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Gonadal expression of c-*kit* encoded at the *W* locus of the mouse

KATYA MANOVA[1,2], KARL NOCKA[3], PETER BESMER[3] and ROSEMARY F. BACHVAROVA[1]*

[1]*Department of Cell Biology and Anatomy, Cornell University Medical College, 1300 York Avenue, New York, NY 10021, USA*
[2]*Permanent address: Institute of Cell Biology and Morphology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Molecular Biology Program, Sloan Kettering Institute and Cornell University Graduate School of Medical Sciences, New York, NY 10021, USA*

*To whom correspondence should be addressed

Summary

Recently, it has been shown that the c-*kit* proto-oncogene is encoded at the *white spotting* (*W*) locus in mice. Mutations of this gene cause depletion of germ cells, some hematopoietic cells and melanocytes. In order to define further the role of c-*kit* in gametogenesis, we have examined its expression in late fetal and postnatal ovaries and in postnatal testis. By RNA blot analysis, c-*kit* transcripts were not detected in late fetal ovaries but appeared at birth. The relative amount reached a maximum in ovaries of juvenile mice, and decreased in adult ovaries. c-*kit* transcripts were present in increasing amounts in isolated primordial, growing and full-grown oocytes, as well as in ovulated eggs. Little was detected in early 2-cell embryos and none in blastocysts. *In situ* hybridization revealed c-*kit* transcripts in a few oocytes of late fetal ovaries and in all oocytes (from primordial to full-grown) in ovaries from juvenile and adult mice. Expression was also observed in ovarian interstitial tissue from 14 days of age onward. Using indirect immunofluorescence, the c-*kit* protein was detected on the surface of primordial, growing and full-grown oocytes, as well as on embryos at the 1- and 2-cell stages; little remained in blastocysts.

***In situ* hybridization analysis of testes from mice of different ages demonstrated expression in spermatogonia from 6 days of age onward. Using information provided by determining the stage of the cycle of the seminiferous epithelium for a given tubule and by following the age dependence of labeling, it was concluded that the period of expression of c-*kit* extends from at least as early as type A_2 spermatogonia through type B spermatogonia and into preleptotene spermatocytes. Leydig cells were labelled at all ages examined.**

The expression pattern in oocytes correlates most strongly with oocyte growth and in male germ cells with gonial proliferation.

Key words: gonadal expression, c-*kit*, *W* locus, mouse embryo, proto-oncogene, oocyte, spermatogonia.

Introduction

Mice homozygous for mutations at the *white spotting* locus (*W*) are deficient in generation of three classes of cells: germ cells, melanocytes and hematopoietic cells (specifically stem cells, erythrocytes and mast cells) (for reviews, see Russell, 1979; Silvers, 1979). A large number of alleles associated with a range of deficient phenotypes has been described (Geissler *et al.* 1981). Germ cells can first be identified as alkaline phosphatase-positive cells around 8 days of development when 10–100 cells are found at the base of the allantois adjoining the caudal primitive streak. In normal mice the number of germ cells increases rapidly to 2500–5500 over the next four days as they migrate from the hindgut to the gonadal ridge (see Heath, 1978, for review). In mice homozygous for more severe *W* alleles, the germ cells are present at 8 days but fail to increase in number thereafter (Mintz and Russell, 1957). Such mice die of macrocytic anemia around the time of birth, and lack melanocytes and germ cells. Heterozygotes are fertile, display variable degrees of white spotting, and may be mildly anemic. Transplantation experiments have shown the defect to be intrinsic to the melanoblast and the hematopoietic cells (Russell, 1979; Silvers, 1979).

Recently, it has been discovered that the *W* locus encodes the proto-oncogene c-*kit* (Chabot *et al.* 1988; Geissler *et al.* 1988; Nocka *et al.* 1989), the normal cellular homolog of v-*kit*, the oncogene of the HZ4 feline sarcoma virus. Its sequence predicts a tyrosine kinase receptor in the CSF-1 and PDGF receptor family (Besmer *et al.* 1986; Yarden *et al.* 1987; Qiu *et al.* 1988). Antibodies generated to the encoded protein detect glycosylated forms of $124–165\times10^3$ M_r, and a nonglycosylated form of 106×10^3 M_r consistent with the size of the predicted protein; it is a cell surface protein with autophosphorylation activity (Yarden *et al.* 1987; Majumder *et al.* 1988). Characterization of the molecu-

lar defects of several mutant alleles at the *W* locus has shown that they affect kinase activity and other aspects of c-*kit* receptor function (Tan *et al.* 1990; Reith *et al.* 1990; Nocka *et al.* 1990).

Phenotypes very similar to those at the *W* locus are caused by mutations at another genetically defined locus called *steel* (*Sl*) (Russell, 1979; Silvers, 1979). In this case, the defect has been located to the environment of the developing cells, and thus the locus could encode a putative factor that interacts with the c-*kit* receptor or a product affecting the activity of such a factor (Russell, 1979; Chabot *et al.* 1988). In fact, wild-type fibroblasts are able to produce a factor that interacts with the c-*kit* receptor, but Sl/Sl^d fibroblasts do not (Jarboe *et al.* 1989; Fujita *et al.* 1989).

Phenotypic analyses indicate that the *W* gene may function not only during early development of precursors of a disparate set of cell types, but also may act at different points along the developmental pathway of an individual cell type. In the haematopoietic system, *W* mutations affect early pluripotential precursors (see Russell, 1979), erythroid precursors (i.e. the transition from BFU-E to CFU-E (Gregory and Eaves, 1978; Iscove, 1978; Nocka *et al.* 1989), and the proliferation and/or differentiation of mast cells (Kitamura and Go, 1978; Fujita *et al.* 1988). In melanocytes, *W* mutations affect melanoblast development, resulting in a lack of pigmentation of the skin. For mice heterozygous for some alleles, melanocytes are produced but the coat color is diluted. This was shown in the case of $W^v/+$ to be a reduction in pigment granule number and size, indicating an ongoing or renewed function of the gene in the differentiated melanocyte (Russell, 1949). Primordial germ cells require the gene during early proliferation as described above. For *W* and *Sl* genotypes with mild phenotypic effects, some germ cells survive, but show deficiencies in postnatal development (Coulombre and Russell, 1954; Nishimune *et al.* 1980; Kuroda *et al.* 1988; Geissler *et al.* 1981) (see Discussion).

Consistent with the cell autonomous character of *W* mutations, there is evidence for expression of c-*kit* in the three major cell lineages that are affected by such mutations (Nocka *et al.* 1989). c-*kit* gene products have been detected in fetal liver, bone marrow and mast cells. Expression is observed in differentiated melanocyte lines, consistent with its function at multiple stages in the development of this cell type. Finally, the mRNA is detected in both ovary and testis. In addition, c-*kit* is expressed in several tissues not known to be affected by *W* mutations: brain, placenta, lung and lymphoid tissue (Yarden *et al.* 1987; Qiu *et al.* 1988; Nocka *et al.* 1989).

The present work was undertaken to define the time course of expression of c-*kit* in germ cells, providing a basis for further studies on the function of the gene. Here we describe the appearance of c-*kit* mRNA and protein in oocytes during late fetal and postnatal development, and of c-*kit* mRNA in postnatal male germ cells. We find that the gene is expressed in the female before, during and after the major growth phase of the oocyte, and in the male primarily in spermatogonia. In addition, we observe expression in specific somatic cells: interstitial gland cells of the ovary and Leydig cells in the testis.

Materials and methods

Mice

Mice used in these studies were derived from mating of ICR females to $CB6F_1$ males. C57BL/6 $W^v/+$ mice were obtained from the Jackson Laboratory.

Preparation of RNA and Northern blots

RNA was extracted from ovaries of mice of different ages as described (Kaplan *et al.* 1985). Oocytes of different stages were collected as follows. Primordial oocytes less than 20 μm diameter were collected one day after setting up an *in vitro* culture of pronase-digested ovaries from 8–10 day old mice (Bachvarova *et al.* 1980). Growing oocytes from 8 day old mice were obtained directly from collagenase-digested ovaries (Eppig, 1976) disrupted by stirring. Full-grown oocytes were released by pricking ovaries of 20–22 day old females stimulated 2 days previously with 4 units of PMS. Unfertilized ovulated eggs were collected after superovulation of prepubertal or adult mice. Two-cell embryos were obtained about $1\frac{1}{2}$ days after fertilization from superovulated $CB6F_1$ females mated to $CB6F_1$ males; for late blastocysts, 2-cell embryos were cultured for 3 days in HTF fluid plus 5 mg ml^{-1} BSA (Quinn *et al.* 1988). RNA was extracted from oocytes and embryos as described (Paynton *et al.* 1988).

^{32}P-labeled riboprobes were derived by transcription with SP6 polymerase of appropriately linearized pGEM7 plasmids containing c-*kit* inserts (Krieg and Melton, 1987). One probe was transcribed from a 3.6 kb insert including the entire coding region of the message. A second 1.1 kb probe, derived from a 1.6 kb insert, extended from the *Pvu*II site in the region encoding the transmembrane segment to the *Bss*HII site near the 5′ end of the region encoding the extracellular domain. This second probe lacks the more conserved region encoding the kinase domain. Hybridization of the blots was carried out as described (Paynton *et al.* 1988) at 60 °C.

In situ *hybridization*

Mouse ovaries and testes of various ages were fixed in 4 % paraformaldehyde in 0.1 M phosphate buffer pH 7 overnight, washed, dehydrated and embedded in 56°–57 °C paraffin (TissuePrep). Sections 2–5 μm thick were mounted on poly-L-lysine-coated slides and dried. ^{35}S-labeled antisense and sense riboprobes (4×10^8 cts min^{-1} μg^{-1}) were synthesized with SP6 and T7 polymerase, respectively, and hydrolysed in mild alkali to a length of 100–200 nucleotides (see Wilkinson *et al.* 1987*a*). The slides were hydrated, postfixed in paraformaldehyde followed by treatment with proteinase K and acetic anhydride (Wilkinson *et al.* 1987*a*) and prehybridization (Mutter and Wolgemuth, 1987). Slides were hybridized at 65 °C overnight in a moist chamber as described (Lehnert and Akhurst, 1988) using 0.3 M NaCl and 60 % formamide in the mix and a probe concentration of 0.25 ng μl^{-1}. The 1.1 kb probe described above was used in most experiments. The sense strand probe was derived from the 3.6 kb insert. Slides were washed as described (Wilkinson *et al.* 1987*b*) except that the RNase conditions were 50 μg ml^{-1} in 0.3 M NaCl, 0.01 M Tris pH 8, 1 mM EDTA, for 30 min at 37 °C. Slides were dipped in NTB2 emulsion, exposed for 1–4 wks, developed and stained with hematoxylin and eosin according to standard techniques.

