High NaCl causes Mre11 to leave the nucleus, disrupting DNA damage signaling and repair

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Dmitrieva, Natalia I., Dmitry V. Bulavin, and Maurice B. Burg. High NaCl causes Mre11 to leave the nucleus, disrupting DNA damage signaling and repair. Am J Physiol Renal Physiol 285: F266–F274, 2003.—High NaCl causes DNA double-strand breaks and cell cycle arrest, but the mechanism of its genotoxicity has been unclear. In this study, we describe a novel mechanism that contributes to this genotoxicity. The Mre11 exonuclease complex is a central component of DNA damage response. This complex assembles at sites of DNA damage, where it processes DNA ends for subsequent activation of repair and initiates cell cycle checkpoints. However, this does not occur with DNA damage caused by high NaCl. Rather, following high NaCl, Mre11 exits from the nucleus, DNA double-strand breaks accumulate in the S and G2 phases of the cell cycle, and DNA repair is inhibited. Furthermore, the exclusion of Mre11 from the nucleus by high NaCl persists following UV or ionizing radiation, also preventing DNA repair in response to those stresses, as evidenced by absence of H2AX phosphorylation at places of DNA damage and by impaired repair of damaged reporter plasmids. Activation of chk1 by phosphorylation on Ser345 generally is required for DNA damage-induced cell cycle arrest. However, chk1 does not become phosphorylated during high NaCl-induced cell cycle arrest. Also, high NaCl prevents ionizing and UV radiation-induced phosphorylation of chk1, but cell cycle arrest still occurs, indicating the existence of alternative mechanisms for the S and G2/M delays. DNA breaks that occur normally during processes such as DNA replication and transcription, as well as damages to DNA induced by genotoxic stresses, ordinarily are rapidly repaired. We propose that inhibition of this repair by high NaCl results in accumulation of DNA damage, accounting for the genotoxicity of high NaCl, and that cell cycle delays induced by high NaCl slows accumulation of DNA damage until the DNA damage-response network can be reactivated.

DNA damage to chromosomal DNA induces a complex cellular response designed to repair the DNA and delay cell cycle progression until it is repaired (37). Damage to DNA activates DNA repair systems and triggers checkpoints that prevent cell cycle progression. The Mre11 complex heads the DNA damage response. It is a multisubunit nuclease that includes Mre11, Rad50, and Nbs1/Xrs2. This complex tethers the ends of DNA molecules and possesses a variety of DNA nuclease, helicase, ATPase, and annealing activities. It detects DNA damage and processes DNA ends for subsequent activation of DNA repair and cell cycle checkpoints (reviewed in Ref. 9). All the members of Mre11 complex are essential in vertebrates (20, 31, 38) and defects in this complex lead to hypersensitivity to DNA damage and defects in triggering cell cycle checkpoints (reviewed in Ref. 9).

DNA damage detection is followed by DNA repair that is accompanied by cell cycle arrest (37). DNA repair involves recruitments of repair factors specific to different types of DNA damage. In the case of double-strand breaks, phosphorylation of histone H2AX is an important initial step in the recruitment of the repair factors and activation of the DNA repair (6, 26). The protein kinase Chk1 is activated in response to a variety of genotoxic agents and has an essential role in transducing the delay signal from damaged DNA to cell cycle machinery (5, 19, 21, 35, 36).

The recent discovery that high NaCl causes DNA double-strand breaks added it to the list of genotoxic stresses known to damage DNA (16). However, the mechanism by which high NaCl causes DNA damage is not known. The response to DNA damage caused by high NaCl includes induction of cell cycle checkpoints (11, 12, 17, 25), induction of the tumor suppressor p53 (10) and of the GADD45 growth arrest, and DNA damage-inducible proteins (7, 17).

Mre11 normally is a nuclear protein. However, in the present study, we show that high NaCl causes Mre11 to translocate out of the nucleus. In the presence of high NaCl, Mre11 remains in the cytoplasm even after UV or ionizing radiation (IR) that is known to induce DNA damage. Exclusion of Mre11 from the nucleus by high salt disrupts DNA damage signaling that is associated with failure of the DNA damage-repair network and leads to DNA damage accumulation. Also, Chk1 is not activated, but cell cycle delays still occur, evidently independent of chk1. When the level of NaCl is returned to normal, Mre11 returns to the nucleus and DNA repair is activated accompanied by chk1 phos-
porylation, indicating activation of DNA damage response. We propose that 1) by inhibiting DNA repair, high salt causes accumulation of the DNA breaks that normally occur during processes such as DNA replication and transcription or induced by genotoxic stresses and ordinarily are rapidly repaired and 2) by inducing chk1-independent cell cycle delay, high salt reduces accumulation of DNA damage until the DNA damage-response network can be reactivated.

