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Repression of c-*fos* Transcription and an Altered Genetic Program in Senescent Human Fibroblasts

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Normal cells in culture invariably undergo senescence, whereby they cease proliferation after a finite number of doublings. Irreversible changes in gene expression occurred in senescent human fetal lung fibroblasts: a non–cell cycle–regulated mRNA was partially repressed; an unusual polyadenylated histone mRNA was expressed; although serum induced c-H-*ras*, c-*myc*, and ornithine decarboxylase mRNA normally, ornithine decarboxylase activity was deficient; and serum did not induce mRNA for a replication-dependent histone and for the c-*fos* proto-oncogene. The loss of c-*fos* inducibility was the result of a specific, transcriptional block. The results suggest that senescent fibroblasts were unable to proliferate because of, at least in part, selective repression of c-*fos*; moreover, the multiple changes in gene expression support the view that cellular senescence is a process of terminal differentiation.

A FUNDAMENTAL FEATURE OF NORmal cells is their limited ability to proliferate in culture. Cells capable of proliferation in vivo often go through an initial mitotic period in culture, but invariably there is a gradual decline in cell division; in cells from humans and certain other species, the decline is virtually irreversible and complete (*1*). This progression has been termed the finite life-span phenotype or cellular senescence.

Cellular senescence has been studied most extensively in cultures of human fibroblasts. Generally, human fibroblasts senesce after 20 to 60 population doublings (PDs). Because the PD at which senescence occurs depends inversely on the age of the tissue donor (*1, 2*), it has been suggested that, at the cellular level, senescence in culture reflects aging in vivo. Another view of senescence suggests that it constitutes a mechanism for curtailing tumorigenic transformation and may attenuate the establishment of metastases. Human cell cultures never spontaneously give rise to immortal variants (cells having an unlimited life-span in culture), whereas cells from several rodent and other species give rise to immortal cells at low but readily measurable frequencies. Relative to rodent fibroblasts, human fibroblasts are exceedingly resistant to transformation by chemical carcinogens, radiation, and oncogenic viruses (*3, 4*).

Senescence is not programmed cell death. Senescent fibroblasts remain viable for long periods (many months), during which they synthesize RNA and protein (*5, 7*). Early passage fibroblasts enter a reversible, proliferatively quiescent state (G_0) when deprived of growth factors; they resume proliferation when appropriate growth factors are resupplied (*8*). However, senescent cells cannot be stimulated to enter the S phase of the cell cycle by any combination of growth factors or physiological mitogens (*9*). This failure to synthesize DNA in response to mitogens is not due to a breakdown in growth factor signal transduction. Senescent fibroblasts possess apparently normal receptors for major mitogenic growth factors (*9, 10*), and serum growth factors fully induce the mRNAs for several growth factor–inducible genes (*6*). Several lines of evidence suggest that senescent human fibroblasts express one or more dominant inhibitors of proliferation (*7*) and that the finite life-span phenotype is dominant (*11*).

The link between senescence, aging, and cancer is not clear. Many studies have focused on the inability to proliferate and have viewed the senescent fibroblast as a special or extreme example of a quiescent cell. Within this context, one or more growth-related proto-oncogenes might be under dominant repression in senescent cells. However, quiescent and senescent fibroblasts showed similar basal and serum-inducible expression of the c-*myc* and c-H-*ras* proto-oncogenes (*6*), both of which stimulate proliferation and are required for the ability to leave G_0 (*12*). Because serum generally induces these genes in the early and midportions of the interval preceding DNA synthesis (the G_0/G_1 interval), it has been suggested that senescent fibroblasts arrest growth in late G_1 (*6, 9*). On the other hand, based on morphology and the pattern of proteins synthesized throughout the proliferative life-span, it has also been suggested that senescent fibroblasts have undergone terminal differentiation (*11*). The growth arrest therefore may be one manifestation of a more complex phenotypic change.

Here, we describe several differences in gene expression between quiescent and senescent fibroblasts. These include gene repression and novel gene induction that occur only in senescent cells. Most striking, c-*fos*, which in quiescent fibroblasts is induced early in the G_0/G_1 interval (*14*) and is essential for proliferation (*15*), is repressed at the level of transcription in senescent cells. Our results suggest that the growth arrest shown by senescent fibroblasts is distinct from growth-arrest states described for early passage cells, and c-*fos* repression may at least partially explain why senescent cells fail to proliferate.

