Functional Analysis of the Fibrinogen-Related scabrous Gene
From Drosophila melanogaster Identifies Potential Effector
and Stimulatory Protein Domains

E-Chiang Lee,*† Sung-Yun Yu,* Xiaoxi Hu,* Marek Mlodzik† and Nicholas E. Baker*†

*Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, New York 10461 and †Developmental Biology Program, European Molecular Biology Laboratory, 69117 Heidelberg, Germany

Manuscript received February 12, 1998
Accepted for publication June 18, 1998

ABSTRACT

The scabrous (sca) gene encodes a secreted dimeric glycoprotein with putative coiled-coil domains N-terminally and a C-terminal region related to the blood clot protein fibrinogen. Homozygous sca mutants have extra bristle organs and rough eyes. We describe a GAL4-based expression system for testing rescue of the sca mutant phenotype by altered SCA proteins and for misexpression. We find that deletion of the fibrinogen-related domain (FReD) greatly decreases SCA function, confirming the importance of this conserved region. SCA function could not be restored by FReDs from human fibrinogen chain genes. However, proteins lacking any FReD still showed some function in both rescue and misexpression experiments, suggesting that putative effector-binding regions lie outside this domain. Consistent with this, proteins expressing only the FReD had no rescuing activity but were recessive negative; i.e., they enhanced the phenotype of sca mutations but had no phenotype in the presence of a wild-type sca allele. This suggests that the FReD contributes to SCA function by binding to other components of the bristle determination pathway, increasing the activity of the linked N-terminal region.

SCABROUS (SCA) belongs to an expanding class of secreted proteins related in part to the blood clot protein fibrin (Baker et al. 1990). These proteins share a C-terminal fibrinogen-related domain (FReD), and they differ in their N-terminal regions, which often contain coiled coils (Doolittle 1992). In SCA, N-terminal apparent coiled coils are required for dimerization, and they are linked to the C-terminal FReD by a short proline-rich region (Hu et al. 1995; Lee et al. 1996). Other class members include fibrinogen itself, the extracellular matrix proteins of the tenasin group (Erickson 1993), all of which are hexameric, and the newly described angiopoietins, which are ligands interacting with tyrosine kinase receptors and are of unknown multimerism (Davis et al. 1996). An evolutionary tree constructed for FReD sequences implies a common ancestor for the various FReDs (Pan and Doolittle 1992). In none of these proteins is the specific function of the FReD known, despite its conservation and the medical importance of fibrin as the protein directly responsible for myocardial infarction and stroke (Doolittle 1994).

The sca gene is expressed during neural development in Drosophila (Mlodzik et al. 1990). Mutations in sca alter the number and positioning of peripheral sensory organs. Many genetic interactions have been documented between sca and mutations in the Notch pathway (Brand and Campos-Ortega 1990; Rabinow and Birchler 1990; Baker and Zitron 1995; Hu et al. 1995). The Notch gene (N) encodes a transmembrane receptor that restricts neural differentiation to subsets of cells within the competent proneural primordia (Artavanis-Tsakonas et al. 1995). Biochemical studies have yet to define any direct interaction between SCA and N proteins, so it is unclear whether SCA acts in the Notch pathway, or perhaps its function is to position neural differentiation in parallel to Notch (Lee and Baker 1996).

Molecular analysis of sca mutant alleles indicates that the FReD contributes to sca function in vivo. Amino acid substitution within the FReD or truncation before the FReD led to failure to complement sca null alleles (Hu et al. 1995). If fibrinogen genes evolved from protein domains present in invertebrate proteins, including SCA, there might be some functional connection between SCA and fibrinogen. Much of human fibrinogen is extravascular, and fibrinogen is required for cell growth during wound healing (Al-Mondhiry and Ehmann 1994; Suh et al. 1995). Roles have also been suggested in leukocyte adhesion at inflammation sites (Languino et al. 1993), in atherosclerosis (Bini and Kudryk 1995), and as a barrier to defense mechanisms against tumor growth (Dvorak 1986; Constantini and Zacharski 1992). It is an attractive hypothesis that fibrinogen functioned originally in wound healing and...
later adopted the role of limiting blood loss. The hypothesis gains support from the expression of FReD-containing tenasin proteins at wound sites (Erickson 1993) and the role of the FReD-containing angiopoietins in vascular development (Suri et al. 1996; Maisonnier et al. 1997). It is largely unknown whether the FReDs are important for these processes.

