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Gene expression pattern

Differential expression of the *Drosophila* zinc finger gene *jim* in the follicular epithelium

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Abstract

The *Drosophila* gene *jim* was identified by an enhancer trap line showing asymmetric dorso-ventral expression in the follicular epithelium. It gives rise to the *jim-1* and *jim-2* transcripts that contain distinct 5′-UTRs but encode the same nine C_2H_2 zinc finger protein. From stage 10A onward, *jim-1* RNA is transcribed in squamous cells while *jim-2* RNA is specific to all non-antero-dorsal columnar cells as the result of repression in antero-dorsal cells by the DER pathway. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Results and discussion

The jim^{OVK} line was selected during a screening for enhancer trap lines showing specific expression of the *lacZ* reporter gene in various subpopulations of follicle cells. From stage 10 of oogenesis, this line displays specific expression of the reporter gene in a population of non-antero-dorsal columnar (NADC) cells complementary to the antero-dorsal columnar (ADC) cells where the DER pathway is activated (Nilson and Schüpbach, 1999) (Fig. 1). In situ hybridization studies, using RNA probes derived from subclones of genomic DNA fragments flanking the P[*lac;w*] insertion, provided evidence for the existence of a transcription unit, *jim*, expressed in NADC cells in stage 10 follicles (Fig. 2A, C–E). A combination of northern blot analysis (Fig. 3 and data not shown), cDNA sequencing and transcript mapping showed that the *jim* locus gives rise to two transcripts, *jim-1* (5.5 kb) and *jim-2* (6.5 kb), from alternative transcription start sites (Fig. 2B). Both RNAs are expressed in ovaries and in late (8- to 20-h-old) embryos (Fig. 3A,B), and encode the same nine zinc finger protein (Fig. 4A,B) (sequence accession no. AJ133756).

A single 115 kDa Jim protein was detected by western blotting of ovarian protein extracts (Fig. 4C). At all stages of oogenesis, the pattern of accumulation of the Jim protein paralleled the expression of the *lacZ* reporter gene in the jim^{OVK} line (Fig. 5) with the exception that the Jim protein was also detected in squamous cells from stage 9 onward (Fig. 5C–E) and in a single row of 10–15 ADC cells in stage 12 follicles (Fig. 5E). In stage 15 embryos, Jim accumulated in the anterior midgut and hindgut and in glossopharyngeal muscles (Fig. 5I).

In ovaries from homozygous jim^{OVK} females, because the *jim-2* RNA was not detectable (Fig. 3C) due to transcription arrest within transposon sequences (Fig. 3D), it can be concluded that Jim accumulation resulted almost exclusively from the translation of the *jim-1* transcript. In the germarium and in stage 1 to 6/7 egg chambers, the pattern of Jim accumulation was similar to that in wild-type ovaries (data not shown), but it differed dramatically at later stages with Jim being undetectable in posterior follicle cells at stages 8 to 9 (data not shown), and in NADC cells at stages 10A to 12 (5F–H). These data strongly suggest that *jim-1* RNA is expressed in squamous cells from stage 9 and in the row of ADC cells in stage 12 follicles and that *jim-2* is transcribed in NADC cells from stage 10A (Fig. 2C–E). In homozygous jim^{OVK} females, Jim accumulation was also observed in ADC cells between stages 10A to 11 (Fig. 5F,G) where no expression of the protein was detected in the wild-type context (Fig. 5C,D). This correlated the ectopic expression of *jim-1* RNA in the ADC cell region in stage 10A/B follicles (Fig. 2F).

