# SHORT COMMUNICATION

# Mouse Chromosomal Location of Three EGF Receptor Ligands: Amphiregulin (*Areg*), Betacellulin (*Btc*), and Heparin-Binding EGF (*Hegfl*)

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**The products of five distinct loci, *Egf*, *Tgfa*, *Areg*, *Btc*, and *Hegfl* act as ligands for the epidermal growth factor receptor. *Egf* and *Tgfa* have previously been mapped to mouse chromosomes 3 and 6, respectively, but the mouse chromosomal locations of *Areg*, *Btc*, and *Hegfl* have not been reported. Here, we show that *Areg* and *Btc* are tightly linked on mouse chromosome 5, while *Hegfl* maps to mouse chromosome 18. We also provide evidence that a putative sixth family member, *Sdgf*, is in fact a species variant of *Areg*. These results confirm and extend known relationships between mouse and human chromosomes, and they also provide new information regarding the evolution of this important gene family.** 

Five distinct gene products have been identified as ligands for the epidermal growth factor receptor (EGFR). These include EGF, transforming growth factor-alpha (TGF$\alpha$), amphiregulin, heparin-binding EGF (HB-EGF), and betacellulin (reviewed in Ref. 8). Another putative member, schwannoma-derived growth factor (SDGF) (7) is highly homologous to amphiregulin, suggesting that it may be a species variant. As a first step toward investigating the physiological roles of the different EGFR ligands, the mouse *Tgfa* gene was inactivated (10, 11). Although TGF$\alpha$ is expressed in a wide array of developing and adult tissues, TGF$\alpha$-deficient mice displayed only hair, hair follicle, and eye anomalies. Similar aberrations were previously reported with the waved-1 (*wa1*) and waved-2 (*wa2*) mutations, and *wa1* and *wa2* have been mapped to the vicinity of the *Tgfa* and *Egfr* genes on mouse chromosomes 6 and 11, respectively. Indeed, the *wa1* mutation was recently shown to be allelic with the *Tgfa* gene and to be associated with reduced expression of apparently normal TGF$\alpha$ mRNA and protein (10, 11). The *wa2* phenotype, on the other hand, results from a point mutation in the EGFR kinase domain (9). Collectively, these results support a role for TGF$\alpha$/EGFR interactions in normal skin and eye development. To determine whether the remaining EGFR ligands might also be associated with known mutations, we have mapped their chromosomal locations.

The mouse chromosomal locations of amphiregulin, betacellulin, and HB-EGF were determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × *Mus spretus*)$F_1$ × C57BL/6J] mice (2; N. A. Jenkins and N. G. Copeland, unpublished results). A total of 205 $N_2$ progeny were obtained; a random subset of these $N_2$ mice was used to map the *Egfr* ligand loci. C57BL/6J and *M. spretus* DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphism (RFLPs), using cDNA probes corresponding to each locus (Table 1). In each case, the inheritance of *M. spretus*-specific alleles was followed in backcross mice since C57BL/6J-specific polymorphisms could be typed only by hybridization intensity. The strain distribution pattern of each RFLP was then determined and used to position the loci on the interspecific backcross map (Fig. 1).

Two loci, *Areg* and *Btc*, cosegregated and mapped to the middle region of chromosome 5, while *Hegfl* mapped to the proximal region of chromosome 18. Using a full-length SDGF cDNA probe (7), *Sdgf* was shown to cosegregate with *Areg* in 183 backcross mice typed in com-



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**TABLE 1**

***Egfr* Ligands Mapped in Interspecific Backcross Mice**

| Locus | Probe[b] | Enzyme | Fragment sizes (kb)[a] | |
|---|---|---|---|---|
| | | | C57BL/6J | *M. spretus* |
| *Areg* | ~900-bp *Xba*I fragment of mouse cDNA | *Puu*II | 3.8, 2.5, 2.3 | 6.3, 2.4 |
| *Btc* | ~660-bp *Eco*RI fragment of mouse cDNA | *Bgl*II | 11.5, 4.5, 4.2 | 15.0, 4.2, 3.3 |
| *Hegfl* | 1.7-kb *Eco*RI/*Kpn*I fragment of mouse cDNA | *Bam*HI | 7.3, 5.4 | 9.6, 5.6 |

[a] The restriction fragments followed in backcross analysis are underlined.
[b] Probes for *Areg* and *Btc* spanned the entire open reading frame. The *Hegfl* probe corresponded to the cytoplasmic domain and the 3′ untranslated region.

mon, and the sizes of the restriction fragments hybridizing with the *Sdgf* and *Areg* probes were virtually identical (data not shown). These results strongly suggest that SDGF and amphiregulin are species variants derived from the same gene.

*Areg* and *Hegfl* have been previously localized to human chromosome 4q13–q21 and chromosome 5, respectively, while *Egf* and *Tgfa* map to human chromosomes 4q25 and 2p13. These locations are all consistent with the positions of these genes on mouse chromosomes (Fig. 1). The human *Btc* locus has not been mapped. However, the close linkage of *Btc* and *Areg* in mouse implies that it will also map to human chromosome 4q13–q21.

