The Ca\(^{2+}\)-induced methyltransferase xPRMT1b controls neural fate in amphibian embryo

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We have previously shown that an increase in intracellular Ca\(^{2+}\) is both necessary and sufficient to commit ectoderm to a neural fate in *Xenopus* embryos. However, the relationship between this Ca\(^{2+}\) increase and the expression of early neural genes has yet to be defined. Using a subtractive cDNA library between untreated and caffeine-treated animal caps, i.e., control ectoderm and ectoderm induced toward a neural fate by a release of Ca\(^{2+}\), we have isolated the arginine N-methyltransferase, xPRMT1b, a Ca\(^{2+}\)-induced target gene, which plays a pivotal role in this process. First, we show in embryo and in animal cap that xPRMT1b expression is Ca\(^{2+}\)-regulated. Second, overexpression of xPRMT1b inhibits the expression of early neural genes such as Zic3. Finally, in the whole embryo, antisense approach with morpholino oligonucleotide against xPRMT1b impairs neural development and in animal caps blocks the expression of neural markers induced by a release of internal Ca\(^{2+}\). Our results implicate an instructive role of an enzyme, an arginine methyltransferase protein, in the embryonic choice of determination between epidermal and neural fate. The results presented provide insights by which a Ca\(^{2+}\) increase induces neural fate.

In the early *Xenopus* gastrula, ectodermal cells can develop along either an epidermal or a neural pathway. The neural fate is conferred during gastrulation, by a tissue interaction between the dorsal mesoderm (Spemann Organizer) and the overlying dorsal ectoderm (1). Neuralizing factors secreted by the Spemann Organizer, including noggin, chordin, and follistatin, act by sequestering bone morphogenetic protein 4 (BMP-4), which otherwise would direct ectoderm toward an epidermal fate (2, 3). Neural induction is therefore regarded as a default pathway (4).

However, in amphibian embryo, we have previously shown that calcium (Ca\(^{2+}\)) plays an active role in neural induction: spontaneous Ca\(^{2+}\) transients are recorded in embryo in the dorsal ectoderm from the late blastula stage until the end of gastrulation (5). Moreover, the blockade of Ca\(^{2+}\) transients by L-type voltage-sensitive Ca\(^{2+}\) channels (LTCs) antagonists or Ca\(^{2+}\) chelator inhibits neural induction (6, 7). In animal caps (ACs), the neural inducing factor noggin causes an influx of Ca\(^{2+}\) via the activation of LTC (5). Activation of LTC and caffeine treatment, which induces a release of Ca\(^{2+}\) from internal stores, triggers neural induction. In open-face Keller explants, planar signals generated by the mesoderm induced Ca\(^{2+}\) transients in the ectoderm. The accumulated Ca\(^{2+}\) pattern correlated with the expression of the early proneural gene, Zic3 (8). Thus, an increase in Ca\(^{2+}\) is both necessary and sufficient to commit ectoderm cells to the neural pathway. However, the molecular mechanism by which Ca\(^{2+}\) orients the cells toward a neural fate remains poorly understood.

To identify Ca\(^{2+}\) target genes involved in neural induction, we constructed a subtractive cDNA library between untreated (i.e., ectodermal) and short time (15–45 min) caffeine-treated (i.e., neutralized) AC to release Ca\(^{2+}\). Here, we describe the characterization of one of the Ca\(^{2+}\) target genes, xprmt1b (*Xenopus* protein arginine methyltransferase type I b), which encodes an arginine N-methyltransferase. PRMT1 constitutes the major type I arginine methyltransferase activity present in mammalian cells and tissues (9). We present functional analysis of xPRMT1b in *Xenopus* embryo and demonstrate that it is a direct Ca\(^{2+}\) target gene required for neural induction. Our results indicate that xPRMT1b mediates Ca\(^{2+}\) signaling by playing a key role in the control of the epidermal versus neural cell fate decision.