In *gurken* mutant follicles in which the DER pathway was not activated, Jim was expressed throughout the columnar epithelium with the exception of a small region at the posterior pole populated by cells having acquired an anterior

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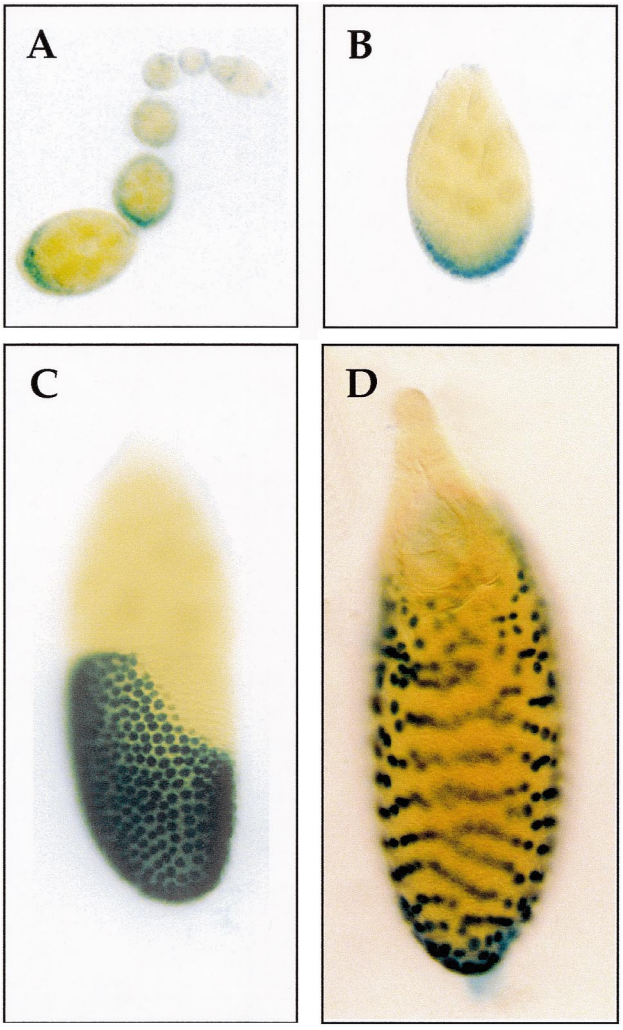

Fig. 1. X-Gal staining pattern of the jim^{OVK} line during oogenesis. (A) *lacZ* staining was detected in all follicle cells from region 2b of the germarium to oogenic stages 5/6. (B) Between stages 6 to 8, X-gal staining was restricted to posterior follicle cells surrounding the oocyte. Between stages 10A/B (C) to 13 (D), β-galactosidase expression was restricted to the NADC cells. Homozygous jim^{OVK} flies were viable and fertile, but 17% of the eggs laid did not hatch as compared to 7% in the case of a wild-type stock. This reduced fertility was not associated with any detectable phenotype.

identity because of the lack of Gurken signal (Gonzàlez-Reyes et al., 1995; Roth et al., 1995) (Fig. 6B). In *fs(1)K10* mutant follicles, Jim accumulated throughout columnar follicle cells except for a 4 to 5 cell-wide domain surrounding the anterior region of the oocyte (Fig. 6C).

2. Experimental procedures

2.1. Drosophila stocks

The jim^{OVK} line was identified among a collection of 700 independent lines containing autosomic insertions of the P[*lac;w*] transposon (Bier et al., 1989). The grk^{DC} (Schüpbach, 1987), grk^{2B6} (Neuman-Silberberg and Schüpbach, 1993) and $fs(1)K10^{LMOO}$ (Cheung et al., 1992) stocks were used. The w^{1118} stock was used as wild-type control.

2.2. Molecular techniques

Genomic DNA fragments flanking the transposon insertion site were cloned by plasmid rescue (Pirrotta, 1986). Screening of ovarian cDNA libraries (Stroumbakis et al., 1994; Hazelrigg and Tu, 1994; Couderc and Laski, unpublished data) was carried out according to standard procedures (Sambrook et al., 1989). In addition, the EST clone GM 05638 (Berkley Drosophila Genome Project) was identified as a *jim* cDNA.

Poly A^+ RNA was isolated using RNeasy and Oligotex mRNA kits (Qiagen). Northern analysis was carried out as described in Yanicostas et al. (1989) except that RNA probe synthesis, and northern blot hybridization and dehybridization were performed using the Strip-EZ kit (Ambion). RACE experiments were carried out using the Marathon cDNA Amplification kit (Clontech). RT-PCR experiments were performed using random hexamers and Expand MMLV reverse transcriptase (Boehringer Mannheim). PCR amplified fragments were cloned into the pGEM-T easy vector (Promega). *jim* cDNA and genomic DNA subclones were sequenced using the Thermosequenase cycle sequencing kit (Amersham Pharmacia Biotech).