Immunofluorescence

Growing and primordial oocytes were collected 1 day after setting up cultures of pronase-digested ovaries (Bachvarova *et al.* 1980). Full-grown oocytes and 2-cell embryos were obtained as described above. Some samples were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min on ice, and washed twice in PBS containing 5% calf serum. Immune serum was derived from a rabbit immunized with vaccinia virus expressing full-length c-*kit* protein (Nocka *et al.* 1990). Preimmune and immune sera were passed through DEAE Affi-gel Blue (BioRad) to enrich for immunoglobulins. For absorption, antibody at a dilution of 1:10 was incubated with an equal volume of mast cells from either *W/W* mice or from +/+ mice (Nocka *et al.* 1990). Fresh or fixed oocytes and embryos were incubated in a 1:100 dilution of antibody for 30 min on ice. Cells were transferred through PBS containing 5% calf serum, and incubated in FITC-conjugated goat anti-rabbit antibody. Samples were washed and mounted under supported coverslips for analysis in a Nikon Microphot microscope with epi-fluorescence attachment.

Results

RNA blot analysis of c-kit *expression in ovaries and oocytes*

An initial survey of the expression of c-*kit* mRNA in ovaries was carried out by RNA blot analysis. Expression was not detected in ovaries from 17½ day fetal ovaries, but a band with a mobility corresponding to the 5.5 kb c-*kit* mRNA first appears at about the time of birth (Fig. 1A). Expression relative to that of actin mRNA reaches a peak in ovaries from young juvenile mice (8–17 days old), and is reduced in adult mice (Fig. 1A).

Oocytes pass through meiotic prophase in late fetal life, reaching the diplotene stage around the time of birth (Speed, 1982). Oocytes are stored in primordial follicles, which initiate growth at any time from birth to the end of fertile life. A large cohort of oocytes enters the growth phase at approximately the day of birth and reaches full size at about 15 days of age (see Bachvarova, 1985, for review). From this point the bulk of the ovarian tissue consists of multilayered and antral follicles and, after the first ovulation, corpora lutea. The time course described above is consistent with the expression of c-*kit* in primordial and growing oocytes.

To explore this possibility further, RNA samples prepared from primordial to full-grown oocytes, and ovulated eggs, were analyzed on blots, and found to contain c-*kit* mRNA (Fig. 1B,C). Using sense strand RNA as a standard in two separate experiments, the amount was estimated roughly as 15 fg per full-grown oocyte or egg and 0.5 fg per primordial oocyte (Fig. 1C). After fertilization, the amount of c-*kit* transcripts declines several fold by the early 2-cell stage, and is undetectable in blastocysts (Fig. 1B).

In situ *hybridization analysis of* c-kit *expression in ovaries: germ cells*

In situ hybridization was used to confirm and extend the investigation of expression of c-*kit* RNA in oocytes and

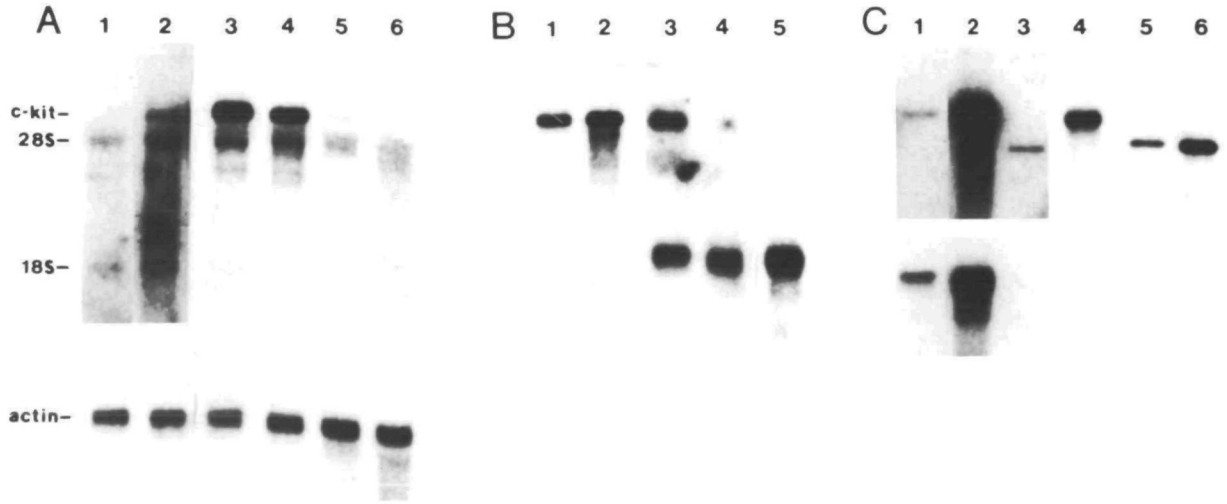

Fig. 1. Expression of c-*kit* transcripts in ovarian, oocyte and embryo RNA. (A) Blot of cytoplasmic ovarian RNA hybridized to the 3.6 kb c-*kit* probe. Panel below shows the same blot rehybridized to a mouse β-actin probe. Lanes 3–5 contain 2 μg and lane 6 contains 10 μg RNA. Lanes 1 and 2 were exposed 4 times longer than lanes 3–6. Lane 1: 17½ day fetal ovary. Lane 2: newborn (0 da) ovary. Lane 3: ovary of an 8 day old mouse (8 day ovary). Lane 4: 17 day ovary. Lane 5: adult ovary. Lane 6: adult liver. (B) Blots of RNA from isolated oocytes and embryos hybridized to the 3.6 kb c-*kit* probe. Panel below shows lanes 3–5 rehybridized to an 18S rRNA probe. Lane 1: 250 oocytes from 8 day old mice. Lane 2: 250 oocytes from 14 day old mice. Lane 3: 400 ovulated eggs. Lane 4: 392 early 2-cell embryos. Lane 5: 305 late blastocysts. (C) Blots of RNA from primordial oocytes and ovulated eggs run in parallel with c-*kit* 3.6 kb sense strand RNA, hybridized to the 1.1 kb c-*kit* probe. Amounts of the sense strand were calculated from radioactivity after three precipitations and the specific activity of the precursor, and corrected for length. Panel below shows the same blot rehybridized to an 18S rRNA probe. Lanes 1–3 exposed for 7 days, lanes 4–6 for 21 h. Lane 1: 299 primordial oocytes. Lanes 2 and 4: 369 ovulated eggs. Lanes 3, 5, and 6: 0.025 pg, 0.5 pg, and 2.5 pg of sense strand c-*kit* RNA, respectively.