Materials and Methods

Embryo and Explants Manipulation, Microinjection, and Ca\(^{2+}\) Measurements. Embryos were obtained by *in vitro* fertilization and staged according to (6, 10). ACs were dissected at stage 8–9 and harvested at chosen stages. Open-face Keller explants were prepared at early stage 10 (11). Caffeine (Sigma) was applied at 10 mM. For Ca\(^{2+}\)-blocking experiments, 20 \(\mu\)M of the intracellular Ca\(^{2+}\) chelator BAPTA-AM (Molecular Probes) was added 30 min before the assay and left for the duration of the experiment. Nifedipine (Sigma), a specific antagonist of the LTCs, was used at 300 \(\mu\)M (6). Cycloheximide (Sigma) was used at 10 \(\mu\)g/ml as described (12). Cycloheximide efficiency was confirmed by the reduced expression level of H4 histone mRNA after 26 cycles (13), instead of 35 cycles for all of the other markers. For microinjection experiments, 5 \(\mu\)l of capped RNAs were injected per blastomere at two- or four-cell stages. We routinely used the GFP tracer expressed from the pCS2-GFP reporter vector at the concentration of 50 pg per embryo. For noggin injection experiments, the CHO-B3 cell line was kindly provided by Richard Harland (University of California, Berkeley), and Xnoggin was purified from conditioned culture medium according to ref. 2 and used at 1 \(\mu\)g/ml. Ca\(^{2+}\) measurements were performed with aequorin according to ref. 5. For BMP signaling inhibition, 1 ng of RNA of a dominant-negative form of BMP type I receptor (tBR) (14) was injected either at two-cell stage for AC assay or in the two ventral blastomeres of a four-cell embryo for Ca\(^{2+}\) imaging.

xPRMT1b Cloning. A subtractive library (PCR-Select cDNA Subtraction kit, Clontech) was constructed between untreated AC and ACs neuralized by caffeine-triggered Ca\(^{2+}\) release (15). To obtain the expression of only early neural genes, we have chosen to pool AC incubated with 10 mM caffeine for 15, 30, and 45 min. These incubation times are sufficient to neutralize the ectoderm (7). Inserts specific to the Ca\(^{2+}\)–induced cDNA population were cloned into the pGEM-T-Easy vector and screened with the PCR-Select Differ-
Plasmid Constructs in Vitro Transcription and Morpholino (Mo) Oligonucleotides. pGEX-xPRMT1b vector was constructed by PCR-amplifying the 1,056-bp ORF from the complete cDNA with BamHI (5’-CCGGATCCATGGCTAGCCAGAAGCTGC-3’) and XbaI (5’-CCTCTAGATCAACGATCTTAT-AGTCTGTG-3’) and by introducing the PCR product in frame into pGEX-2TK at BamHI/XbaI sites. Mo antisense oligomers were obtained from Gene Tools (Philomath, OR). xPRMT1b-Mo (Mo1b) had the sequence 5’-CAGGTCTTCGCTAGCCATCTTTTC-3’ (translational start codon underlined). Control Mo (MoC) against Zebravid ash 1b, 5’-ATGGAGGGCAACTGTCGTCGAG3’- presents no homology with any Xenopus database sequences. For in vivo expression studies, pCS2-xPRMT1b was constructed by transferring xPRMT1b ORF from pGEX-xPRMT1b into pCS2 at BamHI/XhoI sites. pCS2-xPRMT1b was linearized at the Nof site, and capped synthetic RNAs were generated by using SP6 mMessage mMachin (Ambion). To test the specificity of Mo1b, a resistant form of xPRMT1b to Mo1b generated by using SP6 mMessage mMachine kit (Ambion). To test xPRMT1b into pCS2 at BamHI combination with Mo1b or MoC in two-cell embryos. RT-PCR and in Situ Hybridization. Results

Isolation of Genes Induced by a Ca\(^{2+}\) Increase. In Xenopus, Ca\(^{2+}\) transients at gastrulation regulate neural gene expression such as Zic3 (6). The mechanism by which an increase in internal Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) controls early neural gene expression is yet unknown. To solve this question, we constructed a subtractive cDNA library between noninduced and Ca\(^{2+}\)-induced ACs, cultivated in absence or in presence, respectively, of 10 mM caffeine for 15–45 min (15). This treatment, which triggers neural induction through an increase in [Ca\(^{2+}\)]\(_i\), (7), allows the differential isolation of the earliest Ca\(^{2+}\)-dependent genes involved in neural determination. We isolated 32 cDNAs; one clone, called 3E10, was analyzed in more detail.

3E10 Encodes the Arginine Methyltransferase, xPRMT1b. The partial cDNA clone 3E10 was used to isolate the corresponding full-length cDNA from a lithium-dorsalized gastrula library (16). Sequence comparison indicated that the putative protein encoded by this full-length cDNA belongs to the PRMT1 protein arginine methyltransferase family, sharing the four conserved regions that are specific for this class of enzymes (domains I, post-I, II, and III) as found in yeast Hmt1p, mouse PRMT1, rat PRMT1 (rPRMT1), and human PRMT1L2 (22, 23). Amino acid sequences and that of the recently described Xenopus sequence, xPRMT1 (23), is shown in Fig. 5A. Given the high level of sequence homology, we refer to this clone as xPRMT1b.