2.3. Anti-Jim antibodies and Western blotting

Polyclonal antibodies were raised in New Zealand female rabbits against a non-zinc finger domain of the Jim protein (amino acids 134–308) fused to the GST protein in the pGEX bacterial expression vector (Amersham Pharmacia Biotech) and purified on a Glutathione Sepharose 4B matrix (Amersham Pharmacia Biotech). Antibodies were immunoaffinity purified through GST and GST-Jim coupled Sepharose 4 (Amersham Pharmacia Biotech) columns. Chemiluminescent western blot detection of the Jim protein in ovarian and embryonic extracts was performed with the ECL kit (Amersham Pharmacia Biotech). Anti-Jim and goat anti-rabbit horseradish peroxydase-conjugated secondary antibodies (Vector) were used at 1:750 and 1:1600 dilutions, respectively.

2.4. X-Gal staining, whole-mount in situ hybridization and immunocytochemistry

X-gal staining of whole mount ovaries were carried out as described previously (Yanicostas et al., 1995). Whole-mount RNA in situ hybridization of ovaries and embryos was carried out according to Tautz and Pfeifle (1989) with minor modifications (Suter and Steward, 1991). Digoxigenin-labelled antisense RNA probes were synthesized using the RNA Genius kit (Boehringer Mannheim). Immunodetection of the Jim protein in whole-mount ovaries was performed according to Ségalat et al. (1992). Anti-Jim and