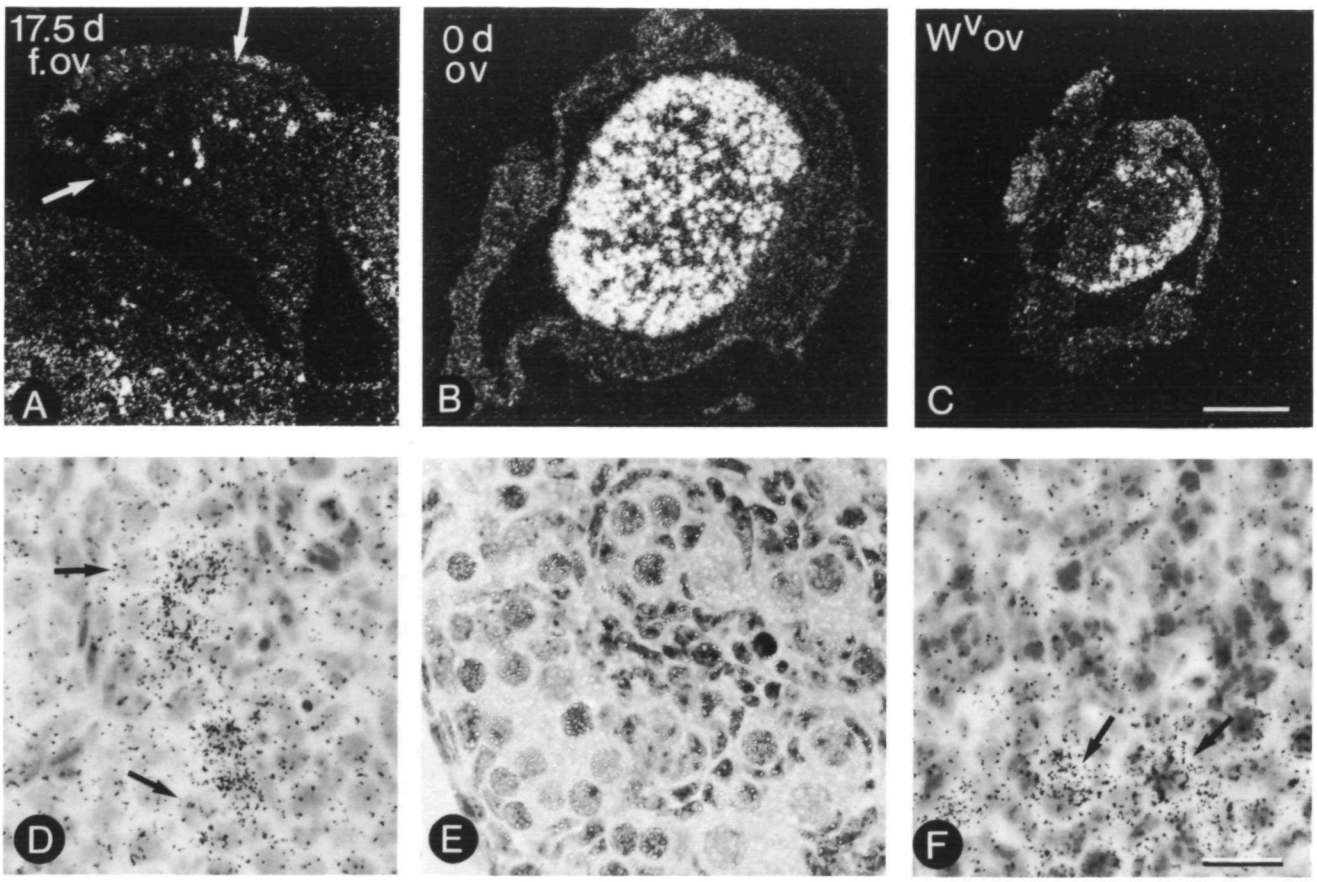

Fig. 2. Expression of c-*kit* RNA in sections of fetal and newborn ovaries hybridized to the c-*kit* antisense probe. (A) Dark-field image of ovary (arrows) and kidney (lower left) of a 17½ day fetus. Some cells in ovary and kidney are labeled. (B) Dark-field image of ovary of a newborn (0 day) mouse. Labeled cells are scattered throughout. (C) Dark-field image of ovary of a newborn mouse derived from a cross of $W^v/+$ parental mice. Label appears in some regions only. Scale bar=200 μm for panels A–C. (D) Bright-field image of a 17½ day fetal ovary. Four labeled oocytes are shown; two of the many unlabeled oocytes are indicated by arrows. (E) Bright-field image of ovary of a newborn mouse, phase-contrast optics, grains appear white. Many oocytes in nests are labeled. (F) Bright-field image of ovary of the newborn mouse carrying W^v, showing the border between the region with labeled oocytes (arrows) and unlabeled region devoid of oocytes in the upper region of the micrograph. Scale bar=20 μm for panels D–F.

to determine whether somatic cells make any contribution to expression in the ovary. The level of label over the region of tissue displaying the lowest grain density, while containing closely packed cells, was taken as the background level; a similar density of labeling was observed using the sense strand probe on slides processed in parallel (see below). The 1.1 kb probe and 3.6 kb probes gave similar results; the 1.1 kb probe corresponding to most of the extracellular domain of the c-*kit* protein was used for all data presented here, except as indicated in the legends.

Little expression was observed in 17½ day fetal ovaries, except in a few cases in which a few oocytes were labeled (Fig. 2A,D). These are interpreted as being the most advanced oocytes, which have probably reached the early diplotene stage. Newborn ovaries viewed at low magnification displayed numerous scattered points of high labeling closely packed in the periphery and distributed more sparsely in the central region (Fig. 2B). At high magnification abundant grains were observed over nests of diplotene oocytes in the periphery (Fig. 2E) and over oocytes isolated in primordial follicles, which tended to be located more centrally. Little expression above background could be detected in somatic cells. This was confirmed by examining the ovary of a W^v/W^v or $W^v/+$ newborn mouse in a litter derived from a cross between $W^v/+$ heterozygotes. The number of oocytes was substantially reduced and grains were confined to the regions containing oocytes (Fig. 2C,F).

Abundant expression of c-*kit* RNA was seen in primordial and growing oocytes in ovaries from juvenile mice 5, 8 and 10 days old (Fig. 3A,B,E), and in oocytes of all sizes up to those in large antral follicles of ovaries from 17 day old and adult mice (Fig. 4A,C,D). Hybridization to the sense probe resulted in little label (Figs 3C, 4B). The grain density in oocytes of different sizes from 8, 10, 17 day and 4½ week old mice declined only 40 % during the approximately 100-fold increase in volume from primordial to full grown oocytes

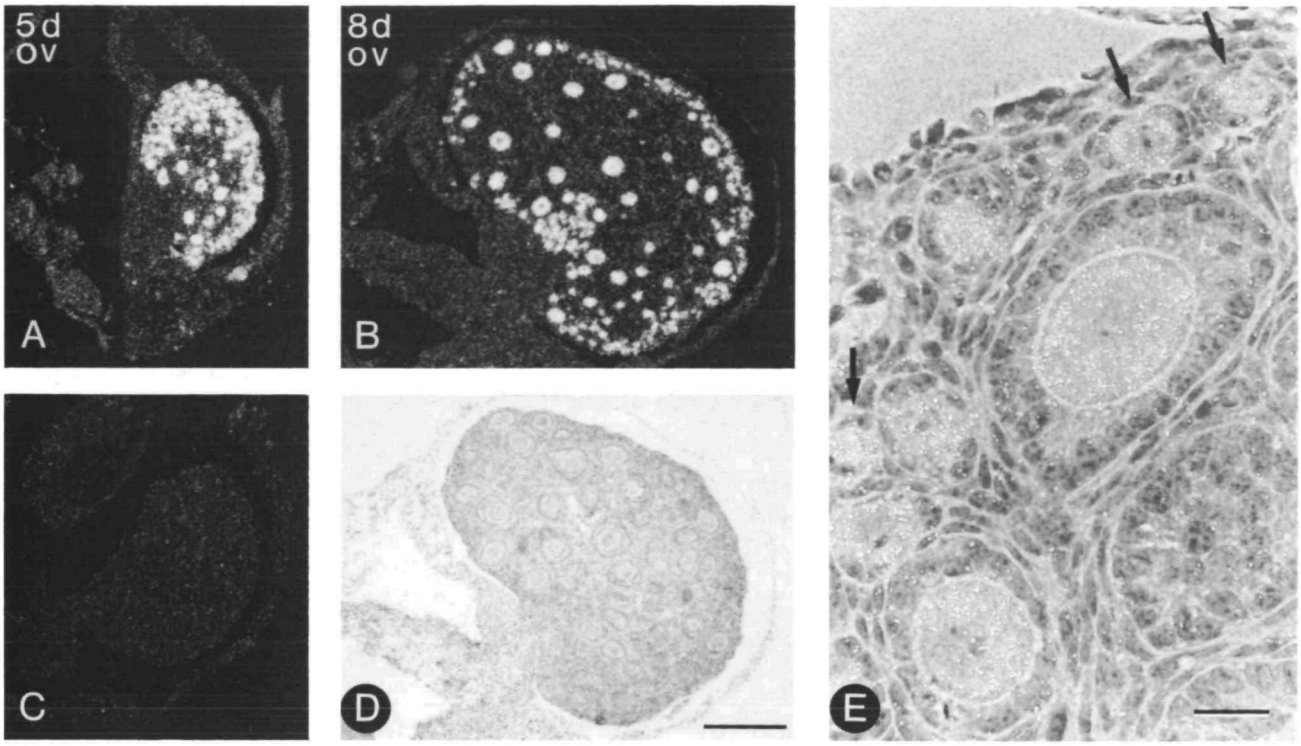

Fig. 3. Expression of c-*kit* RNA in primordial and growing oocytes in ovaries of juvenile mice. (A,B,D,E) Hybridized to the c-*kit* antisense probe. (A) Dark-field image of 5 day ovary. (B) Dark-field image of 8 day ovary. Primordial oocytes are abundant in the periphery and growing oocytes are scattered throughout. (C) Dark-field image of 5 day ovary hybridized to the c-*kit* sense probe. (D) Bright-field image corresponding to B. Scale bar=200 μm for panels A–D. (E) 10 day ovary, phase-contrast optics. Oocytes in primordial (arrows) and growing follicles are labeled. Scale bar=20 μm.

Table 1. c-kit *transcripts in oocytes of different sizes*

Oocyte stage	Number counted	Oocyte diameter (average in μm)	Grain density (arbitrary units±S.E.M.)
Primordial	45	14	100±5
Small growing	26	30	84±5
Mid-growth	28	44	72±6
Large growing	16	58	77±7
Full-grown	21	67	59±5

Samples were taken from sections of ovaries hybridized to the c-*kit* antisense probe. Grains were counted over the whole area of the oocyte on its largest cross section. Diameter was the average of two diameters measured at right angles. The average grain density for the groups of oocytes on a given slide were normalized to the value for primordial oocytes on that slide. No significant difference was observed for the relative values obtained from ovaries of 8 day old through adult mice, so the data were combined.

(Table 1). This indicates a 60-fold increase in content of transcripts per oocyte, reasonably similar to the 30-fold increase estimated from RNA blots (see above).

In situ *hybridization analysis of* c-kit *expression in ovaries: somatic cells*

Significant somatic cell expression could first be seen in ovaries of 14 day old mice, and became more pronounced at 17 days, located primarily over interstitial tissue (Fig. 4A). This pattern was maintained in adult ovaries, where accumulating interstitial tissue makes a substantial contribution to the overall expression (Fig. 4C,D,H). A few labeled thecal cells surrounding follicles judged to be healthy or only in early stages of atresia were consistently present (Fig. 4F,G). These correspond to the distribution of lipid-containing cells in the theca interna as viewed in plastic sections. In addition, cells of the corpus luteum in cycling adults showed a moderate level of labeling lower than that in interstitial tissue (Fig. 4D,E), as well as corporal lutea at 10 days of pregnancy (not shown). Labeling over follicular cells was at background level at all stages. Hybridization to the sense probe resulted in a background level of labeling (Fig. 4B).

