Developmental expression of c-kit, a proto-oncogene encoded by the W locus

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Summary

Developmental expression of the c-kit proto-oncogene, a receptor tyrosine kinase encoded by the W locus, was investigated by in situ hybridization in normal mouse embryos. Early after implantation transcripts were detectable only in the maternal placenta (6½–7½ days p.c.). Subsequently (8½ days p.c.) numerous ectodermal (neural tube, sensory placodes) and endodermal (embryonic gut) derivatives expressed c-kit. Later transcripts were detected also in the blood islands of the yolk sac and in the embryonic liver, the main sites of embryonic hemopoiesis. Around midgestation, transcripts accumulated in the branchial pouches and also in primordial germ cells of the genital ridges. This complex pattern of expression remained characteristic also later in gestation, when c-kit was expressed in highly differentiated structures of the craniofacial area, in presumptive melanoblasts and in the CNS. In the adult ovary, maternal c-kit transcripts were detected. They were present in the oocytes of both immature and mature ovarian follicles, but not in the male germ line, where c-kit expression may be down regulated. Thus, c-kit activity is complex and appears in multiple tissues including those that also display defects in mutations at the W locus where c-kit is encoded. Correlation between W phenotypes and c-kit expression, as well as the regulation of the complex and multiple expression of polypeptide growth factors and receptors, is discussed.

Key words: receptor tyrosine kinase, in situ hybridization, W alleles, murine embryogenesis.

Introduction

The c-kit proto-oncogene encodes a receptor tyrosine kinase with a yet unknown ligand (Yarden et al. 1987). This class of receptors and their ligands, which are polypeptide growth factors, are thought to have important roles in development. This was demonstrated by the analysis of Drosophila and C. elegans mutations, which affect sequences homologous to polypeptide growth factors (Wharton et al. 1985; Greenwald, 1985; Padgett et al. 1987), or protein tyrosine kinase receptors (Hafen et al. 1987; Price et al. 1989; Schejter and Shilo, 1989; Sprenger et al. 1989).

In vertebrates, functional evidence exists for the role of polypeptide growth factors in mesoderm induction. Gene products belonging to the transforming growth factor (TGF beta) and to the fibroblast growth factor (FGF) families respectively mediate this activity in amphibian embryos (for review see Smith, 1989). In mammals, a number of recent in situ hybridization studies report the developmental expression of genes encoding polypeptide growth factors. Genes belonging to the TGFbeta family were shown to be expressed at multiple sites in the early mouse embryo. Their activity in the development of skeletal structures and the integument of later embryos was reported by Sandberg et al. (1988), Pelton et al. (1989) and by Lyons et al. (1989). Complex multiple expression was also found when the FGF-like int-2 gene was investigated. int-2 is expressed in the mesoderm and extraembryonic endoderm during gastrulation and neurulation of early mouse embryos (Wilkinson et al. 1988), and later in the mesenchyme of the teeth and retina and in the cerebellum (Wilkinson et al. 1989). More limited developmental expression was detected of the colony stimulating factor, CSF-1, in the uterine epithelium, whereas transcripts of its receptor, the proto-oncogene receptor tyrosine kinase, c-fms, was found in the maternal placenta (Regenstreif and Rossant, 1989). Thus considerable evidence is accumulating for the role of polypeptide growth factors in mammalian development. Less data are, however, available regarding their receptors. With these general considerations in mind, we decided to investigate the developmental expression of the putative receptor, c-kit.

Special interest in c-kit arose recently when it was...
shown to be genetically connected to the W locus of mice. W and c-kit were closely connected on the physical gene map (Chabot et al. 1988), and in certain W alleles c-kit rearrangements have been detected (Geissler et al. 1988). Moreover, defects were found in the c-kit-associated protein kinase activity of mast cells from W/W mutant mice (Nocka et al. 1989). Most recently a point mutation was reported in the kinase domain of c-kit in the W^42 mutant allele (Tan et al. 1990). In order to evaluate the developmental expression of c-kit and to assess whether it could be responsible for multiple W phenotypes, we have decided to study first its expression in normal, wild-type mouse embryos.