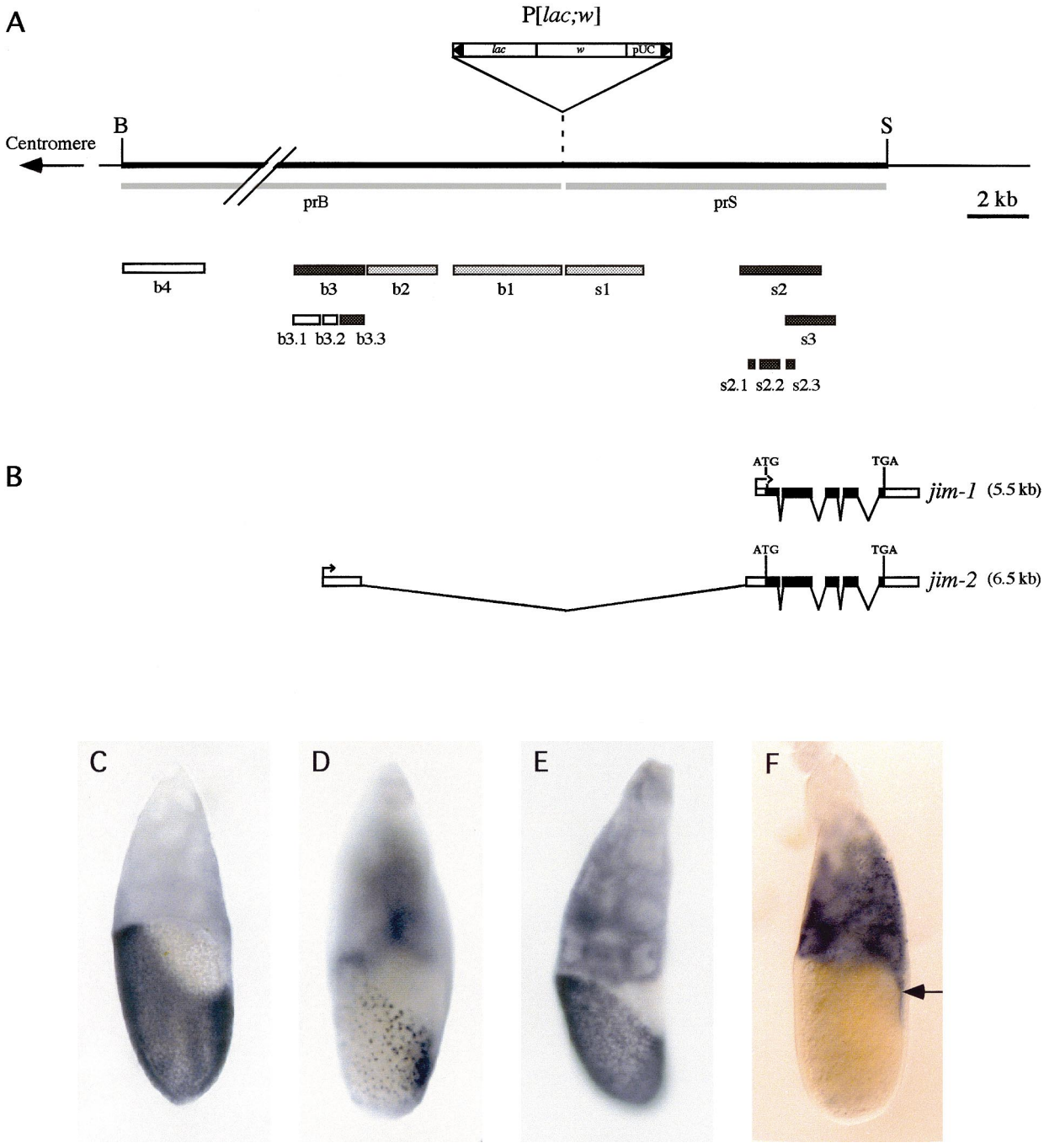

Fig. 2. Genomic organization of the *jim* locus. (A) *Bam*HI (B) and *Sac*II (S) were used for plasmid rescue of genomic DNA fragments prB and prS (grey lines) flanking the insertion site of the P[*lac;w*] transposon at 79F1-2. Subclones (b4, b3.1, b3.2) used for synthesizing RNA probes, that gave no signal above background by in situ hybridization of whole-mount ovaries, are indicated as empty boxes. All probes from b3 to s3 hybridized to sequences transcribed in NADC cells in stage 10 follicles and displayed either a cytoplasmic [b3, b3.3, s2, s3, s2.1, s2.2 and s2.3 (black boxes)] or a nuclear pattern of transcript accumulation [b2, b1 and s1 (grey boxes)] according to their exonic or intronic nature. (B) Structure and direction of transcription (broken arrows) of the *jim-1* and *jim-2* RNA deduced from ovarian cDNA sequencing and transcript mapping data. Untranslated exonic sequences (empty boxes), the putative ORF (black boxes) and introns (broken lines) are indicated. The 0.9 kb RNA detected by northern blotting using the b3.3 probe is not represented. While the *jim* RNAs differ by their 5′-UTRs (351 bp for *jim-1* and 1880 bp for *jim-2*), the possibility that they also differ by their 3′-UTR could not be ruled out. (C–F) Whole-mount in situ hybridization of stage 10A/B follicles from w^{1118} (C–E) or jim^{OVK}/jim^{OVK} (F) females using RNA probes synthesized from subclones b3.3 (C), b1 (D) and s3 (E,F). The staining observed in the nurse cells with the b1 and s3 probes corresponds to non-specific background signal. In (F), the arrow indicates ectopic accumulation of the *jim-1* RNA in the ADC cell region.