To gain insight into the nature of cellular senescence, we examined the pattern of gene expression in human fibroblasts at the extremes of their proliferative life-span. Human fetal lung fibroblasts [strain WI-38 (*1*)] were used at early passage, when the cells had undergone <30 PDs and >70% (typically 75 to 85%) were capable of DNA synthesis, and at late passage (senescence) >48 PDs, when <10% (typically 4 to 8%) were capable of DNA synthesis (*16*). The cells were studied under three conditions in order to identify differences between the quiescent and senescent states. Subconfluent cells in 10% serum were considered exponentially growing; the proliferating fraction was >70% at early passage and <10% at late passage. Confluent cells were given 0.2% serum for 72 hours to generate quiescent cells; the proliferating fraction was <5% for both early- and late-passage cells. Finally, serum-deprived cells were stimulated with fresh 15% serum; >40% of the early-passage cells resumed DNA synthesis, whereas, in senescent cultures, DNA synthesis remained below 10%.

RNA was isolated from the cells and

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analyzed for the expression of c-*fos*, c-*myc*, and c-H-*ras* proto-oncogenes; ornithine decarboxylase (odc); actin; histone 3; and pHE-7. In quiescent fibroblasts, the c-*fos*, c-*myc*, c-H-*ras*, odc, and actin mRNAs are induced by growth factors a few minutes to a few hours after stimulation (*6, 14, 17*), whereas histone 3 mRNA is expressed only during S phase (*18*). The pHE-7 was cloned from a HeLa cell cDNA library and detects a prevalent, 1- to 1.5-kb mRNA whose translation product is unknown (*19*); in several cell systems, pHE-7 mRNA does not change with growth state or position in the cell cycle (*6, 19*).

Senescent cells expressed about ten-fold less pHE-7 mRNA than at early passage (Fig. 1). As expected, the level of pHE-7 mRNA did not differ whether the cells were in 10% serum or were serum-deprived, nor did the concentration change between 30 min and 28 hours after serum stimulation. The decline in pHE-7 mRNA in senescent cells was not due to a general decrease in mRNA expression (Fig. 1). The c-H-*ras* mRNA was present at nearly identical levels and was induced about threefold between 8 and 16 hours after stimulation at both early and late passage (*17*). Actin mRNA was slightly decreased in senescent cells, but only by about twofold. Finally, c-*myc* and odc mRNAs were present and inducible to similar extents in both cultures. Although the induction was slightly delayed in senescent cells, c-*myc* mRNA rose 15- to 20-fold within 2 hours of stimulation, whereas odc mRNA rose 5- to 7-fold within 8 hours, in essential agreement with Rittling *et al.* (*6*). Thus, senescent fibroblasts fully express at least four mRNAs and retain the signaling pathways for their induction by serum. Nonetheless, the constitutively expressed pHE-7 mRNA declined sharply when the cells became senescent.

Despite equal basal and induced levels of odc mRNA at early and late passage, odc enzyme activity was at least twenty-fold lower in senescent cells cultured in 10% serum; after serum deprivation and stimulation, the induced activity was two- to threefold lower (Table 1). Regardless of passage number, odc activity could not be increased by guanosine triphosphate (GTP) (Table 1), which in some cell lysates activates the enzyme (*20*). Recently, a disparity between odc mRNA and enzyme activity was shown in senescent IMR-90 cells, another human fetal fibroblast strain (*21*). The results suggest that, despite normal mRNA abundance, odc is down-regulated in senescent cells by a translational or post-translational mechanism.

In contrast to the near normal induction of c-H-*ras*, c-*myc*, and odc mRNA in senescent cells, there was no induction of the S phase–specific histone 3 mRNA. At early passage, this mRNA was regulated as expected (*18*). It was scarcely expressed in quiescent cells and increased >30-fold 16 to 24 hours after serum stimulation (Figs. 1 and 2A), coincident with S phase (*22*). During S phase, all four replication-dependent histones (H3, H2A, H2B, and H4) were synthesized at a high rate (Fig. 2B). In senescent cells, histone 3 mRNA was nearly undetectable, regardless of the culture conditions (Figs. 1 and 2A). Moreover, none of the replication-dependent histones were synthesized at a high rate at any time tested, although other nuclear proteins were synthesized at comparable rates at early and late passage (Fig. 2B). These results support the finding that DNA synthesis and histone expression remain coupled as neonatal human fibroblasts senesce (*23*) and suggest that, like pHE-7, the replication-dependent histone genes are repressed in senescent cells.

