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Not all lunatic fringe null female mice are infertile

A recent paper in *Development* by Hahn and colleagues reports that female mice homozygous for a targeted null mutation of the lunatic fringe (*Lfng*) gene are infertile (Hahn et al., 2005). In 1998, our laboratory and Randy Johnson's laboratory published independent papers on the construction and analysis of *Lfng* knockout mice (Evrard et al., 1998; Zhang and Gridley, 1998). The construct design of the targeted mutant alleles was very similar. In both alleles, the first coding exon of the *Lfng* gene was deleted, and embryos homozygous for either *Lfng* mutant allele exhibited severe defects in somite formation. Hahn and colleagues obtained *Lfng* mice from the Johnson laboratory for studies on oocyte development and fertility. They reported that $Lfng^{-/-}$ female mice did not mate, exhibited disorganized ovarian morphology and were all infertile.

In their paper, Hahn et al. state that we reported previously that the targeted *Lfng* mutation our group constructed 'results in complete embryonic lethality of $Lfng^{-/-}$ offspring'. Indeed, they give that as the reason they obtained the Johnson *Lfng* mice for their studies on folliculogenesis. However, we stated quite clearly, both in the initial and in subsequent publications on these mice, that a large number of our $Lfng^{-/-}$ null homozygotes are viable into adulthood (Zhang and Gridley, 1998; Zhang et al., 2000; Zhang et al., 2002).

In addition, contrary to the findings of Hahn et al., at least some of our female $Lfng^{-/-}$ null homozygotes are fertile. Although we generally maintain our *Lfng* line by backcrossing or intercrossing $Lfng^{+/-}$ heterozygous mice, in a number of instances in the past 5 years we have mated $Lfng^{-/-}$ female homozygotes. While not every breeding pair set up becomes productive, many of these matings have proven to be fertile. We have frequently used matings to $Lfng^{-/-}$ females to generate mice for double-mutant studies. For example, we have mated $Lfng^{-/-}$ females to male mice containing mutations in the *Notch1*, *Jag1*, *Dll3* and *Rfng* genes. In all of these cases, we had instances of productive matings in which $Lfng^{-/-}$ female homozygotes had multiple litters. Although we do not dispute that there is probably a role for Notch signaling in general, and the *Lfng* gene in particular, during ovarian follicle development in mice, the paper by Hahn et al. states that $Lfng^{-/-}$ female homozygotes are all infertile. Our results unequivocally demonstrate that, at least with respect to our *Lfng* mutant allele, this assertion is not true.

What then are the possible reasons for the discrepancy between the fertility of $Lfng^{-/-}$ female mice of the two groups? One possibility is that the two *Lfng* targeted alleles are functionally different. This is possible, but seems quite unlikely given the similar designs of the two *Lfng* alleles and the essentially identical somite defects exhibited by the null homozygotes (Evrard et al., 1998; Zhang and Gridley, 1998). We suggest that more likely possibilities include differences in genetic background, and/or differences in mouse husbandry and colony conditions. However, the assertions of Hahn et al. that mice homozygous for our *Lfng* targeted mutation cannot survive postnatally and that $Lfng^{-/-}$ female mice are obligatorily infertile are incorrect.

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RESPONSE

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A loss of lunatic fringe is associated with female infertility

The Wilson-Rawls laboratory was the first to describe the dynamic expression pattern of genes of the Notch signaling pathway in mammalian ovarian follicles (Johnson et al., 2001). In a recent report (Hahn et al., 2005), we described a completely penetrant infertility phenotype in female mice that are homozygous null for a lunatic fringe (*Lfng*) mutant allele, previously described by Evrard et al. (Evrard et al., 1998). Furthermore, we characterized defects in follicle development and meiotic maturation that correlate with a loss of expression of the Notch downstream target effector genes in follicles (Hahn et al., 2005). These data are consistent with our conclusion that Lfng and the Notch signaling pathway have an important role to play in ovarian folliculogenesis.

Xu and colleagues now report anecdotal evidence that some mice homozygous for a *Lfng* mutant allele ($Lfng^{lacZ}$) generated in their laboratory are fertile and were used occasionally in the generation of more $Lfng^{lacZ/lacZ}$ mutants, as well as for the generation of compound mutations of *Lfng* and other genes in the Notch pathway (Xu et al., 2006). We note that this breeding strategy is not described in papers published by the Gridley laboratory. In fact, where a breeding strategy was described, the authors have bred $Lfng^{lacZ/+}$ animals to generate homozygous $Lfng^{lacZ/lacZ}$ mutants (Zhang and Gridley, 1998; Zhang et al., 2002). However, Xu et al. concede that not all of the $Lfng^{lacZ/lacZ}$ matings are productive. In other words, consistent with our observations, a lack of lunatic fringe is

associated with infertility. These observations do not dispute our central conclusion that Lfng is important in ovarian folliculogenesis, but instead provide new information regarding the penetrance of the infertility phenotype in the two mutant alleles of *Lfng*.

Genetic mutations in mice are powerful tools for examining the contribution of specific genes to complex biological processes, such as folliculogenesis. Interpreting specific gene mutations can be confounded by variations in the penetrance of phenotypic defects in the same genetic background, as well as in different genetic backgrounds (Beck et al., 2000; Chia et al., 2005). Furthermore, variations in the severity of the phenotype can occur between mutant alleles generated in different laboratories. In the case of *Lfng*, mice homozygous for either mutant allele experience radical disruptions in the organization of the axial skeleton owing to a failure of the proper segmentation of the somites (Evrard et al., 1998; Zhang and Gridley, 1998). Despite similarities in their gross morphology, there was a greater loss of somite epithelialization in the $Lfng^{-/-}$ embryos when compared with the $Lfng^{lacZ/lacZ}$ embryos (Evrard et al., 1998; Zhang and Gridley, 1998). Perhaps it is not surprising that additional studies with these mice are revealing additional differences. Indeed, a careful analysis of fertility and fecundity rates in the $Lfng^{lacZ}$ mice would offer an excellent comparison and contrast to the strain of mice used in our studies. None of this, however, refutes our conclusion that Lfng and, by extension, Notch signaling are important regulators of folliculogenesis.

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