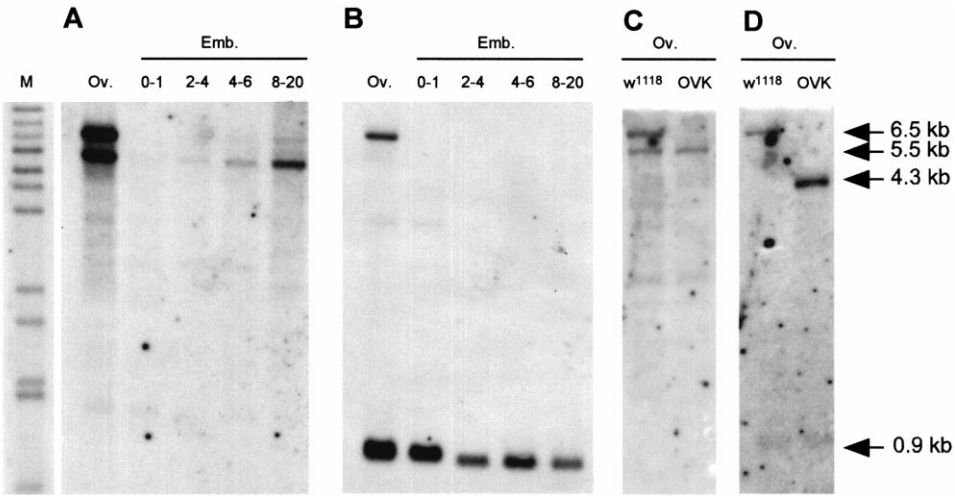

Fig. 3. Northern blot analysis of the *jim* transcripts in wild type and jim^{OVK}/jim^{OVK} females. Samples (10 μg) of poly $(A)^+$ RNA from w^{1118} (w^{1118}) or jim^{OVK}/jim^{OVK} (OVK) ovaries (Ov.), 0- to 1-, 2- to 4-, 4- to 6- and 8- to 20-h-old embryos (Emb.) were analyzed using RNA probes synthesized from s2.2 (A,C) or b3.3 (B,D) subclones (position in Fig. 2A). The 6.5 kb *jim-2* RNA was absent in ovaries from homozygous jim^{OVK} females (C) and was replaced by a truncated 4.3 kb transcript (D). (M) Labelled *Bst*EII-digested λ phage DNA molecular weight marker.

goat anti-rabbit horseradish peroxydase-conjugated secondary antibodies (Vector) were diluted 1:750 and 1:400, respectively.

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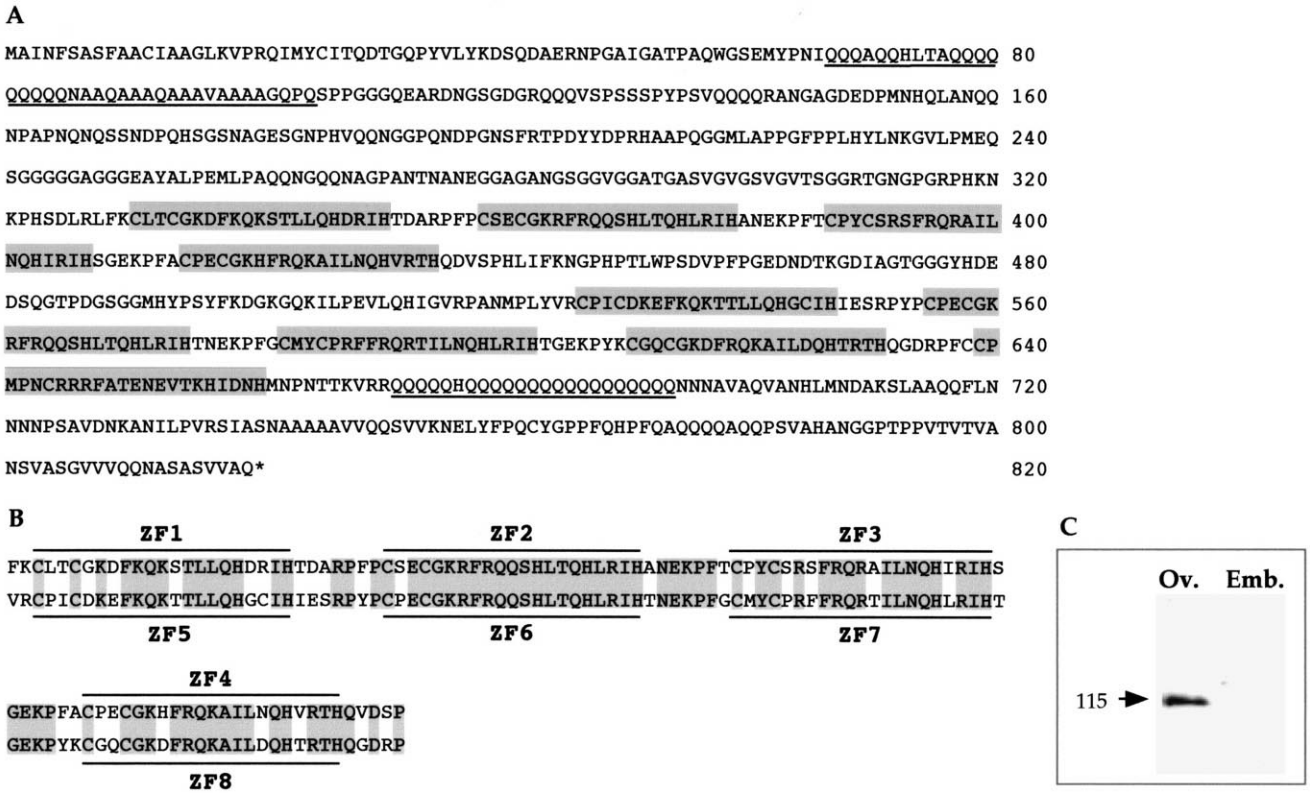