Previous genetic analysis could not establish the functional importance of the unconserved N-terminal region of SCA. Expression of the coiled-coil region only in a truncation allele had the negative effect of increasing bristle number more than sca null mutations. This negative property implies that the coiled-coil region must be capable of interfering with other components of bristle determination pathways and suggests three models for SCA (Hu et al. 1995). First, both coiled coil and FReD domains might bind independently to one or more receptors or other proteins to regulate neural differentiation. Second, the FReD domain alone might interact with a receptor, the negative effect of the truncation mutant being neomorphic and misleading about the normal function of the coiled-coil domain. Third, the coiled-coil domain might interact with a receptor, the FReD being an important regulatory domain. We sought to discriminate among these alternatives using Drosophila lines in which transposon insertions replaced endogenous sca expression with the expression of an inserted GAL4 gene, which could then drive the expression of diverse forms of SCA protein introduced into the genome under UAS control. This approach was made necessary by difficulty in identifying sca promoter sequences convenient for expression in the normal pattern (Rojewski 1993).

In this paper, we show that GAL4-driven expression of a sca cDNA rescues the sca mutant phenotype and use this system to assess the role of each sca domain. We find that the coiled-coil domain exhibits the same properties as full-length SCA in this assay (and in other misexpression assays) and infer that this N-terminal region must include a putative receptor-interacting site. Rescue is greatly enhanced by addition of the FReD domain, which is therefore important for the level of function. The sca FReD cannot be substituted by homologous FReD sequences from human fibrinogen chain genes. Expression of the FReD alone enhances the sca mutant phenotype, even in the absence of endogenous protein, implying that the FReD must bind to other proteins in the absence of the N domain or endogenous sca gene products.

MATERIALS AND METHODS

Drosophila strains: The mutations sca¹¹2, sca¹¹3, and sca¹¹4 were described previously (Hu et al. 1995). Other mutations and chromosomes are described in Lindsey and Zimm (1992).

Plasmid construction and transformation: Drosophila transformant flies were described previously (Ellis et al. 1994). For psca¹¹4d54N, the sca¹¹2 mini-gene was constructed in a three-way ligation of an 1.8-kb fragment (Xhol {102} EcoRI {732}) from the sca cDNA plasmid ps64 (Mlodzik et al. 1990), a 1.1-kb sca¹¹2 genomic DNA fragment [EcoRI (732) XbaI (6 codon at position +12 of Mlodzik et al. (1990))], and Xhol XbaI-digested Bluescript; the 2.9-kb minigene was excised and transferred to Xhol XbaI-digested pUAST. For psca¹¹1513-774, psca¹¹4d14, and psca¹¹4-541, deletion constructs described previously (Lee et al. 1996) were transferred to pUAST as XhoI XbaI fragments.

In each case, the indicated amino acids are predicted to be replaced by Gly Thr encoded by a KpnI site. These proteins were segregated by Sca73-1 cells (Lee et al. 1996). For psca¹¹2135K, the minigene described previously (Hu et al. 1995) was transferred to pUAST as an XhoI fragment. This protein was secreted by S2 cells (Hu et al. 1995). For the sca¹¹ fibrinogen chimeras, PCR was used to replace bases 1842 to seq. from the ps64 cDNA with a KpnI site, and this was inserted into the EcoRI site of pB5 (Stratagene, La Jolla, CA) to give pscaK512. Fibrinogen FReDs were amplified by PCR and cloned into KpnI XbaI-digested pscak512. For the a chain, we amplified 877 bp from the cDNA for R611 to nucleotide 3468 in the 3' UTR; positions were according to Fu et al. (1992). For the chain, 854 bp was amplified from the cDNA template (Chung et al. 1983b) from the cDNA for K209 to position 1558 in the 3' UTR. For the chain, 992 bp was amplified from the cDNA template (Chung et al. 1983a) from the cDNA for K151 to position 1600 in the 3' UTR. Each of the chimeric genes was then transferred to XhoI XbaI-digested pUAST.