To confirm the functional homology between xPRMT1b and the mammalian PRMT1, we performed in vitro methylation assays using xPRMT1b, rPRMT1 (24), and another member of the PRMT family, the coactivator arginine methyltransferase CARM1/PRMT4 (25). xPRMT1b methyltransferase activity was assayed on histones by performing methylation reactions that were then analyzed by SDS/PAGE followed by fluorography. We found that, as expected, xPRMT1b specifically methylated histone H4 and, to a lesser extent, histone H2B, as described for rPRMT1 (26, 27), whereas CARM1 specifically methylated histone H3 (Fig. 5B and C) (27). Taken together, these data confirm that xPRMT1b belongs to the PRMT family and is the functional Xenopus homologue of mammalian PRMT1.

Noggin Up-Regulates xPRMT1b Expression by a Ca\(^{2+}\)-Mediated Pathway.

We confirmed that xPRMT1b expression depends on an elevation of [Ca\(^{2+}\)]\(_i\) in AC using RT-PCR assay. ACs were treated with 10 mM caffeine for 30 min. Under these conditions, xPRMT1b expression was specifically induced (Fig. 1A). Caffeine did not trigger the expression of Xbra, a mesodermal marker, showing that xPRMT1b expression is not a secondary consequence of mesodermal induction. No expression of xPRMT1b was detectable in the absence of caffeine treatment or when the increase in [Ca\(^{2+}\)]\(_i\) triggered by caffeine was inhibited with 20 μM of the Ca\(^{2+}\) chelator BAPTA-AM. Interestingly, xPRMT1b expression was still triggered by caffeine when the ACs were incubated in the presence of 10 μM cycloheximide (Fig. 1A). Control experiment indicates that, on AC, cycloheximide alone does not induce xPRMT1b or Xbra expression (Fig. 1B). These data indicate that xPRMT1b expression is an early response to a Ca\(^{2+}\) increase that does not require de novo protein synthesis.

ACs treated with conditioned medium containing noggin also expressed xPRMT1b (Fig. 1C). The induced expression of xPRMT1b by noggin is blocked by 20 μM BAPTA-AM. These data were further confirmed by RT-PCR analysis for xPRMT1b expression of various GST fusion proteins (GST-PRMT1, GST-CARM1, and xPRMT1b) were performed as described (22) with 2 μg of histones as substrates.

In Vitro Methylation Assays. pGEX vectors allowing the expression of recombinant GST fusion proteins, GST-CARM1 and GST-rPRMT1 were kind gifts from M. R. Stallcup and H. R. Herschman (University of California, Los Angeles). GST fusion proteins were produced as described (22). HMT assays with the various GST fusion proteins (GST-PRMT1, GST-CARM1, and xPRMT1b) were performed as described (22) with 2 μg of histones as substrates.
ACs dissected from embryos injected at the two-cell stage with 50 pg of noggin mRNA (data not shown).

We next wondered whether, in *Xenopus* like in *Pleurodeles* (5), noggin may trigger an increase in \([\text{Ca}^2+]\). Indeed, noggin provoked, on AC, a 30-fold increase above the resting level in light emitted by aequorin (Fig. 1D). Noggin inactivated by several cycles of freeze-thawing and heating at 65°C, did not trigger any significant increase in \([\text{Ca}^2+]\) (data not shown). This noggin-induced \([\text{Ca}^2+]\) increase was absent when LTC activation was inhibited by 300 \(\mu\text{M}\) nifedipine, a specific dihydropyridine antagonist of LTC (Fig. 1D Inset). Nifedipine was previously shown to block the expression of early neural markers in *Xenopus* (6). To test the relationship between \([\text{Ca}^2+]\), increase, noggin, and BMP inhibition, 1 ng of tBR RNA was coinjected with aequorin in the two ventral blastomeres at four-cell stage. Measurements were performed from stage 6 to 25. A sustained increase in \([\text{Ca}^2+]\) was recorded (Fig. 1E). This increase is strongly reduced when the injected tBR embryos were incubated in presence of 20 \(\mu\text{M}\) BAPTA (Fig. 1E Inset). In addition, xPRMT1b was expressed in ACs injected with tBR, whereas xPRMT1b expression is decreased in similar caps treated with BAPTA (Fig. 1F).

