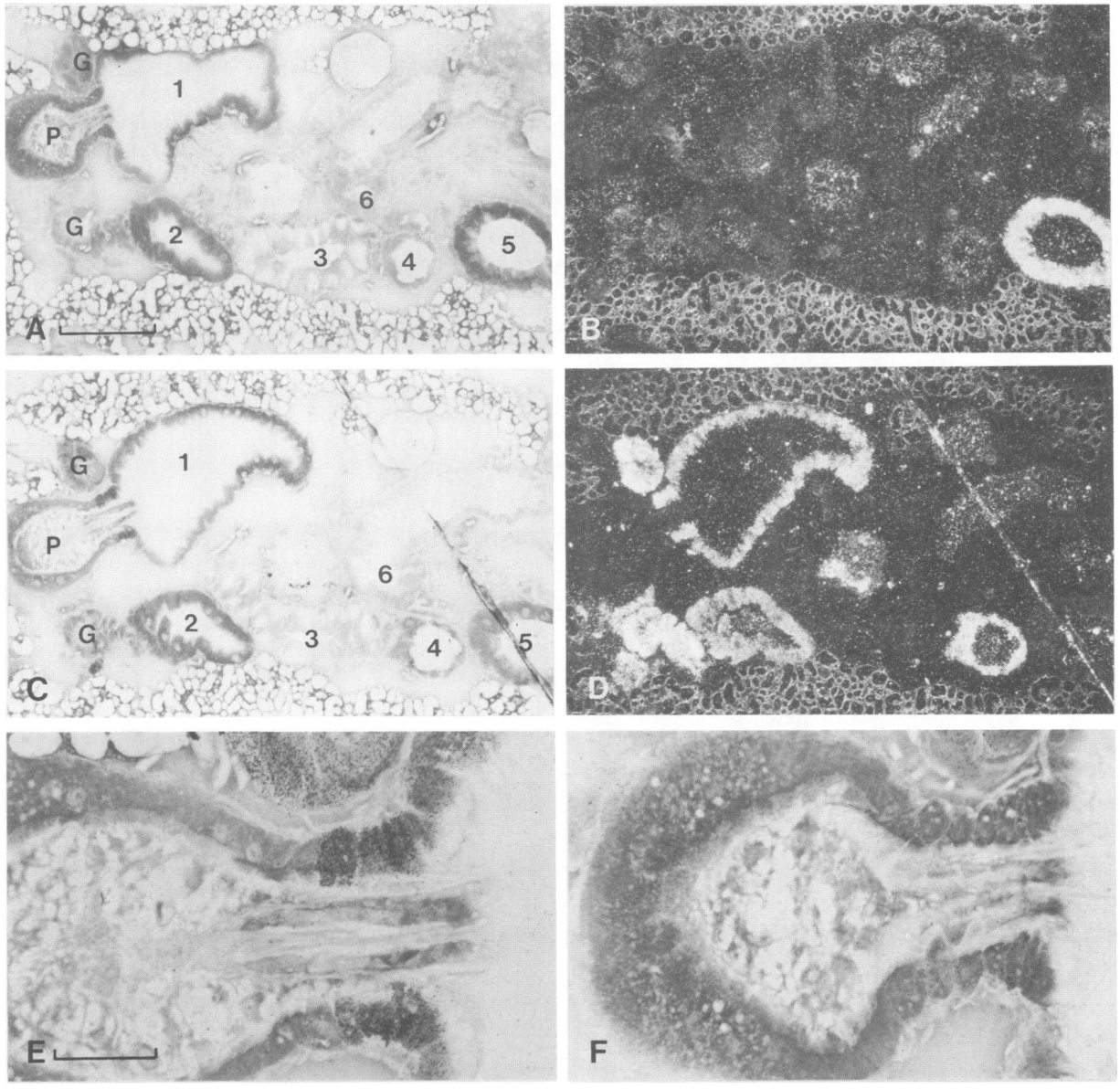

Fig. 6. RNAs homologous to *Jon65Aiv* and *Jon99Cβ* have different distributions. **Panels A–D:** sections of larva through proventriculus (P), stomach (1), gastric caecae (G) and several loops of more posterior gut (labelled in sequence 2–6: 1,2 = anterior midgut; 3,4 = middle midgut; 5 = anterior part of posterior midgut; 6 = posterior midgut at level of fusion with the malpighian tubes. **Panels A,B:** section probed with *Jon99Cβ*. Only loop 5 is strongly labelled, visible most clearly under darkfield optics **(panel B). Panels C,D:** adjacent section of the same individual, probed in parallel with *Jon65Aiv*. The loop of gut (5) which labelled heavily with *Jon99Cβ* is not significantly labelled with this probe. Regions 1, 2 and 4, which are labelled, are separated by region 3, which is unlabelled. Neither probe labels the most posterior region of the midgut (6). **Panels E** and **F:** enlargement of the neck of the proventriculus from **C** and **A**, respectively. There is an abrupt transition between cells which do and cells which do not hybridize to the 65Aiv probe. Labelling of one gastric caecum is visible adjacent to the proventriculus in **E**. 99C probe: aDm3201, whole plasmid; 65Aiv probe: adm135A8, whole plasmid. Both at 1.5 x 10^8 d.p.m./μg; 6 x 10^4 d.p.m./μl. Exposure = 24 days. **Panel A–D:** scale = 200 μm; **panel E,F:** scale = 50 μm.

D. melanogaster, multiple *Jonah* genes are present in the genomes of other *Drosophila* species, and no repeat structures have been identified at their ends (Carlson, 1982).

Jonah RNA has been shown to encode a 28 000 dalton translation product (Carlson, 1982), and it seems most likely that this abundant class of RNA encodes a family of proteins which are specific to the gut, and which are required in large amounts at all stages when the organism is feeding. The prime candidates for such proteins are the structural proteins of the lining of the gut (including those of the peritrophic membrane), and digestive enzymes.