Here we report the embryonic expression of c-kit in hemopoietic cells of the yolk sac and embryonic liver, in presumptive subepidermal melanocytes, as well as in primordial germ cells. These data correlate well with the deficiencies in hemopoietic stem cells, melanocytes and primordial germ cells displayed by various W mutants (Russell, 1979). In addition, c-kit expression was also observed in the developing brain and spinal cord, and also in connection with numerous craniofacial structures as well as in the intestinal tract, organs that are not affected in W mutants. Strong accumulation of c-kit transcripts were observed in the oocytes of both primary and mature ovarian follicles, suggesting that c-kit may have role in oogenesis and as maternal message in early embryogenesis. The multiple roles of c-kit in embryogenesis and gametogenesis and their correlation with mutations at W will be discussed.

Materials and methods

Embryos

(C57BL/6J BALB/cJ)F1 female mice were mated to C57BL/J males. The time of gestation was calculated by considering the morning after mating as day 0.5 p.c. (post coitum, Hogan et al. 1986). Early embryos (64-84 days p.c.) were sectioned within the decidua. Some 84 days old and all 94 days p.c. and older embryos were dissected from the embryonic membranes. All embryos were prefixed in 4% paraformaldehyde at 4°C overnight.

Probes

Probe preparation is described in the first section of the text. T3 antisense or T7 sense (control) polymerase transcripts synthesized in the presence of [35S]-UTP were used as probes. Alkaline hydrolysis of the probe was not found to be necessary. The specific activity of the probes was 8.5-2×10^6 cts min^-1 µg^-1 RNA.

Northern blot hybridization

F9 cells were grown according to Hogan et al. (1981). RNA was isolated from F9 cells and from 124 (p.c.) days old embryos by the LiCl-urea method of Auffray and Rougeon (1980). After electrophoresis in formalin gels, the RNA was blotted onto nylon membranes and hybridized to double-stranded DNA probes derived from the plasmid used also for in situ hybridization.

In situ hybridization

Embedding, sectioning, post fixation and hybridization was performed according to Hogan et al. (1986). 7-10 µm thick cryostat sections were made. Post-hybridization washing was in 2xSSC, 50% formamide and 0.1 M dithiothreitol at 65°C for 30min (high-stringency wash). This was followed by RNase digestion and by a repeated high-stringency wash. For autoradiography, Kodak NTB2 emulsion was used and the slides were exposed for 3-5 weeks. Finally, the sections were stained in Giemsa stain. The slides were photographed either in a Wild/Leitz microscope for small magnifications or in a Zeiss photomicroscope for higher magnifications. Kodak Technical Pan negative film was used for bright-field, and Kodak T Max 400, for dark-field microscopy.

Alkaline phosphatase reaction

The diazonium salt reaction of Sigma Diagnostics, Procedure 86 (St Louis, MO, USA), was performed on sections of prefixed embryos.

Results

Analysis of c-kit expression in teratocarcinoma and embryo RNA

The probe used in this study was derived from a mouse c-kit cDNA clone, which was isolated by screening a mouse brain cDNA library with two oligonucleotides (42mers each) derived from the c-kit nucleotide sequence of the mouse (Qiu et al. 1988). A 3.3 kbp long cDNA clone was subcloned into the Bluescript vector (Stratagene, La Jolla, CA, USA), and the partial nucleotide sequence showed that it contains the entire coding region. The upstream-most 276 bp fragment of this subclone, starting 30bp upstream of the initiator ATG, a SacI–BamHI fragment, was subcloned into Bluescript (KS) and T3 polymerase generated 35S-UTP-labeled antisense transcripts were used as probes. The T7-polymerase-generated sense transcript of the same insert served as control. This fragment was chosen as the hybridization probe because the 5' end of receptor tyrosine kinase genes is less conserved than other areas of the genes in this gene family (Yarden and Ullrich, 1988).