In senescent cells only, the murine histone–3.2 probe (which detects murine and human histone 3 variants) detected a slower migrating RNA, faintly detectable in total RNA. Its abundance did not change after serum deprivation or stimulation (Fig. 2A), and it was 5- to 20-fold less prevalent than replication-dependent histone 3 mRNA in early passage cells. It appeared to be closely related to the replication-dependent mRNA because the hybridization signal persisted

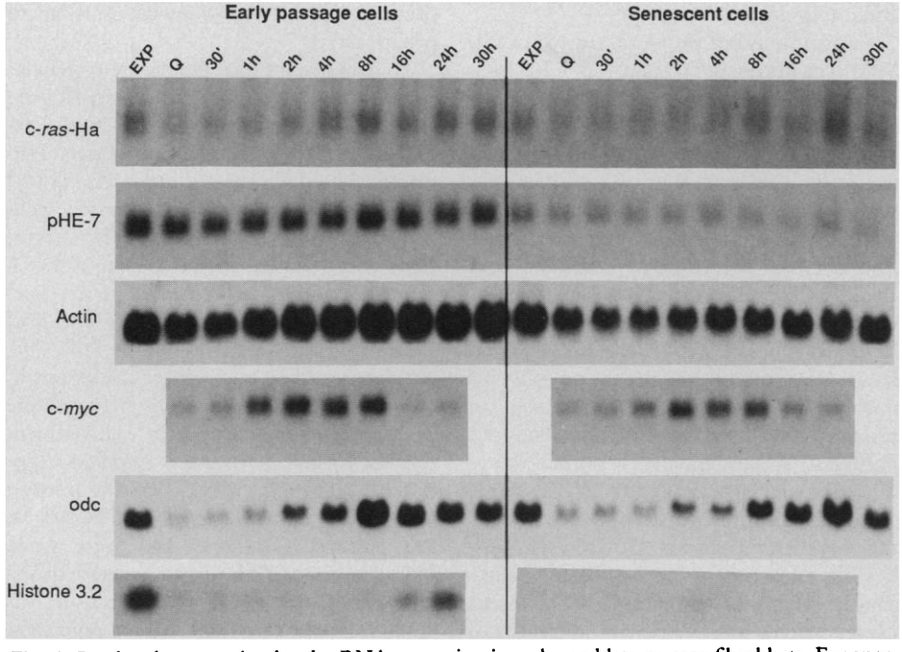

Fig. 1. Basal and serum-stimulated mRNA expression in early- and late-passage fibroblasts. Exponentially growing (EXP), serum-deprived (Q) and serum-stimulated [30 min to 30 hours (h)] WI-38 human fetal lung fibroblasts at early and late (senescent) passage were prepared as described in the text. Total cellular RNA was isolated from 5×10^6 to 1×10^7 cells on 150-mm dishes and analyzed by Northern blotting with DNA probes prepared by random priming or RNA probes prepared by in vitro transcription (*17, 25, 35–37*). Relative differences in mRNA abundance were quantitated by laser densitometry of autoradiograms of the Northern blots (*17, 36*).

Table 1. Activity of odc in early- and late-passage fibroblasts. Lysates were prepared from early- and late-passage cells and assayed for odc activity as described (*21*). When harvested, the cells were either subconfluent in 10% serum (Exponential), serum-deprived (Serum-deprived), or serum-stimulated after serum deprivation (Stimulated), as described in the text. Where indicated, 0.1 m*M* GTP was added to the lysate before addition of the substrate. Odc activity is expressed as picomoles of [^{14}C]CO_2 released from [^{14}C]ornithine per hour per milligram of protein. Three experiments with slightly different time points gave similar results. Shown are the data from one experiment; triplicate determinations varied by less than 10%.