Together, these data suggest that xPRMT1b expression is induced by BMP inhibition (noggin or tBR) through a direct or indirect activation of a \([\text{Ca}^2+]\)-dependent pathway.

**Developmental Expression of xPRMT1b.** To determine the kinetics of xPRMT1b expression, RT-PCR analysis was performed during early *Xenopus* development. xPRMT1b is maternally expressed and subsequently transcribed zygotically throughout all of the developmental stages tested (Fig. 2A). To analyze the spatial expression of xPRMT1b during development, whole-mount ISH was performed. xPRMT1b mRNA was detected from the onset of gastrulation, with an expression in dorsal mesoderm and in dorsal and ventral ectoderm (Fig. 2B). As shown on dorsal sagittal section, xPRMT1b was particularly highly expressed in the inner ectodermal layer (Fig. 2B), which corresponds to the inducible ectodermal layer (28). At the neurula and tail bud stages, xPRMT1b mRNA was restricted to the neururectoderm, with the highest expression in the anterior neural plate (Fig. 2B). The spatial and temporal patterns of xPRMT1b expression are consistent with the hypothesis that xPRMT1b plays a role in the early steps of neural determination.

We next wanted to know whether the early expression of xPRMT1b is controlled by LTC in the whole embryo. Embryos were treated, from stage 8 to 10, with 300 \(\mu\text{M}\) nifedipine, and analyzed at stages 10.5 (Fig. 2C Left) and 12.5 (Fig. 2C Right). The expression of xPRMT1b is strongly reduced in nifedipine treated embryos (\(n = 45\) for each stage) compared to untreated embryos. These data indicate that early expression of xPRMT1b in the embryo depends on \([\text{Ca}^2+]\) signaling mediated by the activation of LTC.

**Overexpression of xPRMT1b Activates Neural Gene Expression.** The expression pattern of xPRMT1b in neural tissue and its \([\text{Ca}^2+]\)- and noggin-dependent expression suggest that it may regulate neural induction. Therefore, we examined the expression of neural and mesodermal markers by RT-PCR in AC from embryos injected with xPRMT1b mRNA. xPRMT1b induced the expression of Zic3 (proneural gene), En2 (mid-hindbrain marker), Neurogenin (neural commitment gene), N-CAM (pan-neural marker), and N-tubulin (pan-neuron marker), whereas MyoD (late mesoderm) transcript was not induced. No expression for any of these markers was
transcription requires protein synthesis. Therefore, whereas Zic3 is a direct target of xPRMT1b, we analyzed whether it regulates the expression of neural markers in the presence of cycloheximide, the expression of the later neural marker N-tubulin was not detected in control AC (Fig. 3A). These results indicate that xPRMT1b leads to the induction of neural markers in the absence of mesoderm.

To test whether xPRMT1b directly induces neural genes expression, AC from xPRMT1b mRNA-injected embryos were cultured, until stage 10, in presence or absence of cycloheximide. As shown in Fig. 3B, whereas the early marker Zic3 is still expressed in ACs in the presence of cycloheximide, the expression of the later neural gene En2 is inhibited (Fig. 3B, lane 3). This finding demonstrates that, whereas Zic3 is a direct target of xPRMT1b activity, En2 transcription requires protein synthesis.

To confirm the inductive activity of xPRMT1b on neural genes, we analyzed whether it regulates the expression of Zic3 and N-tubulin in vivo. xPRMT1b mRNA was injected into only one cell at the two-cell stage; this resulted in an increase in Zic3 expression on the injected side at the neurula stage (n = 12; Fig. 3C2). This observation was further confirmed on anterior to posterior cross sections (Fig. 3D Left). The N-tubulin expression pattern was also affected (n = 14; Fig. 3C4) and expanded as shown on anterior to posterior cross-sections (Fig. 3D Right). Together, these data demonstrate that xPRMT1b can induce the expression of neural genes both in vitro and in vivo.