Expression of c-kit *protein in oocytes*

To determine whether c-*kit* was expressed at the protein level, live oocytes and early embryos were examined by

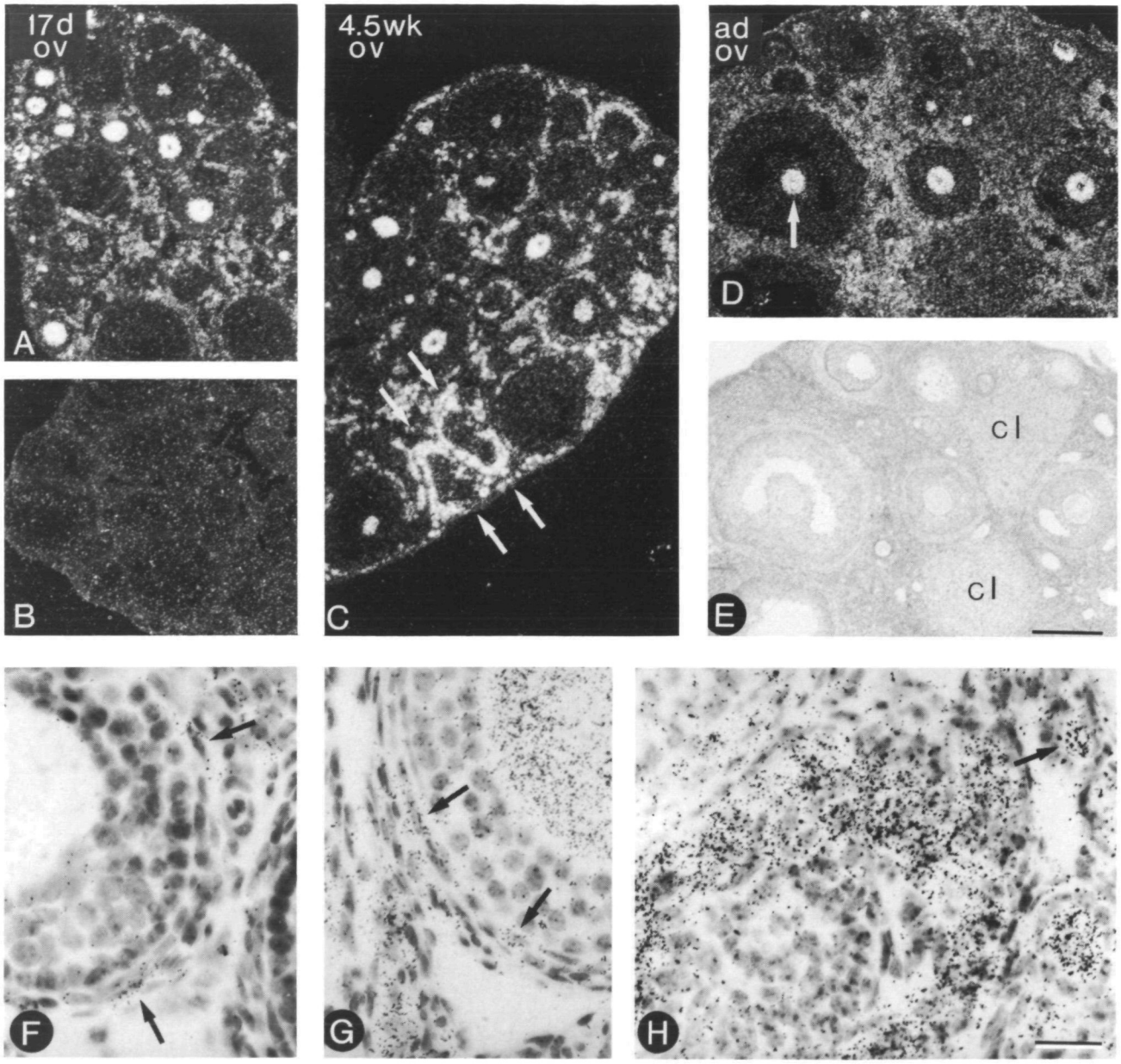

Fig. 4. Expression of c-*kit* RNA in oocytes and somatic ovarian cells of 17 day and older mice. All panels except B hybridized to c-*kit* antisense probes. The 3.6 kb probe was used for panels A and F. (A) Dark-field image of 17 day ovary. Oocytes and interstitial tissue surrounding follicles are labeled. (B) Dark-field image of ovary from a 4½ wk old mouse hybridized to the sense probe. (C) Dark-field image of a ovary from a 4½ wk old mouse. Arrows indicate two follicles in advanced state of atresia, surrounded by highly labeled interstitial tissue. (D) Dark-field image of adult ovary. A full-grown oocyte in an antral follicle is indicated by the arrow. (E) Bright-field image corresponding to D. cl: corpora lutea. Scale bar=200 μm for panels A–E. (F) Bright-field image of a 17 day ovary. A follicle with several layers has a few labeled cells in the theca interna (arrows). The grains over the oocyte are out of focus. (G) Bright-field image of ovary from a 4½ wk old mouse. A follicle with several layers has a few labeled cells in the theca interna (arrows). (H) Bright-field image of ovary from a 4½ wk old mouse. Interstitial cells are highly labeled, as well as oocytes in a small growing follicle (lower right) and in a primordial follicle (arrow). Scale bar=20 μm for panels F–H.

indirect immunofluorescence, using a rabbit immune serum raised against the c-*kit* protein (Nocka *et al.* 1990). Oocytes of all stages showed strong surface fluorescence with immune serum (Fig. 5A,D), but not with preimmune (Fig. 5B,C). The fluorescence on primordial oocytes is patchy and/or less intense compared to growing oocytes (Fig. 5A). Fluorescence is maintained in ovulated eggs and in 1- and 2-cell embryos (Fig. 6A,C). Absorption with +/+ mast cells decreased the fluorescence to near background levels (Figs 5E,F, 6B), while absorption with *W*/*W* mast cells, which lack the c-*kit* receptor (Nocka *et al.* 1990), did not (Figs 5D, 6A,C). This result confirms the specificity of the anti-c-*kit* antiserum. In 8-cell embryos, the fluor-

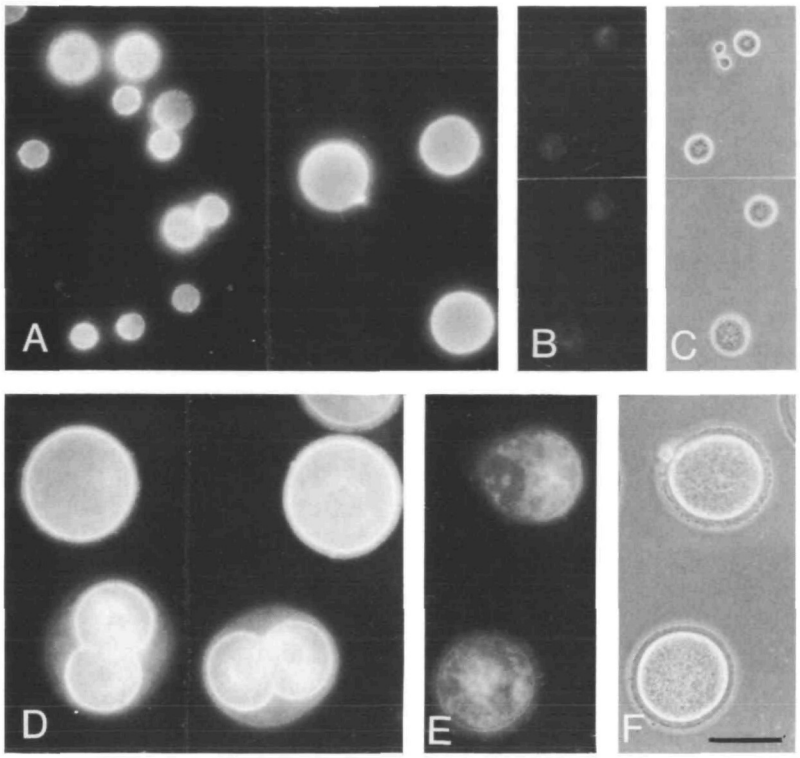

Fig. 5. Expression of c-*kit* protein on the surface of oocytes and 2-cell embryos. (A) Fluorescence image of primordial and growing oocytes incubated with immune serum. (B) Primordial and growing oocytes incubated with preimmune serum. (C) Corresponding phase micrograph. (D) Full-grown oocytes and 2-cell embryos incubated with immune serum absorbed with *W/W* mast cells. (E) Full-grown oocytes incubated with immune serum absorbed with +/+ mast cells. (F) Corresponding phase micrograph. Scale bar=50 μm for all panels.

escence is reduced and in blastocysts, in which only the outer surface is exposed to the antibody, it is near the level seen in controls (Fig. 6A,C). Fixed oocytes and embryos displayed a distinct but lower level of fluorescence (not shown).

In summary, c-*kit* transcripts appear approximately at the time that oocytes enter the diplotene stage of meiosis in late fetal life, and is maintained in primordial, growing, full-grown, and maturing oocytes. The protein is present on primordial oocytes (the earliest stage analysed), and persists during growth, ovulation, and through the 2-cell stage of embryogenesis, disappearing thereafter.

In situ *hybridization analysis of* c-kit *RNA in postnatal testis*

To test the generality of c-*kit* expression in postnatal developing germ cells, *in situ* hybridization was carried out on testes from newborn to adult mice. *At birth*, testicular cords contain quiescent nonproliferating gonocytes (T_1 prospermatogonia) located centrally, and a continuous layer of Sertoli cells located basally. At this stage, no significant label above background was observed (not shown). Further develoment and establishment of the cycle of the seminiferous epithelium have been described (Clermont and Perey, 1957; McKinney and Desjardins, 1973; Bellve *et al.* 1977; Sutcliffe and Burgoyne, 1989). At 3–4 days of age, a few spermatogonia were labeled, presumably the most advanced type A, and a few interstitial cells (not shown). *At 6 days of age*, the progeny of prospermatogonia have become the definitive spermatogonia that will be maintained throughout life, including proliferating undifferentiated type A, type A_{1-4} and a few type B spermatogonia. These have migrated basally and make up about 16 % of the cells in tubular cross sections (Bellve *et al.* 1977). *In situ* preparations of testes from 6 day old mice demonstrated moderate to high labeling of some basal tubular cells. The labeled cells could often be seen to have round nuclei and a halo of clear cytoplasm, presumably type A spermatogonia (Fig. 7A). Many of the unlabeled cells could be identified as Sertoli cells. Interstitial cells, apparently Leydig cells, were also labeled (Fig. 7A). Sections hybridized to the sense strand showed background levels of grains (not shown). *At 9 days of age*, type A spermatogonia are still the predominant germ cell. In some cases the labeling defined a distinct basal layer of spermatogonia (not shown).