Growth condition	Odc activity	+GTP
Early passage		
Exponential	45	42
Serum-deprived	1	1
Stimulated, 4 hours	2	1
Stimulated, 8 hours	141	146
Stimulated, 16 hours	131	130
Stimulated, 24 hours	7	6
Late passage		
Exponential	1	1
Serum-drprived	<1	<1
Stimulated, 4 hours	<1	<1
Stimulated, 8 hours	13	11
Stimulated, 16 hours	56	48
Stimulated, 24 hours	8	7

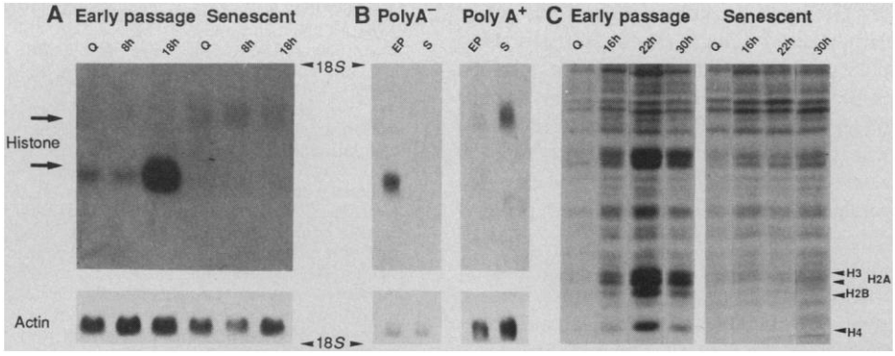

Fig. 2. Histone expression in early- and late-passage human fibroblasts. (**A**) Total RNA was isolated from serum-deprived (Q) or serum-stimulated (8 hours, 18 hours) early- and late- (senescent) passage WI-38 cells and analyzed for the abundance of histone 3 and actin mRNA as described in the legend to Fig. 1. The position of the 18*S* ribosomal RNA is indicated on the right side of the autoradiogram; the positions of the histone variant mRNA (upper arrow) and the replication-dependent histone mRNA (lower arrow) are indicated on the left. (**B**) Total RNA was isolated from early-passage (EP) and senescent (S) cells that had been serum-deprived and then stimulated with serum for 18 hours. RNA (200 μg) was separated by oligo(dT) affinity chromatography (*38*) into a polyA$^+$ and a polyA$^-$ fraction. All of the poly(A)$^+$ RNA and 10 μg of poly(A)$^-$ RNA were analyzed for histone and actin mRNA. (**C**) Serum-deprived (Q) or serum-stimulated (16, 22, and 30 hours) early-passage and senescent cells (1×10^6) on 60-mm dishes were labeled for 2 hours with 5 mCi/ml [^{14}C]lysine (270 mCi/mmol) in lysine-free medium containing 10% dialyzed serum where appropriate. The cells were lysed and acid-soluble nuclear proteins were extracted and analyzed by polyacrylamide gel electrophoresis and fluorography onto preflashed film (Kodak XAR), and the histone protein bands were assigned, as described (*39*); 4×10^5 cpm were loaded in each lane.

after a higher stringency wash [0.5× standard saline citrate (SSC) and 0.1% SDS at 65°C] and was lost only when hybridization to the replication-dependent mRNA was also lost (0.1× SSC and 0.1% SDS at 65°C). In contrast to the replication-dependent mRNA, the slow migrating RNA was polyadenylated [poly(A)$^+$], as judged by its complete retention on an oligo(dT) column and absence from the poly(A)$^-$ RNA fraction (Fig. 2C). Thus, a histone 3–related mRNA was expressed uniquely by senescent cells, and it had properties of a replacement or variant histone mRNA, of which several have been described (*24*).

The most striking change shown by senescent cells was a suppression of c-*fos* expression (Fig. 3). At early passage, c-*fos* mRNA was nearly undetectable in quiescent cells, and serum stimulation caused a large (>20-fold), rapid (within 30 min), transient (undetectable within 2 hours) induction (Fig. 3A), as reported for many fibroblasts (*14, 25*). At late passage, by contrast, c-*fos* was barely induced: the peak in mRNA was at least ten-fold lower than the peak at early passage (Fig. 3A). The low level of induced c-*fos* mRNA in senescent cells was again undetectable within 2 hours (Fig. 3A) and remained so up to 24 hours after stimulation. Thus, in contrast to c-*myc*, c-H-*ras*, and odc mRNA, serum-inducible c-*fos* expression was markedly suppressed in senescent cells (Fig. 1).