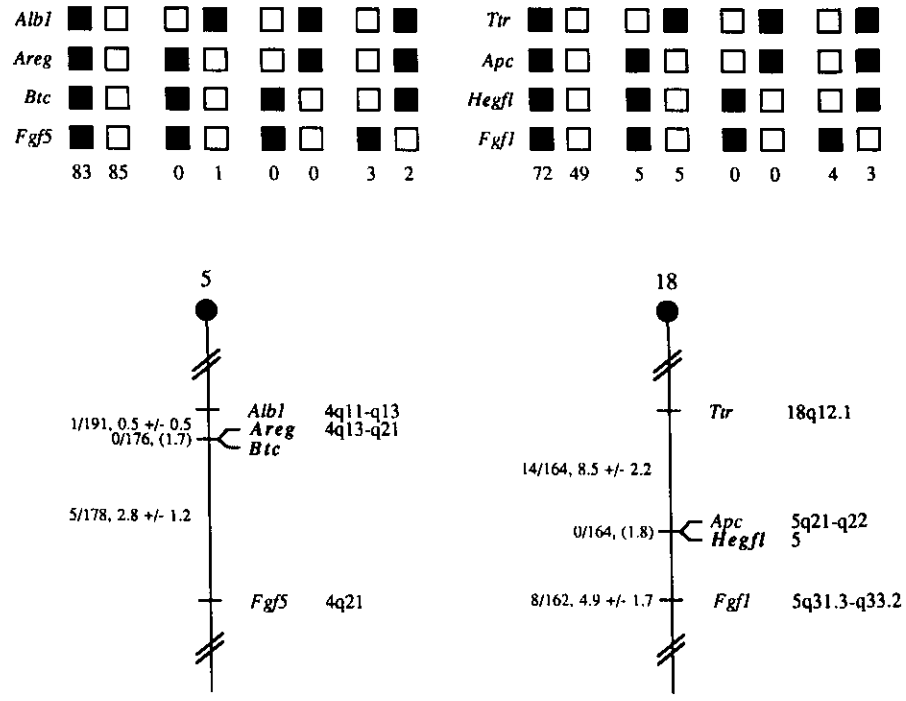


**FIG. 1.** Linkage maps showing the chromosomal locations of *Areg, Btc,* and *Hegfl* in mouse. The loci were mapped by interspecific backcross analysis. The probes and RFLPs for loci used to position the *Egfr* ligands in the interspecific backcross have been described. These loci include albumin 1 (*Alb1*) and fibroblast growth factor 5 (*Fgf5*) on chromosome 5 (1) and transthyretin (*Ttr,* formerly *Palb*), adenomatosis polyposis coli (*Apc*), and fibroblast growth factor 1 (*Fgf1*) on chromosome 18 (6). The segregation patterns of the *Egfr* ligands and flanking genes for animals typed for all loci are shown at the top of the figure. Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*)$F_1$ parent. The black boxes represent the presence of a C57BL/6J allele, and white boxes represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. Partial chromosome linkage maps showing the locations of the *Egfr* ligands in relation to linked loci is shown in the bottom panel of the figure. The number of recombinant $N_2$ animals over the total number of $N_2$ animals typed plus the recombination frequencies, expressed as genetic distance in centimorgans (± one standard error), is shown for each pair of loci to the left of the chromosome maps. Where no recombinants were found between loci, the upper 95% confidence limit of the recombination distance is given in parentheses. The positions of loci in human chromosomes, where known, are shown to the right of the maps. References for the map positions of human loci described in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, MD). Recombination distances were calculated as described (3) using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of recombination events to explain the allele distribution patterns.

The tight linkage between *Areg* and *Btc* in mouse suggests that they may have arisen through a tandem gene duplication event. The observation that the *Areg/Btc* gene cluster and *Hegfl* are each linked to a fibroblast growth factor gene (*Fgf5* and *Fgf1*, respectively) (Fig. 1) raises the additional possibility that they originated from a larger chromosomal duplication event. Consistent with this possibility are the findings that *Areg, Btc,* and *Hegfl* are similar with respect to both gene size and organization. Since *Hegfl* does not cosegregate with any known *Egfr* ligand, the chromosome duplication event may have preceded the tandem gene duplication.

Finally, we have compared our interspecific linkage map of chromosomes 5 and 18 with composite linkage maps that report the location of many uncloned mouse mutations (provided from Mouse Genome Database, a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME). Given the association between *Tgfa* and *wa1*, we were particularly interested in mutations affecting hair growth and/or texture. The comparison between maps revealed that a spontaneous recessive mutation, plucked (*pk*), maps near *Hegfl* on chromosome 18. Homozygous *pk* mice display a thick coat of irregular hairs as a result of disruption of the hair shaft in the follicle (summarized in Ref. 4). However, preliminary characterization did not reveal reduced expression of *Hegfl* mRNA in *pk* mice, nor were any mutations identified within the *Hegfl* coding region (data not shown). Hence, *pk* is unlikely to result from a defect in *Hegfl*. Another hair mutation, angora (*go*), maps near *Areg/Btc* on chromosome 5, but it was recently shown to be caused by a deletion of exon 1 in the *Fgf5* gene (5). Finally, another spontaneous recessive mutation affecting hair, marcel (*mc*), also maps near *Areg* and *Btc*. Homozygous *mc* mice have waved coats by 14 to 21 days of age, and mutant females are sterile (summarized in Ref. 4). The latter phenotype suggests that *mc* could be a good candidate for a mutation in *Areg* since *Areg* expression is highest in placenta and ovary (12). Unfortunately, the *mc* mutation is probably extinct. Hence, confirmation that *mc* and *Areg* share phenotypes in common awaits the outcome of *Areg* gene targeting experiments.

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