**EXPERIMENTAL PROCEDURES**

**Cell cultures.** Mouse inner medullary collecting duct cells (mIMCD3) were a gift from S. Gullans (27). Mouse embryonic fibroblasts (MEF) were isolated from 13.5-day-old embryos. Each embryo with 1.0 ml trypsin was placed into syringe with an 18-gauge needle attached. The content was expelled, and this procedure was repeated twice. After incubation for 45 min at 37°C, trypsin was inactivated by complete medium (DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin). Isolated cells were harvested and cultured at 37°C in a humidified atmosphere of 95% air-5% CO₂. All our primary culture preparations were conducted in conformity with the Guiding Principles in the Care and Use of Animals of the American Physiological Society according to protocol approved by the Institutional Animal Care and Use Committee (protocol 2-KE-32).

Primary dermal fibroblasts (DFs) were isolated from the skin of newborn mice. The skin was incubated in 0.25% trypsin at 4°C for 18 h. Dermis was separated from epidermis and incubated in 0.35% collagenase (Worthington Biologicals) for 30 min at 37°C with gentle agitation. The suspension was pipetted several times to break the dermis apart. The cells were resuspended in the complete medium, plated, and cultured at 37°C in 5% CO₂. Cells were grown in 45% DME low glucose and 45% Coon’s Improved Medium mF-12 (Irvine Scientific) with 10% fetal bovine serum (HyClone) added. Hypertonic (high NaCl) medium was prepared by 10.220.33.4 on June 17, 2017 http://ajprenal.physiology.org/ Downloaded from www.ajprenal.org

**Replication, indicating activation of DNA damage response. We propose that 1) by inhibiting DNA repair, high salt causes accumulation of the DNA breaks that normally occur during processes such as DNA replication and transcription or induced by genotoxic stresses and ordinarily are rapidly repaired and 2) by inducing chk1-independent cell cycle delay, high salt reduces accumulation of DNA damage until the DNA damage-response network can be reactivated.**

**Experimental Procedures**

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IR. Cells were exposed to 5–8Gy from a 137Cs in a Shepherd Mark I irradiator. The medium was replaced with a fresh one before return to the incubator.

UV. Before treatment with UV, culture medium was removed and reserved. Cells were rinsed with cold PBS of the same osmolality as experimental medium and exposed to 15 J/m² of UV light in a Stratalinker UV Crosslinker (Stratagene, no. 400071). The reserved medium was replaced before return to the incubator.

**Protein sample preparation, Western blot analysis, and immunodetection.** Total cell extract: cells were rinsed with PBS adjusted with NaCl to the same osmolality as the medium and then lysed and processed as previously described (12). Nuclear and cytoplasmic cell extracts were prepared and grown as previously described (34). Osmolality of isotonic (control) medium was 320 mosmol/kgH₂O. Hypertonic (high NaCl) medium was prepared by adding NaCl to the control medium.

**Number of viable cells.** Cell number was estimated based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenase in viable cells. The cell proliferation reagent WST-1 was used according to the company’s instructions. The amount of BrdU incorporated in newly synthesized DNA was measured and analyzed by laser-scanning cytometry as previously described (11).

**Analysis of DNA damage by alkali comet assay.** A comet assay kit (Trevigen, no. 4250–050-K) was used according to the manufacturer’s instructions. Cells were rinsed with PBS, scraped off the dish, resuspended in low melting-point agarose, and spread on microscope slides. Slides were incubated for 1 h in lysis solution and then for 1 h in alkaline solution. Electrophoresis was performed at 4°C for 45 min in a horizontal apparatus at 1 V/cm and 300 mA in the alkaline solution. DNA was stained with SYBR Green. Distribution of DNA between the tail and the head of the comet was analyzed with Scion Image software (Scion, Frederick, MD).

