

Investigating the stage-specific expression of Dazl and Boule during fetal ovarian germ cell development

Introduction

The DAZ (deleted in azoospermia) gene family encodes mRNA binding proteins (DAZ, DAZL and BOULE) that are almost exclusively expressed in germ cells. Mutations in these genes lead to abnormal or failed germ cell development which is a key cause of infertility. The DAZ family proteins are believed to promote translation by binding to the poly-A tail of mRNA targets. Professor Anderson's lab at the QMRI, Royal Infirmary of Edinburgh, has been investigating the expression of DAZL and BOULE in human fetal ovary germ cells, to attempt to identify mRNA targets (targets have already been identified in mice but no definitive targets have been identified in humans) and in doing so have observed that DAZL and BOULE are expressed at different stages of meiosis. DAZL is expressed briefly at meiosis initiation, and is later identified in oocytes in the primordial follicles. BOULE was found to be expressed in germ cells undergoing meiosis but was not established in the primordial follicles. Furthermore, there was minimal co-expression of the RNA-binding proteins. These findings suggest that DAZL and BOULE may regulate different stages of meiosis in humans.

Aims

The aim of this project was to investigate if the pattern of expression previously mentioned is conserved between humans and mice.

Methods

Single and double immuno-staining were carried out, first in mouse testes and then mouse ovaries. As mouse gestation is only 20 days long, the stages of meiosis are markedly faster than they are in humans, so mouse ovaries collected over a range of gestations from 13.5 days to 20.5 days were to be examined (from early meiosis, to postnatal mouse primordial follicle formation). This project was focused on e18.5 ovaries.

Results

1. Dazl and Boule are expressed in adult mouse testis

DAB immunohistochemistry single staining was carried out on slides of WT mouse testes, three different concentrations (1/20, 1/50 and 1/100) for both Dazl and Boule to optimise (figure 1A/B). Dazl expressed in cells at an earlier stage and Boule expressed later on in the secondary spermatocytes.

Single and double fluorescence immunohistochemistry was also carried out on WT testes samples and they all coincided with the results in DAB staining. There was some co-expression of Dazl and Boule as shown in figure 1C.

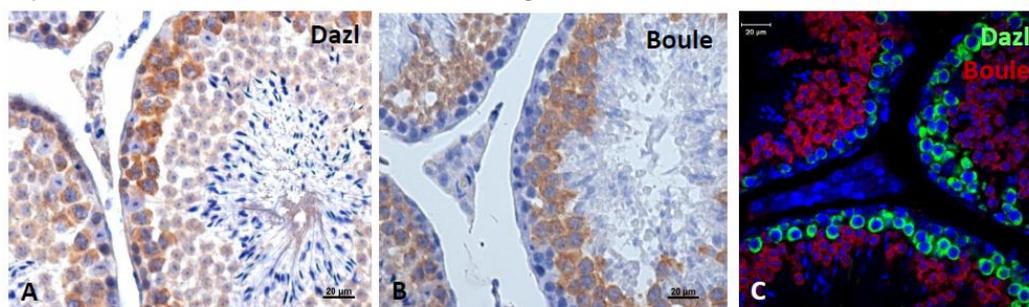


Fig. 1. **A)** Dazl single DAB IHC staining of WT mouse testis at concentration 1/50 40x magnification. The brown DAB stain is distributed around the edge of the testis tubule where the cells are less mature. **B)** Boule single DAB IHC staining of WT mouse testis at concentration 1/50 40x magnification. The brown DAB stain is distributed around the edge of the testis tubule, slightly further in than in the left image, suggesting Boule is expressed more centrally than Dazl, in more mature germ cells (spermatocytes). **C)** Tyramide enhanced fluorescence Dazl/Boule double staining of WT mouse testis, NBF fixed 40x magnification. Shows some overlap of Dazl and Boule suggesting co-expression.

2. Dazl, but not Boule is expressed in mouse fetal ovary

Single fluorescence immunohistochemistry staining of mouse ovaries at stage e18.5 was then carried out. There was GFP staining present in the Dazl slides but no staining in the Boule slides (see figure 2), perhaps signifying that Boule is expressed at other stages than 18.5 days in mouse ovaries or is not expressed in mouse fetal ovary.