Immunocytochemical analysis suggested that the sca-α and sca-γ proteins were secreted but that sca-β was arrested in the secretory pathway. The sca-β protein was inactive in initial misexpression experiments (data not shown). The β chain has a distinctive disulfide bond between C201 and C286, two residues that are not conserved in other FReD proteins (Doolittle 1992) and that are necessarily disrupted in the sca-β protein, from which C201 is absent. Site-directed mutagenesis (Clontech, Palo Alto, CA) was used to change β chain codon 286 from TGT to GCA, encoding an Ala residue and introducing a Stul restriction site. The sca-β(C286A) construct was used for the rescue experiments described in this article.

Expression studies: The arrangement of dorsal thoracic macrochaetes was recorded for each fly on a standardized diagram. Data from males and females were analyzed separately, although all constructs had similar effects on bristle numbers in each sex. The number of bristles varies from fly to fly, but the population distribution is reproducible for each genotype. This is most concisely represented by cumulative frequency graphs where y is equal to the proportion of the population bearing ≤x bristles.

Transformants on the third chromosome were convenient for rescue assays. Strains of the form w; cn sca¹¹2 Bc p[UAS-sca]/ T(2;3)SM5,TM6B were established for several independent transformants of each construct and crossed to w; sca¹¹2/Cyo at 25°C to obtain w; cn sca¹¹2 Bc/sca¹¹2; p[UAS-sca]/ + offspring. The control genotype w; cn sca¹¹2 Bc/sca¹¹2 was also prepared and scored in parallel for every experiment described, although the extra bristle distributions for these controls were indistinguishable from one experiment to another (compare control data in different experiments). w; sca¹¹2 T(2;3)SM5,TM6B siblings showed normal bristle patterning without exception.

The hairyGal4 (h¹¹) and dppGal4 driver lines have been described before (Brand and Perrimon 1993; Staehling-Hampton et al. 1994). The experiment using dppGal4 and shown in Figure 8 was performed at 29°C.
Plasmid rescue: The pGAWB insertion from sca\textsuperscript{73-1} was recovered along with flanking genomic DNA after digestion of genomic DNA with either Bgl II or Nde I, ligation, and transformation of Escherichia coli.

Immunocytochemistry: The mAbsca1 and scaR antibodies were used as described previously. mAbsca1 recognizes an epitope within the L41-Ser275 region. The scaR antiserum recognizes the W698-A774 region (Lee et al. 1996).

RESULTS

pGAWB insertional mutagenesis: The sca locus is a frequent target of P-element insertion (Mlodzik et al. 1990), and several pGAWB (Gal4 enhancer trap) insertions that map near sca and confer sca-like expression patterns have been identified (Y. Hiroi, personal communication; C. Klambt, personal communication; E. Giniger, personal communication). All complemented sca mutations (not shown). As P-element transposition favors reinsertion close to the site of excision (Tower et al. 1993), P elements were mobilized in the background of an insertion near sca, and the progeny were screened for novel sca mutant alleles to detect "local hops" that disrupted sca gene function. Two recessive sca alleles, designated sca\textsuperscript{73-1} and sca\textsuperscript{8-1}, were recovered that failed to complement sca mutations and expressed no SCA protein detectable in eye discs using antibodies (V. Wiersdorff and M. Mlodzik, unpublished results). When combined with UAS-LacZ reporter transgenes, β-galactosidase expression resembled that described for enhancer trap insertions in the sca gene promoter. These alleles provide the basis for functional assays of SCA protein function because they can drive Gal4-dependent gene expression in a similar pattern to the endogenous sca gene and inactivate endogenous sca function.