Histochemical staining indicates that a wide range of digestive enzymes is present in the gut of most Diptera, and that the regions of the midgut within which we find *Jonah* transcripts contain many of these activities. In some cases the distributions of well characterized enzyme activities have been mapped. In none of these does the histochemical distribution of activity match precisely that of either class of *Jonah* RNA (Walker and Williamson, 1980; Dickinson and Gaughan, 1981; Doane, 1969). However, such comparisons may be misleading as enzymes may not be active at the site of synthesis: they may be transported through the gut, passively or in association with the peritrophic membrane.

Gene families encoding gut enzymes have also been studied by mapping loci responsible for enzyme polymorphisms. The chromosomal distribution of *Jonah* genes does not match in

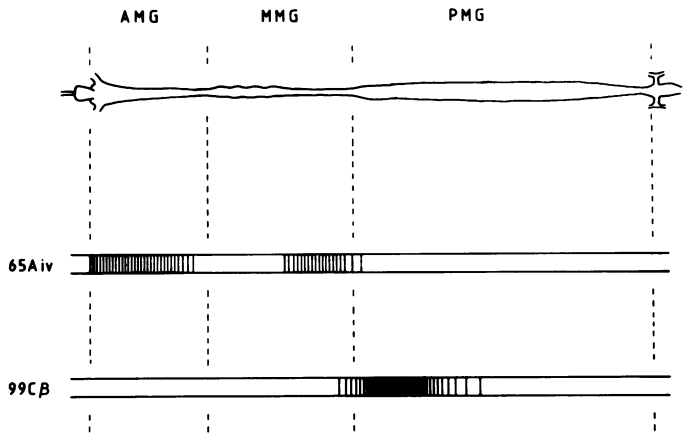

Fig. 7. Diagram summarizing the distribution of RNAs homologous to *Jon65Aiv* and *Jon99Cβ*. The proventriculus is indicated on the left, the malpighian tubules on the right. AMG = anterior midgut; MMG = middle midgut; PMG = posterior midgut. The boundaries between these regions have been assigned somewhat arbitrarily, following the diagram of Doane (1971), and may not correspond precisely with the regions of the gut defined by embryological origin (Poulson, 1950). Some cells in the region of the junction between the MMG and the PMG label with both probes.

detail the distribution of loci encoding known peptidases (Laurie-Ahlberg, 1982) amylases (Abraham and Doane, 1978) or any other gene family with which we are familiar (Dickinson and Sullivan, 1976; Courtwright, 1976).

