Dynamic expression of a glutamate decarboxylase gene in multiple non-neural tissues during mouse development

ABSTRACT
Some of the sites of Gad1 expression are tissues that emit signals required for patterning and differentiation (AER, vibrissal placodes). Other sites correspond to proliferating stem cell populations that give rise to multiple differentiated tissues (tail bud mesenchyme, pharyngeal endoderm and mesenchyme). The dynamic expression of Gad1 in such tissues suggests a wider role for GABA signaling in development than was previously appreciated.

INTRODUCTION
Background Glutamate decarboxylase (GAD) catalyzes the formation of the inhibitory neurotransmitter γ-amino butyric acid (GABA) from glutamate. In mammals, the two isoforms of this enzyme, GAD67 and GAD65, are expressed from two separate genes, Gad1 and Gad2 respectively. GABA signaling plays several roles in neuronal development. Early in CNS development, GABA can modulate neuron progenitor proliferation as well as neuron migration, survival and differentiation. In some classes of neural progenitors GABA stimulates these processes while in others it has an antagonistic activity. For example, recent work has demonstrated that GABA acts in the developing neocortex to stimulate the proliferation of progenitors in the ventricular zone while inhibiting the proliferation of progenitors in the subventricular zone. Later, during postnatal development, normal GABAergic input is required for activity-dependent plasticity in the visual cortex as shown in the Gad2 knockout mouse. In addition to these functions in the developing CNS, GABA signaling is also required for the normal development of non-neural tissues. Targeted mutations of the Gad1 gene lead to defective development of the secondary palate. The cleft palate phenotype of the Gad1 mutants suggests the involvement of GABA-mediated signals in the normal development and differentiation of a structure derived from the oral epithelium and neural crest ecto-mesenchyme. This conclusion is further supported by the similar cleft palate defect seen in mice with a deletion or targeted mutation in the β3 subunit of the GABAA receptor. This intriguing genetic evidence indicates a role for GABA-mediated signaling in the development of a non-neural structure, the secondary palate. The potential for this pathway to be involved in the early development of additional non-neural tissues has not yet been thoroughly explored. To address this question, we surveyed Gad1 transcript distribution in the non-CNS tissues of the embryo. Using a whole mount in situ hybridization approach, we found that Gad1 is indeed expressed in a number of different regions and tissues. A notable feature of this expression pattern is that Gad1 transcripts accumulate in the specialized ectodermal structures that are involved in the formation of the mystacial vibrissae and in limb outgrowth. These specialized ectodermal tissues are known to be sources of developmental signals. In addition, transcripts are expressed in the mesenchymal stem cell population of the tailbud and in the pharyngeal endoderm and mesenchyme. The expression patterns show that Gad1 is expressed in several non-CNS structures that are derived from each of the three germ layers of the embryo.

CONCLUSION
Conclusions The mouse gene encoding the 67 kDa isoform of glutamate

decarboxylase (Gad1) is expressed in the tail bud mesenchyme, vibrissal placodes, pharyngeal arches and pouches and the apical ectodermal ridge (AER), mesenchyme and ectoderm of the limb buds in mouse embryos from E9.0-E14.5. Some of the Gad1 expressing tissues (vibrissal placodes, AER) are known sources of developmental signals. Other sites of expression correspond to stem cell populations that give rise to multiple differentiated tissues (tail bud mesenchyme, pharyngeal endoderm and mesenchyme). The localized and dynamic expression pattern of Gad1 suggests a wider role for GAD and GABA in the development of non-neural tissues than was previously known.