**xPRMT1b Is Necessary for Ca²⁺-Induced Neural Commitment in AC.** To evaluate the importance of xPRMT1b during neural induction, we used antisense Mo to interfere with xPRMT1b translation in Xenopus embryo. Rescue experiments were undertaken to test the specificity of Mo1b against xPRMT1b (Fig. 6, which is published as supporting information on the PNAS web site). Embryos injected at the one-cell stage with Mo1b alone (6.25 ng) displayed a delay in gastrulation at stage 10.5 and a neural tube defect at stage 19. In contrast, coinjection of Mo1b and 2 ng of a resistant form of xPRMT1b (r-xPRMT1b) significantly reduced the phenotypes observed at stage 10.5 and at stage 19 (Table 1 and Fig. 6A). The expression of the pan-neural marker Sox3 and of N-tubulin was partially restored in this rescue experiment (Fig. 6B). In addition, the specificity of Mo1b was tested with a -GFP fusion gene containing a partial sequence of xPRMT1b specifically recognized by Mo1b (referred to as ΔxPRMT1b-GFP). When the ΔxPRMT1b-GFP fusion mRNA is coinjected with Mo1b at the two-cell stage, the expression of GFP is abolished at neurula, whereas coinjection with the control MoC had no effect (Fig. 7, which is published as supporting information on the PNAS web site). Together, these...
expression of the potent epidermal inducer, bmp4, remained robustly expressed in AC from Mo1b-injected embryos. These results indicate that xPRMT1b is an essential factor needed for the epidermal to the neural switch, induced by Ca\textsuperscript{2+} in ACs.

The proneural gene Zic3 is induced by noggin (18). The observation that noggin induced xPRMT1b expression, which in turn activates Zic3 transcription, led us to ask whether the impairment of xPRMT1b activity by Mo1b could block noggin-induced Zic3 expression in AC. As shown in Fig. 4B, noggin induced Zic3 expression in AC. This induction was suppressed in ACs explanted from Mo1b-injected embryos, whereas Zic3 expression was not affected in ACs from MoC-injected embryos. This finding suggests that xPRMT1b is required for noggin-induced Zic3 expression.

xPRMT1b Inhibition by Mo Disturbs Neural Fate in the Whole Embryo. Finally, we investigated the role of xPRMT1b in the whole embryo by loss-of-function experiments using Mo. When injected into one blastomere of a two-cell embryo, Mo1b, but not MoC, reduced the expression of Zic3 (n = 20) at neurula stage on the injected side (Fig. 4C Left). Furthermore, the expression of En2 (n = 10) and N-tubulin (n = 15) was completely abolished on the Mo1b injected side, whereas MoC had no effect (Fig. 4C Center and Right). These results strongly suggest that the expression of neural markers such as Zic3, En2, and N-tubulin require the expression of xPRMT1b.

xPRMT1b Is Not Involved in Gastrulation Movements. The preceding data support the conclusion that Zic3 is a direct target of xPRMT1b. At early gastrula, Zic3 is expressed not only in the neurectoderm but also in the involuting mesoderm (29), where Xbra regulates its expression (30). Xbra acts for the proper anterior–posterior development of the embryo during convergent extension movement at gastrulation (31). Therefore, we carried out two types of experiments to confirm that xPRMT1b inhibition did not impair cell movement. Mo1b was injected at the four-cell stage, into the two dorsal blastomeres and the expression of Zic3 and Xbra analyzed at late gastrula. Under these conditions, Zic3 expression was strongly reduced (Fig. 4D Lower), whereas Xbra pattern was unaffected (Fig. 4E). Neither Zic3 nor Xbra expression were affected by MoC (Fig. 4D Upper and 4E). Furthermore, Mo1b did not significantly alter mesoderm elongation and Xbra expression of open-face Keller explants (Fig. 4F). Together, these results suggest that xPRMT1b is not involved in gastrulation movements but acts only on anterior neural induction.

Discussion

In an attempt to find new Ca\textsuperscript{2+} targets, we have isolated from a Ca\textsuperscript{2+}-dependent subtractive cDNA library the functional Xenopus homologue of the mammalian arginine methyltransferase PRMT1, called xPRMT1b. In the whole embryo, xPRMT1 is expressed in neural territories. In the mouse, PRMT1 is also expressed in the developing central nervous system (32). This finding suggests that the role of methyltransferase activity is conserved in vertebrates during neural development.

AC assays showed that the expression of xPRMT1b is controlled by an increase in [Ca\textsuperscript{2+}], after the application of noggin or by the inhibition of BMP signaling with tBR. This effect is blocked by the Ca\textsuperscript{2+} chelator, BAPTA. Calcium imaging confirmed that, in Xenopus, as in Pleurodeles (5), noggin triggers an increase in [Ca\textsuperscript{2+}] via the activation of LTC. The early expression of xPRMT1b at gastrula also occurs through a Ca\textsuperscript{2+}-dependent mechanism mediated by the activation of LTC.