Nuclear run-on assays showed that the suppression was due to a transcriptional mechanism. Serum-inducible c-*fos* transcription was about ten-fold lower in senescent cells, relative to early passage cells (Fig. 3B). In contrast, serum-inducible actin transcription was less than twofold lower in senescent cells. In fibroblasts, serum stimulation often coordinately induces actin and c-*fos* transcription, and the actin and c-*fos* promoters contain similar regulatory sequences (serum response elements, SRE) that confer serum inducibility and that bind the same cellular factor (serum response factor, SRF) (*26*). Our results suggest that the c-*fos* gene is under specific transcriptional repression in senescent cells.

Paulsson *et al.* (*27*) reported that platelet-derived growth factor (PDGF) induced c-*fos* mRNA in senescent neonatal human fibroblasts. We have not used PDGF, but serum contains substantial quantities of this growth factor (*8*). The low induction of c-*fos* in senescent cells (Fig. 3) could be misleading if early- and late-passage RNAs are not probed on the same Northern (RNA) blot. This low induction may derive from the few proliferating cells in the population, an idea that is testable by in situ hybridization. Finally, although our preliminary data suggest that c-*fos* repression is not confined to senescent human fetal lung fibroblasts, cells from different sites or donor ages may behave differently.

Our results indicate that senescent fibroblasts do not behave like quiescent fibroblasts that are arrested at any point in the G_0/G_1 interval. First, in senescent cells there was repression of at least two temporally separated, growth-related genes: one normally induced early in the G_0/G_1 interval (c-*fos*) and one normally induced at the G_1/S boundary (histone 3). Second, a non–growth-related gene (pHE-7) was repressed in senescent cells. Third, a unique poly(A)$^+$ histone mRNA was expressed only in senescent cells and not in quiescent early-passage cells. Fourth, odc activity, but not the mRNA, was depressed in senescent cells. In IMR-90 fibroblasts, the discrepancy be-

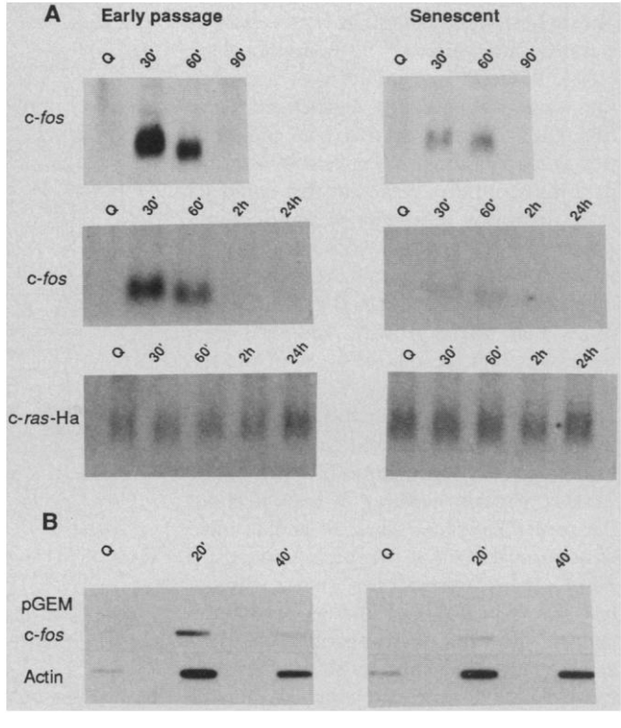

Fig. 3. Expression of c-*fos* in early- and late-passage fibroblasts. (**A**) Total RNA was isolated from serum-deprived (Q) or serum-stimulated (30 min, 60 min, 90 min, 2 hours, and 24 hours) early- and late- (senescent) passage cells and analyzed for the abundance of c-*fos* and c-H-*ras* mRNA as described in the legend to Fig. 1. (**B**) Nuclei were isolated from 5×10^6 to 1×10^7 serum-deprived (Q) or serum-stimulated (20 min, 40 min) early- and late-passage cells, labeled with [^{32}P]UTP and analyzed for run-off transcripts hybridizable to cloned human c-*fos* (*35–37*) or rat actin sequences, essentially as previously described (*36*). The pGEM cloning vector serves as a control for nonspecific hybridization. Signals were quantitated by laser densitometry.

tween odc mRNA and activity was caused by a decrease in mRNA translation (*21*), and it has been reported that senescent cells express a unique translational elongation factor (*28*). Thus, senescent cells may acquire a modified translational machinery that affects the synthesis of some but not all proteins (Fig. 2C).