By 13 days of age, the progeny of type B spermatogonia have entered the next interphase, and some have reached the pachytene stage of meiotic prophase. The 12 stages of the cycle of the seminiferous epithelium (Oakberg, 1956; Clermont, 1972) can be recognised in tubules by the meiotic and postmeiotic cell types present and their arrangement in the layers of the epithelium. This information in turn permits identification of the gonial types. By 13 days of age, four subdivisions of the cycle can be recognised based on the meiotic stages present and their arrangement in the layers of the epithelium (see McKinney and Desjardins, 1973 for a description of 10–21 day old mice and Clermont and Perey, 1957, for the 15 day old rat). Patterns of labeling were characterized as discrete (label over only the basal layer of cells) or diffuse (label over more than one cell layer of the epithelium (Fig. 7B)). The discrete pattern was seen to correspond

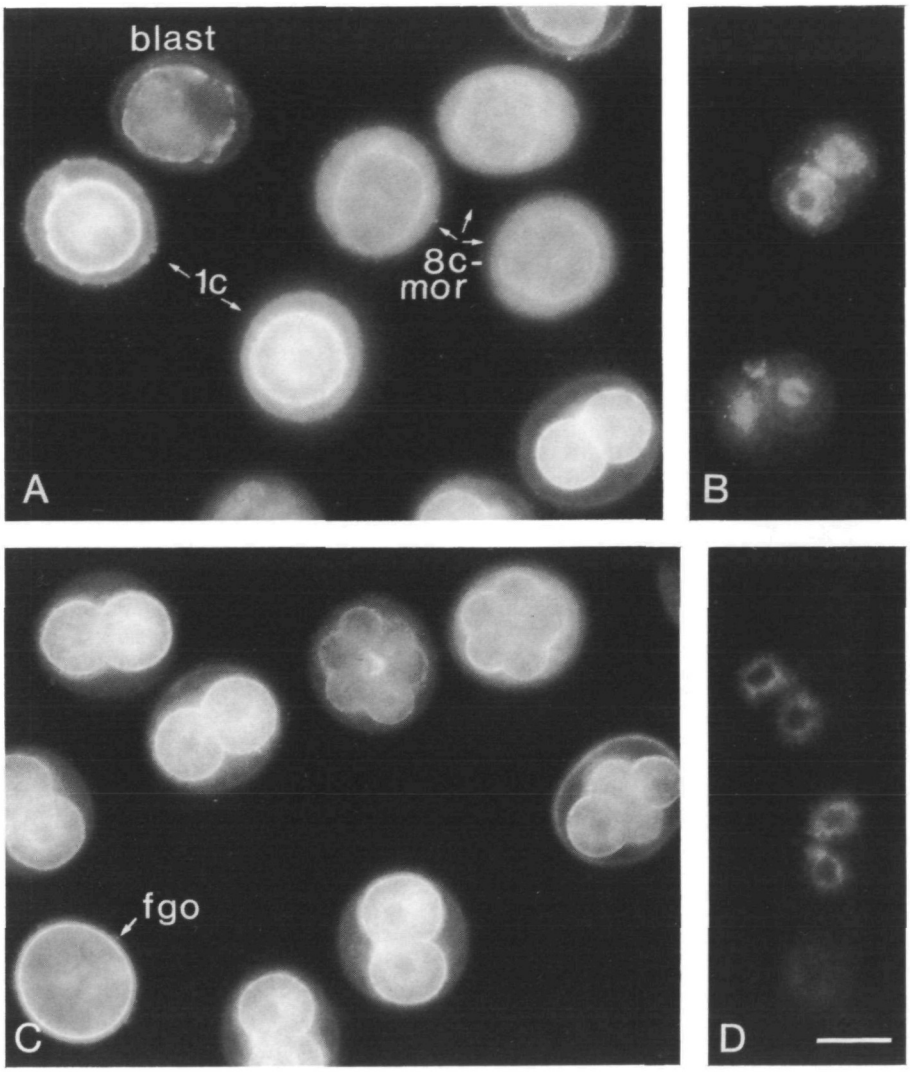

Fig. 6. Expression of c-*kit* protein on the surface of full-grown oocytes and embryos. Fluorescence images of embryos obtained $1\frac{1}{2}$, $2\frac{1}{2}$, or $3\frac{1}{2}$ days after fertilization. (A) Embryos incubated with c-*kit* immune serum absorbed with *W/W* mast cells. 1c: 1-cell fertilized embryo. 8c-mor: compact 8-cell embryos or morulae. blast: early blastocyst. (B) Two-cell embryos incubated with immune serum absorbed with +/+ mast cells. (C) A full-grown oocyte (fgo) and 2-cell to 8-cell embryos incubated with c-*kit* immune serum absorbed with *W/W* mast cells. (D) Two-cell embryos incubated with second antibody only. Scale bar=50 μm for all panels.

to tubules with early pachytene spermatocytes and labeled type A spermatogonia (Fig. 8A) (the stage of the cycle of the epithelium is indicated in the legends). It was also seen in tubules with a few pachytene spermatocytes and labeled intermediate or type B spermatogonia (Fig. 8B). The type A spermatogonia appear to have the highest grain density. The diffuse pattern was seen in tubules containing primarily moderately labeled preleptotene spermatocytes (Fig. 8C). Preleptotene cells were identified by their small size and presence in both the basal and inner layers. Sertoli cells were not labeled (e.g. Fig. 8B). No significant label was observed with the sense strand probe (Fig. 7E).

By 19 days of age, all stages of spermatocytes are present. Labeled basal cells (Fig. 7C) were identified as spermatogonia of types A_4 to B (Fig. 8D,E) and types A_{2-3} (Fig. 8F) (see Huckins, 1971, for placement of types A_{1-4} in the cycle). Preleptotene spermatocytes, now located basally, were also labeled (Fig. 8E). Leydig cells continue to show abundant grains (Fig. 8E).

In adults, the cross-sectional area of the tubules has expanded. A large fraction of the epithelium is occupied by developing spermatids, and the spermatogonia are more sparsely distributed. Identification of the approximate stage of the cycle of the seminiferous epithelium was facilitated by the use of such parameters as the number of layers of spermatids, their position in the epithelium and their nuclear shape (Roosen-Runge and Giesel, 1950; Leblond and Clermont, 1952). At low magnification, two patterns of labeling were most obvious: isolated basal cells with variable numbers of grains and a quite continuous band of basal cells with a low density of grains (Fig. 9A). From the stage of the cycle, it was determined that the former consist of type A_4, intermediate and type B spermatogonia (Fig. 9E,F). The latter were preleptotene spermatocytes, seen most typically in tubules at stage VII (Fig. 9G); their identity was confirmed by their small size and high number. Rare type A_{1-3} spermatogonia could be found labeled in tubules with unlabeled leptotene/zygotene spermatocytes (Fig. 9H). Pachytene spermatocytes at all stages were not labeled above background. Leydig cells were highly labeled (Fig. 9A,D) and unlabeled cells could often be ident-

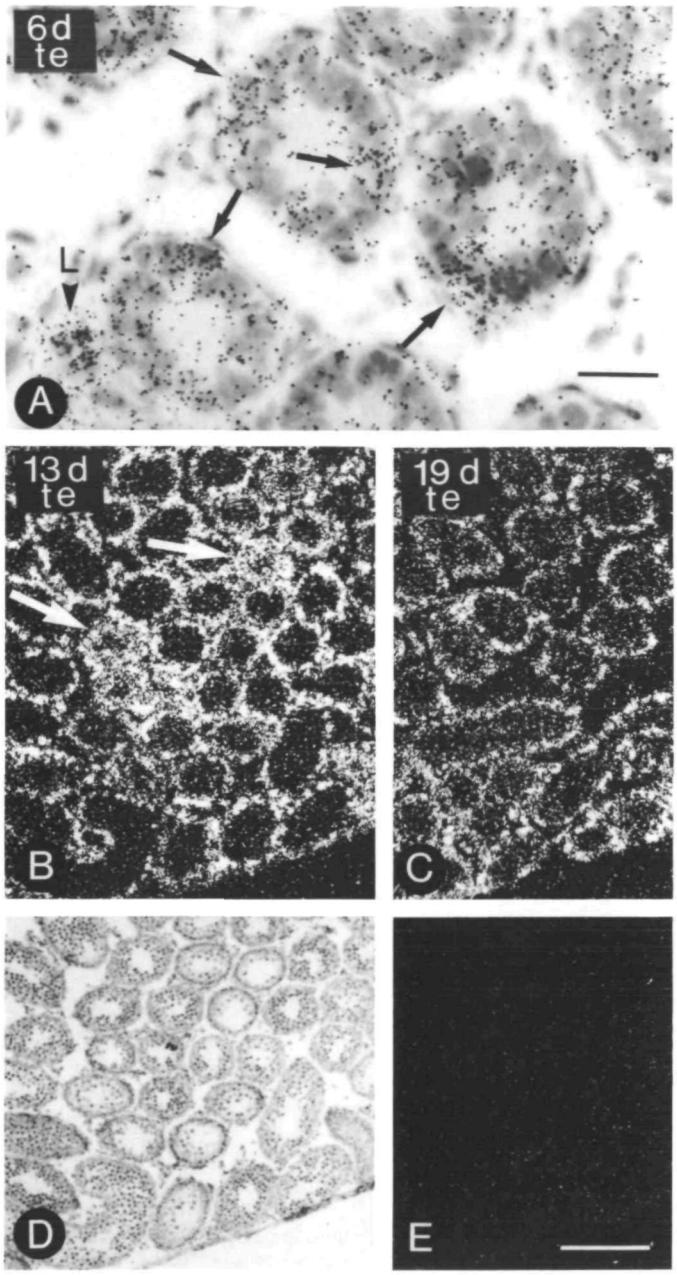

Fig. 7. Expression of c-*kit* RNA in juvenile testis. All panels except E hybridized to the c-*kit* antisense probe. (A) Bright-field image of 6 day testis. Arrows indicate labeled spermatogonia. L: a small cluster of labeled Leydig cells. Scale bar=20 μm. (B) Dark-field image of 13 day testis. Arrows indicate diffusely labeled tubules. (C) Dark-field image of 19 day testis. (D) Bright-field image corresponding to B. (E) Dark-field image of 13 day testis hybridized to the sense strand probe. Scale bar=200 μm for panels B–E.

ified as Sertoli cells (Fig. 9F). The sense strand probe resulted in background levels of labeling (Fig. 9C).