To obtain preliminary information on c-kit expression, poly-A-rich RNA isolated from F9 teratocarcinoma cells and from 124 days old (p.c., post coitum) embryos was investigated. F9 cell cultures were treated with retinoic acid according to the schedules of Hogan et al. (1981). Additional cultures were left untreated and served as controls. Fig. 1 demonstrates an increase of c-kit expression following both treatments, suggesting that c-kit synthesis is activated during F9 cell differentiation. Both F9 cells and cells of 124 days old embryos contained a single 5.8 kb long RNA species which hybridized with c-kit in agreement with previous reports on the size of the c-kit transcript (Yarden et al. 1987; Qiu et al. 1988).

C-kit expression during early embryonic development

To investigate further the role of c-kit in mouse embryogenesis, in situ hybridization experiments were performed. Cryostat sections prepared at different stages of embryogenesis were hybridized with the c-kit probe.
Early following implantation abundant c-kit transcripts were present in the decidua but not in the embryo itself. This can be seen in Fig. 2A and B showing hybridization in sections of a 7½ days p.c. embryo where the transcripts of c-kit localize in the middle of the maternal placenta. Essentially the same picture was obtained in 6½ days old embryos (not shown). Another receptor tyrosine kinase, c-fms, is also expressed in the maternal placenta; however, transcripts of c-fms tend to surround the embryo and, in contrast to c-kit, they are not expressed in the embryo proper even at later stages of development (Regenstreif and Rossant, 1989).

In our studies, c-kit expression could be first detected in the embryo at 8½ days p.c. At this stage, the transcripts were present in elements of the embryonic placenta and in the embryo itself. A crescent-shaped signal, well separated from the maternal placenta in Fig. 2D represents c-kit expression in the choioallantoic placenta, which is of embryonic origin (Hogan et al. 1986). The signal in the yolk sac, however, was less clear at this stage; thus no correlation could be made with the previously mentioned teratocarcinoma differentiation data. Fig. 2C through F demonstrate that, at 8½ days of embryonic development, c-kit transcription also appears in multiple tissues that later contribute to the mature body. Transcripts are detectable in the neural tube, in the otic vesicle, in the endothelium of the foregut and also in or under the surface of the mandibular arch, but not in the developing heart (Fig. 2E).

To investigate further the expression of c-kit in early embryogenesis, coronal and parasagittal sections were prepared from 8½ and 9 days old embryos. Fig. 3 demonstrates that c-kit activity in the neural tube is restricted to specific areas. Hybridization in the midbrain region, but not in the hindbrain, is visible in these sections (Fig. 3 A–F). As in the previous figure (Fig. 2), Fig. 3C–F shows that the gene is expressed in the surface ectoderm of the head corresponding to the first two branchial arches. Branchial-arch-associated c-kit activity is also demonstrated in Fig. 3 G and H, where hybridization in the surface ectoderm surrounding the cross-sectioned branchial arches is apparent. In this parasagittal section of a 8½ days old embryo, a row of somites is visible (Fig. 3G), which, however, display no detectable c-kit expression.

A similar pattern of c-kit expression was seen in 9½ days old embryos (Fig. 4). Here also the surface ectoderm of the branchial arches and in addition the endoderm of the branchial pouches accumulated transcripts (Fig. 4 A–E). It is noteworthy that this expression concentrates at the posterior surface of the branchial pouches. c-kit transcripts are also evident on the ventrolateral surface of the frontal bulge of the head, which probably represents c-kit expression in the developing olfactory epithelium.

At these stages, no c-kit expression could be detected in the heart (Fig. 4 B and G) or in the somites. Other mesodermal structures, however, like the somatic and the splanchnic mesoderm surrounding the developing
aorta, are strongly labeled at this stage (Fig. 4 F-I). In addition weak hybridization signals are visible also above the forelimb bud at 9½ days p.c. (Fig. 4H and I).

The pathologically most significant defect in W mutant embryos, macrocytic anemia, is connected to the migration and proliferation of hematopoietic precursors, which derive from the extraembryonic mesoderm and first appear in the blood islands of the yolk sac and later, around day 11 of embryogenesis, in the liver (Russell, 1979). c-kit expression in the 9½ day p.c. yolk sac is demonstrated in Fig. 5. Most cells contain a moderate but significant amount of transcripts, as can be concluded from a comparison between the signals obtained with antisense (Fig. 5B) and sense (Fig. 5C) probes.

