The Bloom's syndrome helicase stimulates RAD51 DNA strand exchange activity through a novel ATPase-independent mechanism

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Supplementary Figure S1

Supplementary Figure Legends

Supplementary Table S1

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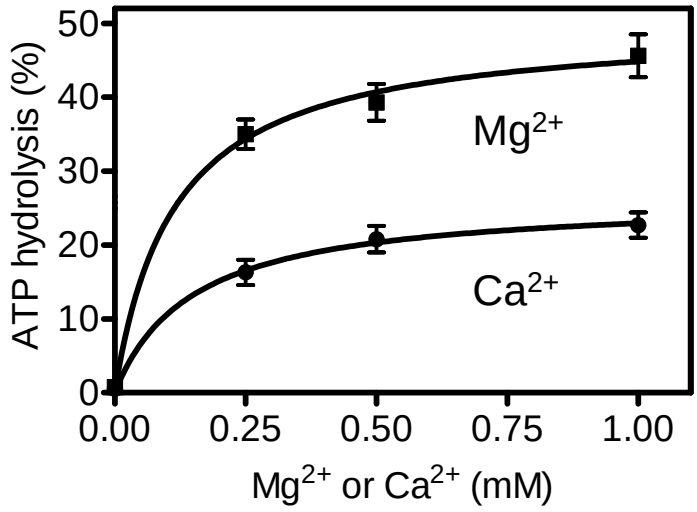
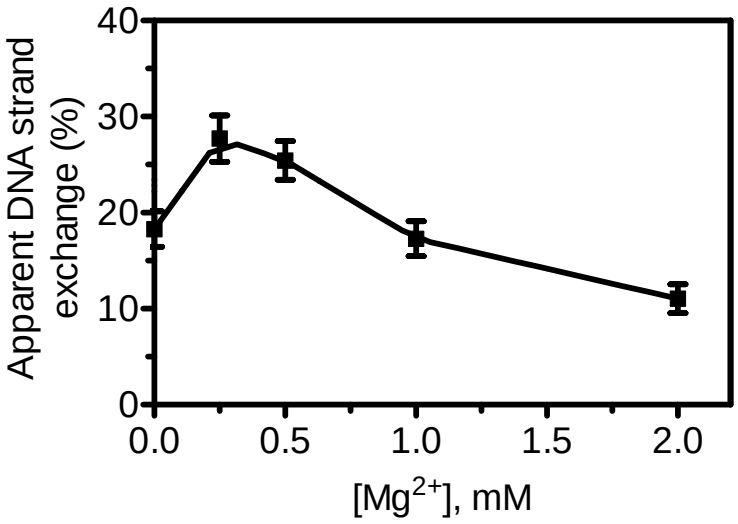
Supplementary Figure S1

A



B



SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. The effect of Mg^{2+} concentration on the biochemical activities of BLM. (A) The ATPase activity of BLM. The BLM protein (10 nM) was incubated with polydT ssDNA (10 μM) and 1 mM ATP, 0.2 μCi of [γ-32P]ATP (6000 Ci/mmol) in reaction buffer (10 μl) containing 25 mM Tris acetate, pH 7.5, 2 mM DTT, 25 mM KCl, 100 μg/ml BSA, and indicated $MgCl_2$, or $CaCl_2$ concentrations at 37 °C for 30 min. The level of ATP hydrolysis was determined using TLC on PEI-cellulose plates in running buffer containing 1 M formic acid and 0.3 M LiCl. The products of ATP hydrolysis were quantified using a Storm 840 PhosphorImager (Molecular Dynamics). (B) Apparent DNA strand exchange promoted by BLM. BLM (100 nM) was mixed with ssDNA (#71; 32 nM, molecules) in buffer containing 25 mM Tris acetate, pH 7.5, 1 mM ATP, 2 mM DTT, BSA (100 μg/ml), 20 mM phosphocreatine, and creatine phosphokinase (30 units/ml) and indicated concentrations of magnesium acetate. To start the reaction ^{32}P-labeled dsDNA (#5/#6; 32 nM, molecules) was added followed by 15 min incubation at 37 °C. The reactions were terminated and deproteinized by addition of stop buffer up to 1.5% SDS and proteinase K (800 μg/ml) for 5 min at 25 °C, mixed with a 0.1 volume of loading buffer (70% glycerol, 0.1% bromophenol blue) and analyzed by electrophoresis in 10% native polyacrylamide gels in TBE buffer (90 mM Tris-borate, pH 8.3, and 1 mM EDTA) at 135 V for 1.5 hr.

SUPPLEMENTARY TABLE S1. Sequences of the oligonucleotides used in this study

N	Length, nt	Sequence (5'→3')
5	32	CCATCCGCAAAAATGACCTCTTATCAAAAGGA
6	32	TCCTTTTGATAAGAGGTCATTTTTGCGGATGG
71	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGA TTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTT ATCAAAAGGA
90	90	CGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAG ATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCA CAGATGCGT
422	90	CGGGTGCCGGGGCTAGCTTAACTTTGCGGCATCAGCGCAG ATTGTACTTAGAGTGCACCCTATGCGTTGTGAAATACCACAC AGATGCGA
423	90	CGGGTGCCGGGGCTGGCTTAACTTTGCGGCATCAGAGCAG ATTATACTGAGAGTGCACCCTATGCGGTGTGAAATACCACAC AGATGCGT