Of the changes that we find in senescent cells, repression of c-*fos* transcription is the only one that is a good candidate for being causative in the irreversible growth arrest. Several studies show that inhibition of c-*fos* expression prevents the initiation of DNA synthesis by serum-stimulated fibroblasts (*15*). An exception has been noted in immortal rodent fibroblasts transformed by oncogenic *ras* (*29*). In these cells, PDGF stimulates proliferation, but does not induce c-*fos* mRNA. Thus, *ras* may abrogate the need for c-*fos* in proliferating immortal rodent fibroblasts. This is not the case in normal human fibroblasts. Senescent human fibroblasts do not enter S phase after microinjection of an oncogenic c-H-*ras*, nor do human fibroblasts transfected with this gene escape from senescence (*4, 30*). Immortalization may permit or entail mechanisms for bypassing the requirement for one or more growth regulatory gene product.

The block to c-*fos* transcription in senescent human fibroblasts was selective and had features of a dominant repression. First, several genes were induced normally, including actin, which, like c-*fos*, is regulated by the SRF. This suggests that senescent cells are not deficient in SRF or in the signals that activate it. Second, we find (*31*) that c-*fos* is not induced in senescent cells by phorbol esters, epidermal growth factor, or elevated adenosine 3′,5′-monophosphate (cAMP), agents that induce c-*fos* transcription through sequences distinct from the SRE (*32*). We suggest that c-*fos* transcription is blocked in senescent cells by a dominant transcriptional repressor that could act as a dominant inhibitor of proliferation. However, the genetics and frequency of immortality (*11*) suggest that there may be multiplicity and redundancy in the mechanisms that arrest growth, and thus c-*fos* repression may be one of a set of mechanisms.

We do not know whether or how the other senescence-associated changes in gene expression affect proliferation. In the case of pHE-7, virtually nothing is known about the protein. Hybrid selection and in vitro translation show that the mRNA encodes a 30-kD basic protein (*33*). In the case of histones, repression of the replication-dependent histones is compatible with the growth arrest, but unlikely to be a primary cause. These histones are generally induced only after cells are committed to and have nearly entered S phase (*8, 18, 24*). It is also unlikely that a variant histone can inhibit proliferation. In a number of cell types, poly(A)$^+$ histone mRNAs code for replacement histones and are expressed after terminal differentiation (*24*). Finally, the rate of protein synthesis can certainly affect proliferation (*8*), and there is evidence that a modest reduction in the synthesis of a few proteins can inhibit proliferation (*34*). Thus, altered translation could have a causative role in the inability of senescent cells to proliferate.

Our results indicate that senescent human fibroblasts express and regulate some genes in a manner typical of fibroblasts, but that they also show striking, irreversible changes in the expression of other genes, only some of which may have a role in the block to proliferation. We do not know whether or how these changes are related. However, the data indicate that senescent fibroblasts arrest growth in a state that is distinct from the growth-arrest states shown by early-passage cells. Therefore our data provide molecular evidence for the theory that cellular senescence is a process of terminal differentiation.

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The Dominant W^{42} *spotting* Phenotype Results from a Missense Mutation in the c-*kit* Receptor Kinase

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The murine *white spotting* locus (*W*) is allelic with the proto-oncogene c-*kit*, which encodes a transmembrane tyrosine protein kinase receptor for an unknown ligand. Mutations at the *W* locus affect various aspects of hematopoiesis and the proliferation and migration of primordial germ cells and melanoblasts during development to varying degrees of severity. The W^{42} mutation has a particularly severe effect in both the homozygous and the heterozygous states. The molecular basis of the W^{42} mutation was determined. The c-*kit* protein products in homozygous mutant mast cells were expressed normally but displayed a defective tyrosine kinase activity in vitro. Nucleotide sequence analysis of mutant complementary DNAs revealed a missense mutation that replaces aspartic acid with asparagine at position 790 in the c-*kit* protein product. Aspartic acid–790 is a conserved residue in all protein kinases. These results provide an explanation for the dominant nature of the W^{42} mutation and provide insight into the mechanism of c-*kit*–mediated signal transduction.

