

## THE BLOOM SYNDROME PROTEIN BLM IS SELECTIVELY CLEAVED DURING APOPTOTIC CELL DEATH

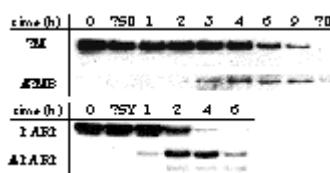
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**INTRODUCTION.** Bloom syndrome (BS) is an autosomal recessive disorder characterized by a high incidence of cancer and genomic instability. BLM, the protein defective in BS, is a RECQL-like helicase that is presumed to function in mammalian DNA replication, recombination or repair. Because of its importance in maintaining genomic stability, we asked whether BLM was among the proteins targeted for selective degradation during the execution phase of apoptosis as has been shown recently for PARP and ATM (1, 2), among others. We show here that BLM is rapidly cleaved in cells undergoing apoptosis.

**RESULTS.** BLM was cleaved to 47 and 110 kDa major fragments, with kinetics similar to the apoptotic cleavage of poly-ADP ribose polymerase (only  $\Delta$ BLM of 47 kDa is shown in the Fig.). BLM cleavage was prevented by a caspase 3 inhibitor, and did not occur in caspase 3-deficient cells. Moreover, recombinant BLM was cleaved to 47 and 110 kDa fragments by caspase 3, but not caspase 6, *in vitro*. The caspase 3 recognition sequence 412TEVD415 was verified by mutating aspartate-415 to glycine, and showing that this mutation rendered BLM resistant to caspase 3 cleavage. Apoptotic cleavage did not abolish the BLM helicase activity, but abolished BLM nuclear foci and the association of BLM with condensed DNA and the insoluble matrix. The results suggest that BLM is an early selected target during the execution of apoptosis.



**DISCUSSION.** Caspases are important for both the initiation and execution phases of apoptosis (3-5). At present, there is a need to identify caspase substrates in order to understand how caspases execute apoptosis. In recent years, a number of caspase substrates have been identified (5). Here, we show that BLM is a substrate for the executioner caspase 3, *in vitro*

and *in vivo*. Immunostaining and biochemical fractionation showed that cleaved BLM lost its characteristic punctate nuclear localization, detached from an insoluble substructure, and dissociated from condensed DNA. Cleavage and loss of localization very likely obliterates the *in vivo* function of BLM. The potential function of BLM in DNA repair suggests that its cleavage and redistribution may aid nuclear disassembly and prevent the complex in which it resides from participating in the repair of fragmented DNA molecules generated by caspase-activated DNase I.

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**REFERENCES.**

1. Lazebnik, Y.A., Kaufmann, S.H., Desnoyers, S., Poirier, G.G., and Earnshaw, W.C. (1994) *Nature* 371, 346-347
2. Smith, G.C., di Fagagna, F., Lakin, N.D., and Jackson, S.P. (1999) *Mol. Cell. Biol.* 19, 6076-6084
3. Cohen, G.M. (1997) *Biochem. J.* 326, 1-16
4. Budihardjo, I., Oliver, H., Lutter, M., Luo, X., and Wang, X. (1999) *Annu. Rev. Cell Dev. Biol.* 15, 269-290
5. Earnshaw, W.C., Martins, L.M., and Kaufman, S.H. (1999) *Annu. Rev. Biochem.* 68, 383-424