Taken together c-kit early after implantation is expressed in the maternal placenta, but not in tissues of embryonic origin. Significant amounts of transcripts first appeared in the embryo at 8½ days p.c. and then already at multiple sites. The gene was expressed in special areas of the neuroectoderm, in the sensory ectoderm and in the surface ectoderm and endoderm...
associated with the branchial arches and pouches. The lateral plate mesoderm as well as the blood islands of the yolk sac also displayed transcripts hybridizing with the c-kit probe.

Expression of c-kit in 12½ days old embryos
At day 12½ the mouse embryo is at the peak of organ development. Sagittal (Fig. 6A–C), transversal (Fig. 6D and E) and parasagittal (Fig. 6F) sections were made. c-kit expression in the CNS is quite specialized at this stage. In addition to the hindbrain and the spinal cord, where c-kit was already expressed at 9½ days p.c. (Fig. 4G and I), transcripts became visible also in the marginal zone of the developing forebrain (Fig. 6B). Specific hybridization signals were detectable in the spinal cord (Fig. 6B, E and F). They accumulated mostly into the dorsal portion of the spinal cord, as is apparent in Fig. 6B and E. Another longitudinal structure expressing c-kit is the chain of dorsal ganglia along the spinal cord, as could be seen both in parasagittal (Fig. 6F) and transversal sections (Fig. 6E). Dorsal ganglia belong to the peripheral nervous system together with other ganglia, like the cranial ganglia, which also express c-kit (not shown) and derive from the neural crest (Le Douarin, 1982).

The largest mass expressing c-kit at this stage was the liver (Fig. 6B, E and F). The specificity of this expression was supported by hybridization with the sense strand (Fig. 6C). Hybridization signals in the liver appeared above the parenchyme. Much less specific
Fig. 4. Expression of c-kit in 94 days old embryos. (A–E) Parasagittal sections, (F and G) sagittal section; (H and I) transversal section. A,D,F,H, bright-field; B,E,G,I, dark-field; C, dark-field, hybridization with the sense transcript (control). Size bars: 200 μm. Abbreviations: bp, branchial pouches; cc, coelomic cavity; da, dorsal aorta; fg, foregut; fl, forelimb; gt, gut; ha, hyoid arch; hb, hind brain; hg, hindgut; ht, heart; ltp, lateral plate; ma, mandibular arch; mc, mesencephalon; op, olfactory placode; ov, otic vesicle; sc, spinal cord; so, somites; 3a, third visceral arch; bold arrow points to caudal neural groove.
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Fig. 5. Expression of c-kit in the blood islands of a 9½ days old embryo. (A) Phase contrast; (B and C) dark-field; C, hybridization with the sense transcript (control). Size bar: 200 μm.

signal was visible in more mature blood cells residing in the liver sinuses.

Another organ visualized by c-kit hybridization in 12½ days old embryos was the genital ridge. Sections shown in Fig. 6E and F include the genital ridge, which contains a high concentration of c-kit transcripts. In the same transversal section, weak, but probably significant, accumulation of c-kit transcripts is visible in the hindlimbs.

An additional major feature of our results was c-kit expression in the digestive tract. Cross-sections of the intestines and the stomach are visible in all sections shown in Fig. 6. Strong hybridization signals appear in the muscular layer and somewhat weaker in the endothelium of these organs. In addition, the endothelium of the mouth, pharynx, oesophagus, as well as the colon and the rectum also show c-kit expression (Fig. 6B). These signals most likely indicate c-kit expression in derivatives of the endoderm and in smooth muscle derivatives of the lateral mesoderm.

