stealing. There is a clear message for policymakers and police officers: Early disorder diagnosis and intervention are of vital importance when fighting the spread of disorder. Signs of inappropriate behavior like graffiti or broken windows lead to other inappropriate behavior (e.g., litter or stealing), which in turn results in the inhibition of other norms (i.e., a general weakening of the goal to act appropriately). So once disorder has spread, merely fixing the broken windows or removing the graffiti may not be sufficient anymore. An effective intervention should now address the goal to act appropriately on all fronts.

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Germ Cell–Intrinsic and –Extrinsic Factors Govern Meiotic Initiation in Mouse Embryos

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Retinoic acid (RA) is an essential extrinsic inducer of meiotic initiation in mammalian germ cells. However, RA acts too widely in mammalian development to account, by itself, for the cell-type and temporal specificity of meiotic initiation. We considered parallels to yeast, in which extrinsic and intrinsic factors combine to restrict meiotic initiation. We demonstrate that, in mouse embryos, extrinsic and intrinsic factors together regulate meiotic initiation. The mouse RNA-binding protein DAZL, which is expressed by postmigratory germ cells, is a key intrinsic factor, enabling those cells to initiate meiosis in response to RA. Within a brief developmental window, *Dazl*-expressing germ cells in both XX and XY embryos actively acquire the ability to interpret RA as a meiosis-inducing signal.

Diploid eukaryotes generate haploid cells via meiosis, a program of two successive cell divisions preceded by one round of DNA replication. The onset of this program is referred to as meiotic initiation. In mammals, debate has focused on whether meiotic initiation is promoted by factors extrinsic or intrinsic to germline cells (*1*–*6*). Meiotic initiation in female mice, commencing at embryonic day 12.5 (E12.5) (*7*, *8*), is induced by an extrinsic factor, retinoic acid (RA) (*8*–*10*), but RA alone cannot account for the exquisite temporal and cell-type specificity of meiotic initiation. Although diverse somatic cell types are exposed and respond to RA during mammalian development (*11*), meiotic initiation is limited to the germ line. Indeed, embryonic germ cells do not respond specifically to RA until their migration ends, at the developing gonad. Does meiotic initiation in mammals also require an intrinsic competence factor expressed in germ cells? Consider the yeast *Saccharomyces cereviseae*, in which meiosis is induced by a nutrient-depleted environment (*12*). For an *S. cereviseae* cell to be competent to initiate meiosis in response to this extrinsic cue, the cell must express the a/α mating-type heterodimer (*13*). We wondered whether an analogous interplay of extrinsic and intrinsic factors governs meiotic initiation in mammals.

We considered the possibility that the *Dazl* (*Deleted in azoospermia-like*) gene might be an intrinsic meiotic competence factor, given the location and timing of its expression. In both XX and XY mouse embryos, germ cells begin to express *Dazl* at about the time of their arrival at the gonad, between E10.5 and E11.5 (*14*). No somatic lineage has been shown to express *Dazl* (*15*). Furthermore, *Dazl*-deficient mice are infertile because of germ cell–differentiation defects (*16*–*19*). These defects are more consistent and pronounced in inbred C57BL/6 mice (*19*) than in noninbred mice (*16*–*18*). Accordingly, we analyzed *Dazl* function in inbred C57BL/6 animals.

We began by testing whether germ cells survive in *Dazl*-deficient embryonic ovaries as germ cells of *Dazl*-deficient C57BL/6 embryonic testes undergo apoptosis, beginning by E14.5 (*19*, *20*). We detected two germ cell markers—endogenous alkaline phosphatase (AP) activity (*21*) and mouse *vasa* homolog (MVH) protein (*22*)—in the ovaries of wild-type and *Dazl*-deficient embryos (fig. S1, A and B). We also found MVH protein in wild-type and *Dazl*-deficient neonatal ovaries (fig. S1C), which indicates that *Dazl*-deficient ovarian germ cells survive embryonic development (fig. S1, A and B) and persist through birth (fig. S1C).

We then compared the nuclear morphology of germ cells in wild-type and *Dazl*-deficient ovaries at E15.5. By this stage of development, many germ cell nuclei in wild-type ovaries exhibit the chromosome condensation that characterizes early meiotic prophase (Fig. 1A). By contrast, germ cells in *Dazl*-deficient ovaries do not display such condensation (Fig. 1A), which suggests that *Dazl* function might be required for meiotic prophase to occur. We then examined the expression of *Stra8*, which is required for premeiotic DNA replication and the subsequent events of meiotic prophase in germ cells of embryonic ovaries (*8*). As expected,

