

The C-terminal domain of the Bloom syndrome DNA helicase is essential for genomic stability

ABSTRACT

The helicase activity and the C-terminal domain of BLM are critical for maintaining genomic stability as measured by the sister chromatid exchange assay. The localization of BLM into the nucleolus by the C-terminal domain appears to be more important to genomic stability than localization in the nuclear bodies.

INTRODUCTION

Background BLM is a member of the RecQ family of DNA helicases Bloom syndrome (BS) is a rare cancer-prone autosomal recessive disorder characterized by genomic instability, immunodeficiency, infertility and small stature. BS cells have a distinctive genomic instability: a high frequency of sister chromatid exchange (SCEs) and quadriradial formation. BLM, the gene mutated in BS, encodes a DNA helicase (BLM) of the RecQ family. BLM shares the most identity in the helicase domain to the mouse and *Xenopus* orthologs, to a predicted *C. elegans* protein CAB05609, and to *D. melanogaster* dmBLM. BLM can partially complement phenotypes of mutations in the *S. cerevisiae* SGS1 gene. There are two published reports of BLM knock-out mice: one strategy used a single deletion allele and found that the homozygous null mutants are embryonic lethals; the second strategy used two different deletion alleles and recovered full sized, fertile compound heterozygote mice with an elevated incidence of cancer. The second mouse model was made with ES cells that have a high frequency of SCEs before injection and recapitulates the BS phenotypes more accurately. There are four other human genes in the RecQ family: RecQL / RecQL, WRN, RecQ4, RecQ5. WRN is the gene mutated in Werner syndrome, a premature aging disorder; WS cells also show features of genomic instability. WRN encodes an exonuclease activity and shares many similar in vitro helicase activities with BLM. Mutations in the RECQ4 gene have been found in persons with Rothmund-Thomson syndrome, a rare premature aging and cancer-prone disorder. Previous work from this laboratory and others demonstrated the DNA helicase activity of BLM in vitro on a variety of DNA substrates. Transfection of the normal BLM cDNA (but not missense alleles lacking helicase activity) into BS cells reduces the frequency of SCEs, indicating that the DNA helicase activity of BLM is essential for genomic stability. BLM is localized in nuclear bodies and the nucleolus The BLM DNA helicase is found in two distinct nuclear structures in normal human cells, ND10 or PML nuclear bodies (NBs) and the nucleolus. The NBs are dynamic PML-dependent depots of multiple proteins disrupted upon viral infection and in certain human malignancies. BS cells have NBs of normal morphology, and cells lacking PML destabilize the NBs and have a two fold increase in the frequency of SCEs. NBs have been implicated in the regulation of apoptosis although their precise function is still unknown. BLM expression is cell-cycle regulated, showing a marked increase in S phase and peaking in G2. The increase in BLM mRNA and protein expression coincides with its appearance in the nucleolus. This report uses a series of inducible cell lines containing deletion alleles of BLM to investigate the role of the N-terminal and C-terminal domains of BLM in nuclear localization and in the maintenance of genomic stability. We find that the N-terminal domain directs BLM for packaging in NBs, while the C-terminal domain is required for efficient nucleolar localization. Compared to the normal BLM protein, deletions of the C-terminus and mutation of the helicase domain have a strong negative

effect on genomic stability whereas deletions of the N-terminus have less effect.

CONCLUSION

Conclusions The GFP-BLM fusion protein has helicase activity in vitro and functions to reduce the SCE frequency in BS cells. Deletions of BLM expressed as GFP fusion proteins demonstrate that the N-terminal domain directs BLM into nuclear bodies and the C-terminal domain is necessary for efficient nucleolar localization. The stable nucleolar localization of BLM is not due to over-expression but rather to a function encoded in the C-terminus of BLM. Mutations in the helicase domain and deletions in the C-terminal domain have a dominant negative effect on the SCE frequency and cause an increase in chromosome abnormalities, whereas N-terminal deletions have relatively little effect. These data demonstrate that the helicase activity and C-terminal domain directed nucleolar localization of BLM is essential to maintain genomic stability. The NBs appear to be storage or regulatory sites for BLM and are not required for BLM activity.