We interpret these results as follows. c-*kit* mRNA is present in spermatogonial stages from about type A_1 through type B, and in the earliest preleptotene spermatocytes. There was no detectable label in later spermatocytes or spermatids. We have not attempted to characterise the labeling of earlier undifferentiated type A spermatogonia directly. Sertoli cells do not contain detectable c-*kit* RNA, while Leydig cells are labeled from 6 days of age through adult life.

Discussion

The major finding presented here is that c-*kit* is expressed in type A and B spermatogonia and highly expressed in oocytes. These results imply that the c-*kit* gene may have a second period of function in germ cells of postnatal gonads, in addition to the well-known requirement for *W* in primordial germ cells.

In oocytes, c-*kit* transcripts first appear at the diplotene stage close to the time of birth. They are maintained in primordial oocytes, accumulate during oocyte growth, and persist through oocyte maturation. The c-*kit* protein is present on the oocyte surface at all stages tested from primordial oocytes through full-grown oocytes, and is still abundant on 2-cell embryos. In male germ cells, expression correlates with the period of spermatogonial proliferation. Transcripts are absent in gonocytes of newborn mice, but are present in proliferating type A and B spermatogonia from their first appearance in juvenile mice, and are retained in the earliest preleptotene spermatocytes. No expression was seen in later meiotic or postmeiotic stages. In addition, c-*kit* is expressed in some somatic cells of the gonads.

The phenotypes of mice carrying *W* alleles provide some evidence for a function of c-*kit* in postnatal female and male germ cells. In addition to a large decrease in the number of germ cells found in juvenile W/W^v and W^v/W^v mice, it was found that the rate of development of both oocytes and spermatogenic cells was slower than in normals in most cases (Coulombre and Russell, 1954). In addition, in some *W* genotypes, female and male germ cell development varies independently (Geissler *et al.* 1981), suggesting a function after the primordial germ cell stage.

The close correspondence of phenotypes seen in *W* and *Sl* mutant mice indicates that they affect the same cellular target. We can therefore infer some aspects of c-*kit* receptor function by analyzing *Sl* phenotypes. Histological examination of juvenile ovaries of infertile Sl/Sl^t females reveals numerous primordial follicles, but no follicles with growing oocytes (Kuroda *et al.* 1988), suggesting a function in initiation and possibly maintenance of oocyte growth. Growth can begin at any time from days to months after reaching the primordial oocyte stage. Thus, if the c-*kit* receptor is involved in oocyte growth, it appears well before it carries out this function. In Sl/Sl^t:+/+ chimeras, oocyte growth can be initiated and maintained even in follicles comprised entirely of Sl/Sl^t cells; thus, the *Sl* product may be produced outside the follicle (Kuroda *et al.* 1988). Nevertheless, the principal source of the *Sl* product could normally be intrafollicular, since only a small contribution of wild-type factor may be sufficient to reinforce the mildly defective factor released by

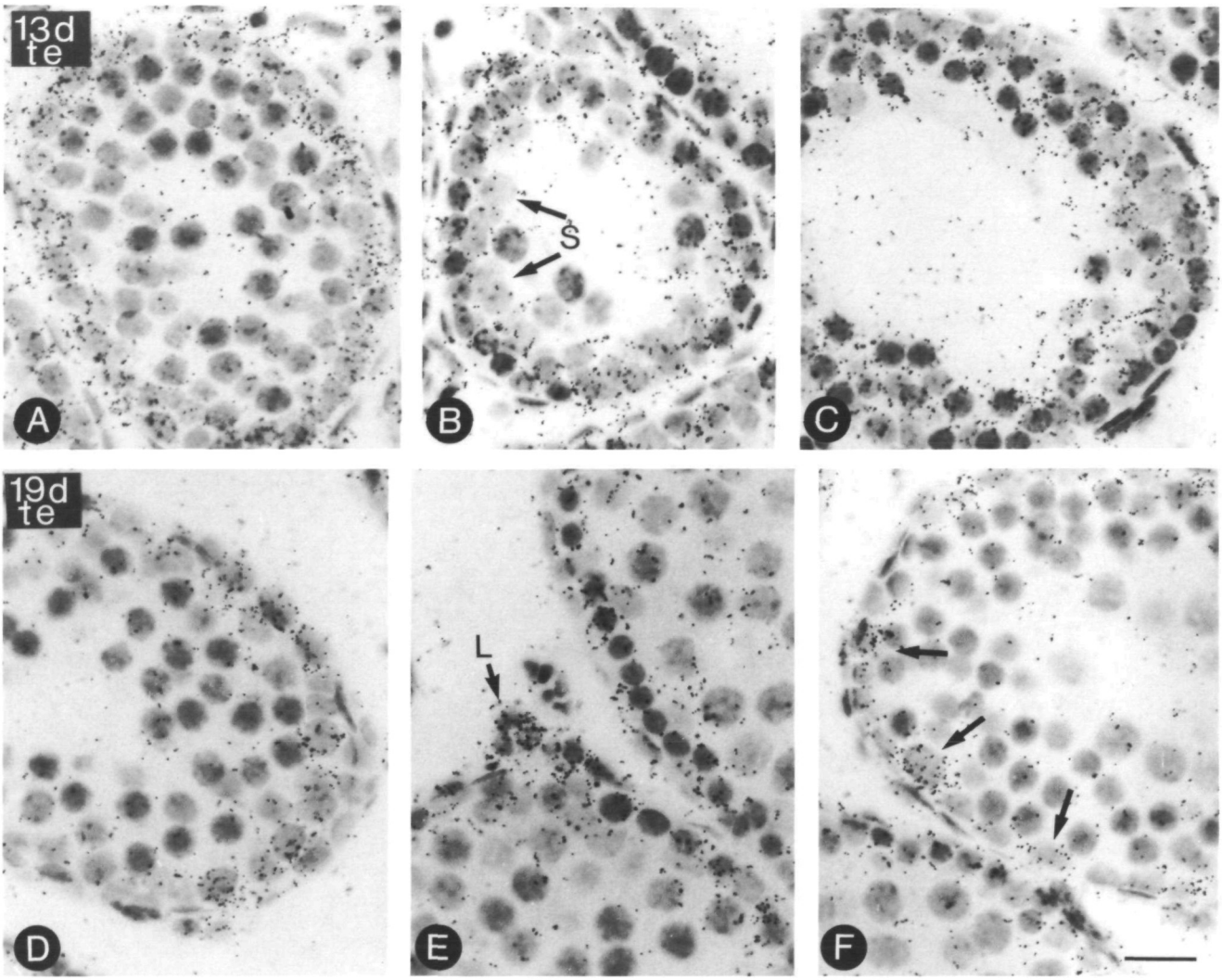

Fig. 8. Expression of c-*kit* RNA in individual cells of seminiferous tubules of 13 and 19 day old mice, shown in high-magnification bright-field images of sections hybridized to the c-*kit* antisense probe. (A–C) 13 day old mice; (D–E) 19 day old mice. (A) Tubule with zygotene or early pachytene spermatocytes, and large labeled basal type A spermatogonia. Approximately stage I of the cycle of the seminiferous epithelium. (B) Tubule with a few pachytene spermatocytes, and basal moderately labeled intermediate or type B spermatogonia. Stage III–V of the cycle. Two unlabeled Sertoli cells are indicated at S. (C) Diffusely labeled tubule with labeled preleptotene spermatocytes in both basal and more apical layers at this age. Approximately stage VII of the cycle. (D) Tubule with early pachytene spermatocytes and labeled type A_4 spermatogonia. Approximately stage I of the cycle. (E) Below: tubule with pachytene spermatocytes and moderately labeled intermediate or type B spermatogonia. Stage III–V of the cycle. Above: tubule with pachytene spermatocytes and labeled preleptotene spermatocytes. Approximately stage VII of the cycle. A small cluster of labeled Leydig cells is indicated at L. (F) Tubule with leptotene/zygotene and pachytene spermatocytes, and a few large labeled type A_{2-3} spermatogonia (arrows). Stage IX–XI of the cycle identified by two layers of meiotic cells. Scale bar=20 μm for all panels.

Sl/*Sl*^{t} cells (Kohrogi *et al.* 1983). Although oocyte growth can be maintained *in vitro*, it has been difficult to assess the role of extracellular factors in this process, since oocytes are highly dependent on communication through gap junctions with follicle cells for growth (see Schultz, 1986, for a review; see Buccione *et al.* 1987).

The c-*kit* receptor is still present on full-grown oocytes, raising the possibility of a function in the resumption of the cell cycle at the onset of meiotic maturation. The presence of the c-*kit* receptor through the 2-cell stage of embryogenesis can be accounted for by continued synthesis of the protein on maternal message present in eggs and stability of the protein into the 2-cell stage. It is difficult to speculate on its role during this period.