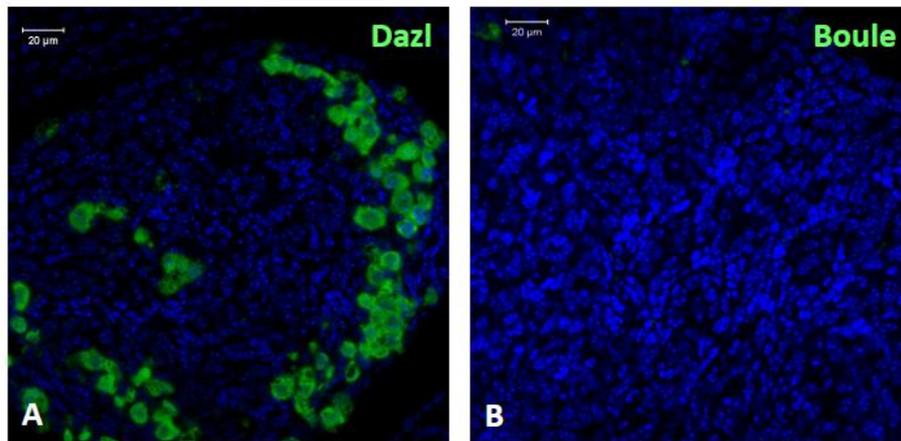


Fig. 2. **A)** Dazl staining in e18.5 mouse ovary, NBF fixed 40x magnification. Dazl is expressed in the packaging cells of the ovary. **B)** Boule staining in e18.5 mouse ovary, NBF fixed 40x magnification. There is no GFP stain for Boule suggesting there is no Boule present at 18.5 days in the mouse ovary.

3. Boule is not expressed in germ cells at diplotene stage in mouse fetal ovary

Lastly, Dazl/Msy2 and Boule/Msy2 double staining was carried out to confirm what stage the stained cells were at. Diplotene is the fourth stage of the prophase of meiosis, when the chromosomes separate into chromatids. Msy2 is a diplotene-specific gene.

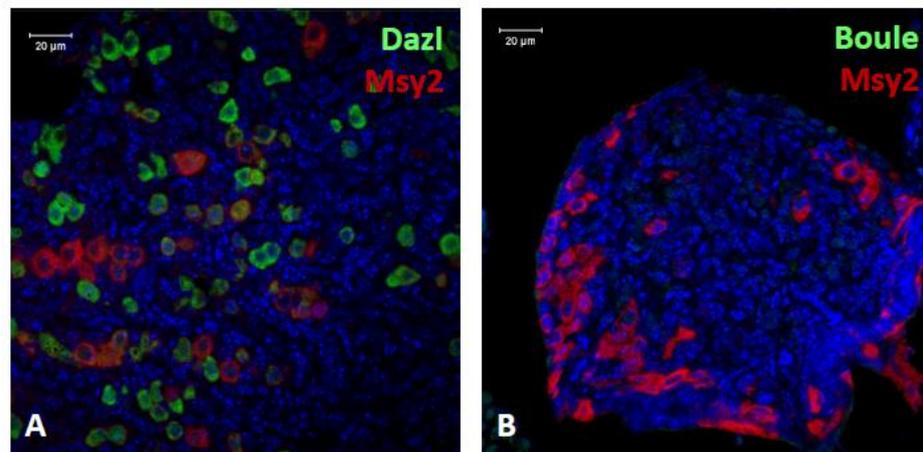


Fig.3 **A)** expression of Dazl at the diplotene stage of meiosis in the mouse fetal ovary is shown. 40x magnification **B)** No expression of Boule at the diplotenene stage of meiosis in the mouse fetal ovary. 40x magnification

Conclusion

These results have lead to the conclusion that Dazl is expressed in the slightly earlier stages of germ cell development in mouse testis than Boule. There is some overlap which may suggest that Boule could compensate for Dazl and vice versa. The pattern of DAZL/BOULE expression in human fetal ovaries may not be conserved in mice. Mouse ovaries at different stages of gestation must still be looked at in the same way as we looked at the 18.5 days stage, to see if Boule appears at a different stage. The results from this study were presented at the Cold Spring Harbor Germ Cell conference in October 2012.