The non-specific lethal (NSL) complex prevents R-loop dependent DNA replication stress

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The non-specific lethal (NSL) complex is a chromatin remodeling complex that regulates the transcription of housekeeping genes. It consists of at least 9 proteins, including the core members KANSL1, KANSL2 and KANSL3, as well as the catalytic subunit MOF. NSL/MOF has acetyltransferase activity directed against lysine 5 and 8 of histone 4 at promotors of housekeeping genes, which is pivotal for transcription of these genes. Intriguingly, NSL has also been implicated in genome stability maintenance, yet how its role therein is linked to its function in transcription regulation is unclear. We found that loss of the NSL complex not only renders cells hypersensitive to hydroxyurea-induced replication stress, but also to endogenous replication stress as indicated by increased phospho-RPA (S33) levels, reduced DNA synthesis and an accumulation of cells in G1 and G2/M phase. Accordingly, DNA fiber analysis revealed that replication fork speed is reduced in unperturbed conditions, while replication fork stalling/collapse is increased in hydroxyurea-treated cells. Since NSL is important for transcription and impaired transcription can lead to the formation of DNA-RNA hybrids, we assessed whether these structures could be the cause of the increase in replication fork stalling/collapse. Indeed, we found that DNA-RNA hybrid levels increased dramatically after NSL loss and that this results in increased collisions between replication forks and DNA-RNA hybrids. These collisions likely cause increased DNA damage as indicated by the observed elevated levels of γH2AX and p-RPA (S4/S8). We conclude that the NSL complex suppresses DNA-RNA hybrid formation, probably at promotors of NSL-regulated housekeeping genes, thereby preventing collisions between replication forks and DNA-RNA hybrids. This couples NSL's role in transcription regulation to preventing replication stress-induced genomic instability.