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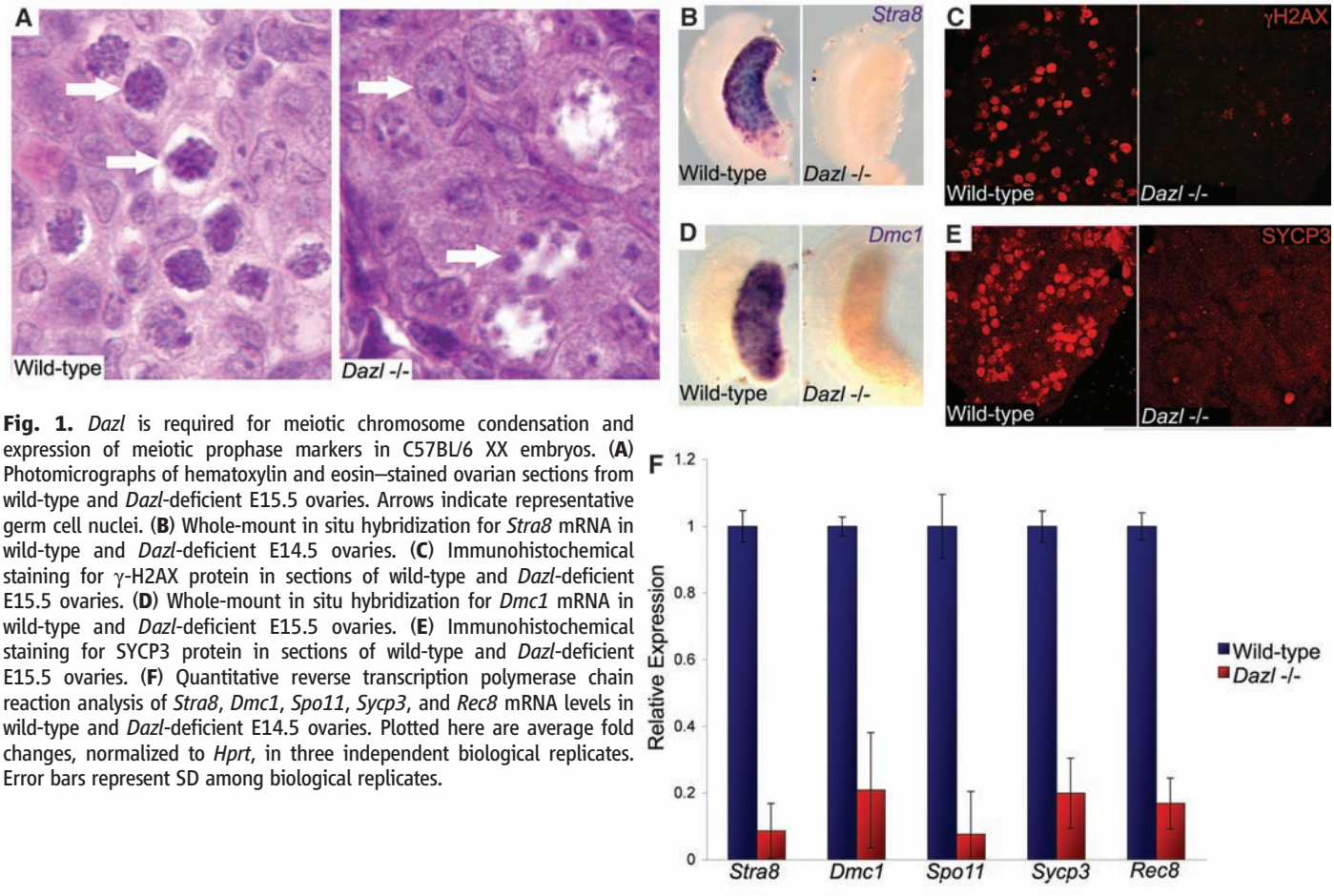

Fig. 1. *Dazl* is required for meiotic chromosome condensation and expression of meiotic prophase markers in C57BL/6 XX embryos. (**A**) Photomicrographs of hematoxylin and eosin–stained ovarian sections from wild-type and *Dazl*-deficient E15.5 ovaries. Arrows indicate representative germ cell nuclei. (**B**) Whole-mount in situ hybridization for *Stra8* mRNA in wild-type and *Dazl*-deficient E14.5 ovaries. (**C**) Immunohistochemical staining for γ-H2AX protein in sections of wild-type and *Dazl*-deficient E15.5 ovaries. (**D**) Whole-mount in situ hybridization for *Dmc1* mRNA in wild-type and *Dazl*-deficient E15.5 ovaries. (**E**) Immunohistochemical staining for SYCP3 protein in sections of wild-type and *Dazl*-deficient E15.5 ovaries. (**F**) Quantitative reverse transcription polymerase chain reaction analysis of *Stra8*, *Dmc1*, *Spo11*, *Sycp3*, and *Rec8* mRNA levels in wild-type and *Dazl*-deficient E14.5 ovaries. Plotted here are average fold changes, normalized to *Hprt*, in three independent biological replicates. Error bars represent SD among biological replicates.