**Repair of Renilla luciferase reporter vector.** pRL-CMV vector (Promega, no. E2261) was damaged by UV in a Stratalinker UV Crosslinker (Stratagene, no. 400071). mIMCD3 cells were placed in high NaCl (total osmolality 600 mosmol/kgH₂O) for 1 h. Normal or UV-damaged pRL-CMV vectors were then transfected into the cells using TransFast Transfection Reagent (Promega, no. E2431). One hour later, the transfection reagent was washed out and cells were placed in either control or high-NaCl medium. Luciferase production was quantified after 16 h with a Renilla luciferase assay system (Promega, no. E2820). To estimate the repair efficiencies, data were normalized to values obtained from undamaged plasmids.

**Analysis of DNA replication rate by BrdU labeling.** Cells were pulse labeled for 45 min with 10 µM BrdU and stained for BrdU using BrdU labeling and a detection kit (Roche, no. 1644807). High NaCl causes accumulation of DNA damage and inhibits the classic DNA damage response. Inspired by the report that osmotic stress in form of high NaCl used specific antibodies against Mre11 (Oncogene, PC388), phospho-H2AX (Ser139) (Upstate, 07–164), and phospho-Histone H3 (Upstate, 06–570). Bound primary antibodies were detected with Alexa 488 goat anti-rabbit IgG (green fluorescence) (Molecular Probes, no. A-11034). DNA was stained with propidium iodide (PI; red fluorescence). The slides were analyzed with a laser-scanning cytometer (CompuCyte, Cambridge, MA) as previously described (12). Briefly, integral green fluorescence from the nuclear area (defined by PI staining) was recorded as a measure of phospho-H2AX (Ser139), Mre11, or phospho-H3 content. Green maximal pixel brightness within nuclei was recorded to identify phospho-H2AX (Ser139)-positive cells. Integral red fluorescence was recorded as a measure of DNA content to identify cells in different phases of cell cycle. Regions of the cytograms that included (Mre11 cytograms) or excluded (phospho-H2AX cytograms) the majority of cells (90–100%) in control samples were delineated by eye and percentage of cells in that region was determined for all experimental conditions.

**RESULTS**

High NaCl causes accumulation of DNA damage and inhibits the classic DNA damage response. Inspired by the report that osmotic stress in form of high NaCl...
induces DNA double-strand breaks (16), we examined the behavior of some major components of the DNA damage network that are usually activated in response to genotoxic stresses.

Mre11 is a nuclear protein that ordinarily accumulates at sites of the DNA damage (23). However, this does not occur following DNA damage induced by high NaCl. In fact, high NaCl causes Mre11 to translocate reversibly from the nucleus to the cytoplasm both in mIMCD3 and MEF cells (Fig. 1A). This causes a large and reversible reduction in nuclear Mre11 abundance (Fig. 1, A and B). p53 abundance and phosphorylation on Ser15 are increased by high NaCl in mIMCD3 cells (10). To test for a possible role of p53 in the high NaCl-induced translocation of Mre11, we examined p53-null cells. The translocation also occurs both in p53−/− MEFS and in p53−/− DF cells (Fig. 1A), excluding a role for p53 in this process. High NaCl has been reported to kill mIMCD3 cells by apoptosis (24, 25, 29). Consistent with this, elevated NaCl reduces mIMCD3 cell number (Fig. 1C). However, if the cells are returned to the control level of NaCl after 2 h, cell number decreases much less (Fig. 1C), associated with translocation of Mre11 back into the nucleus (Fig. 1, A and B).

Histone H2AX is usually phosphorylated in response to DNA double-strand breaks (28) and recruits repair factors (6, 26). However, it is not phosphorylated in response to high NaCl (Fig. 2A). Genomic stress also usually results in phosphorylation of chk1 kinase, signaling cell cycle arrest (5, 19, 33, 36). However, high NaCl reduces its phosphorylation in mIMCD3, which is independent of p53 as it also occurs in p53−/− DF cells (Fig. 2E).

NaCl-induced translocation of Mre11 and inhibition of phosphorylation of histone H2AX and Chk1 suggest that high NaCl might inhibit repair of the DNA that it damages. When the high-NaCl concentration is reduced back to the control level, not only does Mre11 return to the nucleus (Fig. 1, A and B), but histone H2AX and chk1 become phosphorylated (Fig. 2, A and E), evidently in response to the as yet damaged DNA.