Functional assay for SCA protein function: We focused on the thoracic macrochaete pattern to assess SCA protein function. The number of bristles can be counted, and the severity of phenotypes can be readily quantified. As a result, partial rescue or even inhibitory effects are detectable. To facilitate comparison between genotypes, bristle numbers were summarized as cumulative frequency distributions (Figure 1). Graphs show the number of extra bristles plotted against the percentage of the population with at least this many extra bristles.

pGAWB insertional mutagenesis: The sca locus is a frequent target of P-element insertion (Mlodzik et al. 1990), and several pGAWB (Gal4 enhancer trap) insertions that map near sca and confer sca-like expression patterns have been identified (Y. Hiroi, personal communication; C. Klambt, personal communication; E. Giniger, personal communication). All complemented sca mutations (not shown). As P-element transposition favors reinsertion close to the site of excision (Tower et al. 1993), P elements were mobilized in the background of an insertion near sca, and the progeny were screened for novel sca mutant alleles to detect "local hops" that disrupted sca gene function. Two recessive sca alleles, designated sca\textsuperscript{73-1} and sca\textsuperscript{8-1}, were recovered that failed to complement sca mutations and expressed no SCA protein detectable in eye discs using antibodies (V. Wiersdorff and M. Mlodzik, unpublished results). When combined with UAS-LacZ reporter transgenes, β-galactosidase expression resembled that described for enhancer trap insertions in the sca gene promoter. These alleles provide the basis for functional assays of SCA protein function because they can drive Gal4-dependent gene expression in a similar pattern to the endogenous sca gene and inactivate endogenous sca function.

We present results using the Gal4 insertion allele sca\textsuperscript{73-1}. Similar results were obtained with another allele, sca\textsuperscript{8-1}. Various UAS transgenes were crossed into the sca\textsuperscript{73-1} background (often abbreviated scaG4) to assess transgene function. In comparison to sca\textsuperscript{73-1} sca\textsuperscript{8-1} flies, sca\textsuperscript{73-1} sca\textsuperscript{8-1}; UASsca\textsuperscript{73-1} + flies are much closer to wild type. Their eyes are less rough, and the number of bristles is reduced, often to the normal pattern (Figures 2 and 3). For comparison, sca\textsuperscript{73-1} sca\textsuperscript{8-1}; UASsca\textsuperscript{054N} + was used as a control. The D654N substitution is the same as that in the hypomorphic sca\textsuperscript{UN2} mutation. Distinct UASsca\textsuperscript{054N} insertion lines gave either no rescue or

Figure 1.—Numbers of extra bristles for sca mutant alleles. Numbers of extra thoracic bristles were recorded for different genotypes. Graphs represent the proportion of flies with x or fewer extra bristles. The null alleles sca\textsuperscript{BP2} and sca\textsuperscript{OB7} lead to similar bristle distributions. The hypomorphic mutation sca\textsuperscript{UM2} leads to fewer extra bristles. The sca\textsuperscript{MSKF} allele increases bristle number more than null alleles.
Figure 2.—Effect of transgenes on bristle number in the sca^{73} mutant. Shown are the sca^{73}/sca^{bp2} genotype (abbreviated scaG4) and sca^{73}/sca^{bp2}; UAS-sca/+ (abbreviated scaG4/UASsca). These two distributions hardly overlap at all, as the sca mutant phenotype is substantially rescued by expression of the UASsca transgene. Also shown are results with four independent insertions of a scaD654N transgene, the same protein that is encoded by the hypomorphic sca^{um2} allele. These show intermediate degrees of rescue, ranging from insignificant to about half that of the wild-type transgene. This indicates that SCA function in the rescue assay quantitatively parallels that of proteins encoded at the endogenous sca locus.