Even less is known of the genes encoding structural proteins specific to the gut. In *Drosophila*, chitinous components of the peritrophic membrane are secreted by a band of cells in the head of the proventriculus (Rizki, 1956; Peters, 1976). These cells do not contain *Jonah* RNAs for which we have probed. However, in many insects, components of the peritrophic membrane are secreted by a large region of the anterior midgut, and it is not clear in *Drosophila* which cells secrete the abundant protein components of this membrane (Richards and Richards, 1977).

Our results do not therefore provide any strong indication of the function of the *Jonah* genes. However, the differential distribution of two *Jonah* RNAs suggests that the different members of the family may be functionally as well as structurally differentiated from one another.

One striking feature of the distribution of the *Jonah* RNAs is the abrupt transition at the neck of the proventriculus between cells which do and do not contain RNA homologous to *Jon65Aiv*. Neither we nor previous observers have identified any structural or histological discontinuity at this point (Strasburger, 1932; Rizki, 1956). It is close to the junction of cells derived from the foregut and midgut rudiments. This is said to be at the anterior end of the proventriculus, where the outer cell layer derived from the midgut wraps around the projection of the foregut into the lumen of the proventriculus. If this is correct, then the discontinuity which we see lies entirely within cells derived from the anterior midgut rudiment.

A similarly abrupt discontinuity is observed in the distribution of aldehyde oxidase in the anterior midgut, but in this case the discontinuity lies at the base of the gastric caecae (Dickinson and Gaughan, 1981), a few cells distant from that seen for the *Jonah* RNA and in a region where the *Jonah* distribution is continuous. Thus in this region of the gut there are at least two cryptic boundaries separating clearly different but superficially similar cell populations.

The midgut is formed by the fusion of two or three separate embryonic primordia: the anterior midgut invagination, the posterior midgut invagination, and cells of the yolk sac which may contribute to the middle midgut (Poulson, 1950). The boundaries of the derivatives of these primordia have not been determined precisely, and therefore it is not clear whether any of these boundaries correspond precisely to zones defined by the distribution of *Jonah* RNA. However, differences in embryological origin alone cannot be sufficient to account for the differentiation of regions of the midgut. As well as apparent discontinuities in cell type, there are gradual changes in morphology, and presumably function, throughout lengths of the gut. We find these to be associated with gradual changes in the abundance of specific RNA species. The origins and genetic control of this essentially one dimensional pattern deserve further investigation.

Materials and methods

Jonah genes

The different genes of the *Jonah* family are denoted by the chromosomal location of the cluster within which they reside (e.g., 99C) and by Roman numerals to distinguish among genes within a cluster. Thus *Jon65Aiv* refers to the fourth gene in the 65A cluster, the order of genes being that shown in Figure 1. Greek letters are used to distinguish between clusters deriving from the same chromosomal site; thus 99Cα and 99Cβ denote the two clusters, apparently polymorphic variants, which derive from 99C (Carlson and Hogness, 1985a, 1985b).

The 99Cβ cluster contains three genes homologous to a reference *Jonah* cDNA probe under standard filter hybridization conditions. Two of these genes constitute an inverted repeat and the third constitutes a direct repeat of sequences within the left half of the inverted repeat as depicted in Figure 1. We have used as a *Jon99Cβ* probe a clone, aDm3201, which carries *Jonah* sequences derived from this arrangement; owing to an apparent duplication of sequences in the genome we cannot determine whether aDm3201 sequences derive from *Jon99Cβi* or *Jon99Cβii* and for simplicity we refer here to these sequences as *Jon99Cβ*. The plasmid aDm3201 was constructed by subcloning the genomic 1.8-kb *Hind*III fragment of λbDm2306 (Carlson, 1982) into the *Hind*III site of pBR322 (Bolivar *et al.*, 1977); this 1.8-kb fragment corresponds to that of aDm2353 (Carlson, 1982).

The gene cluster at 65A contains four *Jonah* genes (Figure 1). The cDNA clone adm135A8 derives from the gene at the far right, *Jon65Aiv*, as indicated by hybridization data and the correspondence of diagnostic restriction sites within the cDNA clone and within this genomic region.