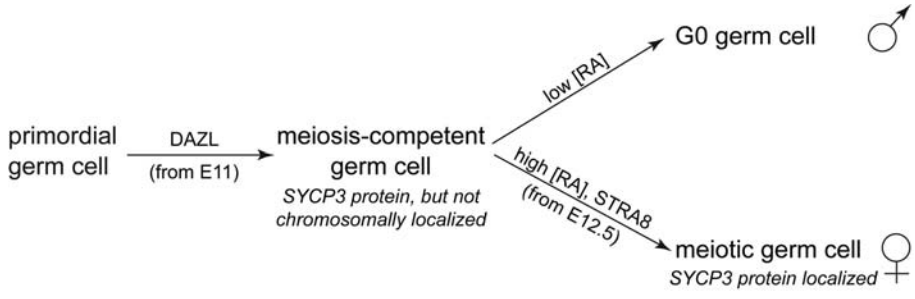

Fig. 2. Embryonic germ cell states: a proposed path by which primordial germ cells acquire meiotic competence (in both XX and XY embryos) and subsequently initiate meiosis (in XX embryos) or undergo G_0 arrest (in XY embryos). "Low [RA]" and "high [RA]" are consequences of differential expression of RA-inactivating enzyme CYP26B1 in embryonic testes and ovaries (*9*, *10*).

Stra8 is expressed abundantly in wild-type ovaries at E14.5 (Fig. 1, B and F). In *Dazl*-deficient ovaries, *Stra8* expression is dramatically reduced if not eliminated (Fig. 1, B and F), which suggests that *Dazl* might have an obligatory function upstream of meiotic initiation; this would account for the absence of meiotic chromosome condensation (Fig. 1A).

If *Dazl* is required for *Stra8* expression and meiotic initiation in embryonic ovaries, then germ cells in *Dazl*-deficient ovaries should not undertake meiotic recombination. We assayed whether *Dazl*-deficient female germ cells form DNA double-strand breaks (DSBs), which initiate meiotic recombination. Cells respond to DNA DSB formation by phosphorylating H2AX, a histone H2A variant, to generate γ-H2AX (*23*). As expected, immunostaining for γ-H2AX revealed the presence of DNA DSBs in many cells of wild-type ovaries at E15.5 (Fig. 1C). In contrast, *Dazl*-deficient ovaries are negative for γ-H2AX, indicating that DNA DSBs have not formed (Fig. 1C). In addition, we asked whether *Dazl*-deficient female germ cells express *Spo11* and *Dmc1*, which encode, respectively, a topoisomerase required to form meiotic DSBs and a recombinase functioning in meiotic DSB repair (*24*). In previous studies, *Stra8* was shown to be required for expression of *Spo11* and *Dmc1* in germ cells of embryonic ovaries (*8*). We found that, in *Dazl*-deficient ovaries, expression of *Dmc1* and *Spo11* is markedly reduced if not eliminated (Fig. 1, D and F). The absence of H2AX phosphorylation and the absence of *Spo11* and *Dmc1* expression indicate that *Dazl*-deficient female germ cells do not engage in meiotic recombination.

These findings are consistent with *Dazl* being required upstream of *Stra8*'s function in meiotic initiation. Does *Dazl* deficiency simply recapitulate the *Stra8* null phenotype (*8*), or does it cause additional abnormalities? We assayed the expression in *Dazl*-deficient embryonic ovaries of the *Sycp3* and *Rec8* genes, which encode, respectively, a component of the synaptonemal complex and a meiosis-specific cohesin (*25*). Both SYCP3 and REC8 proteins function through their loading onto chromosomes. In *Stra8*-deficient female germ cells, as previously reported, *Sycp3* and *Rec8* are transcribed and translated, but the encoded proteins do not load onto chromosomes and therefore do not perform their meiotic functions (*8*). We discovered that, in germ cells of *Dazl*-deficient embryonic ovaries, *Sycp3* function is disrupted at an even earlier step: SYCP3 protein and mRNA levels are markedly reduced as compared with those in wild-type ovaries (Fig.

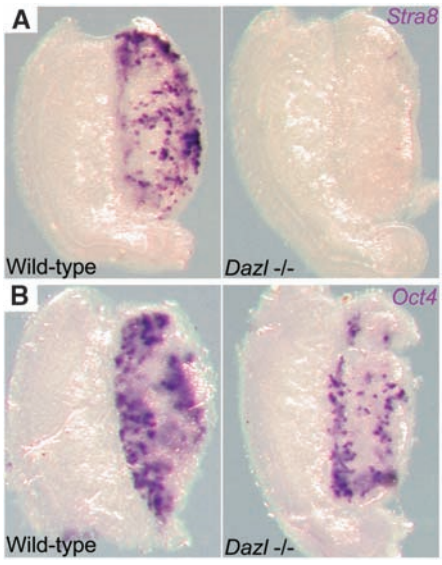

Fig. 3. *Dazl* is required for RA-induced expression of *Stra8* in embryonic testes. Whole-mount in situ hybridization for (**A**) *Stra8* mRNA and (**B**) *Oct4* mRNA in wild-type and *Dazl*-deficient testes dissected at E12.5 and cultured for 48 hours in the presence of 0.7 μM RA is shown.