Figure 3.—Thoraces of flies expressing sca gene constructs. Similar results were obtained with male flies (data not shown). (A) Hemithorax from a typical female scaG4 fly. Arrows indicate three ectopic macrochaetae. (B) Hemithorax from a typical female scaGa/UASsca fly. There are no ectopic macrochaetae. (C) Hemithorax from a typical female scaG4/2 UASsca^{513-774} fly. Arrow points to one ectopic macrochaete. (D) Hemithorax from a typical female scaG4/2 UASsca^{41-514} fly. Arrows point to seven ectopic macrochaetae.

though the FReD was absent. The degree of rescue seen approached that of some scaD654N lines; i.e., it was about half as good as expressing one copy of the wild-type UASsca transgene (compare with Figure 2).

Complementary constructs encoding the C-terminal FReD were also expressed. Two proteins, sca^{41-463} and sca^{41-514}, differed in the presence or absence of a Pro-rich region of the sequence (residues 463–514). Expression of either sca^{41-463} or sca^{41-514} failed to rescue but, instead, increased the number of bristles over that seen in the sca^{73}/sca^{bp2} background (Figure 3). This effect was more severe when two copies of either transgene were present (Figure 5, B and C). Whereas 80% of sca^{73}/sca^{bp2} flies have eight or fewer extra bristles, 80% of sca^{73}/sca^{bp2}; UASsca^{41-514}/UASsca^{41-514} flies have eight or more, including striking individuals with as many as 14 extra thoracic macrochaetae.
Table 1. Proteins tested for rescue and ectopic expression. Shown are the predicted structure of the plasmids expressed in this study. Results are summarized on the right: +++++, strong effects; ++, moderate; +, weak; --, slightly increased bristle numbers; ---, moderately increased bristle numbers.

<table>
<thead>
<tr>
<th>GENE</th>
<th>STRUCTURE</th>
<th>RESCUE</th>
<th>MISEXPRESSION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-domain</td>
<td>FReD</td>
<td>dppG4</td>
</tr>
<tr>
<td>sca-αE</td>
<td></td>
<td></td>
<td>+++++</td>
</tr>
<tr>
<td>sca-βC286A</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>sca-γ</td>
<td>GT</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>sca-MSKF</td>
<td>RELQPATRTLRLPPDKHCYL</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 4.—Proteins tested for rescue and ectopic expression. Shown are the predicted structure of the plasmids expressed in this study. Results are summarized on the right: +++++, strong effects; ++, moderate; +, weak; --, slightly increased bristle numbers; ---, moderately increased bristle numbers.

To test whether effects of coiled-coil domain and FReD expression were independent, these domains were coexpressed. The individual effects canceled out, leading to bristle patterns similar to that of the sca<sup>73/1</sup> sca<sup>BP2</sup> flies (Figure 6D).

Replacement of FReDs from human proteins: The findings that the N-terminal coiled-coil region of SCA only partially rescued the sca mutant phenotype, whereas the FReD domain is required for full function, were consistent with previous characterization of sca mutations that identified a requirement for the FReD. Results for the sca-αE protein are shown in Figure 6A. Bristle numbers were reduced by about the same extent as by expression of the scaA513-774 protein that lacks the FReD domain. The sca-β(C286A) protein appeared to be intracellular and was inactive in initial experiments. The β chain normally has an intra-chain disulfide bond between C286 in the FReD and C201. As C201 was absent from the chimera with SCA, C286 would have no partner. In an effort to compensate for this, an Ala residue was substituted for C286 using site-directed mutagenesis, and this sca-β(C286A) gene was used for further experiments, although much of the sca-β(C286A) protein also seems to be intracellular (not shown).