In agreement with our *in situ* hybridization results on male germ cells, c-*kit* RNA expression has been detected in purified spermatogonial cell populations by RNA blot analysis (as well as in Leydig cells) (Sorrentino, V., personal communication). Transcription may be limited to premeiotic cells and the mRNA detected in preleptotene spermatocytes retained from the preceding interphase of type B spermatogonia. In *Sl*/+ mice the transition from type A to type B

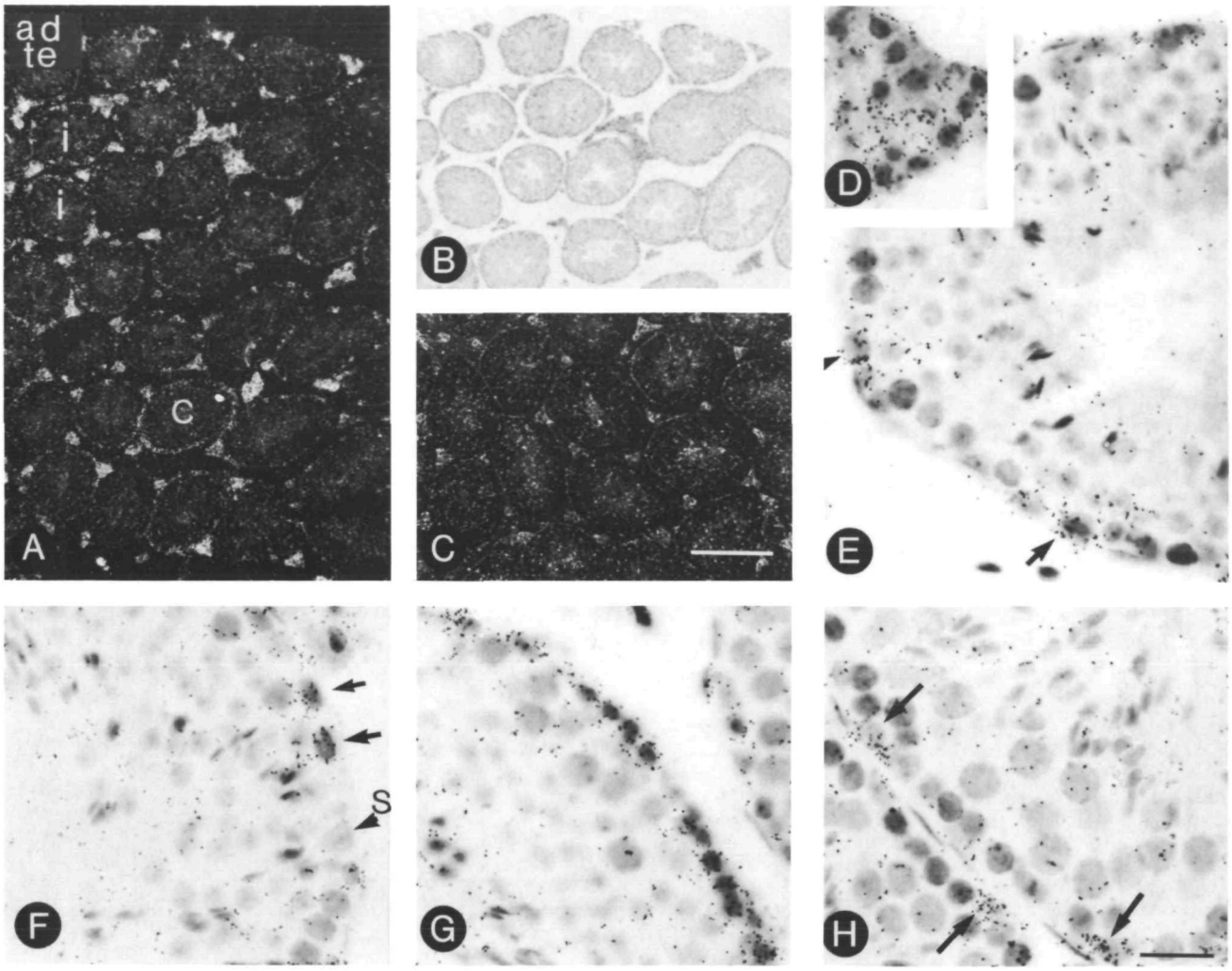

Fig. 9. Expression of c-*kit* RNA in adult testis. All panels except C hybridized to the c-*kit* antisense probe. (A and B) Dark- and corresponding bright-field images of adult testis (6 mon old). A tubule with an almost continuous band of label in the basal layer is marked c, and two with isolated labeled basal cells are labeled i (upper left). (C) Dark-field image of adult testis hybridized to the sense strand probe. (D) High-magnification bright-field image of a cluster of labeled Leydig cells. (E–H) High-magnification bright-field images of seminiferous tubules. (E) Tubule with labeled type A_4 spermatogonia (arrows) at approximately stage I of the cycle of the seminiferous epithelium. (F) Tubule with labeled intermediate or type B spermatogonia (arrows) at stage III–V of the cycle. s: an unlabeled Sertoli cell. (G) Tubule with labeled preleptotene spermatocytes. Stage VII of the cycle of the epithelium identified by the presence of whirls of sperm tails in the lumen (not visible in the photomicrograph). (H) Two tubules with labeled A_{2-3} spermatogonia (arrows). Stage IX–XII of the cycle identified by the presence of only one layer of spermatids. Scale bar=20 μm for all panels.

spermatogonia is defective (Nishimune *et al.* 1980). In testicular tubules of Sl/Sl^d:+/+ chimeras, normal regions alternate with undifferentiated regions, presumably corresponding to clones of wild-type and mutant cells (Nakayama *et al.* 1988). Also, transplantation has shown that the environmental effect of *Sl* is endogenous to the tubules (Kuroda *et al.* 1989). These results implicate Sertoli cells as the site of the defect, and thus the source of the *Sl* gene product.

Developmental transitions of late type A spermatogonia are sensitive to temperature as in cryptorchidism (Haneji *et al.* 1984), as well as genetic lesions (Sutcliffe and Burgoyne, 1989). The expression of a set of proto-oncogenes is increased in type B compared to type A spermatogonia (Wolfes *et al.* 1989), suggesting a new developmental program. If the c-*kit* receptor is involved in mediating a specific transition at this point, it is present before it carries out this function and availability of its ligand could control the process.

Expression of the c-*kit* receptor correlates with proliferation of spermatogonia and potential for growth of oocytes. Activation of tyrosine kinase receptors often results in intracellular signals that promote cell proliferation (including growth) (Rosen, 1987; Yarden and Ullrich, 1988; Ullrich and Schlessinger, 1990). The most economical hypothesis is that the same signal can promote the cell cycle when presented to spermatogonia and can promote growth when present to oocytes in meiotic prophase. An additional or alternate possibility is a role in cell survival, analogous to low

level stimulation of macrophages by CSF-1 (Tushinski *et al.* 1982).

The role if any of c-*kit* in ovarian interstitial tissue and Leydig cells of testis is difficult to assess. The phenotypes of *W* and *Sl* mutant mice have not provided evidence of effects in somatic cells of the gonads (see Kuroda *et al.* 1989; Terada *et al.* 1986).

In summary, we have found expression of c-*kit* transcripts in male and female germ cells starting at or shortly after birth, and maintained to the beginning of meiosis in males and through meiotic maturation in females. Genetic data indicate a functional role for the *W* and *Sl* pair of genes at least for a brief period during germ cell development in postnatal gonads.

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References

BACHVAROVA, R. (1985). Gene expression during oogenesis and oocyte development in the mammal. In *Developmental Biology*, vol. 1 (ed. L. W. Browder), pp. 453–523. New York: Plenum Press.

BACHVAROVA, R., BARAN, M. M. AND TEJBLUM, A. (1980). Development of growing naked mouse oocytes in vitro. *J. exp. Zool.* **211**, 159–169.

BELLVE, A. R., CAVICCHIA, J. Ç., MILLETTE, C. F., O'BRIEN, D. A., BHATNAGAR, Y. M. AND DYM, M. (1977). Spermatogenic cells of the prepuberal mouse. *J. Cell Biol.* **74**, 68–85.

BESMER, P., MURPHY, P. C., GEORGE, P. C., QUI, F., BERGOLD, P. J., LEDERMAN, L., SNYDER, H. W., BRODEUR, D., ZUCKERMAN, E. E. AND HARDY, W. D. (1986). A new acute transforming feline retrovirus and relationship of its oncogene v-*kit* with the protein kinase family. *Nature* **320**, 415–421.

BUCCIONE, R., CECCONI, S., TATONE, C., MANGIA, F. AND COLONNA, R..(1987). Follicle cell regulation of mammalian oocyte growth. *J. exp. Zool.* **242**, 351–354.

CHABOT, B., STEPHENSON, D. A., CHAPMAN, V. M., BESMER, P. AND BERNSTEIN, A. (1988). The proto-oncogene c-*kit* encoding a transmembrane tyrosine kinase receptor maps to the mouse *W* locus. *Nature* **335**, 88–89.

CLERMONT, Y. (1972). Kinetics of spermatogenesis in mammals: Seminiferous epithelial cycle and spermatogonial renewal. *Physiol. Rev.* **52**, 198–236.

CLERMONT, Y. AND PEREY, B. (1957). Quantitative study of the cell population of the seminiferous tubules in immature rats. *Am. J. Anat.* **100**, 241–268.

COULOMBRE, J. L. AND RUSSELL, E. S. (1954). Analysis of the pleiotropism at the W-locus in the mouse. The effects of W and W^v substitution upon postnatal development of germ cells. *J. exp. Zool.* **126**, 277–295.

EPPIG, J. J. (1976). Analysis of mouse oogenesis in vitro. Oocyte isolation and the utilization of exogenous energy sources by growing oocytes. *J. exp. Zool.* **198**, 375–381.

FUJITA, J., NAKAYAMA, H., ONOUE, H., KANAKURA, Y., NAKANO, T., ASAI, H., TAKEDA, S.-I., HONJO, T. AND KITAMURA, Y. (1988). Fibroblast-dependent growth of mast cells in vitro: Duplication of mast cell depletion in mutant mice of W/W^v phenotype. *J. cell. Physiol.* **134**, 78–84.

FUJITA, J., ONOUE, H., EBI, Y., NAKAYAMA, H., KANAKURA, Y. AND KITAMURA, Y. (1989). In vitro duplication and in vivo cure of mast cell-deficiency of Sl/Sl^d mutant mice by cloned 3T3 fibroblast. *Proc. natn. Acad. Sci. U.S.A.* **86**, 2888–2891.

GEISSLER, E. N., MCFARLAND, E. C. AND RUSSELL, E. S. (1981). Analysis of pleiotropism at the dominant white-spotting (*W*) locus of the house mouse: A description of ten new *W* alleles. *Genetics* **97**, 337–361.

GEISSLER, E. N., RYAN, M. A. AND HOUSMAN, D. E. (1988). The dominant-white spotting (W) locus of the mouse encodes the c-*kit* proto-oncogene. *Cell* **55**, 185–192.

GREGORY, C. J. AND EAVES, A. C. (1978). Three stages of erythropoietic progenitor cell differentiation distinguished by a number of physical and biologic properties. *Blood* **51**, 527–537.