After NaCl concentration returns to normal, H2AX rapidly becomes phosphorylated, remains phosphorylated for several hours (Fig. 2C), and returns to basal level simultaneously with levels of DNA damage as measured by comet assay (Fig. 2D). The cells resume growth and survive much better (Fig. 1C). As seen from Fig. 2B, the DNA damage was accumulated with time and took place mostly in S-G2/M cells. The same time course and cell cycle position were obtained for chk1 phosphorylation in mIMCD3 cells (data not shown).

It is noteworthy that similar results were obtained with all the other types of cells that were tested in addition to mIMCD3 cells, including MEFS [wild type (wt) and p53−/−; Fig. 1A], DF (p53−/−; Figs. 1A and 2E), p2mIME cells (94) from wt mice (H2AX and chk1 phosphorylation were tested, data not shown), and p2mIME cells from p53−/− mice (H2AX phosphorylation was tested, data not shown).

These results provide an explanation for how high NaCl might cause DNA double-strand breaks. We pre-}

Fig. 1. High NaCl causes reversible translocation of Mre11 from nucleus to cytoplasm. A and B: medium was changed from control (320 mosmol/kgH2O) to high NaCl or urea (600 mosmol/kgH2O) and then 2 h later back to control. A: Western blot analysis of Mre11 abundance in total cell extracts, cytoplasmic (cyto) and nuclear (nucl) fractions. wt, Wild type. B: nuclear Mre11 content in mouse inner medullary collecting duct cells (mIMCD3), immunocytochemistry, analyzed by laser-scanning cytometry. C: viable mIMCD3 cells estimated from cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenase. Cell number is relative to that (= 1) before NaCl was added at time 0. Survival is greater when cells are returned to 320 mosmol/kgH2O medium after 2 h in high NaCl than when they remain in high-NaCl medium. wt MEFS, wild-type mouse embryonic fibroblasts.
Fig. 2. In high NaCl (600 mosmol/kgH2O), DNA damage accumulates mostly in S and G2/M phases of the cell cycle, but DNA repair is not activated nor is chk1 phosphorylated until cells return to 320 mosmol/kgH2O. A–C: mIMCD3 cells were immunostained with anti-P-H2AX antibody (green), also stained with propidium iodide (for DNA content) (red), and analyzed by laser-scanning cytometry. A: representative cytograms showing no increase in the number of P-H2AX-positive cells (above the line) after change from 320 to 600 mosmol/kgH2O by adding NaCl for 2 h and greatly increased number of P-H2AX-positive cells on return to 320 mosmol/kgH2O medium for 0.5 h. B: cells were exposed to high NaCl (600 mosmol/kgH2O; ●). At indicated times, they were returned to 320 mosmol/kgH2O medium for 0.5 h (○). The number of cells that become P-H2AX positive after return for 0.5 h to 320 mosmol/kgH2O (○) increases with more previous hours in high NaCl (600 mosmol/kgH2O). The positive cells are mainly in S and G2/M phases of the cell cycle. C: cells were exposed to high NaCl (600 mosmol/kgH2O; ●). After 2 h (indicated by arrows), some of them were returned to 320 mosmol/kgH2O and fixed at indicated time points (○). The level of H2AX phosphorylation increases sharply after return to control medium but returns to basal after several hours. D: single-cell gel electrophoresis (comet) assay of DNA damage in mIMCD3 cells. Damaged DNA appears in the “tails” of the “comets.” Left: representative nuclei stained with SYBR Green. Right: ratio of DNA in comet tails/comet heads. High NaCl damages DNA, and the damage is repaired after return to 320 mosmol/kgH2O medium. E: chk1 phosphorylation on Ser345 decreases in high NaCl (600 mosmol/kgH2O) and then greatly increases after return to control (320 mosmol/kgH2O). Western blot analysis of total chk1 and P-chk1 (Ser345).
viously showed that the lethality of high NaCl is greatest in the S phase of the cell cycle, while DNA is replicating (11). Mre11 complexes form not only at sites of exogenously induced DNA damage but also at sites of DNA replication (22). Removal of the Mre11 from frog extracts in which DNA is replicating induces double-strand breaks in the newly replicated DNA (8). Thus high NaCl might induce accumulation of DNA double-strand breaks in S phase cells under otherwise normal conditions, because breaks that normally occur during DNA replication and are rapidly repaired are not repaired in the absence of nuclear Mre11.