1, E and F). Similarly, *Rec8* exhibited little or no expression in *Dazl*-deficient ovaries (Fig. 1F). Thus, *Dazl* is required for both *Stra8*-mediated initiation of meiosis in female germ cells and *Stra8*-independent expression of *Sycp3* and *Rec8* there.

We propose a pathway by which embryonic germ cells advance from a primordial state to the initiation of meiosis (Fig. 2). This pathway includes a newly posited cell state, the meiosis-competent gonocyte, whose derivation from a primordial germ cell requires the germ cell–intrinsic factor *Dazl* and whose progression to meiotic initiation and prophase in the female germ line requires the extrinsic meiosis-inducing factor RA and *Stra8*. We propose that this meiosis-competent cell state exists in both male and female embryonic germ lines, despite the fact that meiosis does not initiate in male embryos. The posited meiosis-competent gonocyte contains SYCP3 protein not yet loaded onto chromosomes (Fig. 2), which is consistent with the observation (*6*) that both male and female embryonic germ cells express SYCP3 protein before the sexes take different paths: Female germ cells advance to meiotic prophase, where SYCP3 functions, whereas male germ cells down-regulate SYCP3 and arrest in G_0.

Embryonic testicular germ cells express *Stra8* when exposed to exogenous RA, even though they normally express *Stra8* only after birth (*9*, *10*). Our model predicts that ectopic expression of *Stra8* in RA-treated embryonic testes should require *Dazl* function. We dissected testes from wild-type and *Dazl*-deficient E12.5 embryos, cultured them in the presence of 0.7 μM RA for 48 hours, and assayed *Stra8* expression by whole-mount in situ hybridization. As previously reported (*9*, *10*), RA induced robust expression of *Stra8* in wild-type testes (Fig. 3A). In contrast, in *Dazl*-deficient testes, no induction was observed (Fig. 3A). To confirm that this failure to induce *Stra8* expression in *Dazl*-deficient testes was not due to germ cell apoptosis (*19*), we performed a control in situ hybridization for *Oct4* (Mouse Genome Informatics ID *Pou5f1*), a gene expressed in embryonic germ cells but not in gonadal somatic cells (*26*). We observed abundant *Oct4* expression in RA-cultured testes, both wild-type and *Dazl*-deficient (Fig. 3B). Thus, expression of *Stra8* in response to RA requires *Dazl* in embryonic testis and ovary alike, confirming that *Dazl* is a competence factor for meiotic initiation in embryos of both sexes.

In *S. cereviseae* cells, expression of the a/α mating-type heterodimer is a prerequisite to launching the meiotic initiation program in response to an extrinsic cue. Our findings demonstrate that *Dazl* plays an analogous role in embryonic mice. In both a unicellular eukaryote and a complex animal, meiotic initiation is governed by a cell-intrinsic competence factor and an extrinsic inducing signal.

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Fig. S1
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Traction Dynamics of Filopodia on Compliant Substrates

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Cells sense the environment's mechanical stiffness to control their own shape, migration, and fate. To better understand stiffness sensing, we constructed a stochastic model of the "motor-clutch" force transmission system, where molecular clutches link F-actin to the substrate and mechanically resist myosin-driven F-actin retrograde flow. The model predicts two distinct regimes: (i) "frictional slippage," with fast retrograde flow and low traction forces on stiff substrates and (ii) oscillatory "load-and-fail" dynamics, with slower retrograde flow and higher traction forces on soft substrates. We experimentally confirmed these model predictions in embryonic chick forebrain neurons by measuring the nanoscale dynamics of single—growth-cone filopodia. Furthermore, we experimentally observed a model-predicted switch in F-actin dynamics around an elastic modulus of 1 kilopascal. Thus, a motor-clutch system inherently senses and responds to the mechanical stiffness of the local environment.

Recent work has demonstrated the importance of substrate stiffness on cell motility, morphology, and fate (*1*). For instance, fibroblasts display a behavior known as durotaxis, preferentially migrating toward regions of higher stiffness (*2*). Softer substrates have been shown to promote branching in primary mouse spinal cord neurons while suppressing the growth of associated glia (*3*, *4*). A recent study has also shown that mesenchymal stem cell fate can be determined by the stiffness of the

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