EXTENDED EXPERIMENTAL PROCEDURES

Chemicals

Aphidicolin (Sigma), hydroxyurea (Sigma), leptomycin B (Sigma).

Cells and Cell Culture

Cells were grown in DMEM media (GIBCO) supplemented with 10% FBS, 2 mM L-Glutamine, 100 units/ml Penicillin, and 100 μg/ml Streptomycin.

MEFs

FMRP KO MEFs were created with an insertion in exon 5 resulting in FMRP protein null MEFs with low level of FMRP mRNA (The Dutch-Belgian Fragile X Consortium, 1994). DOT1L mutant MEFs, which have a significant reduction in H3K79 methylation levels, were created using a gene trap approach (Steger et al., 2008).

Antibodies

Mouse anti-FMRP (Chemicon, MAB2160), rabbit anti-FMRP (Abcam, ab17722), rabbit anti-H3 (Abcam), mouse anti-H2A.X phosphorylated on Ser 139 (anti-γH2A.X) (Abcam), mouse anti-H2A.X phosphorylated on Ser 139 (anti-γH2A.X) (Millipore), rabbit anti-H2A.X (Abcam), mouse anti-Actin (Abcam), goat anti-ATR (Santa Cruz), rabbit anti-BRCA1 (Bethyl), rabbit anti-mouse BRCA1 (gift of S. Namekawa), rabbit anti-phospho-BRCA1(Ser1423) (Abcam), rabbit anti-phospho-BRCA1(Ser1423) (Mybiosource) (recognizes mouse epitope), rabbit anti-RPA32 (Bethyl), mouse anti-CENTB (Santa Cruz), mouse anti-SYCP3 (Santa Cruz), rabbit anti-SYCP1 (Abcam), goat anti-SYCP1 (Santa Cruz), rabbit anti-DMC1 (Santa Cruz), mouse anti-MLH1 (Millipore), mouse anti-SPO11. Anti-SPO11 hybridoma cell line 180 was generated by M.P. Thelen at Lawrence Livermore National Laboratory, California; the purified monoclonal antibody was provided by S. Keeney, Memorial Sloan-Kettering Cancer Center, New York.

Western Blot Band Intensity Quantification

For western blot quantification we measured band intensity using ImageJ software. In the case of BRCA1 phosphorylation we used Photoshop software. For γH2A.X induction we quantified the γH2A.X band in the "treatment" lane and then compared the value to control treatment, e.g., DMSO or the 0 time point.

Histone MLA Binding Reactions

The binding reactions were performed in binding buffer (50 mM Tris pH 7.5, 1 M NaCl, 2 mM $MgCl_2$, 0.5% Triton X-100) at +4C° for 1 hr with rotation. After incubation with glutathione agarose beads (GE Healthcare) the reactions were washed 5 times with binding buffer.

Calculation of Chromatin-Associated FMRP

In quantification of FMRP chromatin recruitment, the y axis represents the ratio of chromatin-associated FMRP to total FMRP. It was calculated based on the level of FMRP on chromatin divided by total FMRP. The data are from 3 independent experiments. The relative fold increase in FMRP loading on chromatin in response to aphidicolin (APH) is compared to the levels of chromatin-associated FMRP upon DMSO treatment, which was designated as 1 (DMSO).

Aphidicolin Treatment

APH treatment was performed for 24 hr.

γH2A.X Analysis in MEF Cells after Replication Inhibition by Aphidicolin

MEFs were treated with DMSO or APH (0.5μM) for 24 hr. Cells were then lysed in SDS loading buffer and samples were subjected to western analysis for γH2A.X and total H2A.X. Three independent experiments were performed.

pS1423 BRCA1 Analysis after Replication Inhibition by Aphidicolin

For HeLa cells, the APH treatment was 0.5μM for 24 hr. For 293 and MEF cells, the treatment was 5μM for 24 hr. Cells were then lysed in SDS loading buffer and samples were subjected to western analysis for pS1423 BRCA1 and total BRCA1. The quantification was performed as a ratio of phospho-BRCA1 to total BRCA1. Three independent experiments were performed.

FMRP RNAi Analysis in HeLa Cells and Treatment with Aphidicolin for γH2A.X Induction

HeLa cells were transfected with two different FMRP shRNA constructs (FMRP shRNA1 and FMRP shRNA2) or control scramble shRNA. After 3 days cells were treated with DMSO or aphidicolin (0.5μM) for 24 hr. Cells were then lysed in SDS sample buffer and subjected to western blot using FMRP, actin, γH2A.X, and H2A.X antibodies. Three independent experiments were performed.

