

# Mouse *amnionless*, which is required for primitive streak assembly, mediates cell-surface localization and endocytic function of cubilin on visceral endoderm and kidney proximal tubules

Sharon Strope<sup>1,2</sup>, Roberta Rivi<sup>2</sup>, Thomas Metzger<sup>2</sup>, Katia Manova<sup>2</sup> and Elizabeth Lacy<sup>1,2,\*</sup>

<sup>1</sup>Molecular Biology Graduate Program, Weill Graduate School of Medical Sciences of Cornell University, New York, NY 10021, USA

<sup>2</sup>Developmental Biology Program, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10021, USA

\*Author for correspondence (e-mail: e-lacy@mskmail.mskcc.org)

Accepted 7 July 2004

Development 131, 4787-4795

Published by The Company of Biologists 2004

doi:10.1242/dev.01341

## Summary

Impaired primitive streak assembly in the mouse *amnionless* (*amn*) mutant results in the absence of non-axial trunk mesoderm, a derivative of the middle region of the primitive streak. In addition, the epiblast of *amn* mutants fails to increase significantly in size after E7.0, indicating that middle primitive streak assembly is mechanistically tied to the growth of the embryo during gastrulation. *Amn*, a novel transmembrane protein, is expressed exclusively in an extra-embryonic tissue, visceral endoderm (VE), during the early post-implantation stages. We show that *Amn* is also expressed in kidney proximal tubules (KPT) and intestinal epithelium, which, like the VE, are polarized epithelia specialized for resorption and secretion. To explore whether *Amn* participates in the development or function of KPT and intestinal epithelia and to gain insight into the function of *Amn* during gastrulation, we constructed *Amn*<sup>-/-</sup> ES cell ↔ +/+ blastocyst chimeras. While chimeras form anatomically normal kidneys and intestine, they exhibit variable, selective proteinuria, a sign of KPT malfunction. In humans, *AMN* has been genetically connected to Cubilin (*CUBN*), a multi-ligand scavenger receptor expressed by KPT, intestine and yolk sac. Loss of

*CUBN*, the intestinal intrinsic factor (IF)-vitamin B12 receptor, results in hereditary megaloblastic anemia (MGA1), owing to vitamin B12 malabsorption. The recent report of MGA1 families with mutations in *AMN* suggests that *AMN* functions in the same pathway as *CUBN*. We demonstrate that *Cubn* is not properly localized to the cell surface in *Amn*<sup>-/-</sup> tissues in the embryo and adult mouse, and that adult chimeras exhibit selective proteinuria of *Cubn* ligands. This study demonstrates that *Amn* is an essential component of the *Cubn* receptor complex *in vivo* and suggests that *Amn/Cubn* is required for endocytosis/transcytosis of one or more ligands in the VE during gastrulation to coordinate growth and patterning of the embryo. Furthermore, as *AMN* is apparently not required for gastrulation in humans, the developmental requirements for *Amn/Cubn* function may not be evolutionarily conserved, possibly reflecting differences between species in the role and organization of extra-embryonic tissues.

Key words: Amnionless, Visceral endoderm, Kidney proximal tubules, Cubilin, Gastrulation