THE PROTO-ONCOGENE C-*kit* ENcodes a transmembrane tyrosine protein kinase receptor which belongs to a family of receptors that includes the receptors for platelet-derived growth factor (PDGF) and colony-stimulating factor–1 (CSF-1) (*1–3*). The c-*kit* gene has recently been determined to be allelic with the dominant *white spotting* locus (*W*) on chromosome 5 of the mouse (*4, 5*). Mutations at the *W* locus affect various aspects of hematopoiesis and the proliferation and migration of primordial germ cells and melanoblasts during development (*6–8*). A large number of independent mutations are known at the *W* locus which give rise to phenotypes that vary in severity in the heterozygous and the homozygous states (*6, 9*). These distinct *W* mutations provide the opportunity to characterize both the consequences of the mutations in different cell types and the molecular basis for the developmental defects that result from them. Mutations that affect the function of the c-*kit* receptor could facilitate the definition of structure-function relations of the c-*kit* receptor in vivo.

The W^{42} allele is a dominant mutation at the *W* locus with severe effects on pigmentation, gametogenesis, and hematopoiesis. Mice homozygous for the W^{42} allele die perinatally of macrocytic anemia (*9*). Mice heterozygous for the W^{42} allele are viable, although they have severe macrocytic anemia, lack virtually all coat pigment, and their gonads are reduced in size. We determined the molecular basis of the W^{42} mutation. In mast cells of homozygous mutant mice, the c-*kit* protein product was expressed normally but displayed a defective tyrosine kinase activity in vitro. Nucleotide sequence analysis of mutant cDNAs revealed a point mutation that resulted in the substitution of an evolutionarily conserved amino acid in the kinase domain of the c-*kit* protein product.

To investigate the c-*kit* protein products in homozygous W^{42} mutant animals, we cultured mast cells from the liver of 14-day-old fetuses, that were W^{42}/W^{42}, $W^{42}/+$, or $+/+$, obtained by breeding $W^{42}/+$ animals. We showed elsewhere (*8*) that mast cells express high levels of c-*kit*, and the *W* protein product is known to be essential for fibroblast-mediated proliferation and terminal differentiation of tissue culture mast cells in the absence of interleukin-3 (IL-3) (*10*). In order to identify homozygous embryos, we determined the proliferation potential of the fetal liver–derived mast cells. One fetus (No. 9) of ten in the first litter, four of nine in the second litter, and none of seven in a third litter appeared to be homozygous, as judged from the mast cell proliferation assay (Table 1). The positive proliferation numbers can be grouped into intermediate and high values. On the basis of the proliferation potential of mast cells from $W^{42}/+$ and from $+/+$ mice, the intermediate values were derived from heterozygous genotypes and the high values from normal $+/+$ genotypes, in agreement with the dominant nature of the W^{42} mutation (Table 1).

Table 1. Proliferation of mast cells on fibroblasts. The proliferation of mast cells, derived from the liver of individual fetuses of litters generated by mating $W^{42}/+ \times W^{42}/+$ mice and from the bone marrow of adult $W^{42}/+$ and $+/+$ mice, was determined after 7 days of coculture with Balb-3T3 cells in the absence of IL-3 (*20*). $+/+$ BM, normal bone marrow; Bgd, background value is the number of round cells observed in a well containing Balb-3T3 cells with no mast cells added.

Litter 1		Litter 2	
Fetus No.	Cells per field	Fetus No.	Cells per field
1	73	12*	3
2	210	13*	3
3	64	14*	4
5	167	15	35
7	167	16	37
8	194	17*	3
9*	6	18	118
10	89	$+/+$ BM	115
Bgd	6	Bgd	3
$W^{42}/+$ BM	107		
$+/+$ BM	300		
Bgd	3		

*W^{42}/W^{42} mast cells.

Our earlier work showed that c-*kit* RNA is expressed in liver, head, and placenta of 14- to 15-day-old embryos (*8*). In order to determine whether the W^{42} mutation affects c-*kit* RNA expression, we prepared RNA from the liver, the head, and the placenta of the ten fetuses in the first litter and subjected it to blot analysis. Equal amounts of c-*kit* RNA were detected in the head and the placenta of all fetuses as well as in homozygous mutant and normal fetal liver–derived mast cells; in contrast, a lower level of c-*kit* RNA was detected in the W^{42}/W^{42} liver (No. 9) when compared with the other liver samples (Figs. 1 and 2a). These results suggest that the W^{42} mutation does not affect c-*kit* RNA expression in the fetal head, in the placenta, and in mast cells. The reduced level of c-*kit* expression in the fetal liver is in agreement with our earlier conjecture that there is a reduction of the number

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