Hydroxyurea Treatment Experiments

MEFs were treated with DMSO or hydroxyurea at 2 mM final concentration and incubated for 0, 30, 60, and 120 min. Cells were then lysed in SDS sample buffer and samples were analyzed by western blotting using γH2A.X antibody and H2A.X antibody. Three independent experiments were performed.

UV Irradiation

MEF cells were UV irradiated with a dose of 50 J/m^2 and cell samples were collected 0, 30, 60, and 120 min postirradiation in SDS sample buffer. Samples were then western blotted for γH2A.X and H2A.X. Three independent experiments were performed.

Ionizing Radiation Treatment

MEFs were treated with 5Gy of ionizing radiation and samples were collected in SDS loading buffer 30 min postirradiation. Western blots were performed using γH2A.X and H2A.X antibodies. Experiments were performed 3 times.

Colony Survival Assay

For the colony survival assays we used HeLa cells stably expressing FMRP RNAi vector, which were complemented with stably expressing rescue vector, either wild-type FMRP-Flag-HA or Flag-HA alone. Cells were seeded at 200 cells/dish density and allowed to attach. The media was then changed to aphidicolin (APH) containing media (0.05μM, 0.1μM, and 0.3μM) or control media containing DMSO. Cells were incubated for 2 weeks until the appearance of colonies. The colonies were then rinsed in PBS, fixed in methanol and stained with Coomassie stain. Survival was determined by comparing the amount of colonies in APH containing media to the number of colonies in control media. Statistical analysis of data from 3 independent experiments was done using 2-way ANOVA. For MEF cells, the protocol was the same except that the cells were treated with 0.01μM or 0.05μM of APH, or 0.01 or 0.03 mM of hydroxyurea (HU).

Lentivirus Production

Lentiviral particles were produced as previously described (Lois et al., 2002). Briefly, HEK293FT cells were transfected using the calcium phosphate method with FIV-CMV-pEGFP-FMRP (gift, G.J. Bassel, Emory University) and packaging vectors. The supernatants containing the viral particles were collected 48 hr after transfection and concentrated using ultracentrifugation. Viral titers were determined by serial dilution of HEK293FT cells as 10^8 per ml. Primary cell cultures were transduced by adding concentrated lentivirus to the growth media.

Hippocampal Neuron Cell Culture and Infection

Hippocampal primary neurons were dissected from WT and *Fmr1* KO mice at embryonic day 16.5 and cultured as described previously (Bassell and Warren, 2008) in accordance with the Institutional Animal Care and Use Committee guidelines. Cells were plated (2000 cells per cm^2) on poly-l-lysine-coated Biotechs coverslips (1 mg/ml) in MEM with FBS (10%) for 2 hr, supplemented with B-27 and Gluta MAX-1 (Invitrogen, San Diego, CA). Neurons were transduced with lentivirus at 14 days in vitro (DIV). Six hours posttransduction, live neurons were processed for the AMPAR internalization assay. FMRP KO MEFs were reconstituted with FMRP-Flag-HA constructs using pMSCV-based viral vector system.

Constitutive AMPAR Internalization Assay

The assay was performed as described previously for rat neurons (Nakamoto et al., 2007) with modifications (Bhattacharyya et al., 2009). Surface AMPARs in live *Fmr1* knockout mouse neurons were labeled with a rabbit polyclonal antibody against the N terminus of the GluR1 subunit (Calbiochem, 1:5 in conditioned media) for 15 min at 37°C, 0.5% CO_2. After incubation with antagonists (1 μM TTX, 10 μM NBQX, and 50 μM APV; Tocris) in conditioned media for 15 min at 37°C, 0.5% CO_2, cells were fixed in 4% FA for 15 min on ice, and labeled surface GluR1s were visualized by saturating with Alexa 546 conjugated goat-anti-rabbit secondary antibody (Invitrogen). After permeabilization with 0.1% Triton X-100 for 30 min at room temperature, internalized GluR1s were saturated with Alexa 633-conjugated donkey anti-rabbit secondary antibody. Raw images were taken with a Zeiss confocal microscope and LSM software, and were compared between samples in the same culture preparation. Signal in identical areas of thick distal dendrites (>20 μm from the soma) were measured. p values were determined using one-way ANOVA ($\alpha = 0.05$) with Bonferroni's post hoc test.

