A regulatory model for Xist expression during X chromosome inactivation

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For dosage compensation of X-linked genes, female mammalian cells inactivate one of their X chromosomes early in development in a process called X-chromosome inactivation. A major regulator of this process is the long non-coding RNA Xist which is expressed exclusively from the future inactive X chromosome and which mediates chromosome-wide gene silencing in cis. We combine experimental approaches and mathematical modeling to elucidate how monoallelic and female-specific Xist expression is ensured. A stochastic Gillespie model of the underlying regulatory network was developed and its parameters were varied to achieve monoallelic Xist upregulation. This model consists of a cis-acting positive feedback loop together with a trans-acting negative feedback and can recapitulate monoallelic Xist upregulation. The negative feedback loop is mediated by a dose-dependent X-linked activator that acts in trans to activate Xist, thus restricting Xist expression to female cells. The X-linked activator is silenced in cis by Xist RNA to prevent Xist upregulation from the second chromosome after X inactivation. The positive feedback is required to stabilize Xist expression once it has been upregulated and is mediated by transcriptional interference between Xist and Xist's repressive antisense RNA Tsix. Mutual inhibition of Xist and Tsix must occur at two levels to allow stable Xist upregulation: Direct transcriptional interference through polymerase collisions during antisense transcription must be combined with a repressive effect of the Xist RNA on Tsix initiation. To characterize the model rates and to experimentally distinguish alternative models, simulation results are compared to quantitative single-molecule RNA FISH data. The mature RNA and the nascent transcript of Xist were quantified together with its antisense transcript Tsix. To test the model, mutants in which single modules of the feedback loops are disturbed, will be constructed and changes in Xist and Tsix expression will be quantified.