The plasmid adm135A8 was isolated from *Drosophila* third instar larval cDNA (Wolfner, 1980). It consists of a cDNA segment inserted by $(dG)_n \cdot (dC)_n$ joints into the *Pst*I site of pBR322. The *Drosophila* fragment excisable with *Pst*I is ~950 bases.

Other *Jonah* gene plasmids are described in Carlson (1982). adm2375 and adm2371 are cDNA clones derived from adult and larval RNA, respectively. aDm2351 and aDm2352 are genomic subclones. The *Jonah* sequences on each plasmid have been ascribed to specific chromosomal loci by *in situ* hybridization, and in the case of cDNA clones by comparing restriction sites present in the cDNA with those present in localized genomic clones. Their inferred derivations are: adm2375, 99Cα; adm3201, 99Cβ; αDm2371, 25B; aDm2352, 66C.

Other plasmids

The plasmids adm124E8 and adm129E7, used to prepared control probes, derive from the same experiment as that used for the *Jon65Aiv* probe, adm135A8, and are constructed in the same way. Plasmid adm124E8 contains a cDNA insert of 700 bases derived from the transcript encoding the *Sgs-3* polypeptide (Meyerowitz and Hogness, 1982). The insert in adm129E7 has not been identified, but it is not homologous to an abundant RNA (Wolfner, 1980).

Plasmid DNA was isolated by CsCl banding as in Wensink *et al.* (1974). Fragments containing *Drosophila* sequences were excised by digestion with restriction enzyme and purified on neutral gradients.

Preparation of probes

We prepared ^{3}H-labelled probes from *Drosophila* genomic or cDNA clones by nick-translation (Rigby *et al.*, 1977) to give a final specific activity of $0.5-1.5 \times 10^8$ d.p.m./μg (= $0.2-0.5 \times 10^8$ c.p.m./μg). Standard reactions

contained 0.4 μg of plasmid DNA or excised fragment in 50 μl, with 50 μCi each of [^{3}H]dCTP (50 Ci/mmol) and [^{3}H]TTP (80–100 Ci/mmol), (New England Nuclear). After nick-translation and gel filtration on Biogel P60, probe was reduced to a mean single strand length of ~50 bases by DNase I digestion in the presence of carrier DNA; 0.2–0.4 μg of probe and 50 μg of salmon sperm DNA in 500 μl of 50 mM Tris-Cl, pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, were incubated with a titrated amount of DNase I (~0.3 μg/ml) at 37°C for 15 min.

The reaction was stopped by phenol extraction, ethanol precipitated and taken up in a 50% formamide based hybridization buffer as described elsewhere (Akam, 1983).

To obtain a series of more closely sized probes, aliquots of the nick-translation reaction before and after the 15 min DNase digestion were mixed and fractionated on an alkaline sucrose gradient (5–30% sucrose in 900 mM NaCl, 100 mM NaOH, spun in an SW 50.1 rotor at 39 000 r.p.m., 20°C for 16 h). Fractions from the gradient were ethanol-precipitated and resuspended to give approximately the same final concentration of label in each fraction.

To monitor the single strand length of probes, aliquots were denatured in 50% formamide and compared with restriction fragments of known length after electrophoresis on 4% acrylamide/7 M urea gels.

Preparation of sections

For these experiments we used late third instar larvae from the *Drosophila melanogaster* strain Oregon R, reared on cornmeal-agar medium at 18 or 25°C. Cryostat sections were cut at 8 μm, fixed in ethanol/acetic acid and dehydrated through ethanol (see Akam, 1983 for further details).

Section pre-treatment and hybridization

Immediately before use the sections were pre-treated as described by Brahic and Haase (1978) to remove some of the tissue proteins and to reduce non-specific binding of label (0.2 N HCl for 20 min at 20°C, 2 x SSCPE for 30 min at 70°C, 1 μg/ml Proteinase K in 20 mM Tris/Cl, 2 mM $CaCl_2$ for 15 min at 37°C, rinsing with distilled water between each step and finally dehydrating in 70% and 95% ethanol and air drying). Hybridization and subsequent washing steps were performed as described elsewhere (Akam, 1983). Autoradiography was carried out as described by Gall and Pardue (1971) using Kodak NTB 2 or Ilford K2 emulsion. After exposure the sections were stained with Giemsa stain.

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