Taken together during organogenesis, c-kit is expressed in the embryonic hematopoietic system and in the developing germ line, derivatives of which are defective in various W mutants. Besides these, however, c-kit is also expressed in two organ systems in which no defects were reported in W mutants, the CNS and the digestive tract. No c-kit expression was detected in numerous mesodermal elements, like prevertebrae (Fig. 4B), the heart and large blood vessels or skeletal muscle.

c-kit expression in late organogenesis

This stage was studied in 14½ and 17½ days old embryos. No basic change took place in the distribution of transcripts at this period. In general, the expression pattern became more specialized. In the CNS, c-kit expression was detectable in the marginal layer of the telencephalon, in the hindbrain, in the primordium of the cerebellum (at day 17½) and in the dorsal part of the spinal cord. The intestines and liver remained positive, and hybridization signals could be detected also in other derivatives of the developing gut, like lungs and the pancreas (not shown). Fig. 7 demonstrates the expression of c-kit in various craniofacial and cutaneous structures of 14½ days p.c. embryos. In Fig. 7B, c-kit expression can be seen in the nasal epithelium and in the tooth bud of an upper incisor. The dental papilla and the odontoblasts, which derive from the neural crest, as well as the enamel-forming cells (ameloblasts), which are ectodermal derivatives, both express c-kit (Fig. 7B). Teeth are formed through interactions between the oral epithelium and the mesenchyme of the first branchial arch. It appears that c-kit is expressed in both tissues.

c-kit expression in the area of the pharynx and the mouth can be seen in Fig. 7C. Strong accumulation of transcripts takes place in the epithelium of the pharynx, in the thyroid duct, in the thymus, in structures surrounding the prospective thyroid cartilage, and in the intrinsic muscles of the tongue. The thymus derives from the branchial pouches, which already at 8½–9 days of gestation contained c-kit transcripts (Figs 3 and 4). Abundant transcripts in these organs at 14½ days p.c. may represent the specialization of the c-kit expression in the craniofacial area observed already at 8½–9½ days p.c. Thus it is possible that the activity of c-kit may be continuously required during the development of these organs.

Punctuate label was observed in subepidermal spaces mainly in the tail and in the nose already at 12½ days p.c. (see Figs 6B and 7B). This type of hybridization signal was most apparent in a parasagittal section of the buccal area of a 14½ days old embryo (Fig. 7F and G). High-power views of this and other sections revealed that this label derives from individual cells that accumulate exceptionally high levels of c-kit transcripts. They are most likely tissue mast cells and/or melanocyte precursors. Fig. 7E shows such cells in the subepidermal space.
of the face. Similar c-kit expressing cells were also seen in the root of sensory hairs in the snout, a localization characteristic for melanocytes (Fig. 7D). Expression of c-kit in subepidermal melanocytes, which are derivatives of the neural crest, connects this gene to the dominant white spotting trait, which gave the name to the W locus. Dominance of the pigment defect (see Silvers, 1979 for review) indicates that the gene dosage of c-kit/W may be important in coat colour patterning.

c-kit expression in the germ line

It has been already shown (Fig. 6) that c-kit is expressed in the genital ridge of 12½ days old embryos. Thus, it may be active in primordial germ cells. Genital ridges are mesodermal swellings in the roof of the abdominal cavity. Primordial germ cells, which are first detectable at 8 days p.c. in the root of the allantois, migrate between days 10 and 11 (p.c.) through the hindgut and the mesenterium to the genital ridges (Clark and Eddy, 1975; Hogan et al. 1986). Therefore the question arose whether the mesodermal cells of the genital ridge or the immigrant primordial germ cells were responsible for c-kit hybridization.

Primordial germ cells can be detected through their high alkaline phosphatase content. Fig. 8A and C shows the localization of primordial germ cells in the genital ridges as shown by this histo-chemical reaction. A comparison of high-power dark-field (Fig. 8B) and bright-field views (Fig. 8C) of two adjacent serial sections demonstrated that the alkaline phosphatase reac-