Fig. 4. (A) Jim protein sequence. The nine C_2H_2 zinc fingers are boxed and polyglutamine/polyalanine or polyglutamine rich regions are underlined. Amino acid numbers are indicated on the right. (B) Sequence alignment of the two zinc finger (ZF) domains suggests that they arose from the duplication of a single ancestral domain. Identical amino acids (72%) are boxed. (C) Anti-Jim polyclonal immune serum recognized a single 115 kDa protein in ovaries (Ov.) that was not detected in embryonic protein extracts (Emb.).

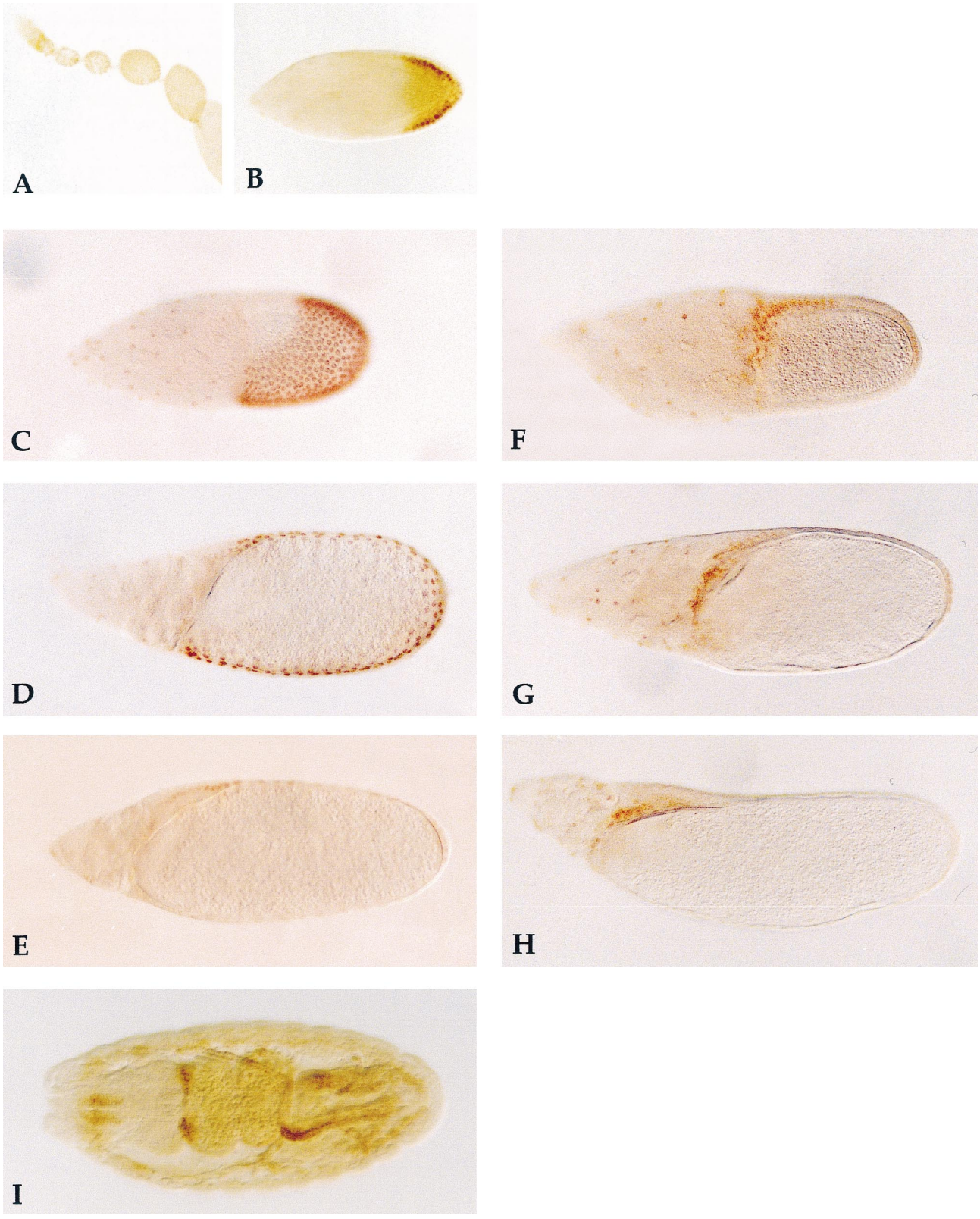