Overexpression of xPRMT1b induced neural markers both in AC and embryos; these include the early proneural gene Zic3 and the neuron differentiation marker N-tubulin. PRMT1 class of enzymes is known to methylate histone H4 (33). Their role in controlling the transcriptional state of specific genes has not yet been explored in a developing organism. Here we show that xPRMT1b directly

data show that Mo1b specifically interferes with xPRMT1b to block its function.

RT-PCR analysis of caffeine-treated ACs from Mo1b-injected embryos shows that Zic3 expression was inhibited, whereas it is not affected in caffeine-treated ACs from MoC-injected or control embryos (Fig. 4A). Conversely, despite caffeine treatment, the

**Fig. 4.** xPRMT1b loss of function by Mo antisense. (A) Mo1b blocks the ability of caffeine to induce an epidermal to neural switch. ACs were excised at stage 8 from embryos injected into two cells at the two-cell stage with either Mo1b or MoC (6.25 ng total) and cultured to stage 9. RT-PCR gene analysis was performed for Zic3, BMP4, Xbra, and ODC (loading control). ACs were treated for 30 min with 10 mM caffeine (+) or untreated (−). (B) Whole-mount ISH of AC with Zic3 probe. ACs were dissected at stage 9 from embryos injected with 6.25 ng of either Mo1b (3) or MoC (2), treated with noggin (1 pg/ml), and cultured to stage 12.5–13. Control AC was treated with noggin (A) or untreated (B). (C) Expression of Zic3, En2, and N-tubulin at neurula stage of embryos injected into one cell at the two-cell stage with 6.25 ng of MoC (Upper) or Mo1b (Lower). The injected sides are on the right (is) in these dorsal views (anterior to the top). Mo1b reduces the expression of Zic3 and abolishes En2 and N-tubulin. (D) Late gastrula embryos (stage 12–12.5) injected at the two-cell stage with either MoC (Upper) or Mo1b (Lower) (6.25 ng total) and probed for the expression of Zic3. Mo1b strongly reduces Zic3 expression. (E) Late gastrula embryos injected at the two-cell stage with Mo1b (Upper) (6.25 ng total) and probed for the expression of Xbra. Mo1b does not affect Xbra expression. (Lower) Control embryos. (F) Whole-mount ISH of open-face Keller explants with Xbra probe. Explants were dissected at stage 10 from either Mo1b (Upper) or MoC (Lower) injected embryo and cultured until stage 12.5.
controls the expression of the proneural gene Zic3 in AC in the absence of protein synthesis. Thus, xPRMT1b might regulate transcription by modifying proteins involved in general transcriptional coactivation. Alternatively, xPRMT1b may methylate specific transcriptional activators, bound to the Zic3 promoter, which in turn triggers Zic3 expression at the onset of neural induction. The transcription factor YY1 has recently been shown to recruit PRMT1 to an YY1-activated promoter (34). Several YY1 consensus sequences are present in the Xenopus Zic3 promoter, one of which is conserved in human (35). Thus, xPRMT1b might facilitate translation by antisense Mo

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Table 1. Rescue experiments to test the specificity of Mo1b Mo against xPRMT1b

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<tr>
<th>Phenotype, n (%)</th>
<th>Stage/Injected (n)</th>
<th>Wild type</th>
<th>Abnormal blastopore formation</th>
<th>Neural tube defect</th>
<th>Neural tube enlarged</th>
<th>Blocked at gastrulation</th>
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<tr>
<td>10.5</td>
<td>Mo1b (12)</td>
<td>112 (53)</td>
<td>100 (47)</td>
<td>27 (45)</td>
<td>21 (19)</td>
<td>29 (48.3)</td>
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<tr>
<td></td>
<td>Rescue (23)</td>
<td>160 (75)</td>
<td>53 (25)</td>
<td></td>
<td></td>
<td>26 (24)</td>
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<tr>
<td></td>
<td>MoC (200)</td>
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<td>40 (20)</td>
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<td></td>
<td>13 (14.4)</td>
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<tr>
<td>19</td>
<td>Mo1b (60)</td>
<td>4 (6.6)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Rescue (108)</td>
<td>48 (45)</td>
<td></td>
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<tr>
<td></td>
<td>MoC (90)</td>
<td>73 (81)</td>
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Embryos were injected at the one-cell stage with 6.25 ng of Mo1b or MoC alone. To obtain the rescue, embryos were co-injected with 2 ng of a Mo1b-resistant form of xPRMT1b, r-xPRMT1b, and 6.25 ng of Mo1b. The number (n) and percentage (%) of the different phenotypes obtained at gastrulation and neurulation are shown.