Fig. 6. c-kit expression in 12½ days (p.c.) old embryos. (A and D) Bright-field; (B,E,F) dark-field, antisense transcript hybridization; (C) dark-field; hybridization with the sense transcript (control). A–C sagittal, D and E transversal, F parasagittal section. Bars: 1 mm. Abbreviations: bl, bladder; bv, blood vessel filled with birefringent erythrocytes (not a hybridization signal); cr, colorectal junction; da, dorsal aorta; dg, dorsal ganglia; fb, forebrain; gr, genital ridge; hb, hindbrain; hl, hindlimb; ht, heart; in, intestine; lv, liver; mb, mandible; nc, neural canal; ph, pharynx; pv, prevertebrae; sc, spinal cord; st, stomach; ar, contamination.
Fig. 7. Localized expression of c-kit in 14½ days old embryos. (A) Bright-field; (B and C) enlarged dark-field views of A; (D and E) high-power views showing localization of silver grains in the epidermis of the nasal area of the section in F and G (their position is shown by small arrows). G is the dark-field view of F. Bars: A, F and G: 1 mm; B and C: 500 μm; D and E: 25 μm. Abbreviations: hr, hair root; ht, birefringent erythrocytes in heart; ls, lens; ne, nasal epithelium; ph, pharynx; sec, subepidermal cells; tb, tooth bud; th, thymus; to, tongue; ty, thyroid duct.
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Fig. 8. Expression of c-kit in primordial germ cells in the genital ridge (12½ days p.c.) (A) Transversal section, bright-field. The frame shows the area enlarged in B and C. (B) Dark-field, hybridization; (C) alkaline phosphatase reaction. Bars: A: 1 mm; B and C: 200 µm. Abbreviations: ms, mesonephros; sc, spinal cord. Arrows in B and C point to identical cells.

tivity and the c-kit hybridization signal colocalize in identical cells of the genital ridge. It follows that c-kit is expressed in cells of the developing germ line.

Adult gonads were also investigated (Fig. 9). Significant c-kit accumulation was detectable in the ovaries, both in primary and antral follicles of a 2 weeks old mouse, and in mature Graafian follicles from a 2 months old mouse (Fig. 9A–D). Highest concentrations were in the oocytes themselves. In contrast are our findings on c-kit expression in the testis. Fig. 9E and F show that c-kit is present in interstitial Leydig cells, but not in spermatagonia and Sertoli cells. Thus it appears that the gene is down-regulated in the adult male germ line in contrast to that of female.

Discussion

Transcripts of c-kit could be first detected in the developing embryo at the beginning of organogenesis at 8½ days p.c. They appeared in specific areas of the neural tube, in association with the branchial arches, in the sensory ectoderm, in the intestinal tract and in the lateral plate mesoderm. These diverse areas remained main sites of c-kit expression later in development. Specific areas of the mid- and hindbrain, as well as of the spinal cord, expressed the gene also at late gestation. Moreover, c-kit transcripts were detectable in the adult brain, specifically in the hippocampus and in the molecular layer of the cerebellum (unpublished). Similarly, c-kit expression in the sensory ectoderm, of 8½–9 days old embryos may be connected to its later expression in the olfactory epithelium at 14½ days of gestation. Such consistency was also observable in the expression of c-kit in the intestinal tract. The appearance of c-kit transcripts in the branchial arches at 8½–9½ days p.c. and in various craniofacial elements in late gestation also belongs to this category.

No c-kit expression was observed in the dorsal mesoderm and its derivatives, like the somites and the somitic system including bones and muscles. In contrast, the lateral plate mesoderm accumulated high concentrations of c-kit transcripts in early gestation, and later signals were detectable in the limbs (Fig. 4H, I, Fig. 6D, and unpublished).

Additional areas of c-kit expression included the hematopoietic system, represented by the blood islands of the yolk sac and the embryonic liver. Because c-kit hybridization in the yolk sac at 8½–9 days p.c. is not strong enough, we could not define the start of c-kit expression in this system.

Strong c-kit expression could be observed in the primordial germ cells of the genital ridges from 12½ days old embryos. We could detect primordial germ cells, as highly alkaline-phosphatase-positive cells in 8½ and 9½ days old embryos, both in the hindgut and in the mesentery. c-kit transcription, however, could not be unequivocally associated with these cells, because numerous other highly labeled cells were also present in these areas (see Fig. 4G and I). In contrast, c-kit transcripts were easily detectable in the mature germ line. Oocytes in both immature and mature ovarian follicles contained abundant c-kit transcripts, raising the possibility that c-kit may also function as a maternal message. In the adult male, however, c-kit was not expressed in the germ line. In the testis only the Leydig cells, not the spermatagonia, contained c-kit transcripts. Thus there may be a down-regulatory step in c-kit expression connected with testis determination.