Results for the sca-αE protein are shown in Figure 6A. Bristle numbers were reduced by about the same extent as by expression of the scaA513-774 protein that lacks the FReD domain. The sca-β(C286A) protein had similar effects. In contrast, the sca-γ protein did not rescue and may slightly increase bristle numbers (Figure 6B). These results show that none of the FReDs from the...
Figure 5—Bristle number after expression of individual SCA domains. (A) Fewer ectopic macrochaetae were observed as increasing numbers of sca\(_{513-774}\) transgenes were expressed. Females shown, and comparable results were obtained with males. (B) More ectopic bristles were observed as increasing numbers of sca\(_{41-514}\) transgenes were expressed. Males shown, and comparable results were obtained with females. (C) More ectopic bristles were observed after expression of sca\(_{41-463}\). (D) Coexpression of sca\(_{513-774}\) and sca\(_{41-514}\) leads to macrochaetae distributions similar to that of the scaG4 genotype.

human \(\alpha_E, \beta, \text{ or } \gamma\) chain genes can substitute for the sca FReD, although the sca-\(\alpha_E\), sca-\(\beta\) (C286A), and sca-\(\gamma\) proteins did not behave equivalently to one another.

**A minigene based on the sca\(_{\text{MSKF}}\) mutant allele:** In previous studies, SCA truncation caused by insertion of the transposable element hobo (sca\(_{\text{MSKF}}\)) led to a more extreme bristle phenotype than SCA null mutations, even though we now find that a truncated protein including similar N-terminal, coiled-coil domain sequences can supply partial sca function (Figure 1). To test whether negative properties of the sca\(_{\text{MSKF}}\) protein were associated with novel amino acid sequences encoded by hobo, a minigene encoding exactly the same protein as sca\(_{\text{MSKF}}\) was tested for rescue. The sca\(_{\text{MSKF}}\)-encoded protein did not enhance the sca\(_{73-1}\)/sca\(_{\text{BP2}}\) phenotype. In fact, multiple copies of a sca\(_{\text{MSKF}}\) transgene slightly reduced bristle number, although not to the same extent as sca\(_{513-774}\), sca-\(\alpha_E\), or sca-\(\beta\) (C286A) proteins (Figure 7). Therefore, negative properties of the sca\(_{\text{MSKF}}\) mutation were not intrinsic to the protein encoded by this allele and appeared to map outside of the coding sequence.

**Ectopic expression of SCA and derivatives:** Ectopic expression of SCA proteins using other Gal4 drivers led to phenotypic effects (Ellis et al. 1994). Expression of SCA using dppGAL4 to drive transcription in a stripe of cells along the anterior side of the compartment boundary in the developing wing resulted in nicked wings, even in a sca\(_{\text{BP2}}\) mutant background (Figure 8F). The same phenotype was seen on expression of the sca\(_{513-774}\), sca-\(\alpha_E\), sca-\(\beta\) (C286A), or sca\(_{\text{MSKF}}\) proteins, and to a lesser extent with sca-\(\gamma\) or sca\(_{\text{D654N}}\) proteins. Expression of sca-\(\beta\), sca\(_{41-463}\), or sca\(_{41-514}\) had no effect (Figure 8). Expression of sca using h\(_{13}\) (a Gal4 enhancer trap) to drive expression in the developing
Figure 6.—Flies expressing proteins containing fibrinogen sequences. (A) Fewer ectopic macrochaetae were observed as increasing amounts of sca-fbgα fusion protein were expressed. (B) Slightly fewer extra macrochaetae were observed after expression of a sca-fbgβ fusion protein, and slightly more were observed after expression of a sca-fbgγ fusion protein.

wing blade led to expansion of the wing veins in a characteristic “delta” shape. This effect also was seen with the scaα1513-774, scaα(E), scaβ(C286A), or scaMSKF proteins, to a lesser extent with scaγ or scaαβ64K proteins, and not with the scaβ, scaα141-443, or scaα141-514 proteins (Figure 9). In general, the effectiveness of different SCA proteins, when misexpressed, paralleled their ability to rescue the sca731 mutant phenotype (Figure 4).