Microscale Thermophoresis from Nanotemper Technologies

The process involves measurement of the fluorescence distribution of fluorescently labeled molecules inside a capillary upon laser irradiation. A temperature gradient is generated by an IR-Laser focused into the capillary. Binding affinities are calculating measuring a temperature jump in the initial stage of irradiation, thermophoretic movement of the molecules within the gradient at later stages, or both (Jerabek-Willemsen et al., 2011; Wienken et al., 2010). Independent experiments were performed a minimum of 3 times for each histone modification. In MST experiments using MLA histones, the Agenet domain was labeled. Histone MLAs were prepared as described (Simon et al., 2007) and purchased from Active Motif. Histone binding reactions with the Agenet domain were performed in binding buffer (50 mM Tris pH 7.5, 1 M NaCl, 2 mM $MgCl_2$, 0.5% Triton X-100) at room temperature for 30 min. In the case of the nucleosome MST reaction recombinant H3K79me2 nucleosome was labeled. Mononucleosomes were prepared as described (Lu

et al., 2008), and incubation in binding buffer (50 mM Tris pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 0.5% Triton X-100) was for 3 hr at room temperature. Data were plotted using GraphPad Prizm software.

Fmr1 KO Mice

Fmr1 KO mice (allele $tm1^{Cgr}$/J, congenic on a C57BL/6J background; same mutation as used for *Fmr1* KO MEFs) were a kind gift from Mark Bear. Mice were kept under standard conditions and all experiments were conducted in compliance with the Animal Welfare act and approved by the Animal Care and Use Committee at the appropriate institution.

Dot1L cKO Mice

Dot1L$^{fl/fl}$ conditional KO mice were a kind gift from Scott Armstrong. To generate a germ-cell-specific *Dot1L* deletion, a germline-specific *Cre* transgene (*Mvh-Cre*, [Hu et al., 2013]) was crossed into the *Dot1L*^{fl} background. *Mvh-Cre;Dot1L*$^{fl/+}$ male mice (germ cell genotype *Dot1L*$^{\Delta/+}$) were mated to *Dot1L*$^{fl/fl}$ females, so that F_1 males were either *Dot1L*$^{fl/\Delta}$ or *Dot1L*$^{fl/+}$. Conditional KO (cKO) animals were *Cre*-positive *Dot1L*$^{fl/\Delta}$ males (germ cell genotype *Dot1L* $^{\Delta/\Delta}$), and *Cre*-negative *Dot1L*$^{fl/+}$ males were used as littermate controls.

SUPPLEMENTAL REFERENCES

Bächner, D., Manca, A., Steinbach, P., Wöhrle, D., Just, W., Vogel, W., Hameister, H., and Poustka, A. (1993). Enhanced expression of the murine FMR1 gene during germ cell proliferation suggests a special function in both the male and the female gonad. Hum. Mol. Genet. *2*, 2043–2050.

Bassell, G.J., and Warren, S.T. (2008). Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron *60*, 201–214.

Bhattacharyya, S., Biou, V., Xu, W., Schlüter, O., and Malenka, R.C. (2009). A critical role for PSD-95/AKAP interactions in endocytosis of synaptic AMPA receptors. Nat. Neurosci. *12*, 172–181.

Bishop, D.K., Park, D., Xu, L., and Kleckner, N. (1992). DMC1: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell *69*, 439–456.

Blanco-Rodríguez, J. (2012). Programmed phosphorylation of histone H2AX precedes a phase of DNA double-strand break-independent synapsis in mouse meiosis. Reproduction *144*, 699–712.

Bostelman, L.J., Keller, A.M., Albrecht, A.M., Arat, A., and Thompson, J.S. (2007). Methylation of histone H3 lysine-79 by Dot1p plays multiple roles in the response to UV damage in Saccharomyces cerevisiae. DNA Repair (Amst.) *6*, 383–395.

Brown, V., Jin, P., Ceman, S., Darnell, J.C., O'Donnell, W.T., Tenenbaum, S.A., Jin, X., Feng, Y., Wilkinson, K.D., Keene, J.D., et al. (2001). Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. Cell *107*, 477–487.

Conde, F., Refolio, E., Cordón-Preciado, V., Cortés-Ledesma, F., Aragón, L., Aguilera, A., and San-Segundo, P.A. (2009). The Dot1 histone methyltransferase and the Rad9 checkpoint adaptor contribute to cohesin-dependent double-strand break repair by sister chromatid recombination in Saccharomyces cerevisiae. Genetics *182*, 437–446.

Costelloe, T., Fitzgerald, J., Murphy, N.J., Flaus, A., and Lowndes, N.F. (2006). Chromatin modulation and the DNA damage response. Exp. Cell Res. *312*, 2677–2686.