HANEJI, T., MAEKAWA, M. AND NISHIMUNE, Y. (1984). Vitamin A and Follicle-stimulating hormone synergistically induce differentiation of type A spermatogonia in adult mouse cryptorchid testes in vitro. *Endocrinol.* **114**, 801–805.

HEATH, J. K. (1978). Mammalian primordial germ cells. In *Development in Mammals*, vol. 3 (ed. M. H. Johnson), pp. 267–298. Amsterdam: North Holland.

HUCKINS, C. (1971). The spermatogonial stem cell population in adult rats. I. Their morphology, proliferation and maturation. *Anat. Rec.* **169**, 533–558.

ISCOVE, N. N. (1978). Committed erythroid precursor populations in genetically anemic W/W^v and Sl/Sl^d mice. In *Aplastic Anemia* (ed. S. Hibino, S. Takaku and N. T. Shahidi), pp. 31–36. Univ. of Tokyo Press, Tokyo.

JARBOE, D. L., MARSHALL, J. S., RANDOLPH, T. R., KUKOLJA, A. AND HUFF, T. F. (1989). The mast cell-committed progenitor. I. Description of a cell capable of IL-3-independent proliferation and differentiation without contact with fibroblasts. *J. Immunol.* **142**, 2405–2417.

KAPLAN, G., JELINEK, W. R. AND BACHVAROVA, R. (1985). Repetitive sequence transcripts and U1 RNA in mouse oocytes and eggs. *Devl Biol.* **109**, 15–24.

KITAMURA, Y. AND GO, S. (1978). Decreased production of mast cells in Sl/Sl^d anemic mice. *Blood* **53**, 492–497.

KOHROGI, T., YOKOYAMA, M., TAGUCHI, T., KITAMURA, Y. AND TUTIKAWA, K. (1983). Effect of the Sl^t mutant allele on the production of tissue mast cells in mice. *J. Hered.* **74**, 375–377.

KRIEG, P. A. AND MELTON, D. A. (1987). In vitro RNA synthesis with SP6 RNA polymerase. *Methods Enzymol.* **155**, 397–413.

KURODA, H., TERADA, N., NAKAYAMA, H., MATSUMOTO, K. AND KITAMURA, Y. (1988). Infertility due to growth arrest of ovarian follicles in Sl/Sl^t mice. *Devl Biol.* **126**, 71–79.

KURODA, H., NAKAYAMA, H., NAMIKI, M., MATSUMOTO, K., NISHIMUNE, Y. AND KITAMURA, Y. (1989). Differentiation of germ cells in seminiferous tubules transplanted to testes of germ cell-deficient mice of W/W^v and Sl/Sl^d genotypes. *J. cell. Physiol.* **139**, 329–334.

LEBLOND, C. P. AND CLERMONT, Y. (1952). Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann. N.Y. Acad. Sci.* **55**, 548–573.

LEHNERT, S. A. AND AKHURST, R. J. (1988). Embryonic expression pattern of TGF β type-1 RNA suggests both paracrine and autocrine mechanisms of action. *Development* **104**, 263–273.

MAJUMDER, S., BROWN, K., QIU, F.-H. AND BESMER, P. (1988). c-*kit* protein, a transmembrane kinase: Identification in tissues and characterization. *Molec. cell. Biol.* **8**, 4896–4903.

MCKINNEY, T. D. AND DESJARDINS, C. (1973). Postnatal development of the testis, fighting behavior, and fertility in house mice. *Biol. Reprod.* **9**, 279–294.

MINTZ, B. AND RUSSELL, E. S. (1957). Gene-induced embryological modifications of primordial germ cells in the mouse. *J. exp. Zool.* **134**, 207–237.

MUTTER, G. L. AND WOLGEMUTH, D. J. (1987). Distinct developmental patterns of c-*mos* proto-oncogene expression in female and male mouse germ cells. *Proc. natn. Acad. Sci. U.S.A.* **84**, 5301–5305.

NAKAYAMA, H., KURODA, H., ONOUE, H., FUJITA, J., NISHIMUNE, Y., MATSUMOTO, K., NAGANO, T., SUZUKI, F. AND KITAMURA, Y. (1988). Studies of $Sl/Sl^d \leftrightarrow +/+$ mouse aggregation chimeras. II. Effect of the steel locus on spermatogenesis. *Development* **102**, 117–126.

NISHIMUNE, Y., HANEJI, T. AND KITAMURA, Y. (1980). The effects

of Steel mutation on testicular germ cell differentiation. *J. cell. Physiol.* **105**, 137–141.

NOCKA, K., MAJUMDER, S., CHABOT, B., RAY, P., CERVONE, M., BERSTEIN, A. AND BESMER, P. (1989). Expression of c-*kit* gene products in known cellular targets of *W* mutations in normal and *W* mutant mice – Evidence for an impaired c-*kit* kinase in mutant mice. *Genes and Dev.* **3**, 816–826.

NOCKA, K., TAN, J. C., CHIU, E., CHU, T. Y., RAY, P., TRAKTMAN, P. AND BESMER, P. (1990). Molecular bases of dominant negative and loss of function mutations at the murine c-*kit*/white spotting locus: W^{37}, W^{v}, W^{41}, *W*. *EMBO J.* **9**, 1805–1813.

OAKBERG, E. F. (1956). A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. *Am. J. Anat.* **99**, 391–409.

PAYNTON, B. V., REMPEL, R. AND BACHVAROVA, R. (1988). Changes in state of adenylation and time course of degradation of maternal mRNAs during oocyte maturation and early embryonic development in the mouse. *Devl Biol.* **129**, 304–314.

QIU, F., RAY, P., BROWN, K., BARKER, P. E., JHANWAR, S., RUDDLE, F. H. AND BESMER, P. (1988). Primary structure of c-*kit*: Relationship with the CSF-1/PDGF receptor kinase family – Oncogenic activation of v-*kit* involves deletion of extracellular domain and C terminus. *EMBO J.* **7**, 1003–1011.

QUINN, P., KERIN, J. F. AND WARNES, G. M. (1988). Improved pregnancy rate in human in vitro fertilization with use of a medium based on the composition of human tubal fluid. *Fertil. Steril.* **44**, 493–498.

REITH, A. D., ROTTAPEL, R., GIDDENS, E., BRADY, C., FORRESTER, L. AND BERNSTEIN, A. (1990). *W* mutant mice with mild or severe developmental defects contain distinct point mutations in the kinase domain of the c-*kit* receptor. *Genes and Dev.* **4**, 390–400.

ROOSEN-RUNGE, E. C. AND GIESEL, L. O. (1950). Quantitative studies on spermatogenesis in the albino rat. *Am. J. Anat.* **87**, 1–30.

ROSEN, O. M. (1987). After insulin binds. *Science* **237**, 1452–1458.

RUSSELL, E. S. (1949). Analysis of pleiotropism at the W-locus in the mouse: Relationship between the effects of W and W^{v} substitution on hair pigmentation and on erythrocytes. *Genetics* **34**, 708–723.

RUSSELL, E. S. (1979). Hereditary anemias of the mouse. *Adv. Genet.* **20**, 357–459.

SCHULTZ, R. M. (1986). Molecular aspects of mammalian oocyte growth and maturation. In *Experimental Approaches to Mammalian Embryonic Development* (eds J. Rossant and R. A. Pedersen), pp. 195–237. New York: Cambridge University Press.

SILVERS, W. K. (1979). *The Coat Colors of Mice*, New York: Springer-Verlag.

SPEED, R. M. (1982). Meiosis in the foetal mouse ovary. I. An analysis at the light microscopic level using surface-spreading. *Chromosoma* **85**, 427–437.

SUTCLIFFE, M. J. AND BURGOYNE, P. S. (1989). Analysis of the testes of H-Y negative XOSxr^{b} mice suggests that the spermatogenesis gene (*Spy*) acts during the differentiation of the A spermatogonia. *Development* **107**, 373–380.

TAN, J. C., NOCKA, K., RAY, P., TRAKTMAN, P. AND BESMER, P. (1990). The dominant W^{42} *spotting* phenotype results from a missense mutation in the c-*kit* receptor kinase. *Science* **247**, 209–212.

TERADA, N., KURODA, H., NAKAYAMA, H., MATSUMOTO, K. AND KITAMURA, Y. (1986). Effect of genetically defined oocyte depletion on production of androgens and oestrogens by ovaries of suckling mice. *J. Steroid Biochem.* **25**, 83–89.

TUSHINSKI, R. J., OLIVER, I. T., GUILBERT, L. J., TYNAN, P. W., WARNER, J. R. AND STANLEY, E. R. (1982). Survival of mononuclear phagocytoes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. *Cell* **28**, 71–81.

ULLRICH, A. AND SCHLESSINGER, J. (1990). Signal transduction by receptors with tyrosine kinase activity. *Cell* **61**, 203–212.

WILKINSON, D. G., BAILES, J. A., CHAMPION, J. E. AND MCMAHON, A. P. (1987*a*). A molecular analysis of mouse development from 8 to 10 days *post coitum* detects changes only in embryonic globin expression. *Development* **99**, 493–500.

WILKINSON, D. G., BAILES, J. A. AND MCMAHON, A. P. (1987*b*). Expression of the proto-oncogene *int*-1 is restricted to specific neural cells in the developing mouse embryo. *Cell* **50**, 79–88.

WOLFES, H., KOGAWA, K., MILLETTE, C. F. AND COOPER, G. M. (1989). Specific expression of nuclear proto-oncogenes before entry into meiotic prophase of spermatogenesis. *Science* **245**, 740–743.

YARDEN, Y., KUANG, W.-J., YANG-FENG, T., COUSSENS, L., MUNEMITSU, S., DULL, T. J., CHEN, E., SCHLESSINGER, J., FRANCKE, U. AND ULLRICH, A. (1987). Human proto-oncogene c-*kit*: A new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO J.* **6**, 3341–3351.

YARDEN, Y. AND ULLRICH, A. (1988). Growth factor receptor tyrosine kinases. *Annu. Rev. Biochem.* **57**, 443–478.

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