Fig. 5. Tissue distribution of the Jim protein during oogenesis (A–H) and embryogenesis (I). Whole-mount ovarian follicles from w^{1118} (A–E) or jim^{OVK}/jim^{OVK} (F–H) females. Note the nuclear accumulation of the Jim protein. (I) Stage 15 w^{1118} embryos.

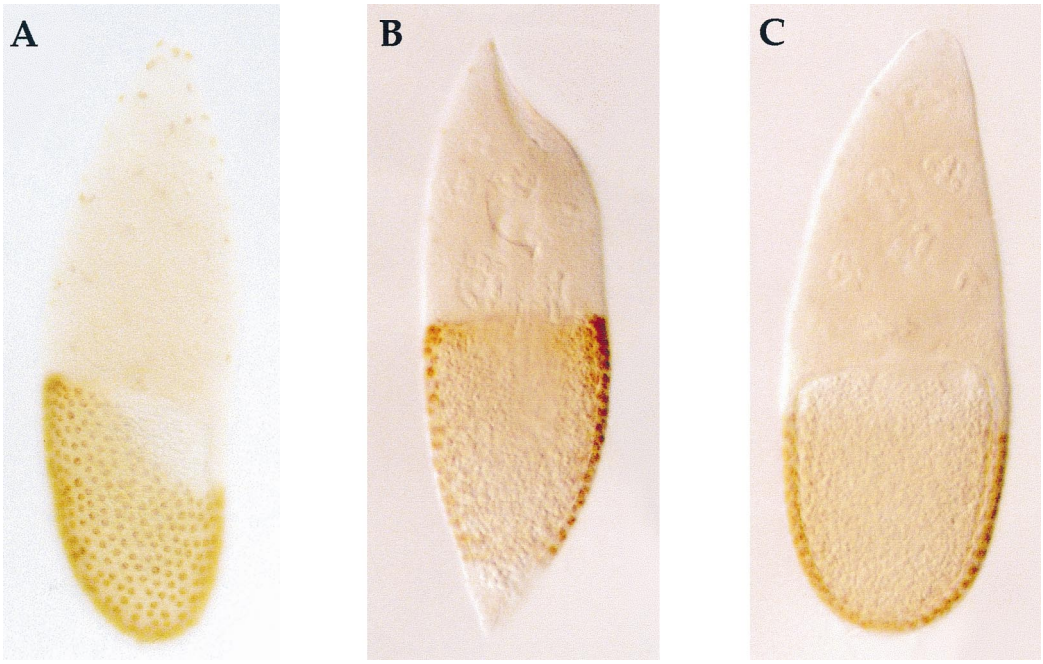

Fig. 6. Regulation of *jim* expression by the Gurken-DER pathway. Immunodetection of the Jim protein on whole-mount ovaries from w^{1118} (A), grk^{DC}/grk^{2B6} (B) and $fs(1)K10^{LM00}$ (C) females.

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