Gagné, J.P., Bonicalzi, M.E., Gagné, P., Ouellet, M.E., Hendzel, M.J., and Poirier, G.G. (2005). Poly(ADP-ribose) glycohydrolase is a component of the FMRP-associated messenger ribonucleoparticles. Biochem. J. *392*, 499–509.

Garber, K.B., Visootsak, J., and Warren, S.T. (2008). Fragile X syndrome. Eur. J. Hum. Genet. *16*, 666–672.

Gatei, M., Zhou, B.B., Hobson, K., Scott, S., Young, D., and Khanna, K.K. (2001). Ataxia telangiectasia mutated (ATM) kinase and ATM and Rad3 related kinase mediate phosphorylation of Brca1 at distinct and overlapping sites. In vivo assessment using phospho-specific antibodies. J. Biol. Chem. *276*, 17276–17280.

Haince, J.F., Kozlov, S., Dawson, V.L., Dawson, T.M., Hendzel, M.J., Lavin, M.F., and Poirier, G.G. (2007). Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents. J. Biol. Chem. *282*, 16441–16453.

Handel, M.A., and Schimenti, J.C. (2010). Genetics of mammalian meiosis: regulation, dynamics and impact on fertility. Nat. Rev. Genet. *11*, 124–136.

Helleday, T., Bryant, H.E., and Schultz, N. (2005). Poly(ADP-ribose) polymerase (PARP-1) in homologous recombination and as a target for cancer therapy. Cell Cycle *4*, 1176–1178.

Hu, Y.C., de Rooij, D.G., and Page, D.C. (2013). Tumor suppressor gene Rb is required for self-renewal of spermatogonial stem cells in mice. Proc. Natl. Acad. Sci. USA *110*, 12685–12690.

Jerabek-Willemsen, M., Wienken, C.J., Braun, D., Baaske, P., and Duhr, S. (2011). Molecular interaction studies using microscale thermophoresis. Assay Drug Dev. Technol. *9*, 342–353.

Kedar, P.S., Stefanick, D.F., Horton, J.K., and Wilson, S.H. (2008). Interaction between PARP-1 and ATR in mouse fibroblasts is blocked by PARP inhibition. DNA Repair (Amst.) *7*, 1787–1798.

Krum, S.A., la Rosa Dalugdugan, Ed., Miranda-Carboni, G.A., and Lane, T.F. (2010). BRCA1 forms a functional complex with γ-H2AX as a late response to genotoxic stress. J. Nucleic Acids *2010*, pii: 801594.

Liu, S., Bekker-Jensen, S., Mailand, N., Lukas, C., Bartek, J., and Lukas, J. (2006). Claspin operates downstream of TopBP1 to direct ATR signaling towards Chk1 activation. Mol. Cell. Biol. *26*, 6056–6064.

Lois, C., Hong, E.J., Pease, S., Brown, E.J., and Baltimore, D. (2002). Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. Science *295*, 868–872.

Lu, X., Simon, M.D., Chodaparambil, J.V., Hansen, J.C., Shokat, K.M., and Luger, K. (2008). The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure. Nat. Struct. Mol. Biol. *15*, 1122–1124.

Méndez, J., and Stillman, B. (2000). Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. Mol. Cell. Biol. *20*, 8602–8612.

Moens, P.B., Kolas, N.K., Tarsounas, M., Marcon, E., Cohen, P.E., and Spyropoulos, B. (2002). The time course and chromosomal localization of recombination-related proteins at meiosis in the mouse are compatible with models that can resolve the early DNA-DNA interactions without reciprocal recombination. J. Cell Sci. *115*, 1611–1622.

Nakamoto, M., Nalavadi, V., Epstein, M.P., Narayanan, U., Bassell, G.J., and Warren, S.T. (2007). Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. Proc. Natl. Acad. Sci. USA *104*, 15537–15542.

Pei, H., Zhang, L., Luo, K., Qin, Y., Chesi, M., Fei, F., Bergsagel, P.L., Wang, L., You, Z., and Lou, Z. (2011). MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. Nature *470*, 124–128.

Peters, A.H., Plug, A.W., van Vugt, M.J., and de Boer, P. (1997). A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. Chromosome Res. *5*, 66–68.

Pittman, D.L., Cobb, J., Schimenti, K.J., Wilson, L.A., Cooper, D.M., Brignull, E., Handel, M.A., and Schimenti, J.C. (1998). Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. Mol. Cell *1*, 697–705.

Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S., and Bonner, W.M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J. Biol. Chem. *273*, 5858–5868.