However, failure to repair transient breaks that occur during DNA replication does not explain the increased levels of H2AX phosphorylation in G2/M and G1 cells upon return to isotonic medium (Fig. 2B).

Nevertheless, high NaCl does exclude Mre11 from nuclei in those phases of the cell cycle, which could cause DNA double-strand breaks by interrupting other processes involving Mre11. Also, additional processes, not dependent on Mre11, might contribute to the DNA damage. That the DNA damage depends on the tonicity of NaCl and is mediated by Mre11 is supported by the observation that high urea does not affect Mre11 localization (Fig. 1A, bottom) and does not cause DNA double-strand breaks (16).

**High NaCl impairs repair of DNA damage caused by UV and IR.** We next asked if high NaCl also impairs the response to DNA damage induced by UV and IR. After UV and IR, Mre11 normally remains in the nucleus (Figs. 3A and 4A), and histone H2AX (Figs. 2B and 4B) and Chk1 (Figs. 3C and 4C) become phosphor-

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**Fig. 3.** High NaCl inhibits repair of UV-induced DNA damage and prevents UV-induced chk1 phosphorylation. A–C: after 1 h in control medium (320 mosmol/kgH2O) or high-NaCl medium (600 mosmol/kgH2O), cells were exposed to UV. One hour later, some cells were returned to control medium for 30 min. A: amount of Mre11 analyzed by immunoblot. In the presence of high NaCl, most Mre11 is cytoplasmic, rather than nuclear, even after UV radiation. B: amount of Mre11 (top) and phosphorylated H2AX (bottom) in nuclei of mIMCD3, analyzed by immunocytochemistry and laser-scanning cytometry. In the presence of high NaCl, nuclear abundance of Mre11 and phosphorylated H2AX is reduced. C: mIMCD3 cell extracts were analyzed by immunoblot for total chk1 and P-chk1 (Ser345). High NaCl prevents UV-induced phosphorylation of Chk1. D: number of mIMCD3 cells, estimated from cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenase 24 h after UV radiation. The cells were radiated in the presence of the levels of NaCl that are shown. Survival of cells that remain in high NaCl (left) falls progressively with increments in NaCl concentration. Many more cells originally in high NaCl survive if they are returned to control osmolality 2 h after the UV radiation (right). E: expression of luciferase by transfected pRL-CMV-luciferase vector (means ± SE of 3 independent experiments, with triplicate measurements in each; *P < 0.05, NaCl vs. control, t-test). Because the vector was damaged by the doses of UV radiation that are shown, luciferase expression depends on DNA repair within the cells. DNA repair is impaired by high NaCl (600 mosmol/kgH2O) both in mIMCD3 (left) and p53-null MEFs (right).
Fig. 4. High NaCl inhibits repair of ionizing radiation (IR)-induced DNA damage and prevents IR-induced chk1 phosphorylation. mIMCD3 in control medium (320 mosmol/kgH2O) or after the first hour in high-NaCl (600 mosmol/kgH2O) medium were exposed to 5 Gy of IR. After 0.5 h, some cells in high-NaCl medium were returned to control medium for 0.5 h. A: amount of Mre11 analyzed by immunoblot. In the presence of high NaCl, most Mre11 is cytoplasmic, rather than nuclear, even after IR. B: amount of phosphorylated H2AX in nuclei of mIMCD3, analyzed by immunocytochemistry and laser-scanning cytometry. In the presence of high NaCl, nuclear abundance of phosphorylated H2AX is reduced. C: mIMCD3 cell extracts were analyzed by immunoblot for total chk1 and P-chk1 (Ser345). High NaCl prevents IR-induced phosphorylation of Chk1.

In high NaCl, S and G2/M cell cycle delays induced by DNA damage are independent of chk1. Both Mre11 and chk1 (5, 19, 21, 35, 36) deficiency leads to checkpoint activation defects. However, high NaCl activates checkpoints in all phases of the cell cycle (11, 25) despite suppression of Mre11 and Chk1. Activation and maintenance of G2/M arrest by high NaCl are not dependent on chk1 (12), consistent with the absence of chk1 phosphorylation (Fig. 2E). In the next experiment, we investigated how chk1 inactivation by high NaCl influences checkpoint activation in response to UV and IR.