High levels of c-kit expression were observed in individual cells dispersed in the subepidermal space and in the developing hair follicles of midgestation and older embryos. At least part of these cells are melanocyte precursors.

The various developmental systems and areas that displayed c-kit transcripts do not seem to be connected either by cell lineage or by spatial and anatomical units. This complex expression pattern is not unique for the c-kit gene. It has been mentioned in the introduction that the transcripts of several polypeptide growth factor
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Fig. 9. Expression of c-kit in adult ovaries and testis. (A and B) Bright-field and dark-field view of a young adult (two weeks old) ovary with numerous young antral and primary follicles; (C and D) high-power view of a mature follicle from a 2 months old mouse; (E and F) testis of a 4 months old male. Bars: A–D 100 μm; E and F: 0.5 mm. Abbreviations: af, antral follicles; Lc, Leydig cells; pf, primary follicles; sm, seminiferous tubules.

genes display similar developmental expression. Among receptor tyrosine kinases, the epidermal growth factor (EGF) receptor homologue of Drosophila (DER) (Schejter and Shilo, 1989) and a murine fibroblast growth factor (FGF) receptor, flg, (Safran et al. 1990) have similar complex, but specific patterns of developmental expression. Mutant analysis revealed that DER is active in follicle cell–oocyte interactions, as shown by the torpedo allele (Price et al. 1989), and that its flb alleles are required for the maternal dorsoventral gradient (Schejter and Shilo, 1989). The activity of DER is also required for the development of the fly’s eye via the Elp, dominant mutant allele (Baker and Rubin, 1989). Thus, seen through its mutant alleles, DER is characterized by complex developmental regulation. Our preliminary experiments with the murine flg gene indicate that this locus also has a complex pattern of developmental expression, but in contrast to c-kit, this murine receptor tyrosine kinase is expressed in the developing dorsal mesoderm, including somites, pre-
vertebrae, inter-vertebral discs, and long bones (Safran et al. 1990 and unpublished). It seems to be likely that the complex expression pattern of these genes could be regulated by various spatially specific transcriptional regulators.

The recent special interest in c-kit arose because of its connection to the W locus of mice (Chabot et al. 1988; Geissler et al. 1988; Nocka et al. 1989; Tan et al. 1990). The expression pattern reported here, especially the expression of c-kit in embryonic hemopoietic tissues, melanoblasts and primordial germ cells, is in good correlation with the W phenotypes. Several observations suggest that the pleiotropic W phenotype, which includes macrocytic anemia, sterility and white spotting, may be due to mutations in one gene. Geissler et al. (1981), describing the phenotypes of ten different new W alleles, emphasized that if they compared the effect of the loci by placing them into series from 'weak' to 'strong' alleles, the severity of all the parameters followed the same order. More recent RNA blotting experiments (Nocka et al. 1989) have suggested that c-kit is expressed in certain erythroid and pigment cell tumor lines, as well as in the ovary and the testis. Our data extend and support these results. Together they support the view that c-kit alone could be responsible for numerous, if not most, W phenotypes.

Not all aspects of c-kit expression in the mouse embryo can, however, be connected to the W phenotypes. Its strong expression in certain craniofacial structures, in the nervous system and in the digestive tract belong to this category. W alleles do not display craniofacial anomalies. A closely linked, related allele, Ph (Grunenberg and Truslove, 1960), however, is distinguished by defects in the neural tube and facial structures, in the nervous system and in the digestive tract. Craniofacial anomalies. A closely linked, related allele, Ph (Grunenberg and Truslove, 1960), however, is distinguished by defects in the neural tube and facial structures, in the nervous system and in the digestive tract. Craniofacial malignomas, as well as in the ovary and the testis. Our data extend and support these results. Together they support the view that c-kit alone could be responsible for numerous, if not most, W phenotypes.

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