Molecular characterization of the sca731 mutation:

During the course of these experiments, we realized that sca731/ scaBp2 flies tended to have more bristles than scaBp2 deficiency flies (Figure 10). To explore the basis of this difference from sca null alleles, genomic regions flanking the P element inserted in the sca731 chromosome were subcloned and the sequences were determined (Figure 11). The 3’ end of the P element was inserted after nucleotide –59, upstream of the sca transcription start site, but the 5’ end was inserted next to a previously uncharacterized sequence that was determined by Southern blotting experiments to lie within intron I. Southern blots confirmed that the first sca exon had been deleted from the sca731 genome. In addition, both 5’ and 3’ ends of the P element were mutated and rearranged, and the 8-bp duplication normally found at P-element insertion sites was absent (Figure 11).

Southern blot analysis of the phenotypically similar scaBp2 allele indicated that it was a similar insertion in the sca upstream region associated with a deletion of the first exon.

Although no SCA protein had been detected in eye discs from sca731 mutant larvae, we find low-level expression of proteins in an abnormal pattern in antennal discs using antisera specific for the C-terminal part of SCA; no such proteins are detected by N-terminal antibodies (data not shown). This indicates that either a readthrough P-element transcript or cryptic transcription start site permits some expression of a protein lacking the N terminus, in an abnormal pattern, and may explain the difference in bristle numbers from sca null alleles. This is unlikely to compromise the interpretation of the rescue assay (see discussion).

DISCUSSION

Previous characterization of mutant alleles of sca suggested that each of its constituent domains might inter-
act with other proteins (Hu et al. 1995). The FReD was found to be essential for proper SCA function because its mutation or deletion led to loss of function. This was important because similar FReDs are found in other proteins, including fibrinogen, the precursor to blood clots, but little is known of their specific roles. Three possible roles for the FReD in SCA function were considered. First, both the FReD and N-terminal region might contact other proteins to effect SCA function. Second, the FReD might be the major functional domain that is attached to the N domain for structural reasons. Third, the N domain might be the effector domain, with the FReD playing a supporting or regulatory role.

Our present data favor the third model, in which the FReD supports or regulates activities of the N domain. The N-terminal region of SCA, which lacks the FReD completely, can partially rescue the sca mutant phenotype, as measured by thoracic bristle number. This indicates that sequences capable of mediating sca gene functions, including possible interaction with a hypothesized but unidentified receptor, must lie in the N domain. Because every function observed for wild-type SCA constructs was shown by the N domain to some degree, whereas none were mediated by the FReD alone, the FReD most likely serves to enhance, regulate, or localize N-domain function rather than to perform supplementary functions. The FReD must also be covalently linked to the N domain; coexpression of the FReD as a separate peptide did not enhance rescue by the N domain (Figure 5D).

A potential caveat to the conclusion that the N domain mediates SCA functions might come from the sca73-1 allele used in the rescue assay, which may encode some protein containing FReD sequences despite deleting the first exon of the gene (Figures 11). However, it is unlikely that this alters the results. Positive effects of N-domain expression more than overcame the negative effects of sca73-1 (Figure 5A), and N-domain function was demonstrated after misexpression in null alleles that expressed no protein from the endogenous locus (Figure 8F) and after using other Gal4 lines in tissues that did not express endogenous sca (Figure 9). A further
The argument is that the putative sca\textsubscript{731} protein must resemble the FReD-only sca\textsubscript{41-463} or sca\textsubscript{41-514} protein. Because these proteins did not enhance the function of coexpressed N domain (Figure 5, B and C), it is also unlikely that the sca\textsubscript{731} protein contributes to the observed N-domain function.

The FReD is clearly required for full sca activity, and, therefore, we conclude that it promotes activities that the N domain can perform alone to a lesser degree. Deleting the FReD, substituting Asn for Asp654, or replacement with FReDs from human fibrinogen chain genes all yielded defective proteins with less than half the wild-type activity, as judged from bristle numbers (Figures 2–5).