To assess S phase checkpoint, we measured DNA replication rate by BrdU incorporation. In control medium (320 mosmol/kgH2O; Fig. 5A, left), either UV or IR reduces DNA replication rate. UCN-01, which inhibits chk1 activity, increases BrdU incorporation, whether or not the cells are radiated, consistent with a role for Chk1 in the suppression of DNA replication. In contrast, the inhibition of BrdU incorporation by high NaCl is not prevented by UCN-01 (Fig. 5A, right), suggesting that Chk1 is not involved in high NaCl-induced delay of the cell cycle in S. Furthermore, although UCN-01 inhibits the S phase delay caused by IR or UV in control medium (Fig. 5A, left), it does not when NaCl is high (Fig. 5A, right).

To assess the G2/M checkpoint, we measured mitotic index, which depends on the G2/M transition rate. In control medium (Fig. 5B, left), either UV or IR reduces the percent of cells in mitosis, consistent with activation of the G2/M checkpoint. UCN-01 increases the percent of cells in mitosis in the control condition and after IR, but not after UV. This is consistent with previous reports that UV-induced G2/M delay depends on p38 but not on chk1 (4). In high NaCl, UCN-01 does not abrogate G2/M delay after IR or UV (Fig. 5B, right). Thus, in high NaCl, cells are still able to activate S and G/M checkpoints despite chk1 inactivation.
DISCUSSION

The findings presented here demonstrate that high NaCl dramatically modifies the response to DNA damage, as summarized in Fig. 5C. Mre11 normally resides in the nucleus, where it detects DNA damage and processes DNA ends for subsequent activation of DNA repair and activation of cell cycle checkpoints (reviewed in Ref. 9). High NaCl results in exclusion of Mre11 from the nucleus, disrupting DNA damage signaling, so that DNA repair and chk1 kinase are not activated. In the present study, we assessed only two types of DNA repair, namely repair of double-strand breaks, monitored by H2AX phosphorylation, and nucleotide excision repair, monitored with a UV-damaged reporter vector. However, high NaCl presumably also interrupts additional processes involving Mre11, such as telomere maintenance, sister chromatid association, and homologous recombination (reviewed in Ref. 9), as well as other processes requiring linked binding of two DNA substrates (15). The effect of high NaCl on Mre11 resembles that of hyperthermia. Hyperthermia also induces Mre11 translocation out of the nucleus and sensitizes cells to radiation (32). It would be interesting to know whether the analogy carries further and hyperthermia disrupts repair of DNA damage, as does high NaCl.

Mre11 deficiency usually compromises the triggering of cell cycle checkpoints (9, 30). However, although high NaCl causes Mre11 to exit from the nucleus, cell cycle checkpoints are still activated and maintained for some time (Ref. 11 and Fig. 5, A and B). Stress-induced cell cycle arrest generally requires phosphorylation of Chk1, but in the presence of high NaCl, Chk1 is not phosphorylated in response to DNA damage and S and G2/M cell cycle arrests are not affected by inhibition of chk1 activity. Thus, when NaCl is high, cell cycle checkpoints are activated in response to DNA damage but by mechanisms independent of chk1.

There were previous indications that the mechanisms of cell cycle arrest following high NaCl might differ from those following other stresses. Compare, for example, the G2/M arrest that occurs after IR-induced DNA damage to that caused by high NaCl. In both cases, arrest occurs quickly and involves inhibition of...
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Cdc2 (3, 12). However, although IR acts through the ATM/ATR-chk1 kinase pathway (1, 19), high NaCl does not. Thus caffeine, an ATM/ATR inhibitor, and UCN-01, a chk1 inhibitor, impair the G2/M arrest following IR (1, 19) but not following high NaCl (12). Instead, p38 kinase is necessary for fast activation of G2/M arrest by high NaCl (12), similar to its role in response to UV radiation (4). High NaCl-induced activation of p38 is transient, but the arrest persists, becoming insensitive to p38 inhibition (12). Furthermore, ATM/ATR-chk1 inhibition abrogates IR- or UV-induced arrest (1, 3) but not high NaCl-induced arrest (12). When the NaCl level falls or when cells accumulate sufficient levels of compatible organic osmolytes in response to the hypertonicity (13), the cell cycle resumes.

Taken together, these findings indicate that the DNA damage response is salt sensitive and that disruption of DNA damage signaling by high NaCl impairs DNA repair and threatens genomic stability.

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REFERENCES