San-Segundo, P.A., and Roeder, G.S. (2000). Role for the silencing protein Dot1 in meiotic checkpoint control. Mol. Biol. Cell *11*, 3601–3615.

Schwacha, A., and Kleckner, N. (1997). Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell *90*, 1123–1135.

Simon, M.D., Chu, F., Racki, L.R., de la Cruz, C.C., Burlingame, A.L., Panning, B., Narlikar, G.J., and Shokat, K.M. (2007). The site-specific installation of methyl-lysine analogs into recombinant histones. Cell *128*, 1003–1012.

Slegtenhorst-Eegdeman, K.E., de Rooij, D.G., Verhoef-Post, M., van de Kant, H.J., Bakker, C.E., Oostra, B.A., Grootegoed, J.A., and Themmen, A.P. (1998). Macroorchidism in FMR1 knockout mice is caused by increased Sertoli cell proliferation during testicular development. Endocrinology *139*, 156–162.

Steger, D.J., Lefterova, M.I., Ying, L., Stonestrom, A.J., Schupp, M., Zhuo, D., Vakoc, A.L., Kim, J.E., Chen, J., Lazar, M.A., et al. (2008). DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. Mol. Cell. Biol. *28*, 2825–2839.

Stoll, G., Pietiläinen, O.P., Linder, B., Suvisaari, J., Brosi, C., Hennah, W., Leppä, V., Torniainen, M., Ripatti, S., Ala-Mello, S., et al. (2013). Deletion of TOP3β, a component of FMRP-containing mRNPs, contributes to neurodevelopmental disorders. Nat. Neurosci. *16*, 1228–1237.

Stucki, M., and Jackson, S.P. (2006). gammaH2AX and MDC1: anchoring the DNA-damage-response machinery to broken chromosomes. DNA Repair (Amst.) *5*, 534–543.

The Dutch-Belgian Fragile X Consortium (1994). Fmr1 knockout mice: a model to study fragile X mental retardation. Cell *78*, 23–33.

Tibbetts, R.S., Cortez, D., Brumbaugh, K.M., Scully, R., Livingston, D., Elledge, S.J., and Abraham, R.T. (2000). Functional interactions between BRCA1 and the checkpoint kinase ATR during genotoxic stress. Genes Dev. *14*, 2989–3002.

Turner, G., Eastman, C., Casey, J., McLeay, A., Procopis, P., and Turner, B. (1975). X-linked mental retardation associated with macro-orchidism. J. Med. Genet. *12*, 367–371.

Turner, J.M., Aprelikova, O., Xu, X., Wang, R., Kim, S., Chandramouli, G.V., Barrett, J.C., Burgoyne, P.S., and Deng, C.X. (2004). BRCA1, histone H2AX phosphorylation, and male meiotic sex chromosome inactivation. Curr. Biol. *14*, 2135–2142.

Turner, J.M., Mahadevaiah, S.K., Fernandez-Capetillo, O., Nussenzweig, A., Xu, X., Deng, C.X., and Burgoyne, P.S. (2005). Silencing of unsynapsed meiotic chromosomes in the mouse. Nat. Genet. *37*, 41–47.

van 't Padje, S., Engels, B., Blonden, L., Severijnen, L.A., Verheijen, F., Oostra, B.A., and Willemsen, R. (2005). Characterisation of Fmrp in zebrafish: evolutionary dynamics of the fmr1 gene. Dev. Genes Evol. *215*, 198–206.

Wakeman, T.P., Wang, Q., Feng, J., and Wang, X.F. (2012). Bat3 facilitates H3K79 dimethylation by DOT1L and promotes DNA damage-induced 53BP1 foci at G1/G2 cell-cycle phases. EMBO J. *31*, 2169–2181.

Ward, I.M., and Chen, J. (2001). Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. J. Biol. Chem. *276*, 47759–47762.

Warren, S.T., and Nelson, D.L. (1994). Advances in molecular analysis of fragile X syndrome. JAMA *271*, 536–542.

Wienken, C.J., Baaske, P., Rothbauer, U., Braun, D., and Duhr, S. (2010). Protein-binding assays in biological liquids using microscale thermophoresis. Nat. Commun. *1*, 100.

Wysocki, R., Javaheri, A., Allard, S., Sha, F., Côté, J., and Kron, S.J. (2005). Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9. Mol. Cell. Biol. *25*, 8430–8443.

Yoshida, K., Kondoh, G., Matsuda, Y., Habu, T., Nishimune, Y., and Morita, T. (1998). The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis. Mol. Cell *1*, 707–718.