The role of the FReD in sca could not be performed by similar sequences from the human fibrinogen chain genes (Figure 6). The fibrinogen FReDs probably do not denature the chimeric proteins, which show partial function attributable to the N domain alone, enter the secretory pathway, and can be detectably taken up by other cells. It is possible that the function of each FReD is unique, e.g., if each specifically regulates the distinct N domains in their own proteins. Alternatively, they may be functionally similar, but the binding partners in Drosophila may be too divergent for the human proteins to recognize.

The FReD from wild-type SCA must be able to interact with some other gene product because expression of the FReD alone enhances the sca mutant phenotype, implying an interaction with other components of the bristle determination pathway. There is no formal evidence to prove that this reflects a normal interaction of the FReD as part of the wild-type protein. However, if absence of the N domain permitted neomorphic FReD interactions, these would also be expected to occur in a wild-type SCA background or after ectopic expression of FReD proteins. Instead, no effect is seen from expressing FReDs in other tissues, and bristle number is in-

![Graph](image-url)

**Figure 10.**—Flies expressing sca\textsuperscript{NKF} minigenes. Slightly fewer extra macrochaetae were observed after expression of sca\textsuperscript{NKF} minigenes. (A) Males. (B) Females.

![Sequence](image-url)

**Figure 11.**—Structure of the sca\textsuperscript{731} allele. Sequences of the pGAWB vector (Brand and Perrimon 1993) compared with genomic sequences determined for the insertion in sca\textsuperscript{731}. The 31-bp terminal repeats of the pGAWB P element are underlined. In sca\textsuperscript{731}, the pGAWB element is inserted after base pair-59 from the transcription start site. The terminal repeat, which is from the 3' end of pGAWB, is partially duplicated and contains inserted nucleotides (shaded). The 5' end of the pGAWB element is adjacent to previously unsequenced genomic DNA, determined by Southern blotting to derive from the first intron of the sca gene. The terminal repeat is partially deleted and replaced by a sequence that resembles an inverted copy of part of the 3' end. Deletion of the first sca exon was confirmed by Southern blotting.
creased only when in a sca mutant background. These findings suggest that the FReD interacts with other gene products whose functions are more important in a sca mutant background and, therefore, part of the same pathway of bristle patterning. Such interactions may well be the basis of the FReD contribution to wild-type SCA function. Because the FReDs are presumed to be structurally similar (Doolittle 1992; Pratt et al. 1997; Spraggion et al. 1997), it may now be possible to map the critical regions within them by replacing smaller regions between SCA and fibrinogen chains.

Multiple altered forms of SCA have now shown the intriguing property of enhancing the phenotype of sca null mutations. These include the hobo-truncated scaHsk protein (when expressed from the sca gene promoter but not in the GAL4/UAS system), the scaTsc allele (which must produce an N-terminally deleted protein), the FReD-containing sca41-463 and sca41-514 proteins, and, to a lesser degree, the chimera between sca and the fibrinogen γ-chain FReD. Some of these proteins, such as sca41-514 and scaHsk, share almost no sequence in common. All these behave like the more familiar class of dominant-negative mutations, except that they are recessive, having phenotypic effects only in the absence of the wild-type gene and, therefore, affecting other proteins. We describe them as “recessive negative.” At the present time, we cannot give a molecular explanation for recessive-negative properties, except that they may inhibit other proteins whose functions are redundant with wild-type SCA. The data suggest models in which SCA interacts with multiple proteins (or multiple sites on the same protein) using its different domains.

We thank Drs. D. Chung and G. Greininger for plasmids, V. Weis dorff for communicating unpublished results and fly strains, and Drs. S. Emmons and G. Greininger for comments on the manuscript. This work was supported by a grant from the American Heart Association (New York City Affiliate).

LITERATURE CITED


Suh, T. T., K. Holmbäck, N. J. Jensen, C. C. Daugherty, K. Small...


Communicating editor: R. S. Hawley